Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids

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The antioxidant activities of 18 typical phenolic acids were investigated using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) assays. Five thermodynamic parameters involving hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton-loss electron transfer (SPLET) mechanisms were calculated using density functional theory with the B3LYP/UB3LYP functional and 6–311++G(d, p) basis set and compared in the phenolic acids. Based on the same substituents on the benzene ring, -CH2COOH and -CH=CHCOOH can enhance the antioxidant activities of phenolic acids, compared with -COOH. Methoxyl (-OCH3) and phenolic hydroxyl (-OH) groups can also promote the antioxidant activities of phenolic acids. These results relate to the O-H bond dissociation enthalpy of the phenolic hydroxyl group in phenolic acids and the values of proton affinity and electron transfer enthalpy (ETE) involved in the electron donation ability of functional groups. In addition, we speculated that HAT, SET-PT, and SPLET mechanisms may occur in the DPPH reaction system. Whereas SPLET was the main reaction mechanism in the FRAP system, because, except for 4-hydroxyphenyl acid, the ETE values of the phenolic acids in water were consistent with the experimental results.

Phenolic acids, a class of compounds formed by the substitution of hydrogen atoms on benzene rings by a carboxylic acid group and at least one hydroxyl, are widely found in plants, plant foods, and human metabolites1. Unlike flavonoids, free phenolic acids, such as benzoic, phenylacetic, and cinnamic acids, have high bioavailability and good water solubility2. They can be absorbed in the stomach, whereas flavonoids cannot be absorbed, and only a small amount of flavonoids are transported passively through the intestinal wall into the blood1–5. Most flavonoids are affected by pH, and digestive enzymes and intestinal microorganisms jointly affect C-ring cleavage, which breaks down into phenolic acids before being absorbed into the blood circulation system6–8. Like flavonoids, phenolic acids are considered to be excellent antioxidants that can quench excessive free radical-induced body damage and chronic diseases3. The antioxidant ability center of phenolic acids is phenolic hydroxyl, so the number and position of phenolic hydroxyls are directly related to their antioxidant activity9. Moreover, the methoxy and carboxylic acid groups also have important effects on the antioxidant ability of phenolic acids10,11.

In recent years, with the development of computational chemistry based on density functional theory (DFT), theoretical results are often used to further explain the experimental results or predict the antioxidant activity of phenolic acids12. Three key antioxidant mechanisms involved in the process of quenching free radicals are hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton-loss electron transfer (SPLET). HAT is a one-step reaction related to O-H bond dissociation enthalpy (BDE), whereas SET-PT and SPLET are two-step reactions, the former is related to ionization potential (IP) and proton dissociation enthalpy (PDE), and the latter is related to proton affinity (PA) and electron transfer enthalpy (ETE)13,14. These reaction mechanisms under different micro-environments may occur independently or simultaneously at different rates15.

In this study, to elucidate the structure-activity relationships (SAR) of phenolic antioxidants, 18 typical phenolic acids, hydroxybenzoic acid (6), hydroxyphenylacetic acid (6), and hydroxycinnamic acid (6),
from natural products or/and colon metabolites of polyphenols with corresponding structures, were investigated by experimental and computational methods\textsuperscript{16}. Their antioxidant activities were evaluated using a 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay in an ethanol system and ferric ion reducing antioxidant power (FRAP) assay in a water system. Here, five thermodynamic parameters of phenolic acids, BDE, IP, PDE, PA, and ETE, were calculated under three different micro-environments (ethanol, water, and gas) at the B3LYP/6–311++G (d, p)//UB3LYP/6-311++G (d, p) level. Moreover, the energy of the highest occupied molecular orbital (HOMO) in the three micro-environments was also computed to better describe the radical scavenging reactivity of the studied compounds. Finally, the effects of the methoxy, phenolic hydroxyl, and carboxylic acid groups on the antioxidant activity of phenolic acids and the possible mechanism of these effects will be discussed.

Results and Discussion
Experimental study of phenolic acids. Phenolic acids are considered excellent natural antioxidants, which have potential applications in medicine and health food. In this study, the antioxidant activity of 18 phenolic acids is expressed by the radical scavenging activity (RSA) value of scavenging DPPH* and the trolox equivalent antioxidant capacity (TEAC) value of the FRAP method (Fig. 1 and Table S1). The names of the phenolic acids and abbreviations are shown in Table 1.

