Theoretical Study of the Molecular Properties and Chemical Reactivity of (+)-Catechin and (-)-Epicatechin Related to their Antioxidant Ability

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Theoretical study of the molecular properties and chemical reactivity of (+)-catechin and (−)-epicatechin related to their antioxidant ability

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Abstract

(+)-Catechin and (−)-epicatechin are two flavonoid stereoisomers very abundant in vegetable tissues which present high antioxidant activity in living systems. It is known that the flavonoids exert their antioxidant properties according to the ability of trapping free radicals by donation of the phenolic hydrogen atoms. Nevertheless, the specific mechanism of the antioxidant activity remains far from well understood, due to the lack of information on the intrinsic molecular reactivity. The principal objective of this investigation was to study the molecular structure and chemical reactivity properties of (+)-catechin and (−)-epicatechin by analyzing the structural, chemical potential and energy properties, as well as Fukui indices, HOMO–LUMO distributions and ^{1}H–^{13}C NMR spectrometries using the CHIH(medium)–DFT model chemistry. We found a similar reactivity in (+)-catechin and (−)-epicatechin, although the different sites for electrophilic attack showed by both molecules could mark the difference in the intermediates oxidation products formed, and therefore in their antioxidant ability.

Keywords: Catechin; Epicatechin; Flavonoids; Antioxidant activity; DFT; Fukui indices; HOMO; LUMO; NMR; Chemical reactivity

1. Introduction

Flavan-3-ols constitute one of the five major polyphenolic groups found in tea leaves and various apple varieties [1]. The lack of a 2,3-double bond in their structure results in four stereoisomers, of which (+)-catechin (β-OH in ring C) and (−)-epicatechin (α-OH in ring C) are the most important ones [2,3]. These compounds are considered to exert protective effects against cancer, inflammatory and cardiovascular diseases in the human body [4]. Because of their astringency they can represent a defense system against insects harmful to plants, and they can also function as stress protecting in plant cells by scavenging reactive oxygen species (ROS) by the photosynthetic electron transport system, and due to their favorable UV-absorbing properties protect plants from UV radiation of sun [3,5]. Such effects have been mainly attributed to their antioxidant activities [2], which give them multiple applications in the pharmaceutical, agricultural and food industries, as well as in those industries that utilize or produce oxidants materials. The mechanism by which the flavonoids carry out the antioxidant activity has not been elucidated yet, and even though a series of studies have been performed, most of them at the experimental level and more recently at the theoretical one, there are many controversies in relation to the specific antioxidant mechanism due to a lack in a deep knowledge on both the molecular structure of such compounds and the structure–properties relationship. Most of the problems encountered when describing antioxidant activity in flavonoids are due to the lack of information on the intrinsic reactivity in the whole molecule and in each rings [6].

Antioxidant activity is considered to be the ability to trap the chain-carrying free radicals by donation of the phenolic hydrogen atom in the A and B rings [7]. It is known that the electron and proton transfer (deprotonation) are necessary, but the sequence for the specific reaction mechanism has not been established yet. Flavonoids are molecules that easily respond to polar and magnetic stimulus, and this is the reason for studying the charge distribution (HOMO–LUMO), reactivity (Fukui indices) and magnetic properties (shielding constants) of each atom. One of the most promising methods at experimental level, to shed light on the antioxidant mechanism in relation to the deprotonation sequence, is the NMR spectrometry based in ^{1}H and ^{13}C isotopes [6]. The calculation of NMR can be achieved by theoretical methods, avoiding the problems of solubility and interactions with solvents that are generated when working with flavonoids at the experimental level.

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The principal objective of this investigation was to study the molecular properties and chemical reactivity of (+)-catechin and (−)-epicatechin through the determination of the structural, chemical potential and energy properties, as well as Fukui indices, HOMO–LUMO distributions and ^{1}H–^{13}C NMR spectrometries with the purpose of contributing to a better knowledge of the intrinsic properties of flavan-3-ol related to their antioxidant ability. A secondary objective was to determinate the stereochemical effects of the OH in ring C on the antioxidant properties.

2. Theory and computational details

All the computational studies were carried out using the density functional theory (DFT) methods implemented in the GAUSSIAN 03W suite of programs [8]. The density functional used in this study is a modification of those incorporated in the GAUSSIAN 03W computational package and define the new model chemistry empirically developed for heterocyclic systems (CHIH–DFT). The particular model chemistry used in this work is known as CHIH(medium)–DFT and is described as PBEg/CBSB2**//PBEg/CBSB4. We worked with a functional that includes 16% of Hartree–Fock exchange and 84% of DFT exchange. For a more detailed explanation of this new chemistry model it is recommended to check Refs. [9–15].

The ground-state molecular geometries were established through the gradient technique. The force constants and vibrational frequencies were determined by the frequency calculations on the stationary points obtained after the optimization to check if there were true minima. Both calculations were done using the CBSB2** basis set, this is equal to the 6-31G(d,p) basis set, but with the exponents for the d functions taken from 6-311G(d,p) basis set and it is also called 6-31G†† [16–18]. The calculated electronic properties were: the infrared spectrum (structural property), total energies in neutral and ionized molecules, HOMO–LUMO gap energies and ΔG of solvation (energy properties), electronic affinity, ionization potential, hardness, electronegativity and electrophilicity (chemical potential properties), Fukui indices, HOMO–LUMO distributions and shielding constants by ^{1}H–^{13}C NMR spectroscopy. These properties were calculated with the CBSB4 basis set, which is identical to 6-31+G(d,p) for H–Si and to 6-31+G(d,f,p) on P, S and Cl. The CBSB2** and CBSB4 basis set are part of the CBS-QB3 method [19].

All the calculations were performed in gas phase with the purpose of obtaining the intrinsic properties of the flavonoids studied, free of any interaction. The solvation energy was calculated in the presence of water simulated by using the polarizable conductor calculation model (CPCM) [20] implemented in the GAUSSIAN 03W package. The default UAO set of solvation radii was employed, where the cavity is built up using the united atom topological method (UATM) applied on atomic radii of the universal force field (UFF) [21]. The superficial area and volume of the cavity were 293.710 Å^2 and 322.853 Å^3 for (+)-catechin and 293.629 Å^2 and 322.653 Å^3 for (−)-epicatechin.

