

A DFT and QSAR study of the role of hydroxyl group, charge and unpaired-electron distribution in anthocyanidin radical stabilization and antioxidant activity

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Abstract Density function theory study was conducted to examine the structure, planarity, and charge and unpaired electron delocalization of some anthocyanidins and mono-hydroxy flavylium ions as well as all their possible phenoxyl radicals. Bond dissociation energy of O–H bond was in the order of 3-<5-<4'-<7-OH bond except for in malvidin where 4'-OH has less bond dissociation energy than 5-OH bond because of the extra stability provided by the two *o*-OMe groups. Stabilization by both charge and unpaired electron resonances were suggested for each radical. The present work revealed that the 3-anthocyanidin radicals were stabilized by three types of resonance through 5-, 7- or 4'-oxygen atoms while 5-, 7- or 4'-radicals were stabilized only by resonance through pyrylium or 3-oxygen atom. Positive charge was found delocalized on carbons 2, 5, 7, 9, and 4' by resonance and moves towards the radical oxygen upon radical formation. In all radicals, radical oxygen carries the highest spin density and least negative charge therefore, its charge, spin density and the least bond dissociation energy ($E_{\text{O-H}}$) in each anthocyanidin were well

quantitatively correlated with the experimental radical scavenging activity.

Keywords Anthocyanidins · Anthocyanins · Antioxidants · Radical delocalization · BDE · Resonance stabilization

Introduction

Anthocyanins are an important class of flavonoids; they are glycosides of anthocyanidins, polyphenols of 2-phenylbenzopyrylium ion (C6-C3-C6). Anthocyanins provide the nutritive value to many functional food and exhibit strong antioxidant activity (Azevedo et al. 2010), where some of them are more potent antioxidants than vitamins E (Kong et al. 2003) and C (Bagchi et al. 1998), which could explain their high biological activities and protection effects against many chronic diseases (Bagchi et al. 1998; Kong et al. 2003; Ma et al. 2016). The high activity of anthocyanins was attributed to their cationic structure and low oxidation potential compared to other classes of flavonoids (van Acker et al. 1996). In addition, they give many flowers, fruits and vegetables their characteristic purple (e.g., cauliflower, grapes, and corn) and red (e.g., cabbage, beet, and sorghum) colors and responsible for most colors of berry family fruits.

Hundreds of natural anthocyanins have been reported (Andersen and Jordheim 2006); all of them share in having hydroxyl groups in positions 5, 7, and 4' while most of them have additional hydroxyl group in position 3. Other hydroxyl groups may also occur in positions 6, 3', and 5'. Hydroxyl groups can be free, methylated or glycosylated. The 4'-OH group is not methylated in all natural anthocyanins; interestingly, its methylation (Rahman et al. 2006) or glycosylation (Zhao et al. 2014) reduces the antioxidant activity. Glycosylation can be found in positions 3, 5, 7, 3',

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and 5' but more frequently in positions 3 and 5 (Kähkönen and Heinonen 2003). Therefore, anthocyanidins differ mainly in the number and position of the hydroxyl and methoxy groups on ring B and the presence of 3-hydroxyl group on ring C; furthermore, anthocyanins vary in the number, type and site of glycosylation (Zhao et al. 2014). Anthocyanidin structure is also characterized by the aromaticity of the three rings and by the presence of an electron deficiency with a delocalized positive charge, which distinguishes anthocyanidins from other classes of flavonoids. The structures of the anthocyanidins examined in the present work are presented in Fig. 1.

The antioxidant activity of simple phenols and flavonoids is increased by the presence of a catechol moiety and electron donating groups e.g., hydroxyl and methoxy groups (Kähkönen and Heinonen 2003; Ali et al. 2013). The 3-hydroxyl group also enhances the antioxidant activity of anthocyanins and other flavonoids (Amić et al. 2007; Ali et al. 2016). However, anthocyanidins either lacking 3-free or glycosylated hydroxyl group (3-deoxyanthocyanidins) e.g., luteolinidin or missing both a 3-OH group and a catechol structure e.g., apigeninidin still exhibit good antioxidant activity (Boveris et al. 2001; Carbonneau et al. 2014).

