

## ARTICLE

**DFT Study on Molecular Structures and ROS Scavenging Mechanisms of Novel Antioxidants from *Lespedeza Virgata***

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The molecular structure and radical scavenging activity of three novel antioxidants from *Lespedeza Virgata*, lespedezavirgatal, lespedezavirgatal, and lespedezacoumestan, have been studied using density functional theory with the B3LYP and BhandHLYP methods. The optimized geometries of neutral, radical cation, radical and anion forms were obtained at the B3LYP/6-31G(d) level, in which it was found that all the most stable conformations contain intramolecular hydrogen bonds. The same results were obtained from the MP2 method. The homolytic O–H bond dissociation enthalpy and the adiabatic ionization potential of neutral and anion forms for the three new antioxidants and adiabatic electron affinity and H-atom affinity for hydroxyl radical, superoxide anion radical, and hydrogen peroxide radical were determined both in gas phase and in aqueous solution using IEF-PCM and CPCM model with UAHF or Bondi cavity. The antioxidant activities and reactive oxygen species scavenging mechanisms were then discussed, and the results obtained from different methods are consistent. Furthermore, the antioxidant activities are consistent with the experimental findings of the compounds under investigation.

**Key words:** Molecular structure, Reactive oxygen species scavenging mechanism, Novel antioxidant, Density functional theory

**I. INTRODUCTION**

Reactive oxygen species (ROS) in human body are constantly formed by  $\gamma$ -rays, X-rays, and UV radiations [1–3]. They play important roles in the oxidative damage of nucleic acids, lipids, proteins and carbohydrates that may result in cellular damage, aging, and human diseases [4–8]. In particular, the hydroxyl radical (OH $\cdot$ ), a highly reactive species can cause damages in some proteins [4] and modify purine and pyrimidine bases which result in DNA damage [6]. Antioxidants can counteract the oxidative damage and thus are believed to protect against cancer, coronary heart disease, neurodegenerative diseases, and other diseases [8–13].

*Lespedeza* species, distributed in East Asia and North America, have been used as medicinal plants. As previous reports, the extracts from *Lespedeza capitata* were used to treat kidney disease in France, Germany, and Poland [14–17]. The extracts from stems of *Lespedeza homoloba* showed strong antioxidant activity against lipid peroxidation in the rat brain homogenate test [18, 19]. Recently, it was reported that the extracts from *Lespedeza cuneata* also had strong antioxidant activity against lipid peroxidation *in vitro* [20]. The extracts from *Lespedeza cyrto-*

*botrya* showed the radical scavenging activity in both a 2'-azino-di-3'-ethylbenzthiazoline sulfonate system and a 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl system [21]. *Lespedeza Virgata* (Thunb.) DC. is a Chinese herb, which is used in treatment of various nephropathies in central China. Until now, total fifteen flavonoids, two benzofurans and one coumestan compounds have been isolated and identified by Liu's groups and Deng's [22, 23]. The flavonoids showed strong superoxide anion radical scavenging activity by the hypoxanthine nitro blue tetrazolium and electron spin resonance methods [22]. Therein, two 2-arylbenzofurans, lespedezavirgatal and lespedezavirgatal, a new coumestan, lespedezacoumestan are novel compounds (Fig.1). They showed strong oxygen radical absorbance capacity and potent inhibition toward lipid peroxidation in both plasma and kidney homogenate of kidney failure rats [23].

All the previous experimental studies have contributed positively to the search for potent antioxidants from *Lespedeza* species. There is only one theoretical work to study the antioxidant activities of lespedezaflavonone, another new compound from *Lespedeza Virgata* [24]. As far as we know, none of the previous studies have provided ROS radicals scavenging mechanism and the comparison of the theoretical values and experimental results for the new benzofurans and coumestan antioxidants.

