

Review

A comprehensive review on biological impact of Anthocyanins on human life

Musarat Jabeen^{1,*}, Namra Hussain¹, Hira Noreen¹, Iqra Amjad¹, Amna Zia¹, Maria Manzoor¹, Komal Ashraf¹ and Rabia Mehmood¹

¹ Department of Chemistry, Govt. Sadiq College for Women University, Bahawalpur, Pakistan.

* Correspondence: dr.mussaratjabeen@gmail.com; Tell: +92-333-5207822

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Abstract: Due to the beneficial effects of anthocyanins on plants, animals and human beings, they have become the most interesting topic of research for scientists. They are being used in food industry as well as in pharmaceutical and cosmetic industries. Anthocyanins are present in red, blue, orange, purple, violet and intermediate color mostly. They are non-hazardous natural pigments that have positive impact on human health. They occur in nature since the evolution of flowering plants on earth. As humans were wild in ancient times, they consumed their large concentration through their diet and human digestive system is very active for their digestion. In this review, the chemistry and impact of anthocyanins on human health is discussed briefly.

Keywords: Anthocyanin; Plant pigment; Co-pigmentation; Metal complexation.

1. Introduction

The original word "Anthokyan" was first coined by a German Pharmacist, Ludwig Clamor Marquart in 1853. The word "antho" means "flower" and "kyanos" means "blue" in Greek language [1]. This word was first used to describe the blue color of cornflower, *Centaurea cyanus*. These are the class of phenolic compounds called flavonoids and most abundantly found in nature. Almost 1000 anthocyanins are present in nature. These are only flavonoids that are visible to human eye [1]. Besides angiosperms, they are also found in gymnosperms, ferns and some bryophytes. They are present in flowers, petals, leaves, tubers, seeds, stems and fruits and generally found in peripheral tissues [2]. They occur in 27 families, 73 genera and multitude species [3]. They give colors to plants which is helpful in pollination and seed dispersal [1]. In anthocyanins' structure, conjugate double bonds are present which absorb light at 500 nm wavelength and show a variety of colors [4]. The production of anthocyanins inside plants and their motion are controlled by genes and a little mutation in those genes cause change in their structure and it can be revealed by change in their color [5]. As it is generally accepted that anthocyanins are produced on the cytoplasmic surface of endoplasmic reticulum but how they are transported and accumulated in cell, isn't understood completely. They are the highest consumed pigments by human beings and it can be estimated by the recent survey that anthocyanins are consumed 180-215mg/day while the consumption of other flavonoids is 20-25mg/day [4]. Their consumption differs due to different gender, age, region and food priorities [3]. They are soluble in polar solvents and stable in acidic conditions [1]. That's why, they are stable in stomach and their stability is reduced to 25% in intestine due to change of acidic condition to alkaline condition in intestine [6]. They can be used as natural food colorants and make a food edible visually. As they have a lot of health benefits, they are being used in food as replacement of synthetic food colorants. Europe has given consent to 13 anthocyanins as natural food colorants in their diet [3].

An anthocyanin named as enocyanin extracted from red grape pomace, is the most abundant and oldest anthocyanin which is sold in market in Italy since 1879 [3]. In the structure of anthocyanins, sugar moieties are present. Structure of anthocyanins without sugar molecules are called anthocyanidins [4]. Almost 17 anthocyanidins are present in nature [1] out of which 6 classes of anthocyanidins are most common [4]. They play vital role in health of plants as well human beings. They suppress the photo-inhibition and photo-bleaching of chlorophyll of plants in light stress conditions [7]. They act as radical scavengers,

anti-oxidant agents, anti-inflammatory agents and positive effect on human health [1]. They regulate the human circulatory and nervous system and have positive therapeutic activity in ophthalmology [3]. They can be extracted from natural sources by both old and modern different techniques including solvent extraction, solid phase extraction, liquid chromatography-mass spectrometry technique (LC-MC), liquid chromatography electron impact ionization mass spectrometry (LC-EI-MS), Atmospheric pressure ionization (API) technique, Matrix assisted laser desorption ionization technique and Capillary electrophoresis (CE) technique [1]. Their structural analysis can be done by NMR spectroscopy and mass spectrometry [1].

