J. Chem. Soc. Nigeria, Vol. 49, No. 5, pp 669 - 682 [2024] https://doi.org/10.46602/jcsn.v49i5.1000

THE ANTHOCYANIN METAL INTERACTIONS: AN OVERVIEW

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ABSTRACT

Anthocyanin is one of the phenolic compounds found in various parts of plants. Its study has become an area of scientific exploration due to reports gathered on its reactivity, bioactive properties, and usefulness in pharmaceutics, agriculture and food production. This study investigated recently characterized anthocyanins to identify variations based on plants, plant parts, the ligand characteristics of the isolated anthocyanin, and the need for quick reference for natural product chemists in the areas of isolation and synthesis. The *in vivo* and *in vitro* metal anthocyanin complexes (MAC) were investigated to expand isolated compounds, synthesis, characterization, and study probable kinetics involving new MAC and its applications. The data reviewed was obtained from research reports found on the Web from 2014 to 2023. Results of the reviewed studies indicate anthocyanins have been isolated and characterized from different plants and all plant parts: leaves, pulp, seeds, peels, stems, and roots. The anthocyanidins (aglycone) have been derivatized through hydroxylation, methylation, glycosylation, and acylation due to the presence of many reactive centers, producing over 800 forms of anthocyanins characterized to date. Chelation of anthocyanin *in vitro* has been studied with several metals and metalloids, using the outcome in some cases to monitor and re-produce compounds formed *in vivo*. Several applications of anthocyanins and MAC have been found and are still under investigation, anthocyanins and its derivatives are being screened for ethnobotanical roles and their random applications in other human endeavors.

Key words: anthocyanin, metal-anthocyanin complexes (MAC), *in vivo*, *in vitro*

INTRODUCTION

Anthocyanins are naturally occurring substances found in different plant parts: leaves, stems, roots, flowers, fruits, and grains [1]. Anthocyanins are secondary plant metabolites [2], polyphenolic in nature, which, according to deductions from studies, evolve with chlorophyll degradation [3]. They are naturally placed to perform such functions as attracting insects for pollination and seed dispersal, playing roles in photosynthetic processes, and being a symptom and coping mechanism for plants when under stress [4]. Other biological roles identified with anthocyanin include redox status regulation, antibacterial activity, inhibition of viral enzymes, gene activation, etc. However, the observed variations in the time of appearance, tissue placement and inducibility make it difficult to establish a unified explanation for the presence of anthocyanins in plant tissues [5].

Anthocyanins are water-soluble pigments responsible for the color observed in fruits, vegetables, flowers, etc. The color ranges from orange-red to blue-violet; hence, anthocyanin has been extensively studied in food science, with a focus on wine production [3, 6], in which anthocyanin serves as a natural colorant [7] against synthetics. Researchers have approached investigations on these polyphenolic substances from different perspectives and fields of interest, varying in order: food science and technology, applied chemistry, nutrition, dietetics, and agriculture. Some reviewed articles [5, 8, 9] constituted the data domain on anthocyanins: extraction, isolation, and identification; the roles in plant propagation; and defense mechanisms. Parameters that alter the natural existence and stability factors were the focus of [2], with emphasis on the limitations associated with these investigations, which is the stability of anthocyanins.

The characterization of anthocyanin from different plants has been the subject of several studies [3, 10, 11]. The pigment is composed of an aglycone (anthocyanidin). There are six common anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin), which, when esterified to one or more sugars, give the glycosides (anthocyanin), whose primary structures vary by substitution at the 3’ and 5’ (Figure 1.0) positions [6]. The substitutions: hydroxylation, methylation, glycosylation, and acylation result in over 800 forms, as well as varying the chemistry of anthocyanin in terms of color, susceptibility to oxidation, bathochromic and hypsochromic shifts, stability, and solubility [2].

