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ORIGINAL PAPER

A QSAR study of radical scavenging antioxidant activity of a series of flavonoids using DFT based quantum chemical descriptors – the importance of group frontier electron density

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Abstract In a pursuit of electronic level understanding of the antioxidant activity of a series of flavonoids, quantitative structure-activity relationship (QSAR) studies have been carried out using density functional theory (DFT) based quantum chemical descriptors. The best QSAR model have been selected for which the computed square correlation coefficient r^2=0.937 and cross-validated squared correlation coefficient q^2 =0.916. The QSAR model indicates that hardness (η), group electrophilic frontier electron density (F_A^E) and group philicity (ω_B^+) of individual molecules are responsible for *in vitro* biological activity. To the best our knowledge, the group electrophilic frontier electron density (F_A^E) has been used for the first time to explain the radical scavenging activity (RSA) of flavonoids. The excellent correlation between the RSA and the above mentioned DFT based descriptors lead us to predict new antioxidants having very good antioxidant activity.

This article is dedicated to late Dr. Golam Mostafa, Department of Physics, Jadavpur University

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Keywords Anti-oxidant design · DFT-QSAR · Flavonoid · Group frontier electron density · Quantum chemical descriptor · Radical scavenging activity

Introduction

During natural metabolism and energy production in living beings, free radicals are generated constantly [1]. Among large varieties of free radicals, superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), alkyl radical ($R^{\cdot}$), alkoxy radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), nitric oxide radical ($NO^{\cdot}$) etc. have been widely studied. Owing to the loss of electron, these radicals become unstable and highly reactive leading to a series of undesired reactions causing electron transfer, proton transfer, H-atom abstraction/addition in various parts of our body. It is strongly believed that this kind of undesired reaction which continuously goes on in living systems lead to cell death and aging. Diseases like inflammation, coronary heart disease, cancer, diabetes, cystic fibrosis, rheumatoid arthritis, Alzheimer's disease, Parkinson's disease and many more are now linked to oxy radicals [2–5]. Depending upon the diversified sources and the various substrate attacking mechanisms of free radicals, the antioxidants are divided into three categories, *viz*., enzyme inhibitors, metal chelators and radical scavengers [6, 7]. The former two types of antioxidants prevent the generation of radicals indirectly, while the latter scavenges the radicals directly. Due to this direct nature, the last category of radical scavenging antioxidants has received much attention [8].

Flavonoids, found in fruits and vegetables, grains, bark, roots, stems, flowers, tea, and wine [9–12], can scavenge the radicals directly to prevent injury caused by free

radicals. These antioxidants are oxidized by radicals, resulting in a more stable, less-reactive radical, according to the following reaction scheme

$$R^{\bullet} + FOH \rightarrow RH + FO^{\bullet}, \quad (1)$$

where FOH is flavonoid, $R^{\bullet}$ is free radical and $FO^{\bullet}$ is less reactive free radical.

Recently, much attention has been paid to find antioxidants that prevent radical induced impairments. Considerable effort has been devoted to investigate the structure-activity relationships (SARs) relating physiological activities of antioxidants to their compositions and structures. From SAR analysis, Bors and co-workers pointed out that three structural factors are important for radical-scavenging activity of the flavonoid antioxidants [13] - a) an ortho-dihydroxy (catechol) structure in ring B (Fig. 1); b) a 2,3-double bond in conjugation with a 4-oxo function (1,4-pyrone moiety) in ring C; c) the additional presence of both 3- and 5-hydroxyl groups. That the presence of catechol enhances the radical-scavenging activity of flavonoids and other phenolics has been verified by a large number of studies performed in diverse systems [14–20]. Besides the catechol group, the occurrence of pyrogallol group also exhibits positive effect in raising the antioxidative potential [16, 17]. As a result, catechol and pyrogallol have been regarded as pharmacophore of antioxidants. However, several lines of evidence suggests that 1,4-pyrone and 3- and 5-hydroxyl groups have little effect on the flavonoid antioxidant activity, because of the slight difference in electronic structure among the flavonoids with or without these groups [18]. It has also been shown that low bond dissociation energy (BDE), low ionization potential (IP) and the presence of 1,4-pyrone extends the conjugation in flavonoids raising the stability of flavonoid radical through resonance and it's radical-scavenging activity [13]. Further, the smaller the torsion angle between rings B and C the higher the resonance effect and the flavonoid is more active. The other consequence of introducing an electron-donating group in ring B, C is to reduce the O-H BDE, while the introduction of an electron-withdrawing group is to increase the O-H BDE. Since 1,4-pyrone is an electron-withdrawing group [21], it cannot stabilize the phenoxy radical or reduce the O-H BDE, despite the fact that it extends the conjugation system [22, 23]. The differing nature of these BDEs is of great significance in the rational design of novel antioxidants. In a similar way, electron-donating group decreases the IP, while electron-withdrawing group increases the IP, and so 1,4-pyrone does not enhance the antioxidative activity of flavonoids by the electron transfer or the H-atom transfer pathway. However, 1,4-pyrone with 3- or 5-OH enhances the proton dissociation and subsequent electron donation capability. Therefore, in certain systems, 1,4-pyrone and 3- or 5-OH can show positive effects. It is clear from the above discussion that the catechol and pyrogallol moiety enhances the antioxidant activity, while the role of the conjugation of 1,4-pyrone is still inconclusive as experiments performed in different environments have given controversial results. This controversy prompted us to undertake further studies in this line using DFT methodology as well as a new descriptor to conclusively establish the role of conjugation of the pyrone moiety in the antioxidant activity of flavonoids.

