THEORETICAL STUDY OF THE SPIN DENSITY DISTRIBUTION OF THE GREEN TEA CATECHIN RADICALS

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1. INTRODUCTION

Tea *(Camellia sinensis)* is believed to have a wide range of pharmaceutical properties including being antihypertensive, antioxidative, antiarteriosclerotic, anticarcinogenic and hypochlolesterolemic. These diverse biological activities are thought to be attributed to a group of polyphenol compounds, namely green tea catechins (GTCs), present in tea leaves. The content of GTCs varies among green tea, black tea, and oolong tea. Green tea refers to a nonfermented product in which GTCs are mostly preserved while black tea is oxidized during manufacturing process. Oolong tea is a partially fermented product in which GTCs are partially degraded [1].

The yield of crude GTC extracts was 7.4% of dry tea leaves and it mainly consisted of 51.2% (-) epigallocatechingallate (EGCG), 18.7% (-) epigallocatechin (EGC), 12.3% (-) epicatechin (EC) and 11.8% (-) epicatechin gallate (ECG) [2]. Several studies have suggested that the GTC extracts exhibited strong antioxidative effect.

Green tea polyphenols, i.e., EC, EGC, ECG, and EGCG belong to flavonoid. The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C_6-C_3-C_6), which are labeled A, B, and C (Figure 1).

The function of antioxidants is to intercept and react with the free radicals at a rate faster 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 number of important degenerative diseases including aging itself.

There are two pathways for oxidation in which antioxidants can play a preventive role. The first is H-atom transfer, illustrated below for the important case of lipid peroxidation [6, 7, 11]:

$RH \rightarrow R^{\cdot}$ (initiation) (1)

$R^{\cdot} + O_2 \rightarrow RO_2^{\cdot}$ (addition of O_2) (2)

$RO_2^{\cdot} + RH \rightarrow RH + R^{\cdot}$ (H-atom exchange) (3)

Once a free radical $R^{\cdot}$ has been generated, then reaction 2 and 3 form a chain reaction. As the chain cycles through (2) and (3) many lipid molecules (R-H) are converted into lipid hydroperoxide (ROOH), resulting in oxidation and rancidity of fats. Reaction 2 is very fast, ca. 10^9 $M^{-1}s^{-1}$, whereas (3) is much slower, typically 10^1 $M^{-1}s^{-1}$.

For the phenolic antioxidant (ArOH), the role of the antioxidant is to interrupt the chain reaction according to

$RO_2^{\cdot} + ArOH \rightarrow ROOH + ArO$ (4)

To be effective $ArO^{\cdot}$ must be a relatively stable free radical, so that it reacts slowly with substrate RH but rapidly with $ROO^{\cdot}$, hence the term "chain-breaking-antioxidant".

The rate of reaction of ArOH with peroxyl radicals depends on the barrier height for transfer of an H-atom from ArOH. It is clear that the Bond Dissociation Enthalpy (BDE) in ArOH will be an important factor in determining the antioxidant capacity, since the weaker the OH bond the faster will be the reaction with free radical.

Another possible mechanism by which an antioxidant can deactivate a free radical is electron transfer:

$$ROO^{\cdot} + ArOH \rightarrow ROO^{-} + ArOH^{\cdot +}$$

Again, the radical cation arising from the electron transfer must be stable, so it does not react with substrate molecules. In this case, the ionization potential (IP) is the most significant energetic factor for the scavenging activity evaluation.

In this work, we would like to introduce another parameter which can be used to predict the stable radicals through calculating the spin density distribution. Spin density is the upaired 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 distribute over radical structure. This is synonym with the maximum spin density – MSD at every atom of radical is small. At the doublet state, sum of spin densities is 1.

