J Mol Model (2012) 18:4073–4080
DOI 10.1007/s00894-012-1397-0

ORIGINAL PAPER

The basic antioxidant structure for flavonoid derivatives

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Received: 19 November 2011 / Accepted: 29 February 2012 / Published online: 14 April 2012

Abstract An antioxidant structure–activity study is carried out in this work with ten flavonoid compounds using quantum chemistry calculations with the functional of density theory method. According to the geometry obtained by using the B3LYP/6-31G(d) method, the HOMO, ionization potential, stabilization energies, and spin density distribution showed that the flavonol is the more antioxidant nucleus. The spin density contribution is determinant for the stability of the free radical. The number of resonance structures is related to the π-type electron system. 3-hydroxyflavone is the basic antioxidant structure for the simplified flavonoids studied here. The electron abstraction is more favored in the molecules where ether group and 3-hydroxyl are present, nonetheless 2,3-double bond and carbonyl moiety are facultative.

Keywords Antioxidant · Basic structure · DFT · Electron transfer · Flavonoids · Stabilization energies

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Introduction

Flavonoids are universally present found in fruits and vegetables as groups of polyphenolic compounds. The interest in flavonoid compounds have increased lately due to their broad pharmacological effect, explained by their inhibition of certain enzymes and their antioxidant activity [1].

The protective effects of flavonoid compounds are recognized as potential drug candidates to be used in the treatment of several diseases such as cancer, atherosclerosis, cardiovascular and coronary heart diseases, neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and other age-related diseases. Therefore, all activities are generally known to be associated with anti-oxidative or free radical scavenging properties of flavonoid derivatives [2–8].

The basic flavonoid structure is one flavan nucleus and the various classes of flavonoids differ in the level of oxidation and pattern of substitution. Among many classes of flavonoids are flavones, flavanones, isoflavones, flavonols, flavanonols, flavan-3-ols, and anthocyanidins. Other flavonoid classes include biflavones, chalcones, aurones, and coumarins [9].

Several studies have been shown that antioxidant activity of flavonoids derivatives is related to its hydroxyl (OH) groups which can scavenge free radicals produced in vivo [10–19]. In those works, the antioxidant activity of flavonoids depends on some structural requirements such as the number of phenolic hydroxyl groups; the location of the hydroxyl groups; the 2,3-double bond in conjugation with a 4-oxo function in phenyl ring; the presence of additional OH groups at the 3- and 5-positions enhances the antioxidant activity; the reactivity of the 3-OH-group on 3,5,7-trihydroxyflavone is enhanced by the electron donating effect of the 5- and 7- OH groups; the presence of the *o*-catechol group (3′,4′-OH) is determinant for a high antioxidant

capacity; glycosylated flavonoids showed less antioxidant capacity than their free aglycone; and glycosylation of C-3 reduces the reactivity if the sugar can form two intramolecular H-bonds [10–27].

In fact, the scavenging ability of flavonoid compounds is related to its standard one-electron reduction potential, a measure of the reactivity of an antioxidant as a hydrogen or electron donor [28] and theoretical studies of structure-activity relationship (SAR) are generally determined using bond dissociation energies of hydroxyl group (BDE_{OH}). However, the number and positions of hydroxyl groups are more determinant for antioxidant activity of phenol derivatives and BDE_{OH} studies can show only the local or neighboring influence, underestimating additional important information concerning the contribution of the basic nucleus of molecules for antioxidant activity. As a consequence, ten simplified flavonoid derivatives were used in this work to understand the characteristics that are necessary for better antioxidant activity. Electronic structure calculations were performed here using the density functional theory (DFT) method in order to obtain the ionization potential (IP), highest occupied molecular orbital (HOMO), stabilization energies (ΔEiso), and spin density distribution [29]. The main goal of the present study is to determine the basic structure responsible for antioxidant activity of simplified flavonoids as a useful method to investigate the electron transfer mechanism for the antioxidant property of the molecules studied in this work.

