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EFFICACY OF ANTHOCYANIN IN PRODUCTION OF REMEDIAL TEA

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ABSTRACT
Tea is consumed as a medicinal beverage from centuries, as the medicinal component includes polyphenols, caffeine, and amino acids. Apart from that it also contains flavonoids; compounds reported to have antioxidant properties with many beneficial effects. Anthocyanins belonging to the flavonoid group are naturally occurring compounds that imparts colour to fruits, vegetables and plants. Apart from that it has an array of health promoting benefits. This article has been reviewed to highlight the importance of anthocyanin as well as to motivate research in exploring tea varieties with abundant anthocyanin so that consumption of anthocyanin rich tea or beverage and also use of natural dye made using anthocyanin would replace harmful effects of chemical drugs and also improve country's economy by flourishing the tea industry with increased consumption.

Keywords: *Camellia sinensis*, anthocyanin, pharmacological activity, industrial use

Introduction

Cultivated tea belongs to genus *Camellia*, consisting of three species each with specific plant types viz. *Camellia sinensis* China type, *Camellia assamica* Assam type and *Camellia assamica lasiocalyx* Cambod type (Wight, 1962). Tea is an important agricultural and commercial crop consumed worldwide, mainly as a beverage made from processed tea leaves and it has also been used for medicinal purposes for several centuries (Friedman *et al.,* 2007; Wang *et al.,* 2012). According to varying processing procedures, tea currently made in the world can be classified into six main types including black, green, white, yellow, oolong and reprocessed tea. Of all the types, green tea is mostly favoured as a medicinal tea as many of its medicinal properties like antioxidative, anti-mutagenic, anticarcinogenic, anti hypersensitive, anti bacterial, antiviral and also weight reducing property have been already reported (Saito et al., 2011). Besides green tea, purple tea is gaining much importance since, interest in anthocyanins is growing among researchers owing to their potential health benefits (Kong et al., 2003).

Anthocyanins are the another most important plant pigment besides chlorophyll visible to the human eye belonging to the widespread class of phenolic compounds collectively named flavonoids (Kong *et al.,* 2003). They occur in different colour basically red, blue or purple depending upon their pH. Synthesized through the phenylpropanoid pathway, the water soluble vacuolar pigment has many important roles to play besides imparting colour and contributing to astringent sensation.

It is found in many plant species including red grapes (Rivero-Perez *et al.,* 2008), berries (blueberry, strawberry, raspberry, blackcurrant, bil- berry, cranberry, elderberry) (Nicoue *et al.,* 2007), eggplant (Azuma et al., 2008), purple fleshed sweet potatoes (Oki *et al.,* 2002) and flowers like *Hibiscus* (Lo *et al.,* 2007a). These pigments have been found to be the largest and most important group of water soluble pigments found in nature and they contribute to the attractive colours of fruits, vegetables and flowers imparting red, orange, purple, violet and blue colours (Feild *et al.,* 2001). Anthocyanins in plants normally accumulate in the vacuoles of the epidermal and sub epidermal cells (Steyn *et al.,* 2002). The colours of these pigments are pH dependent (Mazza and Miniati, 1993). Interest in anthocyanins has recently increased owing to their potential health benefits (Kong, Chia, Goh, Chia, & Brouillard, 2003) and their use as an alternative source of synthetic colourants/dyes (Jackman *et al.,* 1987; Kerio *et al.,* 2012).

The anthocyanin pigments that create the

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color (Tsai and Ou, 1996) are responsible for the wide range of coloring in many foods. Recently, the biological activities of anthocyanin, such as antioxidant activity, protection from atherosclerosis and anti-carcinogenic activity have been investigated, and shown to have some beneficial effects in the treatment of diseases.

For instance, there have been reports on the antioxidant activity in grape anthocyanin (Igarashi *et al.*, 1989) and the biological effect of anthocyanin in low density lipoprotein and lecithin-liposome systems (Meyer *et al.*, 1997). Anthocyanins were also found to have many times more activity than common antioxidants such as ascorbate (Wang *et al.*, 1997). Overall, there is now increasing evidence that antioxidants in the human diet are of major benefit for health and well-being. (Tsai *et al.*, 2002)

However, no tea has been demonstrated to contain an abundant amount of anthocyanins. Therefore, 'Sunrouge' was developed, a red leaf tea cultivar that is rich in anthocyanins by natural crossing in 2009. An anthocyanin-rich parental line, 'Cha Chuukanbohon Nou 6', which was derived from *C. taliensis x C. sinensis* in 2004, was previously developed. *C. taliensis* is closely related to *C. sinensis*. However, the anthocyanin content of Cha Chuukanbohon Nou 6'suddenly diminishes as the leaf matures. Therefore, anthocyanin rich tea was developed the cultivar which was higher in anthocyanin content than 'Cha chuukanbohon Nou 6', and in which the anthocyanin content did not diminish after leaf maturation. 'Sunrouge' is an offspring of 'Cha Chuukanbohon Nou 6'. Saito *et al.* isolated six anthocyanins from 'Sunrouge' leaves (Maeda-Yamamoto *et al.*, 2012). Because of the sedentary nature of plants, they are prone to UVirradiation which can cause oxidative stress. Anthocyanins protect plants against such irradiation. Their biosynthesis has been demonstrated to be upregulated when the plant is exposed to UV-B irradiation (Merzlyak *et al.*, 2008). Although anthocyanin is not found abundantly in Camellia sinensis, there are many more purple leaf coloured tea plants that are yet to be explored to make promising anthocyanin rich tea.