2. Chemistry of Anthocyanins

As anthocyanins are natural pigments and impart colors to biological systems, so anthocyanins have complexity in their general simple structure [8]. Chemistry of anthocyanins play a vital role in their color impart and other health-promoting properties. Most of the anthocyanins are monomeric in nature [9]. Anthocyanins belong to a class of flavonoids. Their aglycosylated form is called anthocyanidins [9] which has a core of flavylium cation [10]. The main parts of anthocyanins are aglycone, sugars and in most of the cases acyl groups [9]. Anthocyanins differ from each other due to number of hydroxyl groups, the nature and number of sugars attached to molecule, the position of this attachment and the nature and the number of aliphatic or aromatic acids attached to sugar in a molecule [1]. There almost 35 naturally monomeric anthocyanidins have been discovered but it is observed that 92% of reported anthocyanins have 6 anthocyanidins in their structures [9]. These six most common anthocyanidins in higher plants are pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp) [9]. Anthocyanidins do not assemble in plant tissues [11]. The flavylium cation has C3-C6-C3 skeleton in which one heterocyclic benzopyran ring (ring C), one fused aromatic ring (ring A) and one phenyl constituent (ring B) are present [10]. The positive charge is present is due to the presence of two double bonds in heterocyclic ring and O-atom is bearing a positive charge [10]. The sugar elements are attached to anthocyanidins through the mechanism of glycosylation to the 3-hydroxyl group of ring C in some cases 5-, 7-hydroxyls of ring A [9,10,12].

It is generally accepted that first sugar elements always substitute 3-hydroxyl group and the second sugar element attach to C-5 [11]. Sugar elements are attached to anthocyanidins through O-linkage in anthocyanins and they enhance the stability and solubility of anthocyanins [9]. These sugar elements consist of one or more units of glucose, galactose, rhamnose, arabinose, xylose and glucuronic acid. But glucose is present in majority of anthocyanins [9]. In almost 66% of anthocyanins, acylation of sugar elements takes place with aromatic or aliphatic acids [9]. The functional properties and chemical stability of anthocyanins are greatly influenced by acyl groups [10]. Mostly acylation of sugar takes place at position-6 but 2-, 3- and 4- acylations have also been observed in rare cases [9]. These acylation patterns show the diversity and complexity in anthocyanins [8]. Malonyl is the most common acyl element which is present in 25% of anthocyanins [9]. There are many more factors that effects the stability of anthocyanins, pH and temperature are one of them [12].

Anthocyanins are more stable in more acidic conditions and flavylium cation shows red color in such conditions [9]. By changing pH, the structure of flavylium cation shows reversible structural changes in aqueous phase and four different structures are formed at different pH levels in aqueous phase due to many chemical amendments [9,12–14]. These structures are flavylium cation, quinoidal bases, carbinol and chalcone forms. Phenolic acids can be produced by chemical degeneration of chalcones [9,12,13]. The color of flavylium cation also deviated towards higher wavelength due to enhanced substitutions of ring B [15]. It is observed in in vitro studies that stability of anthocyanins is reduced by enhancing the hydroxylation of B-ring in pelargonidin, cyaniding and delphinidin [12]. Hydroxylation and methylation also influence the hue and color stability of anthocyanins [10]. In recent research, it is noted increase in blueness is related to greater number of free hydroxyl groups and redness boosts by enhancing hydroxyl group methylation [10]. Anthocyanins also show cis-trans geometry in plants and mostly trans form predominates the cis form [16]. In leaves, it happens due to greater sunlight availability [16]. Anthocyanins are more stable in less temperature with heating for short period of time [17].

