

Electronic Description of Few Selected Flavonoids by Theoretical Study

M.Rajendran

Associate Professor
Research and Postgraduate
Department of Chemistry
N.M.S.S.Vellaichamy Nadar
College, Madurai-625 019,
Tamilnadu, India

R.Ravichandran

Research and Postgraduate
Department of Chemistry
N.M.S.S.Vellaichamy Nadar
College, Madurai-625 019,
Tamilnadu, India

D.Devapiriam

Associate Professor
Research and Postgraduate
Department of Chemistry
N.M.S.S.Vellaichamy Nadar
College, Madurai-625 019,
Tamilnadu, India

ABSTRACT

The electronic properties responsible for free radical scavenging and metal chelation of a set of 7 flavonoids compounds were studied. Electronic parameters were calculated using semi empirical PM3 method. The structure activity relationship of OH substitution, C2-C3 double bond, chelation site of flavonoid and related property of antioxidant efficiency, BDE, Mulliken atomic charge, electron current movement among A, B and C-rings were investigated. The relative change in energy (ΔH_f) associated with the formation of phenoxyl radicals and spin density distribution of phenoxyl radicals were determined. The complexation mechanism of seven flavonoids quercetin, morin, apigenin, naringenin, chrysin, taxifolin and fisetin with metal ions, by molecular mechanics (UFF) method was investigated. Flavonoids metal chelation site order on toxic metals Pb, Bi and Cd was investigated. The most likely chelation site for metal ion was 5-OH & 4=O group, followed by 4=O & 3-OH group and the 3' & 4' hydroxyl for all the flavonoid molecules studied. In the absence of C2-C3 double bond (taxifolin) the order of metal chelation site with metal was 3-OH & 4=O, followed by 5-OH & 4=O group and 3' & 4' hydroxyl.

Keywords

Free radical, antioxidants, flavonoid-Metal complex, PM3 method.

1. INTRODUCTION

Flavonoids are common polyphenolic radical scavengers that commonly occur in plants. This class of compounds includes flavones, isoflavones and the 2,3-dihydro derivatives of flavones, namely flavonones, which are interconvertible with the isomeric chalcones [1]. They are naturally synthesized by the secondary metabolism of plants. One of the most remarkable properties of flavonoids rises from their behaviour as antioxidants acting through a variety of ways, including direct inhibition of formation or activity of reactive oxygen species and interaction, inhibition, with enzyme [2,3]. The study of flavonoids is therefore of great practical and theoretical importance. Thus, the detailed knowledge of their molecular structures, both from energetic and geometric view points, is of great importance to gain a deeper insight into their possible therapeutic applications. Computational chemistry methods are one of the most powerful tools to achieve advances in this field, and several studies may be found in the literature [4, 5].

A great number of flavonoids, especially flavones, can efficiently chelate metals. Metal complexation reactions play important and multiple roles in biological systems and provide

in vivo sensitive colour stabilization mechanisms in plants [6]. Metal complexation reactions enhance protection of plants from pathogens and plant eaters. By chelating metal ions, flavonoids generally prevent metal-catalyzed free radical generation and their subsequent reactions and accordingly protect very important biologically active molecules from oxidative system [7].

In general, radical scavenging by flavonoids occurs *via* electron donation from the free hydroxyls on the flavonoid nucleus with the formation of less reactive flavonoid aroxyl radicals [8, 9]. The phenolic groups are also responsible of the metal-chelating activity. In fact it is to be considered that O_2^- and H_2O_2 in the presence of transition metals such as iron and copper may be converted into highly reactive $\cdot OH$. Flavonoids may sequester these metal ions by chelation and preventing the metal-mediated generation of harmful oxidizing radicals, may protect the potential biological targets from oxidative stress [10, 11].

There are three possible metal complexing domains that can interact with metal ions: the 3',4'-dihydroxy group located on the B ring and the 3 or 5-hydroxy and 4-carbonyl group in the C ring. A relatively large number of publications have appeared in which several complexing mechanisms and spatial structures are proposed for metal-flavonoid compounds [12].

The purpose of this study was to better elucidating molecular aspects of beneficial effect of flavonoids, to obtain the preference position of flavonoids in binding metal ions. Our aim is to individually study the capacity of complexation of each flavonoids with metals theoretically using semiempirical PM3 method. The highest binding energy clearly identifies the 3-hydroxyl-4-carbonyl group as the most probable chelation site for metal ion, followed by 4-carbonyl-5-hydroxyl and 3',4'-hydroxyl groups [13]. In the earlier study Mulliken atomic charges are calculated by semiempirical method, and their values are correlated with antioxidant efficiency of flavones [14].