Biochemistry of Anthocyanin

Anthocyanin belongs to the widespread class of phenolic compounds collectively named flavonoids. They are glycosides of poly-hydroxy and poly-methoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Kong *et al.*, 2003). Chemically, anthocyanins are glycoside moieties of anthocyanidins derived from the flavylium (2-phenyl benzopyrilium) cation shown in Fig 1 (Kerio *et al.*, 2012). The differences between individual anthocyanins relate to the number of hydroxyl groups, the nature and number of sugars attached to the molecule, the position of this attachment, and the nature and number of aliphatic or aromatic acids attached to sugars in the molecule (Kong

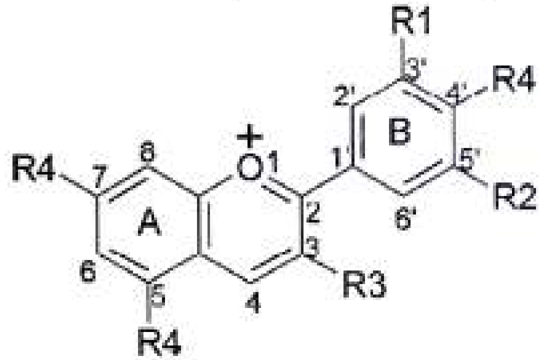

Fig 1: Basic structure of anthocyanidin pigment, the flavylium cation where R1 and R2 are H, OH, or OCH; R3 is a glycosyl or H; and R4 is OH or a glycosyl. *(Kong et al.*, 2003)

There are several anthocyanidins described in nature but among these, six are widespread in fruits and vegetables namely; pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin as shown in Table 1 (Kerio et al., 2012). The various anthocyanidins differs in number and position of the hydroxyl and also methyl ether groups attached on 3, 5, 6, 7, 3', 4' or 5' positions (Table 1). Despite the fact that 31 different monomeric anthocyanidins have been identified (including 3-deoxy anthocyanidins, pyrano-anthocyanidins and sphagnorubins), 90% of the naturally occurring anthocyanins are based on only six structures (30% on cyanidin, 22% on delphinidin,18% on pelargonidin and in summary 20% on peonidin, malvidin and petunidin). Those six anthocyanidins are usually known as common

Table 1: Common anthocyanidins occurring in the nature. Excerpted from Ananga *et al.* (2013)

ANTHOCYANIN COMMON IN HIGHER PLANTS									
Sl. no	Name	Abbreviation	Substitution Pattern						
			3	5	6	7	3′	4′	5′
1	Cyanidin	Cy	OH	OH	H	OH	OH	OH	H
2	Delphinidin	Dp	OH	OH	H	OH	OH	OH	OH
3	Malvidin	Mv	OH	OH	H	OH	OMe	OH	OMe
4	Pelargonidin	Pg	OH	OH	H	OH	H	OH	H
5	Peonidin	Pn	OH	OH	H	OH	OMe	OH	H
6	Petunidin	Pt	OH	OH	H	OH	OMe	OH	OH

anthocyanidins (Ananga *et al.,* 2013).The glycosides of the three non-methylated anthocyanidins (Cy, Dp and Pg) are the most widespread in nature, being present in 80% of pigmented leaves, 69% of fruits and 50% of flowers. The distribution of the six most common anthocyanidins in the edible parts of plants is cyanidin (50%), pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%), and malvidin (7%). The following four classes of anthocyanidin glycosides are common: 3-monosides, 3-biosides, 3,5-diglycosides and 3,7-diglycosides. 3-glycosides occur about two and half times more frequently than 3,5-diglycosides. So, the most widespread anthocyanin is cyanidin 3-glucoside. (Kong *et al.,* 2003)

Because of their polar nature, anthocyanins are soluble in polar solvents, such as methanol (MEOH), ethanol and water. The initial step in their isolation therefore involves solvent extraction, which includes the use of acidified methanol or ethanol. The use of acid stabilizes anthocyanins in the flavylium cation form, which is red at low pH (Mc. Ghie and Walton, 2007).

The color of anthocyanidins differs with the number of hydroxyl groups, attached on their molecules (especially those substituted in ring B). With the increase of attached hydroxyl groups, the visible color of entire molecule shift from orange to violet (Andersen and Jordheim, 2006; Delgado-Vargas et al., 2000; Delgado-Vargas and Paredes-Lopez, 2002; Tanaka *et al.,* 2008).

Glycosylation of anthocyanidins results to additional reddening of obtained anthocyanins, whereas the presence of aliphatic or aromatic acyl moieties causes no color change or slight blue shift and has significant effect on their stability and solubility (Tanaka *et al.,* 2008). Changes in pH can also cause reversible structural transformations in anthocyanins molecules, which has a dramatic effect on their color Delgado-Vargas and Paredes-Lopez, 2002; Wrolstad, 2004). Most of the anthocyanins are *O*-glycosylated at 3 (except those based on 3-deoxyanthocyani-dins and sphagnorubinns), 5 or 7 positions and in some cases at 3′, 4′ and 5′ positions (Ananga *et al.,* 2013). However, 8-*C*-glycosylanthocyanins have been found only in *Tricyrtis formosana* Baker (Saito *et al.,* 2003; Tatsuzawa *et al.,* 2004). Anthocyanins contain two, one or tree monosaccharide units in their molecules. The usual monosaccharide residues are glucose, galactose, arabinose, ramnose, xylose and glucuronic acid. However, anthocyanins containing disaccharides and trisaccharides were also found in nature but no tetrasaccharides have been discovered yet (Ananga *et al.,* 2013).

