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Short communication

A theoretical study on cellular antioxidant activity of selected flavonoids

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ABSTRACT

The antioxidant capacities of the selected flavonoids quercetin, luteolin and taxifolin have been investigated at density functional level of theory with the aim of verifying the cellular antioxidant activity (CAA) values representative of experimental findings. The selected flavonoids were believed to act through the H-atom transfer mechanism. Their potentiality of hydrogen abstraction was evaluated by computing the O—H bond dissociation enthalpy (BDE) in gas-phase and in dimethylsulfoxide solution. Results indicate that the order of antioxidant efficacies calculated in this work is in agreement with that reported by experimental results of CAA. Time-dependent density functional theory (TDDFT) calculations were also performed both in gas-phase and in dimethylsulfoxide to reproduce the electronic UV–vis spectra of the selected flavonoids.

1. Introduction

Flavonoids, as constituents of plant foods, are a class of widely distributed phytochemicals with antioxidant and biological activity. They have structures consisting of two aromatic rings linked by three carbons in an oxygenated heterocycle supplements. Quercetin, a member of flavonols, luteolin, belonging to flavone, and taxifolin, as a flavanone, have been implicated in the reduction of cancer risk. In the Zutphen Elderly Study, flavonoid intake from fruits and vegetables was inversely associated with all-cause cancer risk and cancer of the alimentary and respiratory tract [1,2]. These properties are correlated to their antioxidant activity or, in other words, to their capabilities to scavenge free radicals and to synergistic effects with physiological antioxidants [3–6]. Free radicals can damage biomolecules (proteins, membrane lipids, and nucleic acids); thus, they are involved in several diseases and aging itself [7–10]. It was proposed by Bors et al. [11] that three structural features are important for antioxidant and radical-scavenging activity by flavonoids: (1) an o-dihydroxyl group in the B-ring; (2) a 2,3-double bond combined with a 4-oxo group in the C-ring; and (3) a 3-hydroxyl group in the C-ring. The structure–activity relationships for flavonoids have been investigated in many chemical antioxidant activity assays, and the required structural features for high activity are often those proposed by Bors et al. [11], but not always.

The cellular antioxidant activity (CAA) assay, which was recently developed to measure the inhibition of peroxyl radical-induced oxidation of dichlorofluorescin by antioxidants in cell culture, is more biologically relevant and representative than the chemical antioxidant activity assays and is greatly important for further investigation on the potential bioactivity of foods, antioxidants, and dietary supplements [12–14]. The potentialities of antioxidant activity are highly related to their capabilities to scavenge free radical. The harmful action of free radicals can be alleviated by antioxidants through two main working mechanisms as the H-atom transfer (HAT) and single-electron transfer (SET) that are reported in the literature [7–9] and are widely analyzed. In HAT, a free radical R^{*} removes a hydrogen atom from the antioxidant (ArOH), gives rise to the ArO^{*}. The SET mechanism, according to which the antioxidant gives an electron to the free radical, produces the $ArOH^{*+}$. The stability of radicals from both reactions (ArO^{*} and $ArOH^{*+}$) prevents or slows down the chain radical reactions. In this situation, the antioxidant activity evaluation can be based on the estimation of two parameters as OH bond dissociation enthalpy (BDE) and ionization potential (IP) values [7–11]. The selected flavonoids with the ortho-dihydroxyl group functionality in the B-ring are the most active in donating an H atom, which is attributed to high stability of radical species through H-bond formation. So, the BDE is more indicative of the anti-radical property of the three flavonoids.

The purpose of this work is to rationalize experimental findings carried out in the cellular antioxidant activity field of the selected flavonoids, by employing computational method. Viewing from a theoretical point, no calculations based on density functional level taking into account dimethylsulfoxide (DMSO) solvent effect are performed on the three molecules to our knowledge. The conformational and electronic features were investigated, the BDE values were computed and compared to experimental results of CAA [14].