Several techniques have been employed in extracting plant constituents; the most effective maximizes the recovery of the sample, minimizes the amount of junk, and decreases the degradation or alteration of the natural state of species [5]. Anthocyanin, being polar, was extracted from different sources via alcohol, i.e., methanol or ethanol, at different concentrations in combination with an acid. Hydrochloric acid, acetic acid, and citric acid have been mainly used for the extraction of anthocyanin [2, 12]. The use of acid in the extraction process is to stabilize anthocyanins in the flavylium cation form, which is often red-colored. Color variations are an indicator of the structure of anthocyanins; natural colors have less stability than synthetic colors. Due to the highly reactive nature of anthocyanins, they degrade or react with other constituents in the medium to form colorless, yellow, or brown-colored compounds [1, 2, 4].

Four different secondary structures of anthocyanin are found to exist in equilibrium with one another, with their relative amounts varied and dependent on the pH of the solvent, light, and anthocyanin structure [1, 13, 14]. These factors give rise to proton transfer, hydration, tautomerization, and isomerization, leading to anthocyanin transformations. In strongly acidic conditions (below pH 2), anthocyanidins predominantly occur in the flavylium cation form. In slightly acidic or neutral conditions, deprotonation of the flavylium cation gives rise to quinoidal forms. While still in this weakly acidic solution with the presence of water, the flavylium cation form could be hydrated, giving rise to the hemiketal forms, also known as hemiacetals or carbinol pseudobases. At even higher pH values, these forms can be further deprotonated into quinoidal bases. These various forms create platforms for metal chelation within the natural systems. Although, several factors limit the in – vivo studies [15], metal anthocyanin complexes (MAC) formed in - vivo have been reported [16], in – vitro metal anthocyanin complexes have equally been explored extensively [17, 18]. Among the mineral elements essential to and found in plants are metals; some present on the macro level (1000 mg dry weight) are s-block metals: Ca, Mg, and K, while those in trace amounts (100 mg) found in healthy plants include Fe, Ni, Cu, Mn, Zn, and Mo, which are d-block or transition metals [19]. The accumulation, distribution, and application of adsorbed metals in plant tissues require a complex interaction of chelating compounds. Among these chelating compounds are anthocyanins, which, when modified [2], readily form complexes with metals. Extracts of various plants have been reacted with metals in vitro to form compounds, which have given rise to more information on the metal anthocyanin complexes, which are finding applications in several systems. Metal anthocyanin complexes are self-assembled, supramolecular metal-containing pigments, which comprise 6 anthocyanin molecules, 6 flavone molecules, and 2 metal ions [20].

This review investigated recently characterized anthocyanins in terms of plant and plant parts from which they are derived, types, or forms of anthocyanin, to ascertain the specified structure-activity relationship (SAR) of characterized anthocyanins, gain insights into the sources and structure of already isolated and quantized anthocyanins and their *in vivo* and *in vitro* metal anthocyanin complexes (MAC).

MATERIALS AND METHODS

The data reviewed was obtained from research reports found on the Web. The publication period of the consulted articles ranges from 2014 to 2023. The search was guided by the words anthocyanin, anthocyanin metal interactions, applications of anthocyanin, and applications of metal anthocyanin complexes. There was no discrimination against the article consulted in terms of journal outlets.

RECENTLY CHARACTERIZED ANTHOCYANINS

Anthocyanin in leaves

In leaves, anthocyanins may color the entire blade, but they can also be located only in the margins, stripes, patches, or seemingly random spots on the upper, lower, or both lamina surfaces [4]. Anthocyanin in leaves serves the purpose of revealing the physiological status of the plant and also its stress response. MdGST1 is a gene encoding glutathione-S-- transferases (GST) enzyme found in one of the steps of the anthocyanin biosynthesis pathway, it was isolated from the leaf of the red-fleshed apple variety 'Royalty' by full cDNA library sequencing and the rapid-amplification of cDNA ends method thus supplying information on apple coloration(10). The anthocyanin content in the leaf of Prunus cerasifera [21] was achieved by various calibration methods, the visible spectroscopy and backpropagation neutral network-latent variables (BPNN-LV) model has ascribed the best and a non-destructive determination of anthocyanin content in plants. [22] found the abundances of anthocyanin synthesis-related enzymes: chalcone synthase, chalcone isomerase, dihydroflavonol 4-reductase, and anthocyanin synthetase in the purple leaf of Zijuan Tea, all significantly higher than those in the green leaf. The red variety of Atriplex accumulated more anthocyanins in the leaf than green and rosea ones [23].

