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Evaluation of Antioxidant Activity of Tea Polyphenols by a Quantum Chemistry Calculation Method - PM6

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Abstract Tea polyphenols arepowerful natural antioxidants. To quickly evaluate theantioxidantactivity of these compounds, a quantum chemistry calculation method - PM6 was employed to calculate the heat of formation (HOF). The HOF valuewas negatively correlated well (r= –0.910, RMS=0.12) with the antioxidant activity of flavanols (catechins), flavonols (kaempferol, quercetin, and myricetin), flavanones (dihydromyricetin), anthocyanins (delphinidin, cyanidin, pelargonidin), proanthocyanidins and theaflavins. The number and position of hydroxyl, and the structural groups influencing the conjugation between the B- and C-rings could affect the HOF value and radical scavenging activity of flavonoids. Therefore, the HOF value could be used as a descriptor for modeling antioxidant activity of polyphenols.

Keywords: *antioxidantactivity, tea polyphenols, HOF, PM6*

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1. Introduction

Free radicals have very close relationship with diseases, and play an essential role in the oxidative deterioration of food [1,2]. It is of great interest to scavenging redundant free radicals for human health and food storage. At present, the resources of antioxidant are mainly divided into natural products and the synthetic materials. The natural antioxidants with high efficiency and low toxicity have become more and more popular [3,4]. Numerous studies have reported that tea polyphenols are powerful natural antioxidants [5-10]. It’s known that phenol hydroxyl (PhOH) contributes a lot to theantioxidant activity of polyphenols [11,12]. The essential mechanism is that phenol hydroxyl reacts to ROS (Reactive Oxygen Species) such as the superoxide anion radical $O^{\bullet-2}$, hydroxyl radical •OH, and lipid peroxyl radical LOO•, and form a stable semiquinone-type radicals to terminate the chain action: $PhOH + R^{\bullet} \rightarrow PhO^{\bullet} + RH$[13].

Numerous studies have been conducted to establish the relationship between flavonoid structure and their radical scavenging activity with quantum chemical calculations [14]. However, many molecules of similar structure don’t obey these models [15]. PM6 (parameterized model 6) is a significant improvement and is much more precise than the previously used AM1 (Austin Model 1) and PM3 methods [16,17,18]. PM6 method in the MOPAC 2009^{TM} software package is implemented in Semi- empirical Quantum Chemical Calculations (SQCC). Compared with DFT (Density Functional Theory) method, SQCC can save computer-time, especially in the case of larger molecules such as theaflavins. Many parameters calculated by PM6 method, such as molecular geometry, heat of formation (HOF), dipole moments, and dissociation energies, coincide well with the experimental results. Previous studies indicated PM6 was an efficient method to evaluate the antioxidant activity of flavonoids based on bond dissociation enthalpy (BDE) of OH groups [19]. The HOF value is associated with the ability of polyphenols to donate hydrogen atom, and was found to correlate well with the O-H BDE [20,21]. In this study, HOF values ofthe major series of polyphenols in tea were calculated by the PM6 method, and were used to predict the antioxidant activity of these compounds.

2. Computational Details

All calculations were performed using the MOPAC 2009^{TM} program package with the method PM6. The HOF value was calculated by the following equation:

$$HOF = E_{elect} + E_{nuc} + \Sigma E_{isol}(A) + \Sigma E_{atom}(A) \quad (1)$$

Where E_{elect} is the electronic energy of the final materials, E_{nuc} is the energy of internuclear repulsion in the final materials, ΣE_{isol} (A) is the energy required to remove all the valence electrons from the atoms in the system to an isolated state, and $\Sigma E_{atom}(A)$ is the energy required to isolate the atoms from their standard state to free state [22,23].

3. Results and Discussion

Flavanols, flavonols, flavanones, anthocyanins, proanthocyanidins and theaflavins are major polyphenols intea.In the present work, The HOF values of these compounds were calculated by the PM6 method, and the relevance between the HOF value and antioxidant activity of compounds was studied for the first time.

