PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.1.11

Review Article
Evidence-Based Therapeutic Effects of Anthocyanins from Foods

[1,2]Hock Eng Khoo, [3]See Meng Lim and [1,2]Azrina Azlan

[1]Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Research Centre of Excellence for Nutrition and Non-Communicable Diseases, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Nutritional Sciences Programme and Centre for Community Health, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Abstract
This review covers potential health benefits and efficacies of anthocyanins as well as anthocyanidins, in the prevention of several diseases. Blue, red and purple coloured fruits, vegetables and grains are rich in anthocyanins and have several health benefits, such as prevention of chronic diseases, antimicrobial, antioxidative and anti-inflammatory effects as well as improve vision and memory. Various *in vitro* and *in vivo* studies demonstrated the efficacy of anthocyanins in fruits and vegetables for the prevention of diseases and other health benefits. Most of the studies showed positive results towards the improvement of disease conditions. In short, anthocyanins and the anthocyanin-rich extracts are some of the best remedies used in prevention of several diseases, memory enhancement and behavioural improvement.

Key words: Anthocyanin, antimicrobial, cancer, health benefit, obesity

Received: June 29, 2018 Accepted: September 08, 2018 Published: December 15, 2018

Citation: Hock Eng Khoo, See Meng Lim and Azrina Azlan, 2019. Evidence-based therapeutic effects of anthocyanins from foods. Pak. J. Nutr., 18: 1-11.

Corresponding Author: Azrina Azlan, Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia Tel: +603 8947 2466

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anthocyanins are the coloured pigments found in plant. These pigments are belonging to the phenolic group as they share the same basic flavonoid structure with a positively charged oxygen atom at the C-ring of the structure[1]. The flavylium ion of anthocyanin is stabled at acidic condition, whereas blue quinonoidal species is formed at increasing pH. Anthocyanin appears red while blue hue can be seen in basic solution[2]. The red hue of a plant comes mainly from the flavylium ion of anthocyanins. Among many types of anthocyanidin found in plants, cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin are the most common types of anthocyanidin detected in flowers, fruits and leaves[1].

Anthocyanins play essential roles in plant physiology, in the food industry and human health. Numerous studies have been performed to investigate the health-promoting effects of anthocyanins against some diseases, using *in vitro* and *in vivo* models. Most of the studies only focused on certain types of diseases or the selected health benefits. Although, a review article reported the potential health benefits of anthocyanins from plants, the review did not cover antioxidant capacity, anticancer and antimicrobial effects of anthocyanins. Therefore, this review included the health benefits of anthocyanins reported in the literature, which covered the significant findings on antioxidative and antimicrobial effects, improving coronary heart disease, antiobesity and antidiabetes, anticancer activities, improving neurodegenerative disorders and increasing visual acuity. Table 1 summarises some of the recent studies on the protective effects of anthocyanins from natural products using either animal or human model.

ANTIOXIDATIVE EFFECT

Free radicals are highly reactive and the oxygen-containing molecule has one or more unpaired electrons. These free radicals generated in the body chemically interact with the electrons of the cell components such as deoxyribonucleic acid (DNA), protein or lipid in order to be stabilised[3]. The cumulative oxidative damage that triggered by the free radicals in the body can elicit various diseases including cancer, inflammation, diabetes, rheumatoid arthritis, heart and blood vessel disorders, cataracts and ageing[4]. In fact, an antioxidant is able to scavenge or reduce the effect of free radicals. It also allows other compounds to perform such functions and thus help to maintain a balance between the effect of free radicals and oxidative stress in the body[5].

Anthocyanins are the phenolic compounds that exhibit antioxidative properties. Several studies demonstrated that anthocyanins-rich foods such as berries, blackcurrant and grapes exhibited great antioxidant properties (Table 1). The compounds are able to scavenge free radicals and cease the chain reaction that will cause oxidative damage[6]. The radical scavenging effect and antioxidative activity of anthocyanin, owing to the presence of hydroxyl group in position 3 of the C-ring of anthocyanin molecules as well as the hydroxyl groups in 3', 4' and 5' positions of the B-ring[7]. However, the glycosylated form of anthocyanin decreases the ability of anthocyanin radicals to delocalise electrons. Thus the glycosylated anthocyanin reduces the radical scavenging activity in comparison to its aglycone.

A study reported that cyanidin-3-galactoside extracted from apple peel was more sensitive to hydrogen peroxide (H_2O_2), most active free radical and contributes more to H_2O_2

Table 1: Antioxidative and cardioprotective effects of anthocyanins in foods

Model	Sample	Study design	Outcome
Rats[13]	Red cabbage extract	Atherogenic diet-induced hypercholesterolaemia	• ↑SOD, catalase, reduced GSH and AA levels • ↓Lipid peroxidation • ↓Serum lipids profile and AI • ↓Markers of cardiac (CK-MB and LDH) and hepatic damage (AST and ALT) • ↑Lipids excretion in feces
Cell culture[14]	Defatted dabai peel extract	*In vitro* bioassays	• ↓Lipid peroxidation (plasma MDA) • ↑SOD and GPx levels
White rabbits[15]	Defatted dabai peel extract	High cholesterol diet-induced hypercholesterolaemia	• ↓Atherosclerotic plaque formation • ↓Total cholesterol and LDL-cholesterol • ↑HDL-cholesterol
Human[16]	Anthocyanins (320 mg day^{-1})	Randomised, double-blind, placebo-controlled trial: dyslipidaemic patients	• ↑HDL-cholesterol • ↓LDL-cholesterol
Human[17]	Strawberries (500 g day^{-1})	Human intervention trial	• ↑Plasma lipids profile • ↑Biomarkers of antioxidant status • ↑Antihaemolytic defences and platelet function

AA: Ascorbic acid, AI: Atherogenic index, ALT: Alanine transaminase, AST: Aspartate transaminase, CK-MB: Creatine kinase-MB, GPx: Glutathione peroxidase, GSH: Glutathione, HDL: High-density lipoprotein, LDH: Lactate dehydrogenase, LDL: Low-density lipoprotein, MDA: Malondialdehyde, SOD: Superoxide dismutase scavenging compared with other phenolic compounds such as caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, gallic acid, syringic acid and other phenolic compounds in the sample[8]. Another study also demonstrated that anthocyanin-rich extract of red leaves of *Elatostema rugosum* had higher DPPH radical scavenging activity than the phenolic constituents extracted from green leaves of the plant[9]. Anthocyanins extracted from the red leaves of *E. rugosum* comprised of malvidin (44%), peonidin (23%), cyanidin (17%), petunidin (11%) and delphinidin (5%). The study also reported that these compounds were able to significantly increase superoxide dismutase and catalase activity *in vitro* compared with the phenolic constituents obtained from the green leaves. Therefore, these findings suggested that anthocyanins from plants contributed more to total antioxidant capacity from phenolic compounds.