2. COMPUTATIONAL METHOD

ArgusLab [15] provides molecular modeling functionality that does not utilize the concept of a Z-matrix. Another useful feature is the ability to add hydrogen atoms and molecular mechanics-based cleaning of geometry at each step of structure building. Due to the implementation of the UFF (Universal Force Field), ArgusLab has the ability to handle the entire periodic table. Besides the molecular mechanics, geometry optimization or single-point energy calculations can be performed with semiempirical methods (EHT, PM3, AM1,

MNDO). Additionally, the ZINDO method parameterized specially to predict the excited state properties allows the electronic excitation spectra to be modeled. From semiempirical calculations, electron density is derived and may further be displayed. The visualization of molecular orbitals is provided and the electrostatic potential can be calculated and mapped on to the electron density surface. Although these techniques are reasonably fast on a PC computer, the results are lacking in accuracy and need to be treated on a qualitative basis rather than in a quantitative manner. Similarly, the molecular mechanics approach employing UFF is good for cleaning up structures when sketched in the builder and for refining the initial geometries before using more expensive methods. The molecular mechanics method calculates the energy as the function of the coordinates and energy minimization is an integral part of the method.

The flavonoid structure was generated by ArgusLab 4.0 and geometry optimization was performed with the semi-empirical RHF/ PM3 parameterization (Dewar *et al.*, 1985) [16]. The minimum potential energy is calculated by using geometry convergence function. Surfaces created to visualize ground state properties as well as excited state properties such as orbital electron densities, electrostatic potentials (ESP), spin densities and generated the grid data, used to make molecular orbital surfaces and visualized the molecular orbitals. All geometry optimizations are performed without any symmetry constraints. Geometry optimization is followed by single-point calculation using PM3 method. The ability of flavonoid antioxidants to donate a hydrogen atom is mainly governed by the BDE value of O-H bond. The BDE of OH bond can be calculated by the following equation [17].

$$\text{BDE (Fl - OH)} = \Delta_f\text{H (Fl-O}\cdot\text{)} + \Delta_f\text{H (H)} - \Delta_f\text{H (Fl - OH)}$$

Where $\Delta_f\text{H (Fl-O}\cdot\text{)}$ is the enthalpy of formation of the flavonoid phenoxyl radical generated after H[•] abstraction, $\Delta_f\text{H (H)}$ is the enthalpy of formation of the hydrogen atom and $\Delta_f\text{H (Fl-OH)}$ is the enthalpy of formation of the parent flavonoid molecule.

For metal cation, as well as their metal-flavonoid complexes, the geometries configurations are optimized and the lowest energy structures are chosen for further analysis. The metal-flavonoid binding energies are calculated at MM (UFF) levels in gas phase. The earlier published experimental results find that flavonoids often deprotonate to form (M-O⁻), then the anion interact with metal cations to form coordination complexes [18]. Thus in this study, the metal-flavonoid complexes are obtained by interaction of metal cations with the corresponding anion formed by O3 & O5 and O3' & O4' deprotonation, including O3, O4' and O5, O4'.

3. RESULTS AND DISCUSSION

3.1. HOMO & LUMO and structure property relationship

To determine the theoretical antioxidant efficiencies of flavonoids, various positions of hydroxyl substitution and C2-C3 double bond was first selected for this study. The chemical structure of these compounds is presented in the figure-1. Since π electron configuration is of great importance in understanding the antioxidant capacity of polyphenols, the selected flavonoids should have various structural features. Figure-2 showed the HOMO and LUMO frontier orbitals of

the optimized structure and their charge distribution among the atoms in the molecule. In the absence of C2-C3 double bond, the conjugation was restricted over the entire molecule, as compared to quercetin. Thus in taxifolin, the HOMO and LUMO were strongly separated on the B and A-ring, respectively (figure-2). This lack of conjugation leads to strong hypsochromic shift of the main absorption band near the visible region (e.g at 370 nm like for quercetin); thus taxifolin is colourless in solution [19].