To gain a deeper insight into the problem, molecular descriptors, which are the numerical representation of the molecular structures, are used to perform quantitative structure activity relationships (QSAR) analysis [24]. In the literature, to calculate the molecular descriptors usually quantum mechanical semi-empirical methods such as AM1 [25] and PM3 [26] have been used. However, some recent QSAR studies have revealed that choice of the density functional theory (DFT), instead of AM1 or PM3 shows better correlation between calculated results and experimental data [27–29]. Therefore, to accelerate the discovery of novel antioxidants, the DFT method is expected to lead to statistically more accurate QSAR model.

The aim of our study is to elucidate the relationship between the molecular structure of a series of structurally related flavonoids (flavones, flavonols and flavanones) and their ability to scavenge 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) free radicals (Fig. 2). DFT based molecular descriptors such as hardness, group-philicity, frontier electron density etc. have been used previously but in the present study we introduce a

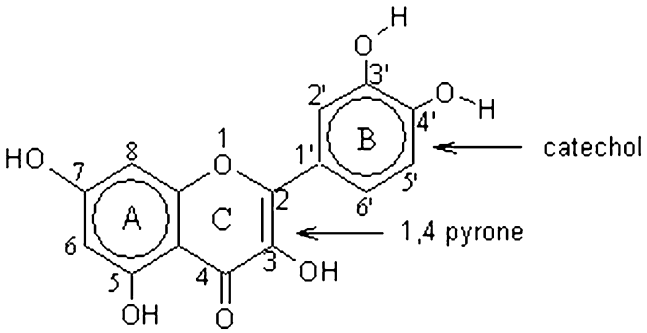


Fig. 1 General structure of flavonoid

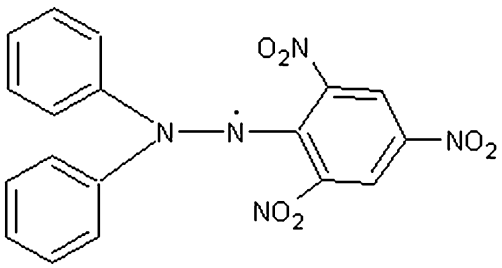


Fig. 2 Structure of 1,1-diphenyl-2-picrylhydrazyl (DPPH•)

new descriptor, namely the *"group frontier electron density"* which takes into account the cooperative effect of a group of atoms on the molecule in their reactivity toward the free radicals. These quantum chemical descriptors are employed to analyze the radical scavenging activity to the best predictive model. The statistical parameters show the QSAR model is highly predictive and based on this model four new molecules with high predicted radical scavenging activities have been proposed.

Methods

Theoretical background

a) Chemical hardness

Based on the density functional theory the chemical hardness (η) has been shown to be a useful global index of reactivity in atoms, molecules and clusters [30, 31]. The concept of hardness was first emphasized by Pearson [32] as an index of reactivity, and was quantified by Parr and Pearson [33]. The global hardness is defined as

$$\eta = \frac{1}{2}\left(\frac{\partial^2 E}{\partial^2 N}\right)_{v(r)}, \tag{2}$$

where E is the total energy, v(r) is the external potential and N is the number of electrons. In a finite difference approximation with the assumption that the energy varies exponentially with the number of electrons, the above equation can be written as

$$\eta = \frac{1}{2}(IP - EA), \tag{3}$$

where IP and EA are the ionization potential and the electron affinity of the chemical species, respectively.