Based on Trolox equivalent antioxidant capacity (TEAC) assay, the antioxidant capacity of 26 different types of sweet cherry cultivars, contained anthocyanins from 4.80-360.90 $\mu g\ g^{-1}$ fresh weight (FW) ranged from 1.53-2.58 nmol Trolox equivalents (TE) mg^{-1} FW[10]. The homemade purple grape juice and concord grape juice were also reported to contain higher antioxidant activity with 4520 mg ascorbic acid (AA) mL^{-1} and 4781 mg AA mL^{-1}, respectively, compared with only 2752 mg AA mL^{-1} in red wine[11]. The processing method of wine compared to grape juice is different which may explain the discrepancy of the antioxidants activities between the red wine and grape juice. Maqui berry, an edible wild berry that grows in south-western Argentina as well as central and southern Chile, contains anthocyanins as the main polyphenolic compounds. The berry extract exhibited 28.18, 18.66, 25.22 g TE kg^{-1} dry weight (DW) and 0.12 g ethylenediaminetetraacetic acid equivalent kg^{-1} DW, which measured using DPPH radical scavenging assay, ABTS radical cation scavenging activity assay, ferric reducing antioxidant power (FRAP) and ferrous ion-chelating ability assay, respectively[12].

Some underutilised plants such as black carrots originate in Turkey and the Middle and the Far East which cultivated for at least 300 years are now getting more attention due to their high anthocyanin content[18]. Based on a previous study, the total antioxidant activity of black carrot juice was 23 and 48 µmol TE mL^{-1}, which assessed using FRAP and cupric ion reducing antioxidant capacity assays[19] respectively. *Canarium odontophyllum* Miq. fruit, also called as dabai by the people of Sarawak, Malaysia, is a highly nutritious underutilised fruit with dark purple/black skin when ripe[20]. The fruit (skin and flesh), contained about 2.49 mg monomeric anthocyanin pigment g^{-1} DW, was showed to have substantial antioxidant activities that determined by TEAC and FRAP assays[21].

Red cabbage and red radish were also found to contain highly conjugated anthocyanins[22], which may exhibit high antioxidant activity. Superoxide dismutase (SOD) and catalase are the enzymes that help break down potentially harmful oxygen molecules in cells, whereas the reduced glutathione (GSH) and ascorbic acid (AA) are important antioxidants in human body[23]. A previous study reported that supplementation of red cabbage extract that contains a high amount of anthocyanins prevented elevation of lipid peroxidation in the treated rats[13]. The extract had a total anthocyanins content of 50.21 ± 3.45 mg 100 g^{-1} leaf, where cyanidin-3-diglucoside-5-glucoside was the major anthocyanin found in the cabbage leaf[24]. A decreased in the SOD and catalase activities and the contents of reduced GSH and AA were also observed for the atherogenic-fed treated rats. The study also showed that the anthocyanin-rich extract helped in restoring the antioxidant status of the rats by acting as an antioxidative agent and as a free-radical scavenger. Similar results were also reported by Khoo *et al.*[14] on defatted dabai peel extract which was showed to inhibit lipid peroxidation and to elevate SOD and glutathione peroxidase levels in the treated white rabbits.

CARDIOPROTECTIVE EFFECT

Cardiovascular diseases are complex diseases that thought to be caused by lifestyle factor. It is linked with numerous disorders involving the heart and blood vessels, which including cerebrovascular disease, congenital heart disease, coronary heart disease, deep vein thrombosis, peripheral artery disease, pulmonary embolism and rheumatic heart disease[25]. The beneficial effects of anthocyanins on cardiovascular disease are believed to be linked with their antioxidative stress ability. Several mechanisms have been proposed for anthocyanin isolates and anthocyanin-rich mixtures in relation to the protection from anti-inflammatory activity, DNA cleavage, enzyme inhibition, estrogenic activity, increased cytokine production, lipid peroxidation as well as decreased capillary permeability and fragility and membrane strengthening.

As shown in Table 1, several studies demonstrated the cardioprotective effect of anthocyanins. Anthocyanin-rich red cabbage extract is reported having hypocholesterolaemia, cardioprotective and hepatoprotective properties on atherogenic diet-induced oxidative stress and tissue injury in rats[13]. The red cabbage extract also increased the excretion of lipids through faeces in the rats. The supplementation of the defatted pulp of dabai also exhibited a positive hypocholesterolaemia with a significant reduction in total cholesterol and low-density lipoprotein cholesterol levels as well as inhibited the formation of atheromatous plaque[15]. The cholesterol-lowering properties of the dabai sample could be due to the action of polyphenol compounds including cyanidin-3-glucoside as the main anthocyanin in the sample, especially in the fruit peel[20].

Anthocyanins are not only being shown to exert benefits in animal models but also have similar beneficial effects in humans. A double-blind, randomised, placebo-controlled trial for 12 weeks in 120 dyslipidaemia patients aged 40-65 years has been performed previously. The result showed that the intake of berry-derived anthocyanin improved lipoprotein profile through cholesteryl ester transfer protein inhibition, an increased in high-density lipoprotein (HDL) cholesterol levels and a decreased in low-density lipoprotein (LDL) cholesterol levels[16]. A few mechanisms on the hypolipidaemic effects of anthocyanins are proposed as the compounds help in improving lipid haemostasis, enhancing energy expenditure, decreasing fat mass, stimulating favourable changes to lipid metabolism associated genes and deferring fat absorption and secretion of chylomicron[26].

