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ORIGINAL PAPER

A DFT and PM6 study of free radical scavenging activity of ellagic acid

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Abstract Reaction enthalpies related to mechanisms of free radical scavenging activity of ellagic acid and its phenoxide anions were calculated by density functional theory and the semiempirical PM6 method. In addition to the gas phase, calculations are performed for water and benzene as the solvents, which may represent biological liquids and the membrane lipids, i.e., a natural environment for antiradical action. The thermodynamically favored mechanism depends on the polarity of reaction media, deprotonation degree of ellagic acid as well as the properties of scavenging radicals. The most acidic 3-OH group of ellagic acid is the active site for radical inactivation. The ellagate monoanions and dianions possess progressively better scavenging potency than unionized ellagic acid. The sequential proton loss electron transfer mechanism is the preferred reaction pathway for the ellagate monoanion and dianion in water. In benzene, ellagic acid inactivates free radicals by the hydrogen atom transfer mechanism. In the gas phase the latter mechanism is favored for all ellagic acid species.

Keywords Ellagic acid · Ellagate anions · Radical scavenging · HAT · SET-PT · SPLET

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Introduction

Ellagic acid is a naturally occurring polyphenolic antioxidant found in a variety of plant species where it forms part of hydrolysable polymeric ellagitannins. Ellagitannins upon hydrolysis produce ellagic acid. They can be found in pomegranates, raspberries, strawberries, cranberries, walnuts, and almonds [1]. Consumption of such fruits and nuts rich in ellagitannins has been associated with a variety of health benefits. Dietary ellagitannins exert many pharmacological properties, including inhibition of LDL oxidation, cardiovascular protection, antioxidant, anti-inflammatory, and anticarcinogenic activities [2, 3]. In vitro experiments suggest that ellagic acid and some of its metabolites (e.g., urolithins) may be the agents responsible for the remarkable biological activities of ellagitannins [4, 5].

The molecular mechanisms explaining the biological activities of ellagic acid are poorly understood and remain to be clarified, but it is possible that several different types of biochemical events are involved [6]. Antioxidant activity of ellagic acid could be a result of direct scavenging of free radicals, sequestration of potential oxidants, regulation of enzyme activity, modulation of cell signaling, and regulation of gene expression [7, 8]. However, despite

the increasing interest in ellagic acid as a potential protective agent against the development of human diseases, the real contributions of this phytochemical to health maintenance is still unclear.

Ellagic acid is a dilactone of hexahydroxydiphenic acid and structurally represents a gallic acid dimer. It is an amphipathic molecule because of the four phenolic and two lactone groups constituting the hydrophilic part and two phenyl rings representing the hydrophobic part of the molecule. Due to its low polarity, ellagic acid is only sparingly soluble in aqueous media [9].

An important influence on the free radical scavenging activity of phenolics is the pH of the surrounding medium [10, 11]. Depending on the acidity, ellagic acid can be partially or fully ionized, meaning that not only the neutral molecule but also the ions could be involved in the antioxidant actions. Ellagic acid contains four phenolic OH groups that may deprotonate. Somewhat controversial are the reports regarding the first deprotonable OH group and related pK_A values. On the basis of spectrophotometric measurements, Priyadarsini et al. [12] published values of pK_{A1} = 6.3 and pK_{A2} = 11.2, and noted that two other pK_A values are in the same pH range, but probably unresolvable. This could be a consequence of the planar symmetric structure of ellagic acid with two equivalent positions of OH groups, i.e., 3 and 3′ as well as 4 and 4′. Hasegawa et al. [13] designated the 4 or 4′-OH group as the most acidic one, which deprotonates in the pH region 0.5–5.6. They stated that first and second dissociation equilibria coexist in the pH region 5.6–7.0 and that in the pH region of 7.6–8.3 monoanion and dianion of ellagic acid exist. Munoz-Munoz et al. [14] derived the 3 or 3′-OH group as the most acidic one and suggest that only two distinguishable pK_A values, related to the dissociation of both the 3 and 3′-OH groups, and both the 4 and 4′-OH groups, exist. Undoubtedly, at physiological pH of 7.4, the predominant form of ellagic acid should be phenoxide anion(s) species.

