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Evaluation of the Radical-Scavenging Properties of Various Flavonols in Ethanol Environment: an *ab initio* Study

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Abstract: The antioxidant properties of six flavonols -fisetin, galangin, gossipetin, kaempferol, morin and myricetin- have been investigated at HF/6-311G+(d,p) level of theory, using ethanol as solvent. Three known antioxidant mechanisms, namely HAT (hydrogen atom transfer), SET-PT (single electron transfer followed by proton transfer) and SPLET (sequential proton loss electron transfer) have been employed in order to evaluate the radical scavenging abilities of the investigated compounds. Thermodynamic parameters like bond dissociation energy (BDE), proton affinity (PA), electron transfer enthalpy (ETE), ionization potential (IP) and proton dissociation enthalpy (PDE) were calculated and the results were associated with the number and the positions of the hydroxyl groups, the geometry of the parent molecule and of the corresponding radicals, as well as with the electron spin distribution. Also, computations of global reactivity descriptors like HOMO-LUMO gap showed that an increased reactivity is related to the presence of the catechol moiety (gossipetin, myricetin, fisetin). The influence of the catecholic OH groups is also outlined by the HOMO energies, highest electron-donor ability being obtained for gossipetin, the flavonol with two catecholic moieties on rings A and B. According to the HAT mechanism, it has been outlined an enhanced antioxidant character of the 3-OH groups, followed by the hydroxyl groups attached to the phenyl ring B. The calculated values of the condensed Fukui functions, computed for a radical attack, are in good agreement with the above-mentioned results.

Keywords: flavonols, radical scavenging, *ab initio*, ethanol solvation, antioxidant mechanisms, HF/6-311G+(d,p), thermodynamic parameters.

INTRODUCTION

WITHIN the last years, there have been performed various studies that have outlined the role of the oxidative stress in neurodegenerative and cardiovascular diseases, metabolic disorders or cancer.[1,2] Defined as the imbalance between prooxidant and antioxidant species,[3] the importance of the oxidative stress in the etiology of the above-mentioned diseases had a significant effect on other research field of great interest, the one of the antioxidants. Taking into account the benefits of a diet rich in antioxidants, there have been investigated various natural compounds and there is a large number of food supplements based on these natural antioxidants.[4] The antioxidant capacity is related to the ability of scavenging the free radicals that can damage important structures like DNA or proteins and transforming them in non-damaging species.[5]

Due to the fact that the antioxidant activity is based on the capacity of donating one electron or proton, there are various classes of compounds that are successfully used within this field. Among them, the polyphenols, the carotenoids, the vitamins A and C are the most frequently encountered.

The flavonoids are substances with phenolic structure that are widely encountered in berries, tea or various plant tissues. They have a pronounced biologic activity, proving antioxidant, anti-allergic,[6,7] antibacterial,[8] antiviral,[9,10] and antitumor[11,12] properties.

The compounds within the class of flavonoids are characterized by the common structure of flavone: two phenyl rings (A and B) connected by a third oxygen-containing cycle (usually, pyran or pyrone).

The flavonols are mostly known for their capacity of scavenging free radicals, thus for their antioxidant

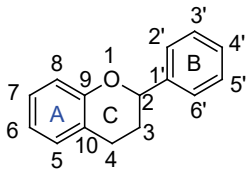

Figure 1. General structure of the flavonoids.

character; meanwhile, they have an important role in various catalytic processes as enzyme inhibition, lipid peroxidation and decreasing the capillary permeability and fragility.