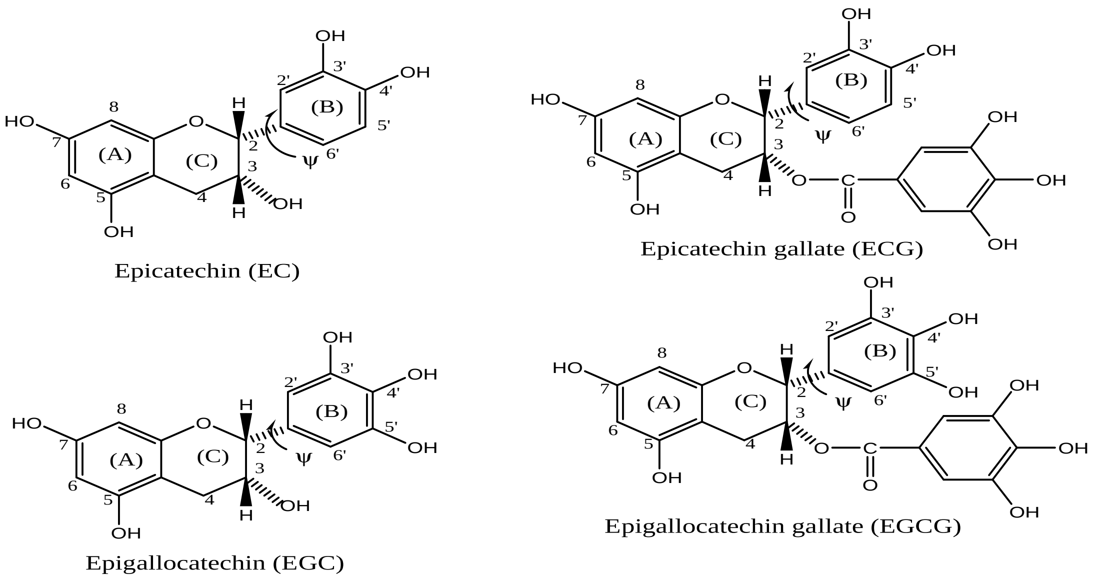
In this paper, we have investigated at the density functional level of the conformation of four catechins: EC, ECG, EGC, and EGCG to predict activity of flavonoids by the MSD values

2. COMPUTATIONAL METHODS

All of the calculations reported in this study were performed using the Gaussian03 code [4]. The B3LYP exchange correlation potential was used for optimizing geometries in connection with 3-21G* basic set. Harmonic vibrational frequencies were computed at HF/3-21G*. Single point energy refinement on the 3-21G* optimized geometries was performed with use of the 6-311++G** basic set.

The unrestricted open-shell approach was used for radical species. Spin contamination was found in accepted limit for radicals, being the $\langle s^2 \rangle$ 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.



Epicatechin (EC)

Epicatechin gallate (ECG)

Epigallocatechin (EGC)

Epigallocatechin gallate (EGCG)

Figure 1. Structures of (-) epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG).

3.RESULTS AND DISCUSSION

The investigated compounds are depicted in Figure 1.

For clarity we will discuss separately the conformational properties and the relative stabilities of radicals for each system and the MSD trend.

3.1.Conformations and Radical Stabilities

Green tea catechins represent the most common and active edible antioxidants. The antioxidant ability was related to the number and mutural position of hydroxyl groups and to conjugation and resonance effects [3].

Epicatechin (EC)

EC contains five phenolic groups (Figure 1) but the 3-OH group on ring C is an alcoholic group to which has no antioxidant ability. The delocalization of the unpaired electron, conjugation effects were determined by a dihedral angle C_3-C_2-$C_{1'}$-$C_{2'}$ (ψ). The values of ψ were from 71.81 to 82.77^o (see Table 1). These values indicated that for EC there is no possibility of conjugation between the rings, due to the saturation of the C ring.

Upon radicalization, EC can give four radicals of which relative energies were within 10kcal/mol (see Table 2). The most stable radical was the radical 4’-OH with the torsion angle ψ was 82.77^o. The 5-OH, 7-OH, and 3’-OH radicals lied at 4.76, 5.71, and 9.62 kcal/mol above the 4’-OH.

Epigallocatechin (EGC)

EGC has the same EC structure but has three phenolic groups on B ring. EGC can give five active radicals of which relative energies are within 10kcal/mol. The delocalization of the unpaired electron, conjugation effects of EGC were stronger than those of EC radicals, the values of ψ were from 71.36 to 106.15^0.