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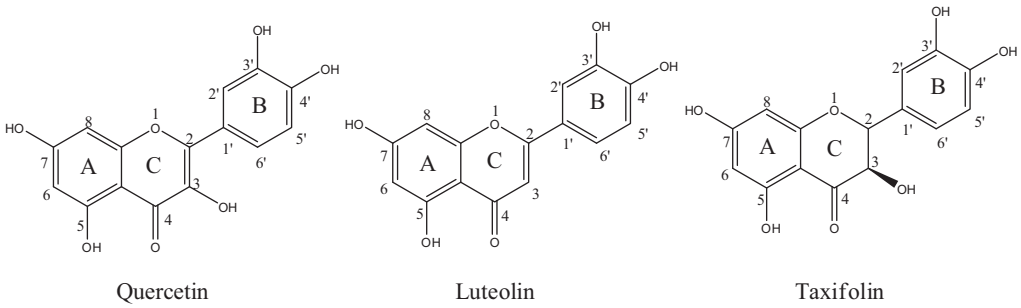

Fig. 1. Structures of the selected flavonoids quercetin, luteolin and taxifolin with atomic numbering.

We hope our data can help the chemists find some indications for exploring high-effective and innocuous antioxidant.

2. Computational methods

All calculations referred in this work were performed with the Gaussian03 software [15]. The geometries of neutral molecules and their radicals were optimized at the B3LYP [16,17] exchange-correlation functional level without constraints, employing the 6-311++G (d, p) basis set [18,19]. Vibrational frequency calculations were performed for both molecule and radicals at the same level of theory, to characterize all their conformations as minima or saddle points and to evaluate the zero-point energy corrections obtained from vibrational analysis which were included in all the relative energy values.

To provide a more complete spectroscopic characterization and for purposes of comparison, UV–vis spectra of the selected flavonoids were computed by using the time-dependent density functional theory (TDDFT) [20] based on the optimized structures at the same level of basis set. Thirty bands of electronic transition were selected and 2500 cm^{-1} of Gaussian band width was assumed. A line spectrum was directly obtained by the output file of Gaussian and a file generated by the Swizard was used to draw the spectrum by the Origin software.

Solvent effects were computed in the framework of the self-consistent reaction field polarizable continuum model (SCRF-CPCM) [21–23] using COSMO model (KLAMT) [24] set of solvation radii to build the cavity for the solute in its gas-phase equilibrium geometry. The dielectric constant of 46.7 was chosen to perform calculations in DMSO solution.

Natural bond orbital (NBO) analysis [25] was used to better characterize the electronic structures of the selected flavonoids. The O—H bond dissociation enthalpy (BDE) was computed at 298.15 K as the enthalpy difference for the hydrogen abstraction reaction:

$$ArOH \Rightarrow ArO^{*} + H^{*}$$

3. Results and discussion

3.1. BDE evaluation

Structures of the selected flavonoids are shown in Fig. 1, which are characterized by the same structures of rings A and B and just differ in ring C. Seen from the equilibrium geometries of the neutral molecules, a torsional $C_{2'}$—$C_{1'}$—C_2—C_3 angle in quercetin is found to be −179.9, while the same torsional dihedral is −160.8 in luteolin. These torsion values indicate that a quite planar disposition of rings B and C is present for both quercetin and luteolin. The 3′-OH and 4′-OH groups are weakly linked by a hydrogen bond whose lengths are 2.162 Å and 2.161 Å. π electrons are delocalized over all rings for these two flavonoids. The same torsional dihedral is found to be −97.2 in taxifolin that indicates rings B and C are almost mutually perpendicular. A hydrogen bond was also found between 3′-OH and 4′-OH groups, whose length is 2.157 Å. The electronic delocalization occurs independently on rings B and A, and there is no possibility of conjugation between rings B and C.

The abstraction of a hydrogen atom from each OH group present in the selected molecules gives rise to four radical species for luteolin, and five radical species for quercetin and taxifolin due to a 3-hydroxyl group substitution. The relative energies of these different radicals in DMSO solution are collected in Table 1. The most stable radical arising from quercetin is the 4′-ArO^{*}, obtained by abstraction of a hydrogen atom from the OH group linked to the C4′-position. The hydrogen bond in the B ring between the 3′-OH group and the 4′-O^{*} from which the hydrogen atom is removed contributes to the radical stability. Energy of the radical 3-ArO^{*} is very near to the absolute minimum (ΔE is 2.51 kcal/mol), followed by radical 3′-ArO^{*}, that is found at 6.13 kcal/mol. The other radical species lie at 11.67 (7-ArO^{*}) and 14.91 kcal/mol (5-ArO^{*}) above the absolute minimum (see Table 1).