Biosynthesis of Anthocyanin in Tea

Anthocyanin molecules are produced via flavonoid pathway. Anthocyanin pigments are assembled like all other flavonoids from two different streams of chemical raw materials in the cell where one stream involves the shikimate pathway to produce the amino acid phenylalanine and the other stream produces three molecules of malonyl - CoA, a C3 unit from a C2 unit (acetyl - CoA). The Shikimate pathway leads to the formation of chorismate, which is the precursor of the aromatic amino acids phenylalanine, tyrosine and tryptophan. Phenylalanine (and in some cases tyrosine, but not in the case of tea plants) is the primary precursor of catechins (Tounekti *et al.*, 2013). The flavonoid pathway starts with phenylalanine, produced via shikimate pathway and trans- formed to 4 coumaroyl - CoA. The key enzymes, chalcone synthase (CHS) produce a naringen in chalcone by condensing one molecule of 4-coumaroyl - CoA and three malonyl - CoA molecules (derived from citrate produced by The Krebs cycle). In this case, the rings A and C are derived from the acetate pathway, whereas the ring B is derived from shikimate pathway (Ananga *et al.*, 2013). Currently there are three isoforms of chalcone synthase (Park *et al.*, 2004). The three genes act to synthesize naringenin chalcone, which is used in the formation of anthocyanins, proanthocyanidins, and other phenolic compounds. According to (Ageorges *et al.*, 2006), the three different CHSs may act in three different pathways to produce different secondary metabolites. In the next step, chalcone isomerase (CHI) converts stereospecifically the naringenin chalcone to its isomer naringenin. Ring B of the naringenin undergoes further hydroxylation by the enzymes flavonoid 3´-hydroxylase (F3´H), flavonoid 3´5´-hydroxylase (F3´5´H) or flavanon 3ß-hydroxylase (F3H) (He *et al.*, 2010). Then, the obtained dihydroflavonols are reduced by the enzyme dihydroflavonol 4-reductase (DFR) to the corresponding leucoanthocyanidins. After this reduction, anthocyanidin synthase (ANS) oxidize leucoanthocyanidins to their corresponding anthocyanidins. Anthocyanidins are inherently unstable under physiological conditions and are immediately glycosylated to anthocyanins by UDP-glucose: Anthocianidin: Flavonoid glucosyltransferase (UFGT) (He *et al.*, 2010). Anthocyanins, containing methylated anthocyani-dins (peonidin 4, petunidin 5 and malvidin 6) as aglycone can be obtained by methylation of hydroxyl groups on the ring B of the cyanidin-3-O-glucoside 7, delphinidin-3-O-glucoside and petunidin-3-O-glucoside by the enzyme O-methyltransferase (OMT). Future acylation of produced anthocyanins is possible by the action of different anthocyanin acyltransferases (ACT).

Extraction of Anthocyanin

Anthocyanins are soluble in polar solvents, and they are normally extracted from plant materials by using methanol that contains small amounts of hydrochloric acid or formic acid. The acid lowers the solution's pH value and prevents the degradation of the non-acylated anthocyanin pigments. However, as hydrochloric acid or formic acid is concentrated during the evaporation of the methanol-hydrochloric acid or methanol-formic acid solvent, pigment degradation occurs (e.g. in the extract of Azalea cv. Alice Erauw, the cyanidin-3monosides are converted into unstable aglycone). Small amounts of acid may also cause partial or total hydrolysis of the acyl moieties of acylated anthocyanins that are present in some plants. One report compared various techniques for the extraction of anthocyanins from red grapes and demonstrated that solvents containing up to 0.12 mol/l hydrochloric acid can cause partial hydrolysis of acylated anthocyanins (Revilla *et al.*, 1998). Acetone has also been used to extract anthocyanins from several plant sources (Garcia Viguera *et al.*, 1998; Giusti *et al.*, 1994). In comparison to acidified methanol, this technique allows an efficient and more reproducible extraction, avoids problems with pectins, and permits a much lower temperature for sample concentration (GarciaViguera *et al.*, 1998). Solid-phase extraction (SPE) on C_{18} (SPE) cartridges or Sephadex is commonly used for the initial purification of the crude anthocyanin extracts. The anthocyanins are bound strongly to these adsorbents through their unsubstituted hydroxyl groups and are separated from unrelated compounds by using a series of solvents of increasing polarity. Sunrouge' tea leaves i.e., anthocyanin rich tea leaves (87.2 g) were extracted with 15% acetic acid (600 mL 3), and additionally extracted with 15% acetic acid-containing 50% EtOH (600 mL 4) (Saito *et al.*, 2011). The anthocyanins were extracted successfully from tea products processed from a number of newly bred purple leaf coloured Kenyan tea cultivars (*Camellia sinensis*) using acidified methanol/HCl (99:1 v/v). (Kerio *et al.*, 2012).

