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Is lecanoric acid a good antioxidant?

Quan V. Vo [a,*], Le Trung Hieu [b], Hoang Thi Cam Hang [a], Vo Huynh Ngoc Diep [a], Nguyen Thi Hoa [a], Uyen T.D. Huynh [a], Nguyen Quang Trung [c,d], Adam Mechler [e]

[a] The University of Danang - University of Technology and Education, Danang 550000, Viet Nam
[b] University of Sciences, Hue University, Thua Thien Hue 530000, Viet Nam
[c] The University of Danang - University of Science and Education, Da Nang 550000, Viet Nam
[d] Quality Assurance and Testing Center 2, Danang 550000, Viet Nam
[e] Department of Biochemistry and Chemistry, LA Trobe University, Victoria 3086, Australia

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ABSTRACT

Lecanoric acid (**LA**), an abundant chemical found in lichens, has demonstrated a wide range of biological activities, including anti-cancer cytotoxic, antibiotic, antimycobacterial, antiviral, and anti-hepatocarcinoma properties. The antioxidant capacity of this molecule, while inferred from certain experimental findings, is doubtful based on structural characteristics and therefore remains to be established. DFT calculations are used in this work to conduct a comprehensive evaluation of the mechanism and kinetics governing the antiradical activity of **LA** in lipidic and aqueous solvent environments. Although the DPPH/$ABTS^{+\bullet}$ assays revealed good antioxidant activity *in vitro*, the modeling yielded mixed results. The data suggests that **LA** is an efficient scavenger of the $HO^{\bullet}$ radical with rate constants of 2.01×10^{10} and 2.80×10^{8} M^{-1} s^{-1} in polar and lipid media, respectively, by the FHT and RAF mechanisms. However, the data also suggests that **LA** exhibits only weak activity against the $HOO^{\bullet}$ radical in all physiological environments. This is consistent with structural features that predict low activity.

1. Introduction

Reactive oxygen species (ROS) are pivotal in the pathogenesis of various human ailments and aging processes, alongside the deterioration of bodily organs. Antioxidants mitigate these detrimental effects by eliminating the ROS in the body through radical reactions. Lecanoric acid (**LA**), depicted in Fig. 1, is a ubiquitous chemical in lichens. **LA** is known to have several biological activities, including pharmacologically significant attributes such as anti-cancer cytotoxic [1], antibiotic [2], antimycobacterial [3], antiviral [4], and in addition to its antioxidative properties [5,6]. **LA** demonstrated substantial and exhibited inhibitory effects in *in vitro* assays against nitric oxide radicals, superoxide radicals, and 2,2-diphenyl-1-picrylhydrazil radicals [5–7]. According to these experimental works, the antioxidant activity of **LA** surpasses that of Trolox, the reference antioxidant, however considering the molecular structure of the target compound and the link between its structure and activity, it is dubious whether lecanoric acid can possess strong antioxidant properties due to the presence of two highly deactivating carbonyl groups.

Among ROS, the hydroxyl radical is a relatively stable and therefore common species. The majority of tissue damage caused by ionizing radiation and the primary oxidative damage to DNA is ascribed to this particular radical [8,9]. Thus, a highly effective strategy for mitigating oxidative stress would be to inhibit the production of hydroxyl radicals [10]. Conducting an inquiry into the kinetics and mechanism of the hydroxyl antiradical activity is essential for assessing the antioxidant activity of organic compounds [11–14]. Furthermore, the $HOO^{\bullet}$ radical has been extensively employed as a model radical in computational calculations to determine radical scavenging activity [10,11,13]. These investigations, however, have not been conducted in **LA**.

Previous studies demonstrated the benefit of using computational approaches to evaluate antioxidant activity and assess structure–activity correlations, aiding the development of new medicines with increased activity [10,11,13–20]. Consistently computational methods emerged as essential tools in medicinal chemistry. In this work, the antioxidant efficacy of **LA** was assessed using a comprehensive approach that included

* Corresponding author.
E-mail address: vvquan@ute.udn.vn (Q.V. Vo).

