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ORIGINAL PAPER

A DFT method for the study of the antioxidant action mechanism of resveratrol derivatives

Ali Benayahoum · Habiba Amira-Guebailia · Omar Houache

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Abstract Quantum-chemical calculations using DFT, have been performed to explain the molecular structure antioxidant activity relationship of resveratrol (RSV) (1) analogues: 3,4-dihydroxy-*trans*-stilbene (3,4-DHS) (2); 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS) (3); 4-hydroxy-*trans*-stilbene (4-HS) (4); 3,5-dihydroxy-*trans*-stilbene (3,5-DHS) (5); 3,3′-dimethoxy-4,4′-dihydroxy-*trans*-stilbene (3,3′-DM-4,4′-DHS) (6); 2,4-dihydroxy-*trans*-stilbene (2,4-DHS) (7) and 2,4,4′-trihydroxy-*trans*-stilbene (2,4,4′-THS) (8). It was found that all compounds studied were effective antioxidants with the exception of 3, 5-DHS. The high antioxidant activity of both 3, 3′-DM-4, 4′-DHS and 3, 4-DHS may be due to the abstraction of the two hydrogen atoms of the para and ortho-position hydroxyls respectively, to form a quinone structure. Our results revealed that the antioxidant pharmacophore of 2,4-DHS and 2,4,4′-THS, exhibiting higher antioxidant activity than resveratrol, is the 2-hydroxystilbene, rather than 4-hydroxystilbene. Experimental observations were satisfactorily explained and commented.

Keywords AIP · Antioxidant activity · DFT method · HOMO and LUMO · Spin density · *Trans*-resveratrol

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Introduction

Polyphenols play an important role in the protection of living cells since they have a wide range of biological activities including anti-inflammatory and chemopreventive. Resveratrol (3, 5, 4′ -trihydroxy-stilbene) is a naturally occurring phytoalexin present in grapes and many other plants. Many studies reported on the biological activities of resveratrol [1], but the most striking biological action, intensely investigated for the last decade is its antioxidant behavior in biological systems [2, 3]. Resveratrol has been reported to possess a potent antioxidant activity against the peroxidation of low-density lipoproteins (LDL) [4, 5] and liposomes [6], and to be a potent inhibitor of lipoxygenase [7]. Moreover, this powerful antioxidant has shown a broad range of biological effects such as neuroprotection, anti-inflammation, and anti-cancer [8, 9]. Resveratrol is able to attenuate neurodegeneration in animal models of Alzheimer's and Parkinson's diseases associated with the neuronal accumulation of β-amyloid and α-synuclein, respectively [10, 11].

Besides resveratrol, Murias et al. [12] studied the antioxidative features of many polyhydroxylated stilbenes.

The antioxidant activity of resveratrol is related to its hydroxyl (OH) groups which can scavenge free radicals produced in vivo [13, 14]. Indeed, Stivala et al. [15] reported that the deletion of all resveratrol hydroxyls as well as the substitution of these hydroxyls by OCH_3 decreased the antioxidant activity. When the 4′-hydroxyl of resveratrol was substituted by OCH_3, the EC50 value of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), used to express the antioxidant activity, was 48.6. Moreover, the EC50 value is 30.1

while the 3- or 5-hydroxyl is replaced by OCH_3. These results have shown that the hydroxyl group in the 4′-position is required for the antioxidant activity, but acts synergistically with the 3 and 5-OH groups. It was also found that some resveratrol derivatives bearing ortho-diphenoxyl functionality possess much higher antioxidative activity against free radical-induced peroxidation in membrane mimetic systems than those that do not possess such functionalities [14].

To find more efficient antioxidants by structural modification Cai et al. [3] synthesized resveratrol and related *trans*-stilbene analogues: 3, 4-dihydroxy-*trans*-stilbene (3, 4-DHS); 4, 4′-dihydroxy-*trans*-stilbene (4,4′-DHS); 4-hydroxy-*trans*-stilbene (4-HS) and 3,5-dihydroxy-*trans*-stilbene (3, 5-DHS) (Fig. 1) and studied in vitro, their antioxidant activity for the free radical-induced peroxidation of rat liver microsomes. They found that resveratrol and its analogues 3, 4-DHS; 4, 4′ –DHS; 4-HS and 3, 5-DHS, are effective antioxidants against both AAPH (a compound used extensively as a free radical generator and it is often used in the study of lipid peroxidation and the characterization of antioxidants) and iron-induced peroxidation of rat liver microsomes with an activity sequence of 3,4-DHS >4,4′ –DHS > resveratrol >4-HS >3,5-DHS. On the other hand, a kinetic analysis of the antioxidation process demonstrated that *trans*-stilbene derivatives are effective antioxidants against AAPH with the activity sequence of 3,3′-DM-4,4′-DHS >3,4-DHS > 2,4,4′-THS > resveratrol >3,5-DHS >4,4′-DHS >2,4-DHS, while the following sequence 3,4-DHS >3,3′-DM-4,4′-DHS >4,4′-DHS > resveratrol >2,4-DHS >2,4,4′-THS >3,5-DHS was obtained for Cu^{2+}-induced low density lipoprotein peroxidation [16].

Recently, Mikulski et al. [17] studied by DFT the antioxidant activity of *trans* and *cis* resveratrol, *trans*-4.4′-dihydroxystilbene (*trans*-4,4′-DHS), *trans*-3,4-dihydroxystilbene