Characterization of Anthocyanin

The characterization of a mixture of anthocyanins usually involves the separation and collection of each compound, and subsequent analysis by nuclear magnetic resonance (NMR) and fast atom bombardment mass spectroscopy (FAB-MS). For the separation and structural analysis, the use of liquid chromatography–mass spectrometry (LC–MS) technique, which combines the separation of LC with the selectivity and sensitivity of the MS detector, permits the identification of individual compounds in a mixture of compounds. Recently liquid chromatography-electron impact ionization mass spectrometry (LC-EI-MS) was also used to identify the anthocyanins of *Catharanthus roseus* extracts (Piovan *et al.,* 1998). LC–MS with an atmospheric pressure-ionization ion-spray interface was used to analyze the anthocyanins contained in the grape skins (*Vitis vinifera* L.). Nineteen derivatives of cyanidin, delphinidin, petunidin, malvidin and peonidin were identified by this ionization technique. The individual mass spectra showed peaks for the molecular ions, together with a fragment corresponding to aglycone; when acylation was present, an additional fragment was detected at mass/charge values corresponding to the loss of acyl moiety from the molecular ion (Baldi *et al.,* 1995). Many new acylated anthocyanins have been found with the help FAB-MS (Saito *et al.,* 1983).

Atmospheric-pressure ionization (API) techniques have several advantages over other MS detection methods. In API-MS the ion source is located outside the MS; the ions are formed at atmospheric pressure, and then sampled into the mass spectrometer. These are soft ionization techniques (only the molecular ion is formed), although the application of a potential at the entrance of the mass spectrometer (fragment voltage) creates suitable conditions for CID, and the production of fragment ions. Two API interfaces are available commercially, namely, the atmospheric pressure chemical ionization interface (APCI) and the ESI interface. LC–MS system equipped with an ESI interface was used to analyze anhocyanins present in extracts of grape skins and red wine (Revilla et al., 1999) Another technique recently used for anthocyanin analysis is capillary electrophoresis (CE) which has excellent mass sensitivity, high resolution, low sample consumption and minimal generation of solvent waste. The separation of a mixture of standards, as well as strawberry and elderberry anthocyanins, by capillary zone electrophoresis (CZE) has already been reported by Bridle et al. (1997). Matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) was used to perform both qualitative and quantitative analyses of anthocyanins in wine and fruit juice, and matrix-assisted laser desorption/ionization time-offlight mass spectrometry (MALDI–TOF–MS) to analyze the content of anthocyanins in various foods (Wang and Sporns, 1999; Wang and Lin, 2000). Purification and isolation of 6 anthocyanins from 'Sunrouge' by chromatography, and identification of them by LC/MS/MS and NMR analysis has already been reported (Saito et al., 2011).

NMR, an important technique is useful for structural elucidation of anthocyanin. Some examples of its use are given where it has been reported that the separation of anthocyanins from red radish (*Raphanus sativus*) and their structural elucidation by one- and two-dimensional NMR.

Four anthocyanins were obtained: pelargonidin 3-O- [2-O-(b-glucopyranosyl) -6-O-(trans-p-coumaroyl) -b-glucopyranoside] 5O-(6-O-malonyl-b-glucopyranoside); pelargonidin 3-O [2-O -(b-glucopyranosyl) -6-O- (trans-feruloyl)-b-glucopyranoside] 5-O- (6-O- malonyl-b-glucopyranoside); pelargonidin 3-O-[2-O-(b-glucopyranosyl) -6-O- (trans-pcoumaroyl)-b-d-glucopyranoside] 5-O- (b-glucopyranoside) and pelargonidin 3-O-[2-O-(b-glucopyranosyl)-6O-(trans-feruloyl)-b- gluco-pyranoside] 5-O- (b-glucopyr-anoside). They also investigated the three-dimensional conformation of the molecule by using NOESY techniques, which showed proximity between the hydrogen from the cinnamic acid acylating group and the C-4 of the pelargonidin (Giusti *et al.,* 1998). Similarly, anthocyanin trisaccharides of *Vaccinium padifolium* were identified by using NMR and other techniques (Cabrita *et al.,* 2000). Anthocyanins from Kenyan teas were purified by C_{18} solid phase extraction (SPE) catridges and characterised by HPLC-UV–Visible. They were identified according to their HPLC retention times, elution order and comparison with authentic standards that were available. Total monomeric anthocyanins were determined by the pH-differential method (Kerio *et al.,* 2012). Anthocyanins content in purple tea was measured by single-pH and the content was much more than that measured by pH-Differential spectrophotometry. The two methods showed good linear relation (R2=0.9927). The effects of interfering substances on quantitative analysis of anthocyanins could be eliminated by using pH-Differential spectrophotometry. The results provided a reliable basis for the measurement of Anthocyanins (Chen and Lu, 2011).