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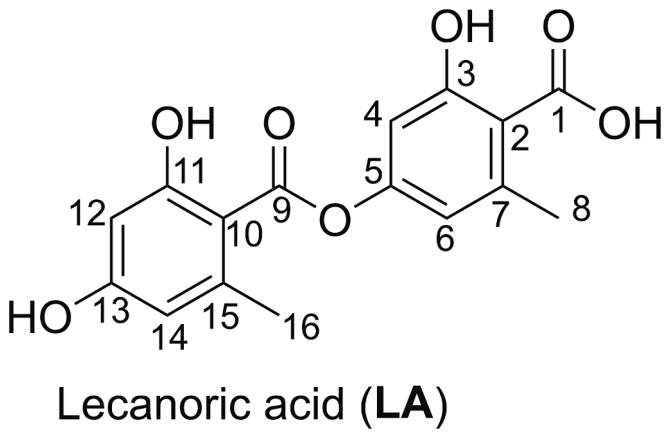

Lecanoric acid (**LA**)

Fig. 1. The structure of **LA.**

both theoretical and experimental assays. **LA**'s radical scavenging capability was assessed using DPPH and $ABTS^{\bullet+}$ antiradical activity assays, while thermodynamic and kinetic simulations were used to elucidate the mechanism and kinetics against $HO^{\bullet}/HOO^{\bullet}$ radicals that underpin its antioxidant activity.

1. Computational and experimental methods

1.1. Computational method

Quantum mechanics-based tests for overall free radical scavenging activity (QM-ORSA) kinetic calculations were performed in this investigation using the solvation model density (SMD) method (for water and pentyl ethanoate solvents) [11,15,21,22]. The rate constant (k) was determined utilizing the conventional transition state theory (TST) at a standard state of 1 M and 298.15 K, in accordance with equation (1) (the methodological information is provided in Table S1, SI) [23–28]:

$$k = \sigma\kappa\frac{k_B T}{h}e^{-(\Delta G^{\neq})/RT} \quad (1)$$

The σ is the reaction symmetry number [29,30], κ contains the tunneling corrections calculated using the Eckart barrier,[31] k_B is the Boltzmann constant, h is the Planck constant, $\Delta G^{\neq}$ is the Gibbs free energy of activation. All calculations were carried out using Gaussian 16 software [32] at the M06-2X/6-311++G(d,p) level of theory [33,34].

1.2. Experimental method

1.2.1. DPPH assay

Varied quantities of purified compounds were solubilized in dimethyl sulfoxide (DMSO) and Tris-hydrochloric acid (Tris-HCl) buffer at a 1:1 vol ratio to generate samples of diverse concentrations (3.14, 6.28, 9.43, 12.57, 15.71 μM). Subsequently, 1 mL of each sample was combined with 1 mL of 100 μM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (prepared in methanol/Tris-HCl buffer at a 5:1 vol ratio) and allowed to react for 30 min at 25 °C. The absorbance was then measured at 517 nm. Trolox served as the standard reference compound, spanning concentrations from 10 μM to 50 μM. The determination of radical scavenging activity was accomplished through the calculation of the half-maximal inhibitory concentration (IC_{50}).

1.2.2. ABTS radical scavenging assay

ABTS (7 mM) was mixed with potassium persulfate (2.45 mM) and incubated in darkness at room temperature for 16 h to generate the ABTS radical. The purified compounds were dissolved in a mixture of dimethyl sulfoxide (DMSO) and 0.01 M phosphate-buffered saline (pH 7.4) at a volumetric ratio of 1:1, resulting in samples with concentrations ranging from 3.14 to 15.71 μM. Subsequently, a volume of 0.1 mL from each sample was combined with 1.9 mL of the $ABTS^{+\bullet}$ working solution, prepared by diluting $ABTS^{+\bullet}$ with 0.01 M phosphate-buffered saline (pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. The absorbance of the resulting mixture was measured at 734 nm. Trolox was employed as a positive control. The scavenging capability towards $ABTS^{+\bullet}$ was calculated using the formula:

$$Scavengingrate(\%) = [1 - ODsample/ODblank] \times 100.$$

The IC_{50} value was used to evaluate the radical scavenging activity.

