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Research Article

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Theoretical Study of Antioxidant Properties of Two Isomers Flavonoids: Kaempferol and Fisetin

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ABSTRACT

By functional B3LYP of the DFT, and in three more and more widened bases set 6-311G, 6-311G (d, p) and 6-311 ++ G (d, p), a theoretical study of the antioxidant properties of two isomeric flavonols (fisetin and kaempferol) was carried out. The electronics parameters as gap HOMO-LUMO, potential of ionization and electronic affinity were calculated, and also thermodynamics parameters as enthalpies of single electron transfert, of proton transfer and of hydrogen atomic elimination. The electronic calculated parameters allowed to estimate the powers electron donors $\bar{\omega}^+$ *and electron acceptors* $\bar{\omega}^-$ *of molecules and to confirm their classifications in the literature according to the oxidizing power. On the basis of the calculated thermodynamics parameters, three various mechanisms of elimination of the peroxide radical (O_2°) were explored for each studied molecules:*

- *Electron elimination followed by proton elimination by the molecule, then trapping of free radical;*
- *Proton elimination followed by electron elimination, then trapping of the free radical;*
- *Elimination of hydrogen atom by homolytic rupture of OH bond, then trapping of the free radical.*

The results of the various calculations have confirmed the classification of molecules according to the antioxidant power, as presented in the literature, and have identified the mechanism through the elimination of atomic hydrogen by homolytic bond breaking, the most likely for the removal of a peroxide radical by each of the two molecules. The theoretical results also confirm that the most important sites of demonstration of the antioxidant activity of both flavonols are especially their catechol hydroxyl groups.

Keywords: Antioxidant; Fisetin; Kaempferol; B3LYP; DFT

INTRODUCTION

Kaempferol and fisetin are polyphenols belonging to the flavonoid group [1], which are natural compounds of plant origin, found in fruits (oranges, grapes, etc.), vegetables (onion, lettuce, etc.), seeds (bean, cocoa), roots and lefts of plant (tea). They are involved vegetables in pigmentation and were daily consumed in the human food [2].

The flavonoids have anti-inflammatory, antiviral, antibiotic, anti-neoplastic, antioxidant, pro-oxidant, vitamins (vascular protection), anti-hepatotoxic and anti-ulcerogenic [3-5].In particular, it is experimentally established that the kaempferol and fisetin have a antioxidant activity.

Indeed, experimental data published in the literature indicate that, due to their low redox potential, these both molecules reduce oxidizing free radicals such as superoxide, peroxyl, alkoxyl and the hydroxyl, by hydrogen transfer [6].They would be able to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) which can react with hydrogen peroxide H_2O_2giving hydroxyl radicals. They can sometimes undergo autoxidation and generation of active oxygen radicals [7]. Furthermore, it was found that their antioxidant activity depends on the number of hydroxyl groups they carry and the position of these groups in the molecule. Thus, the presence of hydroxyl groups on catechol increases

antioxidant activity of this part of the molecule. Theoretical results were also published in the literature on flavonoids such as luteolin and quercetin. For example, a theoretical study of radical forms of different flavonoids, including quercetin, at calculation UHF / STO-3G and UHF / 6 -31G *, conducted by Van Acker et al. (1996), showed that 84% of the total spin density localized was essentially on the oxygen atom from where a hydrogen atom H was removed. These authors have shown that the presence of the double bond C2-C3 in quercetin allows a best electronic relocation than in the case of taxifolin for which the spin density is mainly concentrated on the cycle B. Recently, other authors [9] have proposed, on the basis of DFT calculations(B3P86 / 6-311 + G (d, p) and B3LYP / 6-311 + G (d, p)), a classification of five radical forms of quercetin and taxifolin, in the ascending order of OH bond dissociation enthalpy.

They also justified the better reactivity of the radical site O3of quercetin and also shown that the equilibrium keto enol of O3-H function was less possible in the aqueous phase ($\Delta E \approx 20$ kcal.mol-1) than the enolik form. However, they suggest that in an enzymatic environment this equilibrum should not be neglected. By DFT calculations (B3LYP / 6-311 + G (3df, 3pd)), Song Xiaoli et *al*. (2013) have firstly shown that the 4-oxo sites and O5H favor lutéoline chelation by Cd (+II), and secondly that the deprotonating of luteolin chelated would be easier than no chelated form.