In luteolin, the 4′-ArO^{*} that is still the most stable radical and the 3′-ArO^{*} are separated in energy by 4.81 kcal/mol. The 5-ArO^{*} and 7-ArO^{*} species are found at 16.83 and 11.92 kcal/mol, respectively, above the absolute minimum (see Table 1). Since the radicalization of 5-OH group involves the breaking of the hydrogen bond established with the 4-keto group, 5-ArO^{*} is the least stable radical species for both quercetin and luteolin. The order of stability for luteolin radicals is 4′-ArO^{*} > 3′-ArO^{*} > 7-ArO^{*} > 5-ArO^{*}, which is the same as those obtained for quercetin, and the most stable radicals arise mainly from radicalization on rings B and C.

In the taxifolin, the 3′-ArO^{*} is found to be very near in energy to the absolute minimum 4′-ArO^{*} (ΔE is 3.38 kcal/mol). The other species 5-ArO^{*} and 7-ArO^{*} are found at 15.29 and 24.93 kcal/mol, respectively, above the absolute minimum (see Table 1). The 5-ArO^{*} is more stable than the 7-ArO^{*} which can be attributed to the weaker H-bond interaction than that in quercetin and luteolin between 5-OH and C_4=O caused by the presence of the more flexible saturated C ring. A remarkably high relative energy is found for the radical 3-ArO^{*} (29.49 kcal/mol) above the absolute minimum due to the absence of delocalization.

Table 2 lists the BDE values in gas-phase and in DMSO solution with different OH substitutions calculated for the selected

Table 1
Relative energies (enthalpy values in kcal/mol) of quercetin, luteolin and taxifolin radicals in DMSO solution.

	Quercetin	Luteolin	Taxifolin
3′-ArO^{*}	6.13	4.81	3.38
4′-ArO^{*}	0.00	0.00	0.00
3-ArO^{*}	2.51	–	29.49
5-ArO^{*}	14.91	16.83	15.29
7-ArO^{*}	11.67	11.92	24.93

Table 2
BDE values in gas-phase and DMSO solution for quercetin, luteolin and taxifolin, with different OH substitutions (enthalpy values in kcal/mol).

	Quercetin		Luteolin		Taxifolin	
	Gas	DMSO	Gas	DMSO	Gas	DMSO
3′-OH	83.73	80.16	84.10	80.42	82.94	79.11
4′-OH	72.11	74.03	73.32	75.61	73.56	75.73
3-OH	80.96	76.54	–	–	105.30	105.22
5-OH	95.28	88.94	99.38	92.44	96.46	91.02
7-OH	87.01	85.70	87.60	87.53	101.99	100.66

flavonoids. The magnitude order of BDE values for different OH substitutions in DMSO is the same as that obtained in gas phase (see Table 2). Since the condensed phase seems to be more reflective of real situations as the vacuum phase barely considering ideal state, the BDE values in DMSO are well analyzed.

The BDE values computed with respect to their most stable radical species for quercetin, luteolin and taxifolin are 74.03, 75.61 and 75.73 (in kcal/mol), respectively. A lesser amount of energy is required for breaking the 3′-OH and 4′-OH groups compared with the other ones. This is not surprising if we consider that in the case of 3′-OH and 4′-OH, the hydrogen abstraction produces the two most stable radicals. Hence, the 4′-OH should be the most active radical scavenger and the 3′-OH seems to be good candidates to participate in the antioxidant mechanism because of small energy gaps with the absolute minimum. Since the hydrogen abstraction for 4′-OH produces the most stable radical, the BDE value for 4′-OH is used to characterize the antioxidant activity capacities.