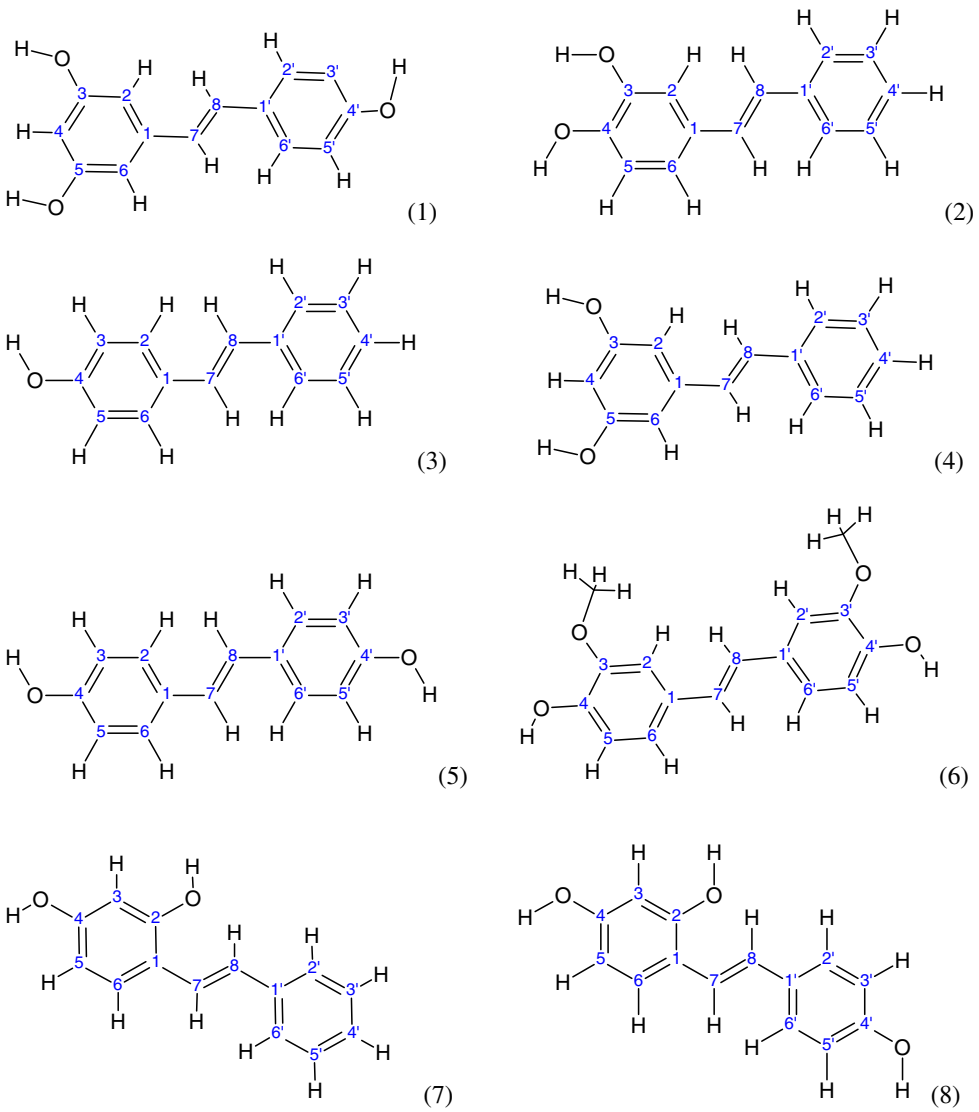

Fig. 1 Molecular structure of RSV (1); 3,4-DHS (2); 4-HS (3); 3,5-DHS (4); 4, 4′- DHS (5); 3,3′-DM-4,4′-DHS (6); 2,4-DHS (7) and 2,4,4′-THS (8)

(*trans*-3,4-DHS), *trans*-3,4,4′-trihydroxystilbene (*trans*-3,4,4′-THS), *trans*-3,4,5-trihydroxystilbene (*trans*-3,4,5-THS) and α, β-dihydro-3,4′,5-trihydroxystilbene (α, β -dihydro-3,4′,5-THS) and found that *trans*-3,4-DHS, *trans*-3,4,4′-THS, *trans*-3,4,5-THS and *trans*-4,4′-DHS exhibit higher antioxidant activity than *trans* resveratrol.

We report herein a DFT study on the antioxidative effect of resveratrol and related *trans*-stilbene analogues, 3,4-dihydroxy-*trans*-stilbene (3,4-DHS) (2); 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS) (3); 4-hydroxy-*trans*-stilbene (4-HS) (4); 3,5-dihydroxy-*trans*-stilbene (3,5-DHS) (5); 3,3′-dimethoxy-4,4′-dihydroxy-*trans*-stilbene (3,3′-DM-4,4′-DHS) (6); 2,4-dihydroxy-*trans*-stilbene (2,4-DHS) (7) and 2,4,4′-trihydroxy-*trans*-stilbene (2,4,4′-THS) (8).

Resveratrol is taken as a reference for stilbenes antioxidant activity, whereas 3,4-DHS and 4,4′-DHS, already reported to be more potent antioxidant than resveratrol by Mikulski et al. [17], are studied here, to illustrate the contribution of ortho- and para-quinone structures, generated from the original structures after H-abstraction, to the antioxidant activity. Mikulski et al. [17], for the same purpose, focused on the contribution of semiquinone structure of the phenoxy free radicals.

3,5-dihydroxy-*trans*-stilbene (3,5-DHS); 3,3′-dimethoxy-4,4′-dihydroxy-*trans*-stilbene (3,3′-DM-4,4′-DHS) (6); 2,4-dihydroxy-*trans*-stilbene (2,4-DHS) (7) and 2,4,4′-trihydroxy-*trans*-stilbene (2,4,4′-THS) are theoretically evaluated here, for the first time, for their antioxidant action mechanism.

This study includes the determination of bond dissociation energy (BDE), adiabatic ionisation potential (AIP), the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), and the single electron density distribution of the radicals. It also, aims to provide a theoretical explanation of the relationship between the antioxidant activity of some stilbenes and their molecular structures. Our calculations were based on 4-HS as a pharmacophore of resveratrol antioxidant activity [18]. We further investigated the influence of the number and position of hydroxyl and methoxy groups, on the antioxidant activity of the stilbene derivatives.

Methods

All calculations were performed using GAUSSIAN 03 program package [19]. Firstly, the original structures of RSV, its analogues and the corresponding free radicals were optimized by AM1 methods [20], then all structures were fully optimized using B3LYP method at 6-31G** basis set (Fig. 2). Our analysis includes the frontier orbitals HOMO

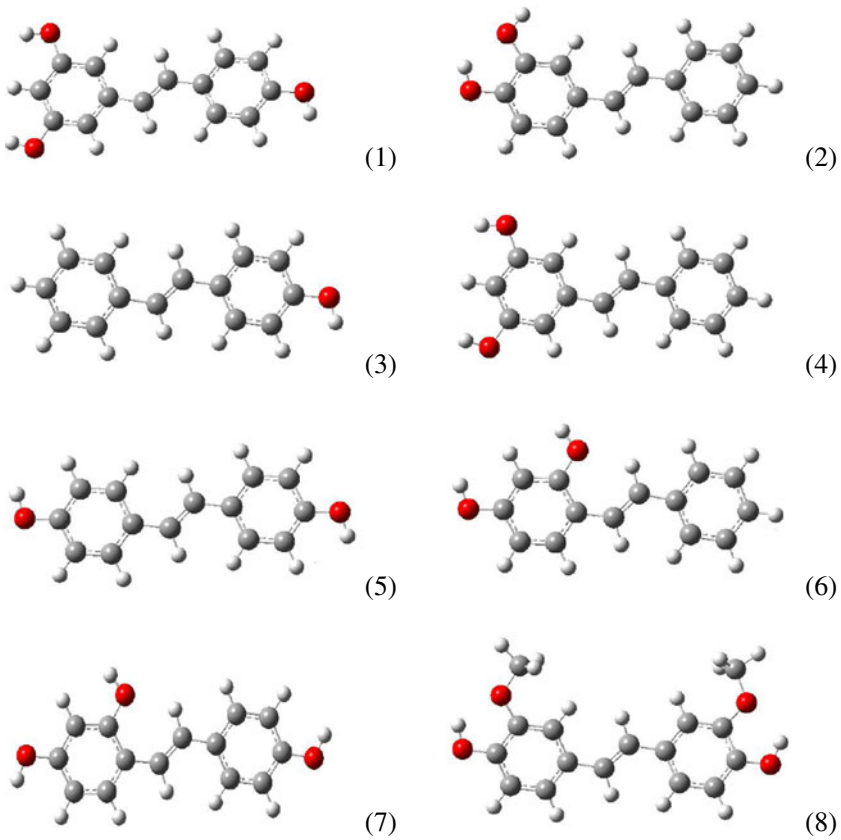

Fig. 2 Geometries of resveratrol and its analogues ground state molecules optimized by B3LYP/6-31G**. (1) RSV;(2) 4-HS; (3) 3,5-DHS; (4) 2,4-DHS; (5) 3,4-DHS; (6) 2, 4, 4′-THS; (7) 4, 4′-DHS and (8) 3, 3′-DM4, 4′-DHS

Table 1 The B3LYP/6-31G** optimized values of the dihedral angles α [°] and θ [°] and hydroxyl bond lengths [Å] of the antioxidants, studied in the gas phase

Compounds	α	θ	4′-OH	3-OH	5-OH	4-OH	2-OH	C=C parent	C=C 4-O-radical
Trans-resveratrol	0.02	0.02	0.963[a] 0.9663[b]	0.962[a] 0.9661[b]	0.962[a] 0.9661[b]	–	–	1.349	1.365
Trans-4,4′-DHS	0.00	0.00	0.962[a] 0.9662	–	–	0.962[a] 0.9662	–	1.349	1.368
Trans-4-HS	0.00	0.00	0.9663	–	–	–	–	1.348	1.366
Trans-3,4-DHS	0.01	0.01	–	0.9662	–	0.9660	–	1.349	1.362
Trans-3,5-DHS	0.01	0.01	–	0.9658	0.9658	–	–	1.347	1.348
Trans-2,4-DHS	0.00	0.00	–	–	–	0.9661	0.9663	1.351	1.371
Trans-2, 4,4′-DHS	0.01	0.01	0.9662	–	–	0.9662	0.9663	1.351	1.372
3,3′-DM- 4,4′-DHS	0.00	0.00	0.9699	–	–	0.9699	–	1.349	1.363

[a] From ref. [17]

[b] From ref. [27]

and LUMO energies, AIP, BDE of phenolic hydroxyl groups on each OH site, and the spin density distribution for the radicals formed after H-removal. The conformer with the lowest electronic energy was used for calculation. Using the standard state enthalpies at 1 atm and 298.15 K, the homolytic BDE values were calculated by the following relationship:

$$\mathrm{BDE} = \mathrm{H_{radical}} + \mathrm{H_H} - \mathrm{H_{molecule}} \quad (1)$$

where $H_{radical}$ is the total enthalpy of the free radical, H_H is the gas-phase total enthalpy of the hydrogen atom, and $H_{molecule}$ is the total enthalpy of the parent molecule.