The present works are also part of the perspective of a study of the antioxidant properties of fisetin and kaempferol, by the methods of quantum chemistry, to establish the theoretical basis underlying the manifestation of these properties and rationalize. The results of work should make it possible to appreciate the relative antioxidant powers of the both molecules, to determine the probable mechanisms of demonstration of their antioxidant activities as well as the principal sites of expression of these activities.

MATERIALS AND METHODOLOGY

The studied chemical systems represent two flavonoids isomers of molecular formula ($C_{15}H_{10}O_6$): fisetin and kaempferol (Figure 1).

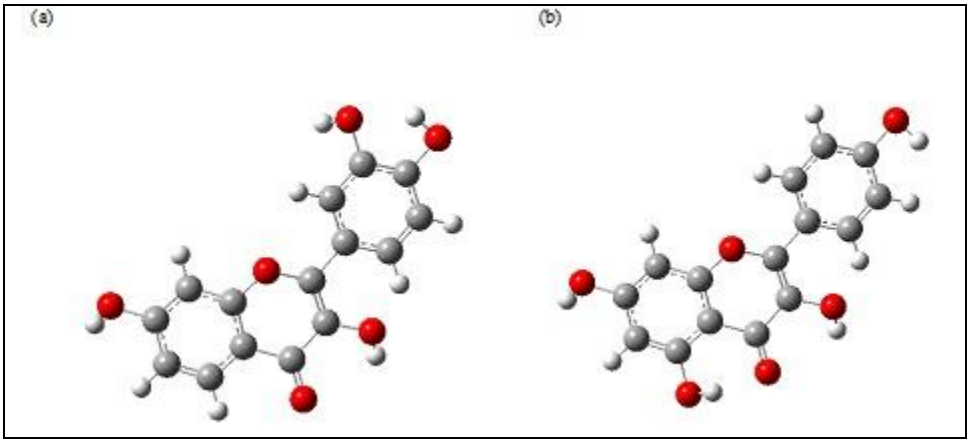

Figure 1: Spatial representations of fisetin (a) and kaempferol (b) molecules

Taking into account the size of molecules, electronic parameters, energy and thermodynamic parameters requested, the calculations were carried out by the functional B3LYP of DFT (Axel D. Becke.J. Chem. Phys., (1993) 98), in three atomic orbitals bases of Pople, increasingly extended: 6-311G, 6-311G (d, p) and 6-311 ++ G (d, p) [12].

- For each molecule (noted ArOH), the calculated electronic parameters were:

the electron affinity $\mathbf{AE = E_{ArOH^-} - E_{ArOH}}$, [13] whose value indicate the ability of a molecule to accept electron or free radical;

- ionisation energy $\mathbf{IE = E_{ArOH^+} - E_{ArOH}}$ [14];
- the $\boldsymbol{Gap_{(HOMO-LUMO)} = AE - EI}$.

The comparison of the antioxidant powers of the studied molecules was carried out on the basis of electron acceptor and electron donor powers ($\bar{\omega}^+ and\ \bar{\omega}^-$respectively), calculated for each molecule:

$\bar{\omega}^{+} = \frac{(EI+3.AE)^2}{16(EI-AE)}$ et $\bar{\omega}^{-} = \frac{(3.EI+AE)^2}{16(EI-AE)}$

The energy and thermodynamic parameters calculated for the determination of probable mechanisms of manifestation of the anti-radical activity of the molecules has been:

- for the path going by electron elimination followed by proton elimination (SET-PT), the ionization potential $IP = H(ArOH^{+}) - H(ArOH)$ and the enthalpy of proton dissociation $PDE = H(ArO^{\circ}) + H(H^{+}) - H(ArOH^{+})$ [15];
- for the path going by proton elimination followed by electron elimination (SPLET), the proton affinity of phenoxyde ion $PA = H\left(ArO^{-}\right) + H(H^{+}) - H(ArOH\ )$ and electron transfer enthalpy $ETE = H(ArO^{\circ}) + H(e^{-}) - H(ArO^{-})$ [16], with

H (ArOH) the enthalpy of the molecule ArOH;
H (ArO°) the enthalpy of the radical ArO°;
H (ArOH°+) the enthalpy of the radical cation $\boldsymbol{ArOH^{\circ+}}$;
H (ArO-) the enthalpy of the phenoxy ion $\boldsymbol{ArO^{-}}$;
H (e^{-}) the enthalpy of the electron (0.752 Kcal/mol) and H (H+) the enthalpy of proton (1.482 Kcal/mol);

- for the path going by the removal of a hydrogen atom by homolytic cleavage of the OH bond (HAT), the dissociation energy of O-H bond $BDE = \Delta H(ArO^{\circ}) + \Delta H(H^{\circ}) - \Delta H(ArOH\ )$ [17,18]

Calculations were performed using the program Gaussian09 [19], and the reading and viewing interface Gauss View5 .0.8.