The calculated BDE values (relative to the most stable radical species) for the selected flavonoids both in gas-phase and in DMSO solution and their corresponding CAA values reported by Wolfe and Liu [14] are listed in Table 3. From Table 3, the BDE values computed in condensed phase are higher than those obtained in the gas phase by ≈2 kcal/mol, which can be due to the minor stability of radical species in the DMSO medium. Quercetin seems to having the largest CAA value (99.1 μmol of QE/100 μmol), or the smallest BDE value (74.03 kcal/mol), indicating a better activity as radical scavenger; the CAA and BDE values are found to be 37.1(in μmol of QE/100 μmol) and 75.61(in kcal/mol) for luteolin; taxifolin has the smallest CAA value which is unquantitative at the concentrations tested, but the BDE value is just a little larger than that of luteolin, which is likely due to the biological components of the CAA assay, because it is not a test tube and accounts for some aspects of cell uptake, distribution, and metabolism of antioxidant compounds. Moreover, seen from Table 3, the order of antioxidant efficacies predicted theoretically is quercetin > luteolin > taxifolin, which is in agreement with that reported by experimental CAA [14], since the smaller the BDE values the stronger the antioxidant activities will be. Results also demonstrate the importance of the H atom transfer mechanism for the three flavonoids to explain their capacities to scavenge free radicals.

Table 3
The Calculated BDE values (in kcal/mol) both in gas-phase and DMSO solution, the experimental CAA values (μmol of QE/100 μmol) for quercetin, luteolin and taxifolin.

	Theoretical BDE		Experimental CAA[a]
	Gas	DMSO	
Quercetin	72.11	74.04	99.1
Luteolin	73.32	75.61	37.1
Taxifolin	73.56	75.73	Low

[a] Data are from Ref. [14].