At least three mechanisms were reported for radi-

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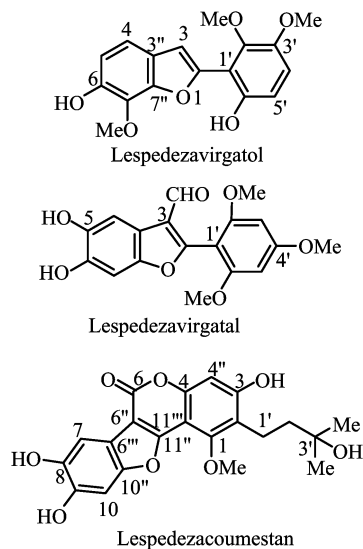


FIG. 1 Structures of new antioxidants from *Lespedeza Virgata*.

cal scavenging processes of antioxidants (AH, Fig.2) [25–32]: one-step H-atom transfer (HAT), stepwise electron-transfer/proton-transfer (ET-PT), and sequential-proton-loss-electron-transfer (SPLET). All of them may occur in parallel with different rates in a certain chemical or biological system [26, 27, 30]. Wherein, bond dissociation enthalpy (BDE), ionization potential (IP) of neutral and anion forms for antioxidants [33–36] and H-atom affinity (HA) and electron affinity (EA) [24, 27] for ROS radicals are the controlling parameters in these mechanisms.

The IP values for antioxidants were computed as the energy differences between a neutral molecule and the respective cation free radical or between an anion and the respective radical. The BDE for antioxidants were calculated as the enthalpy differences between a neutral molecule and the respective radical plus hydrogen radical. The BDE values were computed for all O–H groups in each molecule and the values given and discussed hereafter were those having the lowest absolute values regarding the most active O–H group located in the molecule. The HA for ROS radicals were predicted as the enthalpy differences between a neutral molecule and the respective radical plus hydrogen radical. The EAs for ROS radicals were computed as the energy differences between an anion and the respective radical.

In this work, molecular structures, the BDE and IP values for new antioxidant, and HA and EA values for OH $\cdot$ , superoxide anion radical (O $_2^{\cdot-}$ ), and hydrogen peroxide radical (HO $_2^{\cdot}$ ) are predicted in gas phase. Since water is the main component of all the physiological liquids, the influences of water medium on the physico-chemical parameters were also investigated. On the basis of the values, discussions were then made about the antioxidant activities, ROS scavenging mechanisms, and the comparison with the experimental data

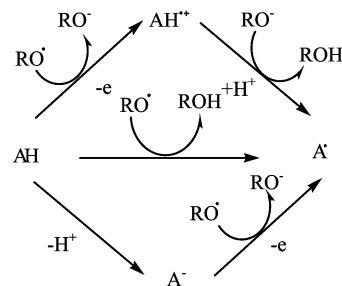


FIG. 2 Three mechanisms, stepwise electron-transfer/proton-transfer (above), one-step H-atom transfer (middle) and sequential-proton-loss-electron-transfer (below), proposed to elucidate radical scavenging of antioxidants.

of compounds. We hope to highlight the potent antioxidant activities of compounds from *Lespedeza* species and stimulate the interest for further studies and exploitation in pharmaceutical industries of extracts in *Lespedeza* species.

## II. COMPUTATIONAL METHODS

All calculations were performed by the Gaussian 03 suite of programs [37]. Conformation analysis and geometry optimizations were carried out using the B3LYP [38, 39] and MP2 [40] methods with the 6-31G(d) basis set. The optimized structures were confirmed as true minima by vibrational analysis at the same level. Zero-point, enthalpy and entropy corrections were extracted from the frequency calculations at the B3LYP/6-31G(d) level. Single-point electronic energies (SPEs) were then calculated using the B3LYP and BHandHLYP [38, 41] methods with the 6-311++G(2df,2p) basis set, and then SPEs were converted to the enthalpy values at 298 K, 101 kPa, by adding the thermal contributions to enthalpy (TCE, in which the vibrational contributions include zero-point vibrational energy).

The solvent effects calculations were carried out using the integral equation formulation-polarized continuum model (IEF-PCM) [42] and the polarizable conductor salvation model (CPCM) [43, 44] with the UAHF or Bondi solvation radii in aqueous solution ( $\epsilon=78.39$ ) at the BHandHLYP/6-311++G(2df,2p) and B3LYP/6-311++G(2df,2p) levels of theory.