Anthocyanin Rich Tea

Sunrouge is the red tea cultivar made by naturally crossing *Camellia taliensis* and *Camellia sinensis* for which an application for registration was made in 2009. An anthocyanin-rich parental line, 'Cha Chuukanbohon Nou 6', was previously developed by crossing *C. taliensis x C. sinensis* in 2004 where *C. taliensis* is closely related to *C. sinensis*. However, the anthocyanin content of 'Cha Chuukanbohon Nou 6 suddenly diminished after maturation of leaves. Therefore Sunrouge was introduced as an offspring of 'Cha Chuukanbohon Nou 6' whose anthocyanin content did not diminish after leaf maturation (Maeda-Yamamoto *et al.,* 2012). The total anthocyanin content of four tea cultivars: 'Sunrouge', 'Cha Chuukanbohon Nou 6', 'Benibana-cha' (*C. sinensis*), the anthocyanin-rich tea cultivar, and 'Yabukita' (*C. sinensis*), the common tea cultivar in Japan was quantified and it has been already reported that the anthocyanin content of 'Sunrouge' was the highest among 4 tea cultivars, and was 8.4 times higher than that of 'Yabukita'. Purification and isolation of 6 anthocyanins from 'Sunrouge' has already been done using chromatography, and also has been identified using LC/MS/MS and NMR analysis, where the four anthocyanins were identified as delphinidin-3-O- ßD- (6-(E)-pcoumaroyl) galactopyranoside(2), delphinidin-3-O-ßD-(6-(E)-p-coumaroyl)glucopyranoside (3), cyanidin-3-O -ß-D- (6-(E)-pcoumaroyl) galacto-pyranoside (4), and cyanidin-3-O-ßD-(6-(E)-p-coumaroyl)glucopyranoside(5), and the other two were estimated respectively as delphinidin-(Z)-p-coumaroylgalactopyranoside (1), petunidin -(E)-p-coumaroyl galacto pyranoside (6). Compound 3 was found in tea for the first time. In general, anthocyanins has been reported to have various bioactivities, including relieving eyestrain and antioxidative effects, so it is expected that drinking 'Sunrouge' tea would bring in the above bioactivities (Saito *et al.,* 2011).

Another anthocyanin rich tea was extracted from tea products processed from a number of newly bred purple leaf coloured Kenyan tea cultivars (*Camellia sinensis*) using acidified methanol / HCl (99:1 v/v). Extracted anthocyanins were purified by C_{18} solid phase extraction (SPE) catridges and characterised by HPLC-UV–Visible. Of the six most common natural anthocyanidins, five were identified in the purified extracts from purple leaf coloured tea, in both aerated (black) and unaerated (green) teas namely; delphinidin, cyanidin, pelargonidin, peonidin and malvidin. The most predominant anthocyanidin was malvidin in both tea products. In addition, two anthocyanins namely, cyanidin-3-O-galactoside and cyanidin-3-O-glucoside were also identified (Kerio *et al.,* 2012).

Anthocyanin and its Utility in Plants

Apart from imparting colour to the plants, anthocyanin play a definite role in the attraction of animals for pollination and seed dispersal, and hence they are of considerable value in the co-evolution of these plant-animal interactions. Anthocyanins and 3-deoxyanthocyanidins however have roles in flowering plants other than as attractants. They can act as antioxidants, phytoalexins or as antibacterial agents. Anthocyanins may be important factors along with other flavonoids in the resistance of plants to insect attack (Harborne, 1988). For example, cyanidin 3-glucoside was shown to protect cotton leaves against the tobacco budworm (Hedin and Hedin, 1983). In photosynthetic tissues (such as leaves and sometimes stems), anthocyanins have been shown to act as a "sunscreen", protecting cells from high-light damage by absorbing blue-green and ultraviolet light, thereby protecting the tissues from photo - inhibition, or high-light stress. This has been shown to occur in red juvenile leaves, autumn leaves, and broad-leaf evergreen leaves that turn red during the winter. The red coloration of leaves has been proposed to possibly camouflage leaves from herbivores blind to red wavelengths, or signal unpalatability, since anthocyanin synthesis often coincides with synthesis of unpalatable phenolic compounds (Sullivan, 1998). Some roles of anthocyanin have been tabulated in Table 2.

Pharmacological Activity

Anthocyanins possess known pharmacological properties and are used by humans for therapeutic purposes. Following the recognition that pigment extracts are more effective than O-(b-hydroxyethyl) rutin in decreasing capillary permeability and fragility and in their anti-inflammatory and anti-oedema activitiest is possible that anthocyanins may replace rutin and its derivatives in the treatment of illnesses involving tissue inflammation or capillary fragility. The crude anthocyanin extracts of *Vaccinium myrtillus* have been given orally and by intravenal or intramuscular injection to reduce capillary permeability and fragility. (Kong *et al.,* 2003)

Anthocyanins were not found effective in suppressing tumor growth (Ghiselli *et al.,* 1998). However, an antioxidant activity study of anthocyanin fractions from Italian red wine showed that the anthocyanin fraction was the most effective both in scavenging reactive oxygen species and in inhibiting lipoprotein oxidation and platelet aggregation (Ghiselli *et al.,* 1998). This result suggests that anthocyanins could be the key component in red wine that protects against cardiovascular disease. The anti-tumor activity of anthocyanins was reported where they found that the anthocyanin fraction from red wine suppressed the growth of HCT-15 cells, which are derived from human colon cancer or AGS cells from human gastric cancer. The suppression rate by the anthocyanin fraction was significantly higher than that of the other fractions (Kamei *et al.,* 1998). The ability of anthocyanin obtained from the petals of *H. rosa-sinensis* was examined which prevented carbon tetrachloride-induced acute liver damage in rats. The results showed that those rats treated with anthocyanin and

Table 2: The role of anthocyanins and 3-deoxyanthocyanidins in plants. Excerpted from Kong *et al.* (2003)