Effect of the carboxylic acid group on antioxidant activity. As shown in Fig. 1, when the other substituents of the benzene ring were the same, the trend in the antioxidant activity of three different phenolic acids in the
two detection systems was as follows: hydroxyphenylacetic acid (\(-\text{CH}_2\text{COOH}\)) > hydroxycinnamic acid (\(-\text{CH} = \text{CHCOOH}\)) > hydroxybenzoic acid (\(-\text{COOH}\)). Similarly, Natella et al. reported that hydroxycinnamic acid had stronger antioxidant activity than hydroxybenzoic acid when other substituents of benzene ring were the same\(^{17}\). Siquet et al. also reported that 3,4-dihydroxyphenylacetic acid (3,4-DH-P) had stronger antioxidant activity than caffeic acid (3,4-DH-C) and protocatechuic acid (3,4-DH-B)\(^{11}\). These results may be related to the electron-donating ability of carboxylic acid groups. The conjugation effect and induction effect together determine that -COOH is a strong electron-withdrawing group, -CH\(=\)CHCOOH is a weak electron-withdrawing group, and -CH\(_2\)COOH is a weak electron-donating group. An electron-donating group can increase the electron cloud density of the benzene ring, decrease the dissociation energy of the phenolic hydroxyl bond and then enhance its free radical scavenging ability. For example, \(-\text{NO}_2\) is considered to be a strong electron-withdrawing group that enhances the dissociation energy of the -OH bond of 3,5-dinitrosalicylic acid, which is about 10 kcal/mol higher than that of 3-methoxysalicylic acid. Similarly, the antioxidant activity of the former is lower\(^{18}\). Therefore, we speculate that the carboxylic acid groups affect the antioxidant activity of phenolic acids according to their electron-donating ability (\(-\text{CH}_2\text{COOH}\) > \(-\text{CH} = \text{CHCOOH}\) > \(-\text{COOH}\)).

However, the reaction systems may interfere with the above rules. Both ABTS and FRAP assays react in a water system, whereas DPPH reacts in an ethanol system. In a study of six dihydrochalcone compounds in \textit{Malus}, the antioxidant activity of phlorizin was found to be the lowest in a DPPH assay, whereas the antioxidant activity of sieboldin was the lowest in an ABTS assay\(^{19}\). In the ethanol system, 4-H-3-M-C is more conducive to scavenging free radicals\(^{20}\). In this study, there is no significant difference in antioxidant activity between the syringic acid (4-H-3,5-DM-B) of the benzoic acid group and the sinapic acid (4-H-3,5-DM-C) of the cinnamic acid group in the DPPH assay \((P > 0.05)\), whereas the former is higher than the latter in the FRAP assay. This may be related to the formation of intramolecular hydrogen bonds between the 4-OH and \(\sigma\)-methoxy groups\(^{10}\). Intermolecular hydrogen bonds between the sinapic acid and ethanol solvent can reduce the role of intramolecular hydrogen bonds, and the polarity of ethanol may not be great enough to completely offset the intramolecular hydrogen bonds formed by the phenolic hydroxyl and \(\sigma\)-methoxy in sinapic acid, so it exhibits a relatively lower antioxidant activity in the DPPH assay compared with the FRAP assay (Fig. 1). Therefore, the effect of the reaction system should be considered when determining the antioxidant activity of compounds.

| Basic structures | Abbreviations | Compounds | Substituents |
|------------------|---------------|-----------|-------------|
| \(\text{CH} = \text{CHCOOH}\) | 3-H-C | 3-Hydroxycinnamic acid | OH H H |
| \(\text{CH}_2\text{COOH}\) | 3-H-P | 3-Hydroxyphenylacetic acid | OH H H |
| 3-H-B | 3-Hydroxybenzoic acid | OH H H |
| 4-H-B | 4-Hydroxybenzoic acid | H OH H |
| 3,4-DH-B | Protocatechuic acid | OH OH H |
| 3-H-4-M-B | Isovanillic acid | OH OCH\(_3\) H |
| 4-H-3-M-B | Vanillic acid | OCH\(_3\) OH H |
| 4-H-3,5-DM-B | Syringic acid | OCH\(_3\) OH OCH\(_3\) |
| 3-H-C | 3-Hydroxybenzoic acid | OH H H |
| 4-H-C | \(p\)-Coumaric acid | H OH H |
| 3,4-DH-C | Caffeic acid | OH OH H |
| 3-H-4-M-C | Isoferulic acid | OH OCH\(_3\) H |
| 4-H-3-M-C | Ferulic acid | OCH\(_3\) OH H |
| 4-H-3,5-DM-C | Sinapic acid | OCH\(_3\) OH OCH\(_3\) |
| 3-H-P | 3-Hydroxyphenylacetic acid | OH H H |
| 4-H-P | 4-Hydroxyphenylacetic acid | H OH H |
| 3,4-DH-P | 3,4-Dihydroxyphenylacetic acid | OH OH H |
| 3-H-4-M-P | Homoisovanillic acid | OH OCH\(_3\) H |
| 4-H-3-M-P | Homovanillic acid | OCH\(_3\) OH H |
| 4-H-3,5-DM-P | 4-Hydroxy-3,5-dimethoxyphenylacetic acid | OCH\(_3\) OH OCH\(_3\) |