## III. RESULTS AND DISCUSSION

### A. Conformational analysis

In phenolic compounds, the behavior of the different OH groups is largely influenced by the neighboring groups and the geometry. Thus, conformation can be regarded as the first parameter of importance to analyze the radical scavenging activity of new phenolic compounds [30]. To compute all the appropriate values

of the selected physiochemical parameters which may characterize the antioxidant potency, all the possible conformations (8 conformations for lespedezavirgatal and lespedezavirgatal, 16 conformations for lespedezacoumestan) were optimized at the B3LYP/6-31G(d) and MP2/6-31G(d) levels to locate the one with the lowest energy. The most stable structures of the three compounds obtained from B3LYP/6-31G(d) method are consistent with those obtained from MP2/6-31G(d) method.

To treat the solvent effect, both specific water molecules and bulk aqueous medium were investigated. Considering the effect of the hydrogen bonds between the water molecules and the three compounds, the structures of four water molecules placed at O1, O6–H, O7–Me, and O6'–H in lespedezavirgatal, O1, C3=O, O5–H, and O6–H in lespedezavirgatal, and O1, O3–H, O8–H, and O9–H in lespedezacoumestan, respectively, were studied. The complexations were optimized at the B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory. The most stable structures of the three compounds remain unchanged whether those are isolated or in complexations, thus water molecules weren't included in the calculations of the physico-chemical parameters [45].

The most stable conformations of isolated compounds optimized at the B3LYP/6-31G(d) level are shown in Fig.3. The most stable conformations were those possessing intramolecular hydrogen bonds (IHBs). Two IHBs are formed in global minimum structure of lespedezavirgatal. One is between the hydroxyl group and the methoxy in A-ring. The other is the hydroxyl group in C-ring and the neutral O-atom in B-ring via a six-membered ring. In global minimum structure of lespedezavirgatal, the O5–H group and the O atom of the O6–H group in A-ring form an IHB. The O9–H group and the O atom of the O8–H group in A-ring form an IHB in most stable conformation of lespedezacoumestan.

## B. O–H bond dissociation enthalpy of antioxidants

As discussed previously, the BDE and the adiabatic IP are the important parameters for the radical-scavenging activity of the antioxidants. We predicted the BDE and IP not only in the gas phase but also in aqueous solution since water is the main component of physiological liquids.

The BDEs computed with different methods for each O–H group of new antioxidants are listed in Table I. To lespedezavirgatal, the BDE of O6–H is lower than that of O6'–H both in gas phase and in solution with different methods, which suggests that the hydrogen atom in O6–H group is easier to be abstracted than that in O6'–H group. In addition, once the hydrogen atom in O6–H group is abstracted by free radicals, a cyclic compound can be formed easily by donating a second hydrogen atom to free radicals (Fig.4) with a very low

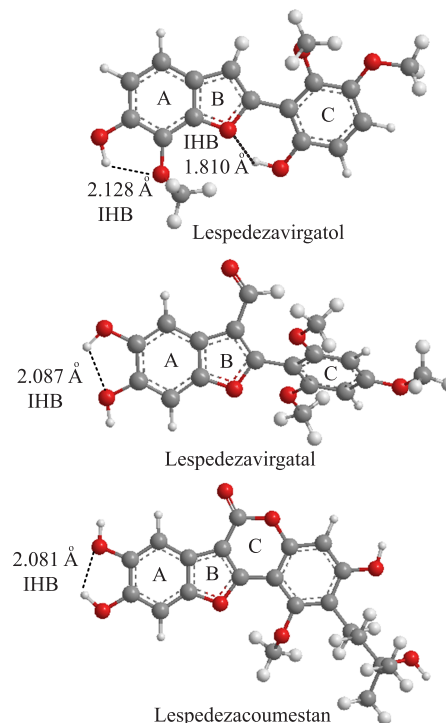


FIG. 3 Optimized structures of the most stable conformations for new antioxidants studied at the B3LYP/6-31G(d) level.

enthalpy value ( $<230.0$  kJ/mol either in gas phase or in aqueous solution with different methods). O6–H BDE is much lower than O5–H BDE obtained from different methods both in gas phase and in aqueous solution for lespedezavirgatal, which shows that the hydrogen atom in O6–H is more active than that in O5–H. In addition, O8–H BDE is the lowest in gas phase and O9–H BDE is the lowest in solution among the four O–H BDEs for lespedezacoumestan. Our data revealed O8 position and O9 position being the most probable sites of H-atom abstraction in gas phase and solution respectively. The consistent results of the three compounds are obtained from different methods. Moreover, the cavities affect the BDE values, but have no effect on the hydrogen donor abilities of hydroxyl groups in various positions.