Plant	Compound	Origin	Function
	Angiosperms		
Senecio cruentus	Cinerarin	Petals	Pollination
Sorghum	Apigeninidin	Leaf sheath	Phytoalexin anti-microbial antioxidants
	Gymnosperms		
Abies concolor	Petunidin-3- glucoside Cyanidin-3- glucoside	Cone	-
Pinus contorta	Anthocyanin	Leaves	Cold tolerance
Pinus banksiana	-	Seedlings	Photoinhibition tolerance
	Ferns		
Davallia divaricata	Pelargonidin-3-p-coumaryl-glc-5-glc (monardein)	Young leaves	-
Ferns species	Apigenidin	Leaves	-
	Mosses		
Bryum, Splachunm	Luteolinidin-5-glc	Leaves	-
	Liverwort		
Cephaloziella exilifolia	Anthocyanin like	Thallus	-

carbon tetrachloride had significantly less hepatotoxicity (P<0.05) than those given carbon tetrachloride alone. This was assessed by measuring the levels of serum aspartate and alanine aminotransferase activities 18 hours after carbon tetrachloride was given. This result suggested that anthocyanin may be protective against carbon tetrachloride-induced liver injury (Obi *et al.,* 1998).

On examining antimutagenicity of water extracts prepared from the storage roots of four varieties of sweet potato with different flesh colors, using Salmonella typhimurium TA 98. Two anthocyanin pigments purified from the purple colored sweet potato 3-(6,6´-caffeyl ferulylsophoroside) 5-glucoside of cyanidin (YGM-3) and peonidin (YGM6), effectively inhibited the reverse mutation induced by heterocyclic amines-mutagen, Trp-P-1, Trp-P-2, and IQ in the presence of rat liver microsomal activation systems (Yoshimoto *et al.,* 1999).

It has been reported that the the administration of anthocyanin dyes from *Aronia melanocarpa* to rats before the intraperitoneal injections of PlateletActivating Factor (PAF) and ceruleine had a beneficial effect on the development of acute experimental pancreatitis in rats (Jankowski *et al.,* 2000). It was revealed that this was due to the reduction of pancreatic swelling and a decrease in lipid peroxidation and adenosine deaminase activity.

They also examined the effect of anthocyanins from Cabernet red wine on the course and intensity of symptoms of experimental diabetes in rats (Jankowski *et al.,* 1999). The results showed that a simultaneous daily administration of anthocyanins obtained from Cabernet red wine and streptozotocin substantially decreased sugar concentrations in the urine and blood serum. These anthocyanins also inhibited the loss of body mass caused by the injection of streptozotocin. Simultaneously, the anthocyanin pigment prevented the generation of free oxygen radicals, and decreased the peroxidation of lipids. The influence of anthocyanins was determined from chokeberries on the generation of autoantibodies to oxidize low density lipoproteins (oLAB) in pregnancies complicated by intrauterine growth retardation (IUGR). An experiment was conducted with a study group of 105 pregnant women (on the turn of trimester two according to LMP) with IUGR (sonographic examination results below the 5th percentile for real gestational age) who were randomly divided into 2 groups. Fifty women were administered anthocyanins and 55 women were given a placebo. There was a control group of 60 healthy pregnant women. They then examined the level of oxidative stress measured by the serum concentration of autoantibodies required to oxidize low density lipoproteins (oLAB). In the anthocyanin group, the oLAB titres decreased from 1104_41 mU/ml before treatment to 752_36 mU/ml in the first month and 726_35 mU/ml in the second month, at P<0.01. In the placebo group, the oLAB titres showed a slightly increasing trend: 1089_37 mU/ml before treatment, 1092_42 mU/ml in the first month and 1115_43 mU/ml in the second month, at P>0.05. The oLAB titres in the control group were 601_49 mU/ml before treatment, 606_45 mU/ml in the first month, and 614_43 mU/ml in the second month, at P>0.05. The results indicated that natural antioxidants (anthocyanins) can be useful in controlling oxidative stress during pregnancies complicated by IUGR (Pawlowicz *et al.,* 2000).

Hibiscus anthocyanins (HAs), a group of natural pigments occurring in the dried flowers of *Hibiscus sabdariffa* L., are used in soft drinks and herbal medicines. Their antioxidant bioactivity has been studied and it appears that HAs can significantly decrease the leakage of lactate dehydrogenase and the formation of malondialdehyde induced by a treatment of tert-butyl hydroperoxide (t-BHP). The *in vivo* investigation showed that the oral pretreatment of HAs before a single dose of t-BHP significantly lowered the serum levels of hepatic enzyme markers (alanine and aspartate amino transferase) and reduced oxidative liver damage. The histopathological evaluation of the liver revealed that *Hibiscus* pigments reduce the incidence of liver lesions including inflammation, leucocyte infiltration, and necrosis induced by t-BHP in rats (Wang *et al.,* 2000).

Their pharmaceutical value has been additionally increased due to their high bioavailability. However, the administration and metabolism of Anthocyanins *in vivo* have been investigated in details mostly in rats, whereas the detailed studies on humans still are scantly presented in scientific literature (He and Giusti, 2010; Yue *et al.,* 2011).

The colorful anthocyanins are the most recognized, visible members of the bioflavonoid phytochemicals. The free-radical scavenging and antioxidant capacities of anthocyanin pigments are the most highly publicized of the modus operandi used by these pigments to intervene with human therapeutic targets, but, in fact, research clearly suggests that other mechanisms of action are also responsible for observed health benefits (Lila, 2004). Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids may provide protection from DNA cleavage, estrogenic activity (altering development of hormone dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses), anti-inflammatory activity, lipid peroxidation, decreasing capillary permeability and fragility, and membrane strengthening (Lila, 2004).