2. Results and discussions

2.1. The radical scavenging activity in the gas phase

2.1.1. Thermodynamic study

The first step in a physicochemical study of antioxidant activity is to establish properties in the reference state, before progressing to specific media. It is well established that the process of free radical scavenging can follow one of three primary mechanisms: sequential proton loss electron transfer (SPLET) [35–38], single electron transfer-proton transfer(SETPT) [39,40], or formal hydrogen transfer (FHT) [33]. The SPLET mechanism exhibits proton dissociation and subsequent single electron transfer, typical in aqueous environments but not favorable in apolar media. In the majority of cases, only the FHT and SETPT processes can take place in low-dielectric media, such as the gas phase. Thus, in this part, the thermochemical characteristics of SETPT and FHT, specifically the ionization energies (IEs) and bond dissociation energy (BDE) were computed and are shown in Table 1.

The lowest BDE values were observed at 90.7 and 91.2 kcal/mol for the C8/16-H bond, respectively. The BDEs of the O3/11/13-H bonds showed a marginal increase of around 3.1–8.2 kcal/mol. In contrast, the BDE of the O1–H bond exhibited a considerably higher value of around 111.9 kcal/mol. The molecular structure of **LA** contains an aromatic with a solitary OH group alongside two deactivating groups (COO), thereby augmenting the BDE(O3-H). The combined impact of the two OH groups on the opposite ring is also diminished: chemically attached to a carbonyl group, the deactivating effect of the ring is maximized due to the location of the OH groups in the meta and para positions. Subsequently, the BDE (O3/11/13-H) values exhibit a magnitude surpassing that of typical phenolic compounds such as Trolox, quercetin, *trans*-resveratrol, and vitamin C (the lowest BDE(O-H) values are 72.1, 75.2, 77.2, and 77.5 kcal/mol, respectively [41–43]), the BDEs of **LA** are characteristically higher. Therefore, the hydrogen abstraction from **LA** by radicals may not be very effective.

The calculated data indicated that the BDE values were considerably reduced in comparison to the IE values (IE = 192.4 kcal/mol). This suggests that the FHT mechanism is likely the preferred pathway for the antiradical activity of **LA** in the gas phase.

To confirm the preferred antioxidant mechanism, the Gibbs free energy changes (ΔG°) for the FHT, single electron transfer (SET), and radical adduct formation (RAF) mechanisms of the **LA** + $HO^{\bullet}/HOO^{\bullet}$ reactions were calculated. Table 2 summarizes the results. The antiradical activity of **LA** is thermodynamically spontaneous in all FHT and RAF reactions ($\Delta G^{\circ} < 0$, −4.9 to − 26.3 kcal/mol), while the SET pathway was not favored ($\Delta G^{\circ} > 0$) under the studied conditions. The

Table 1
The calculated IEs and BDEs (in kcal/mol) in the gas phase of **LA.**

Positions	BDE	IE
O1-H	111.9	192.4
O3-H	98.0	
O11-H	98.9	
O13-H	94.3	
C8-H	90.7	
C16-H	91.2	

Table 2
The calculated ΔG^{o} values (in kcal/mol) of the **LA** + HO$^{\bullet}$ reaction according to the RAF, SET, and FHT pathways in the gas phase.

Mechanisms	Positions	HO$^{\bullet}$	HOO$^{\bullet}$
FHT	O1-H	−5.3	26.0
	O3-H	−19.8	11.4
	O11-H	−19.3	11.9
	O13-H	−23.2	8.0
	C8-H	−26.3	5.0
	C16-H	−25.7	5.5
RAF	C2	−8.1	
	C3	−7.9	
	C4	−10.8	
	C5	−8.8	
	C6	−8.2	
	C7	−13.1	
	C10	−5.4	
	C11	−7.2	
	C12	−12.2	
	C13	−6.2	
	C14	−4.9	
	C15	−11.0	
SET		165.2	169.2

findings from the gas phase analysis validate that **LA** is capable of reacting with HO$^{\bullet}$ radicals at all sites via FHT and RAF reactions. Thus, further investigation in the kinetics study for the **LA** + HO$^{\bullet}$ reaction will be conducted on these reactions. However, the **LA** + HOO$^{\bullet}$ reaction did not exhibit thermodynamic spontaneity in any of the investigated pathways. The data indicates that **LA** has low HOO$^{\bullet}$ radical scavenging activity in the gas phase, and hence, the kinetic investigation for this process should be excluded.

