

Analysis of Conformational, Structural, Magnetic, and Electronic Properties Related to Antioxidant Activity: Revisiting Flavan, Anthocyanidin, Flavanone, Flavonol, Isoflavone, Flavone, and Flavan-3-ol

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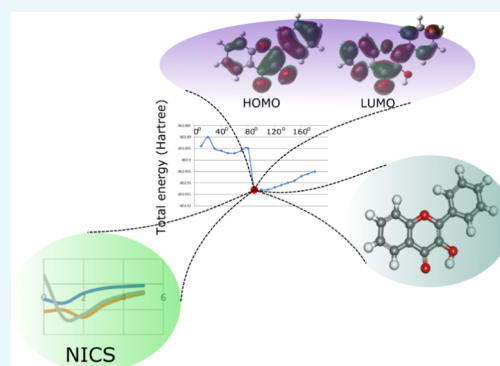
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ABSTRACT: Understanding the antioxidant activity of flavonoids is important to investigate their biological activities as well as to design novel molecules with low toxicity and high activity. Aromaticity is a chemical property found in cyclic structures that plays an important role in their stability and reactivity, and its investigation can help us to understand the antioxidant activity of some heterocyclic compounds. In the present study, we applied the density functional theory (DFT) to investigate the properties of seven flavonoid structures with well-reported antioxidant activity: flavan, anthocyanidin, flavanone, flavonol, isoflavone, flavone, and flavan-3-ol. Conformational, structural, magnetic, and electronic analyses were performed using nuclear magnetic resonance, ionization potentials, electron affinity, bond dissociation energy, proton affinity, frontier molecular orbitals (highest occupied molecular orbital (HOMO)/lowest unoccupied molecular orbital (LUMO)), and aromaticity through nucleus-independent chemical shifts to analyze these seven flavonoid structures. We revised the influence of hydroxyl groups on the properties of flavonoids and also investigated the influence of the aromaticity of these seven flavonoids on the antioxidant activity.



1. INTRODUCTION

Flavonoids are a class of natural compounds characterized by a backbone containing 15 carbon atoms, two phenyl rings A and B linked by heterocyclic (pyran) ring C (Figure 1).¹ Benzo- γ -pyran (chromone) refers to the heterocycle formed by the phenyl ring (A) and the pyran ring (C).² Flavonoids are divided into six subclasses: anthocyanidins, flavanones, flavonols, isoflavones, flavone, and flavan-3-ol.^{3–5}

Flavonoids possess a wide range of biological activities, such as anti-inflammatory,⁶ antibacterial,⁷ anticancer,^{8,9} and antiviral.^{10,11} Different computational studies have investigated the structural properties of these molecules related to their biological activities, especially their antioxidant activity; however, the influence of the aromaticity for their reactivity remains unknown.^{12–19} The antioxidant property is related to the ability to neutralize free radicals that are involved with the oxidative stress in the cell.^{20,21} Free radicals, such as reactive oxygen species, cause serious damages to cell structures, such as lipids, proteins, and nucleic acids, and they are involved in food deterioration and the development of several human diseases.²² Flavonoids are phenolic compounds with proven antioxidant activity that prevent the action of free radicals, thus protecting the cell against their harmful effects.

Investigating the antioxidant activity through electronic properties is important to design novel antioxidant molecules with low toxicity and high activity.^{23,24} Three major mechanisms have been proposed to explain the antioxidant activity of compounds.^{25–27} It has been proposed that flavonoids (FlavOH) can neutralize the free radical by donating a hydrogen atom (eq 1).^{28–30}



Another mechanism consists of the electron transfer, where the antioxidant can donate an electron to the free radical (eqs 2 and 3).



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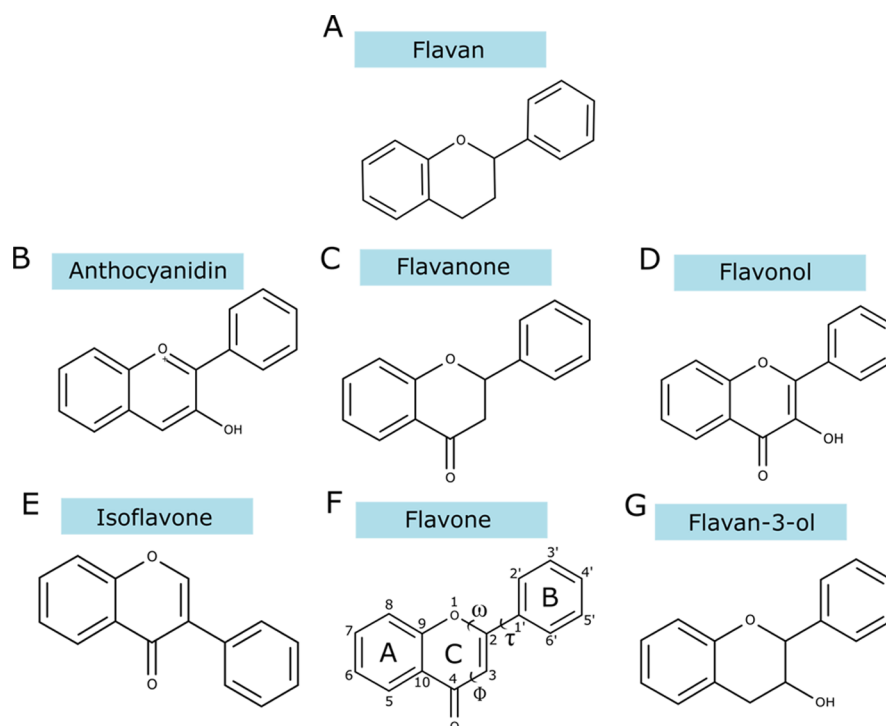
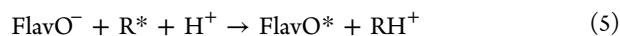


Figure 1. Molecular structures of the investigated flavonoids showing the atoms and ring labels, as well as the studied dihedral angles. (A) Flavan, (B) anthocyanidin, (C) flavanone, (D) flavonol, (E) isoflavone, (F) flavone, and (G) flavan-3-ol.