Anthocyanins are thermally degraded following first order kinetics with respect to temperature and it can be thoroughly illustrated by Arrhenius relation [17]. Pelargonidin 3-glucoside is the lightest common anthocyanin with MW=433g/mol [9]. Deoxyanthocyanins are present in ferns and bryophytes and also occur in angiosperms in rare cases [9]. A group of pyrananthocyanidins is involved in color evolution of wine

[9]. 3-glycosides are present in grapes which is susceptible to readily color lose but the color intensifies in wine maturation and it is due to the fact that 3-substituted anthocyanins are substituted by 4-substituted anthocyanins during maturation through a chemical process [2,18]. The very first C4 substituted anthocyanin extracted from non-fermented plant extract is Rosacyanin B [2].

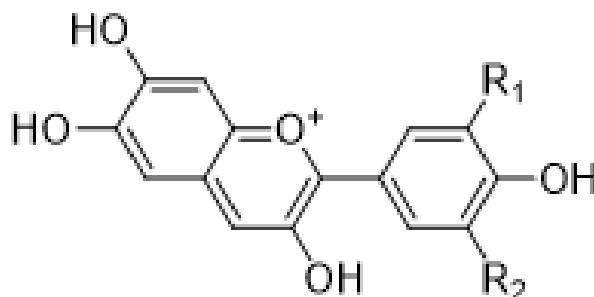


Figure 1. Structure of flavylium cation

Table 1. Substituents of six most common anthocyanidins [13]

Anthocyanidins	R ₁	R ₂
Pelargonidin (Pg)	H	H
Cyanidin (Cy)	OH	H
Delphinidin (De)	OH	OH
Peonidin (Pn)	OCH ₃	H
Petunidin (Pt)	OH	OCH ₃
Malvidin (Ma)	OCH ₃	OCH ₃

3. Co-pigmentation

The phenomenon of piling up of organic molecules called cofactors or co-pigments, on the planar polarizable elements of anthocyanins through molecular inter-relation is called co-pigmentation [19]. It is the spontaneous and exothermic chemical phenomenon [19]. This process was first studied on flower petals [20]. It was also noticed in oenin (malvidin 3-glucoside), a grape pigment, by Willstatter and Zollinger in 1916 as by adding tannin or gallic acid into it, its hue was altered into bluish red. In 1931, Robinson and Robinson discovered that same anthocyanin show different hues and different anthocyanins represent same color in plants through this mechanism [20]. Anthocyanins are highly unstable and lose their color in short time, so it plays a vital role in color stability of anthocyanins in food products [19]. Co-pigmentation leads to diversity and complexity in anthocyanin colors and hues. It can be a worthy natural way to enhance and stabilize the color of anthocyanin rich food products [19]. The type and concentration of anthocyanins and co-pigment determine the variation in color, hue and intensity. So along with color stabilization, co-pigmentation also involves in color modification [19]. Anthocyanins are colorless in plant vacuoles in free state but as they impart colors to flowers and fruits, their color must be stabilized by any other molecules present in cellular fluids of flowers and fruits, commonly referred to as co-pigments [20].

The colorless or slightly yellowish colored molecules possessing a p-electrons rich portion capable of interacting with electron poor flavylium cation of anthocyanins, occurring in plant kingdom are called co-pigments or cofactors [19–21]. More than one co-pigment molecules are involved in co-pigmentation [20]. pH, temperature, concentration, solvents, ionic strength and molecular structure highly influence the co-pigmentation [20]. More amount of co-pigment as compared to concentration of anthocyanins, the more productive co-pigmentation will take place [20]. The size and sterical arrangement of co-pigment also influences the color stability of anthocyanins [19]. Many co-pigments are found in fluids along with anthocyanins but flavonoids and other polyphenols, alkaloids, amino acids and organic acids are the structurally unassociated and most common co-pigments involve in co-pigmentation [20].

