ESTIMATION OF SCAVENGING ACTIVITY OF PHENOLIC COMPOUNDS BY CALCULATING SPIN DENSITY DISTRIBUTION

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ABSTRACT: *The geometries and spin density distribution of phenolic radicals are investigated by density functional theory (DFT) at the B3LYP level. Rusults are indicated that spin density distribution of phenolic radicals is high at the O and C (para) positions. The stabilization of the free radical responds to the decrease of the highest spin density (HSD) at a position of structure radical and enhances antioxidant (radical scavenging) activity. Calculated Highest Spin Density values (HSDs) are in good agreement with experimental cites, thereby estimating of scavenging activity of neutral molecules can be used, especially phenolic antioxidants.*

Key words: *antioxidants, phenolics, flavonoids, spin density*

1.INTRODUCTION

Phenolic antioxidants form an important class of compounds which serve to inhibit the oxidation of materials of both commercial and biological importance. The function of antioxidants is to intercept and react with the free radicals at a faster rate than the substrate, and since free radicals are able to attack a variety of targets including lipids, fats, and proteins, it is believed that they are implicated in a number of important degenerative diseases including aging itself [1].

There are two main mechanisms by which antioxidants can play their protective role. The first is H-atom transfer, in which a free radical $ROO^{\bullet}$ removes a hydrogen atom from the antioxidant (ArOH) [1 - 4]:

$$ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet} \quad (1)$$

The efficiency of the antioxidant ArOH depends on the stability of the radical $ArO^{\bullet}$, which in turn is determined by the number of hydrogen bonds, conjugation, and resonance effects. The bond dissociation enthalpy (BDE) of the O-H bonds is an important parameter to evaluate the antioxidant action, because the weaker the OH bond the easier the reaction of free radical inactivation will be.

Another possible mechanism by which an antioxidant can deactivate a free radical is electron transfer, in which the radical cation $ArOH^{\cdot+}$ is first formed followed by rapid and reversible deprotonation in solution, as followed:

$$ROO^{\bullet} + ArOH \rightarrow ROO^{-} + ArOH^{\cdot+} \text{ (electron transfer)} \quad (2)$$

$$ArOH^{\cdot+} \rightarrow ArO^{\bullet} + H^{+} \text{ (deprotonation equilibrium)} \quad (3)$$

$$ROO^{-} + H^{+} \rightarrow ROOH \text{ (hydroperoxide formation)} \quad (4)$$

The net result from above is: $ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$, the same as in the atom-transfer mechanism. If the radical cation $ArOH^{\cdot+}$ has sufficient lifetime it can attack suitable substrates. Therefore, the radical cation arising from the electron transfering must be stable. In this case, the ionization potential (IP) is the most significant energetic factor for the

scavenging activity evaluation. However, low IP values also enhance the chance of generating a superoxide anion radical through the transfer of the electron directly to surrounding O_2.

Recently, another mechanism has been discovered. This was named sequential proton loss electron transfer (SPLET). It was experimentally confirmed that vitamin E and other phenols can react with DPPH$^\bullet$ (2, 2-diphenyl-1-picrylhydrazil radical) and other electron deficient radicals (ROO$^\bullet$) by two different and nonexclusive mechanisms, H-atom transfer and SPLET. SPLET is not uncommon for ArOH/ DPPH$^\bullet$ reactions in solvents that support ionization. SPLET can be described by these equations:

$$ArOH \rightarrow ArO^- + H^+ \quad (5)$$

$$ArO^- + ROO^\bullet \rightarrow ArO^\bullet + ROO^- \quad (6)$$

$$ROO^- + H^+ \rightarrow ROOH \quad (7)$$

The reaction enthalpy of the SPLET first step corresponds to the proton affinity of the phenoxide anion (ArO^-). In the second step, electron transfer from phenoxide anion to ROO$^\bullet$ occurs and the phenoxyl radical is formed. The reaction enthalpy of this step will be denoted as electron transfer enthalpy. Again, from the antioxidant action viewpoint, the net result of SPLET is the same as in the two previously mentioned mechanisms, i.e., $ROO^\bullet + ArOH \rightarrow ROOH + ArO^\bullet$ [5]

Those mechanisms point to the fact that the predominantly stable phenolxyl radicals determine scavenging activity of phenolic compounds. Quantum thermochemical calculation of the O-H bond dissociation enthalpy (BDE) is known to be successful for characterizing antioxidant activity for a large number of antioxidant. However, there are cases where quantum thermochemical calculations are poor, especially when steric and intramolecular interactions occur [6].