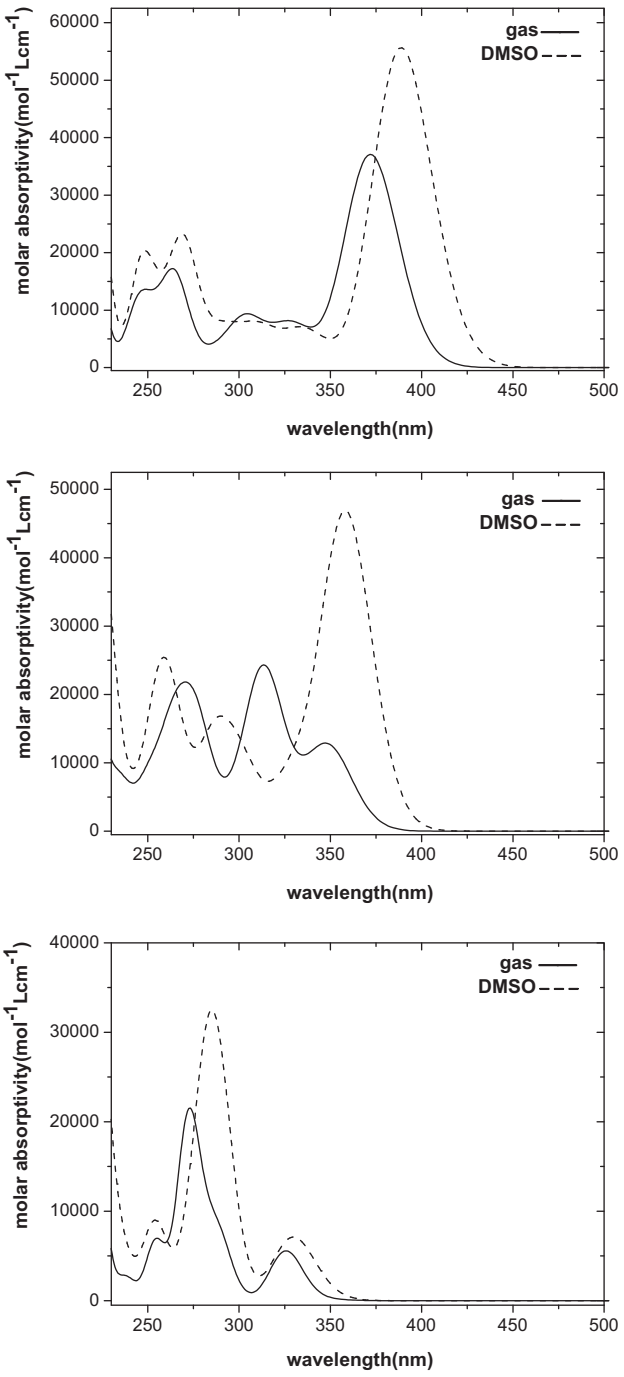

Fig. 2. UV–vis spectra of quercetin (top), luteolin (middle) and taxifolin (bottom).

3.2. UV–vis spectra

UV–vis spectra of the selected flavonoids in gas-phase and in DMSO solution are computed and depicted in Fig. 2. The first three absorption bands (λ), the corresponding oscillator strengths (*f*) together with MO contributions involved in the electronic transitions are collected in Table 4. The Gouterman's HOMO and LUMO frontier orbital compositions for the three flavonoids are depicted in Fig. 3.

The HOMO orbital of quercetin is characterized by a charge distribution mainly on the ring B and on the C_2=C_3 double bond in the ring C, while the LUMO orbital charge density is distributed involving the whole molecule. The most significant HOMO → LUMO transition band (band I) corresponding to a low

Table 4
The calculated absorption bands (λ in nm) and oscillator strengths (*f*), and MO contributions of quercetin, luteolin and taxifolin in gas phase and DMSO solution.

	Gas			DMSO		
	λ	*f*	MO contribution	λ	*f*	MO contribution
Quercetin	372.0	0.4264	H → L(79%)	388.9	0.6404	H → L(85%)
	265.1	0.1528	H → L + 1(47%)	270.2	0.2276	H → L + 1(60%)
			H-3 → L(26%)			H-3 → L(17%)
	244.2	0.1058	H-1 → L + 1(39%)	247.0	0.1825	H-1 → L + 1(30%)
			H-4 → L(26%)			H → L + 3(26%)
			H → L + 3(7%)			H-4 → L(20%)
Luteolin	348.4	0.1441	H → L(83%)	358.5	0.5322	H → L(83%)
	315.1	0.2052	H → L(62%)	286.3	0.1503	H-3 → L(71%)
			H-4 → L(21%)			H → L + 1(7%)
	276.6	0.1775	H-3 → L(62%)	259.1	0.2079	H-1 → L + 1(74%)
			H → L + 3(12%)			H → L + 1(5%)
Taxifolin	325.7	0.0512	H-1 → L(88%)	329.5	0.0801	H-1 → L(88%)
	272.6	0.1918	H-3 → L(65%)	287.4	0.2280	H-2 → L(47%)
			H-4 → L(9%)			H-4 → L(40%)
	254.4	0.0617	H → L + 2(49%)	254.1	0.1016	H → L + 1(62%)
			H → L + 4(25%)			H → L + 2(12%)
			H-2 → L + 5(14%)			H-3 → L + 3(10%)

charge transfer from the ring B to the ring A is localized at 372 and 388.9 nm in gas and in DMSO, respectively. The HOMO-3 → LUMO and HOMO → LUMO + 1 transitions that are considered to be the second absorption band are found at 265.1 (gas) and 270.2 nm (DMSO). Finally, the third absorption band corresponding mainly to HOMO → LUMO + 3 and HOMO-1 → LUMO + 1 transitions is localized at 244.2 and 247.0 nm in the two conditions, respectively.