Consistent consumption of berries also been reported in the literature that has protective effects on cardiovascular disease through improving plasma lipid profile and endothelial function, increasing plasma total antioxidant activity and resistance to LDL oxidation. A study was done to investigate the benefits of eating 500 g of strawberries daily for a month among healthy subjects[17]. The study showed that strawberry consumption helped to improve the status of plasma antioxidant biomarkers and plasma lipids profile, increases antihaemolytic defences and platelet function in the subjects. A high intake of fruit-based anthocyanins associated with a 14% lower risk of non-fatal myocardial infarction in a prospective cohort study of well-characterised men with 24 years of follow-up[27]. A similar result obtained in a prospective cohort study of women aged 25-42 years with 18 years of follow-up showing that a higher intake of anthocyanins from foods was associated with a 32% reduction in myocardial infarction risk[28].

ANTIMICROBIAL EFFECT

Antimicrobial activities of anthocyanin are not well-studied compared to its antioxidative effect. Anthocyanins protect against a wide range of microorganisms including preventing the growth of bacteria and other foodborne pathogens[29]. As reported in the literature, anthocyanins from berry extracts exhibited antimicrobial activity through different pathways, such as inducing damage to the cell wall, membrane and intercellular matrix[30]. Although, anthocyanins appear to have an antimicrobial effect, the inhibition activity is mainly due to the interaction of hydroxyl group of anthocyanin with sulfhydryl groups of the cell walls of the bacteria. This interaction might cause inactivation or loss of functioning of the cell wall or membrane of the bacteria.

Several studies reported that anthocyanin-rich berry extracts have antimicrobial effects. Berry extracts including blueberry, raspberry and strawberry as well as blackcurrant exhibited inhibitory activity on Gram-negative bacteria, however, no inhibition effect on Gram-positive bacteria[31]. Cranberry also had an antibacterial activity which was contributed by its bioactive components such as anthocyanins and flavonols[32,33]. The study also showed that cranberry extract had antibacterial effects towards *Enterococcus faecium* resistant to vancomycin, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

On top of berries, pomegranate is also one of the anthocyanin-rich fruit. The juice of pomegranate is red in colour, which has high anthocyanins content. Pomegranate peel extract was also reported as having the highest antibacterial activity against *Salmonella* strains, followed by its seeds, juice and flower extracts[4]. Although, pomegranate peel contains anthocyanins, the antimicrobial activity could be due the phenolic compounds detected in the peel. Genskowsky *et al.*[12] reported that maqui berry extracts had antibacterial activity, where the highest activities found for the Gram-negative and Gram-positive bacteria, *Aeromonas hydrophila* and *Listeria innocua*, respectively.

On the contrary, anthocyanin-containing dabai pulp extracts were not effective against both tested Gram-positive (Methicillin-susceptible *Staphylococcus aureus* and Methicillin-Resistant *S. aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria but had antifungal effect against *Candida glabrata*[34]. Cisowska *et al.*[35] suggested that the antimicrobial activities of berries and other anthocyanin-containing fruits are due to the synergistic effect of a mixture of phytochemicals other than anthocyanin alone. The antimicrobial effects of anthocyanin-rich fruits could also be due to the several pathways and multiple mechanisms involving anthocyanins, weak organic acids and other phenolic compounds. Thus, anthocyanins are one of the contributors to antimicrobial effect and not solely due to anthocyanins in the fruit.

ANTIOBESITY AND ANTIDIABETIC EFFECTS

Obesity is a condition related to an excessive accumulation of fat tissue in the body that may give detrimental effects. It happens mostly due to the disproportion of energy intake and its expenditure. The increase in the prevalence of the common form of diabetes, such as type 2 diabetes, is closely linked to obesity, where about 90% of type 2 diabetes is attributable to a high body mass index[36].

As shown in Table 2, eight types of berries and drupe (açai, bilberry, blackberry, blackcurrant, crowberry, lingonberry, prune and raspberry) have been determined for their ability to prevent obesity and metabolic abnormalities associated with type 2 diabetes in C57BL/6J mice. The results showed that after 13 weeks of high-fat diet (HFD) supplementation with 20% of bilberries, blackcurrants, lingonberries or raspberries, the rats received either one of the berry extracts gained lesser body weight and had lower fasting insulin levels than the control group[37]. Furthermore, the extracts of lingonberries, blackcurrants and bilberries reduced the percentage of body fat, decreased the accumulation of hepatic lipid and plasma levels of the inflammatory marker plasminogen activator inhibitor (PAI)-1 of the treated rats as well as improved glucose homeostasis. Similar results were obtained by another study which investigated the effect of black chokeberry on the development of obesity in mice fed a HFD[38]. The study showed that supplementation of black chokeberry decreased weight gain and increased adiponectin levels in the mice. Circulating adiponectin levels are inversely related to the body fat percentage, where the lower adiponectin level is considered a risk factor for type 2 diabetes[39].

Anthocyanins are the main bioactive compounds in blueberry and mulberry. The major anthocyanins in blueberry are cyanidin-3-galactoside, delphinidin-3-galactoside and petunidin-3-arabinoside; whereas the predominant anthocyanins in mulberry are cyanidin-3-glucoside and cyanidin-3-rutinoside. Supplementation of either blueberry or mulberry juice containing these anthocyanins on C57BL/6 mice fed with a HFD for 12 weeks was studied by Wu *et al.*[40]. The study shows that blueberry and mulberry juices prevented body weight gain, decreased serum cholesterol and leptin secretion, reduced insulin resistance and inhibited the accumulation of fat in the adipose tissue. Leptin is a hormone produced by adipocytes and links with the homeostasis in body energy and fat stores[43]. Leptin helps to reduce triglycerides formation by enhancing oxidation of free fatty acid and reducing its esterification to triglyceride. Thus reduces insulin resistance and β-cell dysfunction that may lead to obesity and diabetes[44].