The scavenging of free radicals seems to play a notable part in the antioxidant activity of polyphenolic compounds. Antiradical properties of ellagic acid ($EA(OH)_4$) are related to the ability to transfer the phenolic H-atom to a free radical (e.g., alkoxyl radical, RO·). The formal H-atom abstraction from ellagic acid described by:

$$EA(OH)_4 + RO^{\cdot} \rightarrow EA(OH)_3O^{\cdot} + ROH \quad (1)$$

may involve complex processes. It has been recognized that such a reaction could proceed via at least three different mechanisms: hydrogen atom transfer (HAT, Eq. 2), single electron transfer followed by proton transfer (SET-PT, Eqs. 3 and 4), and sequential proton loss electron transfer (SPLET, Eqs. 5 and 6). All three of these mechanisms may take place in parallel, but at different rates [15–18].

$$EA(OH)_4 \rightarrow EA(OH)_3O\cdot + H\cdot \quad (2)$$

$$EA(OH)_4 \rightarrow EA(OH)_4^{\cdot+} + e^- \quad (3)$$

$$EA(OH)_4^{\cdot+} \rightarrow EA(OH)_3O\cdot + H^+ \quad (4)$$

$$EA(OH)_4 \rightarrow EA(OH)_3O^- + H^+ \quad (5)$$

$$EA(OH)_3O^- \rightarrow EA(OH)_3O\cdot + e^- \quad (6)$$

The HAT mechanism is characterized by the homolytic bond dissociation enthalpy (BDE) of the OH group. The HAT is favored for radicals with high H-atom affinity and is preferred in non-polar solvents because it does not involve charge separation. The SET-PT and SPLET mechanisms are favored in polar media because of charge separation. They are preferred for radicals with high electron affinity [19]. The SET-PT mechanism is controlled by the ionization potential (IP) of ellagic acid and the proton dissociation enthalpy (PDE) of the radical cation ($EA(OH)_4^{\cdot+}$). The SPLET mechanism is governed by the proton affinity (PA) of ellagate anion as well as by the electron transfer enthalpy (ETE) of ellagate anion ($EA(OH)_3O^-$). The net result of the SPLET is the same as in HAT and SET-PT—the formation of the corresponding radical species.

Calculated energy requirements (BDE, IP, PDE, PA, and ETE) may indicate which radical scavenging mechanism is thermodynamically preferred and point out the active site for radical inactivation [16, 20–22]. Usually, the energetics related to radical scavenging activity of polyphenolic compounds has been calculated in gas phase for neutral molecules [23]. The objective of the present work is to study mechanisms of free radical scavenging activity of ellagic acid and ellagate anions by the DFT and PM6 methods. As previously shown, the semiempirical fast PM6 method constitutes a valid alternative to the more accurate but time consuming DFT calculations [21, 24].

In addition to the gas phase, the calculations are also performed for water and benzene as the solvents, which may represent biological liquids and membrane lipids, i.e., the natural environment for antiradical action. Water is the main constituent of all physiological liquids, and at physiological pH of 7.4 polyphenols could display complex solution behavior since there can be an equilibrium pool of both the neutral and anionic forms. Since it is well known that as an amphipathic molecule ellagic acid is sparingly soluble in water [9], it could be expected that ellagate anions are better soluble in water than ellagic acid itself. On the other hand, ellagic acid is lipid soluble while ellagate anions are probably not. Bearing in mind these facts, ellagate anions and ellagic acid are analyzed in water (polar media) and benzene (non-polar media) as the solvents. The reason for this choice is that the antioxidant action of polyphenols may proceed between solutions and

multiphase emulsion systems (interfacial antioxidation) [25] as well as near or inside membranes in which ellagic acid and/or ellagate anions may increase its/their local concentration and express antiradical activity [26].

Results and discussion

Conformational analysis of ellagic acid

Figure 1 depicts selected highly stable rotamers of ellagic acid **(1–6)** obtained by the conformational analysis performed here. Analysis of the geometries of ellagic acid rotamers has shown that all of them are planar. Our calculations showed that **1** is the most stable conformer of ellagic acid with a population of 99.1 %. As for other rotamers, the populations of **2** and **5** (0.5 and 0.4 %) are worth mentioning. For this reason, all further discussions in the forthcoming sections are focused on this conformation. Hasegawa et al. [13] have determined that ellagic acid rotamer **1** is involved in complex with pyridine. Sato and Kataoka [27] using the PM3 semiempirical MO method have also found that rotamer **1** is the most stable and has the planar structure. Rotamer **2** has been used in a study of the reaction of ellagic acid with an ultimate carcinogen [28], and rotamer **3** is a component of the ellagic acid-sarcosine and ellagic acid-*N*,*N*-dimethylglycine cocrystals [29]. Rotamer **4** matches the experimentally determined structure of ellagic acid [30–32] and is used in a DFT study of ellagic acid antioxidant activity [33]. On the basis of our calculations, the highest energy conformation is rotamer **6**.

The natural bond orbital (NBO) analysis of ellagic acid

The NBO analysis provides a description of the structure of a conformer by a set of localized bonds, antibonds, and Rydberg extravalence orbitals. Destabilizing interactions between occupied orbitals and stabilizing interactions between occupied and unoccupied orbitals can be obtained from this analysis [34, 35]. The NBO analysis is an efficient method for investigating the hyperconjugative interaction or charge transfer (CT) in ellagic acid molecule. The second-order perturbation theory analysis of the Fock matrix in the NBO basis of the rotamer **1** shows strong intramolecular hyperconjugative interactions, which are presented in Table 1.