In this study, we would like to introduce another method to study scavenging activity of phenolic compounds based on quantitative structure activity/property relationships. The stability of phenolxyl radicals can be predicted through calculating the spin density distribution. Spin density is the unpaired electron density at a position of interest, usually at carbon, in a radical. The electron density $\rho(1)$ at the position r_1 can be described as a sum of a density with α and β spin: $\rho(1) = \rho^\alpha(1) + \rho^\beta(1)$ ($\rho^\alpha(1)$, $\rho^\beta(1)$ corresponds to the probability density of finding an electron with α and β spin at the position r_1). The radical will be stable as the spin densities spread radical structure. This is synonymous with the Highest Spin Density – HSD at every atom of radical is small. At the doublet state, sum of spin densities is 1.

One of the ways to measure spin density experimentally is by electron paramagnetic resonance [EPR, ESR (electron spin resonance)] spectroscopy through hyperfine coupling constants of the atom or attached hydrogen. One is usually however limited to ^1H hyperfine couplings which are an indirect measure of the radicals π-electron spin density. A more direct measure of the π-spin density could in principle be obtained by analysis of the ^{13}C and ^{17}O isotropic or anisotropic hyperfine couplings, but these have not been reported presumably because of difficulty of detection. Without such information, the complete spin density distribution for these phenoxyl-based free radicals cannot be attained experimentally [7, 8]

Recent studies have shown the ability of hybrid density functional methods, in particular, B3LYP, to help characterize free radicals. In this paper, we have investigated the density functional level of the conformation of phenolic radicals to predict activity of their neutral molecules by calculating the HSD values (HSDs).

2.COMPUTATIONAL METHODS

All of the calculations reported in this study were performed using the Gaussian03 code [9]. The B3LYP exchange correlation potential was used for optimizing geometries and computing vibrational frequencies in connection with 6-31G* basis set. Single point energy refinement on the 6-31G* optimized geometries was performed with use of the 6-311++G** basis set.

The unrestricted open-shell approach was used for radical species. Spin contamination was found in accepted limit for radicals, being the $<s^2>$ values about 0.75-0.78 in all cases.

Solvent (water) effects were computed in the framework of the self-consistent reaction field polarized continuum model (SCRF-PCM) implemented on the Gaussian03 package, using the UAHF set of solvation radii to build the cavity for the solute, in the gas equilibrium geometries.

3.RESULTS AND DISCUSSION

3.3.Spin densities and Radical Stabilities

The investigated structures were separated into 3 groups and depicted in Figure 1, 2 and 3. For clarity we will discuss separately the conformational properties and the relative stabilities of radicals for each system and the HSD trend.

- **Group 1: CX_3 radicals** (Figure 1).

Many studies showed that most carbon-center free radical formed by normal oxidative processes readily react with molecular oxygen to form peroxyl radicals. As mentioned in the Introduction, sum of spin densities is 1 at the double state. Calculating the CX3 radicals, we found that the HSDs of radicals were approximative 1 (structures range from 1 to 6). It was shown that the unpaired electron mainly focused on the center carbon atom without distributing over radical structures. Therefore, spin density was high at the C atom position which was removed a hydrogen atom. This was corresponding to these radicals which are very active, especially CH3• .

Because of conjugation system (in structure 7 – 9), the spin distribution was improved by the electron delocalization. Therefore, these radicals were more stable than six previous radicals and there was the decrease in HSDs [the minimum HSD value was 0.822 (in structure 9)].

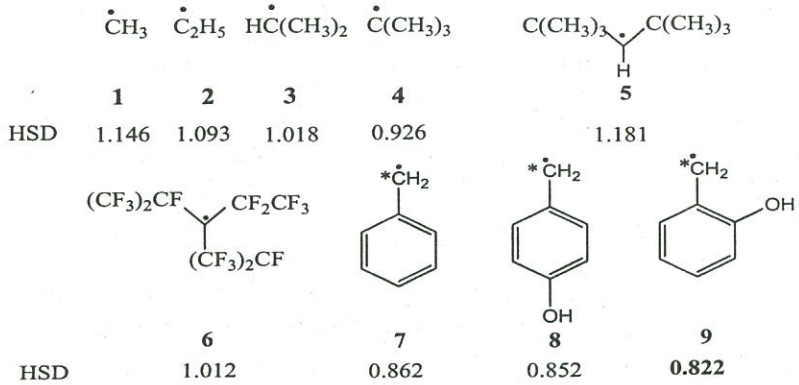


Figure 1. Studied CX_3 radicals and their calculated HSDs.(*) the atom obtained the HSD value.