Flavonoids is the most studied group of co-pigments in which rutin and quercetin show efficient co-pigmentation at pH 3.2 to malvidin 3-glucoside and produce bathochromic shift at 30nm and 28nm,

respectively [20]. Co-pigmentations mainly takes place through four different mechanisms which include intermolecular complex formation, intramolecular complex formation, self-association and metal complexation [20].

Intermolecular complex formation is predominating mechanism in non-acylated anthocyanins present in both vacuolar and extra-vacuolar fluids of cells in which complex formation takes place in 1:1 [20]. It involves hydrogen bonding, hydrophobic interactions and electrostatic interactions between pigment and co-pigment [20]. Intramolecular complex formation is the mechanism associated with co-pigmentation of anthocyanins possessing acylated sugar elements with phenolic acids and it is considered as more efficient as co-pigment becomes part of anthocyanin due to covalent linkage between them [20]. The substitution position of acyl groups effects the color stability of anthocyanins as the acyl group substitution in B ring is more favorable in co-pigmentation as that of A ring [19]. Piling up-like interactions in anthocyanins is parallel to self-association [20]. In some literature, it is also considered as part of intermolecular complexation [20]. Metal complexation mechanism involves the formation of metal chelation of anthocyanins possessing more than one free hydroxyl groups in B ring with different metals like tin (Sn), copper (Cu), iron (Fe), aluminium (Al), magnesium (Mg) and potassium (K) [20]. Ferric ions show dark purple colored complexes in ratio 2:1 with cyaniding 3-glucoside through co-pigmentation, so oxidation state of iron is very important in co-pigmentation [22]. This mechanism shows displeasing contaminants in food products, so it is not favored process in food industry [22].

Co-pigmentation is co-related with unique associated tetrahedral hydrogen-bonded network of water molecules, so it takes place in aqueous media as pigments and co-pigments become closer due to this network [19]. It can also take place in binary mixture of alcohol and water with water as major component but alcohol favors degradation in co-pigmentation [19]. Some authors consider co-pigmentation as platform for production of new pigments with known composition and their concentration [19]. It protects the flavylium ion from hydration and nucleophilic attack of water molecules to stabilize its red color [19,23]. It leads to increase of half-life of anthocyanins [19]. The co-pigmented anthocyanins are more stable chemically and less degradable in ascorbic acid [24]. In fresh red wine, 30% co-pigmented anthocyanins are present but their percentage is reduced during storage due to degradation [21]. Co-pigmentation shows hyperchromic shift by increasing the absorbance intensity and bathochromic shift by positive shift in visible spectrum [23,25]. As anthocyanidins also influence the rate and degree of co-pigmentation through many factors and many other factors increase or decrease the co-pigmentation, so this phenomenon cannot always be predicted and well perceived [23,26]. Just because of possessing wider planar chromophore, pyranoanthocyanins are more favorable to co-pigmentation as compare to their parent anthocyanins [19]. Co-pigmentation enhances both visual and biological activities of food products and it cannot always be predicted visually [27]. Red-colored water soluble co-pigmented Roselle extract can be a favorable substitute of synthetic colorants in food products [28].

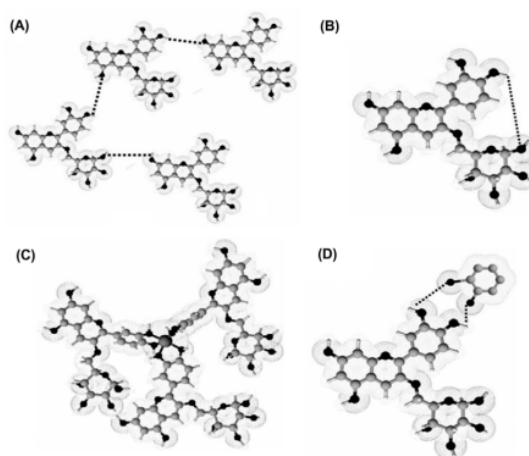


Figure 2. Anthocyanin interactions. (A) self-association, (B) intramolecular co-pigmentation, (C) metal complex formation, (D) intermolecular co-pigmentation