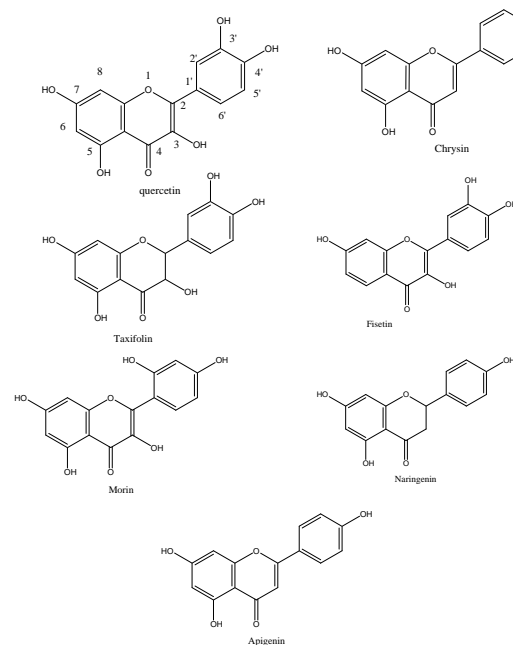


Fig-1 Chemical structure of flavonoids

3.2 UV-Vis spectra and structure activity relationship

The absorption wavelengths of numerous polyphenols depend on number and position of OH groups. When increasing the number of -OH groups in the B-ring of flavonoids, the experimental λ_{max} is systematically red shifted. This is due to mesomeric (+M) effect of hydroxyl group, which extends the π -conjugation in the frontier orbital (figure-2). Consequently, this induces a bathochromic shift, which is formed from the delocalization of MOs, thus rationalizing the experimental results. UV-Vis spectra of flavonoids exhibit at least two absorption bands, one (Band I) ranging from 240 to 280 nm and another (Band II) from 300 to 400 nm [20].

In dihydroflavonols, e.g taxifolin, the absence of C2-C3 double bond destroys the conjugation over the entire molecule, as compared to flavonols, e.g quercetin. Thus, in taxifolin, the HOMO and LUMO are strongly separated on the B and A rings, respectively (figure 2). Absence of conjugation leads to lowering of wavelength of the main absorption band. For example quercetin exhibits absorption at 379 nm, but for taxifolin it appears at 326 nm. Another peak for A-ring was at 320 nm for quercetin, but at 324 nm for taxifolin. C-ring peak was not observed for taxifolin, but for quercetin C-ring peak observed at 270 nm. Similar to taxifolin absorbance behavior, naringenin also shows peak at 322 nm and 225 nm for B-ring and A-ring respectively (Table 7).

The number and position of OH groups, sensitively influences the absorption wavelengths. For example the λ_{max} value was 392 nm, 379 nm, 371 nm, 357 nm and 350 nm for morin (five

OH groups), quercetin (five OH groups), fisetin (four OH groups), apigenin (three OH groups) and chrysin (Two OH groups) respectively [20]. The absorption wavelength is affected by the chemical structure that is conjugation, number and position of substituent. Rice-Evans et al., suggested that an increase in the number of OH or CH₃ substituent leads to a bathochromic (red) shift [21]. This is due to the mesomeric (+M) effect the hydroxyl group which extends the π conjugation in the frontier orbitals, as can be seen from our PM3 calculation in fig-2. In the presence of C2-C3 double bond (flavones and flavonols) the band is assigned to HOMO to LUMO transition. In the absence of the C2 – C3 double bond this transition becomes completely forbidden, due to complete orbital separation.

3.3. Role of C2-C3 double bond

The absence of C2-C3 double bond in dihydroflavonoids (e.g taxifolin) and flavonones substantially reduces the conjugation as compared to flavonols (e.g quercetin) and flavones, and this induces hypsochromic shift. In fact, the shift is due to the loss of the main absorption band observed in flavones and flavonols. In flavonones and dihydroflavonols, this transition becomes completely forbidden, due to complete orbital separation (figure 2).

The molecular structure of flavonoid family is characterized by the large charge delocalization spreading along the studied molecules from A to B ring through the C one. The coplanarity of ring A, B and C are maintained in the presence of C2-C3 double bond (figure 2). In the absence of C2-C3 double bond in the flavonoid the rings should not be planar (figure 2) (e.g Taxifolin, and Naringenin). The conjugation between the B and C ring, is not as high as that of the rest of the molecule due to the non-planarity of B-ring with C-ring, therefore, B-ring can be seen as the weakest part of the delocalized system.

3.4. Electronic properties of studied flavonoid molecules

PM3 functional used in this study to calculate the electronic properties of the flavonoid studied molecules, such as ionization potential (IP), electron affinity (EA), electronegativity (χ), absolute hardness (η), absolute softness (S), electrophilic index (ω). The properties that are displayed in table 1 for each variable are computed based on the differences between the HOMO and LUMO energies of the neutral molecule and is known as orbital-vertical (Koopman's theorem). Table 1 represents the total energy, calculated energy levels of the HOMO, LUMO energy gap in (eV) and dipole moment of the investigated molecules. E_{HOMO} is a quantum chemical parameter which is often associated with the electron donating ability of the molecule. High value of E_{HOMO} is likely to a tendency of the molecule to donate electrons to appropriate acceptor molecule of low empty molecular orbital [22]. From Table 1, it can be clearly seen that the highest value of E_{HOMO} -0.315292 (eV) of morin indicates the better electron donating efficiency. The energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$) is an important parameter as a function of reactivity of the flavonoid molecule towards the free radical. As ΔE decreases the reactivity of the molecule increases were leading to increase in the electron donating efficiency of the molecule [23]. Lower values of the energy difference will render good antioxidant efficiency, because the energy to remove an electron from the last occupied orbital will be low [24]. The calculated energy of HOMO, LUMO