Methods

In this work, the geometry optimization of the flavonoid derivatives have been carried out using density functional theory (DFT) [30], because of its excellent compromise between computational time and description of electronic correlation. The calculations were performed with the Gaussian 03 molecular package [31] and prior to any DFT calculations all structures were submitted to PM3 [32] geometry conformational search. After the PM3 initial optimizations, the structures were reoptimized with the B3LYP hybrid density functional [33, 34] by using a 6-31G* basis sets [35, 36]. The B3LYP optimized structures were confirmed to be real minima by frequency calculation (no imaginary frequency) and to obtain zero-point vibrational energy (ZPVE) corrections. For the species having more conformers, all conformers were investigated. The conformer with the lowest electronic energy was used in this work.

Since our main interest is to understand the role played by the differences between structural and electronic features of these molecules, we adopted a systematic study of simplified structurally related flavonoids, but not including hydroxyl groups. In fact, all previous theoretical studies showed that the number and position of hydroxyl (OH) groups or the glycosilation (sugar) of the aglycone nucleus were determinant for antioxidant activity, despite the contribution of the basic structure of the compounds studied having not been considered [11–28]. In order to achieve this aim, we calculated the following properties: (i) highest occupied molecular orbital (HOMO); (ii) lowest unoccupied molecular orbital (LUMO); (iii) ionization potential (IP); (iv) stabilization energies (ΔEiso); (v) spin density, as described by Queiroz et al. [29].

The IP was calculated as the energy difference between a neutral molecule and the respective cation free radical (Eq. 1).

$$IP = E\mathrm{ArOH}^{\bullet+} - E\mathrm{ArOH} \quad (1)$$

The radical stability was determined by the calculation of stabilization energies (ΔEiso), as shown in Eq. 2 for the electron transfer, where the flavonoid derivatives are represented by ArOH and the phenol molecule is represented by PhOH.

$$\Delta\mathrm{Eiso} = [\mathrm{ArOH}^{\bullet+} + \mathrm{PhOH}] - [\mathrm{ArOH} + \mathrm{PhOH}^{\bullet+}] \quad (2)$$

Results and discussion

The antioxidant activity of ten simplified flavonoid derivatives (see Fig. 1) was theoretically measured. The selection of these compounds was based on their minimal chemical structure characteristics and all compounds are associated with flavan nucleus.

The frontier orbital energies HOMO and LUMO, are important parameters of the molecular electronic structure. The molecule that has the lower HOMO energy has the

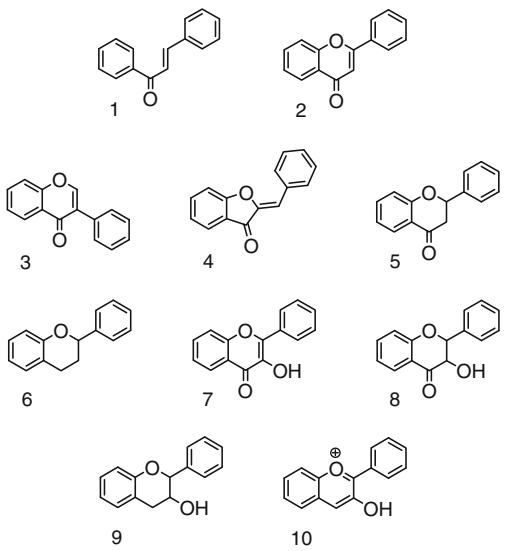

Fig. 1 Structure of the simplified flavonoid derivatives studied

weakest donating electron ability; otherwise, a higher HOMO energy implies that the molecule is a good electron donor [23, 29, 37, 38]. The HOMOs of the flavonoid molecules studied here indicate qualitatively the scavenging free radical mechanism by electron transfer. The calculated HOMO values for the flavonoid molecules studied are shown in Table 1.

The calculated values of HOMO in this work are used for classification of the studied flavonoids into four different groups with relation to their nucleophilic capacity. This energetic parameter is important for evaluating the oxidation potential of these compounds. These four different groups were classified using the lowest standard deviation (SD) values among the calculated HOMO values.