- **Group 2: Phenolic radicals** (Figure 2).

The unpaired spin density for the phenolic radicals was shown in Figure 2. Each radical displayed an alternating pattern of spin density with high spin at the O and C (para) positions. In general, for the phenoxyl free radicals, substituents which can delocalize this spin density would stabilize the free radical. Such an effect is dominant for the para and ortho positions where the spin density is high. An oxygen substituent therefore at the para position can be expected to participate in the π-electron system and delocalize the spin density.

The calculated spin population of Figure 2 gives a more quantitative measure of such effects. The HSDs lied at 0.370 - 0.448, lower than CX3 radicals. It could be observed that the stable radical was correlative with the small HSDs. From these values, it was also found that the phenolic radicals were more stable than CX3 radicals and could be expected to become candidates for antioxidants.

In addition, it should be noted that the radicals were formed by removing a hydrogen atom from the commercial antioxidants (butylate hydroxy anisole BHA and butyl hydroxyl toluene BHT, structure 15 and 16) obtained the lowest HSDs, 0.370 and 0.372, respectively. So, the commercial antioxidants have the trend of forming stable radicals.

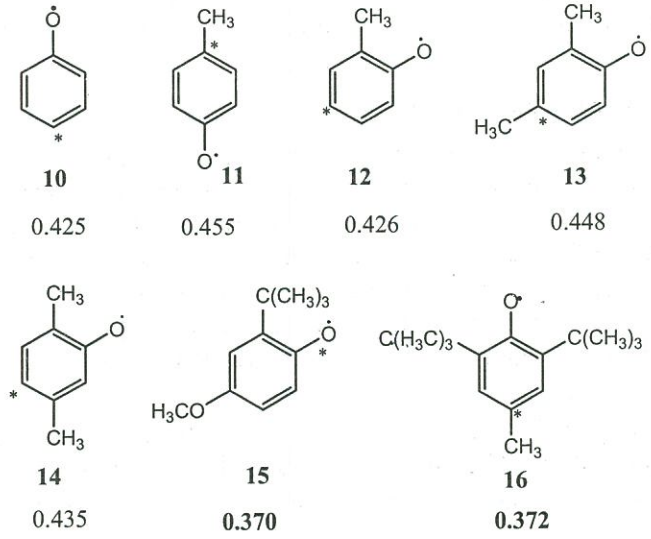


Figure 2. Studied phenolic radicals and their calculated HSDs. (*) the atom obtained the HSD value.

- **Group3: Polyphenolic radicals** (Figure 3)

It was said that the behavior of different OH groups in polyphenolic compounds is largely influenced by the neighboring groups and by the geometry, which are performed by the conjugation systems and electron delocalization. That could be easily quantified through calculating unpaired spin density.

From Figure 3, the HSDs raged from 0.348 to 0.487. It was observed that the presence of a catechol moiety (in structure 18, 23, and 24) probably made the radicals get the small HSDs (values were 0.348, 0.362, and 0.371, respectively). On the other hand, the commercial antioxidant radicals (structure 20 and 21- the tert-butyl hydro quinon TBHQ radicals) also obtained the low HSDs, 0.370 and 0.384, respectively. That was the same results compared to other antioxidant radicals in Group 2, BHA and BHT radicals.

For radicals ranging from 25 to 32, the presence of a saturated (2) ring inhibited the electron delocalization between (1) ring and (2) ring. Therefore, spin density distribution of those radicals were not as good as in catechol radicals. So, the HSDs slightly increased, about 0.430 – 0.487. In addition, the ortho-OH group in (1) ring followed the trend in which forming stable radicals are compared to the para group.

Studying 3 above groups, it could be pointed to the fact that the spin density distribution was correlated with the stability of radicals. The smaller HSDs, the more stable radicals.

