Antioxidant mechanisms of Quercetin and Myricetin in the gas phase and in solution – a comparison and validation of semi-empirical methods

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Abstract: Flavonoids have long been recognized for their general health-promoting properties, of which their antioxidant activity may play an important role. In this work we have studied the properties of two flavonols, quercetin and myricetin, using semi-empirical methods in order to validate the application of the recent Parametric Model 6 and to understand the fundamental difference between the two molecules. Their geometries have been optimized and important molecular properties have been calculated. The energetic of the possible antioxidant mechanisms have also been analyzed. The two studied flavonols do not differ significantly in their molecular properties, but the antioxidant mechanisms by which they may act in solution can be rather different. Moreover, we also show that the Parametric Model 6 can produce reliable information for this type of compounds.

Response to Reviewers: Reviewer #1:

P.3 "Q" and "M" are used before they are defined.
P.5 "Q" and "M" definitions should be given earlier in the paper.
P.5 "... what is of extreme." should be ". which is of extreme ."
P. 7 HAT has already been defined on p.2, BDE has already been defined.
P.4 ". can be stabilized more effectively stabilized ." is very bad English
»»» These issues have been properly adressed.

P.5 ". determining the biological relevance of these compounds." Does not follow from the preceding part of the sentence.
»»» It was an oversimplification of what was meant. We changed it to "indicating that they will be available to interact with surrounding radicals and other species" (pages 5/6).

P.8 The statement, "As it can be seen from Table 6, the absolute values obtained are strikingly different from those obtained by other authors at the DFT level." is utterly useless unless the DFT results are also presented. Do the authors intend the reader to stop, search for suitable DFT publications, do the comparison, and then resume reading?
»»» We have now included these results in the tables and made the necessary comparisons. Table 6 was split in two - Table 6 for M and Table 7 for Q – and now contain the DFT acidities and BDE values

retrieved from the literature. We have also included a new table – Table 8 – which contains the deprotonation and H atom abstraction orders from the literature as well as from our work.

P.8 For the authors to say that ". the calculated heats of formation of H+ and H*, that need to be further refined." is meaningless. They can say that PM6 gives very inaccurate values for these quantities. The implication that further refinement would give better values is not supported by any evidence.
»»» As a consequence of this remark, as well as of the remarks of the Reviewer #2, we have repeated the calculations using the same method but under much more stringent conditions and also using other methods. These new calculations led to heats of formation of H* and H+ of, respectively, 217.99 (which is the accepted reference value, a parameter in the program) and 1303.16 kJ/mol, which differs about 15 % from the accepted reference value of 1530.17 kJ/mol. We have made the energetic analysis using both these values and the reference values, and found out that using the PM6 derived values leads to reactional energy changes that ressemble more closely the DFT ones, presumably by error cancelation.

This reviewer is not willing to re-review this article.

Reviewer #2: This paper addresses interesting and complex quentions concerning the reaction mechanisms of two flavanols. There are 3 significant issues that need to be addressed before publication, however.

1) The calculated homo-lumo gap for M increases in going from the gas phase to SCRF water whereas this number should get very much smaller. I suspect that the problem originates in using orbital energies to approximate IA and EP via Koopman's theorem. For molecules of this size, IP and EA should be evaluated properly from the total energies of the ions. Realistic values for IP-EA for these molecules are in the 2-3 eV range, though semi-empirical methods may overestimate this a bit. I am sure that experimental numbers from Cv are available and should be used for comparison to the calculated values in solution.
2) Semi-empirical methods are not the ones of choice for this problem - they are best for large systems that cant be done by other means. For these molecules I would start with DFT and try to do CCSD. At many points obtained results are compared to published DFT, sometimes they are very similar and sometimes very different. There is no analysis of the cases in which they are different, and no suggestion as to which is the most useful. The comment on page 8 about heats of formation for H and H+ are critical but not explored. I think that the problem is that the calculations have been asked to evaluate a poorly defined quantity and that a better measure should be found for addressing the experimental issues of relevance. In the end a much better case needs to be put for using semiempirical methods than has been presented.

»»» In face of these two pertinent observations, we have repeated all the calculations using the PM6 methods with much more severe restrictions and a couple of other methods, such as the PM6DH (doi: 10.1021/ct9000922) and the semi-empirical methods implemented in Gaussian03 (AM1 and PM3), and the results of all the calculations are nearly coincidental. These new calculations led to heats of formation of H* and H+ of, respectively, 217.99 (which is the accepted reference value, a parameter in

the program) and 1303.16 kJ/mol, which differs about 15 % from the accepted reference value of 1530.17 kJ/mol. We have made the energetic analysis using both these values and the reference values, and found out that using the PM6 derived values leads to reactional energy changes that ressemble more closely the DFT ones, presumably by error cancelation.
The IP and EA were now computed using both the orbital approach and the energetic approach, and the results have been introduced in Table 2. As a consequence, the Results and Discussion section was greatly re-written.

3) The conclusions are very weak. The first paragraph just apologizes becauses the methods used are so bad, and the second paragraph, the one containing the interesting results, is very short. From reading the paper it seems that half of the tasks done in it have been done before and that what this paper offers is a complete view of the whole chemistry of the molecules. It is this positive feature, and the lessons learned, that need to dominate the conclusions.

»»» We have deeply re-written the conclusions to reflect all the comments of the reviewer.

some minor comments;
- use 2 or 3 significant figures throughou
- give the full text along with symbols and acronyms in tables and figure captions
- Q and M are undefined on first use on P 3
- "base" -> "basis" page 4
- "In face of" -> "Given" on page 4

»»» These issues have been properly addressed.

- "As can be seen from Table 6" on page 8 ... cant be seen as the DFT numbers being compared to are not in the table
- page 8 bottom - which experimental results? What about alternative explanations? Its aspects like this that give the paper relevance and meaning and should be expanded.

»»» We have now included these results in the tables and made the necessary comparisons. Table 6 was split in two - Table 6 for M and Table 7 for Q – and now contain the DFT acidities and BDE values retrieved from the literature. We have also included a new table – Table 8 – which contains the deprotonation and H atom abstraction orders from the literature as well as from our work.

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Abstract

Flavonoids have long been recognized for their general health-promoting properties, of which their antioxidant activity may play an important role. In this work we have studied the properties of two flavonols, quercetin and myricetin, using semi-empirical methods in order to validate the application of the recent Parametric Model 6 and to understand the fundamental difference between the two molecules. Their geometries have been optimized and important molecular properties have been calculated. The energetic of the possible antioxidant mechanisms have also been analyzed. The two studied flavonols do not differ significantly in their molecular properties, but the antioxidant mechanisms by which they may act in solution can be rather different. Moreover, we also show that the Parametric Model 6 can produce reliable information for this type of compounds.

Keywords Flavonoids · Antioxidant Mechanism · Semi-empirical methods

Introduction

Flavones and flavonols are two of the most important classes of compounds from the dietary phytochemicals, not only in terms of their greater abundance in the diet, but also due to the recognition of their *in vitro* antioxidant properties [1-3]. Moreover, several studies have shown that flavonoids contribute to the overall antioxidant capacity *in vivo* [4-5]. Besides their antioxidant activity, both flavonoid aglycones and glycosides can act as antifungal, insect antifeedants, antimicrobials and antivirals and *anti*-inflammatory, and are also of particular importance in chemotaxonomy [6-8]. Associated with this, their identification in numerous herbs and infusions has also been the focus of several groups worldwide, particularly from regions where traditional medicine plays an important role [9, 10]. Recently, they have also received considerable interest in the food industry as food additives and as nutraceuticals because of their antioxidant and anticarcinogenic properties, among others [11-14].

Quercetin (3,5,7,3',4'-penta-hydroxyflavone) is one of the most important flavonoids in diet due to its abundance in foods and to its bioavailability [15], and its antioxidant activity has been well studied [16]. On the other hand, several works have shown that myricetin (3,5,7,3',4',5'-hexa-hydroxyflavone) is a stronger antioxidant than quercetin, what has been attributed to the presence of the 5'-OH group that allows a further stabilization of the myricetin derived semi-quinone radical [17, 18]. Briefly, it has been found that myricetin is a stronger brain neuron oxidative stress and liposome oxidation inhibitor than quercetin. Moreover, the pyrogallol moiety present in myricetin is a better superoxide scavenger than the catechol moiety present in quercetin [19]. The structures of these flavonoids are presented in Fig. 1.

Several studies have pointed out the key factors determining the antioxidative potential of a compound, that include i) a low O-H bond dissociation enthalpy (BDE), easing H abstraction; ii) a high ionization potential (IP), hampering oxygen reduction by the antioxidant; and iii) an adequate solubility [20]. Moreover, the stability of the antioxidant-derived radicals must also be considered, as unstable radicals may react with other molecules instead of terminating the radical reactions [20].

In this work we present a semi-empirical study, conducted using the PM6 Hamiltonian, in the gas phase and in the water phase, of quercetin, myricetin and their derived radical and anionic species, where we address the above-mentioned pre-requisites of a high antioxidant activity in order to determine which structural and electronic features are behind the higher antioxidant activity of myricetin and to elucidate the role of each of the three possible mechanisms underlying antioxidant activity, namely a) H atom abstraction (HAT), b) sequential proton loss electron transfer (SPLET) and c) single-electron transfer followed by proton transfer (SETPT) [21]; these mechanisms are depicted in Fig. 2.

Computational details

In the present study, quercetin and myricetin molecules were considered theoretically by performing semi-empirical molecular orbital calculations both in the gas phase and in the water phase. The neutral forms and their anions, the radicals formed by H atom abstraction and the radical cations and radical anions of both flavonols were studied. Pre-optimization was performed under a Dreiding type molecular mechanics force field [22], implemented in the Marvin software and the Calculator plugins [23]. The structures thus obtained were fully optimized using MOPAC2009 version 9.034 [24]. Geometry was optimized using the Baker's Eigenvector Following routine [25]. Geometry was considered to be fully optimized when the gradient norm was less than 0.01. Single point-calculations were then performed to compute the properties of the molecules. All computations were performed using the the restricted Hartree-Fock formalism.