2.1.2. Kinetic study

The kinetics of the favored mechanisms of the **LA** + HO$^{\bullet}$ reaction were assessed using the M06-2X/6–311++G(d,p) method [44] in accordance with the QM-ORSA protocol [11]. The outcomes are summarized in Table 3, while the transition states (TS) that were determined are illustrated in Fig. 2. The overall rate constant ($k_{overall}$) for the HO$^{\bullet}$ + **LA** reaction was 1.29×10^{10} M^{-1} s^{-1}. The HO$^{\bullet}$ antiradical activity of **LA** was significantly enhanced by the RAF reaction, with branching ratios (Γ) of 97.2 % at C12 (Γ = 84.1 %), C6 (Γ = 7.8 %) and C10 (Γ = 3.9 %), C14 (Γ = 1.4 %), C4 (Γ = 0.5 %) and C2 (Γ = 0.3 %) positions. In contrast, the FHT contributed a mere 1.8 % to the HO$^{\bullet}$ + **LA** reaction, primarily through the O13 − H and C16/C8 − H bonds. In the gas phase, the HO$^{\bullet}$ radical scavenging activity of **LA** has no contribution from the remaining reaction pathways. **LA** exhibited a marginally lower HO$^{\bullet}$ antiradical activity compared to analogous acids, namely caffeic acid ($k = 7.29 \times 10^{10}$ M^{-1} s^{-1} at 300 K) [45], and rosmarinic acid (7.28×10^{11} M^{-1} s^{-1}) [14].

Table 3
Calculated $\Delta G^{\neq}$ (kcal/mol), tunneling corrections (κ), and k_{Eck} (M^{-1} s^{-1}) at 298.15 K in the HO$^{\bullet}$ + **LA** reaction in the gas phase.

Mechanisms	Positions	$\Delta G^{\neq}$	κ	k_{Eck}	Γ
FHT	O3-H	14.5	51.5	7.23×10^{7}	0.0
	O11-H	13.5	22.4	1.81×10^{4}	0.0
	O13-H	6.4	1.0	1.20×10^{8}	0.9
	C8-H	7.4	1.7	3.91×10^{7}	0.3
	C16-H	7.0	1.9	8.24×10^{7}	0.6
RAF	C2	7.1	1.1	4.44E + 07	0.3
	C3	11.3	1.4	4.76×10^{4}	0.0
	C4	6.8	1.0	6.63×10^{7}	0.5
	C5	11.9	1.4	1.51×10^{4}	0.0
	C6	5.3	1.2	1.01×10^{9}	7.8
	C7	9.2	1.2	1.33×10^{6}	0.0
	C10	5.7	1.2	5.06×10^{8}	3.9
	C11	13.8	1.4	7.23×10^{2}	0.0
	C12	3.9	1.2	1.08×10^{10}	84.1
	C13	11.1	1.3	6.02×10^{4}	0.0
	C14	6.3	1.2	1.81×10^{8}	1.4
	C15	9.5	1.3	8.43×10^{5}	0.0
$k_{overall}$				$\mathbf{1.29 \times 10^{10}}$	

2.2. The HO/HOO radical scavenging activity in the physiological environment

2.2.1. Acid-base equilibrium

It is crucial to consider the deprotonation of the acidic moieties of **LA**, i.e. the phenolic acids, when evaluating the antioxidant activity in an aqueous solution [10,33]. Evidently, the carboxylic COO–H bond ($\Delta G^{o}(p)$ = 133.6 kcal/mol), which corresponds to pKa1, is where dissociation is most probable, as determined by the calculated Gibbs free energy values for deprotonation ($\Delta G^{o}(p)$. pKa2 and pKa3 are contributed by the O13–H bond ($\Delta G^{o}(p)$ = 141.1 kcal/mol) and O3–H bond ($\Delta G^{o}(p)$ = 152.7 kcal/mol), respectively. As shown in Fig. 3, the corresponding pKa values were calculated in accordance with the literature.[46].

The calculated pKa values are as follows: 3.29 (pKa1), 7.45 (pKa2), and 11.14 (pKa3). Therefore, at a pH of 7.4, **LA** is present in two forms in the aqueous solution: monoanion (H_2A^-, 58.0 %) and dianion (HA^{2-}, 42.0 %). **LA** is present in the lipid medium, more precisely in the pentyl ethanoate solvent, in a neutral state (H_3A). As a result, these conditions were used in subsequent kinetic analyses conducted in the physiological media.