The second mechanism (2) is known as hydrogen atom transfer (HAT), which occurs in (3) single-electron transfer followed by proton transfer (SET-PT). The third mechanism is known as sequential proton loss electron transfer (SPLET), which consists of the following mechanism (eqs 4 and 5)



where occurs the transference of a proton from the antioxidant (4) followed by electron donation. The parameters applied to evaluate the antioxidant activity in the mechanisms of HAT, SET-PT, and SPLET include the calculation of molecular properties, such as bond dissociation enthalpy (BDE), ionization potential (IP), and proton affinity (PA), respectively.³¹ Besides these mechanisms, the free radicals that emerge in both reactions (FlavO* and FlavOH*⁺) must be neutralized to avoid oxidative stress.²⁸

The aromaticity of cyclic structures is a key parameter for their reactivity.^{32,33} Aromatic rings show diamagnetism and increase the stability of the structures. In contrast, antiaromatic rings show paramagnetism and less stability. The aromaticity can be addressed through chemical shielding in ¹H nuclear magnetic resonance (NMR) spectra.^{34,35} Moreover, the antioxidant activity of compounds can be mapped by the frontier molecular orbitals (highest occupied molecular orbital (HOMO)/lowest unoccupied molecular orbital (LUMO)) and ionization potential.³⁶

In the present study, we performed a systematic analysis of seven flavonoid structures—flavan, anthocyanidin, flavanone, flavonol, isoflavone, flavone, and flavan-3-ol—using structural, conformational, and electronic analyses of protonated (neutral) and deprotonated forms. We compared the conformational properties of these structures with previous theoretical studies, analyzing the lower energy conformations,

especially the electron density of the benzo- γ -pyran ring and the influence of the hydroxyl and ketone groups and aromatic rings on their antioxidant activity.

2. RESULTS AND DISCUSSION

Different computational approaches have been used to investigate flavonoid structures.^{3,31,37,38} Recently, Zheng et al. performed a comparative study focusing on the interactions of the hydrogens in the solvated systems of 5,7-dihydroxy-2-(3,4-dihydroxyphenyl)-chrome-4-one (luteolin) using DFT (B3LYP method) and concluded that luteolin presents a nonplanar conformation.³⁷ Similarly, Matteini et al. performed a theoretical study with the flavonoid rutin, using molecular mechanics (MM) and semiempirical calculations using Austin Model 1 (AM1), and they found 16 stable conformations with overall minimum energy due to the formation of intermolecular H-bond interactions between the hydroxyl group and C₁₂ attached to ring B.³⁸ Herein, we optimized the geometries of the seven flavonoid structures using the CAM-B3LYP/def2TZV calculations (Figure 2). The atoms were numbered according to the flavonoid scaffold (see Figure 1).

The dipole moments (Debye), interatomic distances, and dihedral angles for the neutrals and deprotonated structures optimized with CAM-B3LYP/def2TZV are shown in Table 1. The largest dipole moments were found for the flavonol and flavone structures, while anthocyanidin showed the smallest dipole moment. Table 1 shows the dihedral angles τ : O1–C2–C1'–C6', ω : C9–O1–C2–C3, and ϕ : C10–C4–C3–C2.

The interatomic distances obtained in the present study were compared with the luteolin structure calculated at the B3LYP level.³⁷ We noted that there is no significant difference between the calculated interatomic distances of the investigated structures of flavonoids (Table 1) with those previously investigated in the literature.³⁷

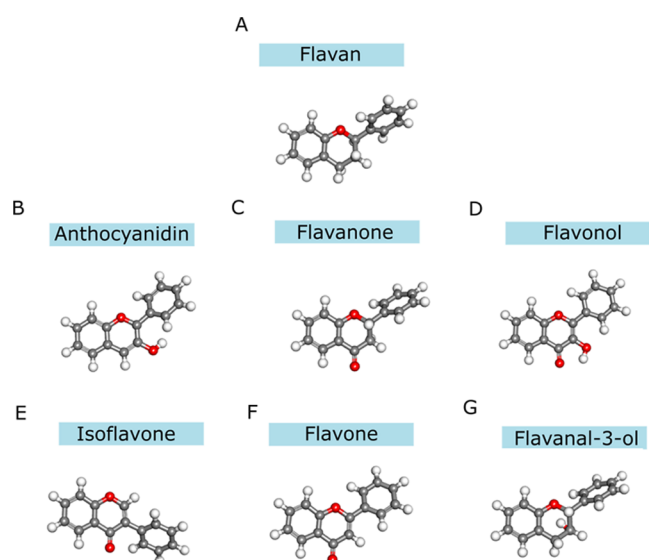


Figure 2. Optimized conformations of the investigated flavonoid structures. (A) Flavan, (B) anthocyanidin, (C) flavanone, (D) flavonol, (E) isoflavone, (F) flavone, and (G) flavan-3-ol.

Lau et al., using B3LYP/6-31G(d) calculations, suggested that the planarities of 5,7-dihydroxyflavone and 7,8-dihydroxyflavone were associated with the induced dipole interaction between O_1 and the hydrogen of C_6 (O_1-HC_6) with variations between 2.32 and 2.46 Å.⁴³ Similarly, Machado et al., investigated flavonoids with a hydroxyl group in rings A and C using B3LYP/6-31G(d,p), and they found the values of the torsion angle τ between 0 and 20° and the distance values of O_1-HC_6 between 2.31 and 2.39 Å.⁴⁴ Aparicio investigated 16 different flavonoid structures using B3LYP/6-311++G(d,p) and associated the planarity of the molecules with the hydroxyl group bonded to C_3 that forms a hydrogen bond with C_6' in ring B.³ Our analyses showed that the protonated structures of anthocyanidin, flavonol, and flavone have a planar conformation with distances of 2.31, 2.31, and 2.40 Å between the hydrogen bonded to C_6' and O_1 , respectively. The distance between O_3H and C_4 is 2.54 Å in anthocyanidin with a torsion angle τ of 171.21°. Flavan-3-ol does show a great conforma-

tional change in its structure with a distance of 2.00 Å between O_3H and C_3 and a torsion angle τ of 147.13°. Previous theoretical and experimental studies obtained a torsion angle τ close to 180°.^{38,39} However, the CAM-B3LYP calculations showed a planar conformation only for the deprotonated anthocyanidin and flavonol; the deprotonated flavan-3-ol showed no planarity between rings B and C. Our computational analysis indicated that the anthocyanidin structure did not show an interaction between hydroxyl and the hydrogen bond to C_6' of ring B as confirmed previously.^{3,44}

The protonated forms of flavonol and anthocyanidin showed planar conformation with a $C_6'-O_1$ distance of 2.30 Å and a $O_1-C_6'-H$ angle of 60°. In contrast, flavan and flavan-3-ol showed conformational changes with atomic distances of $C_6'-O_1$ equal to 2.46 and 2.50 Å, respectively, and angles of $O_1-C_6'-H$ equal to 62.52 and 61.45°, respectively. These results indicate that the stabilization of these molecules is not due to the formation of a hydrogen bond between C_6' and O_1 .