Computational methods have frequently been used to predict and explain the antioxidant activity of anthocyanidins and other flavonoids (Pop et al. 2012; Lu et al. 2014). According to the hydrogen atom transfer mechanism, the antioxidant activity of anthocyanidins in aprotic solvents depends mainly on the stability of the resulting phenoxyl radicals and the spread of the unpaired electron over the entire molecule (Leopoldini et al. 2011; Foti 2012). The resonance stabilization of 3-, 5-, 7- and 4'-phenoxyl radicals of anthocyanidins has been suggested (Ali et al. 2016); however, the effects of detailed structural features of anthocyanins on their radical stabilization and potent

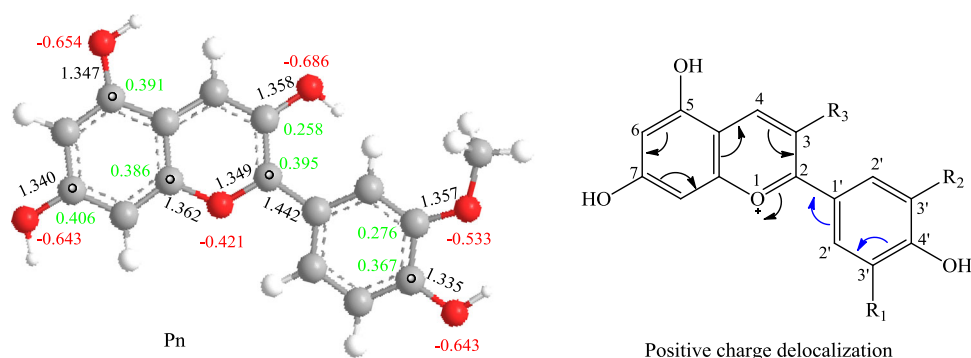
antioxidant activity have not been fully studied; besides, there are still some variations in the literature in the order of $BDE_{(O-H)}$ of anthocyanidins (Pop et al. 2012; Lu et al. 2014; Foti 2012) and absence of quantitative correlations between the anthocyanidin antioxidant activity and their $BDE_{(O-H)}$ and charge and unpaired electron distribution. Since this information may aid in explaining the observed high antioxidant activity of anthocyanidins and the biological activity of many functional food as well as predicting the antioxidant activity of new compounds; the present study involves a density function theory (DFT) calculation of some anthocyanidin structures and the possible resonance pathways caused by the delocalization of both the charge and unpaired electron. In addition, the experimental radical scavenging activity of anthocyanidins is quantitatively correlated with their charge and unpaired electron distribution as well as their least O–H bond dissociation free energy. The studied compounds were 3-, 5-, 7- and 4'-hydroxyflavylium ions and four anthocyanidins bearing no catechol structure (apigeninidin (Ap), pelargonidin (Pg), peonidin (Pn), and malvidin (Mv)) along with all their possible phenoxyl radicals. Anthocyanidins with a catechol structure were excluded from this study to avoid the extra unpaired electron delocalization resulted from the formation of semiquinone radicals (Castañeda-Ovando et al. 2009; Ali and Ali 2015).

Computation methods

DFT calculations

All calculations were performed using Gaussian 09 program package at the level of DFT/B3LYP with a basis set 6-31-G (d,p) while CambridgeSoft Chem 3D package of

Fig. 1 Resonance, charge distribution (positive in green and negative in red) and selected bond lengths (black); atoms carrying the highest positive charge and involved in charge delocalization are marked by a filled circle in Pn model (color figure online)



Anthocyanidin	R ₃	R ₁	R ₂
Apigeninidin (Ap)	H	H	H
Pelargonidin (Pg)	OH	H	H
Peonidin (Pn)	OH	H	OMe
Malvidin (Mv)	OH	OMe	OMe

ChemOffice 12 was used for model visualization and finding the bond lengths and the dihedral angles between the benzopyrylium ring system and each of the 2-phenyl plane (ϕ O1-C2-C1'-C2') and the 3-O-H bond (ϕ C2-C3-O3-H3). Radicals were generated by abstracting a hydrogen atom from the desired hydroxyl group. Calculations of parent compounds were executed at restricted closed shell level while calculations of radicals were accomplished at unrestricted open shell level. Harmonic vibrational frequencies were computed at the same level of theory to characterize saddle points and find the zero-point energies (ZPE) for thermochemical correction of electronic thermal enthalpies of all species. Calculations were performed in the gas-phase at temperature 298.15 K and pressure 1 atmosphere. For all compounds and radicals, energy was minimized and the lowest energy structure was used in all calculations without any geometry or symmetry constraints. Charge of each atom was calculated by natural population analysis with the sum of charges on all atoms is 1.000 (net charge). Spin density was calculated by frequency calculation; spin densities of hydrogen atoms are summed into those of heavy atoms. Sum of spin densities of all atoms is one. Positive charges on carbon atoms and negative or positive charges on oxygen atoms as well as spin densities were placed on the models.