and the energy gap were -0.31958, -0.04009 and 0.27949 eV (Table 1), respectively for quercetin. Quercetin has the lowest ΔE value than the other investigated flavonoid molecules in this study. The small value of energy gap and the distribution of π -bonding electron frontier orbital (HOMO and LUMO) were delocalized through the whole molecule (figure 2). A molecule with a low energy gap is generally associated with the high chemical activity and low kinetic stability and is termed soft molecule [25]. The investigated flavonoid molecule quercetin has highest softness value 7.15589 (table 1). This result showed that quercetin has the lowest energy gap and higher softness than the other investigated flavonoid molecule, this means that the molecule could behave a better antioxidant. The dipole moment is another parameter arise from the electronic distribution in a molecule and is the measure of polarity of a polar covalent bond. Fisetin have a highest value of dipole moment, but no significant relationship between dipole moment values and antioxidant efficiencies is noticed.

Ionization energy is a basic property of a chemical reactivity of atoms and molecule. High ionization energy value indicates high stability and chemical inertness and the lower value of ionization energy indicates high reactivity of atoms and molecule [26]. The low ionization energy 0.315292 (eV) of morin compared to the other selected flavonoids indicates its high antioxidant efficiency. According to Sanderson's electronegativity equalization principle [27], the molecule chrysin with a high electronegativity quickly reaches equalization and hence low reactivity is expected. Table 1 show that the morin has low value of electronegativity among the investigated flavonoids and hence high reactivity was expected. The electrophilicity index ω is a measure of the stabilization in energy after a system accepts additional amount of electron charge from the other species [28]. The results show that naringenin was the strongest nucleophile, while chrysin was the strongest electrophile.

From the optimized flavonoid structures, the calculated excess charges on the atoms on the investigated flavonoids are shown in table 2. The highest atomic charges are available in the fourth position carbonyl oxygen atom. These theoretical values correlates with the metal binding properties of flavonoids as reported previously [29]. The earlier results suggested that binding at the 3-4 sites was stronger than at the 3'-4' sites. The complexation of iron containing one Fe, Fe-quercetin binding strength at different sites has the order 3-4 > 4-5 > 3'-4'; while for complexes containing two Fe atoms, the complex with one Fe bound at the 3,4 site and the second Fe at the 3',4' site [30]. Table 2 showed the mulliken atomic charges of seven investigated flavonoids. The results demonstrate that the order of metal binding was based on mulliken atomic charges on the atom of flavonoid that is 4-5 > 4-3 > 3'-4'. This order of metal chelation was possible only if flavonoids have C2-C3 double bond (e.g. Quercetin). In the absence of C2-C3 double bond the order of charges on atom was changed, hence the metal binding order was 4-3 > 4-5 > 3'-4' (e.g. taxifolin). Based on the presence and absence of C2-C3 double bond in the flavonoids, the metal binding preference was modified.