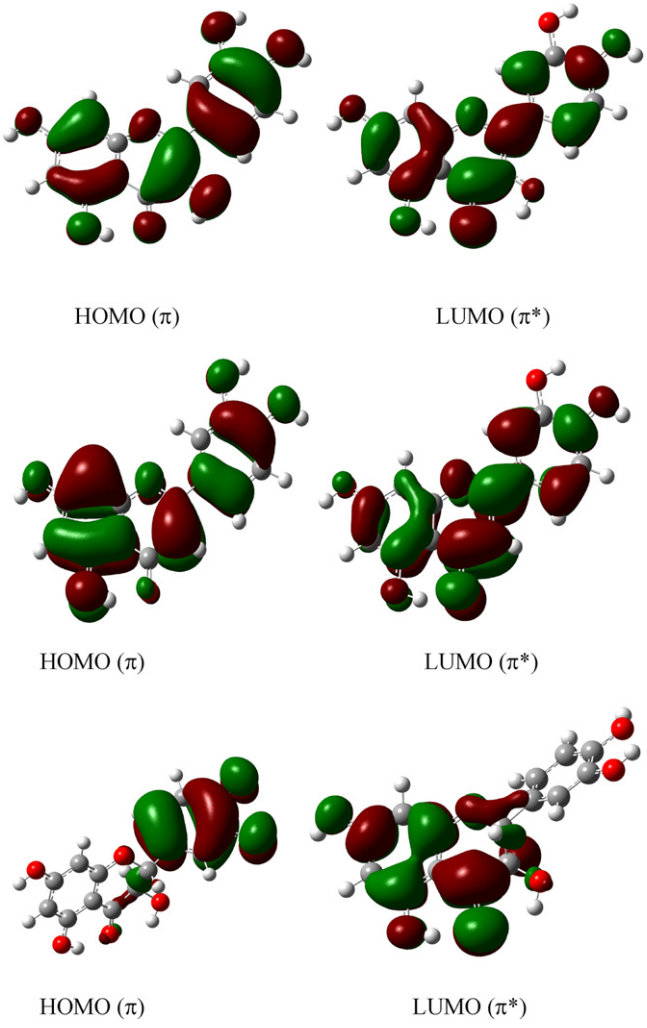

Fig. 3. The two Gouterman's orbital compositions of quercetin (top), luteolin (middle) and taxifolin (bottom).

The HOMO orbital of luteolin presents a charge density localized mainly on the ring A and on the C_2=C_3 double bond in the pyrone moiety. The LUMO orbital is characterized by a charge distribution involving the whole molecule. The HOMO → LUMO (band I) corresponding to a low charge transfer from the ring A to the ring B, charge transition falls at 348.4 (gas) and 358.5 nm (DMSO). The other two absorption bands are localized at 315.1 and 276.6 nm (gas), 286.3 and 259.1 nm (DMSO). In taxifolin, the HOMO orbital presents a charge density localized strongly on the ring B. The LUMO orbital is characterized by a charge density localized on the ring A while a certain degree is found on the ring C (atom C_4 and =O). The HOMO-1 → LUMO transition band whose charge transfer is strongly from the ring B to the rings A and C is localized at 325.7 (gas) and 329.5 nm (DMSO). The maximum absorption band falls at 272.6 and 287.4 nm, in the two conditions. The third absorption band is found at 254.4 (gas) and 254.1 nm (DMSO).

Quantitative comparison cannot be made with the experimental spectra in DMSO solutions, because the experimental UV–vis spectra are not well resolved, considering the theoretical results obtained in this work reliable. The differences concerning the localization of the transition bands in the two different conditions can be attributed to solvent effects. A dielectric constant causes generally a red shift of the main transition bands as verified in the literature [26].

4. Conclusions

The cellular antioxidant capacities of the selected flavonoids have been investigated theoretically by computing the thermodynamic parameter O—H bond dissociation enthalpy (BDE). On the basis of obtained results, we conclude that the order of antioxidant efficacies predicted theoretically is in agreement with that reported by experimental results of CAA, which indicate H-atom transfer seems to be the best indicator of the anti-radical property of the three flavonoids and rationality of cellular antioxidant activity assay representative of experimental findings. It is important to note that having a small BDE value is not equivalent to having a significant CAA action, taxifolin is a good example, which are attributed to the complexity of biological systems. However, knowledge of theoretical BDE values may be helpful in assessing potential in vivo antioxidant activity of flavonoids. TDDFT methodologies can be successfully applied to obtain the UV–vis electronic

spectra of the selected flavonoids, reproducing well the shape of the experimental ones.

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