According to the HOMO values obtained in our DFT calculations, we split the flavonoid molecules studied in four groups, i.e., in group **I** were included flavana **6** and flavonol **7** with HOMO values of −5.75 and −5.79, respectively. Aurone **4**, flavan-3-ol **9**, and isoflavone **3** belong to group **II** and their HOMO values are −5.96, -6.03, and −6.09 eV, respectively. Group **III** includes flavanone **5**, flavanolol **8**, flavone **2**, and chalcone **1**, with HOMO values of −6.33, -6.34, -6.35, -6.56 eV, respectively. Antocianin **10**, which has a HOMO value of −8.58 eV, is located in group **IV**.

In agreement with our calculated HOMO values, the more nucleophilic molecules were flavana and flavonol, respectively.

In addition, the ionization potential (IP) represents the facility of an electron donation and the electron abstraction is the first antioxidant mechanism, the molecules with the lowest IP are the more active ones. The calculated IP values for the flavonoid molecules studied are shown in Table 1 as well.

Through our calculated IP values, we also classified the studied flavonoids into four different groups with relation to their electron donation capacity. Likewise, these four groups were classified using the lowest SD values among the calculated IP values.

Table 1 Theoretical properties obtained for the simplified flavonoid derivatives studied

Derivatives	HOMO (eV)	LUMO (eV)	IP (kcal/mol)	ΔEiso (kcal/mol)
1	−6.31	−2.09	183.32	−1.38
2	−6.35	−1.79	183.34	−1.36
3	−6.09	−1.54	177.53	−7.16
4	−5.96	−2.30	173.84	−10.86
5	−6.33	−1.53	184.19	−0.50
6	−5.75	−0.06	173.89	−10.80
7	−5.79	−1.95	171.36	−13.34
8	−6.34	−1.69	183.79	−0.90
9	−6.03	−0.15	178.43	−6.27
10	−8.58	−6.86	265.11	80.41

Group **I** included the molecules with IP values varying from 171.36 to 173.89 kcal mol^{-1} (flavonol **7**, aurone **4**, and flavana **6**). Group **II** includes the molecules with IP values of 177.53 and 178.43 kcal mol^{-1} (isoflavone **3** and flavan-3-ol **9**, respectively). Chalcone **1**, flavone **2**, flavanolol **8**, and flavanone **5** are included in group **III**, where the IP values vary from 183.32 to 184.19 kcal mol^{-1}. The last group is **IV**, where antocianin **10** has the highest IP value, i.e., 265.11 kcal mol^{-1}.

Our results, according to the calculated IP values, showed that the more nucleophilic molecules were flavonol followed by flavana and aurone. Consequently, these compounds have more electron-donating ability than the other compounds studied.

In general, the combination of a hydroxyl at the 3-position of flavonoid nucleus, the carbonyl, and double bond C_2=C_3 are essential for the molecule to present higher HOMO and lower IP values. The five ring member has equal contribution for HOMO and IP than the six ring member, such as aurone and flavana or flavonol. Therefore, the more effective structures for the simplified flavonoid studied can be separated using HOMO and mainly IP values, according to the inclusion of: chromen opened < translocation of B ring < ether group < five members ring < double bond C_2=C_3< carbonyl moiety <3-hydroxyl.

Furthermore, we have used the stabilization energies (ΔEiso) for the simplified flavonoids. The obtained ΔEiso values were related with phenol and are also shown in Table 1. According to these values it was possible to establish the following relative stability of the involved groups (showed above) for the basic antioxidant structure of flavonoid derivatives.

Previously, we have used the stabilization energies to study the predictions of antioxidant ability for trapping of free radicals or scavenging effects of phenolic derivatives [29]. That study [29] provided further evidence for the importance of the structural differences in the stabilization of radical species by electron abstraction. Our results confirmed a clear classification among different subclasses.

The calculated ΔEiso values supported the classification of the four groups related to the electron donation capacity. Therefore, in group **I** we have included flavonol **7**, flavana **6**, and aurone **4**, with ΔEiso values varying from −13.34 to −10.86 kcal mol^{-1}. Isoflavone **3** and flavan-3-ol **9** belong to group **II** and their ΔEiso values are −7.16 and −6.16 kcal mol^{-1}, respectively. Group **III** includes flavone **2**, chalcone **1**, flavanolol **8**, and flavanone **5** with ΔEiso values varying from −1.36 to −0.50 kcal mol^{-1}, respectively. The same behavior was observed for HOMO and IP values, where antocianin **10** presents the highest ΔEiso value of 80.41 kcal mol^{-1}, showing it is positioned in group **IV** of our