The following semi-empirical Hamiltonians, as implemented in MOPAC2009, were used: Modified Neglect of Differential Overlap (MNDO) [26], Austin Model 1 (AM1) [27] and the derived Recife Model 1 (RM1) [28], and the Parameterized Model 3 (PM3) [29] and the new Parameterized Model 6 (PM6) Hamiltonian [30]. Water phase optimizations and calculations were performed using the Conductor-like Screening Model, a continuum approach to the solvent effect [31]. In order to ensure that the obtained geometries corresponded to absolute energy minimums and not to local ones, quercetin (Q) and myricetin (M) were drawn with dihedral angles (the angle defined by the two planes that contain the B ring and the A and C rings) varying from -180 º to 180º in steps of 10 º and were subject to geometry optimization. All optimizations converged to the same final structure, as presented hereafter. The dihedral angles obtained for all the computed species with the PM6 Hamiltonian vary between -1.7 º and 0.4 º . Both these findings are in agreement with previous results that the rotation around the C2-C1’ bond does not constitute a rate limiting step for the antioxidant activity of these compounds due to the low energetic barrier associated with that rotation [32].

The bond dissociation enthalpy (BDE), the proton affinity (PA), the electron transfer enthalpy from the anion (ETE) and the proton dissociation enthalpy from the radical cation (PDE) were calculated as differences between the heats of formation (H_f) of the products and the reactants (Fig. 2), where F-O$^{\bullet}$ and F-O^{-} are the radical and the anion derived of the antioxidant, F-OH, respectively. These values allow the analysis of the relevance of each of three proposed mechanisms of antioxidant activity, namely H atom transfer (HAT), quantifiable by the BDE, the single-electron transfer followed by proton transfer (SETPT), quantifiable by the ionization potential and the PDE, and the sequential proton loss electron transfer (SPLET), governed by the proton affinity and ETE [21, 33, 34], as shown in Fig. 2. The heats of formation used for $H^{\bullet}$ and H^{+} were, respectively, 217.99 (which is the actual reference value [35]) and 1303.16 kJ mol^{-1}, as calculated with MOPAC2009; although the value for H^{+} differs about 15% from the reference value (1530.17 kJ mol^{-1} [35]), it generates results that are more similar to the DFT results from the literature.

The electronic density-derived properties were computed both from the orbital energies *via* Koopmans’ theorem (and denoted with a subscript *O*) and from the total energies (denoted with a subscript *E*) of the species as follows: $IP_E = H_f(FOH^{\bullet +}) - H_f(FOH)$, $IP_O = -E_{HOMO}$, $EA_E = H_f(FOH) - H_f(FOH^{\bullet -})$ and $EA_O = -E_{LUMO}$, where IP and EA stand for ionization potential and electron affinity, respectively. From these, global hardness (η) was computed as $\eta = (IP - EA)/2$, electronegativity (χ) as $\chi = (IP + EA)/2$ and electrophilicity (ω) as $\omega = \chi^2/8\eta$ [36, 37]. The energy difference between the LUMO and the HOMO was also computed using the orbital energies, as $\varepsilon_{G,O} = E_{LUMO} - E_{HOMO}$, and using the energetic values for electron affinities and ionization potentials, using $\varepsilon_{G,E} = IP_E - EA_E$.

Results and discussion

Comparison of the performance of the different Hamiltonians

Table 1 lists the heats of formation computed using the PM6 Hamiltonians for quercetin and myricetin and their derived radicals and anions; results obtained with the other semi-empirical methods are presented as Supplementary Information. All methods indicate that the most acidic hydroxyl group is the 4’ in both the gas phase and the water phase, and that the H atom more easily abstracted from quercetin is also the 4’ in the gas phase and, in the water phase, either the 3’ or the 4’, though, except in the case of the AM1 method, always with very close heats of formation (with differences inferior to 1 kJ mol^{-1}).

In the case of myricetin, the results with the MNDO, AM1 and RM1 methods show a high dispersion of values for the gas phase radicals and for the water phase anions. On the other hand, and considering the structure of myricetin, the most easily abstractable H atom appears to be the 4’ one as the resulting radical is more efficiently stabilized due to the presence of the two neighboring OH groups. However, the mentioned methods indicate that the H atom to be firstly abstracted is the 3 one.

Results published in the literature [33, 37-44] using several methods (ranging from the semi-empirical AM1 to DFT methods with a 6-311++G(3df,2p) basis, which are presented as Supplementary Information) indicate that the most acidic OH group in quercetin is the 4’ one and that the first H abstraction occurs from the 3-, 3’- or 4’-OH groups, and this variation does not appear to be method-dependent, although the 4’ tends to predominate over gas phase studies and the 3-OH over the water phase studies. For myricetin, the few published results indicate that the 4’-OH group is the most acidic one in both phases. Given these results, we consider that the semi-empirical description of these two flavonoids obtained with the PM6 method, and, partially with the PM3 method, is in broad agreement with what has been published so far. Moreover, the results obtained are in agreement with what is expected from the structure of these flavonoids as both the radical and the anion formed from the 4’-OH group are the ones expected to be more stabilized by charge delocalization and resonance.

Geometry and molecular properties

The closed formulas of quercetin and myricetin are, respectively, $C_{15}H_{10}O_7$ and $C_{15}H_{10}O_8$. The optimized structures of myricetin in the gas and water phases are presented in Fig. 3. The dihedral angles O1C2C1'C2', formed between the plane containing the A and C rings and the plane containing the B ring, are close to zero, showing that both these molecules are very close to being fully planar. The X-ray structure of crystalline quercetin shows that the angle between the two planes is about 7º [45]. Other works at the RHF level with STO-3G basis set [46] and with the semi-empirical PM3 method [38] have found that quercetin is planar; on the other hand, a work using the semi-empirical MNDO method has found it to be non-planar

[47]. Leopoldini *et al.* [41]calculations at the DFT level, using the B3LYP hybrid functional with the 6-311++G** basis set, have found that quercetin and myricetin are both planar, and also that the 4' anion of myricetin and all the quercetin anions are planar, thus allowing charge delocalization, with the concomitant enhancement of their antioxidant activity.

Quercetin and myricetin, being constituted of an hydrophobic part, the phenyl rings, and an hydrophilic part, the hydroxylic groups, display an amphipathic behavior. The negative energies of solvation ($\Delta_{solv}H_f$, Table 1) indicate that these flavonoids are soluble, although sparingly, in water; the derived radicals are also water soluble, which is of extreme importance as most of the reactions in which flavonoids participate occur in water or at water/lipid interfaces [48]. Moreover, the computed permanent dipole moments (μ_0, Table 2) also indicate that both Q and M are relatively polarized systems, reflecting the polarized hydroxyl and carbonyl functions present in the structures and corroborating their water solubility.

The total dipole moment due to the presence of external fields is dependent on, besides μ_0, the polarizability α and the first and second order hyperpolarizabilities, β and γ. The values obtained for Q and M, in water, are α_M = 229.14 a.u., β_M = 1606.44 a.u., γ_M = 123548.86 a.u., and α_Q = 225.03 a.u., β_Q = 1868.56 a.u., γ_Q = 125047.11 a.u., and indicate that these flavonoids are capable of polarizing other atoms or molecules and of accommodating themselves to the surrounding environment, indicating that they will be available to interact with surrounding radicals and other species [49, 50]. However, the direction of the dipole varies strongly from species to species, thus it cannot be used as a descriptor of the activity of these compounds.

The high reactivity of these compounds is also characterized by a small energy gap, ε_G, between the HOMO and the LUMO and also by a low LUMO energy, that indicates that these compounds can behave as soft electrophiles [51-53]. The ε_G values obtained for Q and M are similar, indicating that their reactivity does not differ much. This similar reactivity has also been obtained by DFT computations [54] but the absolute values we obtained are roughly the double of the DFT ones. However, it must be noted that the energy derived ε_G values in water are, as expected, much smaller than those in the gas phase, a trend that is not observed with $\varepsilon_{G,O}$ values.

The ionization potentials and electron affinities of both Q and M have been computed both from the total energies and from the orbital energies (using Koopman's theorem). The results presented in Table 2 show that while all the values computed with the PM6 orbital values are substantially different than those obtained with a CHIH-DFT method, with differences ranging from 10 % to more than twice the DFT value, the PM6 values computed using the energies of the species involved in the processes (the neutral form and the radical cation and anion), the obtained values are in the same range of the values computed at the DFT level. Also, the obtained values also indicate that it is easier for these compounds to yield an electron than to acquire one, thus favoring their action as antioxidants (by reducing other species) than as putative pro-oxidants (by oxidizing other species).

A higher hardness index (η) is associated with a lower reactivity, and the results in Table 2 indicate that the energy-derived hardnesses of Q and M are lower in water than in the gas phase, thus suggesting a higher reactivity in the condensed phase, as expected from a lower ionization potential in water than in the gas phase, or in another words, an higher ability to provide an electron when in the gas phase.

The higher electron affinities of Q and M in water than in the gas phase also indicate that these compounds are more reactive in the condensed phase, where they have a higher ability to accommodate electrons from the solvent of from other species (as expressed from their higher electrophilicity).

However, the nucleophilic and electrophilic delocalizabilities, presented in Table 3, indicate that the reactivity of the two flavonoids, in terms of the most likely sites of electrophilic and nucleophilic attack, presents a striking difference: while myricetin is most likely to be attacked by electrophiles on the O atoms of the B ring OH groups (where Dn(r) hits its maximum values), quercetin is more likely to be attacked by electrophiles on hydroxylic O atoms of the A or C rings. On the other hand, the De($\boldsymbol{r}$) distribution is similar for both flavonoids in either phase.

In general, energy-derived properties present values that are more similar to their DFT analogues than orbital-derived properties. The best example is the ionization potential of Q and M. Also, the variations of the electron-derived properties, when comparing the gas phase and the water phases, are also more coherent with what is expected from the point of organic structure and reactivity and are also coherent with their DFT counterparts.

The IP and EA values of α-tocopherol, a powerful antioxidant ubiquitous in mammal organisms, were also calculated using the PM6 method. In the water phase, the IP for tocopherol is 539.37 kJ mol^{-1} and the EA is 260.68 kJ mol^{-1}. In the gas phase, the calculated values are 684.26 and 26.44 kJ mol^{-1}, respectively.