The deprotonation of anthocyanidin and flavonol did not change the planarity of their structures, which suggests that the hydroxyl group has no influence on the stabilization of planar structures. Machado et al.⁴⁴ indicated an interaction between the oxygen of O_3H and the ketone group of ring C that stabilizes the planar conformation by the electronic redistribution through the molecule. Comparing the results of anthocyanidin, flavonol, and flavan-3-ol (Figure 2), we found an H-bond interaction between the hydroxyl group with ring C. Similar to Machado et al., we also identified an interaction between the hydroxyl group and the oxygen of the ketone group.⁴⁴ Figure 3 shows the relationships between the energy and conformational variations related to the torsion angles τ of the protonated molecules. All conformational analyzes were performed using the CAM-B3LYP/def2TZV protocol.

Flavan and flavanone showed almost the same pattern, with the maximum energy found for torsion angle τ between 80 and 140°, while isoflavone and flavone have the maximum energy at almost 150 and 130°. Table 2 shows the bond distance values obtained using the CAM-B3LYP/def2TZV protocol of the deprotonated flavonoid molecules.

Cappelli et al. performed a conformational analysis of flavonoids analyzing the hydroxyl groups of all rings using

Table 1. Dipole Moments (Debye), Interatomic Distances between the Carbons (Å), and Torsion Angles (Degrees) of the Optimized Structures of Flavonoids at the CAM-B3LYP/def2TZV Level

	dipole	$C_1'-C_2'$	$C_1'-C_6'$	$C_1'-C_2$	C_2-C_3	O_1-HC_6'	τ	ω	ϕ
luteolin ³⁷ experimental		1.41	1.40	1.47	1.35		-160.7	-0.05	-1.50
		1.38	1.42	1.46	1.36 ^c		19.00	55.30 ^c	36.30 ^c
		1.40 ^b	1.40 ^b	1.47 ^b	1.36 ^b		-164.6 ^c		
							-178.4 ^d	0.00 ^d	0.00 ^d
flavan	1.83	1.40	1.40	1.51	1.53	2.46	-147.17	-44.03	-47.84
anthocyanidin	3.44	1.41	1.41	1.45	1.39	2.46	171.21	0.21	1.36
anthocyanidin ^d	5.44						-178.79	-0.01	-0.57
flavanone	2.61	1.40	1.40	1.50	1.53	2.50	144.44	51.21	33.11
flavonol	3.58	1.40	1.40	1.48	1.34	2.32	169.08	0.11	1.56
flavonol ^a	8.52						179.00	-0.19	-0.42
isoflavone	3.03	1.40	1.40		1.34		-177.93	-0.23	-2.46
flavone	4.42	1.40	1.40	1.47	1.35	2.40	157.75	0.28	1.6
flavan-3-ol	1.92	1.39	1.39	1.50	1.53	2.50	147.13	52.43	46.12
flavan-3-ol ^a							116.44	33.96	57.11

^aDeprotonated structures; all values were obtained from the reference structures luteolin.³⁹ ^bQuercetin.⁴⁰ ^cFlavanone derivatives.⁴¹ ^dFlavone derivatives.⁴²

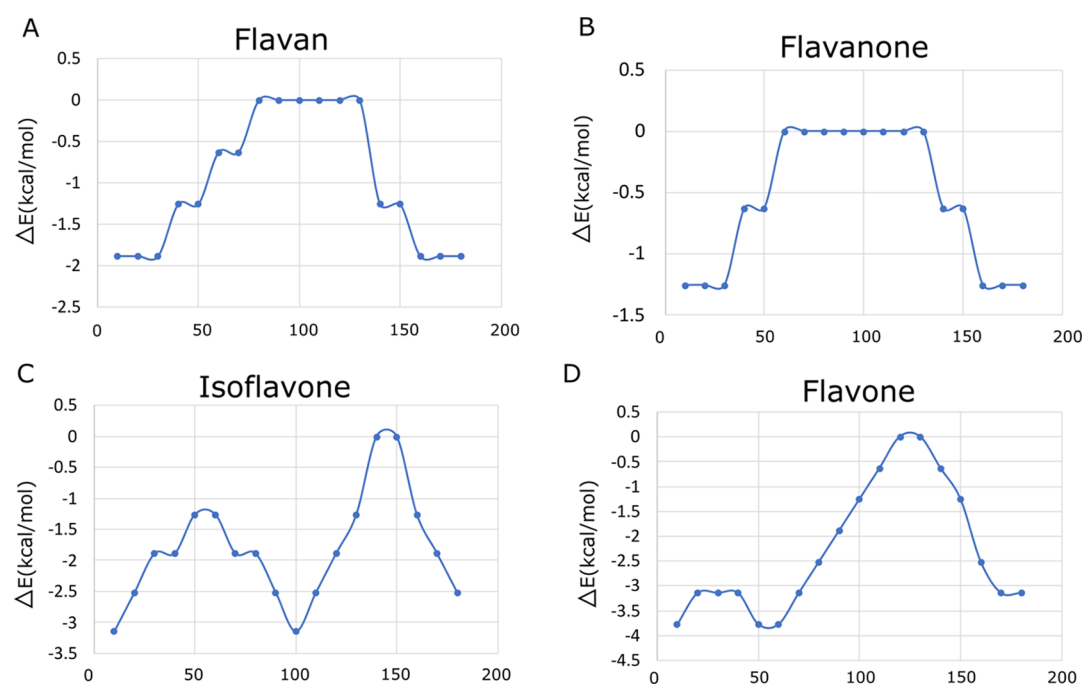


Figure 3. Conformational analysis of the torsion angle τ of the investigated flavonoids (in degrees). (A) Flavan, (B) flavanone, (C) isoflavone, and (D) flavone.

Table 2. Interatomic Distances (Å) and Angles (Degrees) of the Hydrogen Bond were Obtained from the Conformational Analysis of the Flavonoid Structures

	anthocyanidin	flavanol	flavan-3-ol
O3H...O		2.06	
O2-H-O3		115	
HO...HC4	2.66		2.58
HO...H (ring B)	2.09	2.15	2.99
C4-O2-H	86.57		97.69

molecular mechanics and DFT in the gas phase and the methanol solution.⁴⁵ The simulations showed nine different conformations for the investigated structures. In contrast, Machado et al.⁴⁴ did not find interactions between the hydroxyl group and the carbons of ring C in the investigated conformations. However, it is important to highlight that detailed analyses of the electronic properties of the flavonoids will be necessary to corroborate with the aforementioned suggestions.^{44,45} We noted that when the hydroxyl group interacts with the oxygen of the ketone group, it leads to a decrease in the overall energy of flavanol with a dihedral angle τ equal to 169.08°. Additionally, flavanol and anthocyanidin tend to be planar (Table 2).