The most important interaction (n-ε_{σ^*}) energies, related to the resonance in the molecules, are electron donation from the LP2 O atoms to the anti-bonding acceptor σ*(C–O) of the α-pyrone ring (LP2 $O_{9'} \rightarrow \sigma^* O_{8'} C_{7'}$ and LP2 $O_9 \rightarrow \sigma^* O_8 C_7$) = 201.84 kJ/mol. This larger energy shows the hyperconjugation between the electron donating oxygen and the rest of the α-pyrone ring. Also other LP2 O lone electron pairs show a significant electron donation to the anti-bonding acceptor π*(C–C) of the ellagic acid rings. Besides LP2 O → π*(C–C) and LP2 O → σ*(C–C) interactions, strong intramolecular hyperconjugative interactions are formed by the orbital overlap between π(C–C) → π*(C–C) bond orbitals, resulting in intramolecular charge transfer (ICT) causing stabilization of the system. These interactions are observed as an increase in electron density (ED) in the C–C anti-bonding orbital, which weakens their respective bonds. The ED at the conjugated π (∼1.65 e) and π* bonds (∼0.4 e) of the phenyl and α-pyrone rings clearly demonstrates strong delocalization of electrons, leading to stabilization by ∼90–200 kJ/mol. A very strong interaction was observed between the π type orbital containing the lone electron pair, of all OH groups, and neighbor anti-bonding orbitals of the rings. These interactions are responsible for a pronounced decrease of the lone pair orbital occupancy compared to other occupancy, pointing to the hyperconjugation between all oxygen atoms of the OH groups and benzene rings. The natural Mulliken population analysis of the ellagic acid is calculated using the M05-2X/6-311++G(d,p) level [36]. The natural charge distribution in the ellagic acid structure is shown in Fig. 2. As expected, the negative charge is almost uniformly distributed over the oxygen atoms of all OH groups. However, it should be noted that atoms O3 and O3′ are slightly more negative than others, which could mark them as the positions that undergo chemical reactions more easily.

Acidity of ellagic acid

The COSMO/PM6 results [37] have indicated that the most acidic phenolic groups are 3-OH and 3′-OH with pK_A values of 5.73 and 5.75. The same acidity of these OH groups is a consequence of the highly symmetric structure of ellagic acid, i.e., equivalence of 3 and 3′ positions [14]. The least acidic hydrogen atom belongs to the 4-OH and 4′-OH groups with pK_A values of 7.05 and 7.12.

Proton affinity (PA) could be another reliable indicator to ascertain which OH group of ellagic acid deprotonates first. More positive PA values correspond to less acidic phenolic groups. Both DFT and PM6 results indicate that in all studied media the 3-OH (3′-OH) group is the most acidic one because of the lowest PA value (Table 2). This is in accordance with published experimental results [14, 38, 39].

Free radical scavenging mechanisms of ellagic acid and ellagate anions

Reaction enthalpies related to the three mechanisms of free radical scavenging activity (HAT, SET-PT, and SPLET) of