The roles of anthocyanin pigments as medicinal agents have been well-accepted dogma in folk medicine throughout the world, and, in fact, these pigments are linked to an amazingly broad-based range of health benefits. For example, anthocyanins from *Hibiscus* sp. have historically been used in remedies for liver disfunction and hypertension; and bilberry (*Vaccinium*) anthocyanins have an anecdotal history of use for vision disorders, microbial infections, diarrhea, and diverse other health disorders (Rice-Evans and Packer, 2003; Smith *et al.,* 2000; Wang *et al.,* 2000).

But while the use of anthocyanins for therapeutic purposes has long been supported by both anecdotal and epidemiological evidence, it is only in recent years that some of the specific, measurable pharmacological properties of isolated anthocyanin pigments have been conclusively verified by rigorously controlled in vitro, in vivo, or clinical research trials (Tsuda *et al.,* 2003). For example, visual acuity can be markedly improved through administration of anthocyanin pigments to animal and human subjects, and the role of these pigments in enhancing night vision or overall vision has been particularly well documented (Matsumoto *et al.,* 2001). Oral intake of anthocyanosides from black currants resulted in significantly improved night vision adaptation in human subjects (Nakaishi *et al.,* 2000) and similar benefits were gained after administration of anthocyanins from bilberries (Muth *et al.,* 2000). Three anthocyanins from black currant stimulated regeneration of rhodopsin (a G-protein-coupled receptor localized in the retina of the eye), and formation of a regeneration intermediate was accelerated by cyanidin 3-rutinoside (Matsumoto *et al.,* 2003). These studies strongly suggest that enhancement of rhodopsin regeneration is at least one mechanism by which anthocyanins enhance visual acuity. In both in vitro and in vivo research trials, anthocyanins have demonstrated marked ability to reduce cancer cell proliferation and to inhibit tumor formation (Lila, 2004). Anthocyanins inhibit tumorigenesis by blocking activation of a mitogen-activated protein kinase pathway. This report provided the first indication of a molecular basis for why anthocyanins demonstrate anticarcinogenic properties.

The role of anthocyanins in cardiovascular disease protection is strongly linked to oxidative stress protection. Since endothelial dysfunction is involved in initiation and development of vascular disease, four anthocyanins isolated from elderberries were incorporated into the plasma. Crude anthocyanin extracts from bilberry have been administered both orally and via injection to reduce capillary permeability (Kong *et al.,* 2003). Protection from heart attacks through administration of grape juice or wine was strongly tied to the ability of the anthocyaninrich products to reduce inflammation and enhance capillary strength and permeability, and to inhibit platelet formation and enhance nitric oxide (NO) release (Folts, 1998).

Their important function in cognitive decline and neural dysfunction has been investigated and found that fruit extracts (from blueberry) including anthocyanins were effective in reversing age related deficits in several neural and behavioral parameters, e.g. oxotremorine enhancement of a K1 evoked release of dopamine from striatal slices, carbachol-stimulated GTPase activity, striatal Ca buffering in striatal synaptosomes, motor behavioral performance on the rod walking and accelerod tasks, and Morris water maze performance and thus proved to improve neural and behavioral parameters (memory and motor functions). (Joseph *et al.*, 1999).

It has already been reported that the Anthocyanins extracted from purple corn, when provided to mice in tandem with a high-fat diet, effectively inhibited both body weight and adipose tissue increases. Typical symptoms of hyper-glycemia, hyper-insulinemia and hyper-leptinemia provoked by a high-fat diet did not occur when mice also ingested isolated anthocyanins. The experiments suggest that anthocyanins, as a functional food component, can aid in the prevention of obesity and diabetes (Tsuda *et al.*, 2003).

Medicinal Benefits of Anthocyanin Rich Foods

Consumption of anthocyanin adds as a beneficiary source of neutraceuticals and therapeutics and it has various application in the pharmaceutical industry. Recurrent consumption of anthocyanins could provide various health benefits including reduced risk of coronary heart diseases, anti-carcinogenic activity, antioxidant activity, reduced risk of stroke, anti-inflammatory effects etc. (Davies, 2009; Lila, 2004; Stintzing and Carle, 2004; Wrolstad, 2004).

Biological activity of anthocyanin has already been discussed in the above section. Anthocyanin in diet inhibits body weight and adipose tissue increase that could prevent symptoms of hyperglycemia, hyperinsulinemia, and hyperleptinemia provoked by a high-fat diet and thus can aid in the prevention of diabetes and obesity (Tsuda *et al.*, 2003). It improves and cures vision disorders, microbial infections, diarrhea and diverse other health disorders (Rice-Evans and Packer, 2003; Smith *et al.*, 2000; Wang *et al.*, 2000). It has the capacity to modulate cognitive and motor function, to enhance memory, and to have a role in preventing age-related declines in neural function (Lila 2004). It also reduces inflammation and capillary fragility (Kong *et al.*, 2003), scavenges reactive oxygen species and in inhibiting lipoprotein oxidation and platelet aggregation (Ghiselli *et al.*, 1998), provides protection from DNA cleavage, estrogenic activity (altering development of hormone-dependent disease symptoms), enzyme inhibition, boosting production of cytokines (thus regulating immune responses).