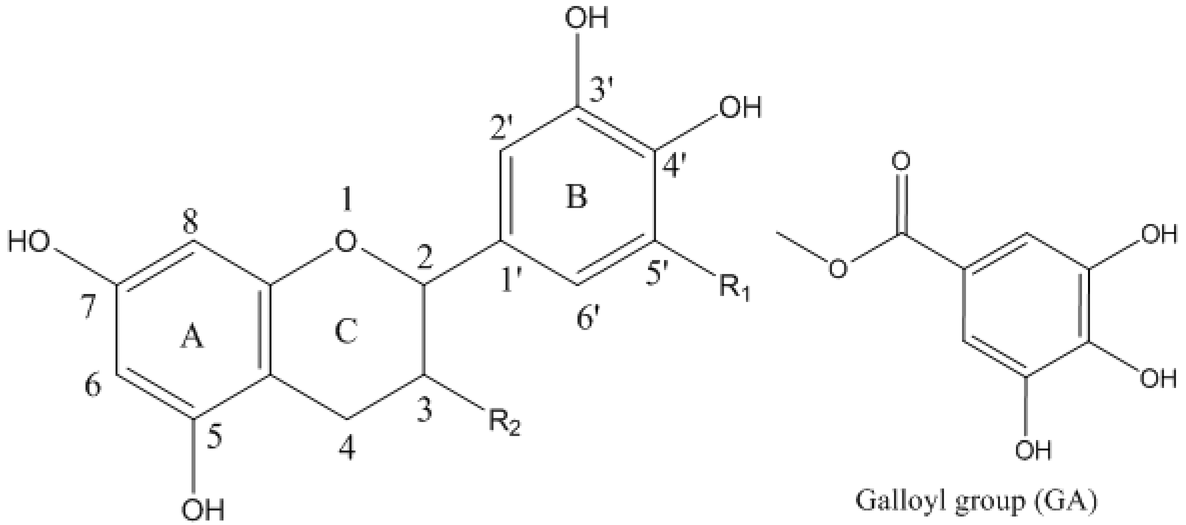
Two structural features of flavonoids can influence their antioxidant potential. The first is the number, position and pattern of hydroxyl substitution in the molecular, and the second is the presence of structural groups required for extended conjugation between the B- and C-rings [11]. It is known that the PhOH on the B ring of flavonoids has strongest radical scavenging ability, and that on the A ring is the weakest. The antioxidant activity of pyrogallol is stronger than that of catechol [24].

Catechins, the primaryflavanols in tea, account for 70 - 80% of tea polyphenols. They mainly consist of epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG) and epigallocatechingallate (EGCG). The HOF values and antioxidant activityof the four catechins were presented in Table 1. These antioxidant data were from previous literatures [11,25,26], and were determined bythe1,1-diphenyl-2-picrylhydrazyl(DPPH), superoxide radical scavenging activity (SRSA) and troloxequivalent antioxidant capacity(TEAC)-ABTS assays.The order of HOF value was EGCG<ECG<EGC<EC, which was opposed to their antioxidant activity sequence. These results showed that the lower HOF value of catechins, the stronger antioxidant activity. As shown in Figure 1, EGC has one more hydroxyl substituent than EC, and EGCG possesses one more C5'-OH than ECG in the B-ring. ECG and EGCG have a pyrogallol moiety in the C3 position. An increased number of OH groups could be related to the increased ability of H atom abstraction and increased scavenging of free radicals.

Table 1. Antioxidant activities of various catechins in different assays and their HOF values

Tea catechins	DPPH[μM][a]	SRSA[μM][b]	TEAC[mM][c]	HOF [kcal/mol]
EC	2.2	203±14	3.16	-221.56
EGC	1.1	32.3±1.4	3.86	-262.07
ECG	0.7	7.2±0.9	4.23	-356.80
EGCG	0.6	4.60±0.43	4.39	-401.82

[a] DPPH radical 50% Scavenging Concentration(IC_{50})(Nanjo *et al.*, 1996). [b] Concentration of catechins for 25% inhibition of superoxide radical $O_2^{\cdot-}$ in non-enzymatic system (IC_{25}) (Furuno *et al.*, 2002). [c] TEAC Value (Cai *et al.*, 2006).