All the BDEs for the most active hydroxyl group of new antioxidants both in gas phase and in aqueous solution are lower than the experimental data of phenol (370.8 kJ/mol) [46]. These data are comparable to those of widely used pigments such as curcumin (359.9 kJ/mol), maritimetin (329.4 kJ/mol) and related analogues (311.4–358.2 kJ/mol), natural polyphenolic flavonoids delphinidin (301.4 kJ/mol), myricetin (303.1 kJ/mol) and quercetin (329.0 kJ/mol), potent natural antioxidants  $\alpha$ -tocopherol (338.2 kJ/mol), the most bioactive component of vitamin E, and epigallocatechin-3-gallate (316.8 kJ/mol), the most active compound in green tea [30, 47–49]. In particu-

TABLE I O–H bond dissociation enthalpy (BDE, kJ/mol) values for lespedezavirgatal (1), lespedezavirgatal (2), and lespedezacoumestan (3) at 298 K in the gas phase and in aqueous solution.

	Gas phase				Aqueous solution						
	B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM		
			UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	
1	O6–H	328.2	325.7	320.6	305.2	321.1	304.7	320.2	303.9	320.2	303.9
	O6'–H	332.8	329.4	324.8	320.2	324.8	320.2	323.2	318.1	323.2	318.1
	Ring <sup>a</sup>	197.2	182.9	207.2	224.0	207.2	224.4	190.0	207.6	190.5	207.6
2	O5–H	342.0	334.9	333.6	321.9	333.6	321.9	330.3	317.7	329.9	317.7
	O6–H	292.6	290.1	321.9	301.4	322.3	301.4	319.0	300.1	321.5	300.6
3	O3–H	336.1	331.5	345.8	329.4	345.8	329.0	347.0	329.9	347.4	329.4
	O3'–H	421.9	416.5	437.0	426.1	437.4	426.1	435.8	425.3	436.2	425.7
	O8–H	309.8	308.5	329.9	310.2	330.3	310.2	330.3	310.2	330.7	310.2
	O9–H	334.5	329.9	324.0	310.2	324.0	309.8	321.9	310.2	321.9	309.8

<sup>a</sup> The enthalpy value of the cyclic compound formation for lespedezavirgatal.

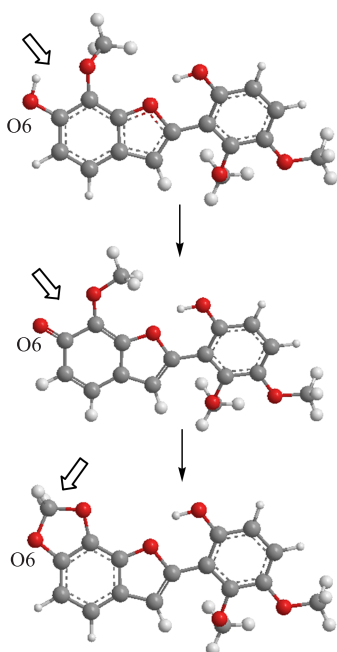


FIG. 4 Formation of a cyclic compound with a low BDE value for lespedezavirgatal.

lar, the enthalpy value (<229.9 kJ/mol) of the cyclic compound formation for lespedezavirgatal is significant lower than that of the above compounds.

### C. Ionization potential of antioxidants

The adiabatic IPs were calculated with different methods for the neutral and anion forms of new antioxidants (Table II). The sequence for the IPs is neutral>anion to three antioxidants both in gas phase and in aqueous solution. The IPs of the neutral antioxidants in gas phase (642.0, 649.1, and 663.8 kJ/mol for lespedezavirgatal, lespedezavirgatal, and lespedezacoumestan, respectively) are comparable to those of widely used food additives such as propyl gallate (701.4 kJ/mol) and butylated hydroxyanisole (638.3 kJ/mol), natural polyphenolic flavonoids delphinidin (627.0 kJ/mol), and epigallocatechin-3-gallate (617.4 kJ/mol) [25, 47, 49]. The solvent effects significantly lower the IPs of the neutrals for the stabilizing effect of the water with the radical cations of the compounds.