The water phase ionization potential of tocopherol is smaller than the corresponding ones of Q and M, indicating that the two flavonoids are able to get an electron from either tocopherol. This agrees with the role of flavonoid on the transference of “radical character” from highly oxidant reactive species to antioxidants like tocopherol (and also ascorbate) that are regenerated from the diet (or, in the case of ascorbate, enzymatically) [55].

Charge and spin delocalization

It is commonly mentioned throughout the literature that planarity is important to the antioxidant activity of flavonoids because it allows charge (in the case of anions) or spin (in the case of radicals) delocalization over the entire molecule, thus contributing to the stabilization of these species. However, as it can be seen in Table 4, and in agreement with other results obtained at the DFT level by Fiorucci *et al.* [33], charge delocalization is restricted to the ring from where the proton is abstracted and the C ring – when deprotonation occurs from the B ring the resulting atomic charges are mainly located on B and C ring atoms, and when deprotonation occurs from the A ring atomic charges are mainly located on the A and C ring atoms. This effect is more evident in the water phase results than in the gas phase results.

Noticeably, the atomic charge of O atoms does not vary considerably between the neutral form and the anionic forms, and the excess charge is distributed mainly over the rings.

In the case of spin distribution (Table 5), spin accumulates essentially on the ring from where the H atom was abstracted, with only a small percentage of it being located on the central ring. The remarkable exception to this is the case of 3-$O^{\bullet}$ radicals, where spin is also distributed throughout the B ring, which agrees with some published results that indicate that the 3-OH group of quercetin can be particularly important in the antioxidant activity of this flavonoid [40, 56].

It must be noted that the position of the OH group from where abstraction occurs is important – abstraction from the 4'-OH group (which is *para* to the attachment position of the C ring) leads to a higher spin delocalization to the C ring than when it occurs from the 3' or 5'-OH groups (which are *ortho* to that position). This higher spin delocalization agrees with the abstraction order, where the 4’-OH H atom is the more prone to be removed. Charge delocalization to the C ring is also more prominent in the case of the 4’-O^{-} anions.

All the above discussed characteristics are in agreement with the HOMO and LUMO distribution of quercetin [38] and myricetin (presented in Fig. 3). The HOMO orbitals are mainly disposed on the C2-C3 double bond, the 3-OH group and the B ring, in good agreement with the many experimental results that sustain the importance of those characteristics as key determinants of the flavonoids antioxidant activity (reviewed by Bors and Michel [3]).

Energetics of the antioxidant processes

Flavonoids have been studied as antioxidants due to the ease with which an H atom can be abstracted from them by a radical, producing a flavonoid radical (F-O$^{\bullet}$) that is more stable and less reactive than the original attacking radical. The HAT mechanism is primarily governed by the O-H bond dissociation enthalpy (BDE, the energy associated with the homolysis of a hydroxylic O-H bond). More recently, other mechanisms have been described that could be important for the formation of the flavonoid derived radicals, particularly the sequential proton loss electron transfer (SPLET) mechanism and the sequential electron transfer proton transfer (SETPT) mechanism [21, 33, 34].

The SPLET mechanism is expected to be relevant in proton-accepting solvents, as is water, and involves deprotonation of the flavonoid (measurable by proton affinity, PA) followed by electron transfer (measured by the electron transfer enthalpy, ETE) to produce the flavonoid radical. Oppositely, the SETPT mechanism involves formation of the flavonoid radical cation by electron loss from the neutral flavonoid (where the ionization potential, IP, becomes important) followed by deprotonation of the radical cation (describable by the proton dissociation enthalpy, PDE). The values computed for these reactions are presented in Table 6 for myricetin and Table 7 for quercetin. Table 8 summarized the orders of deprotonation and H atom abstraction for these compounds obtained with this study and also from published data from other authors.

Comparison of PM6 and DFT values

In the case of myricetin, the computed PA's were compared with the acidities calculated at various DFT levels [41, 56], and the results differ at most 10 % in the gas phase (the average difference is *ca*. 5 %) and at most 30 % in the water phase due to the "outlier" value of the PA for the 3-OH group (the average difference is *ca.* 15 %). More interestingly, the PM6 results have the same behaviour as the DFT ones in the both phases.

The gas phase BDE values of myricetin were also compared to the available DFT ones [57], and show a maximal deviation of 12 % with an average deviation of 6 %, and the values follow have the same behaviour in both cases.

The gas phase PA values of quercetin (Table 7) were compared to the corresponding acidities, enthalpy changes and Gibbs energy changes [33, 41, 56], and a maximal 10 % deviation (with an average 5 % deviation) was found between the different sets of values and the PM6 ones; in the water phase the maximal deviation was of 13 % and the average deviation of 10 %. As before, the data show the same behaviour.

The gas phase BDE values also follow the same trends as the published ones [57, 58], with a global average deviation of 13 % and a maximal of 45 %; however, if we do not consider the ΔG values presented in Table 8, those deviations fall to, respectively, to 10 % and 18 %; the larger deviations found with the Gibbs energy values are likely to come from differences in the entropic part of the Gibbs energy, which, although expectably small, may contribute to the overall deviations. The water phase BDE values also follow the same trends, with the exception of the PM6 value for the 3-OH BDE which is higher than expected from comparison with the other data, and show an average deviation of 5 % with a maximal deviation of 24 %. The distribution of the values discussed above is shown in Fig. 4.

The H atom transfer (HAT) mechanism

The water phase BDE values for quercetin indicate that H atom abstraction occurs primarily from the 3' and 4' OH groups, and the resulting radicals are *ca.* 70 kJ mol^{-1} more stable then the following one, from the 7-OH group. In the case of myricetin, H atom abstraction occurs primarily from the B ring OH groups, being the resulting radicals stabilized by the H bonds established with the oxygen atoms in that ring.

These results are in agreement with the established structure-activity relationships for the antioxidant activity of flavones, which point out the fundamental role of the B ring catechol (or pyrogallol) groups [3]. Strikingly, the DFT results suggest that the 3-OH group of quercetin is the one more prone to suffer H atom abstraction in the water phase, in contrast with the gas phase DFT results that point out the 4' as the one most prone to yield $H^{\bullet}$.

In both flavonoids, the 3-OH H atom is the first one to be abstracted after the B ring H atoms have been removed, explaining experimental results that suggest that the C ring hydroxyl group, when present, potentiates the antioxidant activity that is mainly determined by the B ring OH groups and the C2-C3 double bond [59-62].

The sequential protol-loss electron-transfer (SPLET) mechanism

The SPLET mechanism is primarily governed by the ease of deprotonation, which can be described by the PA values, and secondarily by the ease of electron transfer from the anions, described by the ETE. Concerning the deprotonation step, which, as expected, is more favorable in water than in the gas phase, the PA values presented in Tables 6 and 7 indicate that myricetin deprotonates slightly easier than quercetin. For quercetin, the most acidic proton is the 7-OH one, followed by the 4' one, which is in agreement with the majority of the DFT data available that show that these are two most acidic protons. In the case of myricetin, the PM6 results indicate that the 7-OH proton is also the most acidic one, followed by the 3-OH one in the gas phase and the 5-OH one in the water phase, in clear disagreement with the DFT results indicate that the 4' is the most acidic one followed by the 7 and 3' ones. However, the results of Martins *et al*. [56] indicate that, in solution, flavones deprotonation occurs primarily from the 7-OH group, in agreement with our PM6 data. After deprotonation, the anions may proceed to form the corresponding radicals by electron transfer (measurable by ETE). The ETE values for the flavonoids are in the same order of magnitude, and follow the same trend for both flavonoids.

Considering the set of these results, the SPLET mechanism is expected to be more relevant for myricetin than for quercetin. More, taking into account that the more acidic protons are, in general, the same atoms that are more likely to be abstracted as $H^{\bullet}$, it is expectable that, in aqueous solutions, as are most of the biological environments that surround flavonoids in organisms, the SPLET mechanism will prevail over the HAT one.

The sequential electron-transfer proton-transfer (SETPT) mechanism

Electron removal from the neutral flavonoids, leading to the formation of the radical cations, is the first step of the SETPT mechanism. As it can be seen from the ionization potentials, this process is slightly more favorable for quercetin than for myricetin.

Contrarily to deprotonation, which occurs spontaneously in water, ionization requires an electron acceptor, thus being more likely to occur in the presence of such acceptors, as are proteins, or, in the case of flavonoid-solvent systems, in the presence of polar solvents, preferentially those able to establish H bonds with the flavonoid molecules, thus further stabilizing the radical cation [21].

The radical cations of both flavonoids undergo a favorable deprotonation in aqueous solution, and the protons involved in these deprotonation are the same mentioned in the above analyzed mechanisms – in myricetin, deprotonation of the radical cation occurs primarily from the central 4'-OH group in the B ring, followed by the neighboring two OH groups, and in quercetin it occurs from the 4'-OH and the 3'-OH groups.

Conclusion

In this work we have reported the results of a semi-empirical study of two flavonoids, quercetin and myricetin. Five different semi-empirical methods were employed, and the results obtained with the PM6 method are, in general, in good agreement with other results published using the density functional theory, thus validating the use of this method to study these compounds.

The PM6 calculations led to nearly planar structures of all the analyzed species, either neutral, radicals or anions, in agreement with results from other authors. Quite noticeably, the PM6 method is capable of reproducing the charge delocalization and the resonance characteristics of the flavonoids obtained by other authors using a DFT approach. The ionization potentials and the electron affinities computed using the PM6 results, as well as the other computed molecular properties (electronegativity, HOMO-LUMO gap, hardness, electrophilicity and permanent dipole moment) show trends that accompany the ones observed with DFT derived data. However, the PM6 values quite often differ from the DFT ones by more than 50 %; nevertheless, those differences became smaller when one uses the energies of the species involved instead of the energies of the orbitals.