Table 1. Molecular structures of hydroxybenzoic, hydroxyphenylacetic and hydroxycinnamic acids.
### Table 2. The calculated thermodynamic parameters of 18 tested compounds in gas and solvents at the B3LYP/6-31+G(d,p) level.

| Compounds | Bonds | BDE (kcal·mol⁻¹) | IP (kcal·mol⁻¹) | PDE (kcal·mol⁻¹) | PA (kcal·mol⁻¹) | ETE (kcal·mol⁻¹) |
|-----------|-------|-----------------|----------------|-----------------|----------------|----------------|
|           | gas   | ethanol | water | gas   | ethanol | water | gas   | ethanol | water | gas   | ethanol | water | gas   | ethanol | water |
| 3-H-B     | 3-OH  | 85.9     | 87.4  | 85.5  | 197.1  | 126.9  | 119.3 | 202.5 | 7.0  | 13.3  | 339.8  | 42.3  | 45.5  | 61.3  | 91.6  | 87.2  |
| 3-H-C     | 3-OH  | 84.7     | 86.8  | 82.9  | 189.3  | 123.2  | 114.5 | 209.1 | 10.1 | 15.6  | 336.1  | 43.4  | 45.4  | 63.9  | 89.9  | 84.6  |
| 3-H-P     | 3-OH  | 83.7     | 85.2  | 82.4  | 189.3  | 121.1  | 113.0 | 208.1 | 10.6 | 16.6  | 342.2  | 44.6  | 46.6  | 56.7  | 87.1  | 83.0  |
| 4-H-B     | 4-OH  | 86.2     | 88.9  | 86.7  | 198.1  | 129.2  | 120.6 | 201.8 | 6.2  | 13.3  | 331.5  | 38.9  | 41.5  | 69.9  | 96.6  | 92.4  |
| 4-H-C     | 4-OH  | 82.4     | 83.8  | 81.4  | 182.9  | 118.1  | 110.1 | 213.2 | 12.2 | 18.4  | 326.7  | 43.4  | 45.4  | 63.9  | 89.9  | 84.6  |
| 4-H-P     | 4-OH  | 83.7     | 83.9  | 81.5  | 186.8  | 119.8  | 111.0 | 210.6 | 11.5 | 16.6  | 342.6  | 44.6  | 46.7  | 56.3  | 85.8  | 82.0  |
| 3,4-DH-B  | 4-OH  | 85.0     | 83.3  | 80.9  | 185.1  | 118.8  | 110.9 | 214.9 | 9.3  | 15.5  | 342.9  | 42.9  | 44.6  | 57.3  | 87.0  | 83.5  |
| 3,4-DH-C  | 4-OH  | 85.0     | 83.3  | 80.9  | 185.1  | 118.8  | 110.9 | 214.9 | 9.3  | 15.5  | 342.9  | 42.9  | 44.6  | 57.3  | 87.0  | 83.5  |
| 3,4-DH-P  | 4-OH  | 83.7     | 83.9  | 81.5  | 186.8  | 119.8  | 111.0 | 210.6 | 11.5 | 16.6  | 342.6  | 44.6  | 46.7  | 56.3  | 85.8  | 82.0  |

In phenolic acids the number and position of phenolic hydroxyl groups are directly related to the free radical scavenging ability. When the number of phenolic hydroxyl groups on the benzene ring is less than 4, the antioxidant activity of phenolic acids is proportional to the number of phenolic hydroxyl groups. Moreover, because phenolic hydroxyl groups are electron donor groups they can enhance the antioxidant activity of other phenolic hydroxyl groups. In this study, dihydroxy phenolic acids (3,4-DH) had a higher antioxidant activity than other phenolic acids with corresponding carboxylic acid groups in FRAP and DPPH assays apart from 4-H-3,5-DM-B/C/P.

In general, both phenolic hydroxyl and methoxy groups significantly enhance the antioxidant activity of phenolic acids.

**Computational study of phenolic acids.** To gain further insights into the SAR of phenolic acids, we investigated the mechanistic pathway of the antioxidant activity on the basis of thermodynamic parameters. Previous studies have shown that hydroxyphenol is the antioxidant activity center, and its hydrogen-donating ability is affected by the polarity of the solvent. Moreover, the experimental studies are conducted with an ethanol system (DPPH assay) and a water system (FRAP assay). Here, ethanol or water, and gas (an extreme condition) are used as the micro-environments to calculate the thermodynamic parameters.