The most stable radical was the radical 3’-OH, close in energy to the 4’-OH one (0.00 and 0.94 kcal/mol). The dihedral angle ψ of 106.15^{o} was bigger than other EGC species. The other isomers, generated by the loss the hydrogen atom from the 5’-OH, 5-OH, and 7-OH groups, were found at 8.03, 5.05, and 6.79 kcal/mol, respectively (see Table 1 and Table 2).

Table 1. Structures and total energies for both catechin radicals in gas phase. All calculation at b3lyp/6-311++g**//b3lyp/3-21g*, freq at hf/3-21g*

Compound		**E (HF)**	**ZPE**	**E_{total}**	y
Epicatechin (EC)	5-OH	-1031.0039214	0.275172	-1030.7562666	71.84
	7-OH	-1031.0010950	0.273713	-1030.7547533	71.81
	3-OH	-1030.9686978	0.276691	-1030.7196759	71.22
	3’-OH	-1030.9952564	0.274152	-1030.7485196	76.88
	4'-OH	**-1031.0111722**	**0.274794**	**-1030.7638576**	**82.77**
Epicatechin gallate (ECG)	5-OH	-1601.2330409	0.389749	-1600.8822668	99.10
	7-OH	-1601.2311256	0.389881	-1600.8802327	101.10
	3'-OH	-1601.2342982	0.389243	-1600.8839795	105.67
	4'-OH	**-1601.2424672**	**0.388603**	**-1600.8927245**	**92.53**
	3''-OH	-1601.2264416	0.389771	-1600.8756477	100.45
	4''-OH	-1601.2388684	0.390108	-1600.8877712	99.38
	5''-OH	-1601.2358019	0.388542	-1600.8861141	143.48
Epigallocatechin (EGC)	5-OH	-1106.2526477	0.279607	-1106.0010014	71.41
	7-OH	-1106.2499998	0.279751	-1105.9982239	72.60
	3-OH	-1106.2203770	0.283265	-1105.9654385	71.36
	3'-OH	**-1106.2609896**	**0.279937**	**-1106.0090463**	**106.15**
	4'-OH	-1106.2599331	0.280420	-1106.0075551	78.79
	5'-OH	-1106.2478936	0.279597	-1105.9962563	74.27
Epigallocatechin gallate (EGCG)	5-OH	-1676.4834357	0.391586	-1676.1310083	91.52
	7-OH	-1676.4811357	0.391702	-1676.1286039	92.04
	3'-OH	**-1676.4920751**	**0.392247**	**-1676.1390528**	**85.62**
	4'-OH	-1676.4915579	0.392508	-1676.1383007	97.56
	5'-OH	-1676.4768603	0.391379	-1676.1246192	99.29
	3''-OH	-1676.4753531	0.391478	-1676.1230229	91.63
	4''-OH	-1676.4884617	0.392062	-1676.1356059	93.56
	5''-OH	-1676.4872895	0.391961	-1676.1345246	94.29

Epicatechin gallate (ECG)

ECG is a gallate ester moiety at the 3-position of EC, concludes 3,4,5-trihydroxyphenyl group. This has effects on torsion angle ψ and makes ECG have stranger properties than EC. The values of ψ were from 92.53 to 105.67^{0} (see Table 1). Upon radicalization, ECG can give seven active radicals of which relative energies were within 10kcal/mol. In gas phase, the radical 4’-OH was the most stable one with the minimum dihedral angle ψ was 92.53^{0}. At 6.56, 7.84, 5.49, 10.72, 3.11, and 4.15

kcal/mol above the global minimum, we found the 5-OH, 7-OH, 3’-OH, 3’’-OH, 4’’-OH, and 5’’-OH species, respectively.

Epigallocatechin gallate (EGCG)

EGCG makes up about 40% of the total catechin content and is widely accepted as the major antioxidant ingredient in green tea [5]. EGCG is a gallate ester moiety at the 3-position of EGC, contains 3,4,5-trihydroxyphenyl group. EGCG has 8 hydroxy groups and can give 8 active radicals of which relative energies were within 10 kcal/mol.

In gas phase, the most stable radical was the 3’-OH one, practically isoenergetic with the radical 4’-OH (0.00 and 0.47 kcal/mol, respectively). It was similar to ECG, the most stable radical of EGCG was correlative with the minimum torsion ψ (85.62^0). The radical 4’’-OH was close to the 5’’-OH one (2.16 and 2.84 kcal/mol). The radical 5’-OH, 7-OH, 5’-OH, and 3’’-OH lied at 5.05, 6.56, 9.06, and 10.06 kcal/mol, respectively (see Table 2).