The sites of H atom abstraction and the deprotonation orders are also in general agreement with the DFT data. In the case of quercetin, H atom abstraction is expected to occur from the 3' and 4' sites, with a possible contribution in the gas phase from the 3-OH group, which are the same three sites predicted to be more important by the DFT results. This is accompanied by the trends in the proton affinities (PA) and bond dissociation enthalpies (BDE), which are in general agreement with the trends observed for the same values obtained with the DFT procedures. Noticeably, the PM6-derived PA values are lower than the DFT ones while the PM6 BDE values are higher than the DFT ones, which indicates that the semi-empirical methods tend to overestimate the energetics of the anions and underestimate the energetic of the radicals when compared with the DFT methods. These results indicate that the semi-empirical PM6 method can be used for, at least, a semi-quantitative approach to the energetic and molecular properties of these compounds.

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Table 1 Heats of formation (in kJ mol^{-1}) of quercetin (Q) and myricetin (M) and their derived radicals and anions obtained with the PM6 model. $\Delta_{solv}H_f$ values were calculated as $H_f(X_w)$-$H_f(X_g)$

	H_fQ (g)	**H_fM (g)**
F-OH	-975.55	-1152.72
3-O$^-$	-1184.55	-1377.35
5-O$^-$	-1177.00	-1362.39
7-O$^-$	-1216.78	-1401.10
3’-O$^-$	-1129.74	-1361.34
4’-O$^-$	-1212.07	-1364.81
5’-O$^-$	–	-1321.53
F-OH$^{\bullet+}$	-191.37	-362.05
3-O$^\bullet$	-840.13	-1014.73
5-O$^\bullet$	-748.52	-926.37
7-O$^\bullet$	-768.88	-944.49
3’-O$^\bullet$	-834.88	-1026.54
4’-O$^\bullet$	-856.93	-1025.04
5’-O$^\bullet$	–	-1014.32
	H_fQ (w)	**H_fM (w)**
F-OH	-1052.69	-1235.44
3-O$^-$	-1464.84	-1659.08
5-O$^-$	-1452.84	-1662.03
7-O$^-$	-1486.41	-1664.08
3’-O$^-$	-1448.18	-1650.27
4’-O$^-$	-1468.29	-1642.41
5’-O$^-$	–	-1641.17
F-OH$^{\bullet+}$	-431.44	-611.14
3-O$^\bullet$	-924.67	-1104.30
5-O$^\bullet$	-852.88	-1022.98
7-O$^\bullet$	-865.09	-1028.66
3’-O$^\bullet$	-924.98	-1106.94
4’-O$^\bullet$	-928.46	-1100.94
5’-O$^\bullet$	–	-1111.62
	$\Delta_{solv}H_f$Q	**$\Delta_{solv}H_f$M**
F-OH	-77.14	-82.72
3-O$^-$	-280.29	-281.73
5-O$^-$	-275.84	-299.64
7-O$^-$	-269.63	-262.97
3’-O$^-$	-318.43	-288.93
4’-O$^-$	-256.22	-277.61
5’-O$^-$	–	-319.64
F-OH$^{\bullet+}$	-240.06	-249.09
3-O$^\bullet$	-84.54	-89.57
5-O$^\bullet$	-104.36	-96.62
7-O$^\bullet$	-96.21	-84.18
3’-O$^\bullet$	-90.10	-80.40
4’-O$^\bullet$	-71.53	-75.90
5’-O$^\bullet$	–	-97.30

Table 2 Computed properties for the quercetin (Q) and myricetin (M) neutral molecules in the gas and water phases obtained with the PM6 model; IP – ionization potential (in kJ mol^{-1}); EA – electron affinity (in kJ mol^{-1}); χ – electronegativity; ε_G – energy gap between the highest occupied (HOMO) and lowest unoccupied (LUMO) molecular orbitals (in eV); η – molecular (or Parr and Pople absolute) hardness (in eV) ; ω – molecular electrophilicity (in eV); μ_0 – permanent dipole moment (in Debye). Where available, literature data are included for comparison. The *E* and *O* indexes refer to data computed using an energy approach or an orbital approach, respectively. A – data taken from reference 54, obtained at the B3LYP/6-31G(d, p) level; B – data taken from reference 37, using the CHIHDFT method with a CBSB4 basis set.

	IP_O	IP_E	EA_O	EA_E	χ_O	χ_E	$\varepsilon_{G,O}$	$\varepsilon_{G,E}$	η_O	η_E	ω_O	ω_E	μ_0
M (g)	854.11	790.67	140.58	69.11	5.15	4.46	7.40 (3.74 ^{A})	7.47	3.70	3.74	0.90	0.66	3.97 (1.70 ^{A})
M (w)	872.83	624.30	157.28	137.25	5.34	3.95	7.42	5.05	3.71	2.52	0.96	0.77	6.35
Q (g)	848.90	784.17	129.39	165.86	5.07	4.92	7.46 (3.75 ^{A})	6.41	3.73	3.20	0.86	0.95	4.02 (2.41 ^{A})
Q (w)	863.95 (549.03 ^{B})	621.25 (696.66 ^{B})	147.82 (73.33 ^{B})	396.98 (222.89 ^{B})	5.24 (4.00 ^{B})	5.28 (3.99 ^{B})	7.42	2.32	3.71 (3.23 ^{B})	1.16 (1.69 ^{B})	0.93 (2.47 ^{B})	2.99 (4.72 ^{B})	6.56

Table 3 Nucleophilic – Dn(*r*) – and electrophilic – Dn(*r*) – delocalizabilities of the neutral quercetin (Q) and myricetin (M) molecules in the gas and water phases obtained with the PM6 model

	M (g)		**M (w)**		**Q (g)**		**Q (w)**	
	Dn(*r*)	**De(*r*)**	**Dn(*r*)**	**De(*r*)**	**Dn(*r*)**	**De(*r*)**	**Dn(*r*)**	De(*r*)
O1	-0.27	-0.52	-0.26	-0.53	-0.26	-0.52	-0.26	-0.52
C2	-0.63	-0.36	-0.62	-0.36	-0.63	-0.35	-0.64	-0.34
C3	-0.58	-0.39	-0.57	-0.39	-0.57	-0.39	-0.56	-0.40
O3	-0.21	-0.60	-0.20	-0.60	-0.21	-0.60	-0.20	-0.60
C4	-0.67	-0.25	-0.66	-0.25	-0.66	-0.25	-0.67	-0.24
O4	-0.21	-0.64	-0.20	-0.65	-0.21	-0.64	-0.20	-0.64
C4/C5	-0.44	-0.48	-0.43	-0.49	-0.44	-0.48	-0.44	-0.48
C5	-0.66	-0.26	-0.65	-0.27	-0.66	-0.26	-0.66	-0.26
O5	-0.21	-0.56	-0.20	-0.57	-0.21	-0.56	-0.20	-0.56
C6	-0.41	-0.50	-0.40	-0.51	-0.41	-0.50	-0.40	-0.51
C7	-0.66	-0.27	-0.64	-0.27	-0.66	-0.27	-0.64	-0.27
O7	-0.21	-0.55	-0.20	-0.57	-0.21	-0.55	-0.20	-0.56
C8	-0.42	-0.49	-0.41	-0.50	-0.42	-0.49	-0.41	-0.50
C8/O1	-0.67	-0.28	-0.66	-0.28	-0.67	-0.28	-0.67	-0.28
C1'	-0.54	-0.38	-0.54	-0.38	-0.51	-0.40	-0.51	-0.41
C2'	-0.46	-0.47	-0.46	-0.47	-0.48	-0.44	-0.48	-0.43
C3'	-0.58	-0.34	-0.58	-0.34	-0.56	-0.36	-0.56	-0.36
O3'	-0.20	-0.60	-0.19	-0.60	-0.19	-0.61	-0.19	-0.61
C4'	-0.55	-0.41	-0.54	-0.41	-0.58	-0.37	-0.58	-0.36
O4'	-0.19	-0.61	-0.19	-0.62	-0.20	-0.58	-0.19	-0.60
C5'	-0.58	-0.34	-0.58	-0.34	-0.47	-0.43	-0.46	-0.44
O5'	-0.20	-0.58	-0.19	-0.60	-	-	-	-
C6'	-0.46	-0.47	-0.45	-0.47	-0.49	-0.43	-0.49	-0.42

Table 4 Sum of atomic charges for rings A (Σq_A), B (Σq_B) and C (Σq_C) and for the ring groups AC (Σq_{AC}) and BC (Σq_{BC}) computed for neutral quercetin and myricetin molecules in the gas and water phases using the PM6 model

		Myricetin					**Quercetin**				
		Σq_A	Σq_B	Σq_C	Σq_{AC}	Σq_{BC}	Σq_A	Σq_B	Σq_C	Σq_{AC}	Σq_{BC}
Gas phase	**F-OH**	0,18	0,09	-0,27	-0,09	-0,18	0,18	0,09	-0,27	-0,09	-0,18
	3-O⁻	-0,06	-0,10	-0,84	-0,90	-0,94	-0,06	-0,08	-0,86	-0,92	-0,94
	5-O⁻	-0,58	-0,01	-0,42	-1,00	-0,43	-1,14	-0,21	0,36	-0,78	0,15
	7-O⁻	-0,50	0,01	-0,51	-1,01	-0,50	-0,51	0,02	-0,51	-1,02	-0,49
	3'-O⁻	0,08	-0,78	-0,31	-0,22	-1,08	0,06	-0,75	-0,31	-0,25	-1,06
	4'-O⁻	0,02	-0,60	-0,42	-0,40	-1,02	0,03	-0,62	-0,41	-0,38	-1,03
	5'-O⁻	0,07	-0,77	-0,30	-0,23	-1,07					
	F-OH•+	0,36	0,67	-0,03	0,33	0,64	0,39	0,38	0,03	0,43	0,42
	3-O•	0,19	0,17	-0,36	-0,17	-0,19	0,18	0,18	-0,36	-0,18	-0,18
	5-O•	0,08	0,09	-0,17	-0,09	-0,08	-0,38	-0,11	0,49	0,11	0,38
	7-O•	0,08	0,11	-0,19	-0,11	-0,08	0,07	0,12	-0,19	-0,12	-0,07
	3'-O•	0,20	0,07	-0,27	-0,07	-0,20	0,19	0,08	-0,27	-0,08	-0,19
	4'-O•	0,19	0,08	-0,27	-0,08	-0,19	0,19	0,07	-0,27	-0,07	-0,19
	5'-O•	0,19	0,08	-0,27	-0,08	-0,19					
Water phase	**F-OH**	0,22	0,10	-0,32	-0,10	-0,22	0,20	0,09	-0,33	-0,13	-0,24
	5'-O⁻	0,10	-0,03	-1,07	-0,97	-1,10	0,09	0,00	-1,09	-1,00	-1,09
	4'-O⁻	-0,63	0,06	-0,43	-1,06	-0,37	-0,63	0,07	-0,45	-1,07	-0,37
	3'-O⁻	-0,64	0,07	-0,43	-1,07	-0,36	-0,65	0,08	-0,43	-1,08	-0,35
	7-O⁻	0,20	-0,87	-0,33	-0,13	-1,20	0,20	-0,86	-0,34	-0,14	-1,20
	5-O⁻	0,19	-0,83	-0,35	-0,17	-1,19	0,18	-0,81	-0,37	-0,19	-1,18
	3-O⁻	0,20	-0,87	-0,33	-0,13	-1,20					
	F-OH•+	0,24	0,95	-0,20	0,05	0,76	0,25	0,96	-0,20	0,05	0,76
	5'-O•						0,23	0,27	-0,50	-0,27	-0,23
	4'-O•						-0,51	0,61	-0,10	-0,61	0,51
	3'-O•						-0,60	0,92	-0,32	-0,92	0,60
	7-O•						0,22	0,07	-0,28	-0,07	-0,22
	5-O•						0,22	0,06	-0,28	-0,06	-0,22
	3-O•										