Tea catechins	R_1	R_2
EC	H	OH
EGC	OH	OH
ECG	H	GA
EGCG	OH	GA

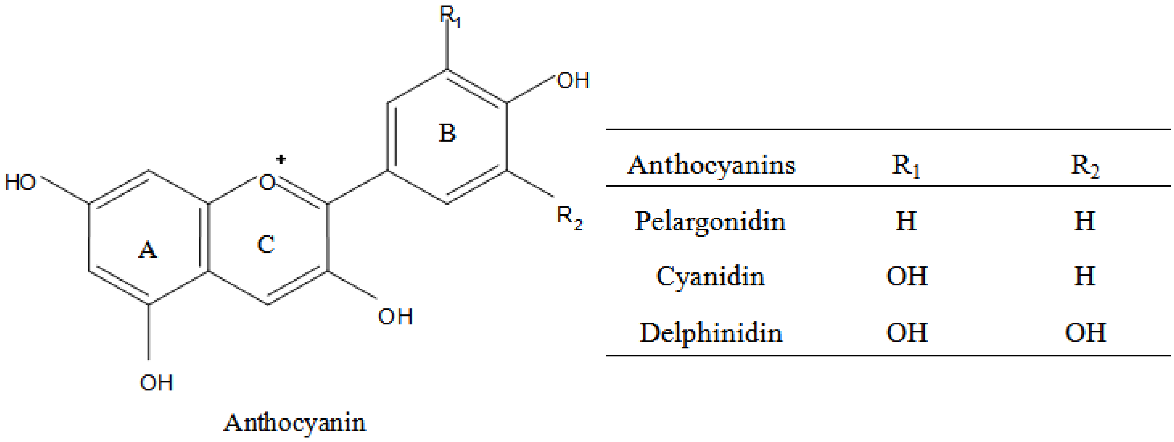
Figure 1. Structures of the tea catechins

Anthocyaninsmainly distributed in shoots of tea plant and account for the bitter taste of tea. Previous studies showed that pelargonidin, cyanidin and delphinidinwere the major anthocyanins in tea [27]. As shown in Table 2, the HOF value order was delphinidin < cyanidin < pelargonidin, and the antioxidant potential sequence of anthocyanins determined by SRSA and TEAC assays was delphinidin>cyanidin>pelargonidin [28,29]. These results suggested that higher antioxidant ability of anthocyanins is associated with lower HOF value. The structure difference of the three anthocyanins is the number and position of hydroxyl at B-ring. Delphinidin possesses three hydroxyls at C3′, 4′ and 5′ positions, cyanidinhas two hydroxylsat C3′ and 4′ positions, and pelargonidin possesses onehydroxyl at C4′ position in the B-ring (Figure 2). These result indicated HOF could serve as a theoretical measure for ranking anthocyanins as antioxidants.

Table 2. Antioxidant activities of various anthocyanins in different assays and their HOF values

Anthocyanins	SRSA[μM][a]	TEAC[mM][b]	HOF [kcal/mol]
Pelargonidin	420	1.30±0.1	-155.67
Cyanidin	22	4.4±0.12	-196.63
Delphinidin	2.4	4.44±0.11	-239.47

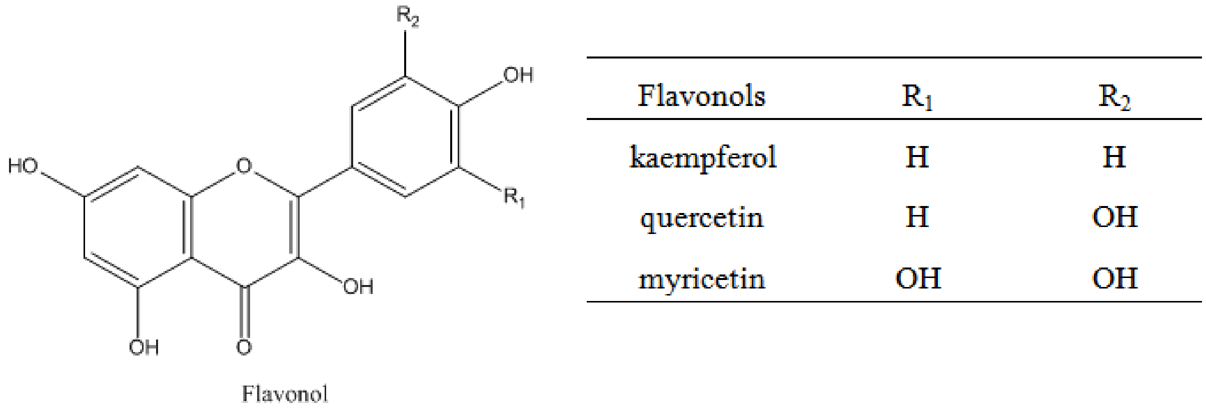
[a]Concentration of anthocyanins for 50% inhibition of superoxide radical $O2^{\cdot -}$ (IC_{50}) (Noda *et al.*, 2002). [b]TEAC value(RiceEvans*et al.*, 1996).