The IPs of the anions for new antioxidants are significantly lower than those of compounds used as food synthetic additives [25, 50]. The IPs of 6' anion for lespedezavirgatal and 5 anion for lespedezavirgatal are lower than that of other anion both in gas phase and in solution, which suggests that 6' and 5 anions are the respectively more active forms in SPLET mechanism for lespedezavirgatal and lespedezavirgatal. For lespedezacoumestan, the IPs of 3' anion in gas phase and 9 anion in solution are the lowest among those of other anion forms which show that 3' and 9 anions are the most active forms in SPLET mechanism in gas phase and in solution respectively. The IPs of the anions in aqueous medium are higher than those in gas phase. Contrary to the influences on the IPs of neutral, solvent effects increase the IPs of the anions for the stabilizing effect of the water with the anions.

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### D. H-atom affinity and adiabatic electron affinity of reactive oxygen species

Three major types of ROS, OH<sup>·</sup>, O<sub>2</sub><sup>·-</sup>, and HO<sub>2</sub><sup>·</sup>, were selected as the scavenged radicals. As mentioned previously, most antioxidants can scavenge ROS radicals through three mechanisms. To determine which pathway is the most possible thermodynamically to scavenge the ROS radicals, we calculated the HAs (Table III) and EAs (Table IV) of the three ROS radicals both in gas phase and in aqueous solution with different methods.

From the Table III, it can be seen that the HAs fol-

TABLE II IP (kJ/mol) values for neutral and anion forms of lespedezavirgatal (1), lespedezavirgatal (2) and lespedeza-coumestan (3) at 298 K in the gas phase and in aqueous solution.

	Gas phase		Aqueous solution							
	B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM	
			UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi
1 Neutral	643.0	647.2	492.7	498.6	492.7	499.0	496.5	502.3	496.9	502.7
Anion, 6	236.1	208.9	406.9	398.9	406.9	398.9	393.5	382.6	393.5	382.6
Anion, 6'	219.3	193.0	403.1	393.5	403.5	393.9	390.6	378.0	391.0	378.4
2 Neutral	650.1	649.2	501.9	511.5	501.9	511.5	503.6	514.5	504.0	514.5
Anion, 5	193.4	154.9	416.5	410.6	417.3	411.1	398.1	388.5	398.5	388.9
Anion, 6	221.0	190.5	418.6	417.3	418.6	417.8	398.9	397.3	401.0	397.7
3 Neutral	664.7	664.3	501.1	515.7	501.1	515.7	499.8	514.5	499.8	514.5
Anion, 3	290.9	270.0	444.6	444.1	444.6	443.7	434.1	430.3	434.1	430.7
Anion, 3'	212.2	164.9	452.9	437.4	454.2	437.9	426.1	403.9	426.1	404.8
Anion, 8	252.4	221.9	421.9	422.8	422.4	422.8	403.9	402.3	404.4	402.3
Anion, 9	239.9	206.8	414.8	412.3	415.3	412.3	396.0	390.1	396.4	390.6

TABLE III H-atom affinity (HA, kJ/mol) values of three ROS at 298 K in the gas phase and in aqueous solution.

	Gas phase		Aqueous solution							
	B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM	
			UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi
OH <sup>•</sup>	-483.9	-453.3	-488.1	-493.5	-488.1	-493.5	-457.5	-463.4	-457.5	-483.9
O <sub>2</sub> <sup>-•</sup>	-247.8	-234.8	-318.6	-277.5	-319.4	-278.0	-306.4	-264.6	-307.3	-247.8
HO <sub>2</sub> <sup>•</sup>	-348.3	-331.5	-372.6	-354.6	-372.6	-355.0	-359.2	-340.3	-359.6	-348.3

TABLE IV Adiabatic electron affinity (EA, kJ/mol) values of three ROS at 298 K in the gas phase and in aqueous solution.