Table 5 Sum of spin densities for rings A (Σs_A), B (Σs_B) and C (Σs_C) and for the ring groups AC (Σs_{AC}) and BC (Σs_{BC}) computed for neutral quercetin and myricetin molecules in the gas and water phases using the PM6 model

		Myricetin					**Quercetin**				
		Σs_A	Σs_B	Σs_C	Σs_{AC}	Σs_{BC}	Σs_A	Σs_B	Σs_C	Σs_{AC}	Σs_{BC}
Gas phase	**F-OH$^{\bullet+}$**	0,03	0,65	0,33	0,35	0,97	0,04	0,51	0,45	0,49	0,96
	3-O$^{\bullet}$	0,05	0,30	0,65	0,70	0,95	0,04	0,30	0,65	0,70	0,96
	5-O$^{\bullet}$	0,86	0,01	0,13	0,99	0,14	0,86	0,01	0,13	0,99	0,14
	7-O$^{\bullet}$	0,97	0,00	0,03	1,00	0,03	0,97	0,00	0,03	1,00	0,03
	3'-O$^{\bullet}$	0,00	0,98	0,02	0,02	1,00	0,00	0,98	0,01	0,02	1,00
	4'-O$^{\bullet}$	0,00	0,88	0,12	0,12	1,00	0,00	0,98	0,02	0,02	1,00
	5'-O$^{\bullet}$	0,00	0,99	0,01	0,01	1,00					
Water phase	**F-OH$^{\bullet+}$**	0,00	0,89	0,10	0,11	1,00	0,00	0,89	0,11	0,11	1,00
	5'-O$^{\bullet}$	0,05	0,29	0,66	0,71	0,95	0,04	0,34	0,62	0,66	0,96
	4'-O$^{\bullet}$	0,08	0,28	0,64	0,72	0,92	0,03	0,56	0,41	0,44	0,97
	3'-O$^{\bullet}$	0,08	0,27	0,65	0,73	0,92	0,01	0,86	0,13	0,14	0,99
	7-O$^{\bullet}$	0,00	0,97	0,03	0,03	1,00	0,00	0,97	0,03	0,03	1,00
	5-O$^{\bullet}$	0,00	0,89	0,11	0,11	1,00	0,00	0,97	0,03	0,03	1,00
	3-O$^{\bullet}$	0,00	0,98	0,02	0,02	1,00					

Table 6 Reaction energies (computed as differences of heats of formation) for the reactions involved in the various mechanisms of antioxidant activity of myricetin (M) in the gas and water phases. IP – ionization potential; PDE – proton dissociation enthalpy; PA – proton affinity; ETE – electron transfer enthalpy; BDE – bond dissociation enthalpy. $\Delta_{ac}H$ – acidity. All values are in kJ mol^{-1}. Where available, literature data are included for comparison

		IP	PDE	PA	$\Delta_{ac}H$ [A]	$\Delta_{ac}H$ [B]	$\Delta_{ac}H$ [C]	ETE	BDE	BDE [D]
M(g)	**3**	790.67	877.49	1305.54	*1397.46*	*1425.91*	*1400.80*	362.62	355.98	*355.64*
	5		965.85	1320.51	*1415.45*	*1455.61*	*1425.91*	436.02	444.34	*416.73*
	7		947.73	1281.79	*1352.69*	*1396.62*	*1369.42*	456.62	426.22	*391.20*
	3'		865.68	1321.55	*1352.27*	*1398.71*	*1366.08*	334.80	344.17	*303.34*
	4'		867.18	1318.09	*1307.50*	*1344.74*	*1317.12*	339.76	345.67	*308.78*
	5'		877.90	1361.37	*1356.45*	*1401.64*	*1367.75*	307.20	356.39	*351.46*
M (w)	**3**	624.30	1037.01	1106.53	*1415.45*			554.78	349.13	
	5		1118.33	1103.59	*1264.82*			639.04	430.44	
	7		1112.65	1101.54	*1240.97*			635.41	424.76	
	3'		1034.37	1115.34	*1248.51*			543.33	346.49	
	4'		1040.37	1123.20	*1224.24*			541.47	352.48	
	5'		1029.69	1124.44	*1249.34*			529.55	341.81	

A – acidity data taken from reference 41, obtained with a B3LYP method and a 6-311++G** basis set; B – data taken from reference 56, obtained at the B3LYP/6-31G(d) level; C – data taken from reference 56, obtained at the B3LYP/6-311G(2d,p) level; D – data taken from reference 61, obtained with a ONIOM-G3B3 method.

Table 7 Reaction energies (computed as differences of heats of formation) for the reactions involved in the various mechanisms of antioxidant activity of quercetin (Q) in the gas and water phases. IP – ionization potential; PDE – proton dissociation enthalpy; PA – proton affinity; ETE – electron transfer enthalpy; BDE – bond dissociation enthalpy. $\Delta_{ac}H$ – acidity. All values are in kJ mol^{-1}. Where available, literature data are included for comparison

		IP	PDE	PA	$\Delta_r H$ ($\Delta_r G$) ^{A}	$\Delta_{ac}H$ ^{B}	$\Delta_{ac}H$ ^{C}	$\Delta_{ac}H$ ^{D}	ETE	BDE	$\Delta_r H$ ($\Delta_r G$) ^{A}	BDE ^{E}	BDE ^{F}
	3		881.41	1321.17	*1385.74 (1387.41)*	*1393.69*	*1432.18*	*1410.01*	344.42	353.40	*322.59 (287.86)*	*357.73*	*350.20 (333.46)*
	5		973.02	1328.71	*1414.19 (1412.52)*	*1411.68*	*1458.96*	*1437.20*	428.48	445.01	*384.93 (247.27)*	*417.98*	*415.47 (396.22)*
Q (g)	**7**	784.17 (682.41[33])	952.66	1288.94	*1349.76 (1351.01)*	*1349.34*	*1392.44*	*1373.19*	447.90	424.65	*347.27 (311.71)*	*389.95*	*370.70 (353.13)*
	3'		886.66	1375.97	*1357.29 (1368.17)*	*1352.69*	*1398.29*	*1366.49*	294.86	358.65	*303.34 (269.03)*	*342.25*	*322.17 (307.94)*
	4'		864.61	1293.64	*1328.42 (1331.35)*	*1324.24*	*1359.80*	*1336.37*	355.14	336.60	*292.88 (259.83)*	*329.28*	*312.13 (297.48)*
	3		1036.94	1118.02	*1213.78 (1206.25)*	*1254.36*			540.17	346.01	*322.59 (276.98)*		
	5		1108.72	1130.02	*1222.98 (1222.56)*	*1264.82*			599.96	340.65	*366.94 (330.12)*		
Q (w)	**7**	621.25 (696.66[36])	1096.52	1096.45	*1199.55 (1201.65)*	*1240.97*			621.32	328.45	*365.68 (323.00)*		
	3'		1036.62	1134.68	*1212.52 (1209.18)*	*1248.09*			523.19	268.55	*332.21 (289.53)*		
	4'		1033.15	1114.57	*1197.88 (1194.53)*	*1254.78*			539.83	265.07	*319.24 (286.19)*		

A – data taken from reference 33, obtained with a B3LYP method and a 6-31G* basis set for C and H atoms and a 6-31+G* basis set for O atoms; B – acidity data taken from reference 41, obtained with a B3LYP method and a 6-311++G** basis set; C – data taken from reference 56, obtained at the B3LYP/6-31G(d) level; D – data taken from reference 56, obtained at the B3LYP/6-311G(2d,p) level; E – data taken from reference 61, obtained with a ONIOM-G3B3 method; F – data taken from reference 62 using the B3P86 and the B3LYP methods with a 6-311+G(d,p) basis set.