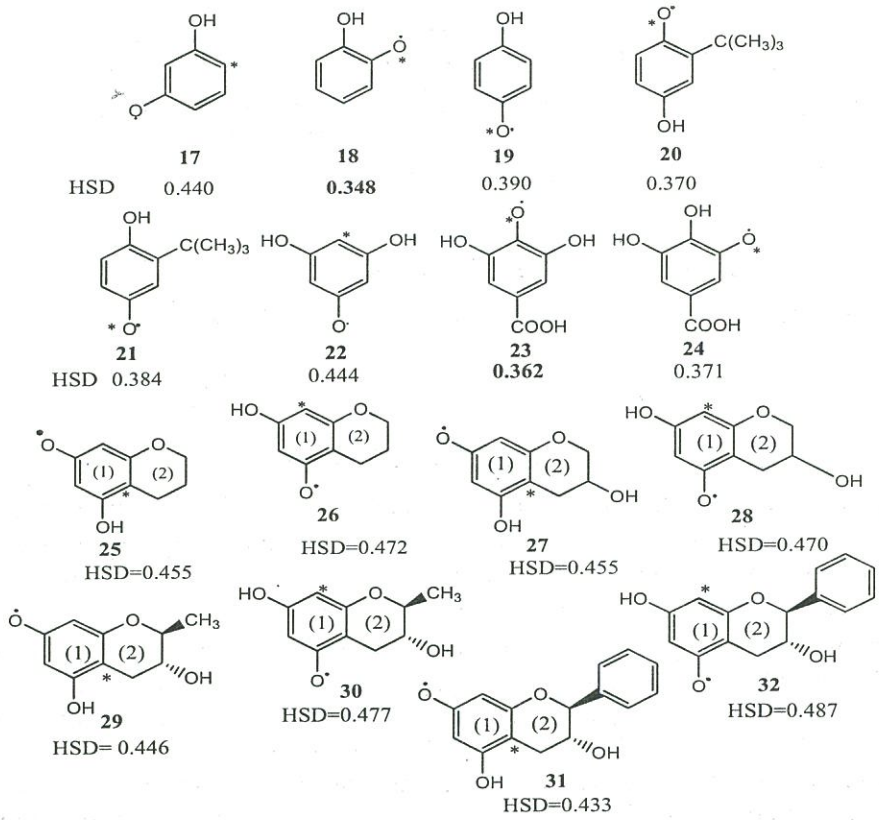


Figure 3. Studied polyphenolic radicals and their calculated HSDs. (*) the atom obtained the HSD value.

3.2.The HSDs of stable flavonoid radicals.

Flavonoids are widespread group of naturally occurring phenolic compounds in common edible fruits, leaves, seeds and other parts of plants. The basic structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6), which are labeled A, B, and C (Figure 4) [10].