Bond dissociation energy (BDE_{O-H}) of all hydroxyl groups in molecules was calculated by subtracting the electronic thermal enthalpy of the parent compound (H_P) from that of the resulted radical (H_R) and hydrogen atom

(H_H) according to the following equation:

$$BDE_{O-H} = [(H_R + H_H) - H_P] \\ \times \text{Correction factor} \times \text{Conversion Factor}$$

Where correction factor suggested by the program manual to eliminate systematic error in thermal energies of frequency calculations is 0.9804 and the conversion factor from Hartree to Kcal/mol unit is 627.51 whereas hydrogen atom enthalpy was -0.4979 Hartree.

Statistical analysis

Regression analyses were performed using SPSS software version 16. The validity of models was assessed by Pearson correlation coefficient (R), standard error of the estimate (SE), the number of data point (N), the least significant difference (p), and the 95% confidence intervals (in parentheses) for each regression coefficient.

Results and discussion

Structure and planarity

The dihedral angle (O1, C2, C1' and C2') of the examined flavyliums (Fv) and anthocyanidins as well as their phenoxyl radicals is listed in Table 1. The angle determines the

Table 1 Thermodynamic parameters of O-H dissociation and dihedral angles (ϕ) of parent compounds and their radicals

Flavylum and anthocyanidin	BDE _(O-H) ^a	ϕ O1-C2-C1'-C2'	ϕ C2-C3-O3-H	ΔG^a	ΔS^b	Flavylum and anthocyanidin	BDE _(O-H) ^a	ϕ O1-C2-C1'-C2'	ϕ C2-C3-O3-H	ΔG^a	ΔS^b
3-OHFv ^c		30.2	18.5			4 [\] -RPg	90.128	22.3	21.1	81.762	28.060
3-RFv ^c	83.484	0.0	–	74.933	28.680	5-RPg	82.500	26.7	19.7	74.502	26.825
4 [\] -OHFv		0.0	–			7-RPg	90.867	23.5	19.1	82.561	27.858
4 [\] -RFv	91.851	0.0	–	83.423	28.268	Peonidin Pn		24.6	27.3		
5-OHFv		0.0	–			3-RPn	74.748	0.0	–	66.504	27.651
5-RFv	81.700	–0.1	–	73.887	26.205	4 [\] -RPn	87.483	22.2	23.4	79.116	28.063
7-OHFv		0.1	–			5-RPn	82.069	27.0	19.4	74.194	26.413
7-RFv	92.651	0.0	–	84.899	26.034	7-RPn	90.067	24.0	18.6	81.762	27.855
Apigeninidin Ap		0.0	–			Malvidin Mv		25.3	24.0		
4 [\] -RAp	90.805	0.0	–	82.315	28.476	3-RMv	74.994	1.2	–	66.627	28.0631
5-RAp	83.607	0.0	–	75.917	25.792	4 [\] -RMv	79.547	23.0	20.2	71.611	26.617
7-RAp	92.958	1.1	–	84.776	27.443	5-RMv	81.823	27.3	17.2	73.948	26.413
Pelargonidin Pg		24.3	27.4			7-RMv	89.636	24.3	17.0	81.331	27.855
3-RPg	76.594	0.0	–	68.227	28.063						

^a Kcal/mol

^b cal/mol K

^c Fv and R stand for flavylum ion and radical respectively

coplanarity of ring B with respect to the benzopyrylium ring required for maximum overlap of the *p*-orbitals of ring atoms and thus increasing the stability of the resulted phenoxyl radicals leading to better antioxidant activity of parent compounds (Leopoldini et al. 2011). There is still controversy concerning the dihedral angle of anthocyanidins depending on the used computational method and the examined compounds. The planar and non-planar conformations of a number of anthocyanidins were examined and found that the former conformer is slightly more stable by 3.0 Kcal/mol for cyanidin and 3.4 Kcal/mol for delphinidin; the dihedral angle of the optimized non-planar conformer was 23.6° in pelargonidin and 25.3° in cyanidin as calculated by DFT/ B3LYP methods (Sakata et al. 2006). Semi-empirical method at AM1 level predicted planar structure of pelargonidin with dihedral angle 0.3° (Rastelli et al. 1993). AM1 calculation predicted also a complete planar structure of 4'-hydroxy flavylum but little deviation from the planarity was found in flavylum ion (9.9°) and some mono and dihydroxylated flavylums on ring B (Pereira et al. 1996, 1997). The same authors found also by using Ab initio methods at the level of HF/3-21 G that flavylum and 4'-hydroxy flavylum are completely planar while pelargonidin has a dihedral angle 15.3°. The difference in planarity among compounds was attributed, by the authors, to the attraction between O1 and H-atom on C2' in one way and the repulsion between H-atoms on C3 and C6' in another way.