Table 8 Order of H atom abstraction and deprotonation of hydroxylic H atoms of quercetin (Q) and myricetin (M) in the gas and water phases obtained with the PM6 model. Data from the literature is also included for comparison

		Method	**Order**	**Ref.**
H atom abstraction	**Q (g)**	UHF/6-31G*	3'-4'-3-5-7	36
		AM1	3-4'-3'-7-5	37
		B3LYP/6-311++G(3df,2p)	4'-3'-3-7-5	40
		B3LYP/6-31+G*	4'-3'-3-7-5	41
		PM6	4'-3-3'-7-5	a)
	Q (w)	B3LYP/6-311++G(3df,2p)	3-4'-3'-…	40
		B3LYP/6-31+G*	3-4'-3'-7-5	41
		PM6	4'-3'-7-5-3	a)
	M (g)	PM6	3'-4'-3-5'-7-5	a)
	M (w)	PM6	5'-3'-3-4'-7-5	a)
Deprotonation	**Q (g)**	B3LYP/6-311+G(3df,2p)	4'-7-3'-3-5	39
		B3LYP/6-311+G(d,p)	4'-3'-7-3-5	42
		MP2/6-311++G(d,p)	4'-7-3-3'-5	43
		B3LYP/6-31+G*	4'-7-3'-3-5	41
		PM6	7-4'-3-5-3'	a)
	Q (w)	B3LYP/6-311++G(3df,2p)	7-3'-3-4'-5	39
		B3LYP/6-31+G*	4'-7-3-3'-5	41
		PM6	7-4'-3-5-3'	a)
	M (g)	B3LYP/6-311+G(3df,2p)	4'-3'-7-5'-3-5	39
		B3LYP/6-311+G(d,p)	4'-7-3'-5'-3-5	42
		PM6	7-3-4'-5-3'-5'	a)
	M (w)	B3LYP/6-311+G(3df,2p)	4'-7-3'-5'-3-5	39
		PM6	7-5-3-3'-4'-5'	a)

a) This work.

Figure captions

Fig. 1 Basic structure of quercetin and myricetin. The ring naming and atom numbering are shown

Fig. 2 Scheme of the analyzed mechanisms of antioxidant activity. SETPT – sequential electron transfer – proton transfer; HAT – H atom abstraction; SPLET – sequential proton loss – electron transfer. IP – ionization potential; PDE – proton dissociation enthalpy; BDE – bond dissociation enthalpy; PA – proton affinity; ETE – electron transfer enthalpy

Fig. 3 Optimized gas phase and water phase structures of myricetin using the semi-empirical PM6 method with superimposed HOMO and LUMO molecular orbitals

Fig. 4 Comparison of the proton affinities (PA) and bond dissociation enthalpies (BDE) values of quercetin and myricetin computed with the PM6 method and the corresponding DFT values retrieved from the literature. **1**) PA values of myricetin in the gas phase; **2**) BDE values of myricetin in the gas phase; **3**) PA values of myricetin in the water phase; **4**) PA values of quercetin in the gas phase; **5**) BDE values of quercetin in the gas phase; **6**) PA values of quercetin in the water phase; **7**) BDE values of quercetin in the water phase.

Legends: ○PM6: values computed with the PM6 method (this work); **A**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-311++G** level [41]; **B**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-31G(d) level [56]; **C**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-311G(2d,p) level [56]; **D**: BDE values obtained with a ONIOM/G3B3 method [61]; **E**: acidity values (expressed as enthalpy – blank squares – or Gibbs energy – blank triangles - changes) at the B3LYP/6-31G* level for C and H atoms and B3LYP/6-31+G* level for O atoms [33]; **F**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-311++G** [41]; **G**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-31G(d) [56]; **H**: acidity values ($\Delta_{ac}H$) at the B3LYP/6-311G(2d,p) [56]; **I**: BDE values obtained with a ONIOM/G3B3 method [61]; **J**: BDE values (expressed as enthalpy – circle – or Gibbs energy – rectangle - changes) using the B3P86 and the B3LYP methods with a 6-311+G(d,p) basis set [62]

Figure 1
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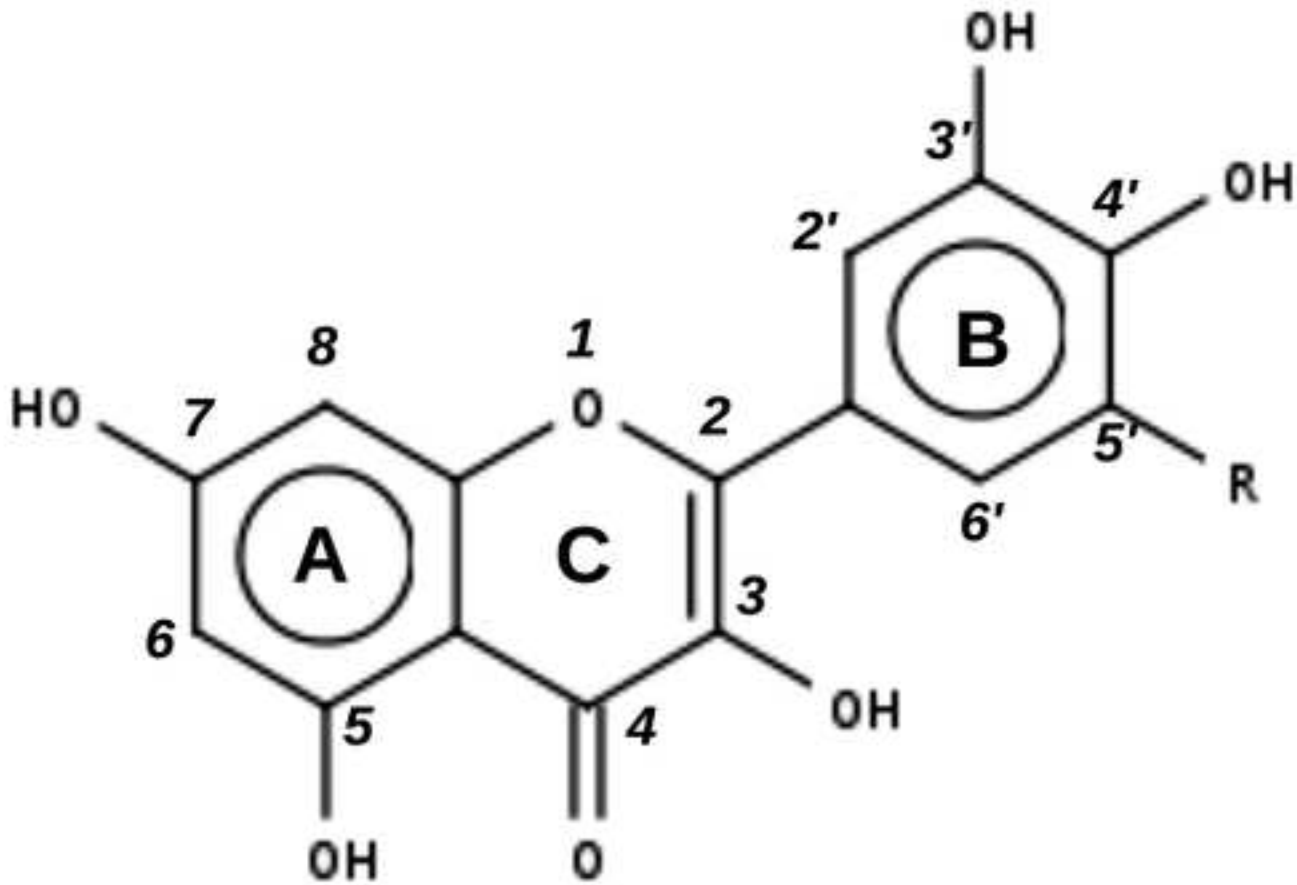

Quercetin: R=H, Myricetin: R=OH

Figure 2

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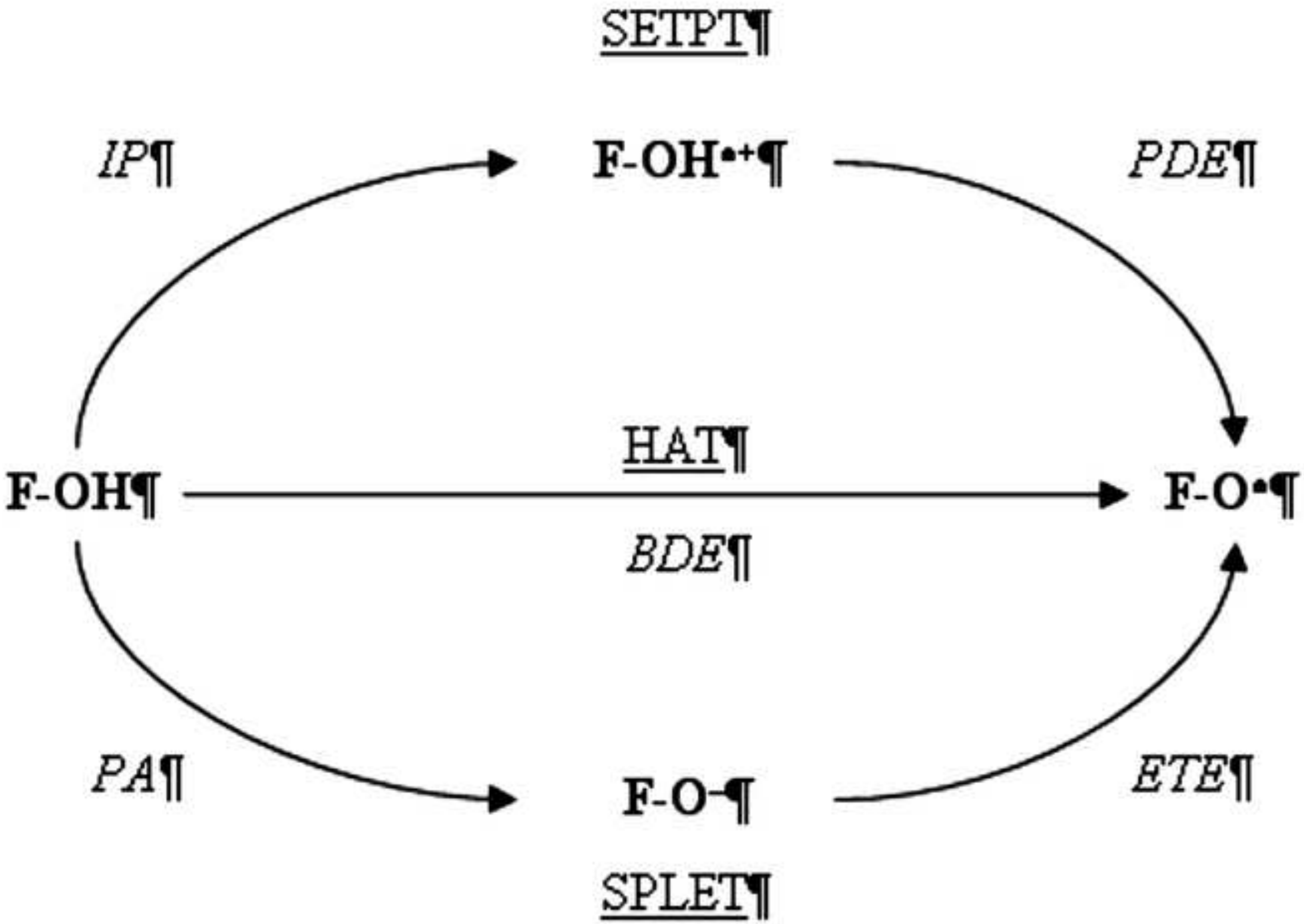