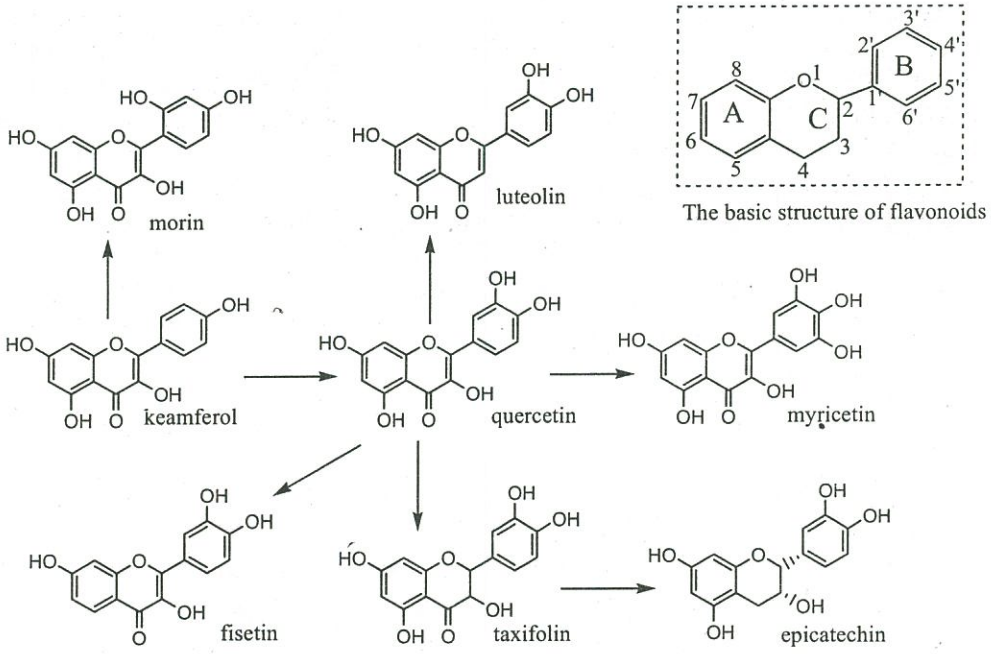
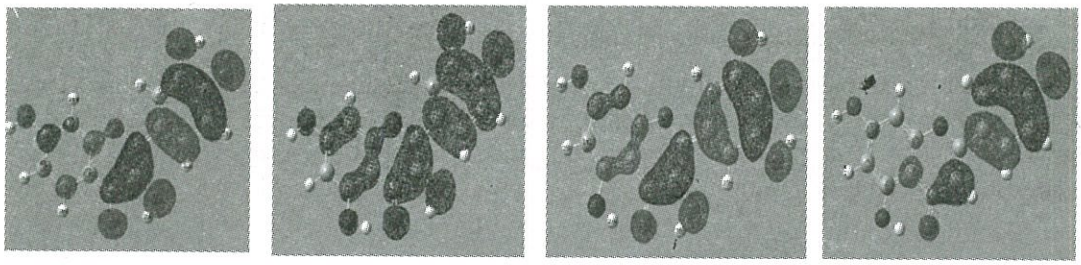


Figure 4. Studied flavonoid antoxidants

Many studies showed that flavonoids are efficient antioxidants because their extensive conjugated π-electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals. It was also shown that upon radicalization, the 4'-OH flavonoid radicals are mainly more stable than other positions.

Calculating spin density distribution of some phenolic radicals above, we found that the polyphenolic radicals which possess dihydroxyl group had the low HSDs, ranging from 0.348 to 0.371. To complete many mentioned studies, we have only regard to the spin density distribution of 4'-OH radicals through HSDs. Because of the large flavonoid molecules, we have employed the 3-21G* basis set for calculating geometry optimization. Sing point energy was performed with the use of the 6-311++G** basis set. The results were shown in Figure 5.

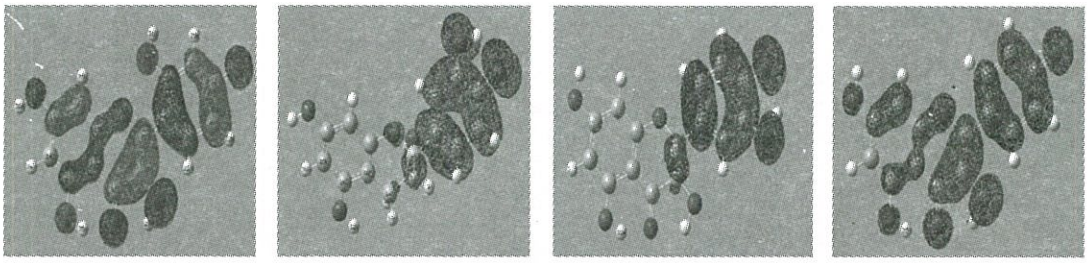


4'-OH Fisetin
HπD = 0.310

4'-OH Quercetin
HπD = 0.311

4'-OH Myricetin
HπD = 0.325

4'-OH Luteolin
HπD = 0.332



4'-OH Morin	4'-OH Epicatechin	4'-OH Taxifolin	4'-OH Keamferol
HSD = 0.373	HSD = 0.374	HSD = 0.374	HSD = 0.375

Figure 5. Unpaired spin density distribution of 4'-OH flavonoid radicals at HOMO (Highest Occupied Molecular Orbital) state and their HSDs