DFT/B3LYP calculations gave also contradicted results (Lu et al. 2014) where some reports suggested planar structure of some anthocyanidins e.g., peonidin, malvidin, petunidin, and cyanidin (Guzmán et al. 2009; Lu et al. 2014), others predicted non-planarity of the same compounds with dihedral angle -10.70 , -7.91 , -28.14 (Woodford 2005) and 5.9° (Woodford 2005; Liu 2008), respectively. The 3-deoxyflavons apigenin and luteolin were predicted planar by DFT/B3LYP calculation (Leopoldini et al. 2004). The limited number of reported X-ray crystallography structures revealed non-planarity of some anthocyanidins where the dihedral angle was found 4.14° in apigeninidin (Woodford 2005), 3.8° in pelargonidin bromide and 10.1° in cyanidin bromide (Sakata et al. 2006).

Figure 1 shows the structure of the examined anthocyanidins and the structural model of peonidin; models of other parent compounds are placed in Supplementary material (S1) while their 3-, 4'-, 5- and 7-radicals are presented in Figs. 2 and 3 and S2–S5. In peonidin, the conformation with methoxy group toward the 3-OH group is preferred over the one with the methoxy group toward the pyrylium oxygen (Fig. 1) by stabilization energy (ΔG) 5.30 Kcal/mol. Models illustrate the charge distribution and selected bond lengths (C2-C1' and C-O bonds) in addition to the spin density distribution of radicals. Table 1 lists the dihedral angles (ϕ O1-C2-C1'-C2') and (ϕ C2-C3-O3-H3) of the parent compounds and the resulting phenoxyl radicals;

Fig. 2 Resonance, charge (positive in green and negative in red), spin density (blue) and selected bond lengths (black) of 3- and 4'-radicals (atoms carrying the unpaired electron in the main resonance are marked by a filled circle in 3-RPg and 4'-RMv models) (color figure online)

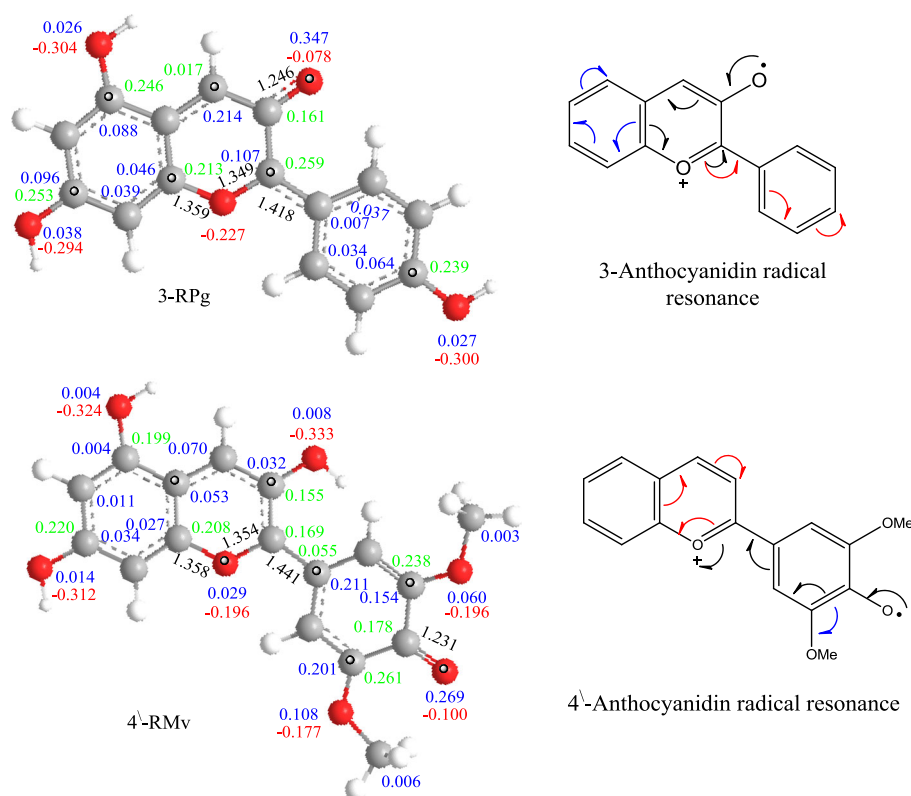
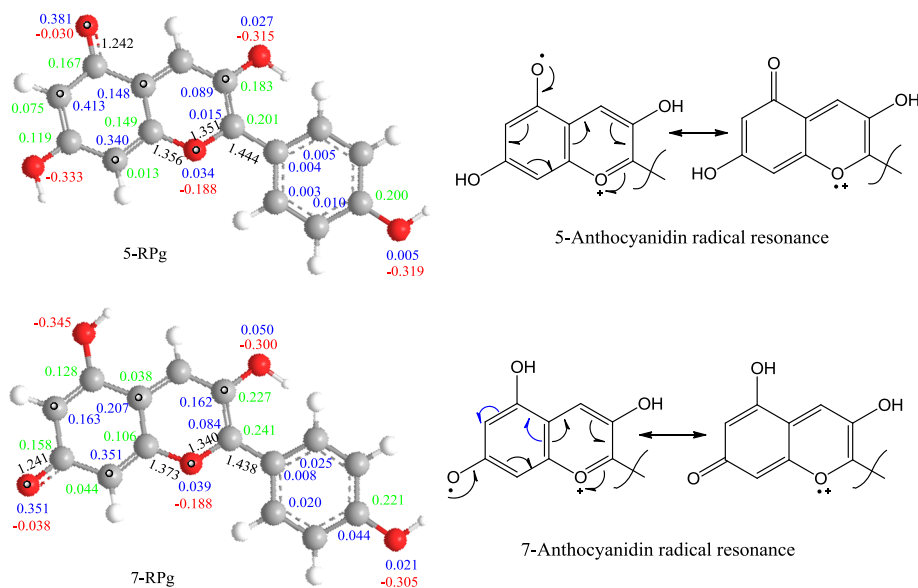