Figure 3

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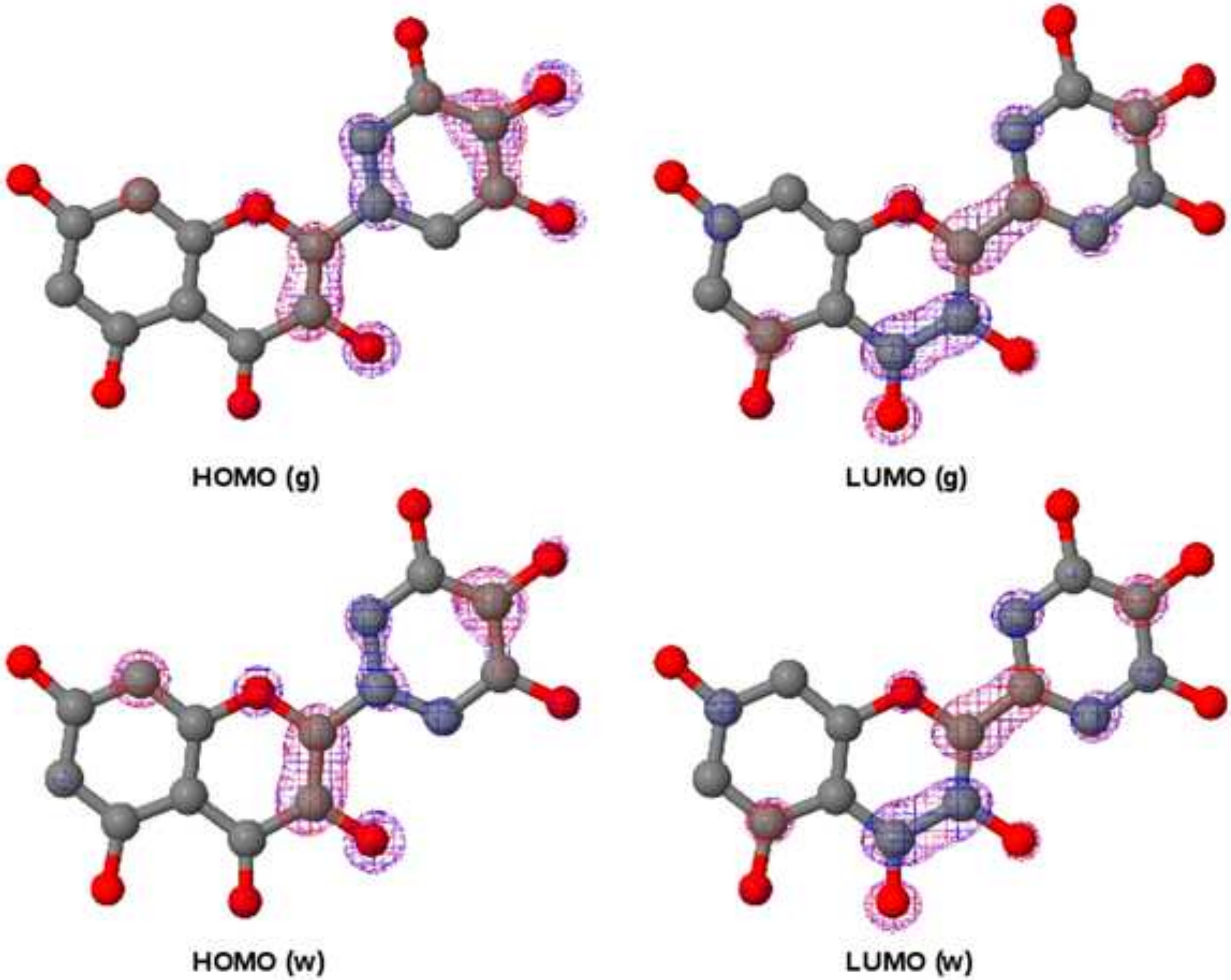

Figure 4

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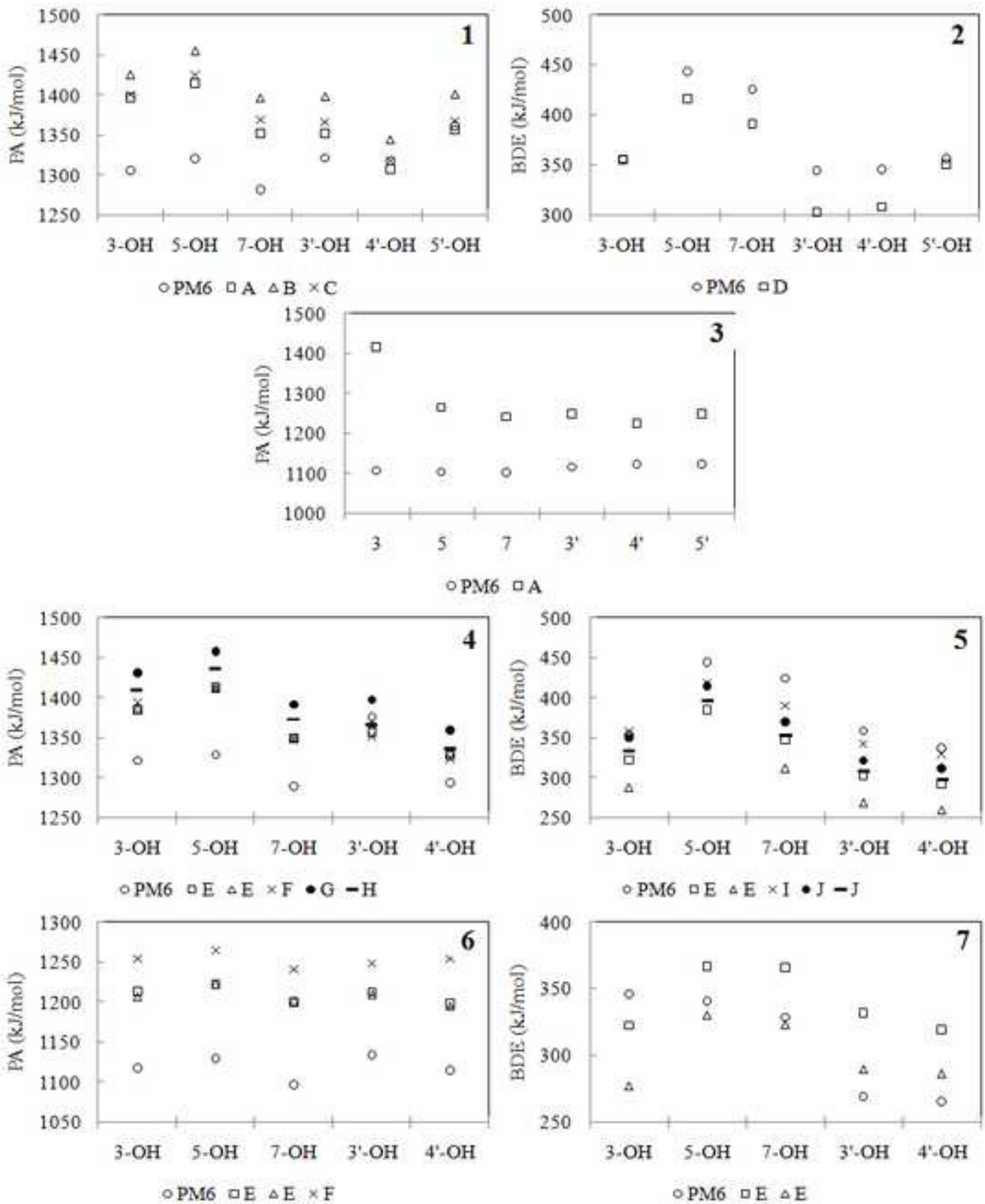

Supplementary Material

Antioxidant Mechanisms of Quercetin and Myricetin in the Gas Phase and in Solution – a comparison and validation of semi-empirical methods

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Table S1. Heats of formation (in kJ/mol) of quercetin (Q) and derived radicals and anions obtained with the various semi-empirical methods employed.

Table S2. Heats of formation (in kJ/mol) of myricetin (M) and derived radicals and anions obtained with the various semi-empirical methods employed.

Table S3. Dihedral angles (in degrees) of quercetin (Q) and myricetin (M) obtained with the semi-empirical methods employed.

Table S1. Heats of formation (in kJ/mol) of quercetin (Q) and derived radicals and anions obtained with the various semi-empirical methods employed. Columns to the right of each method (marked with #) present the ordering of H atom abstraction and of deprotonation. $\Delta_{solv}H_f$ values were calculated as $H_f(X_w)$-$H_f(X_g)$.