Anthocyanins	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH

Figure 2. Structures of the anthocyanins

Tea contains more than 20 kinds of flavonols, which are considered to account for tea infusion color [30]. Our data showed the HOF values of kaempferol, quercetin and myricetin were -179.36, -220.23 and -262.53 kcal/mol, respectively. Figure 3 showed that the three flavonols have different amount of hydroxylin the B-ring, indicating that more hydroxylin the B-ring, lower HOF value. It was reported the activity of scavenging hydroxyl (·OH) radicals decreases in the order: myricetin > quercetin > kaempferol [31], which meant the negative correlation existed between HOF and antioxidant activity of flavonol.



Flavonols	R_1	R_2
kaempferol	H	H
quercetin	H	OH
myricetin	OH	OH

Figure 3. Structures of oligomericproanthocyanidins in tea and their HOF values

Flavanones are another type of flavonoids which contribute to the antioxidant activity of tea [32]. In order to explore the relationship between antioxidant ability and structure except for hydroxyl, EGC and dihydromyricetin were selected because they possess the same number and location of hydroxyl substitute. The HOF values of EGC and dihydromyricetin calculated in this study are -262.07 and -286.22kcal/mol, respectively (Figure 4). Furthermore, we used frontier molecular orbital theory to analyze the energy difference $\triangle E$ ($\triangle E = E_{LUMO}-E_{HOMO}$) of the two compounds. The $\triangle E$ of EGC and dihydromyricetin is 12.99 and 7.16 eV, respectively, indicating dihydromyricetin is less stable and easier deprotonated than EGC [33]. Active electron orbital of EGC mainly focuses on B-ring, whereas that of dihydromyricetin distributes in the C4-carbonyl moiety, C2-3 bond, C3-OH group and adjacent hydroxyl substitute on the A and B-rings (Figure 5). Previous study showed that dihydromyricetin had stronger antioxidant potential than EGC [34]. Compared with EGC, dihydromyricetin contains a carbonyl moiety at C4, which is considered to be an essential structural requirement for flavonoid aroxyl radical stabilization [35,36]. The chromone structure in C-ring can enhancethe dissociation of hydroxyl of C-ring, and promote hydrogen generation of the C3-alcohol hydroxide [12]. In addition, the altered bonding in C ring allows delocalization between the A and B rings, thus stabilize the aryloxyl radical after hydrogen donation [37,38,39]. All these results may explain for the lower HOF value and higher antioxidant potential of dihydromyricetin compared with EGC.