According to Koopman's theorem [30], IP and EA are the Eigen values of highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO), respectively, with change of sign and hence the above equation can be written as,

$$\eta = -\frac{1}{2}(HOMO - LUMO). \tag{4}$$

b) Fukui function (f(r)) and electrophilicity index (ω)

Parr and Yang [30] introduced the term Fukui function indices, $f(r)$, which actually measures the sensitivity of a system's chemical potential (μ) to an external perturbation at a particular site or the sensitivity of the system's electron density at a particular site if an infinitesimally small number of electrons is added or excluded from the system.

$$f(r) = \left(\frac{\partial \mu}{\partial v(r)}\right)_N = \left(\frac{\partial \rho(r)}{\partial N}\right), \tag{5}$$

where $\rho(r)$ is the electron density and $v(r)$is a constant external potential.

In order to describe the reactivity of an atom in a molecule, it is necessary to condense the values of $f(r)$ around each atomic site into a single value (f_k^a) that characterize the atomic contribution in a molecule.

As $\rho(r)$ is a discontinuous function of N, Yang and Mortier [34] have proposed approximated atomic indices $f(r)$ by applying the finite difference approximation to the condensed electronic population on any atom. These can be written as:

$$f_k^+ = \rho_k(N+1) - \rho_k(N) \rightarrow \text{for nucleophilic attacks}, \tag{6}$$

$$f_k^- = \rho_k(N) - \rho_k(N-1) \rightarrow \text{for electrophilic attacks}, \tag{7}$$

$$f_k^0 = \frac{1}{2}\left(f_k^+ + f_k^-\right) \rightarrow \text{for radical attacks}. \tag{8}$$

Parr et al. [35] introduced global electrophilicity index (ω) in terms of chemical potential (μ) and hardness (η) as

$$\omega = \frac{\mu^2}{2\eta}. \tag{9}$$

Recently, Chattaraj et al. [36] have defined a generalized concept of philicity associated with atomic site k in a molecule as:

$$\omega_k^\alpha = \omega . f_k^\alpha, \tag{10}$$

where α=+,-,0 refer to nucleophilic, electrophilic and radical attacks, respectively.

The condensed philicity summed over a group of relevant atoms is also known as "group philicity", and can be written as

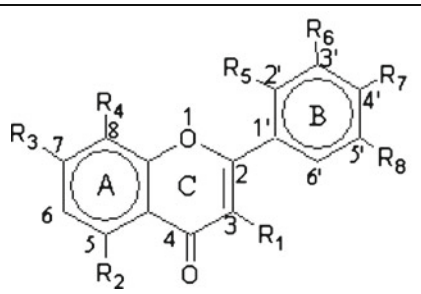
$$\omega_g^\alpha = \sum_{k=1}^{n} \omega_k^\alpha, \tag{11}$$

where the summation is over a group of n relevant atoms and α=+, -, 0 represents nucleophilic, electrophilic, and radical attacks, respectively.

c) Group frontier electron density (F_g)

Frontier electron density refers to the electron distribution associated with the two frontier orbitals, namely the HOMO and the LUMO. Over the years it has transpired that these

Table 1 Molecular structures of flavonoid molecules



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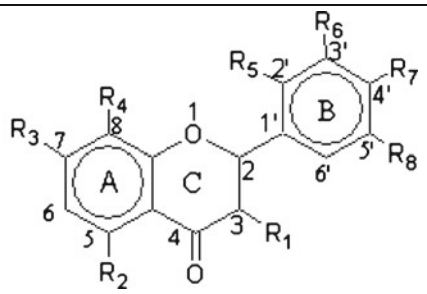
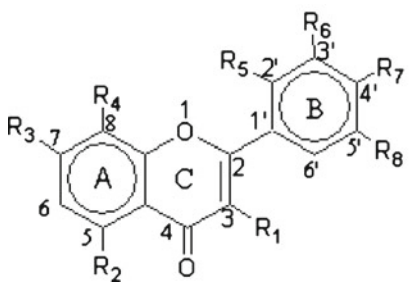


(24-29)