The work was performed in the Laboratoire de Chimie Théorique et de Spectroscopie Moléculaire (LACTHESMO) of Chemistry Department of Sciences and Technology Faculty of University of Abomey Calavi.

RESULTS AND DISCUSSION

Electronic properties of molecules

Electron affinities, ionization energies and Gap (HOMO-LUMO), of molecules were calculated (in eV) and the calculation results are reported in Table 1.

Table 1: Calculated (in eV) of the electron affinities (EA), ionization energies (IE) and Gap (HOMO-LUMO) (EI - EI) of molecules

	AE			EI			AE - EI		
	6-311G	6-311G (d,p)	6-311G++ (d, p)	6-311G	6-311G (d,p)	6-311G++ (d, p)	6-311G	6-311G (d,p)	6-311G++ (d, p)
Fisetin	0.762	0.463	0.762	7.415	7.187	7.382	-10.91	-11.238	-10.748
Kaempferol	0.871	0.544	0.816	7.514	7.27	7.442	-6.666	-6.721	-6.64

According to these results, we can make the following observations:

- electron affinity values were in the order $AE_{Kaempférol} > AE_{Fusétine}$ within three bases 6-311G, 6-311G (d, p) and 6-311 ++ G (d, p). One could then conclude that kaempferol has a stronger antioxidant than fisetin.
- ionization energy values were in the order $EI_{kaempférol} > EI_{Fisétine}$ within three bases 6-311G, 6-311G (d, p) and 6-311 ++ G (d, p). This means that kaempferol have the lowest antioxidant activity.
- the largest values of the Gap (HOMO-LUMO) were obtained with kaempferol in all three bases. This means that kaempferol is less antioxidant than fisetin.

The results of the Gap (HOMO-LUMO) calculations were in agreement with the experimental data published in literature [20]. But, the results of calculations of the electron affinity and the ionization energy, did not coincide with those of the literature [20, 21]. For this reason, the electron acceptor and electron donor powers ($\bar{\omega}^{+}$ and $\bar{\omega}^{-}$ respectively), were calculated (in eV) for each molecule.

The results are shown in table 2.

Table 2: Calculated (in eV) of the electron acceptor ($\bar{\omega}^{+}$) and electron donor ($\bar{\omega}^{-}$) powers of molecules

	$\bar{\omega}^{+}$			$\bar{\omega}^{-}$		
	6-311G	6-311G (d,p)	6-311G++ (d, p)	6-311G	6-311G (d,p)	6-311G++ (d, p)
Fisetin	132.88	120.351	127.854	873.07	888.463	836.682
Kaempferol	42.922	33.251	40.707	229.73	209.624	223.062

The results of Table 2 show that:

- In each of the three bases6-311G, 6-311G (d, p) and 6-311 ++ G (d, p), the electron accept powers were globally put in the order $\bar{\omega}^{+}_{\text{Fisetin}} > \bar{\omega}^{+}_{\text{Kaempferol}}$. The same arrangement order is given in the literature about of experimental data relating to the antioxidant powers of the both molecules [20].
- The same observations made with the donor electron power $\bar{\omega}^{-}$, and that confirms the fisetin as the most oxidative of the both molecules.

Mechanisms and sites of demonstration of anti-radical activity of the molecules

For each of the two studied molecules, three various types of mechanism of free radicals elimination were considered:

- electron elimination followed by proton elimination and trapping of free radical;
- proton elimination followed by electron eliminationand trapping of free radical;
- elimination of hydrogenatom by homolytic rupture of OH bond and trapping of free radical.