		MNDO	#	AM1	#	RM1	#	PM3	#
H_fQ (g)	Neutral	-735.57		-847.56		-845.8		-910.21	
	4’-O^-	-1071.55	1	-1165.5	1	-1126	1	-1216.12	1
	3’-O^-	-1029.87	2	-1144.91	2	-1084.28	2	-1173.32	4
	7-O^-	-939.35	4	-1025.1	4	-1055.43	4	-1178.15	3
	5-O^-	-910.54	5	-1016.87	5	-1024.47	5	-1145.43	5
	3-O^-	-951.95	3	-1027.33	3	-1076.38	3	-1190.47	2
	$Q^{\bullet+}$	114.46		5.1		5.5		-156.86	
	4’-$O^\bullet$	-758.44	1	-852.8	1	-815.65	1	-857.08	1
	3’-$O^\bullet$	-757.54	2	-852.78	2	-814.32	2	-855.47	2
	7-$O^\bullet$	-540.44	4	-659.23	4	-663.39	4	-693.84	4
	5-$O^\bullet$	-538.8	5	-637.26	5	-658.18	5	-689.61	5
	3-$O^\bullet$	-606.31	3	-700.96	3	-737.38	3	-778.08	3
		MNDO	**#**	**AM1**	**#**	**RM1**	**#**	**PM3**	**#**
H_fQ (w)	Neutral	-792.8		-917.87		-900.64		-986.47	
	4’-O^-	-1309.56	1	-1421.63	1	-1357.97	1	-1468.89	1
	3’-O^-	-1294.24	2	-1408.53	2	-1342.3	2	-1455.82	2
	7-O^-	-1166.55	4	-1264.23	4	-1282.99	4	-1421.77	4
	5-O^-	-1160.18	5	-1275.06	3	-1268.19	5	-1414.58	5
	3-O^-	-1172.29	3	-1255.5	5	-1291.56	3	-1428.2	3
	$Q^{\bullet+}$	-138.85		-292.05		-231.58		-318.85	
	4’-$O^\bullet$	-810.85	1	-908.64	2	-864.47	1	-926.03	1
	3’-$O^\bullet$	-810.46	2	-954.65	1	-863.67	2	-925.48	2
	7-$O^\bullet$	-597.93	5	-746.25	5	-724.71	5	-769.55	5
	5-$O^\bullet$	-615.43	4	-765.73	4	-733.61	4	-788.12	4
	3-$O^\bullet$	-666.62	3	-806.19	3	-797.05	3	-856.24	3
		MNDO		**AM1**		**RM1**		**PM3**	
$\Delta_{solv}H_fQ$	Neutral	-57.23		-70.31		-54.84		-76.26	
	4’-O^-	-238.02		-256.13		-231.97		-252.76	
	3’-O^-	-264.36		-263.62		-258.03		-282.5	
	7-O^-	-227.2		-239.13		-227.56		-243.62	
	5-O^-	-249.64		-258.19		-243.72		-269.15	
	3-O^-	-220.34		-228.17		-215.19		-237.72	
	$Q^{\bullet+}$	-253.3		-297.14		-237.08		-161.99	
	4’-$O^\bullet$	-52.4		-55.84		-48.82		-68.95	
	3’-$O^\bullet$	-52.92		-101.87		-49.36		-70	
	7-$O^\bullet$	-57.49		-87.02		-61.32		-75.72	
	5-$O^\bullet$	-76.63		-128.48		-75.43		-98.51	
	3-$O^\bullet$	-60.31		-105.23		-59.67		-78.16	

Table S2. Heats of formation (in kJ/mol) of myricetin (M) and derived radicals and anions obtained with the various semi-empirical methods employed. Columns to the right of each method (marked with #) present the ordering of H atom abstraction and of deprotonation. $\Delta_{solv}H_f$ values were calculated as $H_f(X_w)$-$H_f(X_g)$.

		MNDO	**#**	**AM1**	**#**	**RM1**	**#**	**PM3**	**#**
H_fM (g)	Neutral	-1060.27		-1046.7		-1031.56		-1135.22	
	5'-O^{-}	-1112.31	5	-1241.45	4	-1243.89	3	-1359.98	5
	4'-O^{-}	-1260.98	1	-1340.15	2	-1313.89	1	-1388.94	1
	3'-O^{-}	-1225.25	2	-1298.31	3	-1239.38	5	-1361.35	4
	7-O^{-}	-1128.33	4	-1220.68	5	-1240.23	4	-1385.79	2
	5-O^{-}	-1098.8	6	-1172.76	6	-1208.75	6	-1324.44	6
	3-O^{-}	-1139.76	3	-1364.78	1	-1261.58	2	-1372.39	3
	M$^{\bullet+}$	21.95		42.02		8.28		-333.69	
	5'-O$^{\bullet}$	-682.5	4	-831.61	3	-760.25	4	-731.81	5
	4'-O$^{\bullet}$	-950.58	1	-1045.89	1	-1009.37	1	-1041.04	1
	3'-O$^{\bullet}$	-681.19	5	-831.73	2	-757.54	5	-764.76	4
	7-O$^{\bullet}$	-882.29	2	-567.26	6	-853.9	2	-933.41	2
	5-O$^{\bullet}$	-878.14	3	-574.26	5	-845.91	3	-924.17	3
	3-O$^{\bullet}$	-673.87	6	-787.77	4	-748.69	6	-686.45	6
		MNDO	**#**	**AM1**	**#**	**RM1**	**#**	**PM3**	**#**
H_fM (w)	Neutral	-1137.25		-808.95		-1090.09		-1234.87	
	5'-O^{-}	-1103.35	6	-1619.35	2	-1172.98	4	-1650.86	5
	4'-O^{-}	-1497.14	3	-1629.11	1	-1545.83	1	-1646.46	6
	3'-O^{-}	-1323.78	4	-1532.6	4	-1371.19	3	-1651.3	4
	7-O^{-}	-1508.2	1	-1137.43	6	-1472.28	2	-1669.31	1
	5-O^{-}	-1502.9	2	-1158.69	5	-998.85	6	-1661.56	2
	3-O^{-}	-1108.21	5	-1612.95	3	-1166.23	5	-1660.11	3
	M$^{\bullet+}$	-229.25		-647.01		-544.02		-597.06	
	5'-O$^{\bullet}$	-995.37	3	-1108.83	3	-1047.37	3	-1109.96	2
	4'-O$^{\bullet}$	-1006.36	2	-1108.83	2	-1061.73	2	-1115.98	1
	3'-O$^{\bullet}$	-992.37	4	-1105.57	4	-1045.9	4	-1107.69	3
	7-O$^{\bullet}$	-939.22	6	-1047.4	5	-996.72	6	-1018.17	5
	5-O$^{\bullet}$	-957.07	5	-1134.74	1	-1005.07	5	-1007.22	6
	3-O$^{\bullet}$	-1009.98	1	-854.79	6	-1066.05	1	-1102.69	4
		MNDO		**AM1**		**RM1**		**PM3**	
$\Delta_{solv}H_fM$	Neutral	-76.97		237.75		-58.52		-99.65	
	4'-O^{-}	8.96		-377.9		70.9		-290.87	
	3'-O^{-}	-236.16		-288.95		-231.94		-257.52	
	7-O^{-}	-98.52		-234.29		-131.81		-289.95	
	5-O^{-}	-379.87		83.25		-232.05		-283.51	
	3-O^{-}	-404.1		14.07		209.9		-337.11	
	Q$^{\bullet+}$	31.55		-248.17		95.36		-287.72	
	4'-O$^{\bullet}$	-251.2		-689.03		-552.31		-263.37	
	3'-O$^{\bullet}$	-312.87		-277.21		-287.12		-378.16	
	7-O$^{\bullet}$	-55.79		-62.94		-52.36		-74.94	
	5-O$^{\bullet}$	-311.17		-273.83		-288.37		-342.93	
	3-O$^{\bullet}$	-56.93		-480.15		-142.82		-84.76	

Table S3. Dihedral angles (in degrees) of quercetin (Q) and myricetin (M) obtained with the semi-empirical methods employed.

		Myricetin				Quercetin			
		MNDO	**AM1**	**RM1**	**PM3**	**MNDO**	**AM1**	**RM1**	**PM3**
Gas phase	Neutral	-90	0	0	0.1	89.6	32.3	49.5	25.7
	5'-O$^-$	90.7	0	-57.2	30.2	–	–	–	–
	4'-O$^-$	0	0	29	0	44.5	0.0	31.9	0.0
	3'-O$^-$	0	29.2	55.5	0	91.4	0.0	50.8	0.0
	7-O$^-$	90.1	27.8	48.2	22	92.7	0.0	48.1	22.1
	5-O$^-$	-0.1	-26.3	-46.5	10.7	91.7	0.0	46.4	11.6
	3-O$^-$	-34.9	4.2	-2.9	0	32.0	0.0	0.0	0.0
	Q$^{\bullet+}$	0	0.9	4.7	0.1	0.0	0.0	0.1	0.0
	5'-O$^\bullet$	-90.2	-24.9	49.3	0	–	–	–	–
	4'-O$^\bullet$	0	0	0	0	0.0	0.0	0.0	0.0
	3'-O$^\bullet$	89.3	-31.8	-0.2	0	0.0	0.0	0.0	0.0
	7-O$^\bullet$	0	30.4	-51.1	-27.1	89.0	33.7	50.7	0.3
	5-O$^\bullet$	0	29	-50	24.4	0.0	32.0	48.7	24.2
	3-O$^\bullet$	0	-4.8	0	0	0.1	4.1	37.8	0.2
Water phase	Neutral	-1.6	2.8	-0.1	-9.1	89.7	35.0	49.7	26.4
	5'-O$^-$	80.2	0	0	0	–	–	–	–
	4'-O$^-$	-62.9	0.1	3.9	1.3	98.7	22.9	38.5	4.5
	3'-O$^-$	0	2.3	1.5	3.6	85.3	30.4	47.7	0.1
	7-O$^-$	0	1.8	0	-0.2	87.3	33.7	48.6	25.5
	5-O$^-$	-0.1	2.4	-0.3	0	87.5	33.0	48.3	1.3
	3-O$^-$	53	0.1	0.1	0.1	40.7	7.3	27.7	1.7
	Q$^{\bullet+}$	-1.8	0	0.1	0	82.5	21.3	38.7	29.0
	5'-O$^\bullet$	0.3	0	0	0	–	–	–	–
	4'-O$^\bullet$	0.2	0.1	0.1	0.1	0.0	0.0	0.1	0.1
	3'-O$^\bullet$	0.2	0.1	0.1	0.1	88.8	0.0	0.1	0.0
	7-O$^\bullet$	0.3	-0.7	2.7	0.2	90.6	33.0	52.6	26.8
	5-O$^\bullet$	-72.4	1.7	-0.7	0	84.8	31.3	50.5	24.3
	3-O$^\bullet$	0.2	0	-0.3	0.1	89.0	1.1	41.9	5.8