The chemical structure of flavonols, namely the number of hydroxyl groups, their position on the phenyl rings and the degree of substitution, influence their biologic activity. The role of the polyphenols derivatives against cardiovascular diseases is highly correlated with the protection against oxidative stress.[14] It is well known that the endothelial dysfunction is involved in the early stages of the cardiovascular diseases; in this regard, four compounds with flavonoid structure were included in endothelial cells. The positive effect against oxidative stress that was obtained confirmed their utility as antioxidant agents.[13]

There are three main mechanisms that evaluate the antioxidant activity: HAT (hydrogen atom transfer), SET-PT (single-electron transfer plus proton transfer) and SPLET (sequential proton loss electron transfer).[15–17]

HAT mechanism occurs when an antioxidant scavenge a free radical by means of hydrogen donation:

$$R\cdot + ArOH \rightarrow ArO\cdot + RH$$

SET-PT mechanism implies (I) the transfer of an electron (from the antioxidant molecule) to the radical compound, folllowed by the consequent deprotonation (II):

$$R\cdot + ArOH \rightarrow R^{-} + ArOH^{+\cdot} \quad (I)$$

$$R^{-} + ArOH^{+\cdot} \rightarrow ArO\cdot + RH \quad (II)$$

SPLET mechanim involves two stages: (I) deprotonation of the polyphenolic compound and (II) transfer of the electron towards the radicals species.

$$ArOH \rightarrow ArO^{-} + H^{+} \quad (I)$$

$$R\cdot + ArO^{-} \rightarrow ArO\cdot + R^{-} \quad (II)$$

The theoretical studies regarding the physical and chemical properties of flavonoids play an important role in both the understanding of the structure-properties-biologic activity relationship and the design and syntheses of new derivatives with improved properties.[18–20] Literature data confirm the increased interest in both theoretical and experimental studies regarding the polyphenolic compounds: a study reported by Aparicio[21] dealt with the analysis, at B3LYP/6-311++G** level of theory, of structural and energetic properties of 17 flavones derivatives; also, the structure and barriers to internal rotation of both flavone and flavylium ion have been studied at HF, MP and B3LYP level.[22] A QSAR study performed by Amić et al.[23] correlates an enhanced antioxidant activity of flavonoids with the presence of the 3'-OH, 4'-OH and 3-OH groups. Another study[24] reports the evaluation of structural and antioxidant properties of three flavonols (kaempferol, galangin and morin) at DFT level of theory. Two mechanisms, namely hydrogen-atom transfer and single-electron transfer, have been employed for the evaluation of the antioxidant potential.[24] The conformational analysis of two flavones (chrysin and 7,8-dihydoxyflavone) was investigated both at *ab initio* and DFT level of theory[25] and the thermodynamic parameter BDE was computed for all possible ten isomeric mono-hydroxyl flavones, together with the HOMO energies and the corresponding radicals spin densities.[26] Trouillas et al.[27] performed a DFT study regarding the reactivity of OH groups in quercetin and taxifolin; the results outlined the importance of the B-ring and the 3-OH group in the antioxidant properties. Also, it was showed that the reactivity of the position 3 is enhanced by the presence of the 2,3-double bond.[27] Another study[28] reported the efficiency of PM7 method for evaluating the energetics of free radical scavenging of flavonoids. The double HAT and double SPLET mechanisms have been employed for the evaluation of the radical scavenging ability of quercetin catecholic colonic metabolites.[29] The antioxidant capacity of the flavonoids have been also investigated through electrochemical methods; an investigation of the antioxidant potential of three flavonoids led to the following hierarchy: quercetin>catechin>rutin,[30] while in a different study involving 14 flavonoids[31] a new model for the estimation of the first antioxidant potential has been proposed.

Previous work dealt with both experimental and theoretical investigations of antioxidants; there have been investigated the effects of regarding the anti-hyperglycemic effect of plant extracts on diabetic rats;[32] evaluation of the antioxidant activities of berries extract[33] and small fruits containing anthocyanins,[34] as well as theoretical investigations regarding the antioxidant behavior of anthocyanidins.[35]

The present paper aims to investigate the antioxidant properties of six flavonols with various number of OH groups and substitution patterns: fisetin, galangin, gossipetin, kaempferol, myricetin and morin. Taking into account their frequent use in food supplements and the

solubility of flavonols,[36] ethanol was chosen as solvent throughout the computations.