For the calculations of the shielding constants by ^{1}H and ^{13}C NMR spectrometries the GIAO method [22] was employed. These calculations were performed in gas phase and also in the presence of methanol (CH_3OH) and dimethyl sulfoxide (DMSO) simulated in a similar way to water with the purpose of comparing with experimental results which were determined in the mentioned solvents. The superficial area and volume of the (+)-catechin and (−)-epicatechin cavities in both simulated solvents were:

	(+)-Catechin		(−)-Epicatechin	
	CH_3OH	DMSO	CH_3OH	DMSO
Area (Å^2)	294.40	293.34	292.85	292.59
Volume (Å^3)	325.89	328.70	325.39	327.85

The IR spectra were calculated and visualized using the Swizard program [23].

The different variables of the chemical potential were calculated on the differences of total electronic energies when adding or removing an electron, in relation to the neutral molecule under study and we have called it 'energetic-vertical' procedure [24,25].

The Fukui function is a local property given by [26]:

$$f(r) = \left(\frac{\partial \rho(r)}{\partial N}\right)_{v(r)} \tag{1}$$

where ρ is the electronic density of the system under consideration.

The condensed Fukui functions [27] are found by taking the finite difference approximations from population analysis of atoms in molecules, depending on the direction of the electron transfer, where q_k is the gross charge of atom k in the molecule:

$$f_k^+ = q_k(N+1) - q_k(N) \tag{2}$$

$$f_k^- = q_k(N) - q_k(N-1) \tag{3}$$

$$f_k^0 = \frac{q_k(N+1) - q_k(N-1)}{2} \tag{4}$$

for nucleophilic, electrophilic and radical attack, respectively.

3. Results and discussion

3.1. Structural properties

As can be seen in Fig. 1, the results for the equilibrium conformation of the neutral molecule of (+)-catechin show a non-planar structure, whose torsional angle between rings B and C [$O_{(15)}$–$C_{(1)}$–$C_{(16)}$–$C_{(18)}$] is −31.37°. An intramolecular hydrogen bond is observed in the catechol group of ring B with a bond distance of 2.104 Å $O_{(27)}$–$H_{(26)}$. Similarly, the optimized neutral molecule of (−)-epicatechin shows non-planar structure (Fig. 2), but its torsional angle between rings B and C [$O_{(9)}$–$C_{(18)}$–$C_{(20)}$–$C_{(22)}$], whose value is −45.77°, is higher than that for the (+)-catechin molecule. Therefore, (−)-epicatechin has a more twisted structure. The intramolecular

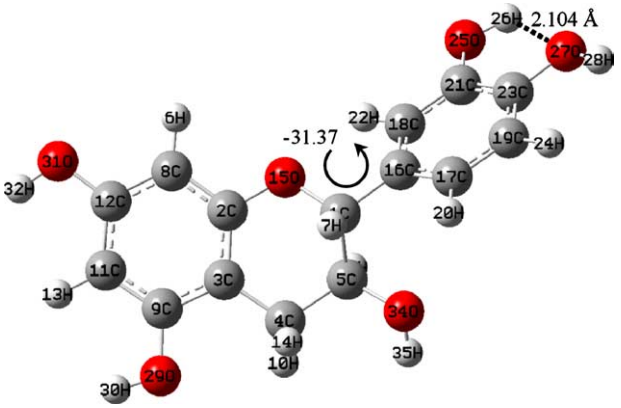

Fig. 1. Molecular structure of (+)-catechin.

hydrogen bond in their catechol group presents a bond distance of 2.097 Å $O_{(30)}$–$H_{(29)}$. For a more complete information about structural properties, a listing of the interatomic bond distances, bond angles, and dihedral angles of (+)-catechin and (−)-epicatechin molecules are available from the authors on request.

The infrared (IR) spectra of both flavonoids are displayed in Figs. 3 and 4, where the main peaks, without scaling, are as follows:

(+)-Catechin

- 240 cm^{-1}: $O_{(27)}$–$H_{(28)}$ and $O_{(34)}$–$H_{(35)}$ out-of-plane bending
- 336 cm^{-1}: $O_{(31)}$–$H_{(32)}$ out-of-plane bending
- 728 cm^{-1}: $C_{(11)}$–$H_{(13)}$ out-of-plane bending
- 1165 cm^{-1}:
  - $C_{(1)}$–$O_{(15)}$–$C_{(2)}$ asymmetric stretching
  - $C_{(8)}$–$H_{(6)}$ and $C_{(12)}$–$O_{(31)}$–$H_{(32)}$ in-of-plane bending
  - slight deformation in rings A and C
- 1345 cm^{-1}:
  - $C_{(1)}$–$C_{(16)}$ and $C_{(21)}$–$O_{(25)}$ stretching
  - $O_{(25)}$–$H_{(26)}$ and $O_{(27)}$–$H_{(28)}$ in-of-plane bending

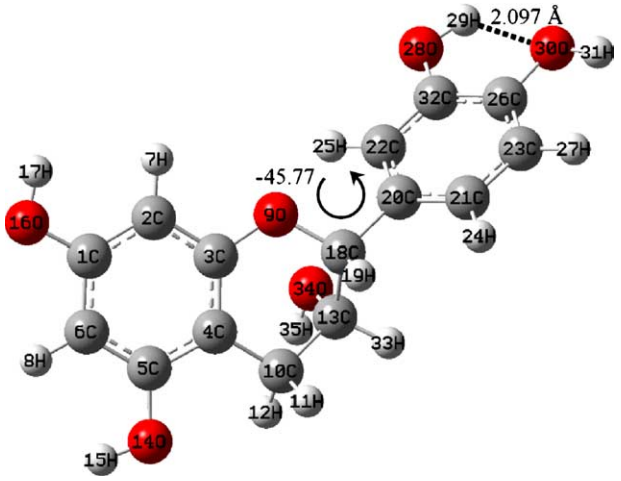

Fig. 2. Molecular structure of (−)-epicatechin.