Anthocyanin in Fruits

In fruits, anthocyanins are mainly located in the peel [24]. The most predominant anthocyanidins found in purple and red potato extracts were petunidin-3-coumaroylrutinoside-5-glucoside and pelargonidin-3-coumaroylrutinoside-5-glucoside, respectively. Steamed purple potatoes was found to retained more anthocyanin than other cooking methods [25], which in turn contained more anthocyanin compared with the fresh sample. Chlorogenic acid and its isomers were found to be the main phenolic compounds in native Andrean potatoes [26]. Malvidine-3-O-glucoside was obtained from *Grewia coriacea* Mast [11]. The efficiency of different extraction techniques was compared in extracting the anthocyanin in *Rubus fruticosus*, *Vaccinium myrtillus*, and *Eugenia brasiliensis* [27] while [14] explored the influence of different organic acids in extracting anthocyanins from barberry, eggplant peel, and red cabbage. The reaction of metal ions with uncharacterized anthocyanin extracts of red dragon skin fruit studied by [28] shows an increase in the antioxidant activity of the samples in the presence of metal ions. Buni (*Antidesma bunius*) fruit extract was tested for its ability to generate a blue color with four different metal ions. The anthocyanin content of the methanol extract of senggani fruit was found by [29], [30] to be 7.516 mg/100 g. The total anthocyanin content in super red dragon fruit rind was 15.66 ± 1.59 mg/L, which increased with temperature and ultra-violet light exposure [31].

Anthocyanin in other Plant Parts

Two red pigments were isolated from liverwort (*Marchantia polymorpha*); structure elucidation shows 2, 3, 6, 8-tetrahydroxybenzofuro [3, 2-b] chromen-5-ium-6-O-(2-O- α-rhamnopyranosyl-β-glucopyranoside and its aglycone. Anthocyanin from was extracted by [32, 33] from red sorghum bran and studied the influence of operating factors on optimizing its yield, concluding that red sorghum bran is a potential source of anthocyanin and its extraction an eco-friendly means of waste valorization. Freeze drying was found by [34] to be the best method to preserve the total monomeric anthocyanin contents, vitamin C, and antioxidant capacity of different parts (the pulp, the seed, and the peel) of the African star apple. Purple Pak Choi (*Brassica campestris ssp. Chinensis Makino*) seedlings were exposed to low light by shading with white gauze and black shading in a phytotron; the anthocyanin content, as well as other parameters, decreased significantly with longer shading time [35].

Metal Anthocyanin Interactions

In-Vivo Metal Anthocyanin Interactions

The hydroxyl groups on anthocyanins are hydrophilic and can establish hydrogen bonds as donors and acceptors. While the phenyl rings are typically hydrophobic but can establish Van der Waals interactions with other similar (weakly polar, polarizable) structures [68], Hence, like other dihydroxy B-ring-substituted flavonoids, anthocyanins can bind metal and metalloid ions. Java plum fruit was analyzed by [16] with the aid of a liquid chromatography-mass spectrophotometer (LCMS). The presence of eight different anthocyanins: C-DG, Dp-G, Mv-DG, Cy-G, Pn-G, Cy-P, Pl-G, and c –Mv - G – Co, was detected, which chelates transition metals as observed in the infrared absorptions: Cu-O at 765–735 and Fe-O at 625–490 and 571.81cm^{-1}. It has been shown that metal ions not only contribute to the color displayed by anthocyanin but are also named among the factors that aid its degradation [17, 18] and constitute stress, especially with the accumulation of heavy metals such as As, Cr, and Cd, which are toxic and contaminate the plants. The presence of Cu, Ni, Pb, and Zn was found to reduce the carotenoid and anthocyanin content of the annual atriplex (*A. hortensis and A. rosea)*[23]. However, studies of in vivo metal anthocyanin interactions are limited by several factors documented by [15].