	Gas phase		Aqueous solution							
	B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM	
			UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi
OH <sup>•</sup>	-170.4	-121.0	-621.6	-530.4	-622.5	-530.4	-581.4	-527.4	-582.7	-170.4
O <sub>2</sub> <sup>-•</sup>	679.0	729.2	-300.6	-242.0	-301.0	-242.0	-272.5	-204.7	-272.5	679.0
HO <sub>2</sub> <sup>•</sup>	-95.9	-63.2	-438.3	-424.9	-438.3	-425.3	-411.1	-396.8	-411.5	-95.9

low the trend both in gas phase and in aqueous solution: O<sub>2</sub><sup>-•</sup> > HO<sub>2</sub><sup>•</sup> > OH<sup>•</sup>. Although the DFT methods and cavities affect the HA values, the orders remain unchanged. The EAs follow the same order as the HAs both in gas phase and in aqueous medium. The DFT methods and cavities also affect the HA values, but they don't influence the order. Although the solvent effect especially significantly decreases the EAs for the stabilizing effect of the water with the anions, it doesn't affect the orders. The hydroxyl radical is a highly reactive species among the three common ROS, which is consistent with experimental observations [4, 6].

### E. Antioxidant mechanisms

According to the results of Zhang [27], the scavenging-radical mechanism of an antioxidant can be inferred by the sum value of its BDE and HA of the scavenged radical and that of its IP and EA of the

scavenged radical. If the former is positive, the HAT is thermodynamically forbidden. Otherwise, HAT is thermodynamically permitted. Likewise, if the latter is positive, SPLET or ET-PT is forbidden. Otherwise, SPLET or ET-PT is permitted.

#### 1. H-atom transfer

The sum values of the lowest BDEs of new antioxidants and HAs of the scavenged radicals both in gas phase and in aqueous solution are listed in Table V. Using the B3LYP and BhandHLYP methods, the lowest O-H BDE of lespedezavirgatal, is -155.5/-125.4, 80.3/90.7, and -20.1/-5.9 kJ/mol lower than the absolute HA of OH<sup>•</sup>, O<sub>2</sub><sup>-•</sup>, and HO<sub>2</sub><sup>•</sup> respectively, which suggests that HAT is thermodynamically permitted in the scavenging of OH<sup>•</sup> and HO<sub>2</sub><sup>•</sup> not O<sub>2</sub><sup>-•</sup> by lespedezavirgatal in gas phase. In addition, solvent effect doesn't affect the scavenging-radical mechanism of lespedezavir-

TABLE V The sum values of BDEs of new antioxidants and HAs of the scavenged radicals at 298 K in the gas phase and in aqueous solution (kJ/mol).

		Gas phase		Aqueous solution							
		B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM	
				UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi
OH <sup>·</sup>	1	-155.7	-127.7	-167.4	-188.4	-167.0	-188.8	-137.3	-159.5	-137.3	-159.5
	2	-191.3	-163.3	-166.2	-192.1	-165.8	-192.1	-138.6	-163.3	-136.0	-162.8
	3	-174.1	-144.8	-164.1	-183.3	-164.1	-183.8	-135.6	-153.2	-135.6	-153.6
O <sub>2</sub> <sup>-</sup>	1	80.4	90.8	2.1	27.6	1.7	26.8	13.8	39.3	13.0	38.9
	2	44.8	55.3	3.3	23.9	2.9	23.4	12.6	35.6	14.2	35.6
	3	62.0	73.7	5.4	32.7	4.6	31.8	15.5	45.6	14.7	44.8
HO <sub>2</sub>	1	-20.1	-5.9	-51.9	-49.4	-51.5	-50.2	-38.9	-36.4	-39.3	-36.4
	2	-55.7	-41.4	-50.7	-53.2	-50.2	-53.6	-40.2	-40.2	-38.1	-39.8
	3	-38.5	-23.0	-48.6	-44.4	-48.6	-45.2	-37.3	-30.1	-37.7	-30.6

TABLE VI The sum values of IPs of the neutral antioxidants and EAs of the scavenged radicals at 298 K in the gas phase and in aqueous solution (kJ/mol).