In Figure 5, the HSDs ranged from 0.310 to 0.375. The spin density was high at the O position which removed a hydrogen atom. It was found that the HSDs of the 4'-OH flavonoid radicals ranged within limit of the HSDs of commercial antioxidants above. As many cites, flavonoids are candidates for good antioxidants when their molecules possess three criteria: the o-dihydroxy structure in the B ring, which confers higher stability to the radical form and participates in electron delocalzation; the 2, 3 double bond in conjugation with a 4-oxo function in the C ring is responsible for electron delocalization from the B ring; the 3- and 5-OH groups with 4-oxo function in C and A rings. Calculating HSDs of strong antioxidants, we found that it corresponds to the decrease in HSDs. The radicals were formed by flavonoids satisfy all the above mentioned determinants obtained the small HSDs. For example, fisetin, quercetin and myricetin satisfy three mentioned criteria, thereby their radicals were displayed small HSDs (0.310, 0.311, and 0.325, respectively). The lack one of the criteria made the radicals increased in HSDs. For example, luteolin lacks the 3-OH group in C ring (HSD = 0.425); morin possess 2 phenolic groups in B ring without the o-dihydroxy structure in B ring (HSD = 0.373); taxifolin lacks the 2, 3 double bond in C ring (HSD = 0.374); epicatechin lacks the 2, 3 double bond and a 4-oxo function in the C ring (HSD = 0.374); keamferol lacks catechol moiety in B (HSD = 0.375).

To study the influence of solvents on the calculated HSDs, we had recalculation of these values in ethanol and water solution (Figure 6). Results showed that HSDs decreased for all 4'-OH flavonoid radicals from the gas phase to ethanol. In the case of water the decrease was slight, compared to ethanol. However, the change of these HSDs was rather low, approximately 0.01 – 0.04 (1% - 4%). It was shown that HSDs changed rapidly with the calculation for different solvents. So, it was useful to study the stabilities of radicals by HSDs compared to other parameters. In general, the 4'OH radicals have the trend of the stability in polar solvents.

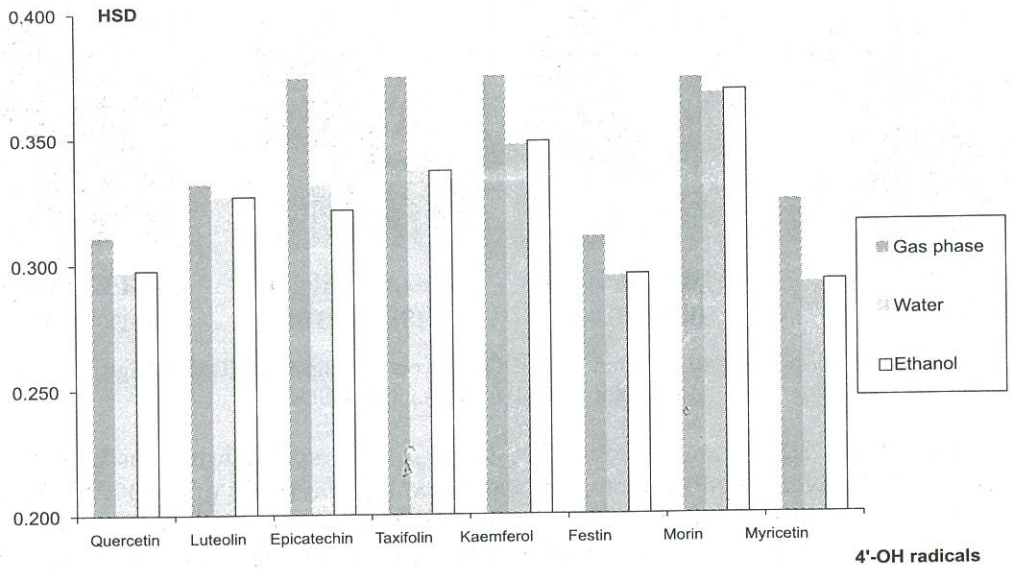


Figure 6. HSDs in gas phase, ethanol and water solution

3.3.Spin density and the activity of antioxidants

As mentioned before, phenolics can play their protective role by donating an H atom or acting as electron donors. A method widely used to predict the ability of flavonoids in experiment is based on the free radicals such as DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]), galvinoxyl (2,6-di-tert-butyl-α-[3,5-ditert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene]-p-toly-loxy),...The antioxidant activity of flavonoids was represented by free radical scavenging activity which is measured by the molar ratio (the molar radical divides the molar antioxidant, $n_{radical}/n_{antioxidant}$). The bigger molar ratio, the stronger antioxidant activity of flavonoid.