Zhang *et al.*[41] used diabetic male KK-A^y mice to study the antidiabetic effect of anthocyanins rich-bayberry fruit extract (200 mg kg^{-1}). The extract significantly lowered the fasting blood glucose levels and improved glucose tolerance and insulin sensitivity in the mice. Serum lipids, inflammation and liver function markers were also significantly reduced. The mice treated with the bayberry fruit extract also have reduced liver weight and accumulation of liver lipid. The hypoglycaemic effect may be explained by the inhibition of hepatic gluconeogenesis, which manifested by reduced in PPARγ coactivator 1-alpha (PGC-1α) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA expressions in the liver of KK-A^y mice. On the other hand,

Table 2: Antiobesity and antidiabetic effects of anthocyanins in foods

Model	Sample	Design	Outcome
Mice[37]	Different types of berries	HFD-induced obesity	• ↓Weight gained • ↓Fasting insulin levels in rats fed with lingonberries, blackcurrants, raspberries or bilberries • ↓Body fat content, hepatic lipid accumulation and • inflammatory marker PAI-1 in rats fed with lingonberries, blackcurrants or bilberries • ↑Glucose homeostasis in rats fed with lingonberries, blackcurrants or bilberries
Mice[38]	Black chokeberry	HFD-induced obesity	• ↓Weight gain • ↑Adiponectin levels
Mice[40]	Blueberry and mulberry	HFD-induced obesity	• ↓Body weight gain • ↓Derum cholesterol and leptin secretion • ↓Insulin resistance • ↓Lipid accumulation.
Mice[41]	Bayberry extract (200 mg kg^{-1})	Diabetic KK-Ay mice	• ↓Fasting blood glucose levels • ↑Glucose tolerance and insulin sensitivity • ↓Serum lipids, inflammation and liver function markers, liver weight and liver lipid accumulation • ↓PPARGC1A and PEPCK mRNA expressions
Human[42]	Cherry juice (69 mg 100 g^{-1})	Randomised clinical trial	• ↑Verbal fluency, short-term and long-term memory • ↓Blood pressure

HFD: High-fat diet, mRNA: Messenger RNA, PAI: Plasminogen activator inhibitor, PEPCK: Phosphoenolpyruvate carboxykinase, PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

Guo and Ling[45] summarised the potential mechanisms of antidiabetic effect of anthocyanins as: (1) suppressed body weight gain, (2) inhibited free radical production and lipid peroxidation, (3) regulated inflammatory response, (4) Reduced blood glucose and lipids levels and (5) improved insulin resistance. A complex interaction of multiple signalling pathways, transcription factors and enzymes might also involve in the protective effects of anthocyanins.

ANTICANCER EFFECT

Anthocyanins may exert their anticancer activity through a few different mechanisms. The anticancer effects of anthocyanins in foods are shown in Table 3. A recent review done by He and Giusti summarised the possible mechanisms of the anticancer activity of anthocyanins. The possible mechanisms are antimutagenic activity, inhibition of oxidative DNA damage and carcinogen activation, induction of phase II enzymes for detoxification, cell cycle arrest and inhibition of cyclooxygenase-2 enzymes as well as induction of apoptosis and antiangiogenesis[6]. Several signalling pathways of anticancer activity of anthocyanins have also been studied such as inhibiting Ras signalling, down-regulating the expressions of cyclin-dependent kinases, stopping ethanol-mediated $p130^{Cas}$/c-Jun N-terminal kinase (JNK) interaction, increasing the Bax/Bcl-2 ratio and inducing signalling by p38/p53 and c-Jun[46].

Extensive studies show that anthocyanins inhibited the development of various types of cancers using animal model. Hui *et al*.[47] investigated the anticancer effect of the anthocyanin-rich extract from black rice on BALB/c nude mice bearing breast cancer cells (MDA-MB-453). The results showed that oral administration of the anthocyanin-rich extract (100 mg kg^{-1} day^{-1}) was significantly suppressed tumour growth and angiogenesis in the mice by suppressing the expression of angiogenesis factors matrix metalloproteinase (MMP)-2, MMP-9 and urokinase-type plasminogen activator (uPA) in tumour tissue. Moreover, the extract also helped to inhibit the vascular endothelial growth factor (VEGF) activated tumour-associated blood vessels formation in the mice model. VEGF is a signal protein that produced by certain types of cells to stimulate vasculogenesis and angiogenesis. These cells are including macrophages, platelets and tumour cells. Inhibition of VEGF activity may help to suppress the tumour growth or tumour regression[48].

Anthocyanins are the coloured pigments that have an anticancer effect on gastric cancer. The cyanidin-3-glucoside, an anthocyanin extracted from red-coloured Chinese bayberry fruit (*Myrica rubra* Sieb. et Zucc.) was used to investigate its antitumour effect on BALB/c nude mice tumour xenograft model[46]. The results showed that cyanidin-3-glucoside inhibited the tumour growth in a dose-dependent manner due to cell cycle inhibition rather than apoptosis induction. The cyanidin-3-glucoside was also up-regulated the Krüppel-like transcription factor (KLF)6 gene expression, a tumour suppressor gene and the downstream effector p21 in a p53-independent manner. Moreover, another study showed that anthocyanin-rich extract from roselle was found to inhibit the progression of N-nitrosomethylurea-induced leukaemia in rats by about 33% compared with the control group[49]. Other studies have shown that anthocyanins have beneficial effects on colon cancer[50], oral cancer[51], oesophageal cancer[52], pancreatic cancer[53], breast cancer[54] and gastric cancer[55].

IMPROVE COGNITIVE FUNCTION AND NEURODEGENERATIVE DISORDERS

Deterioration of cognitive performance and motor abilities with age maybe contributed by oxidative stress and neuroinflammation in the brain[56]. Inflammatory molecules such as cytokines, superoxide and nitric oxide can lead to multiple sclerosis and central nervous system degenerative disease[57]. The evidence of anthocyanins in foods on improving neurodegenerative disorder is shown in Table 4.