Figure 4 shows the conformational analysis of anthocyanidin (Figure 4A), flavanol (Figure 4B), and flavan-3-ol (Figure 4C). We noted a significant reduction in the energy values of the flavanol conformation due to the alterations of O3H orientation. The structures of the lowest-energy conformations of anthocyanidin, flavanol, and flavan-3-ol are related to the positions of the hydroxyl group interacting with the hydrogen of C4, as well as the ketone group. The rotational barrier of flavanol is the highest compared to anthocyanidin and flavan-3-ol, which corroborates the previous findings.³ Additionally, these structures form a hydrogen bond interaction between the hydroxyl groups of C3 (ring C) and O4 (ring C), as well as the

hydroxyl group of C3 (ring C) and the hydrogen of C6' (ring B).

It is expected that neutral structures of anthocyanidin, flavanol, and flavone present high aromaticity when compared with the other analyzed flavonoids. Therefore, the aromaticity of the structures can contribute to understand the antioxidant mechanism. The number and position of hydroxyl groups have been widely studied regarding the antioxidant activity.^{14,46–51} For example, for luteolin, it was demonstrated that by removing the O3H group in ring C, the antioxidant activity consequently decreases.⁴⁶ Moreover, the importance of ring C unsaturation was pointed out, which allows electron delocalization for stabilization of the aryloxy radical.⁴⁶ We have found that isotropic NICS(1) values of anthocyanidin ring C are larger than the values of other studied flavonoid structures, suggesting that the ketone group decreases the aromaticity, while the hydroxyl group increases the aromaticity jointly to the hydrogen bond, as expected from the substituent effects. Besides, high dipole moments were found for the deprotonated molecules; however, in neutral molecules, the highest values were found in flavone, flavanol, and anthocyanidin, which are also the planar structures (Table 1). Additionally, flavanol and anthocyanidin tend to be planar (Table 2). In contrast to flavan-3-ol, the molecular planarity of anthocyanidin and flavanol is characterized by the presence of the O3H group through a weak hydrogen bond with HC6', which is in agreement with the literature.³

Figure 5 shows the NICS plots (ppm per r distance in Å), which are representative of the isotropic, anisotropic, and ZZ (NICS_{zz}) fields. Negative values of absolute shielding computed at the center of the molecule indicate the presence of induced diatropic current (aromaticity), whereas positive values indicate paratropic currents (antiaromaticity).⁵² The r distances were obtained from the neutral structures of the molecules: flavan, anthocyanidin, flavanone, flavanol, isoflavone, flavone, and flavan-3-ol. Our NICS results showed that

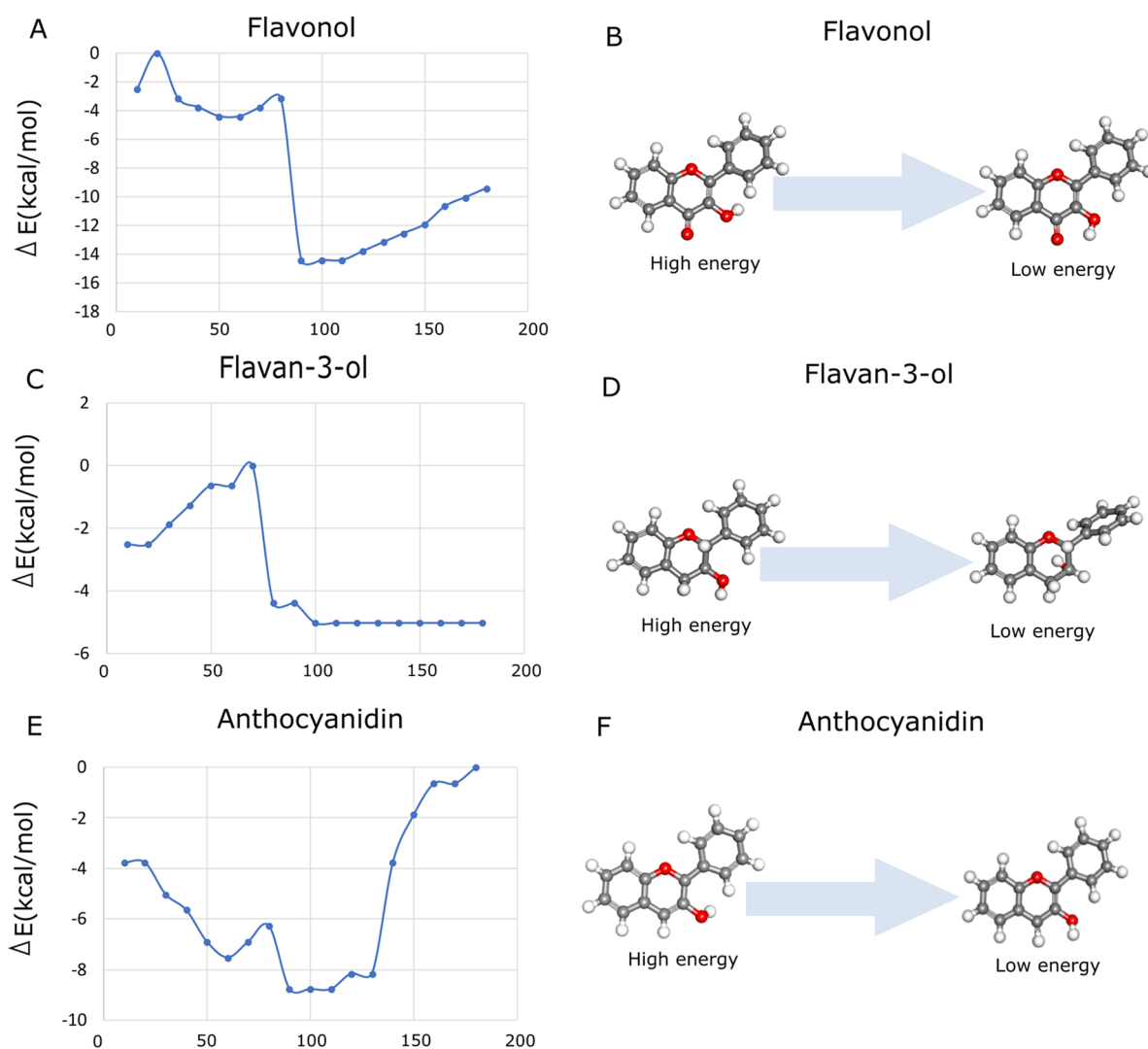


Figure 4. Conformational analysis of flavonoid structures on torsion angle τ (in degrees) and their respective high-energy and low-energy conformational states. (A, B) Flavonol, (C, D) flavan-3-ol, and (E, F) anthocyanidin.