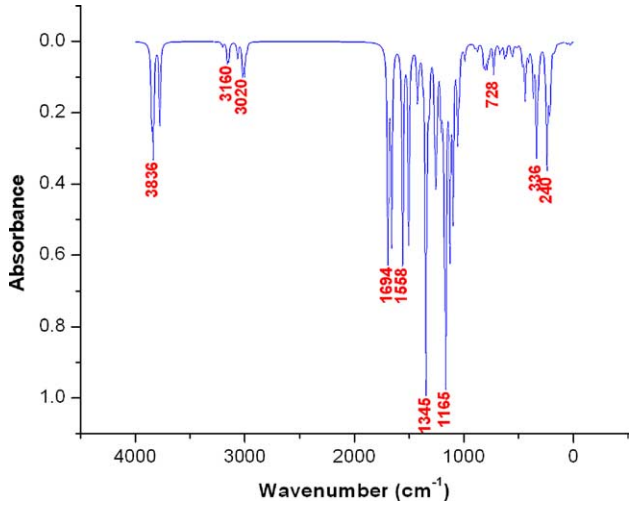

Fig. 3. Infrared spectrum (IR) of the (+)-catechin molecule.

- 1558 cm^{-1}: ring B deformation
- 1694 cm^{-1}: ring A deformation
- 3020 cm^{-1}: $C_{(4)}$–$H_{(10)}$–$H_{(14)}$ symmetric stretching
- 3160 cm^{-1}: $C_{(17)}$–$H_{(20)}$ and $C_{(19)}$–$H_{(24)}$ asymmetric stretching
- 3836 cm^{-1}: $O_{(29)}$–$H_{(30)}$ and $O_{(31)}$–$H_{(32)}$ symmetric stretching

(−)-Epicatechin

- 215 cm^{-1}: $O_{(30)}$–$H_{(31)}$ out-of-plane bending
- 387 cm^{-1}: $O_{(14)}$–$H_{(15)}$ out-of-plane bending
- 773 cm^{-1}:
  - $C_{(21)}$–$H_{(24)}$ and $C_{(23)}$–$H_{(27)}$ out-of-plane bending
  - ring B deformation
- 1187 cm^{-1}:
  - $C_{(1)}$–$O_{(9)}$–$C_{(3)}$ asymmetric stretching
  - $C_{(1)}$–$O_{(16)}$–$H_{(17)}$ and $C_{(2)}$–$H_{(7)}$ in-plane bending
  - deformation in rings A and C
- 1347 cm^{-1}: ring B deformation

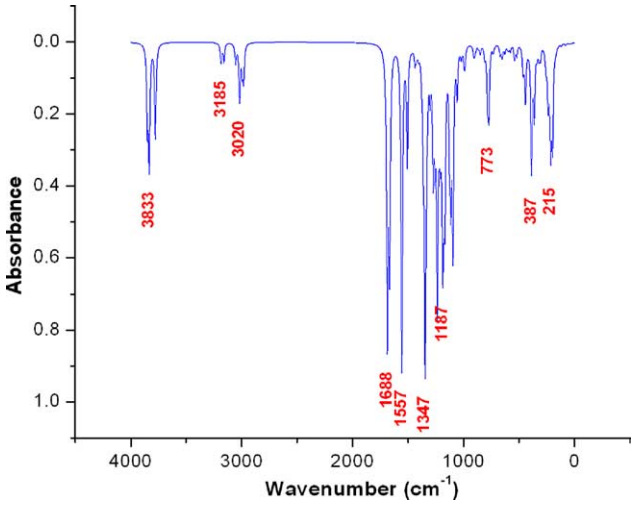

Fig. 4. Infrared spectrum (IR) of the (−)-epicatechin molecule.

Table 1
Chemical potential properties for (+)-catechin and (−)-epicatechin molecules

Property	(+)-Catechin	(−)-Epicatechin
Electronic affinity $A=[E_{(0)}-E_{(-1)}]$ (eV)	−0.40	−0.49
Ionization potential $[I=E_{(+1)}-E_{(0)}]$ (eV)	7.20	7.21
Hardness $[\eta=(I-A)/2]$ (eV)	3.80	3.85
Electronegativity $[\chi=(I+A)/2]$ (eV)	3.40	3.36
Electrophilicity $(\omega=\mu^2/2\eta)$ (eV)	1.52	1.47

- 1557 cm^{-1}: ring A and B deformation
- 1688 cm^{-1}: ring A deformation
- 3020 cm^{-1}: $C_{(10)}$–$H_{(11)}$–$H_{(12)}$ symmetric stretching
- 3185 cm^{-1}: $C_{(6)}$–$H_{(8)}$ and $C_{(2)}$–$H_{(7)}$ asymmetric stretching (very noticeable the first)
- 3833 cm^{-1}: $O_{(14)}$–$H_{(15)}$ stretching
- 3836 cm^{-1}: $O_{(34)}$–$H_{(35)}$ stretching

Basically both flavonoids molecules show the same vibration modes, only the band around 700 cm^{-1}, which is representative of the C–H stretching out-of-molecular plane [28] is more intense in (−)-epicatechin, and this result is in agreement with the presence of a higher torsional angle between rings B and C.

The stereochemistry of hydroxyl group of the ring C is a sign of the differences in the structural properties of the studied compounds. On the one hand, (−)-epicatechin showed a more twisting of the B ring with reference to the C ring, and on the other hand presented a higher intensity in C–H stretching out-of-molecular plane than (+)-catechin.

3.2. Chemical potential properties

These properties are defined by different variables tightly related among them: electronic affinity (EA), ionization potential (IP), hardness (η), electronegativity (χ) and electrophilicity (ω). Such variables have different meanings. Nevertheless, as a group they measure the tendency to give or capture electrons, that is they are an index of the antioxidant potential [16,29,30] as the antioxidant potential or antioxidant activity results from the ability to give electrons [3].