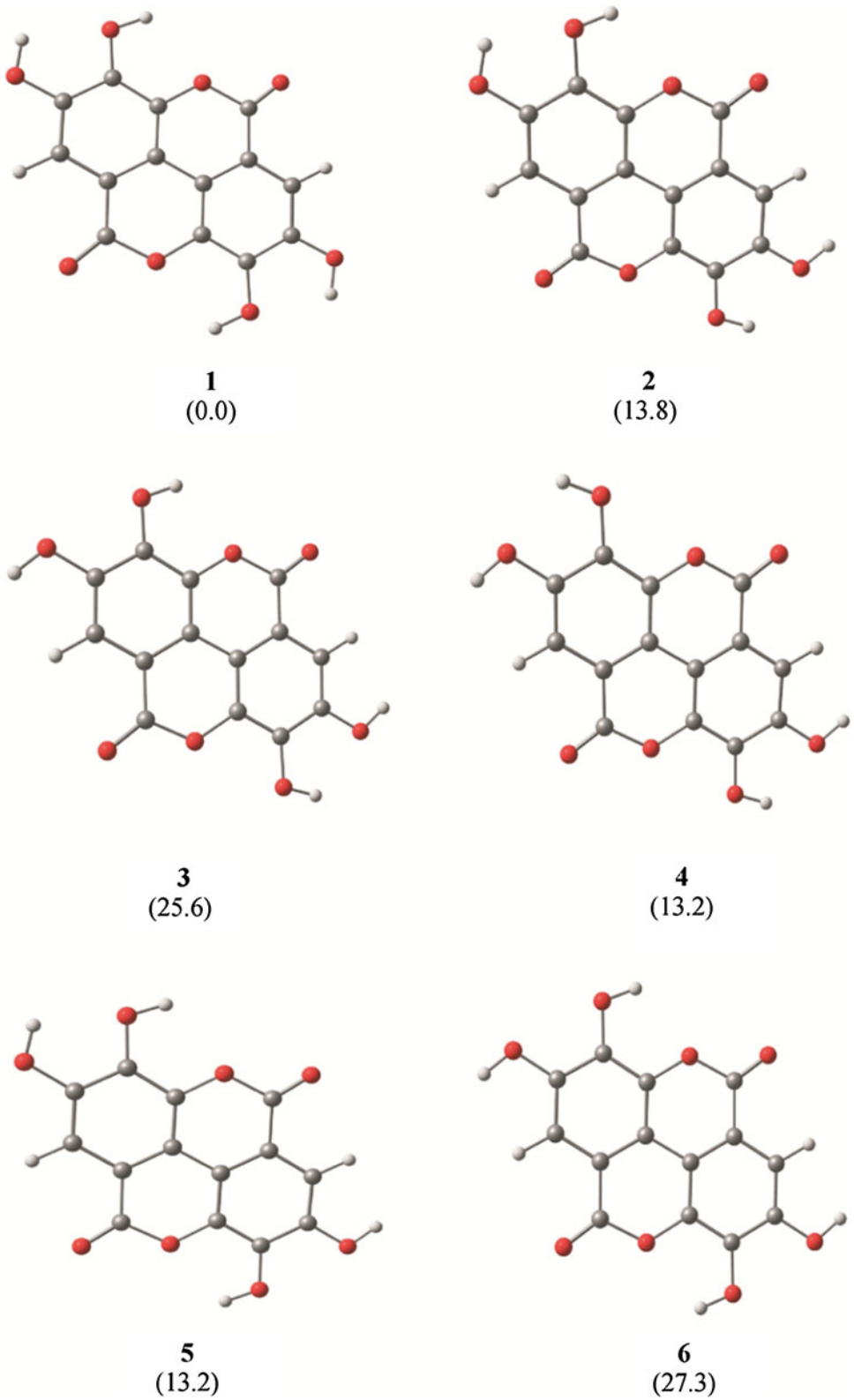

Fig. 1 The most stable rotamer of ellagic acid is conformer **1**; **2**-**6** are less stable rotamers. ΔE values of rotamers are given in parentheses (kJ/mol)

ellagic acid as well as ellagate monoanion and dianion are calculated by the DFT method. Calculations using the PM6 method were also performed and compared to DFT results. The species necessary to perform these calculations are generated from the most stable conformation of ellagic acid, i.e., rotamer **1**.

The preferred mechanism of antiradical activity of phenolics can be estimated from values of BDE (HAT

Table 1 Second order perturbation theory analysis of Fock matrix for ellagic acid

Donor(*i*)	Acceptor(*j*)	ED(*i*)	ED(*j*)	*E*2/kJ mol^{-1}	*E*(*i*)-*E*(*j*)/a.u.	*F*(*i*,*j*)/a.u.
πC_4–C_5	π^*C_6–C_1	1.64	0.47	121.08	0.36	0.094
πC_4–C_5	π^*C_2–C_3	1.64	0.41	119.29	0.35	0.090
πC_6–C_1	π^*C_4–C_5	1.65	0.35	107.57	0.37	0.087
πC_6–C_1	π^*C_2–C_3	1.65	0.41	108.16	0.35	0.086
πC_6–C_1	$\pi^*C_{1'}$–$C_{6'}$	1.65	0.49	88.16	0.36	0.081
πC_6–C_1	$\pi^*C_{7'}$–$O_{9'}$	1.65	0.23	105.77	0.38	0.092
πC_2–C_3	π^*C_4–C_5	1.65	0.35	103.81	0.39	0.088
πC_2–C_3	π^*C_6–C_1	1.65	0.49	126.69	0.38	0.100
$\pi C_{1'}$–$C_{6'}$	π^*C_6–C_1	1.65	0.47	88.16	0.36	0.081
$\pi C_{1'}$–$C_{6'}$	$\pi^*C_{2'}$–$C_{3'}$	1.65	0.41	108.16	0.35	0.086
$\pi C_{1'}$–$C_{6'}$	$\pi^*C_{4'}$–$C_{5'}$	1.65	0.35	107.57	0.37	0.087
$\pi C_{1'}$–$C_{6'}$	$\pi^*C_{7'}$–$O_{9'}$	1.65	0.23	105.77	0.38	0.090
LP2 O_3	π^*C_2–C_3	1.88	0.41	136.65	0.46	0.119
LP2 O_4	π^*C_4–C_5	1.88	0.35	143.30	0.46	0.119
LP2 $O_{8'}$	π^*C_2–C_3	1.80	0.41	123.26	0.46	0.110
LP2 $O_{8'}$	$\pi^*C_{7'}$–$O_{9'}$	1.80	0.23	169.41	0.48	0.126
LP2 $O_{9'}$	$\sigma^*C_{6'}$–$C_{7'}$	1.83	0.06	85.60	0.83	0.120
LP2 $O_{9'}$	$\sigma^*O_{8'}$–$C_{7'}$	1.83	0.12	201.84	0.70	0.166