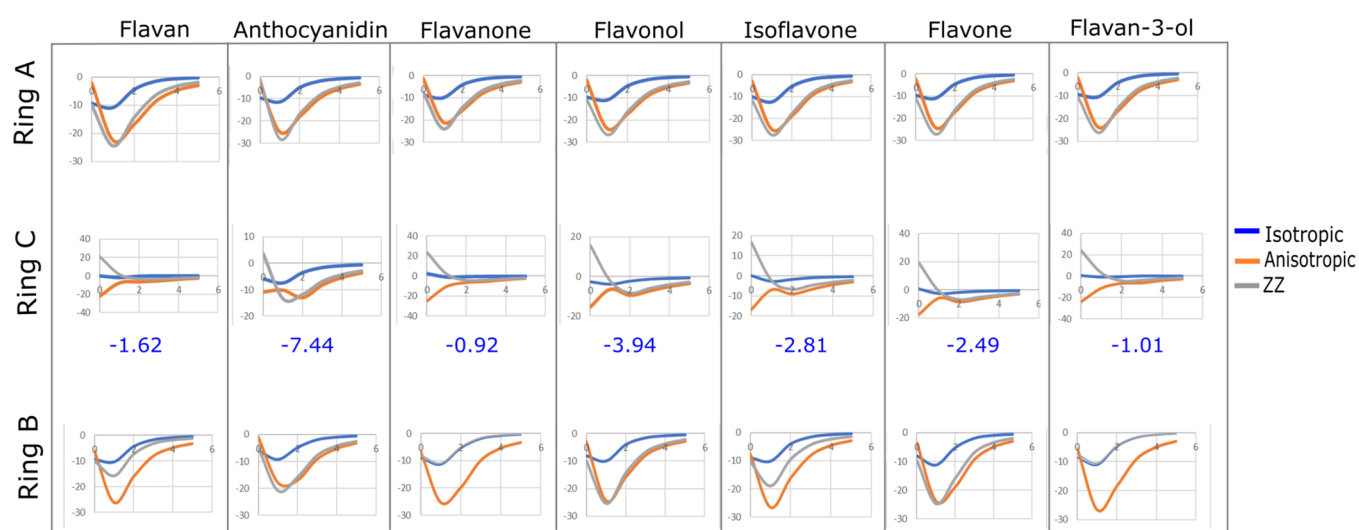


Figure 5. NICS values as a function of the distance r of the neutral and isotropic NICS(1) displayed numerically for ring C. The isotropic NICS(1) value (in ppm) was shown for all analyzed flavonoids.

Table 3. Chemical Shifts (δ) in ppm for ^{13}C Obtained from CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ Calculations Using Tetramethylsilane (TMS) as a Reference

	CAM-B3LYP/def2TZV				HSEh1PBE/cc-pVDZ				
	C3	C4	C8	C10	C3	C4	C8	C10	
	Theoretical								
flavan	37.2	29.0	127.6	132.2	33.7	27.4	114.7	120.5	
anthocyanidin	156.2	143.6	127.8	130.3	146.3	132.1	116.0	121.9	
flavanone	53.0	213.9	127.6	131.6	47.1	194.0	115.3	120.2	
flavanol	148.9	190.1	126.8	130.5	139.9	174.7	115.0	121.1	
isoflavone	137.8	193.2	126.8	134.9	128.8	176.5	114.7	125.3	
flavone	117.7	194.7	126.5	134.8	108.4	177.6	194.7	124.7	
flavan-3-ol	140.6	37.3	128.2	132.5	125.3	34.6	115.3	121.6	
	Experimental								
anthocyanidin ^a	143.9	130.9	94.0	111.1					
flavanol ^b	136.2	176.4	93.8	98.7					
flavanol ^c	135.5	175.7	93.4	102.9					
flavone ^c	103.3	182.2	94.2	104.2					

^aWolnick and Wawer.⁵³ ^bSinha et al.⁵⁴ ^cScoti et al.⁴⁹

the ring C of the analyzed flavonoids exhibited a significant reduction in their aromaticity.

The NICS values demonstrated that only anthocyanidin has an aromatic ring C (Figure 5). The formation of the interaction between the hydroxyl group and C4 could improve the planarity of the structure and also increase the π electrons in ring C. However, it is important to highlight that we should not neglect the influence of ketone oxygen for aromaticity due to the presence of the π electrons associated with this atom. The antioxidant activity of flavonoids was shown to be dependent on the stabilization of the O3H group through unsaturation of ring C.^{14,46–51} Despite the presence of planarity in the molecules, the ZZ term (NICS_{zz}) is well aligned with the anisotropic (NICS_{anisotropic}) term of ring B (Figure 5) and the aromaticity only occurs in the ring C of anthocyanidin (NICS_{zz} and NICS_{isotropic} terms). This result corroborates the high antioxidant activity previously reported for an anthocyanidin analogue.⁴⁶ The chemical shifts (δ) extracted from the NMR of carbon (^{13}C NMR) and oxygen (^{17}O NMR) are shown in Tables 3 and 4. Similar results were found for the calculated ^{13}C NMR concerning previous experimental studies.^{49,53,54}

The analysis of ^{17}O NMR using CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ showed that: (1) the results with CAM-

B3LYP/def2TZV are underestimated concerning the results obtained with HSEh1PBE/cc-pVDZ and (2) in the neutral flavonol structure, the H-bond interaction formed by O3 is stronger than that by O1, and O1 is stronger than O4. Similarly, in the structure of anthocyanidin, the H-bond interaction formed by O3 is stronger than O1.