**HAT mechanism.** It is clear that the BDE is an important parameter in relation to the HAT mechanism. The lower the BDE value, the lower is the stability of the corresponding OH bond, which indicates that the OH bond is easily broken. The calculated OH BDEs of phenolic acids have a similar order in the three micro-environments (Table 2). When the substitution positions of the methoxy and phenolic hydroxyl groups on the benzene ring are the same, the BDE values in hydroxyphenylacetic acid (P) and hydroxycinnamic acid (C) are 1.9–13.3 kcal/mol and 0.9–9.2 kcal/mol lower than the corresponding BDE values of hydroxybenzoic acid (B), respectively. This shows that -CH₂COOH can decrease the dissociation energy of the phenolic hydroxyl bond, thereby enhancing the free radical scavenging ability, which is consistent with the experimental results above. Hydroxybenzoic acid and hydroxycinnamic acid have a stronger antioxidant activity than the corresponding hydroxybenzoic acid in the DPPH and FRAP assays.

The introduction of the methoxy group and phenolic hydroxyl group also reduce the BDE of the phenolic hydroxyl group of phenolic acids, which corresponds to higher antioxidant activities in experimental results (Fig. 1) and in particular, 4-OH BDE of 4-H-3,5-DM-B in ethanol, which is 10 kcal/mol lower than 4-OH BDE of 4-H-B. Compared with 3-H-B and 4-H-B in ethanol, the 3-OH and 4-OH BDEs of 3,4-DH-B decrease to 4.1 kcal/mol and 5.6 kcal/mol, respectively. Moreover, the OH BDE of 4-H-3,5-DM-C and 4-OH BDE of 3,4-DH-C are 4.3 kcal/mol and 6.5 kcal/mol lower than that of 4-H-C, respectively, which is close to the 2.3 kcal/mol and 6.6 kcal/mol calculated by Chen et al.

However, in the DPPH system, the order of RSA values of 4-H-3,5-DM is B ≈ C < P (Fig. 1 and Table S1), whereas the order of the BDE values of 4-H-3,5-DM in the ethanol phase are B (78.9 kcal/mol) > C (75.7 kcal/mol) ≈ P (75.5 kcal/mol) (Table 2). The two results obviously do not correspond with each other. Therefore, we
calculate the ratio of the phenolic hydroxyl group in the active site of phenolic acid to the amount of DPPH⁺ in the DPPH system. The calculation process and formula of the ratio are shown in the Supplementary Data (Table S2). Their ratios are P (2.1) > C (1.6) ≈ B (1.5) (Table S2), which indicates that each -OH of 4-H-3,5-DM-P/C/B scavenged about 2.1, 1.6, and 1.5 DPPH⁺, respectively. When each phenolic hydroxyl scavenged more than one DPPH⁺ in the proton solvent reaction system, quinones may have a nucleophilic reaction with ethanol, resulting in the regeneration of the phenolic hydroxyl structure. Alternatively, after scavenging DPPH⁺, a few semiquinones formed by ferulic acid may couple to form dimers, which increases the scavenging activity of the free radicals. In fact, the formation of a sinapic acid dimer is detected in the DPPH reaction system using HPLC-MS (Fig. S1). Therefore, the final result is B ≈ C < P due to the adverse effects of the possible intramolecular hydrogen bond mentioned above. Moreover, the BDE of 4-H-C (83.8 kcal/mol) is not significantly different from that of 4-H-P (83.9 kcal/mol) in ethanol, but the DPPH⁺ scavenging activity of the former is higher than that of the latter, which is consistent with the DPPH⁺ ratio of 4-H-C > 4-H-P (Table S2). These results imply that the effects of thermodynamics and kinetics should be considered together when the amount of hydroxyl phenol is greater than that of DPPH⁺ and the BDE value is too high. Previous studies also have shown that BDE can only roughly evaluate the antioxidant activity under polar solvent conditions. As a result, in the DPPH reaction system, HAT may not be the main mechanism.

**SET-PT mechanism.** The IP and PDE values are the reaction enthalpies related to the SET-PT mechanism. The PDE value is related to the PA of the phenolic acid cation free radicals. The IP and PDE values are greatly influenced by solvent polarity. As shown in Table 2, while the order of the PDE value is gas > water > ethanol, the PDE value of the protons in gas is generally higher than that in water. The proton dissociation ability of the molecule in ethanol is stronger than that in water, which is mainly influenced by the enthalpy of proton solvation. It was reported that the difference of PDE values between p-phenylenediamine in the gas phase and in methanol was more than 200 kcal/mol, which was similar to the results in this study. The solvation enthalpy of protons in the ethanol system was −249.8 kcal/mol, whereas that of protons in the water system was −244.3 kcal/mol, which were also consistent with our calculations.