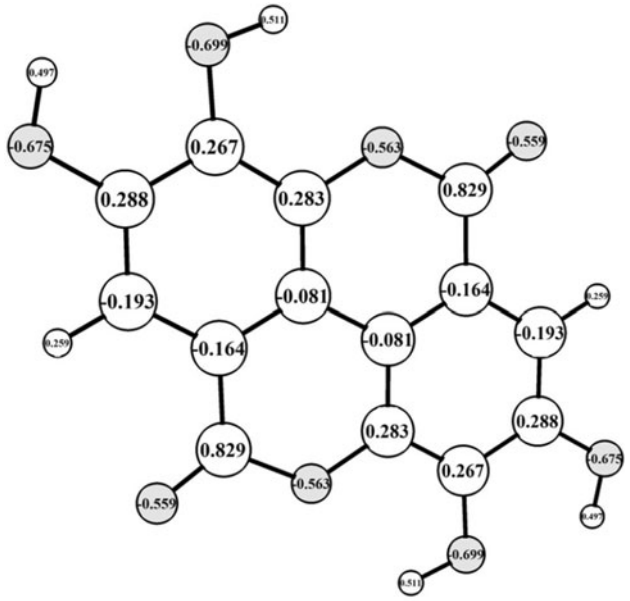

Fig. 2 Natural charge distribution of ellagic acid

mechanism), IP (first step of the SET-PT mechanism), and PA (first step in the SPLET mechanism) [16, 20, 40]. The lowest value indicates which mechanism is thermodynamically the most probable process. On the other hand, the preferred site of antioxidant action can be estimated from the sum of enthalpies involved in a particular free radical scavenging mechanism. For the HAT mechanism it is simply the value of BDE that accounts for one-step H-atom transfer. In the case of the SET-PT mechanism, this sum embraces IP and PDE, and in SPLET mechanism PA and ETE. Recently, we found that these sums are perfectly intercorrelated and that the same OH group is the preferred site for radical inactivation by all three mechanisms, because this particular OH group possesses minimal energy requirements. Consequently, the final product of all free radical scavenging mechanisms is the same, the thermodynamically most stable phenoxyl radical (unpublished work). One of the basic conditions for a phenolic to be an antioxidant is that the resulting phenoxyl radical formed after scavenging must be stable [41]. The antioxidant action of ellagic acid and ellagate anions is related to phenoxyl radicals (obtained after H-atom transfer) showing a planar conformation that allows extended unpaired electron delocalization and consequential stability.

The spin density is considered as a parameter that provides a reliable representation of the free radical reactivity [42]. Phenoxyl radicals with more delocalized spin density are more stable. Spin density delocalization is related to the easiness of phenoxyl radical formation. In general, the more delocalized the spin density in the phenoxyl radical, the easier the radical formation is, and thus the higher the parent phenolic antioxidant activity is. On the other hand, the presence of a high spin density localized on the particular atom implies a high reactivity for that site, a feature that appears only upon formation of the phenoxyl radical [43]. The spin density distributions in the most stable phenoxyl radicals of ellagic acid and ellagate anions are shown in Fig. 3. The best delocalization of spin density is found in the 3O$^-$,4′O· radical anion in water. In this

Table 2 DFT and PM6 calculated parameters of antioxidant mechanisms for ellagic acid/kJ mol^{-1}

	DFT					PM6				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
Gas phase										
		791					817			
3-OH	352		882	1,308	366	316		820	1,264	373
4-OH	368		899	1,364	326	326		830	1,293	354
Water										
		363					400			
3-OH	348		−15	63	285	308		−93	−1	308
4-OH	353		−10	94	260	315		−85	19	295
Benzene										
		682					737			
3-OH	354		86	337	432	321		−2	293	442
4-OH	368		101	388	395	330		8	319	425

structure, the unpaired electron is delocalized over the aromatic rings and both oxygens from which hydrogens have been abstracted. Such good delocalization of spin density was not observed in other radical species presented in Fig. 3.

Reaction enthalpies from DFT and PM6 calculations related to the three mechanisms of antiradical activity of ellagic acid, ellagic acid monoanion, and ellagic acid dianion in different media are presented in Tables 2, 3, and 4. As can be seen from Table 2, the compatibility of PM6 and DFT results in all studied media is reasonable and leads to the same result. The preferred mechanism of ellagic acid action in the gas phase is HAT, because the BDE values of OH groups are lower than the corresponding IP and PA values. Although the DFT calculated BDE value in benzene is slightly higher than the PA values, the high ETE value makes SPLET less probable than the HAT mechanism. Position 3 (or 3′) is the preferred site for radical inactivation because of its lowest energy requirements [lowest BDE, (IP + PDE) and (PA + ETE)]. In benzene PA is significantly lower than gas phase ones mainly because of the large enthalpy of H^+ solvation. On the other hand, there is no such pronounced difference between BDEs in the studied environments.