3.2.Correlation between MSD values and relative energies.

Table 2 reports the relative energy and the maximum spin density (MSD) values in the gas phase and water solution for all green tea phenolics.

For green tea stable radicals, the MSD lied at 0.37 – 0.38 in gas phase. A correlation (r = 0.95) was found between MSD values and relative energies for catechin radicals in gas phase (Figure 2). The radical that had minimum in energy had the small in MSD. It could be observed that the stable radical correlative with the smallest value of MSD.

However, not all compounds followed this trend in water due to the effect of solvent. We found that the correlation coefficient between MSD values and relative energies is 0.75 in water. All green tea catechin radicals EC and EGC radicals were more stable in solution than in gas phase but ECG and EGCG radicals were not. We thought that the presence of 3,4,5-trihydroxyphenyl group in structure of ECG and EGCG caused stranger properties in water.

Example, for EGCG, it was slightly different: the most stable radical has the MSD value close to minimum in gas phase and water. In gas phase, the radical 3’-OH is the most stable one in energy but the radical 4’’-OH is the most stable one in spin density (value is 0.38 and 0.37, respectively). In water solution, the radical 4’-OH is the most stable one in energy but the radical 3’-OH is the most stable one in spin density (value is 0.35 and 0.34, respectively).

Table 2. Relative energies and max spin values for both catechin radicals in gas phase and water solution. All calculation at b3lyp/6-311++g**//b3lyp/3-21g*

Compound		Gase phase			Water solution		
		$E_{relative}$ (kcal/mol)	Max spin	s^2	$E_{relative}$ (kcal/mol)	Max spin	s^2
EC	5-OH	4.76	0.444	0.784	2.30	0.458	0.779
	7-OH	5.71	0.439	0.786	1.18	0.438	0.778
	3-OH	27.72	0.891	0.750	172.40	0.858	0.754
	3'-OH	9.62	0.454	0.782	0.70	0.377	0.774
	4'-OH	**0.00**	**0.374**	**0.769**	**0.00**	**0.332**	0.767
ECG	5-OH	6.56	0.466	0.785	10.44	0.491	0.780
	7-OH	7.84	0.439	0.786	10.98	0.438	0.783
	4'-OH	**0.00**	**0.367**	**0.769**	**0.00**	**0.332**	0.767

	3'-OH	5.49	0.380	0.773	5.61	0.331	0.769
	3"-OH	10.72	0.465	0.778	9.66	0.404	0.773
	4"-OH	3.11	0.370	0.774	4.34	0.340	0.772
	5"-OH	4.15	0.419	0.773	4.13	0.384	0.769
EGC	5-OH	5.05	0.444	0.784	8.01	0.458	0.779
	7-OH	6.79	0.437	0.785	7.79	0.433	0.779
	3-OH	27.36	0.875	0.754	30.78	0.868	0.750
	3'-OH	**0.00**	**0.381**	0.772	4.37	**0.334**	0.768
	4'-OH	0.94	0.391	0.771	**0.00**	0.350	0.768
	5'-OH	8.03	0.431	0.777	4.91	0.364	0.771
EGCG	5'-OH	5.05	0.451	0.785	8.40	0.472	0.780
	7-OH	6.56	0.438	0.786	8.81	0.433	0.779
	3'-OH	**0.00**	0.382	0.771	5.32	**0.342**	0.768
	4'-OH	0.47	0.396	0.771	**0.00**	0.353	0.768
	5'-OH	9.06	0.442	0.779	5.31	0.400	0.773
	3"-OH	10.06	0.469	0.779	8.06	0.405	0.773
	4"-OH	2.16	**0.374**	0.774	3.12	0.355	0.772
	5"-OH	2.84	0.419	0.773	8.19	0.385	0.770