The IP value is influenced by the overall structure of the molecule and the delocalization and conjugation of the pion electrons, which can directly reflect the electron donation ability of the molecule. The lower the IP value, the easier it is for the molecule to donate electrons. In this study, for the same molecule, the order of the IP value in water is P ≈ C < B due to the adverse effects of the possible intramolecular hydrogen bond mentioned above. Moreover, the BDE of 4-H-C (83.8 kcal/mol) is not significantly different from that of 4-H-P (83.9 kcal/mol) in ethanol, but the DPPH⁺ scavenging activity of the former is higher than that of the latter, which is consistent with the DPPH⁺ ratio of 4-H-C > 4-H-P (Table S2). These results imply that the effects of thermodynamics and kinetics should be considered together when the amount of hydroxyl phenol is greater than that of DPPH⁺ and the BDE value is too high. Previous studies also have shown that BDE can only roughly evaluate the antioxidant activity under polar solvent conditions. As a result, in the DPPH reaction system, HAT may not be the main mechanism.

**SPLLET mechanism.** The PA value and ETE value are the enthalpy of the reaction related to the SPLLET mechanism. The PA value represents the degree of difficulty of phenolic hydroxyl dephosphorization, while the ETE value represents the electron donation ability of corresponding polyphenol ions. As shown in Table 2, similar to the PDE values, molecule dephosphorization in ethanol was easier than in water. Due to the direct influence of proton solvation enthalpy, the order of PA values is gas > water > ethanol, whereas due to the influence of electron
The solvation enthalpy, the order of ETE values is ethanol > water > gas. The differences between the average PA of phenolic acids in ethanol and water and the average PA in gas are 293.7 kcal/mol and 291.6 kcal/mol, respectively, which are close to the reported average PA values of polyphenols in methanol and water, 287.5 kcal/mol and 281.5 kcal/mol, respectively. Compared with the IP values, the ETE values are generally smaller, indicating that electrons are donated easier by polyphenol anions than by neutral molecules, for example, catechol anions have stronger electron-donating ability than neutral molecules.

In this study, compared with -COOH, -CH2COOH reduces the ETE value of phenolic acids by 8.1 kcal/mol on average, while -CH = CHCOOH reduces the ETE value of phenolic acid molecules by 1.6 kcal/mol on average. Therefore, the TEAC difference in the reduction ability of Fe(III) between hydroxyphenylacetic acid and hydroxybenzoic acid is significantly greater than that between hydroxycinnamic acid and hydroxybenzoic acid in the FRAP assay (Fig. 1B). Moreover, taking 4-H-3, 5-DM-B, and 4-H-B as examples, the differences in the ETE value between them in gas, ethanol, and water are more than 10 kcal/mol, respectively (Table 2), indicating that the methoxy group decreases the ETE value of the phenolic hydroxyl group.

In fact, the electron donor group increases the acidity of the phenolic hydroxyl group and decreases the pKa value, which is more conducive to proton dissociation and the antioxidant activity. However, the effect of pH on proton dissociation ability is not considered in this calculation. Nevertheless, since the FRAP assay is tested in water with pH 3.6, the consistency between the ETE values and the TEACs of FRAP is greater than the RSA of the DPPH assay. Therefore, we speculate that SPLET is the main reaction mechanism in the FRAP system.

The calculation results show that the BDE values of the HAT mechanism in gas are the lowest relative to the IP values of the SET-PT mechanism and the PA values of SPLET mechanism, so HAT is the most likely mechanism to occur in gas. The PA values in water and ethanol are significantly lower than the IP value, so the SPLET mechanism is prone to occur in the two environments. Moreover, except for monohydroxyphenolic acid, the ETE values of phenolic acids are consistent with the experimental results of the FRAP assay. SPLET may be the main reaction mechanism in the FRAP system. In the DPPH system, although SPLET has thermodynamic advantages, some experimental results can be explained by the HAT and SET-PT mechanisms. Therefore, all three mechanisms may occur in the DPPH system.

**Conclusion**

The experimental results show that -CH2COOH and -CH = CHCOOH promote the antioxidant activities of phenolic acids when the other substituents on the benzene ring are the same, which may be related to the electron donation ability of the functional groups. The introduction of methoxy and phenolic hydroxyl groups can promote the antioxidant activities of phenolic acids. On the other hand, compared with -COOH, the BDE, PA, and ETE of the phenolic hydroxyl group can be reduced due to the introduction of -CH2COOH and -CH = CHCOOH. A methoxy group can reduce the BDE of the phenolic hydroxyl group and enhance the electron-donating ability of phenolic acids by reducing PA and ETE values. Therefore, by comparing the results of the experiment and calculation, we speculate that HAT, SET-PT, and SPLET mechanisms may occur in DPPH reaction system, and different molecules are affected by reaction thermodynamics. Whereas SPLET is considered the main reaction mechanism in the FRAP system because ETE values are consistent with the experiment results, except with the
4-H group. These results may help us to evaluate the reaction mechanism of phenolic acids and screen them for pharmaceutical and food applications based on their structure.