The ^{17}O NMR analysis of flavonol and anthocyanidin oxygen indicated the formation of an interaction between O3H and the oxygen of ketone and C4 of ring C, respectively, thus stabilizing the molecule in a planar conformation. Moreover, the change in the shielding (ppm) of the oxygen atoms of flavonol due to deprotonation occurs along with an increase of 80.09° in the torsion angle τ . Aromatic rings tend to planarity, but a similar conformational change does not occur with ring C of flavan-3-ol that differently from flavonol does not have ketone oxygen. The change in the shielding (ppm) of the oxygen due to deprotonation occurs in anthocyanidin similar to flavonol, but without a change in aromaticity. The energy density at the critical point divided by the electron density distribution ($H(\mathbf{r}_c)/\rho_c$) was obtained from AIM calculations, which suggest for the O3H...HC6' interaction of anthocyanidin and flavonol structures, a hydrogen bond with a partial electrostatic character, showing 0.15 and 0.18 Hartree/electron, respectively.

To better understand the relevance of the structural differences of the investigated flavonoids for their antioxidant activity, we also analyzed the energy of the HOMO/LUMO molecular orbitals using CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ protocols. Both quantum chemistry calculation protocols showed the same profile. Therefore, Figure 6 shows the HOMO and LUMO molecular orbitals of the investigated flavonoid structures, calculated at the CAM-B3LYP/def2TZV level. The HOMO topology of a phenolic compound may indicate its active site of the free-radical elimination due to hydrogen abstraction after electron transfer.⁵⁵

The analysis of the natural bond orbital (NBO) of ring C showed π bonds (1) in C9–C10 of flavan; (2) in C3–C4 and C9–C10 of anthocyanidin; (3) in O2–C4 and C9–C10 of flavanone; (4) in O3–C4, C9–C10, and C2–C3 of flavonol; (5) in C2–C3 and C9–C10 of isoflavone; (6) in O2–C4, C2–C3, and C9–C10 of flavone; and (7) in C9–C10 of flavan-3-ol. Mendes et al. studied the antioxidant activities of

Table 4. Chemical Shifts (δ) in ppm of Oxygen (^{17}O NMR) Obtained from CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ (in Parenthesis) Calculations

compound/ atoms	O1	O3	O4
flavan	197.3 (209.3)		
anthocyanidin	20.5 (59.1)	215.4 (224.9)	
anthocyanidin ^a	14.5 (54.1)	−41.6 (−28.1)	
flavanone	187.6 (201.7)		−348.7 (−274.6)
flavanol	133.6 (153.4)	247.9 (252.8)	−160.2 (−112.8)
flavanol ^a	136.1 (149.6)	−20.27 (−15.0)	−253.4 (−190.3)
isoflavone	118.9 (132.2)		−249.3 (−181.9)
flavone	120.6 (137.9)		−258.3 (−202.7)
flavan-3-ol	181.3 (189.2)	156.8 (181.0)	
flavan-3-ol ^a	178.9 (189.9)	223.7 (207.4)	

^aDeprotonated structures.

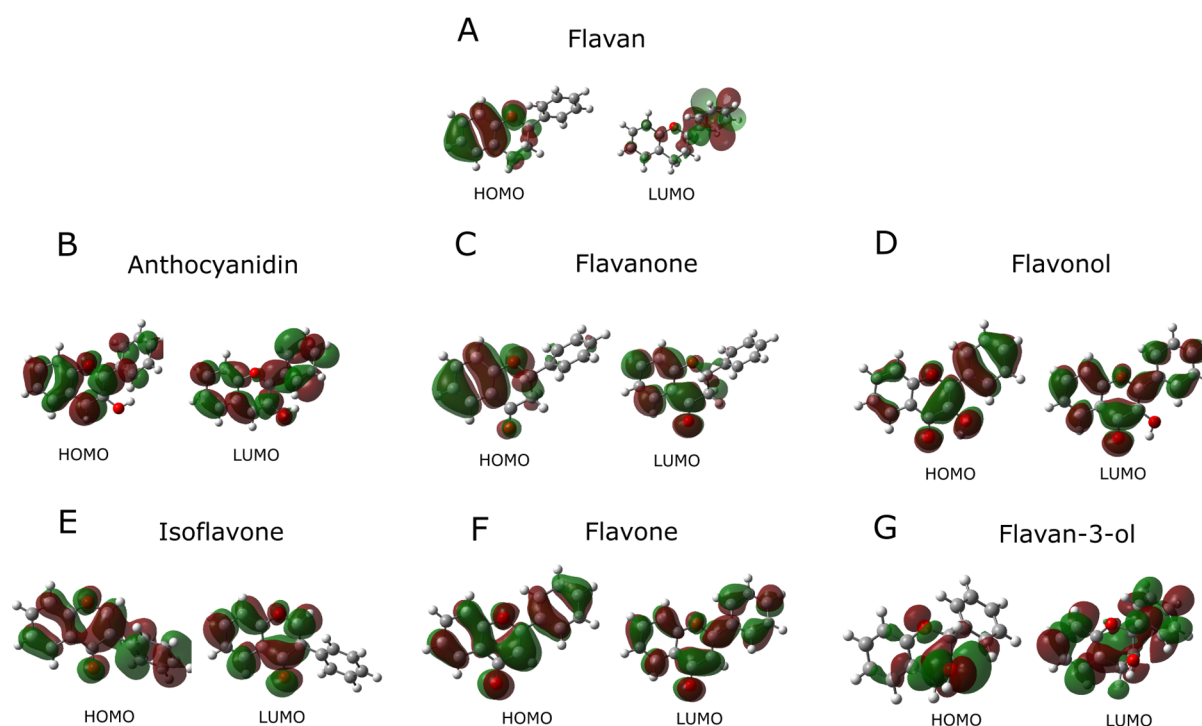


Figure 6. Frontier molecular orbitals obtained using CAM-B3LYP/def2TZV calculations. Negative regions are represented in red color and positive in green. (A) Flavan, (B) anthocyanidin, (C) flavanone, (D) flavonol, (E) isoflavone, (F) flavone, and (G) flavan-3-ol.