Another approach described for determining the priority of radical couples and evaluated the in vitro antioxidant potential of flavonoids on the basis of the one-electron reduction potential at pH 7 (E_7) of the Fl-O$^{\bullet}$/Fl-OH pair. In contrast, the half-peak oxidation potentials ($E_{p/2}$) of flavonoids have been proposed as suitable parameters to evaluate the scavenging activity. This assumes that the electrochemical oxidation Fl- OH $\rightarrow$ Fl-O$^{\bullet}$ + e^{-} + H^{+} and the hydrogen atom donating reaction Fl-OH $\rightarrow$ Fl-O$^{\bullet}$+ H$^{\bullet}$ involve the breaking of the same O-H bond [10, 14]. According to this approach, flavonoids with $E_{p/2} < 0.2$ are defined as readily oxidable and therefore good scavengers.

Calculating the HSDs, we found that all values of HSD were referred to the most stable radical species deriving from the minimum value of each antioxidant radical. The HSDs of 4'-OH flavonoid radicals lied at 0.29 – 0.37 in water solution. Our results were compared to experimental data [10 - 14].

In comparison to the computed HSDs and the scavenging activity of DPPH radical, the molar ratio $n_{DPPH\ radical}/n_{flavonoid\ antioxidant}$ (Table 1 and Figure 7), a good correlation was found (r = -0.93). This showed that good antioxidants which can react with many DPPH radicals generally form stable radicals with small HSDs.

Table 1. Comparison between the HSDs and the ratio $n_{DPPH\ radical}/n_{antioxidant}$

4'-OH Flavonoid radicals	**HSD in water solution** (this work)	Free radical scavenging activity $n_{DPPH\ radical}/\ n_{antioxidant}$ [12]
Myricetin	0.292	-
Fisetin	0.295	5.59
Quercetin	0.297	6.74
Luteolin	0.327	4.73
Epicatechin	0.332	-
Taxifolin	0.337	4.09
Kaemferol	0.347	1.87
Morin	0.367	-

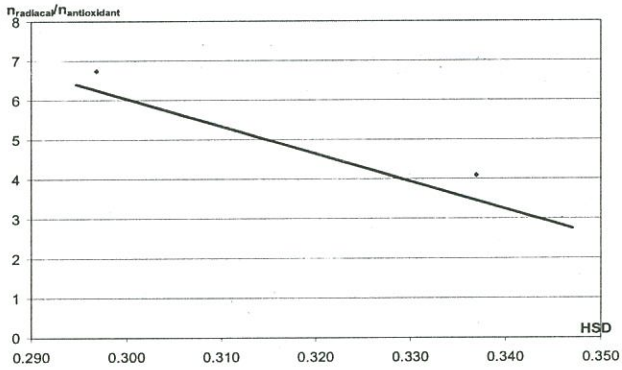


Figure 7. Correlation between the computed HSDs and the ratio $n_{DPPH\ radical}/n_{atioxidant}$, the correlation coefficient is -0.93.

Table 2 and Figure 8 showed that the correlation between the computed HSDs and scavenging activity of galvinoxyl radical. The correlation coefficient was also -0.93.

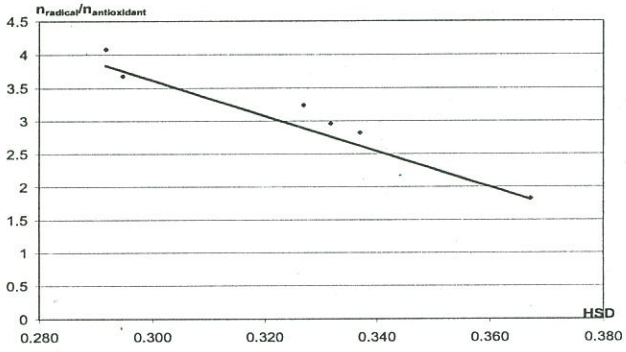


Figure 8. Correlation between the computed HSDs and the ratio $n_{galvinoxyl\ radical}/n_{atioxidant}$, the correlation coefficient is -0.93

Table 2. Comparison between the HSDs and the ratio $n_{galvinoxyl\ radical}/n_{atioxidant}$