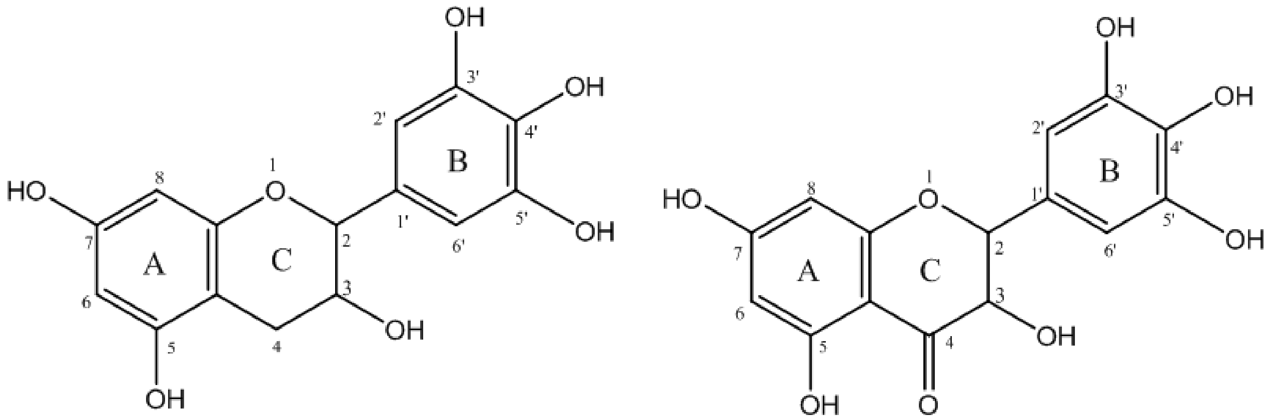
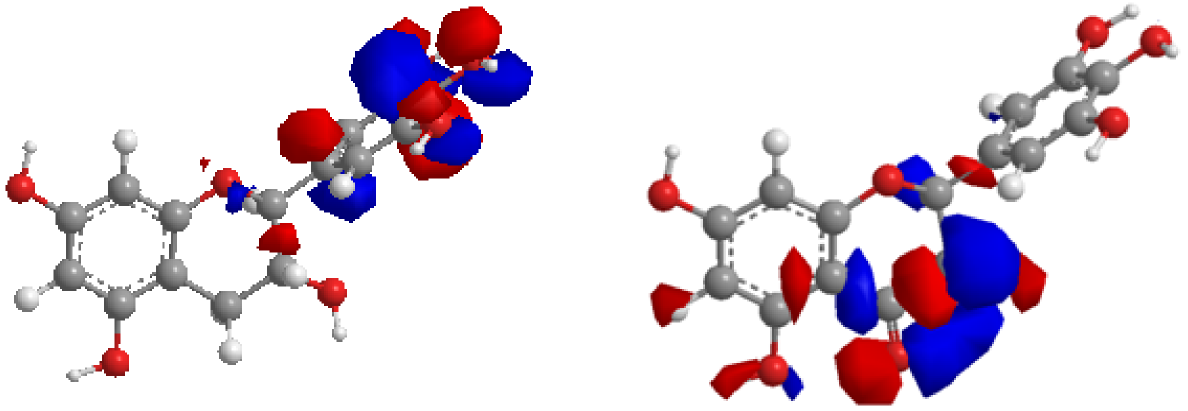
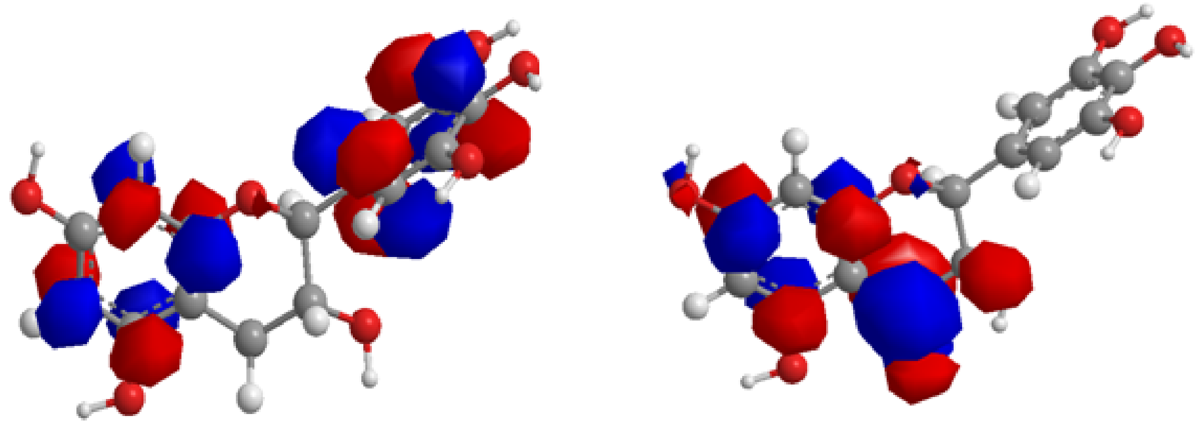


Figure 4. Structures of the major flavonols in tea



EGC HOMO= -10.71 eVDihydromyricetin HOMO= -10.31 eV



EGC LUMO= 2.28 eVDihydromyricetin LUMO= -3.15 eV

Figure 5. Structures of EGC and dihydromyricetin and their HOF values

In addition to the polyphenol monomers, the relevance between HOF value and antioxidant activity of polymers was studied in this work.Our data showed the HOF value sequence was quercetin-Fe complex (3:1)<quercetin-Zn complex (2:1)<quercetin, indicating the order of antioxidant activity was quercetintrimer>dimer >monomer. This prediction was consistent with the previous antioxidant data determined by SRSA assay in Table 3 [40].