In comparison with ellagic acid, energy requirements for radical inactivation by the 3-O$^-$ phenoxide anion are reduced (Table 3). This is in agreement with experimental findings that phenoxide anions are better radical scavengers than neutral molecules [44]. In the gas phase, the BDE value is lower than corresponding IP and PA values, indicating that HAT is the thermodynamically preferred mechanism. In water, the SPLET mechanism is favored because the PA value (as well as the ETE value) is lower than corresponding BDE and IP values. The SET-PT mechanism cannot be discarded as a possible mechanism for the phenoxide anion in water because of a lower IP value than BDE value and equal overall energetics of the SET-PT and SPLET mechanisms (Table 3). Results from PM6 calculations indicate that in both studied media the 4-OH group of the 3-O$^-$ phenoxide anion of ellagic acid possesses the lowest energy requirements and represents the preferred site for radical inactivation. In water as the solvent, the DFT method confirms this finding. Divergence arises in the gas phase where DFT calculations indicate the 3′-OH group of the 3-O$^-$ phenoxide anion as the active site.

The 3,3′-diO$^-$ phenoxide dianion of ellagic acid is symmetrical, and radical inactivation could proceed equally at the 4-OH and 4′-OH group. The energy requirements for radical inactivation by the dianion are furthermore reduced (Table 4). The HAT mechanism is dominant in the gas phase. In water, the preferred pathway of radical scavenging is SPLET. Ellagic acid is poorly soluble in water, but its solubility increases after deprotonation at two hydroxyl group positions [9]. These facts indicate that in a polar environment, at physiological pH of 7.4, ellagate anions may be effective in radical inactivation.

The radical scavenging mechanisms of antioxidants depend not only on their physicochemical properties, but also on the properties of the scavenged radicals [45–47]. The DFT values for BDE indicate that some prevalent radicals in food chemistry, such as lipid peroxyl radicals (LOO·), here represented by MeOO·, cannot abstract an H atom from the ellagic acid molecule in any of the media under investigation because BDE(EA(OH)$_4$) > BDE(MeOO·) (see Tables 2, 5). Therefore, the HAT mechanism seems thermodynamically unfavorable for this