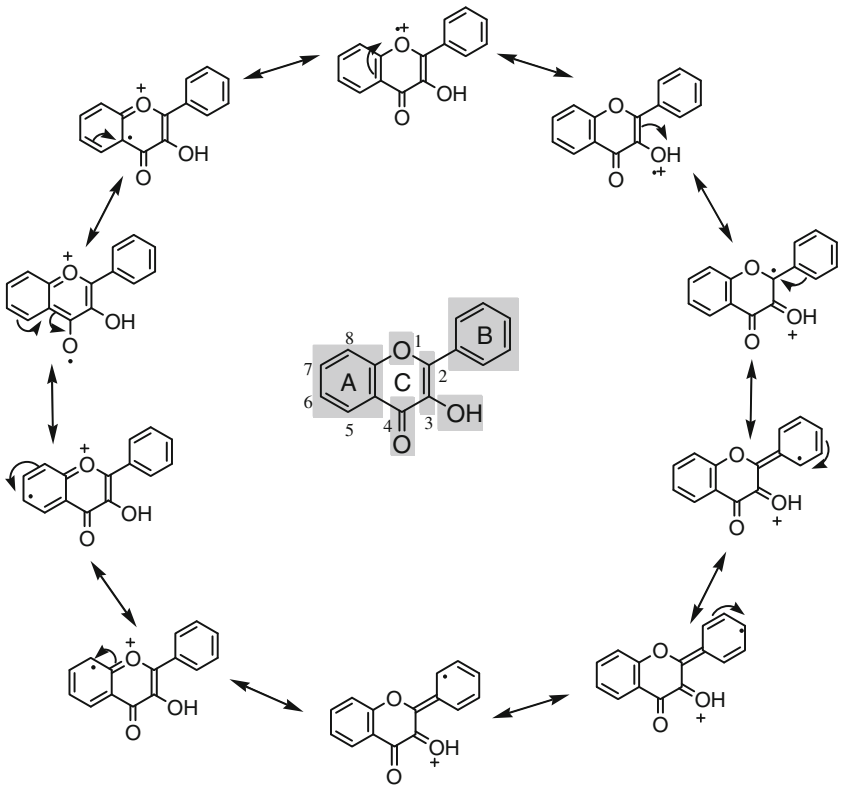

Fig. 2 The resonance structures of the cation free-radical in the simplified flavonoids studied