Fig. 3 Resonance, charge (positive in green and negative in red), spin density (blue) and selected bond lengths (black) of 5- and 7-radicals (atoms carrying the unpaired electron in the main resonance are marked by a filled circle in 5-RPg and 7-RPg models) (color figure online)



while the former angle examines the direction of ring B (i.e., the molecular planarity), the later angle shows the orientation of the 3-O-H bond, both directions are in respect to the benzopyrylium ring. It is believed that the orientation of the 3-OH bond is responsible for ring B direction and thus the planarity of the molecule (Sakata et al. 2006; Lu et al. 2014). In previous DFT calculation, the 3-O-H bond was located away from ring B and thus Pg, Pn, and Mv were found planar (Guzmán et al. 2009). However, in the present study, 3-oxyflavylium and anthocyanidins (Pg, Pn, and Mv) as well as their 5-, 7- or 4'-radicals showed the 3-O-H bond directed towards the B ring with a dihedral angle (ϕ C2-C3-O3-H3) 17.2–27.4° causing ring B to rotate by a dihedral angle (ϕ O1-C2-C1'-C2') 22.2–30.2°. In addition, in all 3-radicals (Figs. 2, S2), the strain between the 3-O-H group and ring B was removed; therefore, the 3-radicals were all found planar with dihedral angle (ϕ O1-C2-C1'-C2') 0.0–1.2°. The planar structure of 3-radicals is also manifested by the π -bond characters of C2-C1' bond where the bond length decreased from (1.442–1.454 Å) in the parent compounds (Figs. 1, S1) to (1.417–1.429 Å) in their 3-radicals (Figs. 2, S2). Moreover, the C2-C1' bond in 3-radicals is shorter than in other corresponding radicals; for example, the bond length in 3-RPg is 1.418 Å while in 4'-RPg, 5-RPg, and 7-RPg are 1.440, 1.444, and 1.438 Å, respectively. In consequence, all compounds lacking 3-OH group (5-, 7- and 4'-OHFv and Ap) and their radicals were also found planar with dihedral angle (ϕ O1-C2-C1'-C2') –0.1–1.1°.

Bond lengths of all parent compounds (Figs. 1, S1) and resulting radicals (Figs. 2–3 and S2–S5) showed that the pyrylium oxygen bonds (O1-C2 and O1-C9, where the fused bond carbons of the benzopyrylium ring are

numbered consequently 9 and 10 in the present article) are single bonds with the former is shorter showing some double bond character (1.333–1.356 Å) than the later (1.356–1.377 Å) consistent with previous AM1 and ab initio calculations of flavylium cation (Pereira et al. 1996). The carbonyl formation of the radical oxygen is expressed by shortening the C-O bond length from (1.335–1.358 Å) in the parent compounds to (1.230–1.248 Å) in all radicals.