EXPERIMENTAL

Materials and Methods

Geometry optimization and vibrational analysis of the six flavonols were performed at HF/6-311+G(d,p) level of theory; the results have confirmed that the obtained structures are true minima. The corresponding radicals, anions and cation radical of the flavonols have been obtained from the initial optimized structures; a subsequent geometry optimization and vibrational analysis has been performed at HF/6-311G level of theory, followed by a single-point computation using the 6-311+G(d,p) basis set. In order to avoid issues regarding the spin contamination, restricted open-shell (ROHF) computations have been performed for all the radical species.

In order to evaluate the antioxidant capacity of the OH groups of the investigated polyphenolic compounds, the following thermodynamic parameters, associated to the HAT (Hydrogen Atom Transfer), SET-PT (Single-Electron Transfer plus Proton Transfer) and SPLET (Sequential Proton Loss Energy Transfer) mechanisms have been computed:

$$\text{HAT: } ArOH \rightarrow ArO\cdot + H$$

$$\text{Bond Dissociation Enthalpy: } BDE = H_{ArO\cdot} + H_{H\cdot} - H_{ArOH} \quad (1)$$

$$\text{SET-PT(I): } ArOH \rightarrow ArOH^{+\cdot} + e^{-}$$

$$\text{Ionization Potential: } IP = H_{ArOH^{+\cdot}} + He^{-} - H_{ArOH} \quad (2)$$

$$\text{SET-PT(II): } ArOH^{+\cdot} \rightarrow ArO\cdot + H^{+}$$

$$\text{Proton Dissociation Enthalpy: } PDE = H_{ArOH\cdot} + H_{H^{+}} - H_{ArOH^{+\cdot}} \quad (3)$$

$$\text{SPLET(I): } ArOH \rightarrow ArO^{-} + H^{+}$$

$$\text{Proton Affinity: } PA = H_{ArOH^{-}} + H_{H^{+}} - H_{ArOH} \quad (4)$$

$$\text{SPLET(II): } ArO^{-} \rightarrow ArO\cdot + e^{-}$$

$$\text{Electron Transfer Enthalpy: } ETE = H_{ArO\cdot} + He^{-} - H_{ArO^{-}} \quad (5)$$

Another parameter that can be used for the evaluation of the antioxidant character is represented by the condensed Fukui functions[37] for a radical attack. The Fukui functions are reactivity indices based on the distribution of the electron density in the frontier molecular orbitals (LUMO orbitals – reactivity towards nucleophiles and HOMO orbitals – reactivity towards electrophiles, respectively). The simplified formula used for the calculation of the condensed Fukui reactivity index is (with neglection of the overlap integral):

$$f_k^{\alpha} = \sum c_{\mu}^{\alpha^2} \quad (6)$$

where c are the coefficients of the frontier molecular orbitals HOMO and LUMO.

The Fukui functions for a radical attack are computed as the averaged value of the nucleophilic and electrophilic Fukui functions:

$$f\cdot = \frac{f^{+} + f^{-}}{2} = \frac{\left|c^{HOMO}\right|^2 + \left|c^{LUMO}\right|^2}{2} \quad (7)$$

Global parameters of reactivity[38] like chemical potential (μ), hardness (η) and electrophilicity (ω) were calculated according to equations:

$$\mu = \frac{(E_{HOMO} + E_{LUMO})}{2} \quad (8)$$

$$\eta = \frac{(E_{LUMO} - E_{HOMO})}{2} \quad (9)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (10)$$

For the computations of the natural charges and of the stabilization energy E2, the NBO (Natural Bond Orbital) analysis implemented in Gaussian 09W[39] software has been carried out. The Gaussian 09W software has been employed throughout the computations within this paper, except for the graphical representation of the HOMO orbitals, which has been performed with Multiwfn_3.3.7.[40] In order to quantify the effects of the solvent (namely ethanol), the IEFPCM (integral equation formalism polarizable continuum model – with the solute characterized by the electronic density) – have been employed.[41] The dielectric constant of ethanol ε = 24.852 was employed throughout the computations. The enthalpy of the hydrogen radical was computed at the same level of theory (HF/6-311+G(d,p) in EtOH), the obtained value of –0.499826H being used throughout the computation. The enthalpies of the proton and electron (in ethanol environment) were taken from literature[42] (–1070.5 kJ mol^{-1} for H^{+} and –45.1 kJ mol^{-1} for e^{-}).