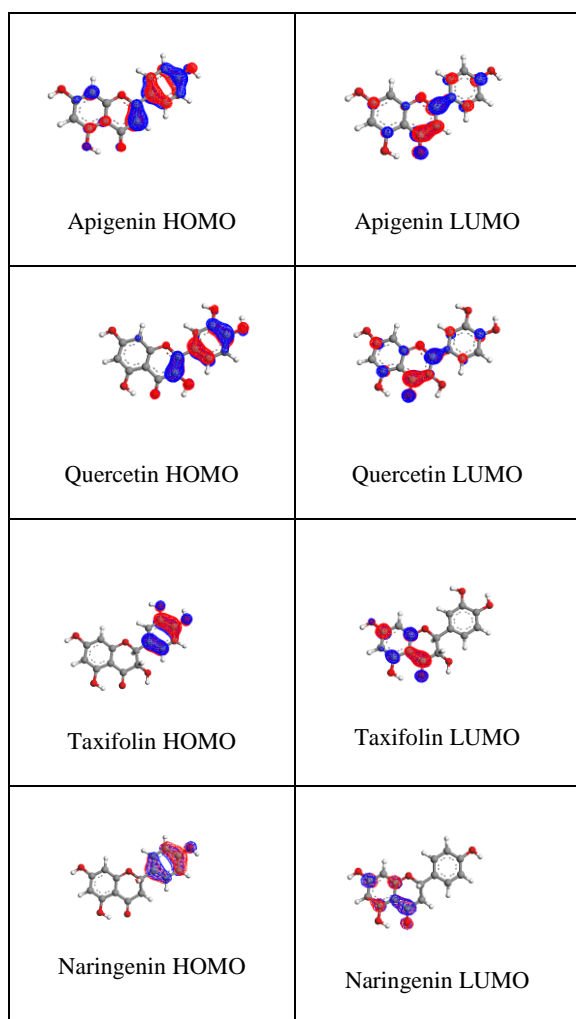


Figure 2. Charge distribution of the HOMO and LUMO in the optimized molecules

3.5. O-H bond distance and intramolecular hydrogen bonding

Table 3 shows the hydroxyl bond distances of various –OH substituted flavonoids and their order of bond lengths. The observed results show that quercetin have the following order of bond lengths, 5-OH > 3-OH > 7-OH > 3'-OH > 4'-OH. In general weaker character of hydrogen bond was in agreement with the shorter O-H bond distance for the OH group, while the longer the OH bond distance, the stronger the hydrogen bonding. As reported in the previous work [31] the quercetin 5-OH have longer bond length than other OH groups, which may be due to stronger hydrogen bond interaction between 5-OH and with the carbonyl group in C-ring. Similarly the interaction between 3-OH and 6'-H sites come next to the 5-OH and C ring carbonyl group interaction. The 7-OH bond length is lower than 3-OH, this may be due to lack of any intramolecular hydrogen bonding through this position. The introduction of a hydroxyl in position 3 also weakens 5-OH interaction with carbonyl group. The hydroxyl groups in B-ring do not affect remarkably the interactions in A and C rings. The hydroxyl group bond distances in B ring are lower than A and C ring hydroxyl groups, discarding strong hydrogen bonding for B ring hydroxyl group, hence it have

longer bond length than A and C ring OH bond. The intramolecular hydrogen bonding tendency was slightly altered for the flavonoids (taxifolin and fisetin) that have no C2- C3 double bond. The OH bond length and intramolecular hydrogen bond have a strong correlation among flavonoid molecule (Table 3).

Table 3 shows the bond distances of 2C-1'C of the entire optimized flavonoid molecules. The charge delocalization from A to B ring via C ring (through the C2-C3 double bond) can also be analysed here. The reported results in Table 3 shows that the charge delocalization between A and B rings are done through the double bond in C ring. The order of 2C-1'C bond length for flavonoids are as follows; Taxifolin > Naringenin > Chrysin > Apigenin > Fisetin > Quercetin > Morin. The resonating electron current movements from ring B to C was through 2C-1'C bond however, in the absence of C2-C3 double bond, the electron current movement was found to decrease. The bond distance between 2C-1'C of all flavonoid are compared with their resonating efficiencies.

The electron current movement from B ring to C was restricted for C2-C3 double bond absent structures. As the flavonoid molecule taxifolin and naringenin were lack of C2-C3 double bond, the resonating electron current movement from B ring to C was restricted. HOMO, LUMO maps and the increase in C2-C3 bond length for taxifolin and naringenin is confirming the role of C2-C3 double bond in flavonoids. Hence the bond distance 2C-1'C bond for these two flavonoids is found to increase compared with other flavonoids. The order of decrease in bond length next to taxifolin and naringenin was chrysin > apigenin > fisetin > quercetin > morin. And this order depends on the amount of electron current movement from ring B to C (Table 3). Chrysin does not have any hydroxyl group in B ring; hence it appears next to naringenin. Apigenin have one hydroxyl group in the B ring hence it comes next to chrysin. Fisetin, quercetin and morin each have two hydroxyl groups in the B ring, but in fisetin and quercetin that are in third and fourth positions. Electron releasing group in the adjacent position the electron current movement was decreased, hence morin 2C-1'C bond length lower than quercetin.