		Gas phase		Aqueous solution							
		B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM	
				UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi
OH <sup>·</sup>	1	472.6	526.2	-128.9	-31.8	-129.8	-31.4	-85.0	-25.1	-85.8	-24.7
	2	479.7	528.3	-119.7	-18.8	-120.6	-18.8	-77.9	-13.0	-78.7	-13.0
	3	494.4	543.3	-120.6	-14.7	-121.4	-14.7	-81.6	-13.0	-82.9	-13.0
O <sub>2</sub> <sup>-</sup>	1	1321.9	1376.4	192.1	256.6	191.7	257.0	224.0	297.6	224.4	297.6
	2	1329.1	1378.4	201.3	269.6	200.9	269.6	231.1	309.8	231.5	309.3
	3	1343.7	1393.5	200.5	273.8	200.1	273.8	227.3	309.8	227.3	309.3
HO <sub>2</sub>	1	547.1	583.9	54.4	73.7	54.4	73.7	85.4	105.5	85.4	105.9
	2	554.2	586.0	63.6	86.7	63.6	86.2	92.5	117.6	92.5	117.6
	3	568.9	601.1	62.8	90.8	62.8	90.4	88.7	117.6	88.3	117.6

gatal. To lespedezavirgatal, the sum values of its BDE and the HA of OH<sup>·</sup>, O<sub>2</sub><sup>-</sup>, and HO<sub>2</sub> are -191.0/-163.0, 44.7/141.2, and -55.6/-41.4 kJ/mol respectively in gas phase. Likewise, HAT is thermodynamically permitted in the scavenging of OH<sup>·</sup> and HO<sub>2</sub> not O<sub>2</sub><sup>-</sup> by lespedezavirgatal both in gas phase and in solution. Furthermore, the same trends are found for lespedezacoumestan. The activities of three antioxidants with the OH<sup>·</sup> and HO<sub>2</sub> both in gas phase and in aqueous solution increased in the order of lespedezacoumestan < lespedezavirgatal < lespedezavirgatal. Moreover, the activity of OH<sup>·</sup> is higher than that of HO<sub>2</sub>. The consistent results are obtained from different methods.

## 2. Stepwise electron transfer-proton transfer

The sum values of IPs of the neutral antioxidants and EAs of the scavenged radicals at 298 K both in gas phase and in aqueous solution are listed in Table VI. Different methods don't affect the signs of the sum values. All the sum values of the adiabatic IP of lespedezavirgatal and the EAs of OH<sup>·</sup>, O<sub>2</sub><sup>-</sup> and HO<sub>2</sub> are positive, which suggests that the electron trans-

fer between lespedezavirgatal and these selected ROS are thermodynamically forbidden in gas phase. Using the different methods, the sum values are also positive for O<sub>2</sub><sup>-</sup> and HO<sub>2</sub> in solution. However, the sum value is negative (-128.7 kJ/mol) for OH<sup>·</sup> in solution. It is inferred that ET-PT is thermodynamically permitted for lespedezavirgatal only in the scavenging of OH<sup>·</sup> in solution. To lespedezavirgatal and lespedezacoumestan, we also find that ET-PT is thermodynamically forbidden in the scavenging of O<sub>2</sub><sup>-</sup> and HO<sub>2</sub> both in gas phase and in solution, and ET-PT is permitted only in the scavenging of OH<sup>·</sup> in solution, but not in gas phase. The activities of the three antioxidants with the OH<sup>·</sup> in aqueous solution increased in the order of lespedezacoumestan < lespedezavirgatal < lespedezavirgatal.

## 3. Sequential proton loss-electron transfer

The sum values of the lowest IPs of the anion form for antioxidants and EAs of the scavenged radicals both in gas phase and in aqueous solution are listed in Table VII. All the sum values of the IPs of anion

TABLE VII The sum values of the lowest IPs of anion form for new antioxidants and EAs of the scavenged radicals at 298 K in the gas phase and in aqueous solution (kJ/mol).

	Gas phase		Aqueous solution								
	B3LYP	BHandHLYP	B3LYP/IEFPCM		B3LYP/CPCM		BHandHLYP/IEFPCM		BHandHLYP/CPCM		
			UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	UAHF	Bondi	
OH <sup>•</sup>	1	49.0	72.0	-218.5	-136.9	-218.9	-136.5	-190.9	-149.4	-191.7	-107.2
	2	23.0	33.9	-205.1	-119.7	-205.1	-119.3	-183.3	-139.0	-184.2	-96.7
	3	41.9	44.0	-206.8	-118.0	-207.2	-118.0	-185.4	-137.3	-186.3	-95.0
O <sub>2</sub> <sup>-</sup>	1	898.3	922.2	102.6	151.5	102.6	152.0	118.0	173.3	118.5	173.3
	2	872.4	884.1	116.0	168.7	116.4	169.1	125.6	183.8	126.0	183.8
	3	891.2	894.1	114.3	170.4	114.3	170.4	123.5	185.4	123.9	185.4
HO <sub>2</sub> <sup>•</sup>	1	123.5	129.8	-35.2	-31.4	-34.7	-31.4	-20.5	-18.8	-20.5	-18.4
	2	97.5	91.7	-21.8	-14.2	-20.9	-14.2	-13.0	-8.4	-13.0	-8.0
	3	116.4	101.7	-23.4	-12.6	-23.0	-13.0	-15.1	-6.7	-15.1	-6.3