In-Vitro Metal Anthocyanin Interactions

Varying quantitative and physicochemical deductions from red cabbage (RC) and purple sweet potato (PSP) as both interacted with metal ions were recorded [17]. The acylated cyanidin glycosides in the two plants are only slightly different in structure; however, at pH 6 and 7, bathochromic shifts induced by Al^{3+} in the presence of RC (50 nm) was greater than PSP (20 nm), Fe^{3+} at similar acidity, exerted much higher shifts for RC; 90 nm at pH 7 and 110 nm at pH 5.7. Thus, the study, with the aid of high-resolution mass spectrometry, suggested the formation of metal-anthocyanin complexes of ratio 1:1, 1:2, and 1:3. Purple potato and black goji were found to contain acylated petunidin derivatives, which when reacted with Al^{3+} and Fe^{3+} at different concentrations in buffers of pH 3–10, within alkaline pH, small metal ion concentrations triggered bathochromic shifts of almost 80 nm, producing vivid blue hues [36]. However, Fe^{3+}caused a larger bathochromic shift producing green colors at pH 8–9 and enhancing the stability of anthocyanin.

Although, the specific anthocyanin in Buni fruit (*Antidesmal bunius*) was not identified in the study by [29] a similar influence was observed, however, in contrast to [36], the stability of the metal-anthocyanin component was lower than that of anthocyanin alone, and the impact made on bathochromic and hyperchromic shift at pH 3–8 by Ca^{2+} and Mg^{2+} was minimal. The anthocyanin content of red dragon skin fruit (*Hylocereus polyrhizus*), as well as the antioxidant activity (IC_{50}), were determined [28], and the latter was found to increase with the addition of Cu^{2+} and Mg^{2+} ions. A red-yellowish complex obtained from the treatment of a concentrated anthocyanin extract of red dragon fruit with hydrated iron (III) chloride was evaluated for its applicability as a photosensitizer on a dye-sensitized solar Cell (DSSC). The observed increased absorption coverage in terms of excitation from the valence band to the conduction band (HOMO-LUMO) and the blue shift of the conduction band by the complex are indications of a good solar cell sensitizer [37]. Metal chelating ability (MCA) in the freeze- and oven-dried samples of African star apple was found by [34] to range from 43.53 (seed) and 51.46% (pulp) to 42.72% (seed) and 47.13% (pulp), respectively. Self-association, co-pigmentation, and metal chelation as a means to expand the color expression of anthocyanin to purple and blue in acidic pH were suggested [18]. The nature of anthocyanin and the extent of acylation prominently affect the metallation of anthocyanin; chemical shifts were greatest with electron-rich metal ions. Total anthocyanin content; 709.3866 mg/mL, antioxidant activity; 0.0046 µg/mL, was obtained from rambutan (*Nephelium lappaceum*) peel extract when reacted with Mg^{2+} in varying concentrations [24], the results showed that the metal has no significant effect on the antioxidant activity of the plant.