2.2.2. Kinetic evaluation

The anti-radical capacity of the HO$^{\bullet}$ species in **LA** is determined in nonpolar environments, specifically the gas phase, by the RAF pathway at the C12, C6, C10, C14, C4, and C2 sites of the hydrogen abstraction of the C8/16-H and O13-H bonds, as described in the preceding section. Therefore, the rate constant of the **LA** + HO$^{\bullet}$ reaction was calculated in this section using the preferred mechanisms (i.e., FHT (C8/16/O13-H) and RAF (C12, C6, C10, C14, C4, and C2) in a lipid medium (pentyl ethanoate). In addition, activity was also investigated in water at pH 7.4 using the dominant states (H_2A^- and HA^{2-}) and all feasible reactions of the ionic states (Table S2, SI).

The results of the thermodynamic analysis (Table S2, SI) revealed that the **LA** + HOO$^{\bullet}$ reaction did not proceed spontaneously in any of the examined pathways involving the existing states (H_2A^- and HA^{2-}) in water at a pH of 7.4. Therefore, there is no need to perform a kinetic study on the interaction between **LA** and HOO$^{\bullet}$ in both water and lipid media, since **LA** has a limited ability to scavenge HOO$^{\bullet}$ radicals in the gas phase (see section 3.2.1). Thus, only the kinetics of **LA** + HO$^{\bullet}$ were computed in the physiological environments and the findings are displayed in Table 4.

The $k_{overall}$ values for the HO$^{\bullet}$ radical scavenging activity of **LA** in pentyl ethanoate and water solvents were 2.80×10^{8} and 2.01×10^{10} M^{-1} s^{-1}, respectively. The HO$^{\bullet}$ antiradical activity of **LA** in the lipid medium was dominated by the RAF reaction (Γ = 75.4 %), while the FHT contributed approximately 24.6 % of the overall rate constant. The RAF and FHT reactions of the anion and dianion states both contributed to the HO$^{\bullet}$ antiradical activity in the polar medium; nevertheless, the SET pathway accounted for approximately 18.0 % of the hydroxyl radical scavenging activity exhibited by **LA**.

In the aqueous solution, the H_2A^- + $^{\bullet}$OH reaction was responsible for approximately 36.4 % of the $k_{overall}$, whereas the HA^{2-} + $^{\bullet}$OH reaction contributed 63.4 % to the overall rate constant. It was observed that the FHT reaction of the O-H bonds (O3-H and O11-H) and the RAF of C12 and C14 in the dianion state against the HO$^{\bullet}$ radical in water did not exhibit any reaction barrier ($\Delta G^{\neq} \sim 0$ kcal/mol). Therefore, the k_{app} values corresponding to these reactions were comparable in magnitude to the diffusion rates (k_D) and comprised approximately 22.5 % of the overall rate constant. The calculated data indicated that **LA** had higher radical scavenging activity than syringic acid (9.77×10^{7} M^{-1} s^{-1} and 4.63×10^{9} in lipid and water solvents, respectively) [13], and is