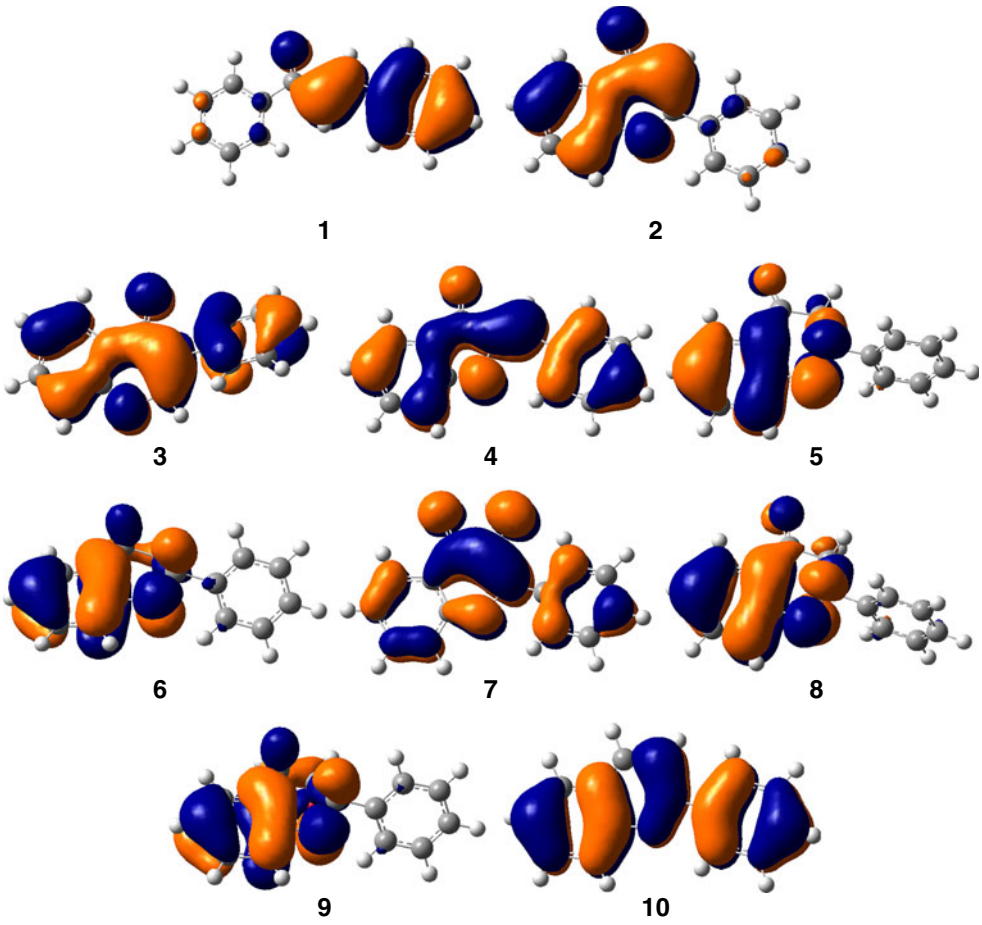

Fig. 3 HOMO of the simplified flavonoid derivatives studied

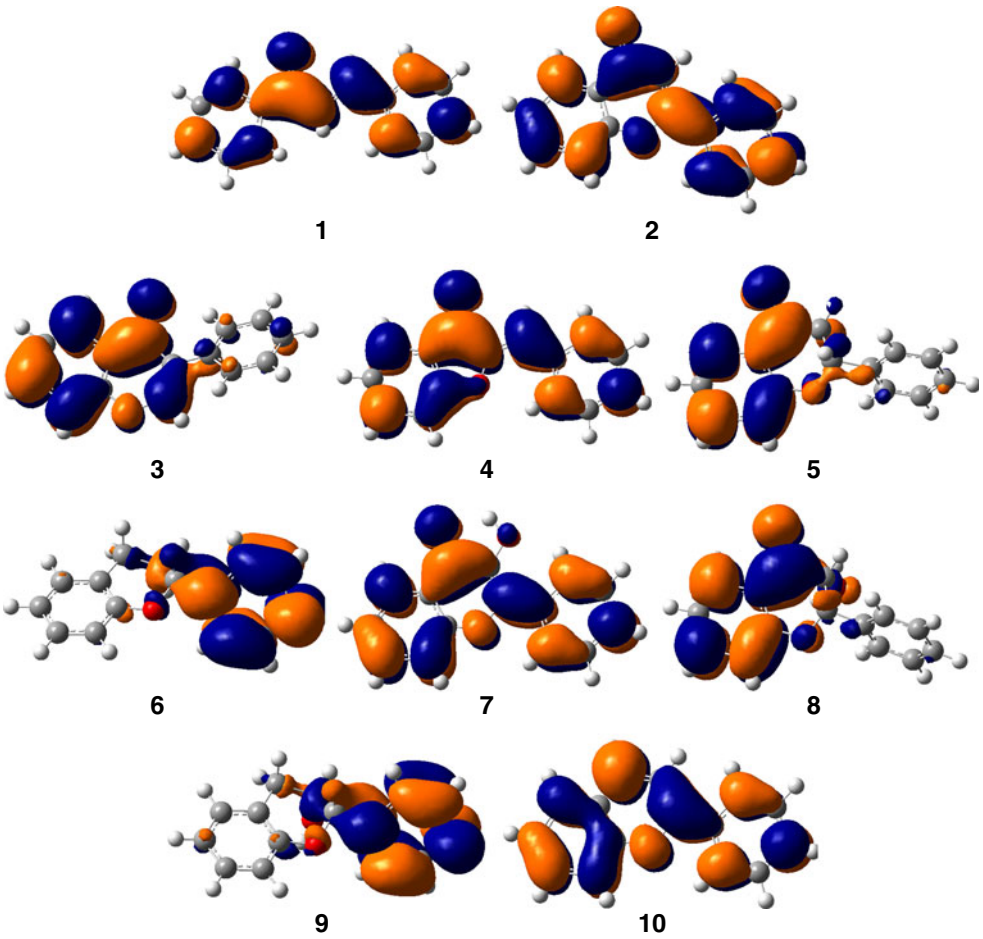

Fig. 4 LUMO of the simplified flavonoid derivatives studied

category. In fact, the HOMO and IP values have a good agreement with the ΔEiso values.