AIP was calculated as the total energy of the radical species after the electron oxidation minus the total energy of the species before it was oxidized:

$$\mathrm{AIP} = \mathrm{E(ArOH^{\bullet +})} - \mathrm{E(ArOH)}. \quad (2)$$

Results and discussion

Molecular structure–antioxidant activity relationships of the studied stilbenes

From the optimized values of the dihedral angles α ($C_{6'}$-$C_{1'}$-C_8-C_7) and θ (C_8-C_7-C_1-C_2) at the B3LYP/6-31G** level (Table 1), it is evident that all resveratrol analogues studied in this work, are strictly planar. It must be pointed out that the strictly planar geometry of these compounds determines the energetically favorable delocalization of π-electrons, and the stacking interaction of the planarity of stilbenes system may also facilitate electron transfer.

In compound 3, 4-DHS there is a weak hydrogen bond between the 3-OH and 4-O with a bond length of 2.12013 and between the 3′-OCH_3 and 4′-OH in compound 3, 3′-DM-4, 4′-DHS, with a bond length of 2.07516 Å. These H-bonds stabilize the ground state molecules and inhibit the H-abstraction reaction. However, the free radicals formed after the H-abstraction reaction in 3- and 4-positions in 3, 4-DHS include stronger intramolecular hydrogen bonds with lengths of 1.94853 and 1.9717 Å respectively, which makes the reaction occur easily. The intramolecular hydrogen bonds can be helpful for the stabilization of the 3 and 4 positions free radicals. Therefore, we can say that the higher antioxidant activity of 3, 4-DHS is due to the intramolecular hydrogen bond effect after H-abstraction.

It seems from the geometrics optimized results (Table 2), and reversible conversion from single to double bond, that all resveratrol analogues studied have a semiquinone structure after H-abstraction.

As shown in Table 2, the bond length of C_4-O in 3, 4-DHS, decreased from 1.3606 Å to 1.2530 Å, while, the bonds C_2=C_3 and C_5=C_6 decreased from 1.3842 Å and 1.3939 Å to 1.37481 Å and 1.3677 Å, respectively. On the contrary, the bonds of C_3=C_4, C_4=C_5, C_6=C_1 and C_1=C_2 increased from 1.40910, 1.39102, 1.40525, and 1.41202 to

Table 2 The B3LYP/6-31G** optimized values of the C-O, C-C and C=C bond lengths [Å] in 3, 4 DHS in the gas phase

Compounds	C3-O	C4-O	C1-C2	C5-C6	C2-C3	C3-C4	C4-C5	C6-C1	C=C
3,4-DHS	1.3772	1.3606	1.4120	1.3939	1.3842	1.4091	1.3910	1.4052	1.349
4-Semiquinone	1.3379	1.2530	1.4369	1.3677	1.3748	1.4732	1.4423	1.4369	1.362
3,4 Quinone	1.2232	1.2183	1.3661	1.3462	1.4599	1.5546	1.4742	1.4763	1.352

1.47328, 1.44239, 1.43698 and 1.43698 Å, respectively. The most stable structure for both 4 and 4′-radicals is the semiquinone form, in which the unpaired electron is disposed on the whole molecule by the double bond. The semiquinone resonance structures are favorable to stabilize the free radicals. The 5-O radicals in RSV and 3, 5- DHS, also have the same resonance structure as the 4-radical, but the unpaired electron is mainly distributed on the 5, 2, 4 and 6 atoms.

HOMO and LUMO

Both HOMO and LUMO are the main orbitals involved in the chemical reaction. The HOMO energy characterizing the ability of electron-giving is suitable for representing the free radical scavenging potential of polyphenols, since the process to impede the auto-oxidation may involve not only the H-atom abstraction but also, the electron-transfer [21]. Higher values of ε_{HOMO} suggest that the molecule is a good electron donor [22]. On the other hand, the atomic sites characterized by high density of the HOMO distribution are very sensitive to the attack of free radicals and other reactive agents. The more HOMO orbital is delocalized, the more numerous are the electron sites, and more redox reactions will occur [17].

Figure 3 (a, a') shows that both HOMO and LUMO are delocalized in the whole 3,3′-DM-4,4′-DHS molecule except for C_3, $C_{3'}$, and $C_{5'}$ atoms and 3, and 3′-O-