The chemical potential properties are shown in Table 1, indicating that (+)-catechin and (−)-epicatechin molecules have close values, which are interpreted as having a low reduction potential or good antioxidant activity [4] due to the similarity with the quercetin chemical potential calculated by Mendoza et al. [13]: EA = 0.76 eV, IP = 7.22 eV, η = 3.23 eV, χ = 3.99 eV and ω = 2.47 eV. Quercetin is considered one of the flavonoids with more antioxidant activity, and, in many cases, the effect of flavan-3ols against free radicals is the same as that of quercetin, or even greater [7].

In agreement with Ref. [31], the molecules which possess more twisting of the B ring with reference to the C ring are regularly weaker or inactive. This idea is supported by Burton et al. [32], who stated that the dihedral angle is an important factor in the orbitals overlapping in aromatic rings. An unfavorable conformation of the heterocyclic ring contributes for a relatively low reactivity. In this context, it was expected a minimum antioxidant potential in (−)-epicatechin compared with (+)-catechin. Nevertheless, their antioxidant potential resulted surprisingly the same, and both molecules were similar to quercetin.

3.3. Energy properties

As can be seen in Table 2, the properties of energy in (+)-catechin and (−)-epicatechin molecules at ground state are the same. These results indicate that the stereochemistry of the hydroxyl group in the ring C of flavan-3-ols has not any effect in the total energy defined by molecular, orbital and solvation energies. The gap value (5.25 eV) shows the same orbital

Table 2
Properties of the energy for (+)-catechin and (−)-epicatechin molecules

Property	(+)-Catechin	(−)-Epicatechin
Total energy of neutral molecule (0) (a.u.)	−28035.12	−28035.12
Charged molecule (+1) (a.u.)	−28027.92	−28027.91
Charged molecule (−1) (a.u.)	−28034.71	−28034.64
HOMO (eV)	−5.63	−5.69
LUMO (eV)	−0.38	−0.44
Gap $(\Delta E=E_{LUMO}-E_{HOMO})$ (eV)	5.25	5.25
Solvation energy (ΔG) (kcal/mol)	−24.06	−24.09

Table 3
Fukui indices of (+)-catechin molecule

Charge population analysis	Fukui indices					
	Atom	Nucleophilic attack	Atom	Electrophilic attack	Atom	Radical attack
NPA	C_{23}	0.09987	C_8	0.09921	C_{11}	0.13399
ESP	H_{28}	0.46543	C_8	0.14142	H_{28}	0.24656

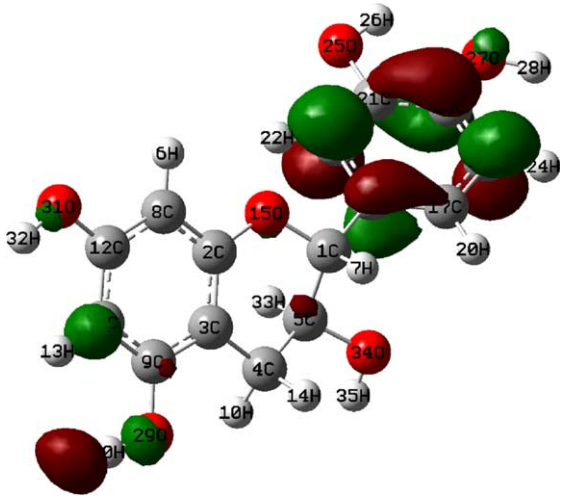

Fig. 5. Charge distribution of the LUMO (isovalue of 0.04) in the optimized molecule of (+)-catechin.

reactivity for both molecules, which are classified as little reactive molecules on the basis of this value. In previous studies, it has been found that there is a large energy difference between HOMO and LUMO, corresponding to stable and little reactive systems, whereas in the opposite case the systems are little stable and highly reactive [16,33]. In other work, the gap value found for quercetin was 3.38 eV, and this flavonoid has been considered as a reactive system [13].

3.4. Fukui indices, HOMO–LUMO distributions and ^{1}H–^{13}C NMR spectrometries

The unpaired electron of the free radicals increases their polar properties, and though they are neutral molecules they present certain tendencies to gain or to lose electrons, that is to say, they can perform as a electrophile or nucleophile in the transition states [34]. On the other hand, the π electrons system of flavonoids is highly polarizable, thus the attacks for electrophiles or nucleophiles lead to charge density variations and appears effective charges in the flavonoids molecules [16,33]. For these reasons the flavonoids reactivity can be determined by Fukui indices and HOMO–LUMO distributions.

The Fukui indices calculation was carried out by considering two types of charges analysis: natural population analysis (NPA or NBO) and electrostatic potential analysis (ESP).

3.4.1. (+)-Catechin

According to the results in Table 3, the preferential sites for nucleophilic attack of the (+)-catechin molecule are suggested to be the C_{23} and H_{28} in the ring B (Fig. 1). These results are supported by the LUMO distribution, which represents the molecular site for the positive charge density, and is presented in Fig. 5. The LUMO distribution also indicates that the remaining carbon atoms of the ring B are potential sites for nucleophilic attack. The site for electrophilic attack is the C_8 of the ring A (Table 4). These results are supported by the HOMO distribution, which represents the molecular site for the negative charge density, and is shown in Fig. 6. The HOMO distribution is observed also on the carbon atoms of the ring B, indicating that these atoms are potential sites for electrophilic attack. It is already known that in the aromatic electrophilic substitution, the presence of an OH favors the electron donation (oxidation) by effect of the resonance [34]. Though the (+)-catechin molecule is little conjugated compared with quercetin and others flavonoids [12], it has many sites for the electrophilic attack. The radical attack can be carried out in the C_{11} of the ring A or in the H_{28} in the ring B.