STRUCTURE ACTIVITY RELATIONSHIP (SAR) of CHARACTERISED ANTHOCYANIN

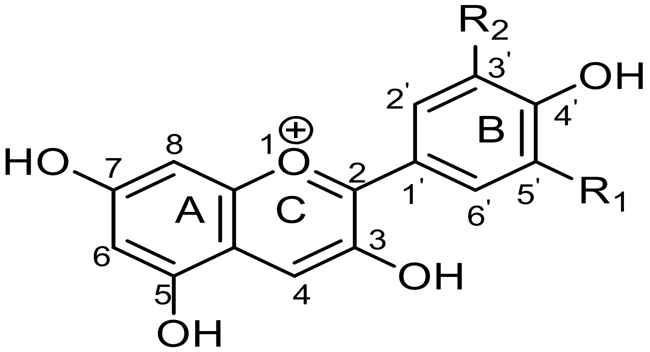

Figure 1.0: Typical Structure of Anthocyanin

Structure and Stability

Around 92% of all anthocyanins occurring in nature are based on just six anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (Figure 1.0), which only differ in the hydroxylation and methoxylation of the 3’ and 5’ positions of the B-ring [1, 38]. The type and number of acylations found in anthocyanins have been linked to which plant family of species the anthocyanins come from [38]. Anthocyanins can be present in four different forms or structures [1], which are in equilibrium with one another, but upon variation of the pH of the environment, the relative amount of the forms changes. The flavylium cation (red color) is the most stable form (stable at $pH \leq 2$) of the molecule under acidic conditions. However, as acidity decreases, the C-ring opens up to give the hemiketal and chalcone (Cis and Trans) forms (both forms are colorless) at pH 3 - 6. The flavylium form undergoes proton loss to give the quinonoid form (blue color) under neutral pH and slightly alkaline conditions (pH 7, 8, and 10). Cy3Glc was more stable than cyaniding at pH 2.0 and 25°C, this observation is reversed with an increase in temperature and pH, as obtained by [6], in which Cyanidin showed greater thermal stability compared to Cy3Glc at pH 7.0 and 55 ± 0.1 °C. the degradation kinetics of total monomeric anthocyanins was studied by [14], and it was revealed that the stability of anthocyanins in solution depends on temperature and other components that are present in the medium. Anthocyanins from blueberries were subjected to simulated gastrointestinal digestion by [39] and found that fewer hydroxyl groups and more methoxyl groups on the B-ring improved anthocyanin stability, while acylation decreased the stability of malvidin 3-O-glucoside [38, 39].

Spectra of Anthocyanin and Metal-Anthocyanin Complexes

Chromophores in anthocyanin are the aromatic rings, in addition to autochromes groups such as C = O, -N = O, = C-NH, -C-S, -N = N-, -CH = N, and -C = C, whose number defines the intensity of the color. In general, pigments of pelargonidin (max = 494) and cyanidin (max = 504–510) derivatives produce red and purple colors, respectively, whereas those of malvidin, delphinidine, and petunidin (max = 520, 522, 546) show purple or blue colors [1, 41, 42]. The intensity and type of the color of anthocyanins are determined by the conjugated double bonds as well as the number of hydroxyl and methoxyl groups. The color observed in metal anthocyanin interactions in vivo and in vitro had been attributed to self, inter, intra, and/or metal co-pigmentations. The strongest color effect and color stabilization were ascribed to co-pigmentation with positively charged alkaline earth metals or with poor metals (+2, +3) [13, 41]. Studies have equally shown that specific metal ions and pH alter or stabilize the color displayed by anthocyanins. Blueness is increased with an increased number of hydroxyl groups, as well as the presence of Mg^{2+} and Al^{3+}, while redness increases with enhanced methylation in the B-ring [3]. The presence of 3-glucosidic residue significantly decreased the color intensity of Cy3Glc compared to cyanidin under given conditions at pH 7.0 and 55 ± 0.1°C, while 3-glucosidic substitution is favorable for color intensity in the alkaline region [43]. The array of applications observed with anthocyanin and or its metal complexes are color-dependent.

APPLICATIONS OF ANTHOCYANIN

Natural Indicators

Several studies have focused on applying anthocyanin extracts obtained from different plants and their parts, such as flowers, fruits, vegetables, seeds, roots, and microorganisms as indicators: *Turmeric Rhizome, Curcuma longa* [44], *Hibiscus sabdariffa, Beta Vulgaris* (beetroot), *hibiscus rosa sinensis*, *Acalypha wilkesia* [45], and *Costus afer* [46]. *Syzygium cumini* (L.) [47]. *Aster chinensis* [48], *Hibiscus rosasinensis*, *Calotropis gingantia, Brassica oleracea-capitata, Rosa chinensis, Brassica oleracea-italica, Ixora chinensis, Cantharanthus roseus* [49], red cabbage and sappanwood, *Hibiscus sabdariffa and Ixora coccinea* [50], and Rose, Allamanda, and Hibiscus flowers [51]. *Acalypha Wilkesia* [52]. The extracts were found to compare favorably with synthetic indicators; however, instability limited their applications.