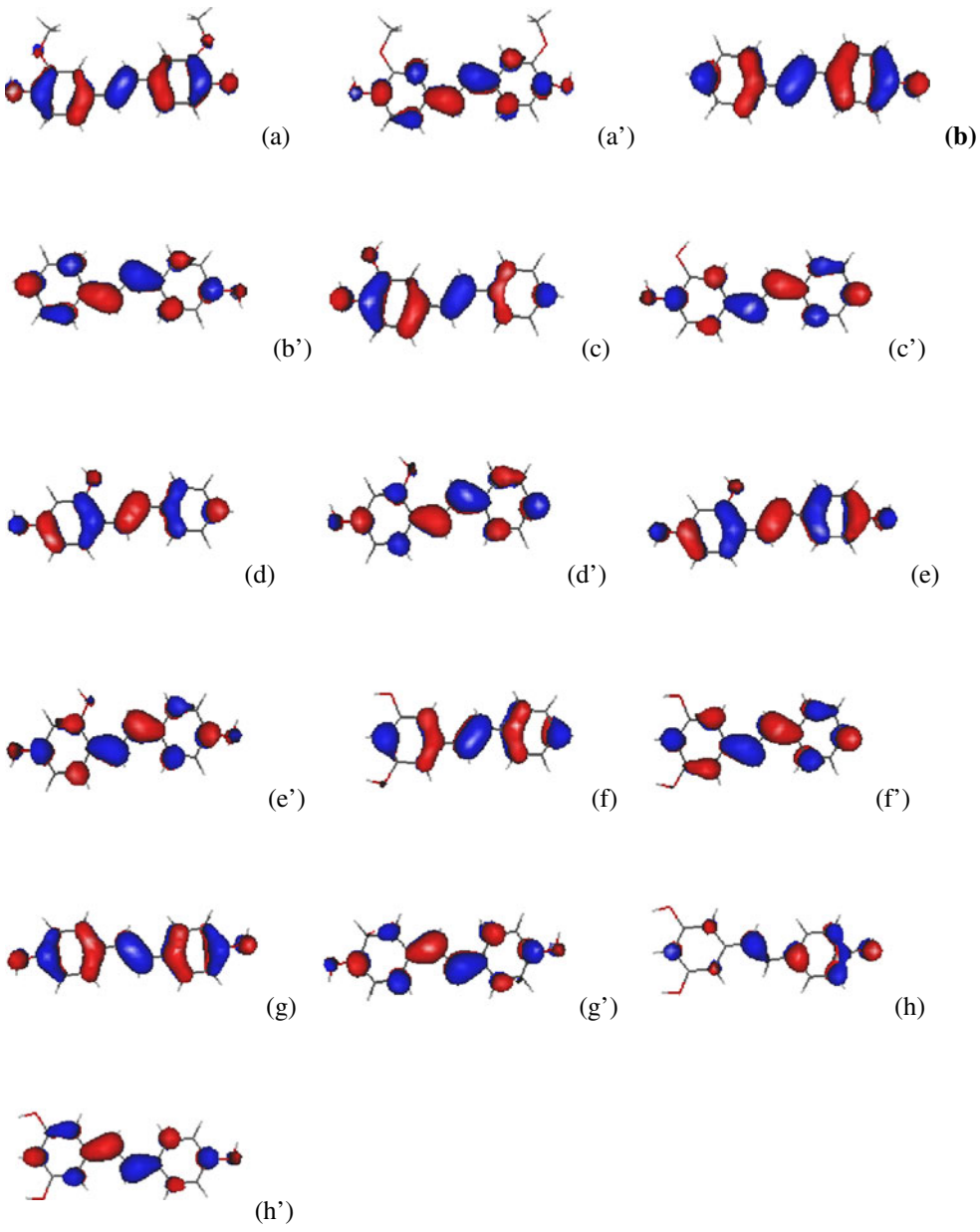

Fig. 3 The density distributions of HOMO and LUMO of 3,3′-DM-4,4′-DHS (**a**,a'); 4-HS (**b**,b'); 3,4-DHS (**c**, c'); 2,4-DHS (**d**,d'); 2,4,4′-THS (**e**,e'); 3,5-DHS (**f**,f'); 4,4′ -DHS (**g**,g') and RSV(**h**,h'), respectively

methoxy groups for LUMO. Considering the disposition of HOMO and LUMO orbitals, O_4 and $O_{4'}$ atoms of the two hydroxyls donate electrons easily so that it is possible to lose an electron during the H-abstraction reaction and form the electropositive free radical, then the proton transfer occurs. The OH groups of both 4 and 4′-positions are easily attacked by either the electrophilic or nucleophilic agents. The HOMO and LUMO electron density (Fig. 3c) in 3, 4 DHS is mainly distributed over the molecule except for the 3-OH position for the LUMO. Regarding 4-HS and 4, 4′- DHS, the hydroxyl positions are very sensitive to the attack of free radicals and other reactive agents, due to the high density of the HOMO distribution. Noting that, the HOMO and LUMO orbitals are distributed in all hydroxyl sites, but the HOMO density is higher in 4 and 4′-OH than

Table 3 Theoretical properties of resveratrol and derivatives

Compounds	E_{total}/Hartree	HOMO (ev)	LUMO (ev)	BDE (O-H) kJ mol^{-1}	AIP kJ mol^{-1}
33′-DM-44′-DHS	−920.222815	−4.8294	−1.0003		586.7466
Semi-quinone form	−919.589188	α=−5.0158 β=−6.0014	α=−1.6117 β=−3.3053	350.1211	
Para-quinone form	−918.967004	−5.6071	−3.4076	670.1976	
3,4-DHS	−691.170654	−5.1698	−1.2503		632.7136
4-O-radical	−690.550713	α=−5.2569 β=−6.3027	α=−1.6582 β=−3.4781	314.1890	
3-O-radical	−690.544007	α=−5.5309 β=−5.7971	α=−1.7050 β=−3.5562	331.7954	
Quinone form	−689.919739	−6.2311	−3.4659	657.3460	
4,4′-DHS	−691.169619	−5.0112	−1.0547		613.6919
Semi-quinone form	−690.536455	α=−5.1606 β=−6.1707	α=−1.4857 β=−3.3559	348.9055	
para-quinone form	−689.915495	−6.2039	−3.7431	665.78047	
4, HS	−615.9493435	−5.2420	−1.2109		640.9185
4-O-radical	−615.314812	α=−5.3927 β=−6.3522	α=−1.6413 β=−3.4963	352.4943	
3,5-DHS	−691.168631	−5.4972	−0.0487	371.5491	666.76398
3-O-radical	−690.5268428	α=−6.0011 β=−5.8256	α=−1.7611 β=−3.5997		
2,4-DHS	−691.16791	−5.0692	−1.0187		622.3615
4-O-radical	−690.53572	α=−5.3181 β=−6.3021	α=−0.0541 β=−3.4661	346.3485	
2-O-radical	−690.53840	α=−5.2822 β=−5.8520	α=−1.3896 β=−3.4577	339.3122	
2, 4,4′-THS	−766.38929	−4.8509	−0.8484		596.5397
4-O-radical	−765.75754	α=−5.0781 β=−6.0545	α=−1.3066 β=−3.3203	345.5608	
2-O-radical	−765.760422	α=−5.0278 β=−5.6406	α=−1.2280 β=−3.3099	337.62663	
4′-O-radical	−765.755869	α=−4.9848 β=−6.0197	α=−1.2751 β=−3.1946	349.5805	
para-quinone form	−765.137772	−6.1484	−3.6596	658.9279	
Resveratrol	−766.389138	−5.2183	−1.1714		635.9039
4′-O-radical	−765.755404	α=−5.4006 β=−6.1851	α=−1.6144 β=−3.5056	350.4023	
3-O-radical	−765.745116	α=−5.6975 β=−5.5209	α=−1.6207 β=−3.5467	377.4134	

in 2-OH position. It was noticed that the HOMO density was absent in both 3–OH and 5 –OH sites in 3, 5-DHS and RSV. If we look closely at Fig. 3, we can see that there is a significant HOMO density contribution from the double bond between C_7 and C_8 connecting the phenyl rings, in all RSV analogues. The vinyl double bond ensures π-electron delocalization between the A- and B-rings, contributes to the stabilization of RO· after H-abstraction, and hence contributes to the antioxidant activity.

The ε_{HOMO} eigenvalue of 4-HS is -5.2420 eV. The substitution of a hydroxyl group in 2, 3 and 4′ positions of the antioxidant pharmacophore 4-HS leads to compounds with εHOMO eigenvalues; −5.0692, −5.1698 and −5.0112 eV, respectively. These values are higher than that of RSV (−5.2183 eV). The most nucleophilic compounds in this theoretical study are 3,3′-DM-44′-DHS and 2, 4, 4′-THS with ε_{HOMO} values of −4.8294 ev and −4.8509 ev, respectively. The results obtained in the present work indicate that the existence of a catechol structure or the addition of the methoxy groups increased HOMO values.

Adiabatic ionization potential (AIP)

Both AIP and HOMO are used to determine the electron donating ability of a molecule. The electron abstraction is the first step of the antioxidant mechanism. Therefore, molecules with a lower AIP are more active. However, low values of AIP do not guarantee high antioxidant potency of the antioxidants studied [23]. A low AIP or a high