Additionally, ether group, double bond C_2=C_3, carbonyl moiety, and 3-hydroxyl are important, since they are groups with π-type electrons that can be donated in order to stabilize the cation free radical. Therefore, the alkenes and phenyl ring may stabilize the radical formed during oxidation by the increase of the conjugation via resonance effect, contributing to the decrease of ΔEiso. These results showed that when we have a hydroxyl group in 3-position a higher number of conjugation structures is possible. Consequently, the 3-hydroxy-flavan or 3-hydroxy-flavone showed more contribution in the stabilization (after the electron abstraction) than other derivatives.

These results showed that antioxidant activity can be determined mainly by the stability of the cation free-radical generated after electron abstraction. In fact, the cation free-radical of 3-hydroxy-flavone is formed with less energy than the other compounds studied, because of the high electron transfer found in the electron abstraction on the oxygen atoms. This abstraction is facilitated by the existence of the π-delocalized system between B- and A-rings (see Fig. 2). Consequently, an electron abstraction causes an increase of the number of resonance structures in the molecules with ether group, double bond C_2=C_3, carbonyl moiety, and 3-hydroxyl. Only flavonol presents these characteristics, therefore its resonance structure is more important to the antioxidant activity than resonance structures of the other flavonoids studied.

The results presented here have a direct influence in the resonance effect between pyrane C-ring and the A- and B-rings as shown in Fig. 2. The number of resonance structures can be related to nucleophilicity and the more nucleophilic positions are determined by prevalent contributions of HOMO of the ether group, double bond C_2=C_3, and carbonyl moiety for compounds **4** and **7** (see Fig. 3). Additional contributions were observed by the A- and B-rings. Nonetheless, other flavonoid compounds showed they do not have coplanar orbital among chromane and B-ring or absent participation of chromane or B-ring (see Fig. 3), while antocianin **10** does not have interactions between these three rings. On the contrary, the LUMO contributions have indicated that antocianin **10** is the most electrophilic compound, mainly at position 4, as shown in Fig. 4.