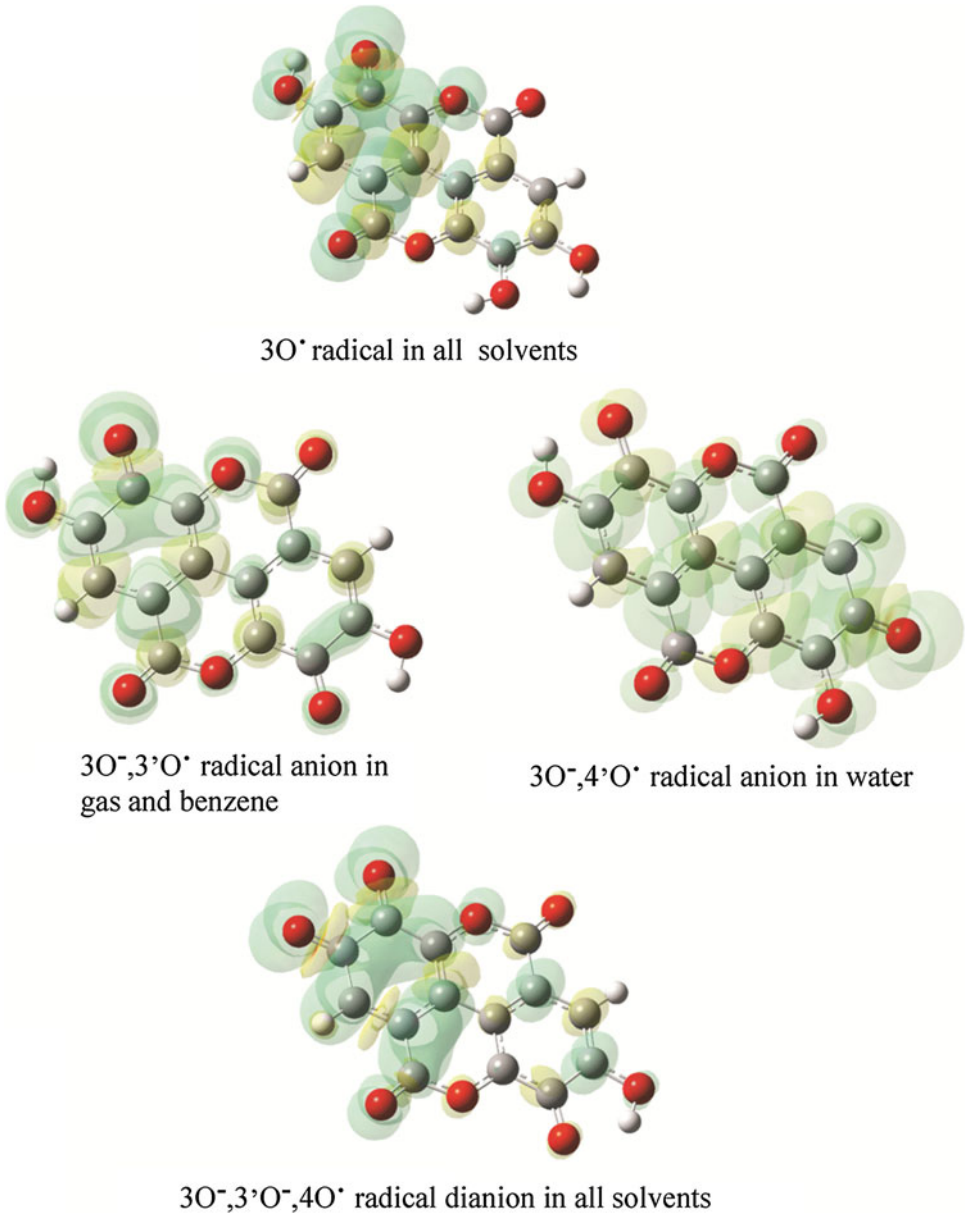

Fig. 3 Spin density distribution in the most stable phenoxyl radicals of ellagic acid and ellagate anions in gas phase, water, and benzene

radical type. Taking into account the upper limit of the BDE value for the methyl peroxyl radical (370 kJ/mol) [48], it is possible to presume that the HAT mechanism is a probable process. This is in agreement with PM6 results in benzene. In water, the reaction energetics indicates that ellagate ions can scavenge MeOO· by all three reaction pathways. Due to overall energetics lower than the corresponding ones for MeOO· (Tables 3, 4, 5), ellagate anion and ellagate dianion are capable of progressively better scavenging of the MeOO· radical, preferably by the SPLET mechanism. The PA values increase with increasing deprotonation, which is accompanied with decreasing ETE values. Overall, as the minimal sum of the (PA + ETE) decreases, the ellagate dianion becomes a progressively better scavenger. It appears that at physiological pH of 7.4, ellagate anions and dianions could be good free radical scavengers for this radical type. Similar conclusions can be drawn from PM6 results presented in Tables 2, 3, 4, 5.

Conclusions

According to the calculated energy requirements (BDE, IP, PDE, PA, and ETE), the favorable free radical scavenging pathway in water (representing physiological liquids) for ellagate anions is the SPLET mechanism. The radical scavenging potency of ellagic acid increases with increasing deprotonation. In the gas phase, the HAT mechanism is preferred. This indicates that results obtained by gas phase calculations, as is usual practice, are not transferable to the

Table 3 DFT and PM6 calculated parameters of antioxidant mechanisms for 3-O^- phenoxide monoanion/kJ mol^{-1}

	DFT					PM6				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
Gas phase										
		366					373			
4-OH	342		1,298	1,761	−98	308		1,256	1,695	−66
3′-OH	331		1,287	1,582	71	310		1,258	1,550	81
4′-OH	338		1,294	1,627	32	318		1,267	1,560	79
Water										
		285					308			
4-OH	325		40	134	191	305		−4	70	234
3′-OH	344		58	71	272	311		2	12	298
4′-OH	349		63	100	248	321		13	24	297

Table 4 DFT and PM6 calculated parameters of antioxidant mechanisms for 3,3′-diO^- phenoxide dianion/kJ mol^{-1}

	DFT					PM6				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
Gas phase										
		71					81			
4-OH	320		1,572	2,026	−384	291		1,532	1,960	−347
Water										
		272					298			
4-OH	321		49	140	180	301		3	75	226

water environment. The lipophilic ellagic acid molecule in non-polar benzene (representing a lipid membrane) may inactivate free radicals by the HAT mechanism. As exemplified in the case of the methyl peroxyl radical, the properties of the scavenged radicals should also be taken into account.

Computational details

DFT calculations

The conformations of ellagic acid species involved in radical scavenging pathways are fully optimized by the local density functional method (M05-2X), developed by the Truhlar group [49], by using the 6-311++G(d,p) basis set, implemented in the Gaussian 09 package [36]. The hybrid meta exchange–correlation functional M05-2X is parameterized so that it includes both nonmetallic and metallic compounds. This functional also yields satisfactory overall performance for the main group thermochemistry and thermochemical kinetics, as well as organic, organometallic, biological, and noncovalent interactions. The M05-2X functional has been successfully used by independent authors [21, 50–55]. In addition, this functional nicely reproduces nonplanarity of the dihedral angle between rings B and C in morin and quercetin [24, 53]. To calculate the thermodynamic properties in the solvent environment (water and benzene), calculations with an implicit solvation model SMD [56, 57], as implemented in Gaussian 09, were used in combination with DFT calculation at the M05-2X/6-311++G(d,p) level. The vibrational frequencies were obtained from diagonalization of the corresponding M05-2X Hessian matrices. The nature of the stationary points was determined by analyzing the number of imaginary frequencies: 0 for minimum and 1 for transition state. Relative enthalpies were calculated at 298 K. The NBO analysis [34, 58, 59] of ellagic acid was performed using the M05-2X density matrice.