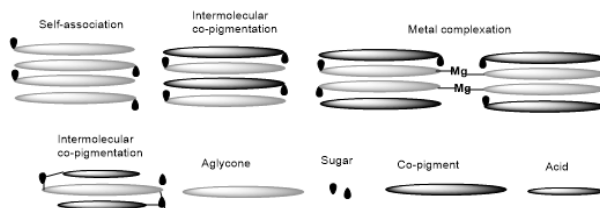


Figure 3. Interactions of co-pigmentation

4. Biological activities of anthocyanins

Anthocyanins are glycosylated polyphenolic compounds, belong to a group called flavonoids. They are the part of human diet for centuries and also used as medicines to cure diseases. They behave like defensive compounds for human body [29]. Besides of being used in food industry as food colorants, they are also use in medicinal industry and behave as anti-carcinogenic compounds and protect against cardiovascular and coronary heart diseases [30–34]. Most of the anti-oxidants are derived and extracted from natural products like flowers, fruits, vegetables, spices, grains, herbs etc [29]. They are efficient natural anti-oxidants and free radical scavengers for human use possessing no harmful effect [35]. Anthocyanins are most beneficent, natural anti-oxidant and free radical scavengers, although, being good anti-oxidants cannot describe the health promoting effects of anthocyanins [36], but they regulate the body system towards normal strengthen the immune system [30]. Free radicals are produced in body during metabolism and if they are produced within normal range, they are consumed by body but if their concentration exceeds, they cause uncontrolled damaging effect and lead to cardiovascular disease, arthritis, aging, cancer, Alzheimer's disease, critical oxidative destruction to biological molecules like DNA, lipids and proteins and many other drastic effects on body [30,35]. In vivo and vitro studies showed that anthocyanins possess better scavenging properties as compared to vitamin C [18]. Anthocyanins produce phenoxy radicals through dehydrogenation which have a strong ability to reduce free radicals in the body [18]. Production of free radical takes place through a chain like process, these molecules interrupt the chain and inhibit the production of free radicals [30].

DNA is a genetic material possessing double helical structure and carry all information and instructions about an individual. It is also protected by anthocyanins from oxidative damage through co-pigmentation as it is very reactive co-pigment and forms complexes through the mechanism of intercalation while proteins are moderate-level co-pigments of anthocyanins [37]. As anthocyanins can easily donate hydrogen, they protect lecithin, low-density lipoprotein, liposome, linoleic acid and ascorbic acid from oxidation by hydrogen donation, metal chelation, protein binding and co-pigmentation interactions [37].

Besides anti-oxidant activities, anthocyanins also possess anti-inflammatory properties and reduce the risk of cardiovascular heart damage. As they possess this property, they inhibit the production of nitric oxides in the body [14], reduce inter cellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) levels, angiotensin level, cholesterol and triglyceride levels and ameliorate the endothelial functions and menopausal symptoms by protecting from osteoporosis [36]. Excessive alcohol consumption can lead to severe chronic hepatic diseases like steatosis, steatohepatitis and hepatocellular carcinoma, but the consumption of anthocyanins can protect liver from alcoholic damage [37]. Plasma monocyte chemoattractant protein 1 is an inflammatory factor present in blood which enhances and promotes the cardiovascular heart damage. So extracts of red grapes like juices are preferred in diet of hemodialysis patients as they reduce the cardiovascular heart damage through anti-inflammatory activity and vasorelaxation [32].