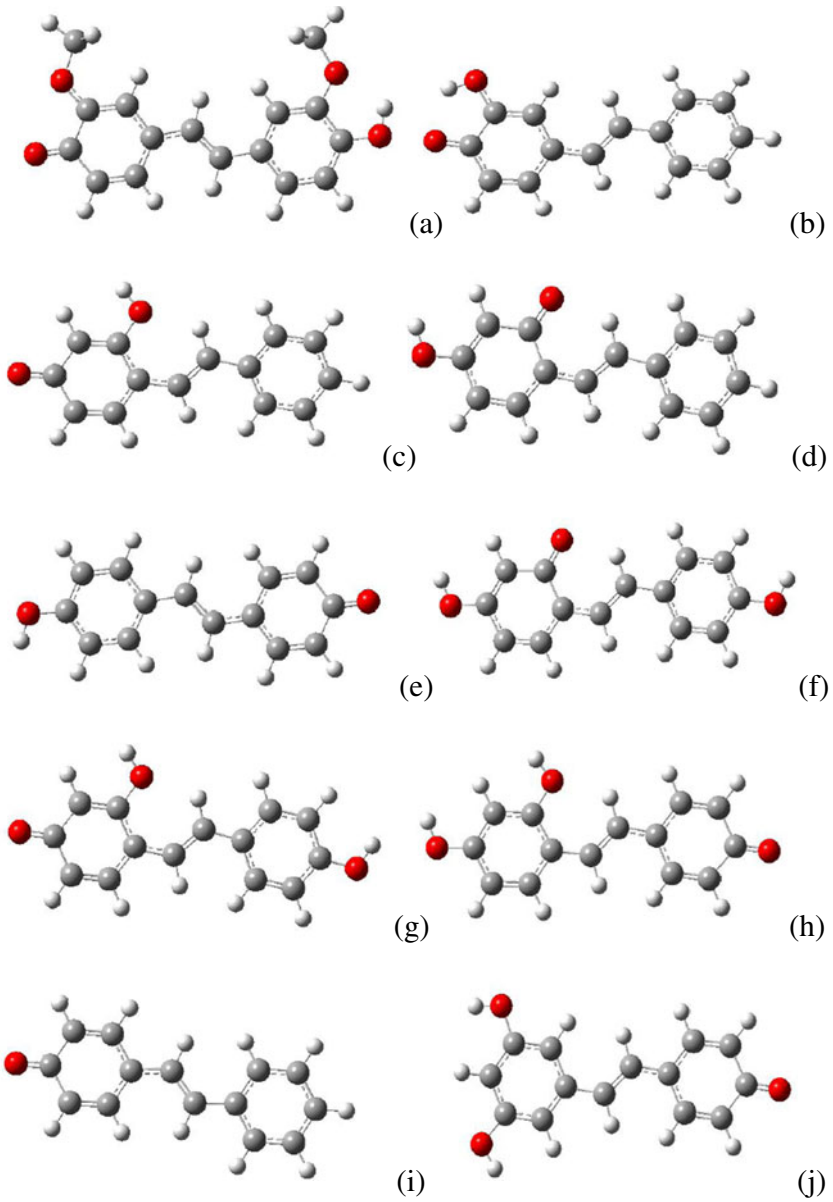

Fig. 4 The B3LYP/6-31** fully optimized structures of the resonance forms of 4-O-radical of 3, 3′-DM4, 4′-DHS (1); 3,4-DHS (2); 2,4-DHS (3); 4, 4′-DHS (5); 2,4,4′-THS(7); 4′-O-radical of 2,4,4′-THS (8); 4-HS (9) and RSV (10); and 2-O-radical of 2,4-DHS (4) and 2,4,4′-THS(6)

HOMO reflects a strong ability to donate the electrons [24]. The results in Table 3 reveal that the highest AIP values correspond to the lowest HOMO values. For example, 3, 3′-DM-4, 4′-DHS has the highest AIP but the lowest HOMO, while 3, 5-DHS has the lowest AIP but the highest BDE.

Bond dissociation energy (BDE)

The antioxidant activity reflected in the calculated BDE values is often attributed to π-electron delocalization, leading to the stabilization of the radicals obtained after H-abstraction. This conclusion is drawn assuming that, if π electron delocalization exists in the parent molecule, it also exists in the corresponding radical [25]. In order to understand the relationship between the electron delocalization and the reactivity of the radicals, we examined the electron distribution in the singly occupied molecular orbital (SOMO), called in this case, the α-HOMO. By comparing the shape of the α-HOMO to that of the first unoccupied β orbital, we found that α-HOMO is the highest-occupied molecular orbital of spin α, and it is delocalized over the entire molecule. The shape of α-HOMO is quite similar for all radicals, and does not exhibit sufficient variations to explain the differences in activity between the OH groups. Therefore, the shape of α–HOMO is not a reliable indicator for our investigation as it does not describe the global electronic behavior of the radical.

The spin density is often considered to be a more realistic parameter and provides a better representation of the reactivity [26]. The importance of the spin density for the description of RSV antioxidant activity has been pointed out by Cao et al. [27]. We have, therefore, decided to analyze the spin density of all radicals and used Eq. (1) to calculate the hydroxyls BDEs (Table 3).

The H-abstraction reactions of hydroxyls occur easily, because of their lower BDEs (Table 3) compared to the bond energy of the covalent O-H bonds in water (498.0 kJ mol^{-1}) [28]. BDE value of 4-HS is 352.494 kJ mol^{-1}. The

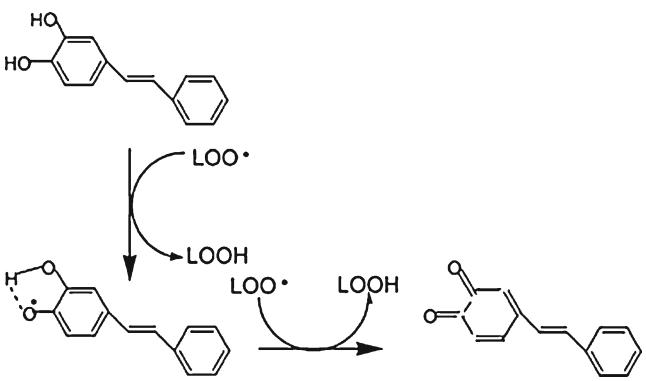

Fig. 5 Mechanism of 3, 4-DHS inhibited peroxidation

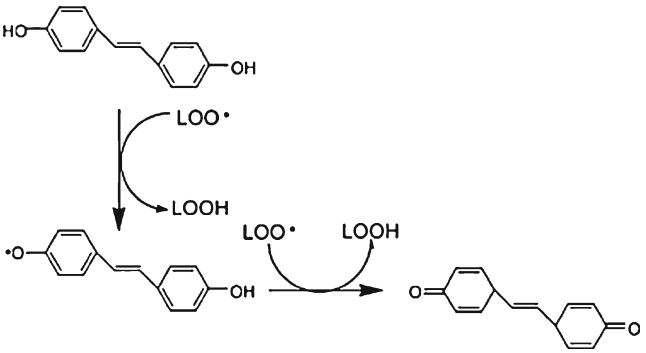

Fig. 6 Mechanism of 4, 4′-DHS inhibited peroxidation

addition of OH in 3, 2, and 4′ positions of 4-HS (to obtain 3,4-DHS; 2,4-DHS; and 4,4′-DHS, respectively) decreased the 4-O-radical BDE values to 314.1890, 346.3485 and 348.9055 kJ mol^{-1}, respectively. These BDE values are lower than that of 4′-O-radical of RSV (352.1 kJ mol^{-1}). On the other hand, the BDE value for the semiquinone form of 4, 4′-DHS was 348.9055 kJ mol^{-1}, the additional OH in 2-position leading to 2,4, 4′-THS decreased BDE value of 4′-O-radical to 345.5608 kJ mol^{-1}. The addition of two methoxy groups in both 3 and 3′ positions of 4, 4′-DHS increased the semiquinone BDE value to 350.1211 kJ mol^{-1}. The BDE of the 4-semiquinone form of 3,3′-DM-4,4′-DHS is higher than that of the 4, 4′DHS, despite that the spin density on the O-atom of the 4-OH radical of the 4, 4′ DHS (0.3124) is higher than that in 3,3′-DM-4;4′-DHS (0.2788). This difference is related to the existence of an H-bond between the hydrogen in the 4-OH and 4′-OH groups with the oxygen atoms of the methoxy groups on C_3, and C_3′ respectively. As a consequence, the BDEs on those sites are higher because the H-removal also implies the breaking of the H-bond. The BDE of 2-O radical in 2, 4 DHS was lower than the 4-O radical, and the BDE of 2-O radical in 2, 4, 4′ THS was lower than that of 4, 4′-O radical. The unpaired electron of the 2-OH radical is on C_1 (of 2, 4 DHS and 2, 4, 4′ THS), so that it can conjugate with the double bond. This indicates that the 2-O radicals are more stable than the 4, 4′-O radicals and the H-abstraction takes place easier in the 2- position than in the 4- and 4′-ones. For other stilbenes such as resveratrol, the 4′-O- radical is the most stable (Fig. 4).