**Methods**

**Chemicals.** All 18 phenolic acids were of chromatographic-reagent grade purchased from Aladdin Industrial Inc. (Shanghai, China). Other chemicals and reagents were of analytical-reagent grade and purchased from Sigma Chemical Co (China).

**DPPH assay.** The DPPH, the stable artificial free radicals, has been widely used for the measurement of free radical scavenging capacity of the phenolic compounds in ethanol and aqueous systems. Briefly, 2 mL DPPH solution (0.2 mM, in 95% ethanol) was incubated with 2 mL different concentrations of phenolic acid solution. Then, the reaction mixture was shaken and incubated in the dark for 40 min at room temperature. The absorbance was immediately recorded at 517 nm against ethanol with a spectrophotometer (Metash, model UV-5200, China). The DPPH free radical scavenging rate was calculated using the equation:

\[
\text{DPPH scavenging activity} \,(\%) = \frac{A_0 - A_1}{A_0} \times 100.
\]

where \(A_0\) was the absorbance of the control reaction (containing all reagents except the tested compound), and \(A_1\) was the absorbance of the test reaction (containing all reagents with the tested compound). The percentage of DPPH radical scavenging activity was plotted against the sample concentration to acquire the IC\(_{50}\) value, defined as the concentration of sample necessary to cause 50% inhibition. Radical Scavenging Activity (RSA) was calculated from the IC\(_{50}\) value as the equation: RSA = \(\text{pIC}_{50} = -\lg[\text{IC}_{50}]\). The smaller the IC\(_{50}\) value, the larger is the RSA value and the higher is the antioxidant activity.

**FRAP assay.** The FRAP assay was used to determine the AC of phenolic acids by the reduction of Fe(III) and Fe (II). Briefly, Fe(III) was reduced to Fe(II), and Fe(II) was mixed with TPTZ to form a blue complex with strong absorption peak at 593 nm at pH = 3.6 condition. Acetate buffer (pH = 3.6), TPTZ solution (10 mM, in 40 mM hydrochloric acid) and FeCl\(_3\) solution (20 mM, in water) were mixed in a ratio of 10:1:1 to prepare FRAP working solution. Phenolic acid solution (0.5 mL) was mixed with 4.0 mL FRAP working solution, and reacted at 37 °C for 30 min in the dark, and the absorbance at 593 nm was immediately recorded with a spectrophotometer. The result was expressed as the equivalent amount of Trolox per mole of phenolic acid (mol TE/mol).

**Computational methods.** Here, the BDE, IP, PDE, PA and ETE values were determined in the gas phase, water and ethanol at 298.15 K and 1 atmosphere based on the following expressions (Eqs. 2, 5, 6, 9 and 10), respectively. The enthalpy values of hydrogen atom in the gas phase was -312.956 kcal/mol and in solvents were from Parker et al. The enthalpy values of protons and electrons in the gas phase and solvents were from Rimarcik et al.

Hydrogen-Atom Transfer (HAT) mechanism

\[
\text{R}^* + \text{ArOH} \rightarrow \text{RH} + \text{ArO}^* \tag{1}
\]

\[
\text{BDE} = \text{H(ArO}^*\text{)} + \text{H(H}^*\text{)} - \text{H(ArOH)} \tag{2}
\]

Single electron transfer followed by proton transfer (SET-PT) mechanism

\[
\text{R}^* + \text{ArOH} \rightarrow \text{R}^- + \text{ArOH}^{++} \tag{3}
\]

\[
\text{ArOH}^* + \rightarrow \text{ArO}^* + \text{H}^+ \tag{4}
\]

\[
\text{IP} = \text{H(ArOH}^{++}\text{)} + \text{H(e}^-\text{)} - \text{H(ArOH)} \tag{5}
\]

\[
\text{PDE} = \text{H(ArO}^*\text{)} + \text{H(H}^+\text{)} - (\text{ArOH}^{++}) \tag{6}
\]

Sequential proton-loss electron transfer (SPLET) mechanism

\[
\text{ArOH} \rightarrow \text{ArO}^- + \text{H}^+ \tag{7}
\]

\[
\text{ETE} = \text{H(ArO}^*\text{)} + \text{H(e}^-\text{)} - \text{H(ArO}^-\text{)} \tag{8}
\]

\[
\text{ArO}^- + \text{R}^* + \text{H}^+ \rightarrow \text{RH} + \text{ArO}^* \tag{9}
\]

\[
\text{PA} = \text{H(ArO}^-\text{)} + \text{H(H}^+\text{)} - \text{H(ArOH)} \tag{10}
\]