The polar effects of the free radicals can create a magnetic field in flavonoids, affecting their reactivity. Under this

Table 4
Absolute shielding constants of (+)-catechin calculated by ^{1}H–^{13}C NMR spectrometries

Atom	Shielding constant (σ) (ppm)		Atom	Shielding constant (σ) (ppm)	
	Isotropic (σ_i)	Anisotropic (σ_a)		Isotropic (σ_i)	Anisotropic (σ_a)
1 C	113.6362	32.6395	19 C	89.5248	119.6467
2 C	43.2830	122.4260	20 H	24.6771	8.1617
3 C	99.3500	109.4187	21 C	53.9549	116.3751
4 C	162.1664	25.6984	22 H	24.1608	10.8671
5 C	125.4075	36.0885	23 C	57.8046	108.5492
6 H	25.2245	6.7228	24 H	24.9112	4.9069
7 H	26.9292	5.6514	25 O	244.9398	86.5282
8 C	102.1099	98.7394	26 H	26.7928	12.5109
9 C	46.0052	122.9301	27 O	248.5123	32.1288
10 H	28.6729	6.8399	28 H	28.1374	13.9424
11 C	108.0295	62.0481	29 O	225.3376	38.4392
12 C	44.4939	127.5885	30 H	28.1412	13.4945
13 H	26.3864	4.5419	31 O	225.1038	58.9840
14 H	29.1314	6.8610	32 H	28.2908	12.6557
15 O	208.2615	88.7240	33 H	27.9032	2.8179
16 C	64.7665	159.3303	34 O	281.0910	35.6905
17 C	78.1170	143.3697	35 H	31.3296	17.9342
18 C	85.1265	137.9048			

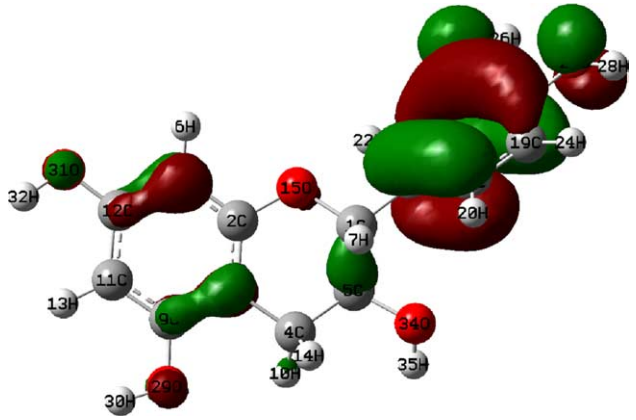

Fig. 6. Charge distribution of the HOMO (isovalue of 0.03) in the optimized molecule of (+)-catechin.

perspective, the study of their magnetic properties can be seen very useful to understand their reaction mechanism.

The π electrons system under the influence of a magnetic field in circulation generates their own magnetic field opposing the applied field, hence, the 'shielding' effect [28]. The diamagnetic anisotropy concept is important in hydrogen atoms of compounds related to benzene (flavonoids), where it is present the 'ring-current effect': The molecule is oriented on the carbon plane, generating a magnetic field whose effect is greatest on carbon atoms (shielding) and weak on hydrogen atoms (deshielding), due to the molecular geometry.

Considering such effects for (+)-catechin and (−)-epicatechin molecules, in this work, we calculated the absolute isotropic shielding constants (σ_i) for carbon atoms (^{13}C) and absolute anisotropic shielding constants (σ_a) for hydrogen atoms (^{1}H). We considered that the application of both NMR spectrometries will allow us to obtain more reliable results for the prediction of the reaction site or deprotonation sequence.

Table 4 shows the absolute shielding constants isotropic (σ_i) and anisotropic (σ_a) for all the atoms of the (+)-catechin molecule. As can be seen, the anisotropic shielding constants of the hydrogen atoms have low values, and are best indicators of the differences between each hydrogen atom compared with isotropic shielding constants. According to the Fukui indices the C_{23} and H_{28} are the reactive sites in the ring B. C_{23} is the site for nucleophilic attack and their $\sigma_i = 57.80$ ppm (deshielded), which is by far minor than C_8 and C_{11} (sites for electrophilic attack). C_{23} is bonded to one hydroxyl that is part of catechol group, where an intramolecular hydrogen bond is formed. H_{28} is the site for nucleophilic and radical attacks ($\sigma_a = 13.94$ ppm, $\sigma_i = 28.14$ ppm deshielded), and is part of the hydroxyl group bonded to the C_{23}, but not a part of the intramolecular hydrogen bond.

Hydrogen bonds decrease the electron density around the proton being the intramolecular less affected by their environment than the intermolecular ones. The OH protons are relatively deshielded due to the fact that the enolic form is strongly stabilized by an intramolecular hydrogen bond [28].

By means of the ring B, (+)-catechin could interact directly with nucleophiles such as structural proteins, enzymes, DNA or glutathione [24,35]. Probably, the ring B reacts in the first stages of the oxidation reactions, inhibiting enzymes and/or chelating metals (Fe, Cu, Zn) that perform as catalyzers in such reactions.

The C_8 in the ring A is the preferential site for electrophilic attack, whose $\sigma_i = 102.11$ ppm (shielded). C_8 is bonded to H_6, whose $\sigma_a = 6.72$ ppm and $\sigma_i = 25.22$ ppm (deshielded). Other reactive site (radical attack) in the ring A is C_{11} ($\sigma_i = 108.03$ ppm, shielded) bonded to H_{13} ($\sigma_a = 4.54$ ppm, $\sigma_i = 26.39$ ppm, deshielded). The previous values are in total agreement with the Fukui function analysis, due to the high electron density (shielding) on the carbon atoms and low electron density (deshielding) on hydrogen atoms. These charge distributions facilitate the hydrogen abstraction for the free radicals. Probably, the ring A of (+)-catechin is an important scavenger of free radicals.

The displacement of ortho, meta and para protons of a substituent in an aromatic ring are correlated to electron densities and to the effects of electrophilic reagents. The ortho and para protons of phenol are shielded due to the high electron density which also account for the predominancy of ortho and para substitutions of electrophilic reagents [28].