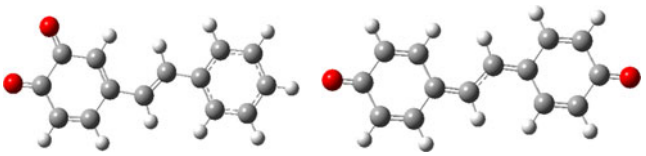

Fig. 7 The 3, 4 ortho- quinone and 4, 4′para- quinone structure of 3,4-DHS and 4, 4′-DHS

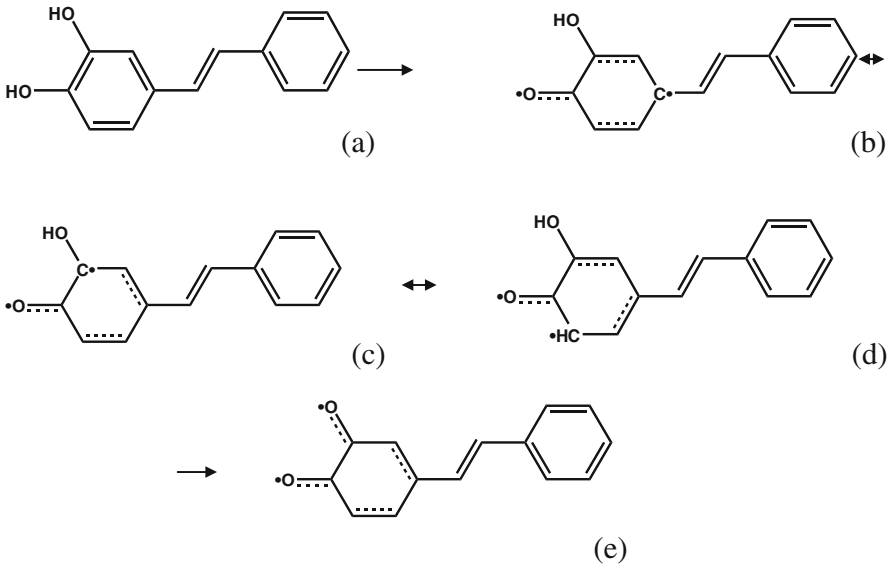

Fig. 8 The formation of semiquinone and quinone structures of 3, 4-DHS

Calculated BDE values for these compounds are in correspondence with the spin density results.

3, 4-DHS bearing ortho-diphenoxyl functionality, like rosmarinic acid [29], is remarkably more active than resveratrol and other ROHs. This is understood in view of the antioxidation mechanism of phenolic antioxidants as exemplified in Fig. 5.

As shown in Fig. 6, the 4′-OH group in 4, 4′DHS, also enhances the activity since it is able to stabilize the phenoxyl radical intermediate by resonance through the trans-double bond and be further oxidized to a para-quinone [3], nevertheless, there has been no experimental or theoretical supporting evidence.

Jovanovic et al. [30] proved experimentally that the ortho-position phenolic hydroxyls of flavonoids could undergo an abstraction of two hydrogen atoms and form a quinone structure, but there is no experimental data proving the abstraction of the second hydrogen in the para position of the 4,4′-DHS and other stilbenes, to give the para-quinone structure. We have calculated the BDE of the quinone formed by losing both hydrogen atoms in both 3, 4-ortho and 4, 4′-para-quinone positions. Figure 7 shows the geometry optimization of both ortho and para quinone structures of 3, 4-DHS and 4, 4′-DHS respectively.

We calculated the BDE of the quinone structure (Table 3) formed by losing the two hydrogen atoms in the ring A of 3,4-DHS. It is 657.3460 kJ mol^{-1} and only 11.3615 kJ mol^{-1} different from the BDE sum of losing 4-H and 3-H, respectively. On the other hand, the BDEs of the para 4, 4′-quinone structure of 3,3′-DM-4,4′-DHS, 4, 4′-DHS and 2,4,4′-THS were 670.1976, 665.7707 and 658.9279 kJ mol^{-1}. The half of these values are 335.0988, 332.8853 and 329.46395 kJ mol^{-1}, respectively, and are lower than those obtained for the semiquinone radicals BDEs (348.9055, 350.1211 and 345.5608 kJ mol^{-1}) (Table 3); this shows the relative stability of the para-quinone structure.

As shown in Table 2, the C_3–O and C_4–O bond lengths in 3, 4-DHS parent molecule decreased from 1.3772 and 1.3606 to 1.22322 and 1.2183 Å, in the 3, 4 quinone form.

The same change was observed in C_1–C_2 and C_5–C_6 bond lengths, decreasing from 1.4120 and 1.3939 to 1.36619 and 1.3462 Å respectively. Furthermore, C_2–C_3, C_3–C_4, C_4–C_5, and C_6–C_1, bond lengths increased from 1.3842, 1.4091, 1.3910 and 1.4052 to 1.4599, 1.5546, 1.4742 and 1.4763 Å respectively.

The HOMO and LUMO energies of the 3,4-DHS ground state molecule are −5.1698 and −1.2503 eV, respectively. After the semiquinone structure formation, the 4-O radical HOMO energy is −5.2569 eV and LUMO is −1.6582 eV, the HOMO energy of the 3-O-radical is −5.5309 eV, and the LUMO is −1.7050 eV. For the quinone structure, the HOMO and LUMO energies are −6.2311 eV and −3.4659 eV, respectively. This indicates that the semiquinone form would provide an electron again to continue the H-abstraction.

As shown in reaction mechanism (Fig. 8), the first H-abstraction in 3,4-DHS occurs in the 4-position and the radical formed will be stabilized by the semiquinone

Table 4 Spin density on the oxygen atoms in phenoxy radicals of *trans*-resveratrol and its analogues

Compounds	4-OH	2-OH	3-OH	5-OH	4′-OH
Trans-resveratrol	–	–	0.4415	–	0.3211
4-HS	0.3205	–	–	–	–
3,5-DHS	–	–	0.4143	–	–
2,4-DHS	0.2928	0.2533	–	–	–
3,4-DHS	0.2853	–	0.3317	–	–
2,4,4′-THS	0.2841	0.2407	–	–	0.3119
4,4′-DHS	0.3124	–	–	–	–
3,3′-DM -4,4′-DHS	0.2788	–	–	–	–

structure (Fig. 8a and b) after passing through the three resonance structures (Fig. 8b–d). The hydrogen atom of the ortho-position hydroxyl is abstracted again and the quinone structure is formed (Fig. 8d and e).

According to the discussion above, it would be inferred that the semiquinone free radical and the 3, 4 ortho-quinone structure are the proper explanations for the higher antioxidant activity of 3, 4-DHS. The same observation was reported for resveratrol by Cao et al. [27]. Concerning the compounds bearing 4, 4′-OH, such as 4, 4′-DHS, an H-abstraction may occur at the 4-position of the A ring and a semiquinone free radical is formed. The semiquinone free radical and the 4, 4′-para quinone structure are the proper explanations for the higher antioxidant activity of 4, 4′-DHS, 3, 3′-DM4, 4′-DHS and 2, 4, 4′-THS. Moreover, the oxygen atoms of the ortho-hydroxyls (4-OH to 3-methoxy and 4′-OH to 3′-methoxy groups for 3, 3′-DM-4, 4′-DHS and 4–OH to 3-OH in 3, 4 -DHS) contribute to the single