All the calculations were carried out using the Gaussian 09 program suite. The geometries were obtained using the B3LYP/6-311++G (d, p) and UB3LYP were used to optimize free radical system. Here, the wave functions of every radical were checked after calculation, and all spin contaminations of radicals were controlled to avoid affecting the calculation of energy value. The Cartesian coordinates of each molecules used in this study were
shown in the Supplementary Data (Figs. S2–S19). The absence of imaginary frequencies confirmed that the optimized structure was a local minimum. The B3LYP/6-311++G (d, p) and M06-2X/6-311++G (d, p) methods were also used to calculate the HOMO energies of molecules. The solvent effects were computed using an integral equation formalism polarized continuum model (IEF-PCM method).

Statistical analysis. All determinations in Fig. 1 represented the means of at least three independent experiments, each conducted in triplicate. The data were expressed as mean ± standard deviation (SD) and assessed with one-way analysis of variance (ANOVA) using SPSS 19.0 software (IBM, New York, USA). Significant differences between means were determined using Duncan’s multiple tests (p < 0.05).

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References
1. Sozen, E., Kandemir, B. & Ozer, N. K. Basic mechanisms in endoplasmic reticulum stress and relation to cardiovascular diseases. Free Radical. Bio. Med. 78, 30–41, https://doi.org/10.1016/j.freeradbiomed.2014.09.031 (2015).
2. Middleton, E., Kandaswami, C. & Theoharides, T. C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52, 673–751, https://doi.org/10.1124/jphs.2000.0734 (2000).
3. Wang, W. Y. et al. The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. Trends Food Sci. Tech. 56, 21–38, https://doi.org/10.1016/j.tifs.2016.07.004 (2016).
4. Kathrin, K. et al. Intestinal transit and systemic metabolism of apple polyphenols. Eur. J. Nutr. 50, 507–522, https://doi.org/10.1007/s00394-010-0157-0 (2011).
5. Barrington, R., Williamson, G., Bennett, R. N. & Davis, B. D. Absorption, conjugation and efflux of the flavonoids, kaempferol and galangin, using the intestinal CaCo-2/TC7 cell model. J. Funct. Foods. 1, 74–87, https://doi.org/10.1016/j.jff.2008.09.011 (2009).
6. Duyhoven, J. V. et al. Metabolic fate of polyphenols in the human superorganism. P. Natl. Acad. Sci. USA 108, 4531–4538, https://doi.org/10.1073/pnas.0809987108 (2011).
7. Thomas, W., Kristina, W. U. & V., H. P. Carbon dioxide is the major metabolite of quercetin in humans. J. Nutr. 131, 2648–2652, https://doi.org/10.1093/jn/131.10.2648 (2001).
8. William, M. et al. Bioavailability of [2-14C]quercetin-4'-glucoside in rats. J. Agr. Food Chem. 56, 12127–12137, https://doi.org/10.1021/jf080275a (2008).
9. Rodriguez-Bonilla, P., Gandia-Herrero, F., Matencio, A., Garcia-Carmona, F. & Lopez-Nicolas, J. M. Comparative study of the antioxidant capacity of four stilbenes using ORAC, ABTS, and FRAP techniques. Food Anal. Method. 10, 2994–3000, https://doi.org/10.1007/s12161-017-0871-9 (2017).
10. Farhoosh, R., Johnny, S., Asnaashari, M., Molahammadibrahamsen, N. & Sharif, A. Structure–AA relationships of o-hydroxyl, o-methoxy, and alkyl ester derivatives of p-hydroxybenzoic acid. Food Chem. 194, 128–134, https://doi.org/10.1016/j.foodchem.2015.08.003 (2016).
11. Siquet, C., Paiva-Martins, F., Lima, J. L. F. C., Reis, S. & Borges, F. Antioxidant profile of dihydroxy- and trihydroxyphenolic acids–a structure-activity relationship study. Food Chem. 275, 354–360, https://doi.org/10.1016/j.foodchem.2018.09.135 (2019).
12. Galano, A. J., Dai, F., Zhou, B., Yang, L. & Liu, Z. L. AA of hydroxynamic acid derivatives in human low density lipoprotein: Mechanism and structure–activity relationship. Food Chem. 104, 132–139, https://doi.org/10.1016/j.foodchem.2006.11.012 (2007).
13. Ammar, R. B. et al. Antioxidant and free radical-scavenging properties of three flavonoids isolated from the leaves of Rhamnus alaternus L. (Rhamnaceae): A structure-activity relationship study. Food Chem. 116, 258–264, https://doi.org/10.1016/j.foodchem.2009.02.043 (2009).