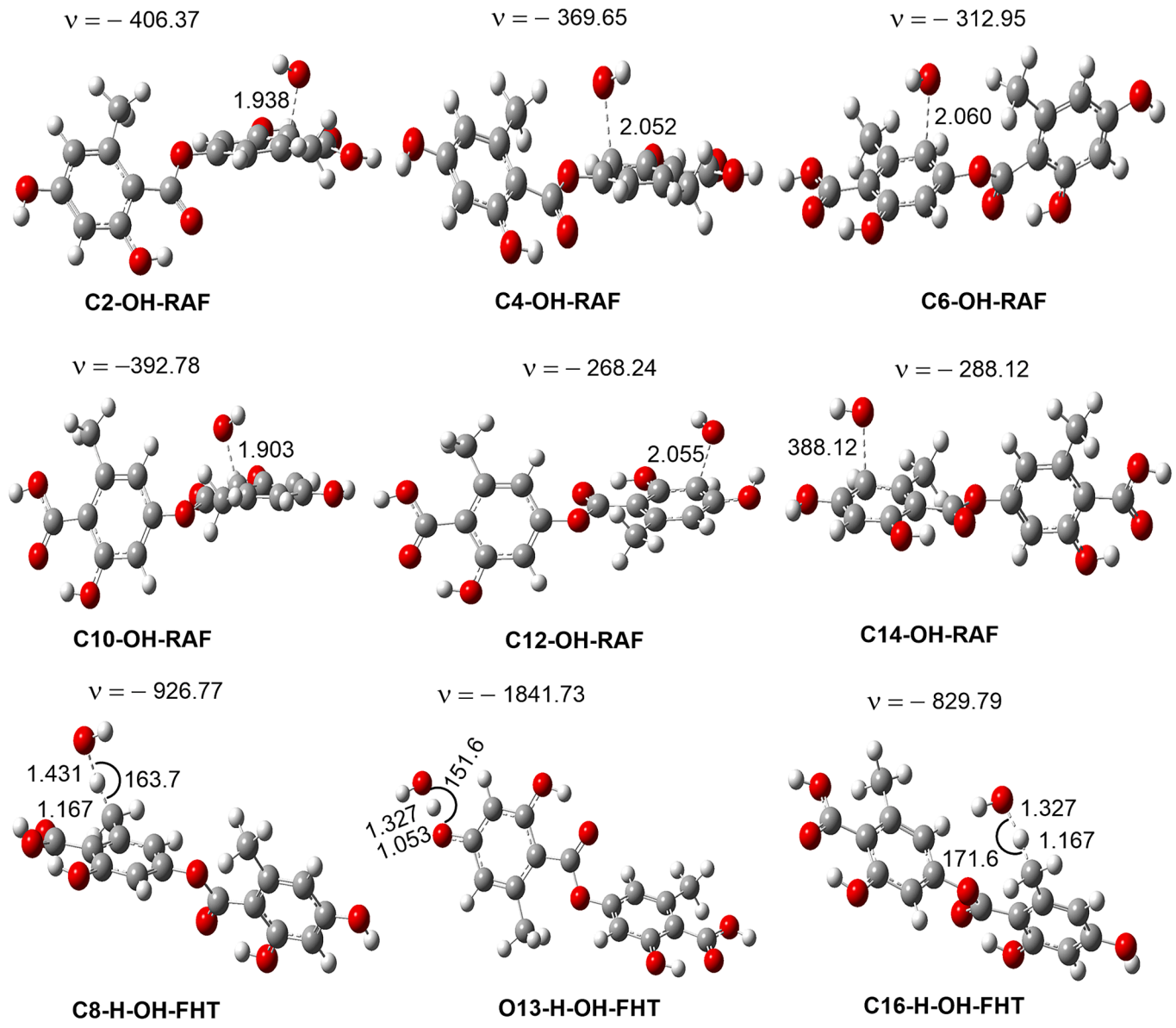

Fig. 2. The selected TS of the **LA** + HO• reactions in the gas phase.

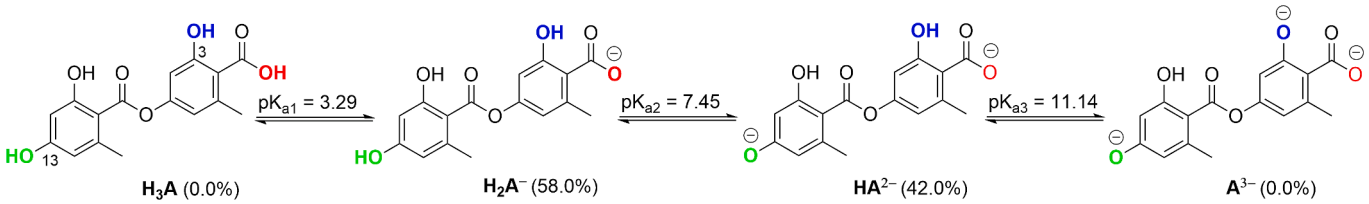

Fig. 3. The deprotonation of **LA** at pH = 7.40.

comparable to gallic acid [12], Trolox [16], and caffeic acid [45,47,48], whereas **LA** exhibited lower HOO• radical scavenging activity compared with Trolox in the physiological environments.

The evaluation of the efficacy of radical trapping in relation to various radicals, such as $CH_3O^•$, $CCl_3O^•$, $CH_3OO^•$, $CCl_3OO^•$, NO, NO_2, $O_2^{•-}$, $SO_4^{•-}$, $N_3^•$, DPPH, and $ABTS^{•+}$ through the primary antioxidant mechanism (the SET reaction) in water (Table S3, SI) indicated that **LA** exhibited low radical scavenging activity in water via the SET reaction against CH_3O, $CH_3OO^•$, NO, $O_2^{•-}$, DPPH, and $ABTS^{•+}$ radicals. Thus, based on the computed data, **LA** exhibited moderate even low antioxidant activity.