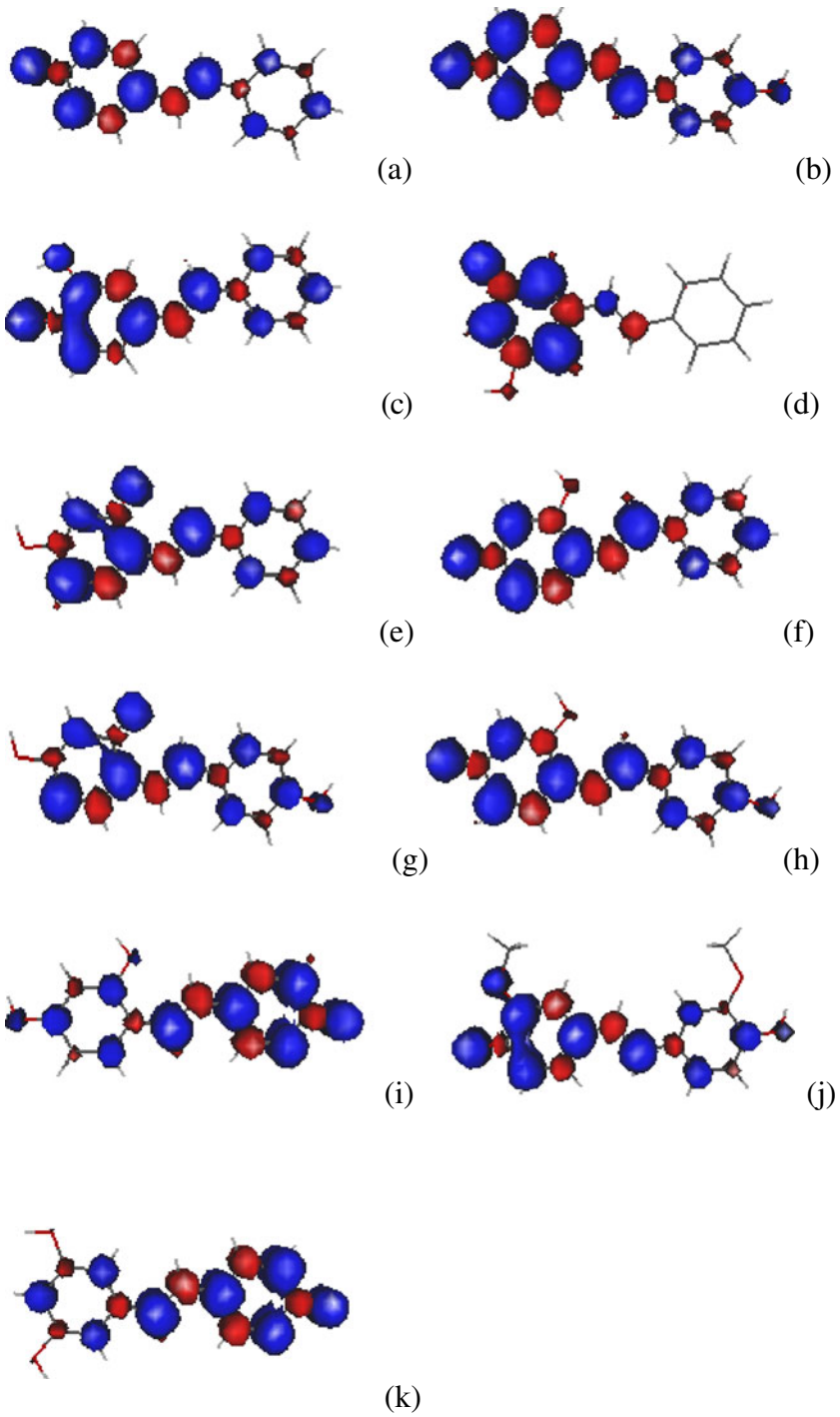

Fig. 9 Distribution of spin densities in the radicals formed by H-removal from the A and B rings for 4-HS (1); 4, 4′-DHS (2); 3,4-DHS (3); 3,5-DHS (4); 2,4-DHS (5, 6); 2,4,4′-THS (7, 8, 9); 3, 3′-DM4, 4′-DHS (10) and resveratrol (11) computed at B3LYP/6-31G**

electron dispersing, this is why the spin density has been reduced from 0.3124 in 4, 4′-DHS to 0.2788 in 3,3′-DM-4,4′-DHS and from 0.3205 in 4-HS to 0.2853 in 3,4-DHS, respectively. The inductive effect has an important contribution in the stabilization of the free radical as well as the hydrogen bond effect. The 2-O-radical of 2,4,4′-THS, displays a low spin density compared to the 4 and 4′-O-radicals because of the resonance effects.

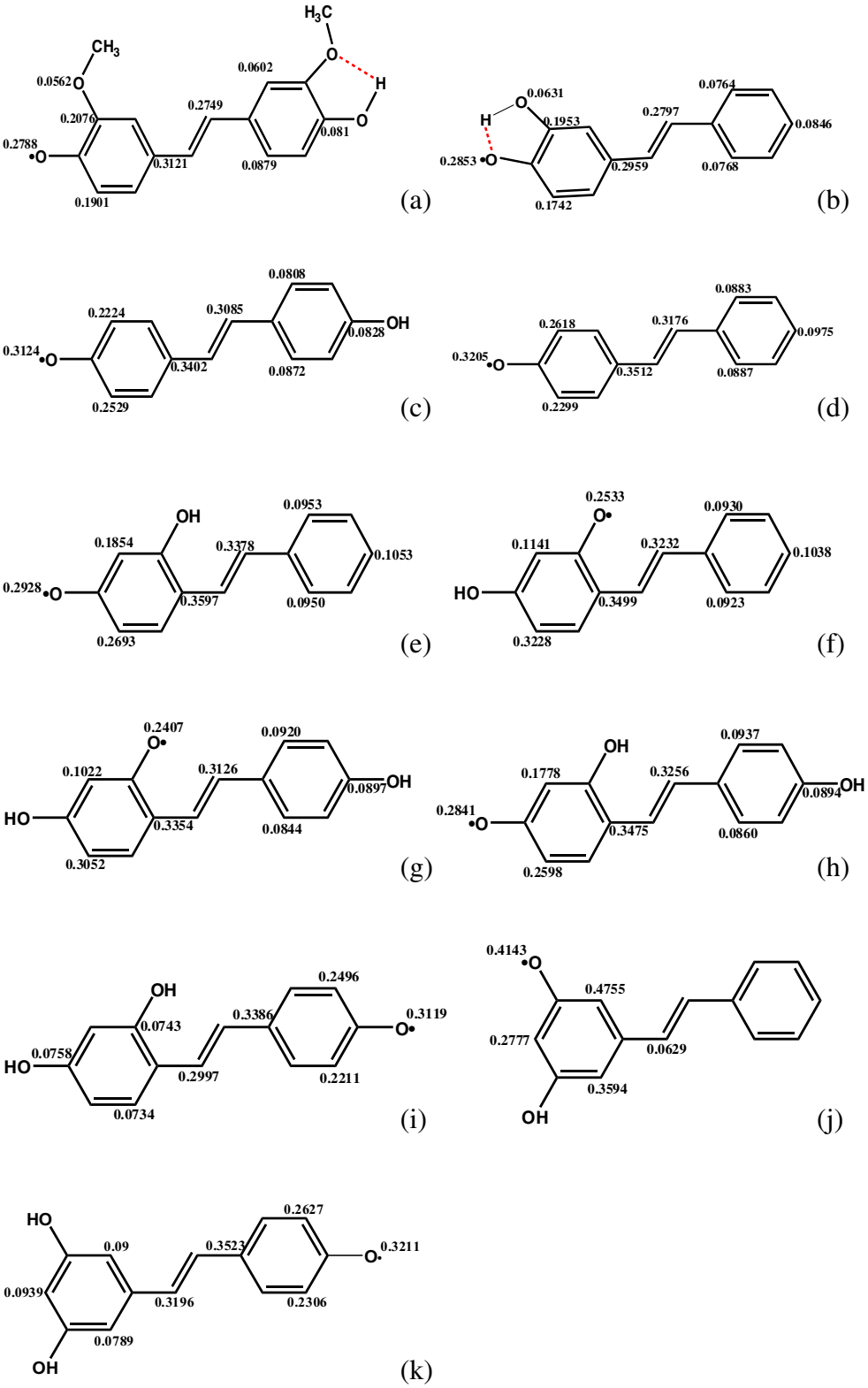

Fig. 10 Spin density in the 4-O-radical of 3, 3′-DM 4, 4′-DHS (1); 3,4-DHS (2); 4, 4′-DHS (3); 4-HS (4); 2,4-DHS (5);, and 2,4,4′-THS(8); 4′-O-radical of 2,4,4′-THS (9) and resveratrol (11); 2-O-radical of 2,4-DHS (6) and 2,4,4′-DHS (7) and 3-O-radical of 3,5-DHS (10) computed at B3LYP/6-31G**