3.6. BDE of OH in flavonoids

Radical scavenging potency of flavonoids is mainly related to the presence of OH groups at specific positions on the flavonoid core. The minimal value of the Bond Dissociation Energy (BDE) of OH bonds indicates which OH group on the flavonoid core posses the most abstractable hydrogen, that is, which OH group is targeted for radical attack. Table 4 shows the BDE order of OH group in flavonoids. The BDE order of OH groups in quercetin is as follows 4'-O < 3'-O < 7-O < 5-O < 3-O. This indicates that vicinal OH groups on the B ring are the preferred sites for hydrogen abstraction as seen in to quercetin most of the tabulated flavonoids has lower BDE for 4'-OH and 3'-OH presence in B ring. Similarly the higher O-H BDE value is noted for 7-O < 5-O 3-O of flavonoids, may be due to interatomic hydrogen bond formed between fourth carbonyl and 5-OH and 3-OH groups. From the differences in the BDE value it is confirmed that reactivity of the B ring higher than that of A-ring.

3.7. Spin density and their reactivity

The more delocalized the spin density in the radical, the easier is the radical formed, and thus the lower is the BDE [32]. The spin population on the remaining O-atom after H removal

from O-H, appears to be delocalized. In the unrestricted scheme, the spin density is often considered to be a more realistic parameter and provides a better representation of the reactivity [33]. Spin density of flavonoid has been an important factor in the description of antioxidant behaviour [34].

The table 5 shows the difference in spin density of molecular and radical species of flavonoids. When comparing the molecular and radical flavonoid, the spin density value has lowered on all atoms in flavonoid molecule. The difference in spin density value of molecular and their radical flavonoids are analysed here. The decrease in spin density value for quercetin was 0.230, 0.224, 0.222, 0.221 and 0.201 for 4'-O•, 3'-O•, 7-O•, 5-O• and 3-O• radical species respectively. This indicates that there was more delocalization for oxygen radical, formed in B-ring. The higher value of 5-O• and 3-O• may be due to hydrogen bonding of 3-OH and 5-OH groups with the fourth carbonyl group oxygen. Morin spin density values are in the decreasing order of 4'-O•, 5-O•, 7-O•, 2'-O• and 3'-O•. The higher value of spin density difference for 4'-O• and lower value of spin density difference for 3-O• and the lower value for other OH was due to the position of OH groups in morin (2'-OH and 4'-OH). In the absence of C2-C3 double bond in naringenin and taxifolin the order of decrease in spin density was modified, because the resonating electron current movement from ring B to ring C was restricted. The OH group which have high spin density difference with -O•, will easily donate hydrogen to free radical species.

Table 6 shows the formation constant values for flavonoid-metal complexes. Mole fraction experiments revealed that a 1:1 stoichiometry for metal-flavonoid complexes. For a single flavonoid molecule, there are several possible sites that could bind a metal atom. The possible binding site for apigenin, naringenin and chrysin was fourth carbonyl oxygen and 5-hydroxyl oxygen. Their formation constant values with Pb, Bi and Cd metals are reported.

Fisetin have two possible binding sites one at 4th carbonyl oxygen and 3-hydroxy oxygen, another one was 3'-O and 4'-O groups. The formation constant value of fourth carbonyl oxygen and 3-hydroxyl oxygen was lower than 3'-O and 4'-O formation constant. Formation constant values are well correlated with the mulliken atomic charges on atoms in molecules (Table 2). Morin also have two possible binding sites one is 4th carbonyl group oxygen and 3-OH oxygen another one is 4th carbonyl group oxygen and 5-OH oxygen. The formation constant value of 4th carbonyl and 5-OH was lower than 4th carbonyl oxygen 3-OH sites. These results are well correlated with the mulliken atomic charges of atoms in morin (Table 2). 5-OH oxygen have higher charges (-0.2698) than the 3-OH oxygen charge (-0.2353). Quercetin have three possible sites for metal chelation, 4th carbonyl oxygen & 3-OH, 4th carbonyl oxygen & 5-OH and 3'-OH & 4'-OH. The formation constant values are in the order of 4=O & 5-O < 4=O & 3-O < 3'O & 4'-O. This order of formation constant correlate with mulliken atomic charges, 5-OH oxygen charge

was higher than 3-OH oxygen charge, hence 5-OH with metal formation constant is lower in value. Taxifolin as similar to quercetin three possible chelation sites, but absence of C2-C3 double bond the formation constant order has changed 4=O & 3-O < 4=O & 5-O < 3'-O & 4'-O. This change in order was due to higher mulliken atomic charge on the 3-OH oxygen and also the structural property of taxifolin. Taxifolin has no conjugation, due to the absence of C2-C3 double bond, hence 3-O has higher charge than 5-O. Some studies report that 3'-4' site is the first chelation site [35]. This may be due to different solvent conditions (acidic or basic). The results suggest that metal binding site order was 4-5, 4-3 and 3'-4' in the presence of C2-C3 double bond, in the absence of C2-C3 double bond the order was 4-3, 4-5 and 3'-4'.