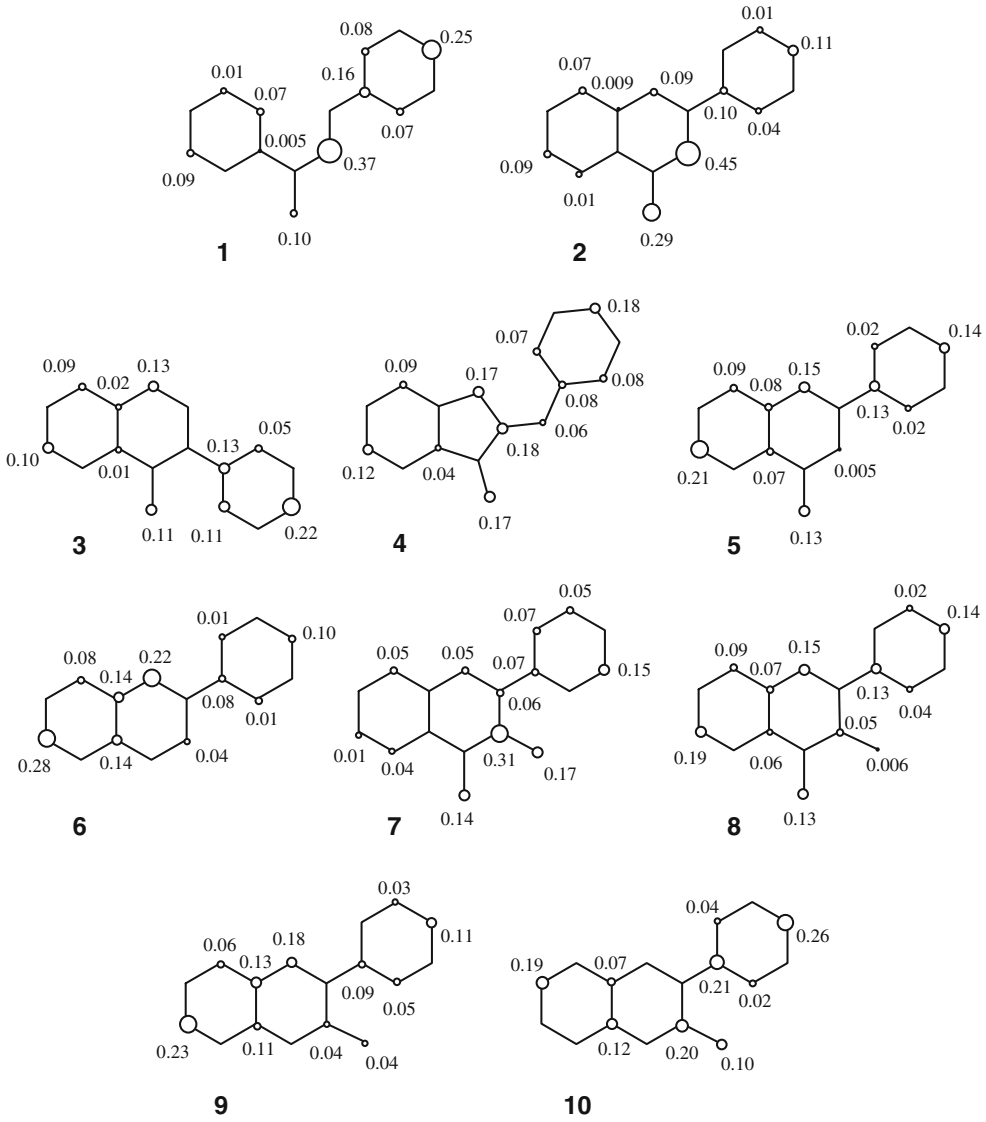

Fig. 5 Spin density contribution of the cation free radical of the simplified flavonoid derivatives studied

The resonance structures of cation free-radicals by electron abstraction can be observed by spin densities distribution for the simplified flavonoids in Fig. 5. These values were obtained with the hybrid functional B3LYP. The spin density distribution has more significant contribution for the hydroxyl moiety, double bond C_2=C_3, oxygen of ether group, oxygen of carbonyl moiety, and A- and B-rings.

In fact, our theoretical results have pointed out mainly that all molecular compounds that present the π-type electron system of the type 3-hydroxy-flavone are the ones responsible for the antioxidant structure of simplified flavonoids. These results are supported by experimental results obtained for DPPH and ABTS radicals and one-electron reduction potentials [18, 19, 23, 26, 39–43]. It is possible that antocyanin acts through a different antioxidant mechanism due to the HOMO, IP and ΔEiso values for its simplified structure compared with other flavonoid classes. In addition, this methodology is a useful and economical method to investigate the antioxidant mechanism of molecules.

Conclusions

Through our calculations, we can state that there is a good correlation between theoretical and experimental methods for the nine simplified flavonoids, except for the antocyanin. The flavonol class is the more antioxidant nucleus, and their unpaired electron cation free-radicals are mainly distributed on several resonance structures. The spin density contribution is determinant for the stability of the free-radical. The number of resonance structures is related to the π-type electron system. 3-hydroxyflavone is the basic antioxidant structure for the simplified flavonoids studied in this work. The electron abstraction is more favored in the molecules

where ether group and 3-hydroxyl are present, nonetheless 2,3-double bond and carbonyl moiety are facultative. Furthermore, our calculations showed that HOMO, IP and ΔEiso are electronic properties responsible for the excellent antioxidant activity of the flavonol class, which is one of the most antioxidant flavonoids.

Acknowledgments The authors would like to thank Conselho Nacional de Pesquisa (CNPq) and Pro-Reitoria de Pesquisa da Universidade Federal do Pará (PROPESP/UFPA) for the financial support.

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