Table 3. The inhibition rates of quercetin and quercetin-metal complexes on O_2^{-}radical (Li*et al.*, 2006)

Material	Concentration (units: $\times10^{-5}$ mol/L)					
	2.5	5.0	6.2	7.5	10	12.5
Quercetin	25.4	37.3	33.3	40.5	52.4	54.8
Quercetin-Zn(2:1)	43.6	55.6	56.0	56.9	57.4	69.8
Quercetin-Fe (3:1)	57.1	69.8	72.5	80.2	88.9	94.4

Proanthocyanidins (PAs) are a class of colorless phenolics characterized by an oligomeric or polymeric structure based on flavan-3-ol units. Flavan-3-ol units are connected via C4→C8, C4→C6, C2→O7 or C2→O5 linkages [41]. EC is considered to be one of most common constitutive units of Pas [27]. Our results showed the HOF value of PAs decreased with the rise ofdegree of polymerization (Figure 7). It was reported that the antioxidant activity of PAs largely depends on the degree of polymerization. Although the antioxidant activity of monomers was inferior to that of oligomers, the antioxidant ability of polymers reduced with the increased degree of polymerization. High polymerization degree may attenuate the dissociation of hydroxyl due to the existence of steric hindrance [42,43]. These results indicated the HOF value may only applies to oligomeric proanthocyanidins.

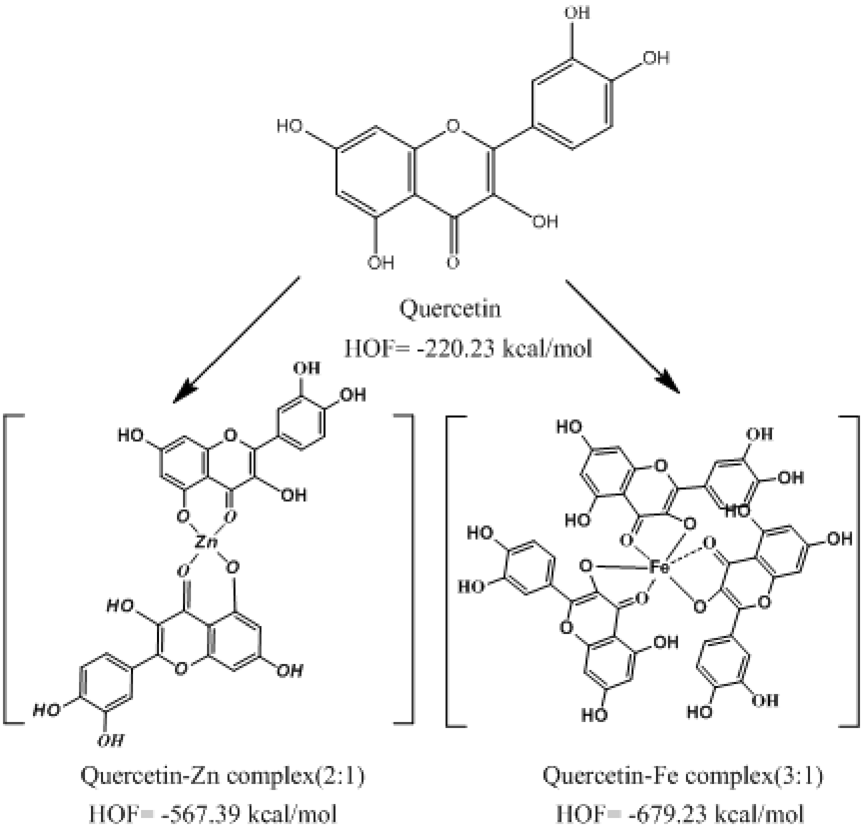


Figure 6. Highest Occupied Molecular Orbital (HOMO) and Lower Unoccupied Molecular Orbital (LUMO) of EGC and dihydromyricetin