Above all anthocyanin has many other health beneficial properties, which include antioxidant (Bae and Suh, 2007), anticarcinogenic (Lee *et al.*, 2009), anti-angiogenic (Bagchi *et al.*, 2004), antimicrobial (Viskelis *et al.*, 2009) antiapoptotic (Elisia and Kitts, 2008) and pro-apoptotic (Lo *et al.*, 2007b) properties.

Utility in Various Industries

The world market of natural food colorants expands with the annual growth rate of 4-6% (Cormier *et al.*, 1996). In USA 4 of the 26 colorants approved by the food administration, that are exempt from certification, are based on anthocyanin pigments (Wrolstad, 2004). In European Union, all anthocyanin-containing colorants are classified as natural colorants under the classification E163 (Socaciu, 2007).

Currently most of the worldwide anthocyanins supply comes from processing of grape pomace, which is a waste product from wine making. But in European Union other plant sources such as red cabbage, elderberry, black currant, purple carrot, sweet potato, and red radish are also allowed (Mortensen, 2006). Anthocyanins, produced by grape cell suspensions can be a promising alternative supply of natural colorants. It has already been demonstrated that the produced pigments by the grape cell suspensions undergo significant structural modifications. Grape cell suspensions accumulates higher levels of metabolically more evolved structures (methylated and acylated anthocyanins). Acylated anthocyanins are suitable for application in food products, mainly because of the improved color stability compared to non-acylated structures (B¹kowska -Barczak, 2005). Moreover, the grape cell suspensions can also produce elevated levels of beneficial phenolic compounds such as flavonoids, stilbenes, phenolics, etc., which are capable of increasing the added value of the final additive. The overall metabolite profile of grape cells in combination with the lack of microbial and toxic contaminations will give the potential for development of new types of food additives if the entire cell suspension biomass is utilized.

The commercial interest of cosmetic companies to apply plant additives, derived by biotechno-logical cultivation of plant cells to their products has increased remarkably in the last few years (Schurch *et al.*, 2008). The addition of plant cell derived extracts in cosmetic products has been considered as a powerful approach used to increase their health benefits. Several plant extracts have been added to various cosmetic products as moisturizers, antioxidants, whitening agents, colorants, sunscreens, preservatives. With the advancement of plant cell biotechnology, more and more cosmetic companies have been attracted for application of additives, based on plant cell suspensions. Recently the application of so-called plant "steam" cells attracts industry's attention (Schurch *et al.*, 2008). In the last few years, the French company "Sederma" launched the product "Re-sistem™" based on application of *in vitro* cultivated plant cells (www.sederma.fr). The other company, "Mibelle Biochemistry", situated in Switzerland, developed a "PhytoCellTec" prod- uct, based on grape cell suspension of *V. vinifera* L. *cv.* Gamay Fréaux, which was processed by high-pressure homogenizer to produce liposomes for application in cream products (www.mi-bellebiochemistry.com). According to the company, the grape cell derived liposomes contained higher amounts of anthocyanins and when applied on skins serve as strong UV protectors and fight photoaging. The presented examples clearly demonstrate the commercial interest to application of grape cell suspension derived products. However, it is a matter of time for the scientists to develop the biotechnological approach of producing anthocyanins by grape cell suspensions from the frame of experimental scale to commercially applicable products (Ananga *et al.*, 2013).

Anthocyanins have also been employed to produce juices and red wine whose natural colour as well as high antioxidant property adds to the quality of product.

Anthocyanins can also be used as pH indicators because their color changes with pH; they are pink in acidic solutions (pH < 7), purple in neutral solutions (pH ~ 7), greenish-yellow in alkaline solutions (pH > 7), and colourless in very alkaline solutions, where the pigment is completely reduced (Michaelis *et al.*, 1936) and thus, it is employed in many chemical or pharmaceutical industry as well as in the fied of research.

Nowadays anthocyanins are being used widely in organic solar cells because of their ability to convert light energy into electrical energy (Cherepy *et al.*, 1997). The many benefits of using dye sensitized solar cells instead of traditional pn junction silicon cells include lower purity requirements and abundance of component materials, such as titania, as well as the fact they can be produced on flexible substrates, making them amenable to roll-to-roll printing processes (Gratzel, 2003).

Conclusion and Future Prospects

Anthocyanins represent a class of important antioxidants, as they are so common in human foods. In recent years, many papers have been published on the *in vitro* antioxidant activity of anthocyanins and their other functions. However, there are still fewer studies on anthocyanin compared to the studies of other flavonoids. On the other hand tea is a pleasant, popular, socially accepted, economical and safe drink that is initially taken as medicine and later as beverage and now, it has proven well as future potential of becoming an important industrial and pharmaceutical raw material. As green tea, the purple coloured anthocyanin rich tea may also be a popular health drink since anthocyanins has many medicinal properties and is particularly known to be beneficial against cardiovascular diseases, for providing anticancer benefits, improving vision, cholesterol and blood sugar metabolism as discussed above in the article. Most importantly it sports much lower caffeine content than black or green tea which is beneficial in beverage. Anthocyanin content in tea in addition to other polyphenols and other medicinal compounds would add a splendid color as well as an enigmatic healing property.

Based on these facts, this review is directed to highlight the importance of anthocyanins in order to improve further research in this field, discovering tea cultivars or wild tea plant, rich in anthocyanin.

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