The hydroxyl groups in position meta (resorcinol group) in the ring A of (+)-catechin intend the electrophilic substitution toward the ortho and para positions (C_8 and C_{11}). In symmetric molecules the two opposite (shielding and deshielding) ^{13}C NMR shifts nearly compensate, but in a non-symmetric molecule like (+)-catechin where preferential deprotonation can exist, the two opposite effects do not compensate, and it predominates one of the two [36], as was seen in our results.

3.4.2. (−)-Epicatechin

This molecule presented a similar behavior to (+)-catechin. Table 5 shows the Fukui indices and, as can be seen, the sites for nucleophilic and radical attacks are exactly the same that in (+)-catechin, but in this case the numeration of the atoms is different (C_{26} and H_{31}) (Fig. 2). These results are supported by the LUMO distribution (Fig. 7) and shielding constants shown in Table 6: C_{26} ($\sigma_i = 57.25$ ppm deshielded) and H_{31}

Table 5
Fukui indices of (−)-epicatechin molecule

Charge population	Fukui indices					
	Atom	Nucleophilic attack	Atom	Electrophilic attack	Atom	Radical attack
NPA	C_{26}	0.11290	C_4	0.13348	C_{26}	0.08648
ESP	H_{31}	0.54593	C_4	0.16776	H_{31}	0.28535

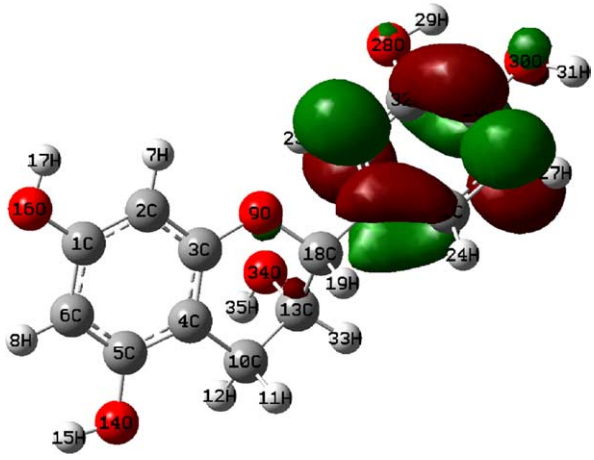

Fig. 7. Charge distribution of the LUMO (isovalue of 0.04) in the optimized molecule of (−)-epicatechin.

($\sigma_a = 14.04$ ppm, $\sigma_i = 28.14$ ppm deshielded). Electrophilic attack is presented in C_4 of the ring A, which constitutes the ortho and para intends for resorcinol group (C_5 and C_1). These results are supported by HOMO distribution (Fig. 8) and shielding constants ($\sigma_i = 100.47$ ppm shielded). In spite of the C_4 not being bonded directly to a hydrogen atom, the hydrogen atom abstracted for to carry out the reaction could belong to the contiguous C_5 ($\sigma_i = 45.93$ ppm deshielded), which is bonded to the OH that is part of the resorcinol group. After the hydrogen abstraction could take place a transposition to form a stable phenoxyl radical. The reaction mechanism presented in this case could be 'neighbor groups effects': intramolecular effects that have influence in the reaction through a direct participation of a group close to the reaction center for a movement inside of the bond length [34].

In an NMR experimental study performed in various flavan-3-ols, it was found that the antioxidant mechanism exerted for (+)-catechin is similar to (−)-epicatechin, and both molecules exert a different antioxidant mechanism compared with other flavan-3-ols molecules [37].

Tables 7 and 8 show the computational ^{13}C and ^{1}H NMR spectral data of (+)-catechin and (−)-epicatechin determined in our investigation, and experimental data obtained in other works with the purpose to establish a comparison. With regard to that a high correlation was found between the experimental and computational data.

The data showed in Tables 7 and 8 are represented as chemical shifts in relation with TMS to standardize experimental and computational results. These data presented the same behavior than the absolute shielding constants isotropic (σ_i) of carbon and hydrogen atoms determined computationally in our study. The comparison with anisotropic shielding constants (σ_a) was not possible because to experimental level only were determined isotropic shielding constants.

Figs. 9 and 10 show the correlation between experimental and computational ^{13}C NMR spectral data of the (+)-catechin and (−)-epicatechin molecules, respectively, comparing methanol, dimethyl sulfoxide and gas phase at the same time. Figs. 11 and 12 show the corresponding correlation for ^{1}H NMR spectral data.

The correlation of experimental vs. computational ^{13}C NMR spectral data of (+)-catechin in methanol presented a tendency line with an R^2 value of 0.9922, and in dimethyl sulfoxide R^2 was 0.9944. The (−)-epicatechin molecule presented similar values: 0.9966 and 0.9964, respectively. In relation to the ^{1}H NMR spectral data of (+)-catechin in methanol the R^2 value was 0.9958 and 0.9929 in dimethyl sulfoxide. The (−)-epicatechin R^2 values were 0.9678 and 0.9869, respectively. A largest difference was observed when a

Table 6
Absolute shielding constants of (−)-epicatechin calculated by ^{1}H–^{13}C NMR spectrometries

Atom	Shielding constant (σ) (ppm)		Atom	Shielding constant (σ) (ppm)	
	Isotropic (σ_i)	Anisotropic (σ_a)		Isotropic (σ_i)	Anisotropic (σ_a)
1 C	44.2616	128.4429	19 H	26.9804	7.6542
2 C	104.2942	80.6611	20 C	65.8968	162.8637
3 C	42.9685	122.2829	21 C	81.1701	131.0685
4 C	100.4742	118.8234	22 C	80.2649	145.1051
5 C	45.9258	122.3075	23 C	89.9804	117.1294
6C	107.2236	78.6299	24 H	25.0313	7.3250
7 H	25.7185	6.9061	25 H	23.8362	10.5978
8 H	25.7286	4.6162	26 C	57.2547	111.5084
9 O	211.3007	128.7409	27 H	25.0444	4.6829
10 C	161.8239	26.7541	28 O	245.5952	93.1507
11 H	28.6339	8.3453	29 H	26.5451	12.8119
12 H	28.8771	7.0302	30 O	249.4129	43.7707
13 C	127.7206	46.6905	31 H	28.1385	14.0367
14 O	225.7606	36.0735	32 C	53.7218	118.4485
15 H	28.0976	13.6388	33 H	27.5390	5.9914
16 O	225.1115	60.33	34 O	285.3176	80.0752
17 H	28.0614	11.9280	35 H	31.9646	18.1707
18 C	114.4817	32.4409			