Charge resonance and distribution

The positive charge in parent compounds (Figs. 1, S1) is located on ring carbons next to oxygen atoms (pyrylium, hydroxyl, or methoxy oxygen atoms) which could stabilize positive charge by the π -electron donating ability of oxygen atoms. The highest positive charge (0.364–0.482) is located on carbons 2, 5, 7, 9, and 4' while carbon atoms next to 3-OH, 3'-OMe or 5'-OMe (in 3-OHFv, Pg, Pn, and Mv) carry the least positive charge (0.258–0.270). The pyrylium oxygen carries the least negative charge i.e., more positive character than other oxygen atoms which is consistent with the previous DFT calculation (Guzmán et al. 2009). The increased resonance through ring B and localization of charge on C4' was observed in 4'-hydroxy flavylium compared to the unsubstituted flavylium (Pereira et al. 1997; Sakata et al. 2006). The proposed resonance (Fig. 1) explains the higher positive charge delocalization on carbons 2, 5, 7, 9, and 4' while the lower charge on C3, C3', or C5' in 3-OHFv, Pg, Pn, and Mv could be due to only the inductive effect of the neighboring oxygen atoms (Seyoum et al. 2006).

In all radicals, the positive charge is still found on the same carbon atoms as in parent compounds indicating that

radicals undergo the paired-electron resonance similar to that described for parent compounds to delocalize the positive charge. In contrary to the parent compounds, the oxygen lost the hydrogen atom (the radical oxygen) carries either positive charge (7-RFv and 7-Ap, S5) or least negative charge among oxygen atoms i.e., able to carry more positive character while the pyrylium oxygen is the second least negatively charged oxygen after the radical oxygen. The low negative charge density observed on radical oxygen atom, despite its high electronegativity reported in simple phenols (Foti et al. 2010), could be attributed, in anthocyanidin radicals, to the different types of resonance and high electron delocalization over the three rings presented in Figs. 1–3. The same observation of increasing positive charge where the radical is formed was also previously reported in other flavonoid systems (Sadasivam and Kumaresan 2011). In addition, atoms in the ring at which the radical is formed carries more positive character than the same ring in other radicals; for example, atoms of ring B in 4'-RPg carry more positive charge than the same ring in 3-RPg, 5-RPg, or 7-RPg while ring A in 5-RPg or 7-RPg carries more positive charge than in 3-RPg or 4'-RPg suggesting a radical stabilization by vicinity to a charge. Accordingly, the more positive (or less negative) charge on radical oxygen should reflect the radical stabilization and the antioxidant activity. Therefore, charge on radical oxygen (of lowest BDE), as shown in Figs. 2, S2, S3 and S4, of 4'-hydroxyflavylium ion (4'-R, -0.012), apigeninidin (5-R, -0.011), pelargonidin (3-R, -0.078), peonidin (3-R, -0.098) and malvidin (3-R, -0.104) was found correlated with scavenging percentage of DPPH (7.12, 10.20, 36.43, 53.49, and 68.64% respectively) and hydroxyl radicals (11.19, 33.33, 47.46, 65.82, and 66.78% respectively) reported previously (Ali et al. 2016). Correlations can be presented by the following equations:

$$\% \text{ scavenging DPPH} = 0.86 (\pm 6.03) - 566.35 (\pm 82.37) \text{ charge on radical oxygen}$$

$$R = 0.970, SE = 7.55, N = 5, \text{ and } p = 0.006$$

$$\% \text{ scavenging OH} = 16.17 (\pm 7.99) - 474.44 (\pm 109.20) \text{ charge on radical oxygen}$$

$$R = 0.929, SE = 10.01, N = 5, \text{ and } p = 0.023$$

These correlations reflect the role of charge distribution in stabilizing the anthocyanidin radicals and thus their potent antioxidant activity.