Consumption of high antioxidants and anti-inflammatory foods is believed to protect our body from deleterious effects such as oxidative stress and inflammation, thus reduce the risk of developing age-related diseases such as Alzheimer disease and Parkinson's disease[58]. Pretreated HAPI rat microglial cells with tart cherry which the cyanidin is the most abundant

Table 3: Anticancer effects of anthocyanins in foods

Model	Sample	Design	Outcome
Mice[46]	Cyanidin-3-glucoside from Chinese bayberry fruit (125 mg kg^{-1} BW)	Nude mouse tumour xenograft	• ↓Tumour growth • ↑KLF 6 gene expression • ↓p21 effector in a p53-independent manner
Mice and cell culture[47]	Anthocyanins extract from black rice (100 mg kg^{-1} BW)	BALB/c nude mice and breast cancer cell lines	• ↓Expression of angiogenesis factors (MMP-2, MMP-9 and uPA) • ↓Vascular endothelial growth factor activated tumour-associated blood vessels formation

BW: Body weight, KLF: Krüppel-like transcription factor, MMP: Matrix metalloproteinase, uPA: Urokinase-type plasminogen activator

Table 4: Anthocyanins in foods improve neurodegenerative disorder

Model	Sample	Design	Outcome
Rats[59]	Anthocyanins (200 mg kg^{-1} BW)	Scopolamine-induced amnesia	• ↓Memory deficit • ↓Na^+, K^+-ATPase and Ca^{2+}-ATPase activities in hippocampus • ↓Acetylcholinesterase activity in both cortex and hippocampus • ↑Anxiolytic effect
Rats[61]	Anthocyanins extract from Korean black soybean (100 mg kg^{-1} BW)	Oxidative stress and neuroinflammation mediated cognitive impairment	• ↑Behavioral performance in Morris water maze and Y-maze tests • ↓Expression of receptor for advance glycation end product • ↓ROS, lipid peroxidation and Alzheimer's disease markers • ↓p-NF-KB, iNOS and TNF-α in the hippocampus and cortex regions
Human[42]	Cherry juice (200 ml day^{-1})	Randomised controlled trial	• ↑Verbal fluency, short-term and long-term memory • ↓Blood pressure

ATPase: Adenosine triphosphatase, BW: Body weight, iNOS: Inducible nitric oxide synthase, p-NF-KB: Phospho-nuclear factor kappa-light-chain-enhancer of activated B cells, TNF-α: Tumour necrosis factor-α

anthocyanins showed a decrement in the levels of nitric oxide (NO), tumour necrosis factor-alpha (TNF-α) and cyclooxygenase-2 in a dose- and time-dependent manner compared with those without pretreatment[56].

The therapeutic efficacy of anthocyanins in cognitive deficits is associated with Alzheimer's disease and induced by scopolamine, which was reported by Gutierres *et al.*[59]. The study also found that with the treatment of 200 mg kg^{-1} anthocyanins was able to regulate cholinergic neurotransmission, to restore Na^+, K^+-ATPase and Ca^{2+}-ATPase activities and to prevent memory deficits in rats induced by scopolamine. In fact, scopolamine is a potent and non-selective muscarinic receptor. It is able to induce memory deficits and to elevate brain oxidative status in animal models[60].

A recent study reported the neuroprotective effect of anthocyanins extracted from Korean black soybean based on an artificial ageing model. The researchers used D-galactose to induce oxidative stress and inflammatory response which resulted in memory and synaptic dysfunction[61]. The study found that the anthocyanins treated rats had improved behavioural performance using Morris water maze and Y-maze tests. The study proved that anthocyanin neuroprotection against D-galactose-induced oxidative stress is one of the potential neuroprotective effects. Anthocyanins from the Korean black soybean also inhibited the activated astrocytes and neuroinflammation via suppression of various inflammatory markers including p-NF-KB, inducible nitric oxide synthase (iNOS) and TNF-α in the hippocampus and cortex regions of treated rats brain.

A 12-week randomised controlled trial was performed to investigate whether daily consumption of anthocyanin-rich cherry juice (200 mL) could change cognitive function in older adults with dementia[42]. The results showed that verbal fluency, short-term and long-term memories improved in the cherry juice groups. A reduction in blood pressure was also exhibited in the intervention group. It has been hypothesised that flavonoids help to improve cognitive function by increasing the number and strength of neuronal signals through the increased brain blood flow as well as the ability to initiate neurogenesis in areas of the brain associated with learning and memory[62].

VISUAL ACUITY

The role of anthocyanins to vision is one of the first health benefits recognised to them[63]. The link between anthocyanins and vision improvements started in part from a body of research published between the 1960s and 1980s on the effects of bilberry on various vision parameters in the *in vitro*, *in vivo* and clinical interventions[64]. Some possible mechanisms of action of anthocyanins on the visual apparatus are the acceleration of re-synthesis of rhodopsin, improvement in microcirculation and modulation of retinal enzyme activity[65].

Previous studies reported that anthocyanins in berries improved vision (Table 5). A rat retinal degeneration model, which induced by N-methyl-N-nitrosourea, was used to investigate the effect of cyanidin-3-glucoside from mulberry at 50 mg kg^{-1}. The study found that cyanidin-3-glucoside treatment helped to reduce photoreceptor damage and to improve scotopic visual functions in the rats[66]. Besides these findings, the maqui berry extract also suppressed ROS formation from lacrimal gland tissue and preserved tear secretion using the rat blink-suppressed dry eye model[67]. Thus, it suggested that maqui berry extract is a nutraceutical for dry eye by regulating tear secretion capacity in the lacrimal gland.

A prospective, randomised, double-blind, placebo-controlled study has been performed to investigate the effect of bilberry extract (480 mg day^{-1}) on eye fatigue induced by acute video display terminal (VDT) loads[68]. The study found that eight weeks of supplementation of bilberry extract

Table 5: Anthocyanins in foods improve vision

Model	Sample	Design	Outcome
Rat[66]	Mulberry (50 mg kg^{-1})	N-methyl-N-nitrosourea-induced retinal degeneration	• ↓Photoreceptor damage • ↑Scotopic visual functions
Rat[67]	Maqui berry extract	Blink-suppressed dry eye	• ↓ROS formation from lacrimal gland tissue • ↑Tear secretion

ROS: Reactive oxygen species

alleviated the VDT load-induced reduction in critical flicker fusion. The treatment also helped in mitigating eye heaviness, ocular fatigue sensation, ocular pain, foreign body sensation and uncomfortable sensation in the subjects studied.