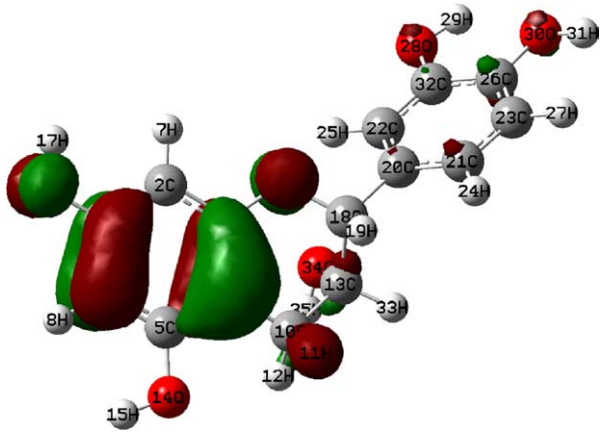

Fig. 8. Charge distribution of the HOMO (isovalue of 0.04) in the optimized molecule of (−)-epicatechin.

comparison between the data determined in solvent and gas phase in both experimental and computational calculation was performed, but R^2 remained with a high value.

The previous results confirm that the computational determination of ^{1}H–^{13}C NMR spectral data, represented as absolute shielding constants or as chemical shifts are very close to the experimental data, and on the whole with Fukui indices and HOMO–LUMO distributions can be a good preliminary tool for studies related to the determination of reactivity and antioxidant activity in neutral molecules of polyphenolic compounds.

4. Conclusions

The reactivity of (+)-catechin and (−)-epicatechin is similar. In both molecules the ring A was the preferential site for electrophilic attack and ring B for nucleophilic attack.

Table 7
^{13}C NMR chemical shifts (δ) in ppm vs. TMS for (+)-catechin and (−)-epicatechin in solution and gas phase by computational and experimental methods

Carbon atom[a]	Experimental		Carbon atom[b]	Computational		
	CD_3OD[c]	DMSO-d_6[d]		CH_3OH[e]	DMSO[f]	Gas[g]
(+)-Catechin						
C_2	77.14	81.00	C_1	86.15	86.22	83.53
C_3	67.05	66.40	C_5	71.90	72.39	71.76
C_4	29.74	27.70	C_4	35.69	35.64	35.00
C_5	158.00	156.10	C_9	153.55	153.35	151.63
C_6	96.58	95.30	C_{11}	92.16	92.12	89.14
C_7	158.05	156.40	C_{12}	154.06	153.84	152.68
C_8	96.19	94.00	C_8	93.37	93.00	95.06
C_9	157.34	155.30	C_2	154.57	154.09	153.80
C_{10}	100.59	99.20	C_3	98.99	98.91	97.82
$C_{1'}$	132.12	130.70	C_{16}	131.83	132.03	132.40
$C_{2'}$	115.33	114.50	C_{18}	113.10	112.90	112.04
$C_{3'}$	145.66	144.80	C_{21}	143.16	142.80	143.22
$C_{4'}$	145.91	144.80	C_{23}	142.41	142.29	139.73
$C_{5'}$	116.00	115.10	C_{19}	110.25	110.48	107.65
$C_{6'}$	119.47	118.40	C_{17}	121.56	121.90	119.05
(−)-Epicatechin						
	79.83	78.10	C_{18}	83.60	83.70	82.69
	67.46	65.00	C_{13}	70.00	69.71	69.45
	29.34	28.20	C_{10}	34.84	34.92	35.35
	157.59	156.50	C_5	152.98	152.80	151.25
	96.45	95.20	C_6	91.75	91.62	89.95
	157.95	156.30	C_1	153.80	153.79	152.91
	95.94	94.20	C_2	92.80	96.68	92.88
	157.34	155.80	C_3	154.45	154.54	154.20
	100.12	98.60	C_4	98.91	98.86	96.70
	132.28	130.70	C_{20}	131.32	131.29	131.28
	115.33	114.90	C_{22}	114.97	114.70	116.91
	145.89	144.40	C_{32}	143.17	143.21	143.45
	145.73	144.50	C_{26}	141.62	141.63	139.92
	115.95	114.80	C_{23}	111.13	111.00	107.19
	119.45	118.00	C_{21}	118.41	118.58	116.00

[a] Experimental numeration.
[b] *Z*-matrix numeration.
[c] Ref. [38].
[d] Ref. [39].
[e] $\delta = 197.72 - \sigma_i$.
[f] $\delta = 197.67 - \sigma_i$.
[g] $\delta = 197.17 - \sigma_i$.