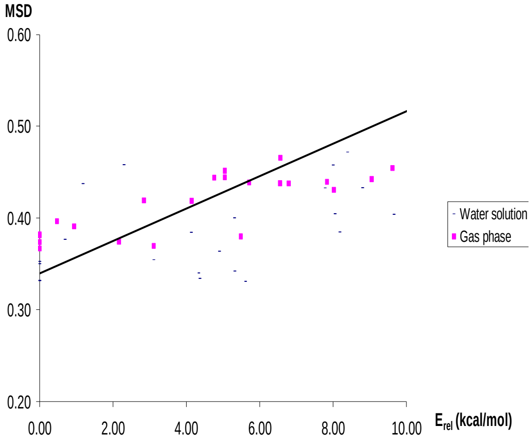


Figure 2. Correlation between MSD values and relative energies in gas phase and water solution. The correlation coefficient is 0.95 in gas phase, 0.75 in water solution

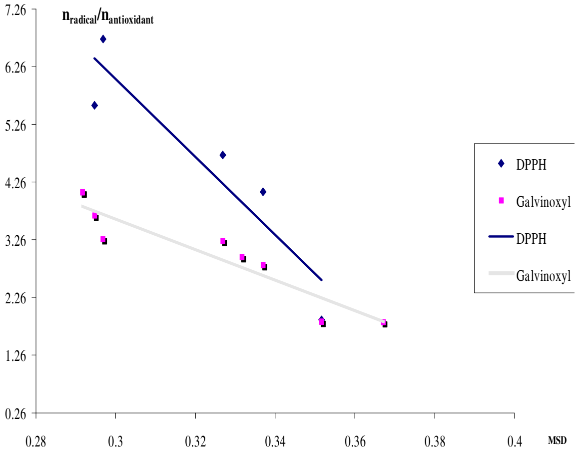


Figure 4. Correlation between computed MSD and experimental values. The correlation coefficient is -0.93 for both DPPH radical and galvinoxyl radical.

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. It is clear that as far as specific molecular properties are concerned, the bond dissociation enthalpy (BDE) for the –OH bond and ionization potential (IP) are of particular importance in deciding which the mechanism is the favored one for the radical scavenging activity. Flavonoids with the dihydroxy functionality are the most active compounds in donating an H atom, as confirmed by their low BDE and IP values [6, 7, 8].

Many studies in experiment showed that upon radicalization, the 4'-OH flavonoid radical was the most stable radical. The antioxidant activity of flavonoid was represented by free radical scavenging activity which is measured by the molar ratio ($n_{radical}/n_{atioxidant}$). The bigger molar ratio, the stronger antioxidant activity of flavonoid.

Calculating the MSD values, we found that all the values of MSD were referred to the most stable radical species deriving from the minimum value of each antioxidant radical (see Table 1 and Table 2). Then, we have calculated the MSD values of some 4'-OH flavonoid radicals (Figure 3) in gas phase and water solution (see Table 3, 4). Our results were compared to computed values [6, 7, 8] and experimental data [9, 10].

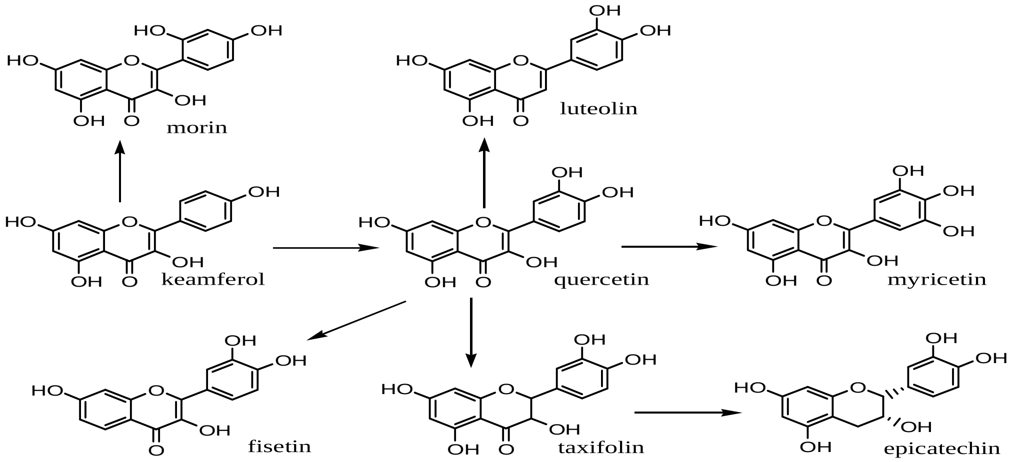


Figure 3. Structure of some studied flavonoids

Table 3. Comparison between MSD values and BDE and IP values in gas phase for some 4'-OH flavonoid radicals(*). All MSD values were calculated at b3lyp/6-311++g**// b3lyp/3-21g*