But in this work, the stage of trapping of free radical has been not examined. For the different OH bonds of each molecule, the calculated values of the various energetic parameters (IP, PDE, PA, ETE, SETPT, SPLET, HAT and BDE), of those three paths of reaction, are consigned in tables 3 and 4.

Table 3: Calculated energy parameters (kcal/mol) of fisetin

		IP	PDE	PA	ETE	SETPT	SPLET	HAT
						IP+PDE	PA+ETE	BDE
6-311G	O-H^{3}		142.44	355.79	56.475	411.64	412.269	97.891
	O-H^{7}	269.2	140.56	342.62	67.77	409.76	410.387	96.008
	O-H^{13}		141.19	349.52	61.495	410.39	411.014	96.636
	O-H^{14}		134.91	354.54	50.2	404.11	404.739	90.36
6-311G (d, p)	O-H^{3}		145.58	363.95	52.083	415.41	416.035	101.66
	O-H^{7}	269.83	144.95	350.77	64.633	414.78	415.407	101.03
	O-H^{13}		137.42	354.54	53.338	407.25	407.877	93.498
	O-H^{14}		143.07	359.56	53.965	412.9	413.524	99.146
6-311++G (d, p)	O-H^{3}		150.6	357.68	58.985	416.04	416.662	102.28
	O-H^{7}	265.43	149.97	306.22	109.81	415.41	416.035	101.66
	O-H^{13}		143.07	347.64	61.495	408.51	409.132	94.753
	O-H^{14}		148.72	352.03	62.75	414.15	414.779	100.4

The results obtained for fisetin (Table 3) show that:

- In the three bases, the reaction pathway passing by elimination of hydrogen atom by homolytic cleavage of OH bond has required the lowest values of energies. This means that, probably, the manifestation of anti-radical activity of fisetin pass by the elimination of hydrogen atom by homolytic cleavage of OH bond and trapping of free radical.
- The lowest enthalpy of homolytic dissociation of OH bond (BDE) is mainly obtained for the elimination of H^{14} and H^{13} atoms. This means that these atoms can be easily dissociated from the fisetin molecule to release a radical capable to trap free radicals. Thus, these positions (H^{14} and H^{13}) are appeared as important sites of manifestation of antioxidant activity of fisétin molecule. Indeed, the proximity of C^{14} and C^{13} carbon atoms of the ring B of the molecule, bearing the hydroxyl groups OH^{14} and OH^{13} (in ortho) is a stabilization factor of the phenoxy radical which could be formed by homolytic rupture of any of two OH bonds.

This stability is firstly due to the relocation of the unpaired electron, and the other hand, to the hydrogen bond formation between the non-dissociated hydrogen and the phénoxy.

- The lowest dissociation enthalpy were obtained in the base 6-311G (d, p) which therefore appears to be the most suitable for these fisetin energy calculations.

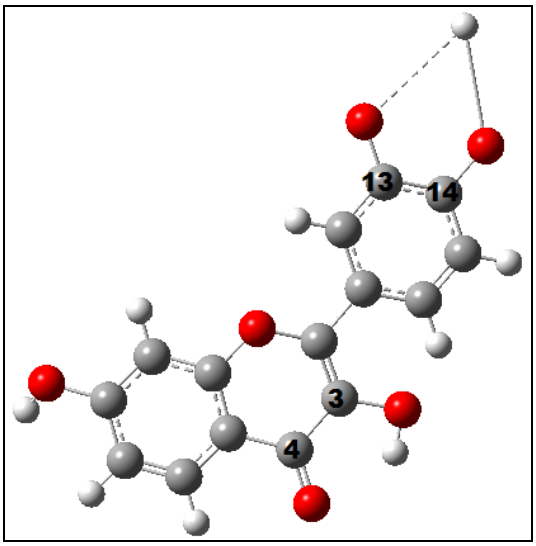

Figure 2: Formation of hydrogen bond between H^{14}atom and phenoxy radical formed after H^{13}homolytic elimination