14. Mateos, R. et al. Synthesis and antioxidant evaluation of isochroman-derivatives of hydroxytyrosol: Structure–activity relationship. Food Chem. 173, 313–320, https://doi.org/10.1016/j.foodchem.2014.04.036 (2014).
15. Wright, J. S., Johnson, E. R. & DiLabio, G. A. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. J. Am. Chem. Soc. 123, 1173–1183, https://doi.org/10.1021/ja02455su (2001).
16. Saito, S. & Kawabata, J. Synergistic Effects of Thiols and Amines on Antiradical Efficiency of Protocatechuic Acid. J. Agr. Food Chem. 52, 8163–8168, https://doi.org/10.1021/jf048970d (2004).
17. Gülcin, I. AA of eugenol: a structure - activity relationship study. J. Med. Food. 14, 975–985, https://doi.org/10.1089/jmf.2010.0197 (2014).
18. Mendes, R. A. et al. Probing the antioxidant potential of phloretin and phlorizin through a computational investigation. J. Mol. Model. 24, 101, https://doi.org/10.1007/s00894-018-3639-9 (2018).
19. Nenadis, N. & Tsimidou, M. Z. Contribution of DFT computed molecular descriptors in the study of radical scavenging activity trend of natural hydroxybenzaldehydes and corresponding acids. Food Res. Int. 48, 538–543, https://doi.org/10.1016/j.foodres.2012.05.014 (2012).
20. Rimarčík, J., Lukei, V., Klein, E. & Ilcín, M. Study of the solvent effect on the enthalpies of homolytic and heterolytic NH bond cleavage in p-phenylenediamine and tetracyano-p-phenylenediamine. J. Mol. Struct. 952, 25–30, https://doi.org/10.1016/j.theochem.2009.04.002 (2010).
21. Xue, Y., Zheng, Y., An, L., Dou, Y. & Liu, Y. Density functional theory study of the structure-AA of polyphenolic deoxybenzoins. Food Chem. 151, 198–206, https://doi.org/10.1016/j.foodchem.2013.11.064 (2014).
30. Karelson, M., Lobanov, V. S. & Katritzky, A. R. Quantum-chemical descriptors in QSAR/QSPR studies. Chem Rev. 96, 1027–1044, https://doi.org/10.1021/cr950202r (1996).
31. Altunkaya, A., Gökmen, V. & Skibsted, L. H. pH dependent AA of lettuce (L. sativa) and synergism with added phenolic antioxidants. Food Chem. 190, 25–32, https://doi.org/10.1016/j.foodchem.2015.05.069 (2016).
32. Piang-Siong, W. et al. Contribution of trans-aconitic acid to DPPH scavenging ability in different media. Food Chem. 214, 447–452, https://doi.org/10.1016/j.foodchem.2016.07.083 (2017).
33. Rajan, V. K. & Muraleedharan, K. A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid. Food Chem. 220, 93–99, https://doi.org/10.1016/j.foodchem.2016.09.178 (2017).
34. Mikuński, D. & Molski, M. Quantum-chemical investigation of the structure and the antioxidant properties of α-lipoic acid and its metabolites. J. Mol. Model. 18, 2907–2916, https://doi.org/10.1007/s00894-011-1306-y (2012).
35. Parker, V. D. Homolytic bond (H-A) dissociation free energies in solution. Applications of the standard potential of the (H+/H·) couple. J. Am. Chem. Soc. 114, 7458–7462, https://doi.org/10.1021/ja00045a018 (1992).
36. Ngo, T. C., Dau, D. Q., Nguyen, M. T. & Nam, P. C. A. DFT analysis on the radical scavenging activity of oxygenated terpenoids present in the extract of the buds of Cleistocalyx operculatus. RSC Advances 7, 39686–39698, https://doi.org/10.1039/C7RA04798C (2017).
37. Zheng, Y. Z. et al. AA of Quercetin and Its Glucosides from Propolis: A Theoretical Study. Sci. Rep. 7, 7543, https://doi.org/10.1038/s41598-017-08024-8 (2017).
38. Jacopo, T., Benedetta, M. & Roberto, C. Quantum mechanical continuum solvation models. J. Chem. Phys. 107, 3032–3041, https://doi.org/10.1063/1.474659 (1997).

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Author contributions
J.Y. and J.C. designed the study; J.C., L.M., J. L., N.S. and C. K. K conducted all the experiments and calculations; J.Y. and J.C. analyzed the data and wrote the manuscript.

Competing interests
The authors declare no competing interests.

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