CONCLUSION

Anthocyanins are coloured pigments found in plants. These coloured pigments have therapeutic effects against inflammation, antimicrobial, several chronic diseases, visual and memory functions. Many studies have proven the efficacies of anthocyanins in the prevention of these diseases and improve general health. Although, most studies did not specify the type of anthocyanin used, these studies reported the efficacy of anthocyanin-rich extracts from fruits and vegetables. However, the efficacy of anthocyanins is not mainly due to the compounds themselves. The synergetic effect of anthocyanins and other phenolic compounds is essential for prevention of diseases as well as their antioxidative activities. In a nutshell, anthocyanins are the coloured pigments isolated from plants with therapeutic effects in the prevention of several diseases and health complications.

SIGNIFICANCE STATEMENT

This review reported on the potential health benefits of anthocyanins in the prevention of several diseases. These purple pigments from plants have antioxidative, cardioprotective, antimicrobial, anticancer and several metabolic diseases including antiobesity and antidiabetes potential. Anthocyanins also improve cognitive function and neurodegenerative disorder as well as visual acuity. These evidence-based reports are useful for the scientific community to further research on anthocyanin pigments and for the general public to fully utilise anthocyanins in the prevention of diseases.

ACKNOWLEDGMENT

All authors are very grateful to all those who helped in searching for information.

REFERENCES

1. Khoo, H.E., A. Azlan, S.T. Tang and S.M. Lim, 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients and the potential health benefits. Food Nutr. Res., Vol. 61, No. 1. 10.1080/16546628.2017.1361779.
2. Khoo, H.E., L.Y. Chew, A. Ismail and A. Azlan, 2013. Anthocyanins in Purple Colored Fruits. In: Polyphenols: Chemistry, Dietary Sources and Health Benefits, Sun, J., K.N. Prasad, A. Ismail, B. Yang, X. You and L.L. Xiangrong (Eds.). Nova Science Publishers, New York, pp: 133-151.
3. Schieber, M. and N.S. Chandel, 2014. ROS function in redox signaling and oxidative stress. Curr. Biol., 24: R453-R462.
4. Routray, W. and V. Orsat, 2011. Blueberries and their anthocyanins: Factors affecting biosynthesis and properties. Comprehensive Rev. Food Sci. Food Saf., 10: 303-320.
5. Ferretti, G., T. Bacchetti, A. Belleggia and D. Neri, 2010. Cherry antioxidants: From farm to table. Molecules, 15: 6993-7005.
6. He, J. and M.M. Giusti, 2010. Anthocyanins: Natural colorants with health-promoting properties. Annu. Rev. Food Sci. Technol., 1: 163-187.
7. Wang, L.S. and G.D. Stoner, 2008. Anthocyanins and their role in cancer prevention. Cancer Lett., 269: 281-290.
8. Bi, X., J. Zhang, C. Chen, D. Zhang, P. Li and F. Ma, 2014. Anthocyanin contributes more to hydrogen peroxide scavenging than other phenolics in apple peel. Food Chem., 152: 205-209.
9. Neill, S.O., K.S. Gould, P.A. Kilmartin, K.A. Mitchell and K.R. Markham, 2002. Antioxidant activities of red versus green leaves in *Elatostema rugosum*. Plant Cell Environ., 25: 539-547.
10. Di Matteo, A., R. Russo, G. Graziani, A. Ritieni and C. Di Vaio, 2017. Characterization of autochthonous sweet cherry cultivars (*Prunus avium* L.) of Southern Italy for fruit quality, bioactive compounds and antioxidant activity. J. Sci. Food Agric., 97: 2782-2794.
11. Callaghan, C.M., R.E. Leggett and R.M. Levin, 2017. A comparison of the antioxidants and carbohydrates in common wines and grape juices. Free Radicals Antioxid., 7: 86-89.