To determine the preferred relative positions of the four OH groups, the conformational space of ellagic acid is investigated as a function of torsional angles (Fig. 4). The

Table 5 DFT and PM6 calculated parameters for methyl peroxyl radical/kJ mol^{-1}

	DFT					PM6				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	BDE	IP	PDE	PA	ETE	BDE	IP	PDE	PA	ETE
Gas phase										
		948					858			
OH	339		713	1,556	105	312		775	1,588	46
Water										
		429					355			
OH	347		−83	141	205	317		−39	133	183
Benzene										
		799					728			
OH	345		−39	522	237	323		9	530	207

Using the DFT/B3P86 functional, Kozlowski et al. [46] obtained for MeOO· radical BDE of 352 kJ/mol (84.1 kcal/mol)

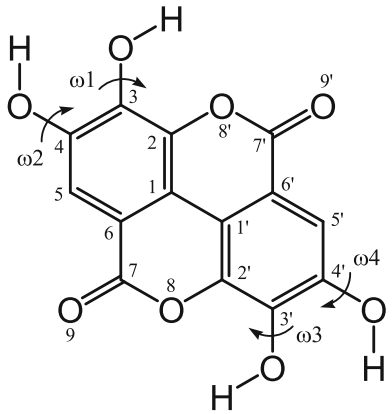

Fig. 4 Atomic numbering for ellagic acid and its main dihedral angles (ω1, ω2, ω3, and ω4)

effects of the following torsion angles rotations are studied: ω1 (H-O3-C3-C2), ω2 (H-O4-C4-C5), ω3 (H-O3′-C3′-C2′), and ω4 (H-O4′-C4′-C5′).

The O–H BDE is calculated by the following equation: BDE = $H(\mathrm{EA(OH)_3O\cdot}) + H(\mathrm{H}) - H(\mathrm{EA(OH)_4})$, where $H(\mathrm{EA(OH)_3O\cdot})$ is the enthalpy of the ellagic acid radical generated after H· abstraction, $H(\mathrm{H})$ is the enthalpy of the hydrogen atom, and $H(\mathrm{EA(OH)_4})$ is the enthalpy of the ellagic acid. The IP is calculated as follows: IP = $H(\mathrm{EA(OH)_4^{+}}) + H(\mathrm{e^-}) - H(\mathrm{EA(OH)_4})$, where $H(\mathrm{EA(OH)_4^{+}})$ is the enthalpy of the ellagic acid radical cation generated after electron abstraction and $H(\mathrm{e^-})$ is the enthalpy of the electron. The PDE is calculated by the equation: PDE = $H(\mathrm{EA(OH)_3O\cdot}) + H(\mathrm{H^+}) - H(\mathrm{EA(OH)_4^{+}})$, where $H(\mathrm{H^+})$ is the enthalpy of the proton. PA is defined by the equation: PA = $H(\mathrm{EA(OH)_3O^-}) + H(\mathrm{H^+}) - H(\mathrm{EA(OH)_4})$, where $H(\mathrm{EA(OH)_3O^-})$ is the enthalpy of the ellagate anion generated after proton abstraction. ETE is calculated by the equation: ETE = $H(\mathrm{EA(OH)_3O\cdot}) + H(\mathrm{e^-}) - H(\mathrm{EA(OH)_3O^-})$ [21, 24]. The solvation enthalpies of the hydrogen atom (H·), proton (H^+), and electron (e^-) for solvents dealt with in this work were taken from the literature [16, 20].

MOPAC calculations

The geometries of ellagic acid species involved in radical scavenging mechanisms were optimized using the PM6 method included in the MOPAC2009^{TM} program package [37]. The eigenvector following the (EF) optimization procedure was carried out with a final gradient norm under 0.00239 kJ mol^{-1} Å^{-1}. The solvent contribution to the enthalpies of formation of ellagic acid species was computed employing the COSMO (Conductor-like Screening Model) calculations implemented in MOPAC2009^{TM}. This approach was used for all structures.

The pK_A values for the phenolic groups of ellagic acid are calculated using the COSMO/PM6 method [37]. In this approach, the pK_A is calculated employing optimized O–H bond lengths and optimized partial charges on the ionizable hydrogen atom in such a way as to reproduce the pK_A of a set of simple organic compounds [60].

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