form for lespedezavirgatal, lespedezavirgatal and lespedezacoumestan with EAs of OH<sup>•</sup>, O<sub>2</sub><sup>-</sup>, and HO<sub>2</sub><sup>•</sup> in gas phase are positive, which shows that SPLET is thermodynamically forbidden for three antioxidants in scavenging the selected radicals in gas phase. The sum values are also positive for O<sub>2</sub><sup>-</sup> but negative for OH<sup>•</sup> and HO<sub>2</sub><sup>•</sup> in solution, which suggests that SPLET is thermodynamically permitted for three antioxidants in scavenging the OH<sup>•</sup> and HO<sub>2</sub><sup>•</sup>, but not O<sub>2</sub><sup>-</sup> in solution. The activities of three antioxidants with the OH<sup>•</sup> and HO<sub>2</sub><sup>•</sup> in aqueous solution increased in the order of lespedezacoumestan < lespedezavirgatal < lespedezavirgatal. The activity of OH<sup>•</sup> is higher than that of HO<sub>2</sub><sup>•</sup>. The results obtained from different methods are consistent.

#### F. Comparison with experimental data

The inhibition of lipid peroxidation for lespedezavirgatal (IC<sub>50</sub>, 0.16±0.002 mmol/L), lespedezavirgatal and lespedezacoumestan in kidney failure rats was reported by Chen *et al.* [23]. It is interesting to investigate whether the theoretical data could shed light on the experimental findings. Although the size of the data sample is too few to establish a valuable QSAR equation, the qualitative comparison of the order of activity with the predicted data could be performed which is helpful for us to understand the scavenging radical mechanisms.

The IC<sub>50</sub> (half maximal inhibitory concentration) values are 0.16±0.002, 0.64±0.016, and 0.45±0.453 mmol/L in kidney homogenate [23]. These data show that the order of the activity is lespedezavirgatal > lespedezacoumestan > lespedezavirgatal. The BDEs of three novel antioxidants don't follow the trend. The IPs of neutral and anion forms both show the same activity order. It was noting that good agreements were achieved by comparing the IPs of neutral and anion forms with the pIC<sub>50</sub> in kidney homogenate, which suggests that the radical scavenging mechanisms

via ET-PT and SPLET may be more likely for new antioxidants.

#### IV. CONCLUSION

In this work, the molecular structures and ROS scavenging activities of three novel antioxidants, lespedezavirgatal, lespedezavirgatal, and lespedezacoumestan were studied by DFT calculation. The most stable conformations of three new antioxidants have characteristic of IHBS. The BDE and adiabatic IP values for three novel antioxidants and HA and EA values for OH<sup>•</sup>, HO<sub>2</sub><sup>•</sup>, and O<sub>2</sub><sup>-</sup> are obtained both in gas phase and in aqueous solution. Different methods have no effect on the antioxidant activities and the active site. The solvent effects significantly lower the IPs of the neutrals but increase the IPs of the anions. For three new antioxidants, HAT and SPLET is thermodynamically permitted in the scavenging of OH<sup>•</sup> and HO<sub>2</sub><sup>•</sup> not O<sub>2</sub><sup>-</sup> both in gas phase and in aqueous solution. ET-PT is thermodynamically permitted only in the scavenging of OH<sup>•</sup> in aqueous solution. The activities of new antioxidants with radicals increased in the order of lespedezacoumestan < lespedezavirgatal < lespedezavirgatal. The experimental antioxidant activity, the pIC<sub>50</sub> in kidney homogenate, and calculated IPs of neutral and anion forms reveal good agreements.

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