RESULTS AND DISCUSSION

Global Descriptors of Reactivity

As previous mentioned, the polyphenolic compounds are characterized by an enhanced biologic activity, mostly due to their capacity of scavenging the free radicals. An increased interest has been devoted in order to establish the correlations between the structure and the properties of different classes of polyphenols. Within the present study, the antioxidant activity of six flavonols with both various number of OH groups on the A and B cycles and different substitution pattern has been evaluated.

General structure of the investigated compounds is depicted in Figure 2, together with the number and the position of OH groups that are presented in Table 1.

A brief summarization regarding the number and the position of the hydroxyl groups of the investigated flavonols outlines that one of the investigated structures (galangin) is characterized by the presence of three OH groups exclusively attached to the rings A and C; two compounds have four OH groups (fisetin and kaempferol; the former presents two OH groups on the ring B and two OH groups attached to the cycles A and C in 3- and 7-positions, while the latter has one OH group on the phenyl cycle B and three OH found on the 4-chromone skeleton. Morin has five OH groups, two hydroxyls attached to the benzene ring B, the other three OH appear on the cycles A and C. There are also two compounds that have six OH groups, on one hand is gossipetin with two OH attached to the ring B and four OH groups on the rings A and C, and on the other hand is myricetin, with three OH on the ring B and three OH on the cycles A and C.

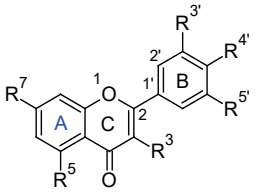

Figure 2. General structure of the investigated flavonols.

Table 1. Structure of the investigated flavonols.

Compound	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	R^{3}	R^{5}	R^{7}	R^{8}
fisetin	H	OH	OH	H	OH	H	OH	H
galangin	H	H	H	H	OH	OH	OH	H
gossipetin	H	OH	OH	H	OH	OH	OH	OH
kaempferol	H	H	OH	H	OH	OH	OH	H
myricetin	H	OH	OH	OH	OH	OH	OH	H
morin	OH	H	OH	H	OH	OH	OH	H

The values of the total energy of the optimized structures, of the frontier molecular orbitals HOMO and LUMO energies and the computed HOMO-LUMO gap depicted in Table 2 outline the importance of the position of the hydroxyl groups on the flavonols skeleton. The relative energy of kaempferol (compared to fisetin, the flavonol with the same number of OH groups) is 0.00821 H, while myricetin is more stable with 0.00972 H than gossipetin (polyphenols with six OH groups). Also, the lowest values of the HOMO-LUMO gap have been obtained for gossipetin, myricetin and fisetin, respectively. The results can be attributed to the existence of the OH groups in catechol position. For gossipetin, the flavonol with the smallest difference HOMO-LUMO among the investigated compounds, there are two catechol moieties: one on the phenyl ring B (3'-OH and 4'-OH) and other on the cycle A (7-OH and 8-OH). Myricetin, that has a slightly increased stability compared to gossipetin, has two catechol moieties on the ring B (3'-OH and 4'-OH, as well as 4'-OH and 5'-OH). A catechol structure appears also on the B ring of fisetin, where there are the two OH groups in 3'- and 4'-position.

The energy of the HOMO orbital is directly related to the antioxidant activity; highest HOMO energy leads to the easiness of the electron-donation. The results depicted in Table 2 show that gossipetin, the flavonol with two catechol moieties on the rings A and B, has a more pronounced electron-donor ability. Smallest values of E_{HOMO} have been obtained for galangin, characterized by the unsubstituted phenyl ring B, as well as for morin, which has a distorted geometry compared to the other five investigated compounds.