No	Name	R1	R2	R3	R4	R5	R6	R7	R8	RSA activity
1	**Kaempferol**	OH	OH	OH	H	H	H	OH	H	95.3
2	**Galangin**	OH	OH	OH	H	H	H	H	H	91.8
3	**Quercetin**	OH	OH	OH	H	H	OH	OH	H	89.8
4	**Kaempferol 3,7-dirh**	Orh	OH	Orh	H	H	H	OH	H	70.6
5	**Robinetin**	OH	H	OH	H	H	OH	OH	OH	82.3
6	**Fisetin**	OH	H	OH	H	H	OH	OH	H	79.0
7	**3-hydroxyflavone**	OH	H	H	H	H	H	H	H	66.0
8	**Laricytrin**	OH	OH	OH	H	H	OH	OH	OMe	84.6
9	**Laricytrin 3'-O-glucoside**	OH	OH	OH	H	H	Ogl	OH	OMe	83.8
10	**Myricetin**	OH	OH	OH	H	H	OH	OH	OH	72.8
11	**3,5,7,3',4',5' hexamethoxy flavone**	OMe	OMe	OMe	H	H	OMe	OMe	OMe	2.6
12	**Quercetin 3-O-glu-7-O-rha**	Ogl	OH	Orh	H	H	OH	OH	H	86.8
13	**Rutin**	Oru	OH	OH	H	H	OH	OH	H	90.9
14	**Morin**	OH	OH	OH	H	OH	H	OH	H	96.5
15	**Flavone**	H	H	H	H	H	H	H	H	01.5
16	**5-hydroxyflavone**	H	OH	H	H	H	H	H	H	00.6
17	**7-hydroxyflavone**	H	H	OH	H	H	H	H	H	02.8
18	**Crysin**	H	OH	OH	H	H	H	H	H	01.1
19	**8-methoxyflavone**	H	H	OMe	H	H	H	H	H	00.7
20	**Apigenin**	H	OH	OH	H	H	H	OH	H	00.7
21	**Vitexin**	H	OH	OH	gl	H	H	OH	H	21.0
22	**Apigenin 7-O-glucoside**	H	OH	Ogl	H	H	H	OH	H	34.8
23	**Luteolin 7-O-glucoside**	H	OH	Ogl	H	H	OH	OH	H	89.8

Table 1 (continued)



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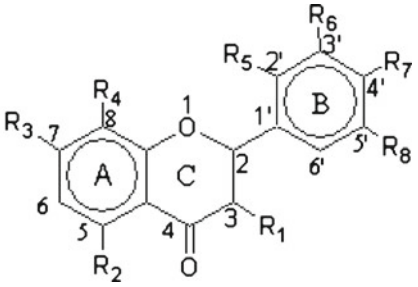

(24-29)

No	Name	R1	R2	R3	R4	R5	R6	R7	R8	RSA activity
24	**Flavanone**	H	H	H	H	H	H	H	H	02.6
25	**Naringenin**	H	OH	OH	H	H	H	OH	H	06.3
26	**Naringin**	H	OH	One	H	H	H	OH	H	04.7
27	**Hesperetin**	H	OH	OH	H	H	OH	OMe	H	30.0
28	**Fustin**	OH	H	OH	H	H	OH	OH	H	91.6
29	**Taxifolin (dih)**	OH	OH	OH	H	H	OH	OH	H	94.8

two molecular orbitals play a very important role in a wide range of chemical reactions of saturated or unsaturated compounds determining the reactivity of these compounds. To quantify the role of electron density of the frontier orbitals, Karelson et al. [37] introduced two reactivity indices, namely electrophilic frontier electron density (F_k^E) and nucleophilic frontier electron density (F_k^N) which are defined as

$$F_k^E = \frac{\sum (C_k^{HOMO})^2}{\Delta E} \times 100, \tag{12}$$

and

$$F_k^N = \frac{\sum (C_k^{LUMO})^2}{\Delta E} \times 100. \tag{13}$$

Here C_k^{HOMO} and C_k^{LUMO} are the coefficients of the atomic orbital of a particular atom in the HOMO and LUMO respectively. ΔE is the HOMO-LUMO energy gap.

The above definition of the frontier electron density is local in the sense that it takes into account the contribution of a single atom in the frontier orbital electron density. It transpired that, in case of flavonoids as the radical scavenging activity would mostly be governed by π-stacking interactions, instead of a single atom, a group of atoms associated with the π-ring will be responsible for the interaction between the favonoids and the radicals. So instead of the frontier electron density which is an atomic reactivity index one should use an index which is relevant for a set of atoms. Hence we extended Karelson's definiton of frontier electron density to a logically related set of atoms (such as a aromatic ring) by defining the group frontier electron density as the sum of the frontier electron densities over a group of atoms. In a sense our reactivity indices are semi-global being relevant not for the whole molecule or for a single atom in it, but it takes into account the contribution from a group of atoms that is part of the whole molecule and is relevant for intermolecular interaction.