4'-OH Flavonoid radicals	HSD in water solution (this work)	Free radical scavenging activity $n_{galvinoxyl\ radical}/\ n_{antioxidant}$ [13]
Myricetin	0.292	4.08
Fisetin	0.295	3.68
Quercetin	0.297	3.27
Luteolin	0.327	3.24
Epicatechin	0.332	2.96
Taxifolin	0.337	2.82
Kaemferol	0.347	1.84
Morin	0.367	1.83

Table 3 showed that the HSDs of selected flavonoids are compared with the reduction potential at pH 7 (E_7) and the half-peak oxidation potentials ($E_{p/2}$). Figure 9 indicated the correlation between computed HSDs and reduction potential of flavonoid. It was found that the strong antioxidant respond to the low in the value of reduction potential at pH 7 (E_7) of the Fl-O$^{\bullet}$/Fl-OH pair and the stability of radical. Our results were in good with experimental data.

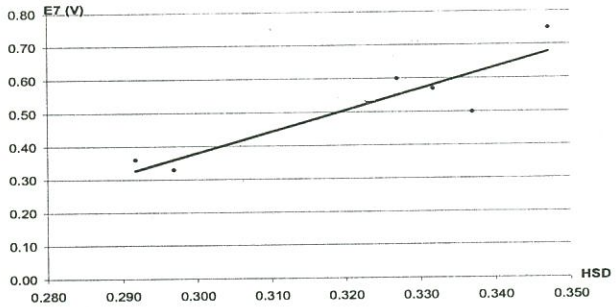


Figure 9. Correlation between the computed HSDs and the reduction potential at pH 7 (E_7), the correlation coefficient is 0.90

Table 3. Comparison between the computed HSDs and the reduction potential at pH 7 [14]

4'-OH Flavonoid radicals	HSD in water solution (this work)	$E_{p/2}$ (V)	E_7 (V)
Myricetin	0.292	-	0.36
Fisetin	0.295	-	-
Quercetin	0.297	0.06	0.33
Luteolin	0.327	0.18	0.60
Epicatechin	0.332	0.16	0.57
Taxifolin	0.337	0.15	0.50
Kaemferol	0.347	0,12	0.75

Morin	0.367	-	-

Since experimental data have not been available for flavonoids, there was a limit to compare calculated HSDs with experimental studies. However, for above results can be infered that all computed values were excellent indicators of free radical scavenging activity. That was useful to estimate quickly the antioxidant activity of phenolic compounds.

4.CONCLUSIONS

In summary, a density functional - based method has been applied to study naturally antioxidant compounds, especially phenolic compounds. The study has concerned the determination of the highest spin density (HSD) according to the stability of radicals and their scavenging activity. The radicals that have the smallest value of HSD are referred to as the most stable radical species and their neutral molecules have strong antioxidant activity.

In general, for the phenolic radicals, substituents which can delocalize spin density at the O and C (para) positions would stabilize the free radical. For flavonoid compounds, the 3', 4'-dihydroxyl groups on B ring, the 2, 3 double bond in conjugation with a 4-oxo function in the C ring, and the 4-oxo function predominantly generate the most stable 4'-OH radicals with the lower in HSDs.

Calculating spin density distribution of phenolic radicals provide a powerful new method of quantitative measure the stability of such radicals, which are often difficult to investigate experimentally and explain by chemical effects. The ability of spin density calculations to accurately model phenolic radical properties opens up a new avenue for understanding and estimating the antioxidant activities of novel species.

DỰ ĐOÁN HOẠT TÍNH QUÉT GỐC TỰ DO CỦA HỢP CHẤT PHENOLIC BẰNG TÍNH TOÁN SỰ PHÂN BỐ MẬT ĐỘ SPIN

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TÓM TẮT: *Sử dụng thuyết phiến hàm mật độ B3LYP tính cấu trúc và mật độ spin của các gốc tự do họ phenolic. Kết quả cho thấy đối với gốc tự do phenolic, mật độ spin cao ở vị trí nguyên tử O và C (para). Sự bền hóa của các gốc tự do có liên quan đến mật độ spin cao nhất ở một vị trí nguyên tử trên cấu trúc gốc tự do và làm tăng khả năng oxy hóa (hoạt tính quét gốc tự do. Tính toán các giá trị HSD phù hợp với các kết quả thực nghiệm khác, từ đó có thể dự đoán hoạt tính quét gốc tự do của phân tử, đặc biệt là các hợp chất chống oxi hóa họ phenolic.*

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