Unpaired electron resonance and distribution

All O–H bond dissociation energy (BDE) of the examined compounds were calculated and listed in Table 1. BDE is frequently used, depending on the hydrogen atom transfer mechanism, to identify the best hydroxyl group as hydrogen atom donor (Guzmán et al. 2009; Foti et al. 2010). In the three tested 3-oxyanthocyanidins (Pg, Pn, and Mv), the 3-O–H bond is the easiest to break relative to other hydroxyl groups in each molecule with BDE 74.748–76.594 Kcal/mol which could be derived by the steric relieve of 3-O–H bond and the stability of the resulted planar radicals discussed above; this finding rationalize the previous suggestion that 3-OH group in anthocyanidins plays an important role in their antioxidant activity (Wang et al. 1997; Lu et al. 2014). There are still some variations in the literature in the order of $BDE_{(O-H)}$ of anthocyanidins. For examples, the order in malvidin was reported as $3 > 4' > 5 > 7$ (Lu et al. 2014; Guzmán et al. 2009) and $3 > 5 > 7 > 4'$ (Pop et al. 2012) while in peonidin the reported orders were $3 > 5 > 7 > 4'$ (Guzmán et al. 2009) and $3 > 4' > 5 > 7$ (Lu et al. 2014). The present calculation showed that the order of $BDE_{(O-H)}$ in Ap, Pg and Pn is $3 < 5 < 4' < 7-OH$; however, in Mv, the 4'-O-H bond has lower BDE than that of 5-O-H bond where the order is $3 < 4' < 5 < 7-OH$. It can be observed that the BDE of 4'-O-H bond decreases in the order of $Pg > Pn > Mv$ (90.128, 87.483 and 79.547 Kcal/mol respectively) which matches the order of their antioxidant activity (Rahman et al. 2006; Ali et al. 2016) and the order of increasing number of neighboring methoxy groups (at 3' and 5') which stabilizes the 4'-radical and explains the obtained order of $BDE_{(O-H)}$ in Mv. Malvidin also exhibited high scavenging activity against singlet oxygen even larger than cyanidin that contains a catechol moiety which was attributed to the role of 4'-OH and the electron donating *o*-OMe groups in favoring the interaction with the singlet oxygen electrophile (De Rosso et al. 2008). These results indicate the important of the BDE of both 3- and 4'-OH in determining the antioxidant activity of anthocyanidins. The order of Gibbs free energy (ΔG) of the O–H dissociation matches that of the corresponding BDE in all the examined flavyliums and anthocyanidins while the entropy change (ΔS) changed only within 2.6 cal/mol K indicating that the process is mainly governed by the dissociation enthalpy. Although BDE of hydroxyl groups is frequently correlated with the antioxidant activity of phenolic compounds, no such quantitative correlation was reported between the antioxidant activity of anthocyanidins and their lowest $BDE_{(O-H)}$ (or $\Delta G_{(O-H)}$). Therefore, this correlation not only shows the dependence of antioxidant activity on the $BDE_{(O-H)}$ but also validates the choice of the eligible hydroxyl group for each anthocyanidin. Correlations of radical scavenging activity (Ali et al. 2016) with the

least $BDE_{(O-H)}$ in each compound are presented by the following equations.

$$\% \text{ scavenging DPPH} = 290.83 (\pm 81.83) - 3.18 (\pm 1.02) \text{ BDE}$$

$$R = 0.875, N = 5, SE = 14.95, p = 0.052$$

$$\% \text{ scavenging OH} = 292.73 (\pm 35.87) - 3.08 (\pm 0.45) \text{ BDE}$$

$$R = 0.970, N = 5, SE = 6.55, p = 0.006$$

The unpaired electron distribution is modeled by the spin density as illustrated in models of flavylum and anthocyanidin radicals presented in Figs. 2–3 and S2–S5. The 3-radicals can be stabilized by resonance through 5-, 7 or 4'-oxygen atom (Ali et al. 2016) as presented in Fig. 2. These resonance pathways are manifested by the location of spin density (>0.025) on the three pathway atoms (O3, C4, C9, C7, C5, and O5), (O3, C4, C9, C7, and O7) and (O3, C4, C9, C2, C2', C4', and O4') respectively. On the other hand, O1 can not be unpaired electron acceptor in any of these resonance pathways and thus does not bear any spin density in all the examined 3-oxyanthocyanidin radicals. It can also note the similarity between the unpaired electron distribution of 3-radicals and charge distribution mentioned above. The planarity and the wide distribution of the unpaired electron of 3-radicals could explain the lower BDE of the 3-OH group and the impotence of this group, discussed earlier, in determining the antioxidant activity of 3-oxyanthocyanidins.

The 4'-RFv radical can be stabilized by resonance through the pyrylium oxygen, O1 (Fig. 2) where alternative atoms in that route (O4', C3', C5', C1', and O1) carry the highest spin density (0.054 (O1)–0.385 (O4')) followed by delocalization of the unpaired electron over ring C then ring A at lesser extent. Similar behavior of higher spin density on alternative atoms of ring B than on atoms of rings A and C can be observed in all other 4'-radicals. Considerable higher spin density is located on the methoxy oxygen of Pn (0.090) and Mv (0.060 and 0.108) than on hydroxyl oxygen atoms (≤ 0.017) indicating the involvement of the *ortho* methoxy oxygen in the unpaired electron resonance as shown in Figs. 2, S3. In addition, the 4'-radical oxygen carries lower spin density in Pn (0.270) and Mv (0.269) than in Pg (0.376) radicals; besides, the C4'-O bond in Pn and Mv has more π -bond character (bond length 1.230 and 1.231 Å, respectively) than in Pg (1.243 Å) which all indicate the strong effect of the *ortho* electron donating methoxy groups in delocalizing the unpaired electron¹⁶ and explains the lower BDE of 4'-O-H than that of 5-O-H in Mv, in contrary to other tested anthocyanidins.