12. Genskowsky, E., L.A. Puente, J.A. Perez-Alvarez, J. Fernandez-Lopez, L.A. Munoz and M. Viuda-Martos, 2016. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry. J. Sci. Food Agric., 96: 4235-4242.
13. Sankhari, J.M., M.C. Thounaojam, R.N. Jadeja, R.V. Devkar and A.V. Ramachandran, 2012. Anthocyanin-rich red cabbage (*Brassica oleracea* L.) extract attenuates cardiac and hepatic oxidative stress in rats fed an atherogenic diet. J. Sci. Food Agric., 92: 1688-1693.
14. Khoo, H.E., A. Azlan, M.H. Nurulhuda, A. Ismail, F. Abas, M. Hamid and S. Roowi, 2013. Antioxidative and cardioprotective properties of anthocyanins from defatted dabai extracts. Evidence-Based Complement. Altern. Med., Vol. 2013. 10.1155/2013/434057.
15. Nurulhuda, M.H., A. Azlan, A. Ismail, Z. Amom and F.H. Shakirin, 2013. Sibu olive inhibits artherosclerosis by cholesterol lowering effect in cholesterol fed-rabbit. Proceedings of the 4th International Conference on Biomedical Engineering in Vietnam, IFMBE Vol. 49, January 2013, Springer, Berlin, Heidelberg, pp: 141-144.
16. Qin, Y., M. Xia, J. Ma, Y.T. Hao and J. Liu *et al*., 2009. Anthocyanin supplementation improves serum LDL-and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. Am. J. Clin. Nutr., 90: 485-492.
17. Alvarez-Suarez, J.M., F. Giampieri, S. Tulipani, T. Casoli and G. di Stefano *et al*., 2014. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. J. Nutr. Biochem., 25: 289-294.
18. Kamiloglu, S., A.A. Pasli, B. Ozcelik, J. van Camp and E. Capanoglu, 2015. Colour retention, anthocyanin stability and antioxidant capacity in black carrot (*Daucus carota*) jams and marmalades: Effect of processing, storage conditions and *in vitro* gastrointestinal digestion. J. Funct. Foods, 13: 1-10.
19. Khandare, V., S. Walia, M. Singh and C. Kaur, 2011. Black carrot (*Daucus carota* ssp. *sativus*) juice: Processing effects on antioxidant composition and color. Food Bioprod. Process., 89: 482-486.
20. Shakirin, F.H., K.N. Prasad, A. Ismail, L.C. Youn and A. Azrina, 2010. Antioxidant capacity of underutilized Malaysian *Canarium odontophyllum* (dabai) Miq. fruit. J. Food Compos. Anal., 23: 777-781.
21. Chew, L.Y., K.N. Prasad, I. Amin, A. Azrina and C.Y. Lau, 2011. Nutritional composition and antioxidant properties of *Canarium odontophyllum* Miq. (dabai) fruits. J. Food Compos. Anal., 24: 670-677.
22. Wu, X. and R.L. Prior, 2005. Identification and characterization of anthocyanins by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry in common foods in the United States: Vegetables, Nuts and Grains. J. Agric. Food Chem., 53: 3101-3113.
23. Maritim, A.C., R.A. Sanders and J.B. Watkins III, 2003. Diabetes, oxidative stress and antioxidants: A review. J. Biochem. Mol. Toxicol., 17: 24-38.
24. Scalzo, R.L., A. Genna, F. Branca, M. Chedin and H. Chassaigne, 2008. Anthocyanin composition of cauliflower (*Brassica oleracea* L. var. botrytis) and cabbage (*B. oleracea* L. var. capitata) and its stability in relation to thermal treatments. Food Chem., 107: 136-144.
25. Wallace, T.C., 2011. Anthocyanins in cardiovascular disease. Adv. Nutr., 2: 1-7.
26. Thompson, K., W. Pederick and A.B. Santhakumar, 2016. Anthocyanins in obesity-associated thrombogenesis: A review of the potential mechanism of action. Food Funct., 7: 2169-2178.
27. Cassidy, A., M. Bertoia, S. Chiuve, A. Flint, J. Forman and E.B. Rimm, 2016. Habitual intake of anthocyanins and flavanones and risk of cardiovascular disease in men. Am. J. Clin. Nutr., 104: 587-594.
28. Cassidy, A., K.J. Mukamal, L. Liu, M. Franz, A.H. Eliassen and E.B. Rimm, 2013. High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women. Circulation, 127: 188-196.
29. Cushnie, T.P.T. and A.J. Lamb, 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.
30. Pojer, E., F. Mattivi, D. Johnson and C.S. Stockley, 2013. The case for anthocyanin consumption to promote human health: A review. Compr. Rev. Food Sci. Food Saf., 12: 483-508.
31. Puupponen-Pimia, R., L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K.M. Oksman-Caldentey, 2001. Antimicrobial properties of phenolic compounds from berries. J. Applied Microbiol., 90: 494-507.
32. Cote, J., S. Caillet, G. Doyon, D. Dussault, J.F. Sylvain and M. Lacroix, 2011. Antimicrobial effect of cranberry juice and extracts. Food Control, 22: 1413-1418.
33. Wafa, B.A., M. Makni, S. Ammar, L. Khannous and A.B. Hassana *et al*., 2017. Antimicrobial effect of the Tunisian Nana variety *Punica granatum* L. extracts against *Salmonella enterica* (serovars Kentucky and Enteritidis) isolated from chicken meat and phenolic composition of its peel extract. Int. J. Food Microbiol., 241: 123-131.
34. Caceres, A., O. Cabrera, O. Morales, P. Mollinedo and P. Mendia, 1991. Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. J. Ethnopharmacol., 33: 213-216.