The distribution of the HOMO orbitals (see Supplementary file) show that fisetin, kaempferol and myricetin are characterized by the presence of the HOMO orbitals mainly on the phenyl B ring and the C2-C3 double bond of the ring C. As regards galangin, gossipetin and morin, the HOMO orbitals are delocalized on the entire flavonols skeleton, including the benzene cycle A.

Taking into account that the energies of LUMO and (especially) HOMO orbitals strongly influence the

Table 2. Absolute energies, HOMO and LUMO energies, computed HOMO-LUMO gap (HF/6-311+G(d,p) in ethanol).

Compound	Total energy / Eh	E_{HOMO} / Eh	E_{LUMO} /Eh	HL gap / eV
fisetin	–1023.30468	–0.30353	0.05523	9.758
galangin	–948.42451	–0.31271	0.05267	9.938
gossipetin	–1173.07261	–0.29815	0.04907	9.444
kaempferol	–1023.31289	–0.30344	0.05712	9.807
myricetin	–1173.08233	–0.30438	0.05427	9.755
morin	–1098.19822	–0.31299	0.06098	10.172

antioxidant properties of the compounds, the global parameters of reactivity defined by these values have been computed. The values of the chemical potential, chemical hardness and electrophilicity of the six flavonols are depicted in Table 3. The results outline that the lack of OH moieties on the ring B leads to a smaller reactivity of galangin; the higher value of the hardness calculated for morin can be attributed to its distorted geometry. Among the six investigated flavonols, the highest electrophilicity value that have been obtained for galangin, suggesting a more likely character of accepting electrons instead of donating them. As regards the other three flavonols substituted with OH groups on both A and B rings, no significant differences have been obtained. Experimental studies reported the evaluation of the total antioxidant capacity of flavonols by FRAP assay:[43] fisetin > myricetin > kaempferol > galangin, while DPPH and ABTS methods[44] led to the following results: galangin < kaempferol < morin < myricetin < fisetin and kaempferol < galangin < morin < fisetin < myricetin, respectively.

Table 3. Global reactivity parameters: chemical potential (μ), chemical hardness (η) and electrophilicity (ω) (6-311+G(d,p) (EtOH).

Compound	μ / eV	η / eV	ω / eV
fisetin	–3.37	4.88	1.16
galangin	–3.54	4.97	1.26
gossipetin	–3.39	4.72	1.22
kaempferol	–3.35	4.90	1.14
myricetin	–3.40	4.88	1.18
morin	–3.43	5.08	1.15

Antioxidant Mechanism HAT: the Radical Approach

According to the HAT mechanism, the hydrogen atom of the OH groups is donated in order to neutralize a free radical, and the thermodynamic parameter BDE (Bond Dissociation Enthalpy) evaluates the easiness of this process. Lower BDE values are attributed to an enhanced antioxidant capacity, and they are strongly influenced by the stability of the newly formed flavonoid radicals.

Data regarding the planarity/non-planarity of the parent compound and the corresponding radicals, namely the values of the dihedral angle between the 4-chromone and the phenyl cycle is depicted in Table 4. There have been obtained values within the range [27.5°–30.5°] for five of the investigated compounds; in the case of morin, due to the substitution in the 2'-position of the ring B, the value of the dihedral angle between the two rings is 60.3°. There can be noticed the similitudes of the geometries of the parent flavonols and the corresponding 3-OH radicals. Based on the obtained geometries, the thermodynamic parameter BDE has been computed, the results being listed in Table 5.

Table 4. Geometric parameters of the optimized structures of the six flavonols and the corresponding radicals.

	Dihedral angle O1-C2-C1'-C2' / °								
Compound	Neutral	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	28.8	–	0	0	–	25.4	–	0	–
galangin	30.5	–	–	–	–	28.1	0	17.5	–
gossipetin	27.5	–	3.6	8.6	–	21.3	24.3	4.6	0
kaempferol	27.5	–	–	11.9	–	22.1	10.5	0	–
myricetin	–27.5	–	4.5	4.	–2.5	–24.1	–8.2	–5.6	–
morin	–60.3	–53.4	–	–68.8	–	–54.8	–64.0	–60.8	–

Table 5. BDE values of the OH groups of the investigated compounds (kcal mol^{-1}) (HF/6-311+G(d,p) (EtOH).