Radicals of 5-OH and 7-OH groups are characteristic by high spin density on alternative atoms of rings A and C i.e.,

O5 or O7 respectively in addition to C6, C8, C10, C3, and O1 suggesting a resonance through the pyrylium oxygen as illustrated in Figs. 3, S4, S5. The resonance can also be extended to O3 in 3-oxyanthocyanidins instead of O1 as indicated by the existence of spin density on O3. On the other hand, the absence of possible resonance between the *meta* 5- and 7-oxygen atoms led to existence of unpaired electron on only either one of them. The resonance stabilization of 5- and 7-anthocyanidin radicals supports the reported significant higher antioxidant activity of Ap than that of 4'-OHFv (Ali et al. 2016). In addition, the location of the double bonds of the benzopyrylium ring (rings A and C) in all 5- and 7-anthocyanidin radicals matches exactly that of the suggested resonance.

It can be observed that the spin density is distributed over the three rings in all radicals even those showed non-planarity indicating that the planarity discrepancy does not prevent the through resonance among the rings; however, the movement of the unpaired electron from the benzo-pyrylium ring to ring B is higher in the planar 3-radicals than in the corresponding 5- or 7-radicals; for example, the sum spin density of ring B is higher in planar 3-RPg radical (Fig. 2) than in the non-planar 5-RPg or 7-RPg radicals (Fig. 3). In all radicals, the radical oxygen retains the highest spin density consistent with previous calculations (Lu et al. 2014). All radicals also form a π -bond character between radical oxygen and neighboring carbon atom (C=O) revealing the initiation of a single-electron resonance for delocalization of the unpaired electron. It is well known that more delocalized unpaired electron reflected by more spin density distribution results in more stable radical and lower BDE (Sadasivam and Kumaresan 2011). In addition, the presence of electron donating hydroxyl groups on carbon atoms of a positive spin density increases radical stabilization (Seyoum et al. 2006). Therefore, the decrease of spin density on the radical oxygen is a measure of the increased resonance and thus can be used as indicator of radical stabilization and antioxidant activity. Correlating the spin density on the radical oxygen (of the lowest BDE_{OH}) with the previously reported (Ali et al. 2016) scavenging of DPPH and hydroxyl radicals gave good correlations.

$$\% \text{ scavenging DPPH} = 257.91 (\pm 41.68) - 624.95 (\pm 116.34) \text{ spin density}$$

$$R = 0.952, N = 5, SE = 9.49, p = 0.013$$

$$\% \text{ scavenging OH} = 216.58 (\pm 64.67) - 481.65 (\pm 180.51) \text{ spin density}$$

$$R = 0.839, N = 5, SE = 14.72, p = 0.076$$

Finally, the 3-radical oxygen of each anthocyanidin carries less spin density and higher negative charge (less positive character) than other radical oxygen atoms; for example, compare spin density (0.347) and charge (−0.078) of Pg (Fig. 2) the 3-radical oxygen with those of other radicals (Figs. 3, S3). Therefore, it could be stated that the less positive charge and spin density on radical oxygen, the higher antioxidant activity since the charge and unpaired electron are then more delocalized over the rings.

Conclusion

A DFT study of the anthocyanidins apigeninidin, pelargonidin, peonidin and malvidin, plus the 3-, 4', 5- or 7 flavylium ions as well as their phenoxy radicals revealed some points concerning their molecular planarity, charge distribution and the resonance stability of their radicals. The existence and direction of the 3-OH bond affects ring B orientation and compound planarity where compounds and radicals lacking 3-O–H bond were found planar. Bond dissociation energy of O–H bond in anthocyanidins was in the order of 3- < 5- < 4' - < 7-OH bond except for in malvidin where 4'-OH has less BDE than 5-OH bond because of the extra stability provided by the two *o*-OMe groups. Positive charge was stabilized by resonance on carbons 2, 5, 7, 9 and 4'. The 3-anthocyanidin radicals were stabilized by unpaired electron resonance through 5-, 7- or 4'-oxygen atom while 5-, 7- or 4'-radicals were stabilized by resonance through pyrylium or 3-oxygen atom. In all radicals, radical oxygen retains the highest spin density and forms a carbonyl group (C=O) with neighboring carbon atom. In addition, delocalization of the unpaired electron was observed through the three rings but was higher in planar radicals. Experimental radical scavenging activity was found well correlated with both charge and unpaired electron delocalization expressed by charge and unpaired electron density on radical oxygen.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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