Table 5. ΔE_{L-H} ($E_{LUMO} - E_{HOMO}$) Values (eV), IP ($\text{kcal}\cdot\text{mol}^{-1}$), EA ($\text{kcal}\cdot\text{mol}^{-1}$), and NBO Atomic Charges of the Benzo- γ -pyran (Chromone) and Ring B for the Neutral and Deprotonated (d) Structures of the Analyzed Flavonoids

	flavan	anthocyanidin	anthocyanidin ^d	flavanone	flavonol	flavonol ^d	isoflavone	flavone	flavan-3-ol	flavan-3-ol ^d
CAM-B3LYP/def2TZV										
HOMO	-7.55	-11.66	-6.68	-8.14	-7.64	-2.30	-7.88	-8.13	-7.90	-1.94
ΔE_{L-H}	8.46	5.50	4.47	7.35	6.34	4.84	7.04	7.09	8.62	6.19
IP	174.00	268.94	153.94	188.19	176.18	52.95	181.71	187.43	182.13	44.68
EA	21.27	142.20	50.73	18.10	30.11	-58.97	19.28	24.08	-16.77	-98.68
NBO _{chromone}	0.36	0.75	-0.83	-0.03	-0.06	-0.86	-0.04	-0.05	-0.02	-0.99
NBO _{ring B}	0.01	0.25	-0.17	0.03	0.06	-0.14	0.04	0.05	0.02	-0.05
HSEh1PBE/cc-pVTZ										
HOMO	-5.81	-10.12	-5.14	-6.40	-5.87	-0.64	-6.20	-8.39	-6.08	0.17
ΔE_{L-H}	5.32	2.83	1.87	4.40	3.50	2.20	4.19	6.17	5.51	2.8
IP	134.12	233.42	118.41	147.27	135.35	17.79	142.83	147.43	140.17	-4.02
EA	11.30	168.03	74.92	45.79	54.56	-35.90	46.14	51.29	13.07	-68.57
NBO _{chromone}	0.048	0.72	-0.07	0.03	-0.02	-0.75	-0.14	-0.04	-0.06	-0.74
NBO _{ring B}	-0.05	0.28	0.15	-0.02	0.02	-0.25	0.00	0.04	-0.02	-0.34

flavonoids using B3LYP/6-31G(d) calculations and they found ΔE_{L-H} ranging from 1.72 to 5.88 eV.³⁶ Payán-Gómez et al. investigated rutin using DFT with M05-2X/6-31+G(d,p) calculations, and they showed a HOMO–LUMO transition of 4.28 eV, which is in the region of ultraviolet electronic transition.⁵⁶ However, it is noteworthy that a transition of 3.88 eV was also previously found for this molecule.⁵⁶ According to Payán-Gómez et al., the HOMO–LUMO transitions were characterized by charge transfers along with the entire flavonoid structure.⁵⁶ Table 5 shows the energies (eV) of the HOMO–LUMO molecular orbitals, the energy values of ΔE_{L-H} , IP ($\text{kcal}\cdot\text{mol}^{-1}$), and EA ($\text{kcal}\cdot\text{mol}^{-1}$); the charges of ring B and benzo- γ -pyran were obtained using CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ calculations.

The energies of the frontier molecular orbitals (HOMO–LUMO) are associated with electron donation and acceptance

through ionization potential and electron affinity, respectively.^{57,58} Although anthocyanidin has a +1 charge, due to the pyrylium ion, the EA value showed to be high when compared with other molecules, showing the known values previously reported for this molecule. Anthocyanidin also showed aromaticity in ring C, but the low values of BDE and PA suggest that the high antioxidant activity is explained by the hydroxyl group. This result is consistent with the sequential proton loss electron transfer mechanism (SPLET).^{25–27} It is important to note that the values with HSEh1PBE/cc-pVTZ are underestimated concerning the CAM-B3LYP/def2TZV protocol.⁵⁹

According to Matteini et al., in the rutin structure, the HOMO–LUMO transitions are characterized by charge transfers in ring B showing a ΔE_{L-H} value of 7.78 eV.³⁸ Ninh The et al. studied isoflavones using B3LYP/6-311(d) and

found a ΔE_{L-H} between 4 and 5 eV.⁶⁰ We found a similar ΔE_{L-H} value for isoflavone using the HSEh1PBE/cc-pVTZ protocol.⁵¹ Regarding the ΔE_{L-H} values obtained using the CAM-B3LYP/def2TZV calculations, the investigated flavonoid structures showed values consistent with those obtained in previous studies.³⁸ Table 4 shows the atomic charges of benzo- γ -pyran and ring B for the investigated structures. Using the CAM-B3LYP/def2TZV calculations, we demonstrated that the charge is shifted from benzo- γ -pyran to ring B (Table 4). The flavan, protonated anthocyanidin, and flavanone structures are consistent with the results obtained using the HSEh1PBE/cc-pVTZ calculations.³ The long-range functional CAM-B3LYP/def2TZV showed different results for the charges when compared with the functional hybrid HSEh1PBE/cc-pVDZ.⁶¹ We noted that the results obtained with the CAM-B3LYP/def2TZV protocol are more accurate due to the additional polarization functions. Table 5 shows bond dissociation energy values and proton affinity calculated at 298.15 K and 1 atm. The calculations were carried out using CAM-B3LYP/def2TZV.

The BDE of O–H bonds is a key concept to analyze the antioxidant activity, while PA is a conceptual parameter to evaluate the deprotonation. It is expected that weaker O–H bonds favor the reaction to inactivate the free radical, as this low value of BDE favors the transfer of hydrogen from the O3H group to the free radical.^{57,58,62,63}

$$\text{BDE} = E(\text{FlavO}) + E(\text{H}) - E(\text{FlavOH}) \quad (6)$$

$$\text{PA} = H(\text{FlavO}^-) + H(\text{H}^+) - H(\text{FlavOH}) \quad (7)$$

Table 6 presents the BDE and PA values calculated at the CAM-B3LYP/def2TZV level. Our BDE values showed to be

Table 6. Bond Dissociation Energy Values (BDE, kcal·mol⁻¹) and Proton Affinity (PA, kcal·mol⁻¹) of Anthocyanidin, Flavonol, and Flavan-3-ol

	anthocyanidin	flavonol	flavan-3-ol
BDE	63.32	91.36	110.55
PA	239.16	337.77	394.32

larger than the values from previous DFT studies of about 85.2 kcal·mol⁻¹ for a flavonol analogue, and 80.9 kcal·mol⁻¹ for quercetin.^{64,65} The present study investigated flavonoid structures with fewer hydroxyls than these cited studies, and it could explain the differences between the obtained BDE values. Anthocyanidin has the lowest BDE value, which is in agreement with the highest antioxidant activity found for this molecule when compared with the flavonol and flavan-3-ol.⁴⁶

It is well known that SPLET is determined by proton affinity.^{46,66} Our PA values of the flavonoid structures corroborate those investigated by Ninh The et al.⁵¹ The low BDE and PA values of anthocyanidin demonstrate that its

structure possesses a strong antioxidant activity that could be explained by the dissociation of the hydrogen bond.