For kaempferol, the results are consigned in table 4

Table 4: Calculated energy parameters (kcal/mol) of kaempferol

		IP	PDE	PA	ETE	SETPT	SPLET	HAT
						IP+PDE	PA+ETE	BDE
6-311G	O-H^3		237.2	352.66	58.985	411.01	411.64	97.263
	O-H^5	173.88	256.02	360.81	69.653	429.84	430.47	116.09
	O-H^7		237.82	343.24	69.025	411.64	412.27	97.891
	O-H^{14}		233.43	345.13	62.75	407.25	407.88	93.498
6-311G (d, p)	O-H^3		246.61	371.48	43.298	414.15	414.78	100.4
	O-H^5	167.54	260.41	358.3	70.28	427.96	428.58	114.21
	O-H^7		249.12	362.07	55.22	416.66	417.29	102.91
	O-H^{14}		244.73	367.09	45.808	412.27	412.9	98.518
6-311++G (d, p)	O-H^3		242.84	353.91	61.495	414.78	415.41	101.13
	O-H^5	171.94	256.02	360.19	68.398	427.96	428.59	114.21
	O-H^7		244.73	344.5	72.79	416.66	417.29	102.91
	O-H^{14}		528.99	345.13	356.42	712.22	540.28	98.518

For kaempferol, the results of Table 4 show that:

- In the three bases, the reaction pathway passing by elimination of hydrogen atom by homolytic cleavage of OH bond has required the lowest values of energies.

This means that, probably, the manifestation of anti-radical activity of kaempferol pass by the elimination of hydrogen atom by homolytic cleavage of OH bond and trapping of free radical.

- The lowest enthalpy of homolytic dissociation of OH bond (BDE) is mainly obtained for the elimination of H^3 and H^{14} atoms. This means that these atoms can be easily dissociated from the kaempferol molecule to release a radical capable to trap free radicals. Thus, these positions (H^3 and H^{14}) are appeared as important sites of manifestation of antioxidant activity of this molecule.

Indeed, phenoxy radical gave by the homolytic cleavage of the O-H^3 bond, and carried by the C^3 carbon of ring A of the molecule, is stabilized by its conjugation with C^2= C^3 bond, and with group carbonyl C= O carried by the adjacent carbon C^4 [22] (Figure 2).

- The lowest dissociation enthalpy were obtained in the base 6-311G which therefore appears to be the most suitable for these kaempferol energy calculations.

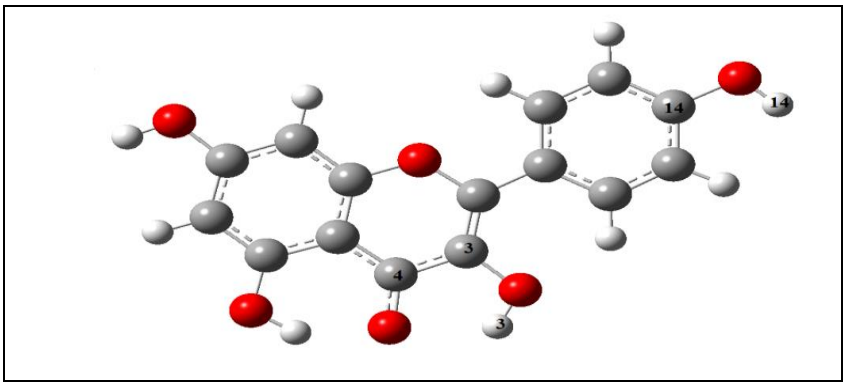

Figure 3: Sites of manifestation of the antioxidant properties of the kaempférol

From the analysis of results of the two tables it was found that the lowest values of bond dissociation enthalpy are given by fisetin. This result confirms more that fasting would be the best molecule antioxidant.

CONCLUSION

By the B3LYP functional of DFT and in three atomic orbital bases (6-311G, 6-311G (d, p) and 6-311 ++ G (d, p)), a theoretical study of antioxidant properties of two isomers flavonols (fisetin and kaempferol) was performed. The results of calculations have confirmed that fisetin has a higher antioxidant power than kaempferol. From the thermodynamic point of view, for the both molecules, the reaction pathway passing by elimination of hydrogen atom by homolytic cleavage of OH bond appeared as the most favourable way for the formation of radical phénoxy likely to trap the noxious free radicals. Also, on the basis of the calculated energy parameters, the hydroxyl groups of the catechol of these flavonoids have been identified as the most important manifestation of sites of the antioxidant activity of the two molecules.

The results were in agreement with the experimental data of the literature. In addition, the results also showed that fisetin is the best antioxidant molecule and the bases 6-311G (d, p) and 6-311G respectively would be the most suitable for the calculations of the dissociation enthalpies of bond of the both molecules respectively.

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