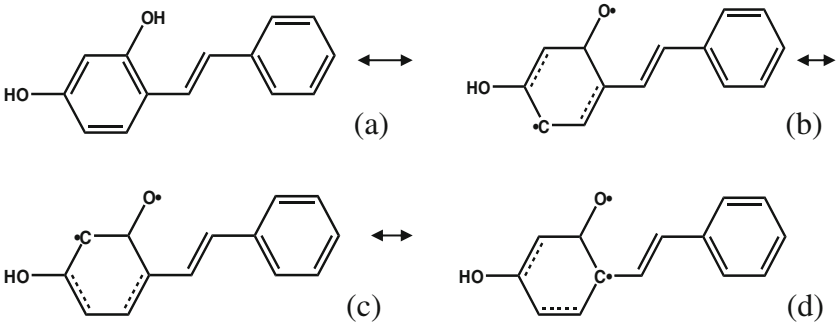

Fig. 11 The resonance structures of the 2, 4-DHS radicals

Spin density

Another molecular parameter correlated with the free radical scavenging activity is the spin density, which was also calculated. Spin density characterizes the distribution of electron spin in the free radicals and is responsible for their stability [17]. It must be stressed that the more delocalized the spin density in the radical, the easier the radical formed and thus the lower the BDE [31]. The spin density on the 4-O-radical in the 3, 4-DHS is 0.2853 (Table 4), whereas it is 0.3317 for the 3-O-radical (Fig. 9). This is a consequence of delocalization effects due to the presence of the double bond between C_7 and C_8, which allows for spin presence on the C_8. Indeed, the dominant structures of the 4-O-radicals are those in which the unpaired electron is disposed on the whole molecule. The 3-O-radical also has the same resonance structure as the 4-O-radical, but the unpaired electron is mainly distributed on the A ring (Fig. 9). Unlike the unpaired electron on C_1 of the 4-O-radical, the unpaired electron on the C_2 of the 3-O-radical can not conjugate with the double bond.

Here, it must be stressed that a hydroxyl group substituted on a carbon with positive spin density increases the stability of a radical and has the opposite effect when substituted on a carbon with negative spin density [32].

Note that the spin density on 4-O- atom in 4- HS is 0.3205 and it is delocalized through the whole molecule as shown in Fig. 9. C_1 (0.3512), C_3 (0.2618), C_5 (0.2299) and C_8 (0.3176) atoms are positive spin density centers, while C_2, C_4, C_6 are negative ones. With the addition of an OH-group on the 3-ortho position of 4- HS, the spin density on 4-O-radical decreases to 0.2853, with the delocalization on C_1 (0.2959), C_3 (0.1953), C_5 (0.1742) and C_8 (0.2797). Moreover, the 3-OH added is substituted on a carbon with positive spin density as shown in Fig. 10, so it increases the stability of the 4-O-radical. The addition of an OH-group on the 4′-para position in 4-HS, decreases the spin densities on the 4-O-radical to 0.3402 (C_1), 0.2224 (C_3), 0.2529 (C_5) and 0.3085 (C_8). In 4, 4′-DHS, 4′-OH is substituted on a carbon with positive spin density which contributes to the stability of the radical. On the other hand, 3,3′-DM-4,4′-DHS is the result of adding two methoxy groups in 4,4′-DHS on 3 and 3′ -positions, the spin density on 4-O-radical decreases to 0.2788, with delocalization on C_1(0.3121), C_3 (0.2076), C_5(0.1901) and C_8 (0.2749). In view of BDEs, AIP and spin density values (Tables 3 and 4), it appears that O_3 and O_5 have almost no contribution to resveratrol antioxidant activity. As a consequence, 3, 5-DHS is not a good antioxidant.

The spin density on 4-O-radical of 2,4-DHS is 0.2928 and localized on C_1 (0.3597), C_3 (0.1854), C_5 (0.2693) and C_8 (0.3378). The addition of an OH group to the 2-position of 4-HS, unlike the other analogues, increases the spin density on C_8. It is noticed that the concentration in B-ring is slightly increased. The same finding was obtained with 2, 4, 4′-THS (addition of an OH to 4, 4′DHS on the 2-position). On the other hand, the spin density value of 2-O-radical is 0.2533 in 2,4-DHS and 0.2407 in 2, 4, 4′-THS respectively. The later spin density values are lower than that of 3,4-DHS 4-O radical (0.2853), despite higher BDE values; 339 and 337 kJ mol^{-1} for 2,4-DHS and 2, 4, 4′-THS respectively, compared with that of 3,4- DHS (314.1890 kJ mol^{-1}). This is the effect of the vicinity of the double bond to the 2-position in both 2, 4-DHS and 2, 4, 4′-THS. The most stable structure for these molecules is that in which the unpaired electron is localized on the ortho position of the 2-O-radical (Figs. 11 and 4). The calculated BDEs of these compounds confirm this result.

The low values of spin density in O-radicals studied show that these compounds are excellent antioxidants.

The experimental observations about some resveratrol derivatives antioxidant activity were satisfactorily explained by the results obtained by Cheng et al. [16] and Cai et al. [3]. Our results confirmed and provided deeper understanding of these experimental observations. Furthermore, we must point out that our theoretical study supports the experimental one about the 2,4,4′-THS antioxidant activity found to be higher than that of resveratrol for AAPH test but not for the Cu^{2+}-induced LDL peroxidation [16].

Conclusions

The results of this DFT computations revealed that *trans*-3, 4-DHS; *trans*-3, 3′-DM4, 4′-DHS and 4, 4′-DHS exhibit

remarkably high antioxidant activity. Moreover, the later mentioned compounds are more active antioxidants than *trans*-resveratrol. However, 3,5-DHS and 4-HS are less able to scavenge free radicals than *trans*-resveratrol. These results are in full accordance with experimental results indicating that molecules bearing ortho-dihydroxyl or 4-hydroxy-3-methoxyl groups possess significantly higher antioxidant activity than those bearing no such functionalities. Our calculations demonstrate that the antioxidant potency depends on: (i) the geometry of the neutral compounds and their phenoxy radicals, (ii) the number and position of the hydroxyl groups, (iii) the semiquinone and (iv) quinone structures. It is worth noting that the inductive effect of both hydroxyl and methoxy groups, and the intramolecular hydrogen bond effect after H-abstraction contribute strongly to the antioxidant activity. The OH addition in the 2-position of 4-HS, unlike the other analogues, increases the spin density on C_8 at the double bond. On the other hand, the electron or hydrogen abstraction in ortho position is more favored than in para position for 2, 4-DHS and 2,4,4′-THS. The low spin density in the 2-position compared with the 4-position, revealed that the antioxidant pharmacophore of these two compounds is 2-hydroxystilbene.

AIP values were significantly higher than those of BDE for all compounds studied. In conclusion, this work demonstrates that H-atom transfer takes the main part in free radicals scavenging activity of the antioxidants studied and this mechanism dominates over the single-electron transfer.

Finally, this work contributes to the understanding of the pharmacological activity of the compounds studied. These findings could be useful to both food and pharmaceutical industries.

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