4. CONCLUSION

The results of semiempirical methods are usually good in a qualitative sense and very useful in the comparison of data obtained for structurally related compounds. A systematic computational study on the properties of flavonoids has been carried out to a deeper insight into their molecular properties. Quantum chemical parameters such as E_{HOMO}, E_{LUMO}, energy gap (ΔE), hardness (η), softness (S), electron affinity (EA), ionization potential (IP), absolute electronegativity (χ) and electrophilicity index (ω) were calculated. The BDE value of OH bond is an important parameter in evaluating antioxidant action, because weaker the O-H bond the faster the radical inactivation. The present semiempirical values clearly demonstrate that H-transfer from B-ring is easier than A-ring and C-ring.

The optimized structures of metal-flavonoid complexes were investigated. The study on complexation of metal with flavonoid in gas phase shows that chelation property depends on charge on the chelating site of flavonoid. The mulliken atomic charge data implicate that 3-OH & 4-carbonyl, 5-OH & 4-carbonyl and catechol structural units are with chelating power. The orders of chelating efficiency of flavonoid depend on charge on the chelating site. The binding energy for the metal atom to a single flavonoid (quercetin) molecule demonstrates that 3-hydroxyl & 4-carbonyl group is optimal chelation site, followed by 4-5 and 3'-4' sites. In the absence of C2-C3 double bond, flavonoid (taxifolin) binding site with metal was found to be 5-4, 3-5 and 3'-4' sites.

5. ACKNOWLEDGEMENTS

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Table-1. The total energy, HOMO, LUMO, ΔE (ev) and the dipole moment (Debye) for the studied molecule

Parameters	Quercetin	Morin	Fisetin	Apigenin	Chrysin	Taxifolin	Naringenin
ΔH _f	-224.373	-223.8852	-175.309	-142.195	-96.6987	-237.1898	-158.8035
HOMO	-031958	-0.315292	-0.31582	-0.33533	-0.33871	-0.327540	-0.341074
LUMO	-0.04009	-0.034085	-0.03434	-0.03696	-0.03815	-0.032046	-0.022956
Dipole Moment (Debye)	2.758272	2.2798268	3.850305	2.576055	2.955009	2.488736	2.169745

ΔE (eV)	0.27949	0.281207	0.28148	0.29837	0.30056	0.295494	0.317218
Ionization Potential (I) (eV)	0.31958	0.315292	0.31582	0.33533	0.33871	0.327540	0.340174
Electron Affinity (A)	0.04009	0.0340085	0.03434	0.03696	0.03815	0.032046	0.022956
Chemical Potential (μ) (eV)	-0.17983	-0.174688	-0.17508	-0.18614	-0.18843	-0.17979	-0.181565
Electronegativity (χ) (eV)	0.179835	0.1746885	0.17508	0.186145	0.18843	0.179793	0.181565
Global Hardness (η)	0.139745	0.140603	0.14074	0.149185	0.15028	0.14774	0.158609
Global Softness (S)	7.15589	7.11222	7.105530	6.703086	6.654245	6.768326	6.304812
Electrophilicity (ω)	0.115708	0.108518	0.108899	0.116130	0.118132	0.109394	0.1039217

Table -2. PM3 optimized flavonoids mulliken atomic charges

Flavonoids	1- O	3- O	4 =O	5- O	7- O	2' - O	3' - O	4' - O	C-2	C-3
Quercetin	-0.1038	-0.2286	-0.406	-0.2661	-0.2341		-0.257	-0.233	0.1122	-0.1299
Taxifolin	-0.2051	-0.3118	-0.3772	-0.2655	-0.2406		-0.2531	-0.2538	0.0752	-0.0869
Fisetin	-0.1060	-0.2312	-0.3571		-0.2335		-0.2576	-0.2339	0.0959	-0.01306
Chrysin	-0.1114		-0.3935	-0.2705	-0.2357				0.1764	-0.4189
Apigenin	-0.1125		-0.3957	-0.2709	-0.2357			-0.2331	0.1885	-0.4283
Naringenin	-0.2117		-0.3753	-0.2694	-0.2427			-0.2446	0.0923	-0.3581
Morin	-0.0155	-0.2427	-0.5045	-0.2698	-0.2432	-0.244		-0.2368	0.1271	-0.0934