2.3. ABTS and DPPH assays

The antioxidant activity of **LA** was also performed in a 7.4 pH aqueous solution with DPPH and ABTS assays in a comparison with Trolox (Table 5). **LA** was considerably more effective than Trolox at scavenging DPPH radicals, as evidenced by its IC_{50} value of 6.89 ± 0.28 μM, which is approximately 2.81 times greater than Trolox's IC_{50} value of 19.39 ± 0.14 μM (Table 5). Furthermore, it can be observed that **LA**

Table 4
The $\Delta G^{\ddagger}$ (kcal/mol); κ, k_{app}, k_f, $k_{overall}$ (M^{-1} s^{-1}) and Γ (%) at 298.15 K, in the **LA** oxidation by HO$^{\bullet}$ radicals in the physiological environment.

Pentyl ethanoate					Water					
Mechanism		$\Delta G^{\neq}$	k_{app}	Γ	States	Mechanism	$\Delta G^{\neq}$	k_{app}	k_f	Γ
FHT	O13-H	8.5	5.90×10^{7}	21.1	**H_2A^-**	**SET**	19.9	1.70×10^{-2}	9.86×10^{-3}	0.0
	C8-H	8.5	3.60×10^{6}	1.2		**FHT** O3-H	18.2	1.20×10^{2}	6.96×10^{1}	0.0
	C16-H	8.4	6.50×10^{6}	2.3		O11-H	8.1	7.40×10^{6}	4.29×10^{6}	0.0
RAF	C2	9.0	1.70×10^{6}	0.6		O13-H	5.9	2.75×10^{8}	1.59×10^{8}	0.8
	C4	8.4	5.20×10^{6}	1.9		C8-H	7.3	2.77×10^{7}	1.61×10^{7}	0.1
	C6	7.0	4.51×10^{7}	16.1		C16-H	7.3	2.77×10^{7}	1.61×10^{7}	0.1
	C10	8.1	6.88×10^{6}	2.5		**RAF** C2	3.9	1.73×10^{9}	1.01×10^{9}	5.0
	C12	5.4	1.05×10^{8}	37.5		C3	6.9	5.80×10^{7}	3.36×10^{7}	0.2
	C14	7.0	4.71×10^{7}	16.8		C4	2.7	2.40×10^{9}	1.39×10^{9}	7.0
						C5	9.6	7.00×10^{5}	4.06×10^{5}	0.0
						C6	1.6	2.48×10^{9}	1.44×10^{9}	7.2
						C7	7.9	1.20×10^{7}	6.96×10^{6}	0.0
						C10	5.2	6.78×10^{8}	3.93×10^{8}	2.0
						C11	8.9	2.10×10^{6}	1.22×10^{6}	0.0
						C12	2.9	2.38×10^{9}	1.38×10^{9}	6.9
						C13	11.0	6.30×10^{4}	3.65×10^{4}	0.0
						C14	1.7	2.48×10^{9}	1.44×10^{9}	7.2
						C15	8.4	5.10×10^{6}	2.96×10^{6}	0.0
					HA^{2-}	**SET**	0.5	8.60×10^{9}	3.61×10^{9}	18.0
						FHT O3-H	0.7	2.70×10^{9}	1.13×10^{9}	5.6
						O11-H	0.0	2.70×10^{9}	1.13×10^{9}	5.6
						C8-H	24.6	5.80×10^{-6}	2.44×10^{-6}	0.0
						C16-H	17.1	1.80	7.56×10^{-1}	0.0
						RAF C2	4.0	1.84×10^{9}	7.71×10^{8}	3.8
						C3	8.6	3.20×10^{6}	1.34×10^{6}	0.0
						C4	4.3	1.62×10^{9}	6.80×10^{8}	3.4
						C5	10.7	9.20×10^{4}	3.86×10^{4}	0.0
						C6	3.5	2.26×10^{9}	9.47×10^{8}	4.7
						C7	2.2	2.56×10^{9}	1.07×10^{9}	5.3
						C10	2.2	2.65×10^{9}	1.11×10^{9}	5.5
						C11	7.5	2.08×10^{7}	8.73×10^{6}	0.0
						C12	0.0	2.70×10^{9}	1.13×10^{9}	5.6
						C13	8.1	7.37×10^{6}	3.10×10^{6}	0.0
						C14	0.0	2.70×10^{9}	1.13×10^{9}	5.6
						C15	6.3	1.49×10^{8}	6.26×10^{7}	0.3
$k_{overall}$			**2.80×10^{8}**						**2.01×10^{10}**	