Compound	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	–	85.37	84.53	–	83.60	–	128.90	–
galangin	–	–	–	–	83.26	107.49	110.70	–
gossipetin	–	86.47	85.88	–	84.30	84.25	77.91	77.97
kaempferol	–	–	103.03	–	83.07	113.24	100.57	–
myricetin	–	88.00	84.84	77.63	85.07	86.95	131.45	–
morin	89.78	–	90.01	–	84.24	112.94	111.97	–

The results presented in Table 5 show, in most of the cases, lower values for the BDE parameter associated to the 3-OH groups. An exception is represented by the catechol groups 7-OH and 8-OH of gossipetin, together with the 5'-OH group of myricetin. For the latter compounds, the results can be attributed to the stabilization of the myricetin and gossipetin radicals due to the formation of the hydrogen bonds (in both of the cases, the catechol moiety is encountered). As regards the former, the lower values obtained for the 3-OH derivatives can be due to the extended conjugation over both phenyl and 4-chromone rings (via the C2-C3 double bond).

For comparison, a recent study[44] performed at M06-2X/6-311+G(d,p) level of theory showed that, in polar solvents like ethanol and acetone, lower BDE values have been obtained for the 3-OH groups of galangin, kaempferol, morin and fisetin and for the 3'-OH group of myricetin, respectively.

As concerns myricetin,[45] computations performed via B3P86 using three basis sets (6-31G (d), 6-311+G (d,p) and 6-311+G (2d,2p)) showed that the radical 4'-O is the most stabilized by intramolecular hydrogen bonds, leading thus to an enhanced radical scavenging capacity. The position of the three OH groups attached to ring B leads, in all of the cases, to highly stabilized radicals; this way, the ring B is attributed with the main contribution to the antioxidant capacity of myricetin.[44]

In order to evaluate the reactivity of each OH group, the condensed Fukui functions ($f^{\cdot}$) for a radical attack have been computed and the obtained values are summarized in Table 6. Calculated as the averaged value of the Fukui functions for an electrophilic and nucleophilic attack (f^{+} and f^{-}, respectively), they show an increased reactivity of the 3-OH groups, followed by the hydroxyl groups attached to the phenyl ring B. It can be noticed the lower reactivity of the 7-OH groups (even in the case of gossipetin, where is a vicinal OH in the 8-position). Also, significantly larger values have been obtained for morin, which can be attributed to its highly distorted geometry (compared to the other five investigated flavonols).

Computations of the spin density values may offer another important insights regarding the antioxidant activity. Table 7 depicts the values of the spin density calculated for each O· radical of the investigated flavonols. Higher values suggest a more reactive site in a radical attack, but also a localization at the specific atom. On the other hand, lower values of spin density can be related with an increased delocalization over the entire molecule, leading thus to a more stable radical. The results outline an increased reactivity of the OH groups attached to the rings C and B (for all the investigated flavonols, the 3-OH group is characterized by a higher value of the spin density). The smaller value obtained for the 5'-OH group of myricetin can be attributed to an extended delocalization and a higher stability of this radical, in good agreement with the BDE values. It may also be noticed the enhanced reactivity of the 8-OH group of gossipetin and the lower value obtained for the single OH group within the phenyl ring B of kaempferol,

Table 6. Condensed Fukui functions for a radical attack (a.u.) (6-311+G(d,p) (EtOH).

Compound	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	–	0.025	0.029	–	0.034	–	0.010	–
galangin	–	–	–	–	0.040	0.021	0.010	–
gossipetin	–	0.008	0.022	–	0.024	0.019	0.011	0.010
kaempferol	–	–	0.026	–	0.039	0.018	0.010	–
myricetin	–	0.019	0.030	0.007	0.038	0.017	0.009	–
morin	0.182	–	0.103	–	0.459	0.344	0.121	–

Table 7. Spin density of the monoradical species (6-311+G(d,p) (EtOH).