The values presented in Table 7 are smaller (absolute value) than those obtained at B3LYP/6-311++G**.³ The values of hydrogens of O3H and the oxygen of ketone (OC4) of the flavonol structure showed differences due to the bond formation. Similar results were obtained for the oxygen of the O3H and hydrogen (HC6') of the flavonol structure. The OC4 and O3H' hydrogen charges also suggest the formation of a hydrogen bond in the flavonol structure.

3. CONCLUSIONS

In the present study, we revised conformational, electronic, and structural properties related to the antioxidant activity of seven flavonoid structures and presented novel information regarding their magnetic properties. The conformational analyzes of anthocyanidin, flavonol, and flavan-3-ol indicate the stabilization of the molecules with increased planarity due to the formation of the H-bond interaction between O1 and HC6'. The analysis of flavonol also indicates the existence of a hydrogen bond between the hydroxyl group (O3H) and the oxygen of ketone that stabilizes its molecular structure, improving the planar conformation. Comparing the aromaticity results of flavonol, flavone, and anthocyanidin, we found that the planarity and the interaction between O3H and HC6' contributed to the aromaticity of ring C. Similar to the expected behavior of the substituent effect, the analysis of the aromaticity of ring C showed that the hydroxyl group improves the aromaticity and the ketone group decreases the aromaticity.⁶⁷ The importance of the ketone group is also seen in the oxygen NMR chemical shift of flavonol, as the δ in O2 shows that it is more strongly bonded than O1. NMR also indicates an H bond between O3H and the oxygen of ketone.

Regarding anthocyanidin, our results demonstrated that its lower PA and BDE values, when compared with flavonol and flavan-3-ol, indicate that it has the highest antioxidant activity. Furthermore, our results indicate that the anthocyanidin aromaticity is not related to electron transfer.

Finally, the NBO atomic charge values are displaced from ring B to benzo- γ -pyran in flavan, neutral anthocyanidin, flavanone, and neutral flavan-3-ol. The values obtained for the charges are related to the interactions between O3H and the oxygen of ketone and between O3H and HC6'.

4. METHODOLOGY

4.1. Flavonoid Structures. Initially, the flavonoid structures were obtained in the PubChem database in the MOL2 format.⁶⁸ The following flavonoid structures from the natural origin were selected due to their reported antioxidant activities: flavan (CID 94156, C₁₅H₁₄O, Figure 2A), anthocyanidin (CID 414159, (C₁₅H₁₁O₂)⁺; Figure 2B), flavanone (CID 10251, C₁₅H₁₂O₂; Figure 2C), flavonol (CID 11349, C₁₅H₁₀O₃; Figure 2D), isoflavone (CID 72304,

Table 7. Calculated Atomic Charge (NBO) of Oxygen (O3H), Hydrogen (HC6'), Hydrogen (O3H), and Carbon-Bound Oxygen (OC4) of the Flavonoids Using the CAM-B3LYP/def2TZV Calculations

atoms	flavan	anthocyanidin	flavanone	flavonol	isoflavone	flavone	flavan-3-ol
O3H(O)		-0.633		-0.655			-0.696
HC6'		0.261	0.213	0.256	0.234	0.221	0.209
O3H(H)		0.503		0.505			0.463
OC4			-0.512	-0.583	-0.541	-0.542	

C₁₅H₁₀O₂; Figure 2E), flavone (CID 10680, C₁₅H₁₀O₂; Figure 2F), and flavan-3-ol (CID 3707243, C₁₅H₁₄O₂; Figure 2G).

4.2. Electronic and Structural Analysis. The self-consistent field calculations of flavonoid structures were performed using DFT.⁶⁹ DFT has been a useful method to study the structural, electronic, and magnetic molecular properties of compounds.^{16,37,58,70–72} The CAM-B3LYP functional was used with the split valence def2TZV basis set of Ahlrichs et al. (CAM-B3LYP/def2TZV).^{73–76} We selected the CAM-B3LYP functional due to its well-established and useful application to investigate chemical structures.⁷⁷ The correlation consistent cc-pVDZ basis set was also applied to study the aromaticity and the oxidation of the aromatic ring in the flavonoid structures.⁷⁸ We used CAM-B3LYP/def2TZV to perform the conformational, electronic, and magnetic analyses. We performed a relaxed scanning for all structural optimizations of the flavonoids. The balanced polarized triple-zeta def2TZV basis set has presented satisfactory structural results in the literature.⁷⁹ Recently, we have used CAM-B3LYP and def2TZV basis sets for structural analyses and the results showed to agree with the experimental data.^{71,80} These computational methods are efficient and less time-consuming to analyze conformations. Besides, we compared the conformational analyses obtained with CAM-B3LYP/def2TZV calculations with the hybrid functional HSEh1PBE/cc-pVDZ. The HSEh1PBE level of density functional theory has been widely used to study the frontier molecular orbitals and NMR spectra.⁸¹

The calculations were carried out in the Gaussian09 package^{82,83} in the gas phase (vacuum). Conformational analyses were performed varying the dihedral angles τ present in the atoms O1–C2–C1'–C6' with steps containing 10° totalizing 18 minimization cycles using CAM-B3LYP/def2TZV. Frontier molecular orbitals HOMO and LUMO were analyzed using the CAM-B3LYP/def2TZV and HSEh1PBE/cc-pVDZ levels. Atoms in molecules (AIM) analysis was carried out using the AIMAll package.⁸⁴

4.3. Magnetic Properties Analysis. The investigation of the aromaticity was carried out through the nucleus-independent chemical shift (NICS) method.³⁵ This method consists of the calculation of ¹H NMR, employing ghost atoms defined preferably in the center of the molecule ring (NICS 0) or at a point outside the plane and perpendicular to the molecule at 1 Å (NICS 1). The NICS values were calculated with the Gauge-independent atomic orbital (GIAO),⁸⁵ using the cc-pVDZ basis set with the hybrid functional HSEh1PBE (HSEh1PBE/cc-pVDZ) and compared with the results obtained from CAM-B3LYP/def2TZV calculations.

The NICS method has been extensively tested on planar and cyclic molecules, but aromaticity can also occur on nonplanar molecules.^{86,87} An NICS_{isotropic}(1) value more negative than NICS_{isotropic}(0) is expected in planar aromatic rings. All calculated NICS values are on the z-axis of the ring. The NICS_z procedure has been used to investigate the aromaticity of cyclic structures.⁸⁸ We applied the NICS(0) plus NICS(1) procedure to explore the aromaticity of the rings of the seven selected flavonoids.

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Notes

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