$k_f = f.k_{app}$; f(H2A) = 0.58; f(HA) = 0.42

Table 5
LA and Trolox IC_{50} (μM) values in the DPPH and $ABTS^{\bullet+}$ assays in water at pH = 7.40.

Inhibition (%) **LA**				Inhibition (%) Trolox		
C (μM)	DPPH	C (μM)	$ABTS^{\bullet+}$	C (μM)	DPPH	$ABTS^{\bullet+}$
15.71	84.69 ± 0.77	12.57	92.39 ± 0.16	50	88.67 ± 0.11	90.24 ± 0.08
12.57	72.66 ± 0.21	9.43	78.50 ± 0.16	40	74.45 ± 0.08	80.28 ± 0.14
9.43	59.62 ± 0.34	6.28	64.73 ± 0.32	30	61.40 ± 0.16	69.79 ± 0.20
6.28	45.69 ± 0.17	3.14	49.77 ± 0.10	20	49.65 ± 0.20	59.56 ± 0.27
3.14	36.82 ± 0.05	1.57	34.80 ± 0.13	10	38.66 ± 0.10	46.32 ± 0.38
IC_{50}	6.89 ± 0.28	IC_{50}	3.80 ± 0.15	IC_{50}	19.39 ± 0.14	25.64 ± 0.21

exhibited a significantly greater capacity to capture $ABTS^{\bullet+}$ radicals, as evidenced by its IC50 value of 3.80 ± 0.15 μM, that is, roughly 6.74 times more potent than the reference compound (IC_{50} = 25.64 ± 0.21 μM). These results, which are consistent with those of Wu et al.,[49] suggest that **LA** would be a good antioxidant exceeding the activity of Trolox based on both the DPPH and $ABTS^{\bullet+}$ assays.

In light of the modest activity predicted by the calculations, these results are highly unexpected. One possible explanation is the regularly overlooked presence of DMSO in the experimental assays, which is used to solubilize the compounds and that likely remains associated with both DPPH and ABTS. DMSO itself can form stable radicals [50] or enhance partial ionization of LA and electron transfer from the phenoxide anion to the DPPH/$ABTS^{\bullet+}$ [51], and thus it may contribute to the observed activity.

3. Conclusion

LA was determined to be an effective HO$^{\bullet}$ radical scavenger by computational results; its rate constants in lipid and polar media were 2.80×10^{8} and 2.01×10^{10} M^{-1} s^{-1}, respectively. The HO$^{\bullet}$ antiradical activity of **LA** in the lipid medium was assessed via the RAF (Γ = 75.4 %) and FHT (Γ = 24.6 %) reactions. Conversely, in the aqueous solution, the activity was determined via the FHT, RAF, and SET reactions utilizing the dianion and anion states. Despite the fact that *in vitro* data indicated good antioxidant activity as determined by the DPPH/$ABTS^{\bullet+}$ assays, the computational results suggest that **LA** is an inferior radical scavenger compared to Trolox. This is consistent with the structure of **LA** and therefore our results cast doubt on the reliability of these common experimental radical scavenging assays. We conclude that **LA** might not be an effective antioxidant in physiological environments.

CRediT authorship contribution statement

Quan V. Vo: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Le Trung Hieu:** Writing – original draft, Methodology, Formal analysis. **Hoang Thi Cam Hang:** Visualization, Validation, Investigation, Formal analysis, Data curation. **Vo Huynh Ngoc Diep:** Visualization, Validation, Formal analysis, Data curation. **Nguyen Thi Hoa:** Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Uyen T.D. Huynh:** Writing – review & editing, Validation, Project administration, Data curation. **Nguyen Quang Trung:** Validation, Methodology, Investigation, Data curation. **Adam Mechler:** Writing – review & editing, Supervision, Software, Project administration, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2024.125336.

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