Compound	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	–	0.855	0.841	–	0.818	–	0.062	–
galangin	–	–	–	–	0.822	0.564	0.402	–
gossipetin	–	0.856	0.844	–	0.814	0.056	0.073	0.785
kaempferol	–	–	0.286	–	0.808	0.897	0.010	–
myricetin	–	0.863	0.815	0.153	0.820	0.042	0.045	–
morin	0.884	–	0.884	–	0.833	0.379	0.381	–

4'-OH. As well as the BDE values computed for kaempferol, the 3-OH group appears to be more reactive than the 4'-OH one.

The energy of the HOMO orbitals is also used as a global descriptor of the antioxidant capacity [46]. In order to evaluate the local antioxidant capacity of the OH groups, the contribution of each oxygen atom to the total HOMO energy has been calculated for each oxygen atom of the hydroxyl groups. The results (see Supplementary File) show that the OH groups attached to the phenyl B ring are favored: 4'-OH (fisetin), 4'-OH (myricetin) and 4'-OH (morin). Similar values of the HOMO energy contributions have been obtained for the 3-OH and 4'-OH groups of gossipetin, while 3-OH is preferred for galangin and kaempferol.

Antioxidant Mechanism SET-PT

According to the SET-PT mechanism, there are two stages of the antioxidant action; at first, the flavonol gives an electron and becomes a cation radical, and then donates the hydrogen atom. The first stage of the process implies the calculation of the ionization potential (IP), which represents a reliable index for evaluating the general antioxidant activity of the compound, not to a specific OH group like BDE. The values presented in Table 8 suggest a more pronounced antioxidant character for gossipetin. Comparing the IP values obtained for fisetin and kaempferol, as well as for gossipetin and myricetin, the two groups of polyphenols with the same number of OH groups, it results that the presence of a larger number OHs on the ring B (fisetin) lowers the value of the ionization potential.

The second stage of the SET-PT mechanism involves the donation of the proton from the cation radical structure, so the PDE parameter has been computed for each hydroxyl group; the results presented in Table 8 are in good agreement with the BDE trend (taking into account that are the same radical structures that are employed throughout this computation). Lowest values have been obtained for the 3-OH radicals, followed by hydroxyls attached to the B ring. Exceptions are the gossipetin structure (with the favored catechol OH groups on the A cycle) and the myricetin, whose 5-OH radical appears to be the most favored.

Antioxidant Mechanism SPLET

As regards the sequential proton-loss electron transfer mechanism, the first stage consists in obtaining the anionic structure by subtracting the proton. A higher acidic character of the OH groups implies an easier donation of the proton, thus lower values for the proton affinity (PA).

The results presented in Table 9 outline an enhanced acidic character for the hydrogen atom of the 7-OH groups. According to the general structure of flavonols depicted in Figure 1, the 3-OH and 5-OH groups can establish

Table 8. Ionization Potential (**IP, kcal mol^{-1}**) and Proton Dissociation Enthalpy (PDE; kcal/mol) (HF/6-311+G(d,p) (EtOH).

Compound	IP	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	110.51	–	22.34	21.50	–	**20.57**	–	65.89	–
galangin	120.75	–	–	–	–	**9.98**	34.21	37.41	–
gossipetin	106.70	–	27.24	26.65	–	25.08	25.02	**18.68**	**18.74**
kaempferol	109.71	–	–	40.80	–	**20.84**	51.01	38.34	–
myricetin	115.16	–	20.29	17.13	**9.92**	17.35	19.23	63.74	–
morin	110.65	26.59	–	26.83	–	**21.05**	49.75	48.78	–

Table 9. Proton affinity **PA** (kcal/mol; bold) and electron transfer enthalpy *ETE* (kcal/mol; italic) (HF/6-311+G(d,p) (EtOH).