Table 8
^{1}H NMR chemical shifts (δ) in ppm vs. TMS for (+)-catechin and (−)-epicatechin in solution and gas phase by computational and experimental methods

Hydrogen atom[a]	Experimental		Hydrogen atom[b]	Computational		
	CD_3OD[c]	DMSO-d_6[d]		CH_3OH[e]	DMSO[f]	Gas[g]
(+)-Catechin						
H_2	4.58	4.51	H_7	4.75	4.82	4.73
H_3	4.27	3.84	H_{33}	4.32	4.23	3.76
H_4	3.33	2.68	H_{10}	3.09	3.09	2.99
H_5	2.80	2.38	H_{14}	2.46	2.45	2.53
H_6	6.05	5.90	H_{13}	6.15	6.15	5.28
H_8	5.99	5.72	H_6	6.43	6.43	6.44
$H_{2'}$	6.89	6.74	H_{22}	7.57	7.47	7.50
$H_{5'}$	6.71	6.70	H_{24}	7.30	7.38	6.75
$H_{6'}$	6.69	6.61	H_{20}	7.17	7.14	6.98
(−)-Epicatechin						
	4.17	4.75	H_{19}	5.09	5.07	4.68
	3.35	4.03	H_{33}	4.38	4.39	4.12
	2.88	2.70	H_{11}	3.01	3.01	3.03
	2.79	2.50	H_{12}	2.85	2.87	2.79
	5.93	5.91	H_8	6.40	6.40	5.93
	5.95	5.75	H_7	6.36	6.35	5.94
	6.97	6.91	H_{25}	7.73	8.11	7.83
	6.77	6.68	H_{27}	7.27	7.25	6.62
	6.73	6.68	H_{24}	7.09	7.12	6.63

[a] Experimental numeration.
[b] *Z*-matrix numeration.
[c] Ref. [38].
[d] Ref. [39].
[e] $\delta = 31.65 - \sigma_i$.
[f] $\delta = 31.64 - \sigma_i$.
[g] $\delta = 31.66 - \sigma_i$.

Though the antioxidant potentials of (+)-catechin and (−)-epicatechin are similar, the different carbon atoms showed as preferential site for electrophilic attack in each molecule imply that intermediate oxidation products formed could mark the difference in their antioxidant activity. Being this result the principal effect attributed to the stereochemistry of hydroxyl group of the ring C.

The results in Fukui indices, HOMO–LUMO distributions and shielding constants determined by ^{1}H–^{13}C NMR spectrometries were coherent. For this reason they are considered good tools for the determination of reactive sites in flavonoids related to their antioxidant activity.

The computational determination of the magnetic properties through ^{1}H–^{13}C NMR spectrometries shows a great similarity

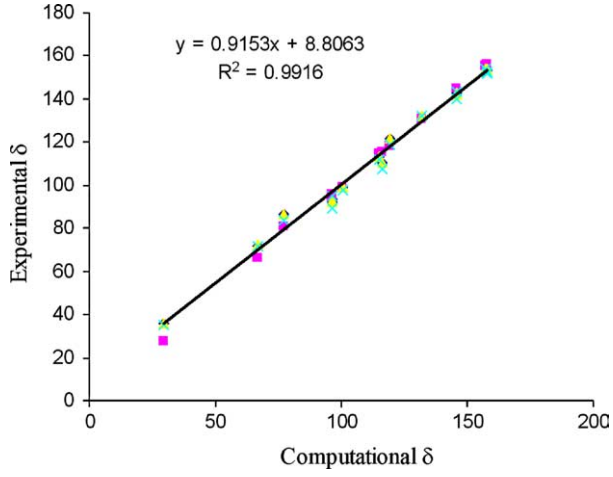

Fig. 9. Experimental vs. computational ^{13}C NMR chemical shifts (δ) of (+)-catechin in solution and gas phase.

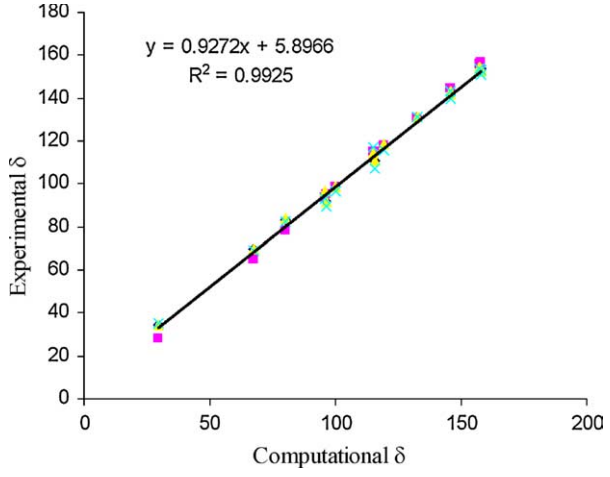

Fig. 10. Experimental vs. computational ^{13}C NMR chemical shifts (δ) of (−)-epicatechin in solution and gas phase.

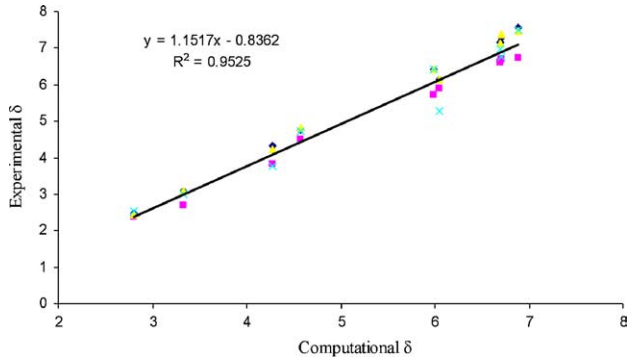

Fig. 11. Experimental vs. computational ^{1}H NMR chemical shifts (δ) of (+)-catechin in solution and gas phase.

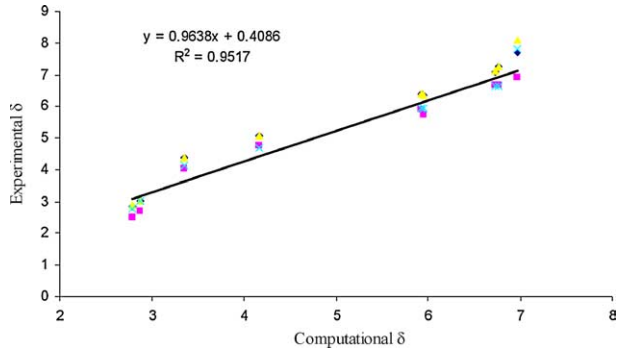

Fig. 12. Experimental vs. computational ^{1}H NMR chemical shifts (δ) of (−)-epicatechin in solution and gas phase.

with experimental results, and could be useful to shed light on the antioxidant mechanism of flavonoids.

Acknowledgements

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