Anthocyanins also possess anti-fungal and anti-microbial properties [32,36,38] and many phenolic compounds like resveratrol protect against pathogenic fungi like *G. candidum* and *C. albicans* when it is used in controlled amount i.e. 10-20 μ L, with no damaging activity in human blood [32,38]. The microbial activity is influenced by the number of free hydroxyl groups in ring B and degree of polarization [32]. 3-deoxyanthocyanin, occur in angiosperms, possess a beneficial anti-bacterial and anti-oxidant properties [38]. Cancer is the highly spreading disease in human beings and thousands of people die every year over the world but yet there is no treatment of chronic cancer has discovered. It may cause either due to any environmental factor or due to genetic disorder. Anthocyanins are very effective cancer prevention and treatment as they suppress the growth of cancer or tumor cells due to its anti-proliferative property [39] and apoptotic and anti-angiogenesis activities when they are dosed in appropriate amount [33]. They also bind with DNA

through co-pigmentation and block its expression in case if tumor cells are produced due to genetic effect [32]. They also show their anti-carcinogenic effect in gastric, breast, colon, prostate, renal, hepatic and leukemia cell lines and among them, leukemia cell line is most susceptible towards cancer [34].

They also act as anti-aging agents by inhibiting the activity of aldose reductase [40] and by preventing the increased level of oxidative DNA damages in neural tissues [32], ameliorate the neural and behavioural activities [38]. Sickle cell anemia is a genetic disease due to abnormal haemoglobin production in blood that affect the children and many children die before their probation due to poor treatment. Many pharmaceutical medicines are discovered to cure this disease but as it is a chronic disease, they also show reverse effects on stomach, kidneys and liver. As DNA is a reactive co-pigment for anthocyanins, pigments interact with normal haemoglobin and compete with abnormal haemoglobin and help in curing this chronic disease with no reverse effect on immune system and other organs through their anti-sickling property [41].

Anthocyanins also show anti-fatigue, anti-obesity and anti-diabetic activities. Fatigue is caused by many factors including physiological and biochemical changes and it can be mental or physical fatigue. Fatigue is mainly caused by oxidative damage and high level of radicals and anthocyanins reduce fatigue through radical scavenging [40]. They show the inhibitory effect towards insulin resistance and increase the insulin production, glycemia level and sensitivity of insulin receptors cells. They inhibit the fat accumulation and protect from obesity and high blood pressure and it is affected by the number of OH groups in ring B [33]. Diabetic cataract is a condition in which diabetes gets complicated and it leads to vision loss and it is caused by an enzyme named as aldose reductase [31]. Almost 20,000 patients in USA and one quarter of total blinded persons in Japan lose their vision due to cataract and retinopathy. Anthocyanins are very effective aldose reductase inhibitors, so they protect from cataract and improve vision [31]. Besides all of these properties, anthocyanins protect from circulatory diseases by reducing capillary fragility and platelet aggregation [33], protect from UV radiations and ulcer [38]. It is accepted that, not anti-oxidant activity is always related to anthocyanins alone, other phenolic compounds show synergistic effect in anti-oxidant activity along with anthocyanins [42].

It is believed from free radical theory that aging is caused by mainly DNA mutation and lipid and protein dysfunction. Moreover, other accumulated oxidatives also take part in aging [40]. The people who consume food rich in anthocyanins and take 2 glass of red wine per day have 27-39% reduced risk of cardiovascular and coronary diseases [14,33]. Excessive intakes of anthocyanins can cause their accumulation in many tissues of body organs even in brain barriers [31].

5. Conclusion

Anthocyanins are glycosylated polyphenolic compounds, found abundantly in nature. They possess a simple basic structure, flavylium cation, but they have very complexity and diversity in their structures as well as functions. They have become a vital dietary supplement due to their enormous functions in food and pharmaceutical industries as health promoting and disease preventing agents. They are being used for centuries to cure diseases in the form of herbs. They possess anti-oxidative, anti-allergic, anti-inflammatory, anti-mutagenic, anti-diabetic, anti-obesity and anti-aging properties and protect against, cardiac, hepatic, renal and circulatory disease and play a significant role in ophthalmology. Yet it is not well understanding that how anthocyanins take part in health promoting activities, through which mechanism they take part and which part of their structure play vital role, so further research is still on progress.

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