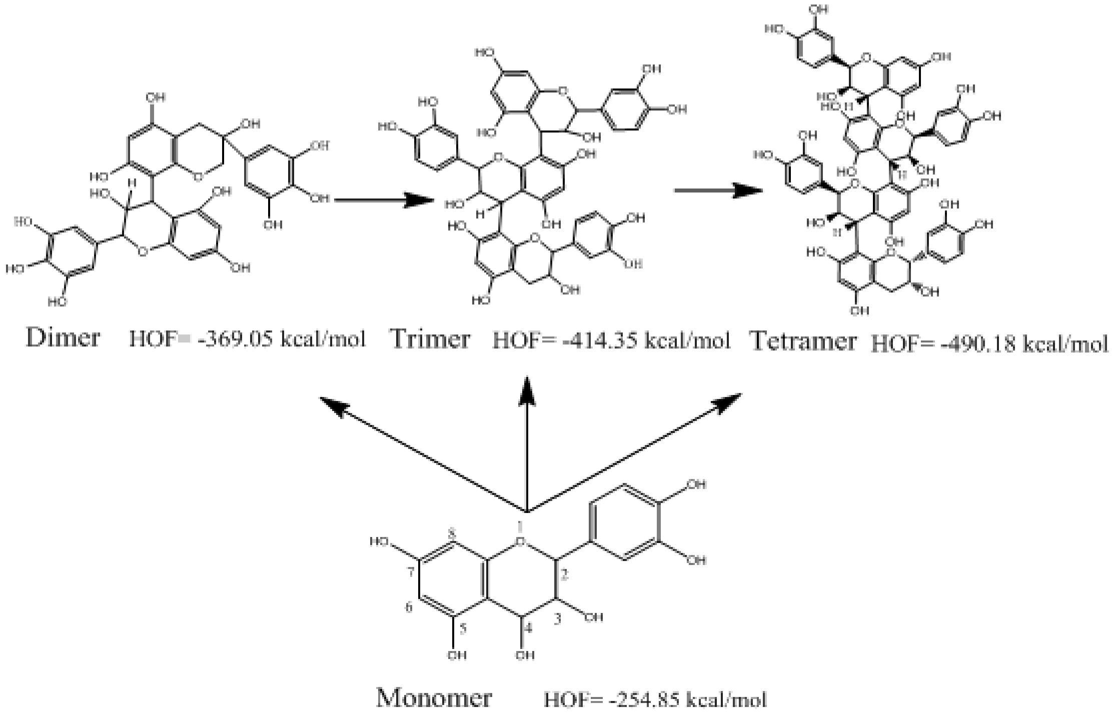


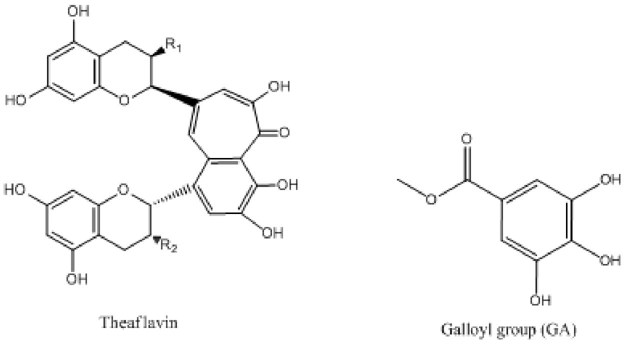
Figure 7. Structures of quercetin-metal complexes and their HOF values

Black tea is the most popularlyconsumed tea in the world, and has excellent antioxidant activity (Tu*et al.*, 2007). During the production of black tea, catechins are enzymatically oxidized to produce polymerized polyphenols including theaflavins and thearubigins. Theaflavins possess a benzotropolone skeleton that is formed from co-oxidation of appropriate pairs of catechins [27]. The major theaflavins in black tea are theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3-gallate (TF2B) and theaflavin-3,3-digallate (TF3). On the other hand, thearubigins are largerand less well-characterized polymers. (Figure 8) [45]. Table 4 showed theHOF valueorderwas TF1>TF2>TF3. TF2A and TF2B had the similar HOF. The antioxidant dataof the four theaflavins determined by TEAC, HRSA and DPPH assays suggested that lower HOF value, higher antioxidant activity [46,47,48]. In addition, all the HOF values of theaflavinsis lower than that of EGCG (-401.82 kcal/mol), which is the strongest antioxidant among catechins. This result was in agreement with the previous report that theaflavins possess stronger antioxidant activity than catechins [49,50]. Theaflavins have two A-rings of flavanols linked by a fused seven-member ring. These structural features may provide more interaction sites with radicals. In addition, the benzotropolone moiety of theaflavins plays an important role in affording antioxidant protection for the preferred oxidation site, and might be responsible for electron donation because of the existence of resonanceforms [51].