Compound	2'-OH	3'-OH	4'-OH	5'-OH	3-OH	5-OH	7-OH	8-OH
fisetin	–	**60.43**	**51.54**	–	**59.61**	–	**50.19**	–
	–	*93.97*	*102.01*	–	*93.02*	–	*147.75*	–
galangin	–	–	–	–	**57.22**	**55.27**	**48.48**	–
	–	–	–	–	*95.07*	*121.24*	*131.23*	–
gossipetin	–	**59.61**	**50.88**	–	**57.38**	**56.82**	**49.87**	**54.75**
	–	*95.78*	*104.02*	–	*95.95*	*96.45*	*97.06*	*92.24*
kaempferol	–	–	**54.85**	–	**59.21**	**56.25**	**49.28**	–
	–	–	*117.20*	–	*92.88*	*126.00*	*120.32*	–
myricetin	–	**60.55**	**55.14**	**54.77**	**58.28**	**56.77**	**50.02**	–
	–	*96.47*	*98.72*	*91.88*	*95.78*	*99.20*	*150.46*	–
morin	**54.85**	–	**55.05**	–	**61.14**	**56.81**	**49.78**	–
	103.95	–	*103.97*	–	*92.11*	*125.14*	*131.20*	–

intramolecular hydrogen bonds with keto C4=O group (larger PA values for 3-OH and 5-OH, depicted in Table 9, have been obtained). Also, intramolecular hydrogen bonds are possible when multiple phenolic groups are attached to the ring B, which resulted in higher values of PA for the respective groups of fisetin, gossipetin and myricetin. For the three compounds that have two OH groups in catechol position (fisetin, gossipetin and myricetin), one of the OH groups is characterized by a low PA value, while the other shows the highest PA values among the OH groups of the flavonol. In the case of 3'-OH radicals of fisetin, myricetin and gossipetin, there are no intramolecular hydrogen bonds. Instead, for the 4'-OH radicals, an intramolecular hydrogen bond appears between the O- and the H atom of the neighbor 3'-OH group (similar situation for the 5'-OH radical of myricetin, which establishes a hydrogen bond with the H atom of the 4'-OH group). The results are in good agreement with the PA computations.

The lower values obtained for the proton affinity of the 7-OH groups can be related with the diminished values of the natural atomic charges (see Supplementary File).

Also, another study[47] showed that for the most part of flavonols, the H atom of the 7-OH group is the most easily donated. Among them, the most acidic molecule is morin, due to the formation of a phenoxide anion stabilized by intramolecular hydrogen bonds with 3-OH group.[46]

The second step of the SPLET mechanism deals with the formation of the radical species, the results being also depicted in Table 9. The same variation of the ETE values as calculated for the BDE and PDE parameters that involved the radical species have been obtained.

The stabilization energy E2, computed within the NBO analysis, may offer some additional information regarding the reactivity of the OH groups. The results (see Supplementary File) summarize only the strongest interactions between the lone pair of electrons of each oxygen atom. The results suggests an enhanced stabilization for the O-5 and O-7 radicals, which is in good agreement with their lower reactivity in radical processes like BDE, PDE or ETE.

CONCLUSION

The antioxidant properties of six flavonols have been investigated at HF/6-311+G(d,p) level of theory, in solvent environment (ethanol, IEFPCM approach). All the three antioxidant mechanisms HAT, SET-PT and SPLET have been taking into account, and their corresponding parameters BDE, IP, PDE, PA and ETE have been computed.

According to the HAT mechanism, an enhanced antioxidant character is shown by the 3-OH groups, followed by the hydroxyl groups attached to the phenyl ring B. Similar results have been obtained for the condensed Fukui functions, computed for a radical attack. The results suggest the increased reactivity of both the 3-OH and the OH groups from the cycle B in case of a radical mechanism (especially the ones that show a catechol configuration).

As regards the computed values of the IP, the presence of a larger number of OHs on the phenyl cycle B leads to the increased antioxidant activity. Concerning the PA values, when there are two OH groups in catechol position (fisetin, gossipetin and myricetin), one of them is characterized by a low PA value, while the other shows the less acidic character among the OH groups of the flavonols at the HF/6-311+G(d,p) level of theory.

Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3449.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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