35. Cisowska, A., D. Wojnicz and A.B. Hendrich, 2011. Anthocyanins as antimicrobial agents of natural plant origin. Nat. Prod. Commun. 6: 149-156.
36. Lazar, M.A., 2005. How obesity causes diabetes: Not a tall tale. Science, 307: 373-375.
37. Heyman, L., U. Axling, N. Blanco, O. Sterner, C. Holm and K. Berger, 2014. Evaluation of beneficial metabolic effects of berries in high-fat fed C57BL/6J mice. J. Nutr. Metab., Vol. 2014. 10.1155/2014/403041
38. Baum, J.I., L.R. Howard, R.L. Prior and S.O. Lee, 2016. Effect of *Aronia melanocarpa* (Black Chokeberry) supplementation on the development of obesity in mice fed a high-fat diet. J. Berry Res., 6: 203-212.
39. Guenther, M., R. James, J. Marks, S. Zhao, A. Szabo and S. Kidambi, 2014. Adiposity distribution influences circulating adiponectin levels. Transl, Res., 164: 270-277.
40. Wu, T., Q. Tang, Z. Gao, Z. Yu, H. Song, X. Zheng and W. Chen, 2013. Blueberry and mulberry juice prevent obesity development in C57BL/6 mice. PLoS One, Vol. 8. 10.1371/journal.pone.0077585
41. Zhang, X., Q. Lv, S. Jia, Y. Chen, C. Sun, X. Li and K. Chen, 2016. Effects of flavonoid-rich Chinese bayberry (*Morella rubra* Sieb. et Zucc.) fruit extract on regulating glucose and lipid metabolism in diabetic KK-A^y mice. Food Funct., 7: 3130-3140.
42. Kent, K., K. Charlton, S. Roodenrys, M. Batterham and J. Potter *et al*., 2017. Consumption of anthocyanin-rich cherry juice for 12 weeks improves memory and cognition in older adults with mild-to-moderate dementia. Eur. J. Nutr., 56: 333-341.
43. Myers Jr, M.G., R.L. Leibel, R.J. Seeley and M.W. Schwartz, 2010. Obesity and leptin resistance: Distinguishing cause from effect. Trends Endocrinol. Metab., 21: 643-651.
44. Meydani, M. and S.T. Hasan, 2010. Dietary polyphenols and obesity. Nutrients, 2: 737-751.
45. Guo, H. and W. Ling, 2015. The update of anthocyanins on obesity and type 2 diabetes: Experimental evidence and clinical perspectives. Rev. Endocr. Metab. Disord., 16: 1-13.
46. Wang, Y., X.N. Zhang, W.H. Xie, Y.X. Zheng and J.P. Cao *et al*., 2016. The growth of SGC-7901 tumor xenografts was suppressed by Chinese bayberry anthocyanin extract through upregulating KLF6 gene expression. Nutrients, Vol. 8. 10.3390/nu8100599
47. Hui, C., Y. Bin, Y. Xiaoping, Y. Long, C. Chunye, M. Mantian and L. Wenhua, 2010. Anticancer activities of an anthocyanin-rich extract from black rice against breast cancer cells i*n vitro* and i*n vivo*. Nutr. Cancer, 62: 1128-1136.
48. Ferrara, N., 2009. Vascular endothelial growth factor. Arteriosclerosis Thrombosis Vasc. Biol., 29: 789-791.
49. Tsai, T.C., H.P. Huang, Y.C. Chang and C.J. Wang, 2014. An anthocyanin-rich extract from *Hibiscus sabdariffa* linnaeus inhibits N-nitrosomethylurea-induced leukemia in rats. J. Agric. Food Chem., 62: 1572-1580.
50. Venancio, V.P., P.A. Cipriano, H. Kim, L.M.G. Antunes, S.T. Talcott and S.U. Mertens-Talcott, 2017. Cocoplum (*Chrysobalanus icaco* L.) anthocyanins exert anti-inflammatory activity in human colon cancer and non-malignant colon cells. Food Funct., 8: 307-314.
51. Fan, M.J., I.C. Wang, Y.T. Hsiao, H.Y. Lin and N.Y. Tang *et al*., 2015. Anthocyanins from black rice (*Oryza sativa* L.) demonstrate antimetastatic properties by reducing MMPs and NF-κB expressions in human oral cancer CAL 27 cells. Nutr. Cancer, 67: 327-338.
52. Peiffer, D.S., N.P. Zimmerman, L.S. Wang, B. Ransom and S.G. Carmella *et al*., 2014. Chemoprevention of esophageal cancer with black raspberries, their component anthocyanins and a major anthocyanin metabolite, protocatechuic acid. Cancer Prev. Res., 7: 574-584.
53. Kuntz, S., C. Kunz and S. Rudloff, 2015. Inhibition of pancreatic cancer cell migration by plasma anthocyanins isolated from healthy volunteers receiving an anthocyanin-rich berry juice. Eur. J. Nutr., 56: 203-214.
54. Luo, L.P., B. Han, X.P. Yu, X.Y. Chen and J. Zhou *et al*., 2014. Anti-metastasis activity of black rice anthocyanins against breast cancer: Analyses using an ErbB2 positive breast cancer cell line and tumoral xenograft model. Asian Pac. J. Cancer Prev., 15: 6219-6225.
55. Lu, J.N., W.S. Lee, A. Nagappan, S.H. Chang and Y.H. Choi *et al*., 2015. Anthocyanins from the fruit of *Vitis coignetiae* Pulliat potentiate the cisplatin activity by inhibiting PI3K/Akt signaling pathways in human gastric cancer cells. J. Cancer Prev., 20: 50-56.
56. Shukitt-Hale, B., M.E. Kelly, D.F. Bielinski and D.R. Fisher, 2016. Tart cherry extracts reduce inflammatory and oxidative stress signaling in microglial cells. Antioxidants, Vol. 5, No. 4. 10.3390/antiox5040033
57. Perry, V.H., 2010. Contribution of systemic inflammation to chronic neurodegeneration. Acta Neuropathol., 120: 277-286.
58. Joseph, J.A., B. Shukitt-Hale and G. Casadesus, 2005. Reversing the deleterious effects of aging on neuronal communication and behavior: Beneficial properties of fruit polyphenolic compounds. Am. J. Clin. Nutr., 81: 313S-316S.
59. Gutierres, J.M., F.B. Carvalho, M.R.C. Schetinger, P. Agostinho and P.C. Marisco *et al*., 2014. Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rats. Int. J. Dev. Neurosci., 33: 88-97.
60. Klinkenberg, I. and A. Blokland, 2010. The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. Neurosci. Biobehav. Rev., 34: 1307-1350.
61. Rehman, S.U., S.A. Shah, T. Ali, J.I. Chung and M.O. Kim, 2017. Anthocyanins reversed D-galactose-induced oxidative stress and neuroinflammation mediated cognitive impairment in adult rats. Mol. Neurobiol., 54: 255-271.

62. Kent, K., K.E. Charlton, M. Netzel and K. Fanning, 2017. Food-based anthocyanin intake and cognitive outcomes in human intervention trials: A systematic review. J. Hum. Nutr. Diet., 30: 260-274.
63. Smeriglio, A., D. Barreca, E. Bellocco and D. Trombetta, 2016. Chemistry, pharmacology and health benefits of anthocyanins. Phytother. Res., 30: 1265-1286.
64. Kalt, W., J.E. McDonald, S.A.E. Fillmore and F. Tremblay, 2014. Blueberry effects on dark vision and recovery after photobleaching: Placebo-controlled crossover studies. J. Agric. Food Chem., 62: 11180-11189.
65. Canter, P.H. and E. Ernst, 2004. Anthocyanosides of *Vaccinium myrtillus* (Bilberry) for night vision-a systematic review of placebo-controlled trials. Survey Ophthalmol., 49: 38-50.
66. Lee, S.H., E. Jeong, S.S. Paik, J.H. Jeon and S.W. Jung *et al*., 2014. Cyanidin-3-glucoside extracted from mulberry fruit can reduce N-methyl-N-nitrosourea-induced retinal degeneration in rats. Curr. Eye Res., 39: 79-87.
67. Nakamura, S., J. Tanaka, T. Imada, H. Shimoda and K. Tsubota, 2014. Delphinidin 3,5-O-diglucoside, a constituent of the maqui berry (*Aristotelia chilensis*) anthocyanin, restores tear secretion in a rat dry eye model. J. Funct. Foods, 10: 346-354.
68. Ozawa, Y., M. Kawashima, S. Inoue, E. Inagaki and A. Suzuki *et al*., 2015. Bilberry extract supplementation for preventing eye fatigue in video display terminal workers. J. Nutr. Health Aging, 19: 548-554.