Medical Applications

Anthocyanins are consumed in appreciable amounts as part of a healthy diet high in fruits and vegetables. Anthocyanin consumption varies across populations and depends on the type of food consumed [22]. Generally, anthocyanin provides beneficial health effects to the human body as an antioxidant, antidiabetic, anticancer, and anti-inflammatory [9]. An inverse relationship was detected between anthocyanin intake and cardiovascular diseases [53] as well as the risk of hypertension, especially when accompanied by dietary and other modifiable cardiovascular risk factors [54]. Anthocyanins control metabolic syndrome and obesity by ameliorating lipid metabolism, increasing energy expenditure, suppressing food intake, and inhibiting lipid absorption [55]. Of the anthocyanin-rich plants available in Nigeria, the most studied for their medicinal application is *Hibiscus sabdariffa* [56, 57, 58]. It is a rich source of anthocyanin, which has been quantified by various studies [59, 60, 61] and associated with the inhibition of several forms of cancer [62, 63, 64] and cardiovascular diseases.

Applications of Metal Anthocyanin Complexes

According to submissions from studies [15], metals bonded to anthocyanin *in vivo* stabilized anthocyanin, and modified the colors of plants *in vivo* and *in vitro*. It serves as a rapid method for determining the concentrations of metals in biological systems [65], and it is an inbuilt system for detoxifying plants of toxic metal accumulation [66]. Metal anthocyanin complexes act as free radical scavengers [16] and increase antioxidant properties [28]. Metal chelation has been suggested to expand the color expression of anthocyanin from commonly observed red to purple and blue colors in acidic pH [17, 18, 29, 36, 67]. The iron-anthocyanin complex is a good candidate for the sensitizer of DSSC [37].

Other Applications

A film based on biodegradable polymers incorporated with roselle anthocyanins was found applicable to monitoring pork freshness and intelligent packaging, having shown high tensile strength, the highest antioxidant activity, and the best color stability [68]. The findings of [31 and 69] demonstrated that gelatin film incorporated with dragon fruit skin extract could be used as a visual indicator of pH variations to monitor the freshness of foods during storage time. Bread fortified with red cabbage anthocyanin kept at 4°C, was stable over 21 days, retaining its antioxidant properties [70]. Anthocyanins in the flower petals attract insects and birds; hence, they function in cross-pollination. While the colorful skins of fruits attract animals that may eat them and disperse the seeds, anthocyanins are important as natural colorants [7]. Photo-protection [8]. Anthocyanin chelated to TiO_2 can convert light energy to electrical energy [41] and is found to be applicable as photosensitizers [72 and 73]. Technological potential uses in different industrial fields was identified and reviewed by [71] and include the textile and food industries, photodynamic therapy, pharmaceuticals, cosmetic industry, sunscreen filters, nail colorants, and skin and hair cleansing products. Anthocyanin applications would be enhanced by subjecting them to nanoencapsulation [74] and varying physical and chemical modification techniques, itemized and discussed by [75].

CONCLUSIONS

Studies of anthocyanin and its applications have been extensively engaged by researchers for decades. The *in-vivo* metal anthocyanin complexes in plants had not only been equally established, but they had found applications *in - vitro* in different fields: biological, medical, pharmaceutical, and electrical. However, its studies are still evolving; its application in various fields has not been optimally analyzed and utilized. Several potential sources in the locality are yet to be investigated, as are its applications in the area of ethnobotanical claims in traditional healing processes; therefore, there is still a need for researchers to engage in active research on this highly reactive class of compounds and their derivatives. They should be applied blindly in other human endeavors so that they can be used optimally.

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