Table-3 Bond Distances in (Å) after optimized with PM3 method, in gas phase

	5-OH	3-OH	7-OH	3'-OH	4'-OH	2'-OH	2C-1'C
Quercetin	0.96644	0.95215	0.951802	0.950606	0.950224		1.472481
Taxifolin	0.965322	0.951273	0.951846	0.950548	0.95009		1.515027
Fisetin		0.950084	0.951488	0.950013	0.950198		1.472724
Chrysin	0.967599		0.961752				1.475984
Apigenin	0.967584		0.951715		0.951360		1.473511
Naringenin	0.965844		0.951759		0.951200		1.512024
Morin	0.966635	0.951938	0.951725		0.951426	0.951669	1.462769

Table-4 Calculated Bond dissociation energy (BDE) orders at the various OH position in flavonoids

Quercetin	4'-O < 3'-O < 7-O < 5-O < 3-O
Morin	4'-O < 7-O < 2'-O < 5-O < 3-O
Apigenin	7-O < 4'-O < 5-O
Chrysin	7-O < 5-O
Fisetin	7-O < 4'-O < 3'-O < 3-O
Naringenin	4'-O < 7-O < 5-O
Taxifolin	4'-O < 3'-O < 7-O < 5-O < 3-O

Table-5 Differences in mulliken atomic charges, after removal of –OH hydrogen atom from the neutral flavonoids.

Quercetin	4 ^o -O 0.230	>	3 ^o -O 0.224	>	7-O 0.222	>	5-O 0.221	>	3-O 0.201
Morin	4 ^o -O 0.224	>	5-O 0.224	>	7-O 0.222	>	2 ^o -O 0.217	>	3-O 0.204
Apigenin	5-O 0.224	>	7-O 0.222	>	4 ^o -O 0.218				
Chrysin	7-O 0.218	>	5-O 0.021						
Fisetin	7-O 0.221	>	4 ^o -O 0.213	>	3 ^o -O 0.211	>	3-O 0.202		
Naringenin	5-O 0.224	>	7-O 0.222	>	4 ^o -O 0.216				
Taxifolin	3-O 0.239	>	3 ^o -O 0.230	>	4 ^o -O 0.229	>	5-O 0.225	>	7-O 0.224

Table-6 Formation constant values of flavonoid-metal complexes calculated by MM(UFF) method

Metal chelating sites	Formation constant kcal/mol	Formation constant kcal/mol	Formation constant kcal/mol
Apigenin 4keto,5hydroxy	Apigenin-pb 67.04354605	Apigenin-Bi 55.10143690	Apigenin-Cd 62.60301647
Chrysin 4keto, 5hydroxy	Chrysin-pb 66.79615555	Chrysin-Bi 54.85756666	Chrysin-Cd 62.44283754
Fisetin 4keto, 3hydroxy	Fisetin-Pb 69.05617333	Fisetin-Bi 58.01115621	Fisetin-Cd 68.41067366
Fisetin 3'4'-hydroxy	Fisetin-Pb 69.61364541	Fisetin-Bi 58.88701525	Fisetin-Cd 68.36815317
Morin 3hydroxy 4keto	Morin-Pb 71.31808457	Morin-Bi 60.12149712	Morin-Cd 70.74884584
Morin 5hydroxy 4keto	Morin-Pb 70.38913704	Morin-Bi 58.36616423	Morin-Cd 66.01514389
Naringenin 5hydroxy 4keto	Naringenin-Pb 51.34232137	Naringenin-Bi 42.38606897	Naringenin-Cd 47.31938598
Quercetin 3hydroxy 4keto	Quercetin-Pb 70.82007459	Quercetin-Bi 59.59937902	Quercetin-Cd 70.25781055
Quercetin 5hydroxy 4keto	Quercetin-Pb 69.98430509	Quercetin-Bi 57.96043958	Quercetin-Cd 65.63366226
Quercetin 3',4'-hydroxy	Quercetin-Pb 71.32394833	Quercetin-Bi 60.59483893	Quercetin-Cd 70.07719294
Taxifolin 3hydroxy 4keto	Taxifolin-Pb 50.90080964	Taxifolin-Bi 40.01854597	Taxifolin-Cd 49.52627828
Taxifolin 5hydroxy 4keto	Taxifolin-Pb 53.09607002	Taxifolin-Bi 44.08780535	Taxifolin-Cd 49.11226105
Taxifolin 3'4'-hydroxy	Taxifolin-Pb 63.35768849	Taxifolin-Bi 52.64166156	Taxifolin-Cd 62.10810148

Table-7 Experimental UV –Visible spectrophotometer data of flavonoids in Phosphate buffer (pH 7.4)

Flavonoids	B ring (nm)	A ring (nm)	C ring (nm)
Quercetin	379	320	270
Naringenin	322	225	---
Morin	392	269	222
Chrysin	350	272	233
Apigenin	357	297	271
Fisetin	371	320	249
Taxifolin	326	224	---

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