Table 4. Antioxidant activities of various TFs in different assays and their HOF values

Theaflavins	TEAC(mM)^a	HRSA(μM)^b	DPPH(μM)^b	HOF[kcal/mol]
TF1	2.94±0.08	3.9	33.2	-415.70
TF2A	4.65±0.16	3.1^c	10.9^c	-547.83
TF2B	4.78±0.19			-549.64
TF3	6.18±0.43	1.6	7.7	-680.27

^{a}TEAC values (Miller *et al.*, 1996); ^{b}Concentration of theaflavins for 25% inhibition of hydroxyl radical OH.$^-$in non-enzymatic system (IC_{25}) and 50% inhibition of DPPH radical (IC_{50}) (Yang *et al.*, 2008);^{c}TF2A and TF2B mixture.



Theaflavins	R1	R2
TF1	OH	OH
TF2A	GA	OH
TF2B	OH	GA
TF3	GA	GA

Figure 8. Structures of the maintheaflavins

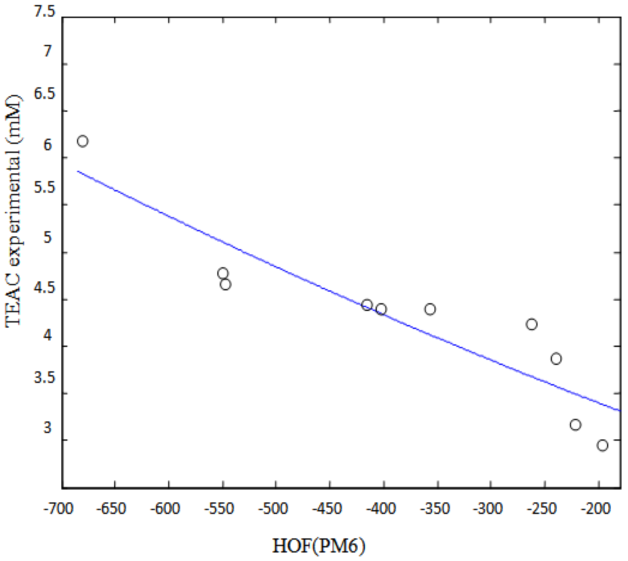


Figure 9. Scatter plot of calculated HOF value versus experimental TEAC values of 11 tea polyphenols (one outlier was omitted)

A scatter plot between the experimental TEAC values for ABTS radical and calculated HOF for a set of 11 polyphenols from Table 1, Table 2 and Table 4 is shown in Figure 9. From this set of TEAC data, the value of TF1 identified as outliers was omitted. A linear relationship indicated a general trend that higher TEAC is associated with lower HOF. A two-descriptor model is given by Eq. 2:

$$TEAC = 2.362 - 0.005HOF \quad N = 10 \qquad (2)$$

The high correlation coefficient of fit (r= –0.910) and the low RMS (0.12) confirmed the significance of this model, and indicated that HOF could be a viable tool for predicting the radical scavenging activity of tea polyphenols.

4. Conclusions

In this study, the relativity of the HOF value and the antioxidant activity of tea polyphenolsincluding flavanols, flavonols, flavanones, anthocyanins, proanthocyanidins and theaflavins, was analyzed by the PM6 method. It was found that the number and position of hydroxyl, and the structural groups influencing the conjugation between the B- and C-rings could affect the HOF value and radical scavenging activity of tea polyphenols.The HOF valuewas negatively correlated well with the antioxidant activity of tea polyphenols.These results suggested that the HOF value might be used to predict the antioxidant activity of polyphenols and other compounds.

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