Compound	**MSD** (this work)	**BDE (*) kcal/mol**	**IP (*) kcal/mol**
Quercetin	**0.31092**	**72.35**	**166.08**
Fisetin	**0.31042**	-	-
Luteolin	0.33199	74.54	174.44
Taxifolin	0.37441	74.73	182.82
Kaemferol	0.37454	80.94	167.99
Epicatechin	0.37395	73.72	170.85
Myricetin	0.32460	-	-
Morin	0.37333	-	-

(*) In reference 6, 7, 8

From Table 3, it could be observed that the compound of which stable radical has the low MSD, BDE and IP value. Our results were in good agreement with other computed values: quercetin could to be good candidate for active antioxidants.

Table 4. Comparison between the MSD values of 4'-OH flavonoid radicals and Free radical scavenging activity ($n_{radical}/n_{atioxidant}$) (**). All computed MSD values at b3lyp/6-311++g**// b3lyp/3-21g*

Compound	**MSD in water solutior** (this work)	**Free radical scavenging activity** (**) $n_{radical}/n_{atioxidant}$	
		DPPH radical	Galvinoxyl radical
Quercetin	**0.29686**	**6.74**	**3.27**
Fisetin	0.29472	5.59	3.68
Luteolin	0.32690	4.73	3.24
Taxifolin	0.33698	4.09	2.82
Kaemferol	0.335169	1.87	1.84
Epicatechin	0.33175	-	2.96
Myricetin	0.29172	-	4.08
Morin	0.36718	-	1.83

(**) In reference 9, 10

In comparison between the computed MSD values and the molar ratio, a good correlation was found (Table 4 and Figure 3). The correlation coefficient is -0.93 for both DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and galvinoxyl (2,6-di-tert-butyl-α-[3,5-ditert-butyl-4-oxo-2,5-cyclohexadien-1-ylidene]-p-toly-loxy) radical (Figure 3). It could be observed that the strong antioxidant correlative with the smallest value of MSD. It also meant that all computed values were excellent indicators of free radical scavenging activity. The flavonoid has strong antioxidant activity for 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 A and C rings. Myricetin and quercetin satisfy all the above mentioned determinants and they have strong antioxidant activity than others. From the table 4, the studied flavonoids appear to be good candidates for active antioxidant as confirmed by their stable radicals has low MSD values, which are less than 0.31 in gas phase and less than 0.29 in water solution.

Studying the MSD values between green tea catechin radicals, we found that the stable radicals have similar values in MSD, so we could not compare their antioxidant activities using MSD values. Because there is no electron delocalization between the A and B rings, the antioxidant activity of green tea catechin responds broadly to the tenet that structure with the most hydroxyl groups exert the greatest antioxidant activity. Therefore, the order of decreasing effectiveness, EGCG ≈ ECG > EGC > EC.

4. CONCLUSIONS

In summary, a density functional - based method has been applied to study naturally antioxidant compounds, especially green tea catechins. The study has concerned the determination of the max spin density according to the stability of radicals and their scavenging activity.

In solution and gas phase, the minimum of MSD values do not always follow the same trends. In particular, some compounds that appear to be good candidates for H-atom transfer in the gas phase are less active in water.

For green tea stable radicals, the MSD lie at 0.37 – 0.38 in gas phase, 0.33 – 0.34 in water. The most active systems able to work through the H atom transfer mechanism are those with the smallest value of max spin density (MSD). Besides, the antioxidant activity of green tea catechins depends on the number of hydroxyl groups.

Studying the antioxidants by calculating the MSD values gives the same results as by calculating the BDE and IP values. It is also in good agreement with experimental data. Thus, the spin density distribution can now be further used to explore the reactivity and scavenging activity of radicals.

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