Two reactivity indices characterizing a group of atoms for electrophilic and nucleophilic attack are named as electrophilic group frontier electron density (F_g^E) and nucleophilic group frontier electron density (F_g^N) given by

$$F_g^E = \sum_{i=1}^{n} F_i^E, \tag{14}$$

$$F_g^N = \sum_{i=1}^{n} F_i^N. \tag{15}$$

Here the summation extends over a group of n relevant atoms.

Table 2 Uncertainties, T-test and P values of Eqs. 16 and 17

	Equation 16			Equation 17		
Variables	Uncertainties	T-test values	P values	Uncertainties	T-test values	P values
Constant	54.48	8.20	0.000	28.43	16.63	0.000
η	1.10	−6.98	0.000	0.57	−13.98	0.000
F_A^E	0.03	−2.48	0.020	0.02	−6.16	0.000
ω_B^+	0.84	−2.96	0.007	0.45	−7.38	0.000

Computational details

A series of 29 flavonoids with the radical scavenging activity (RSA%) tested in a methanolic solution of DPPH ($R^{\bullet}$), were taken from the literature [38] and listed in Table 1. All the geometries of selected flavonoids have been fully optimized (supplementary materials) using the DFT method [39–43] with the help of Becke's three parameter hybrid density functional, B3LYP/6-31 G(d,p), which include both Hartree-Fock exchange and DFT exchange correlation functional

Table 3 DFT-based descriptors and predicted RSA (%) according to Eqs. 16 and 17

No.	Observed activity	Descriptors			Eq. 16		r_j^2	q_j^2	Eq. 17	
		η	F_A^E	ω_B^+	Predicted	Residual			Predicted	Residual
1	93.5	42.890	104.605	8.443	87.53	5.97	0.76	0.69	90.42	3.08
2	91.8	43.706	160.009	10.969	71.25	20.55	0.77	0.70	70.76	21.04
3	89.8	42.357	89.063	14.220	79.85	9.95	0.76	0.69	78.57	11.23
4	70.6	46.624	62.703	8.091	62.86	7.74	0.77	0.70	65.39	5.21
5	82.3	41.949	27.165	15.088	85.86	−3.56	0.76	0.91	85.19	−2.89
6	79.0	42.514	30.323	12.943	86.09	−7.09	0.77	0.70	86.88	−7.88
7	66.0	44.208	43.557	10.235	78.12	−12.12	0.77	0.70	80.20	−14.20
8	84.6	41.510	67.538	14.184	88.12	−3.52	0.76	0.69	87.60	−3.00
9	83.8	42.200	88.377	13.191	83.42	0.38	0.76	0.69	83.05	0.75
10	72.8	41.855	74.695	16.513	79.67	−6.87	0.76	0.69	77.01	−4.21
11	12.6	46.593	45.849	10.863	58.18	**−45.58**	**0.81**	**0.74**	-	-
12	86.8	44.741	0.000	4.633	90.01	−3.21	0.76	0.69	97.22	−10.42
13	90.9	43.267	0.000	8.077	93.61	−2.71	0.76	0.69	98.60	−7.70
14	96.5	41.478	68.181	13.949	88.85	7.65	0.76	0.69	88.52	7.98
15	1.5	51.832	94.379	15.714	3.19	−1.69	0.75	0.67	−3.01	4.51
16	0.6	45.714	404.573	17.178	22.78	−22.18	0.77	0.69	11.95	−11.35
17	2.8	52.272	62.395	15.267	3.31	−0.51	0.75	0.67	−2.11	4.91
18	1.1	46.781	383.863	16.657	17.36	−16.26	0.76	0.68	6.93	−5.83
19	0.7	51.926	119.188	14.873	2.43	−1.73	0.75	0.67	−3.60	4.30
20	0.7	47.471	314.999	17.484	15.56	−14.86	0.76	0.68	5.48	−4.78
21	21.0	45.745	365.331	13.146	34.67	−13.67	0.77	0.69	27.81	−6.81
22	34.8	47.565	328.783	9.685	31.30	3.50	0.77	0.69	27.22	7.58
23	87.6	47.220	270.070	18.601	18.48	**69.12**	**0.89**	**0.86**	-	-
24	2.6	55.315	330.518	0.329	−7.41	10.01	0.76	0.68	−6.94	9.54
25	6.3	52.962	379.316	0.647	6.17	0.13	0.76	0.68	6.38	−0.08
26	4.7	55.786	26.996	2.722	7.24	−2.54	0.75	0.77	11.26	−6.56
27	30.0	51.111	4.358	1.960	46.68	−16.68	0.77	0.70	53.53	−23.53
28	91.9	46.561	2.289	6.021	72.71	19.19	0.79	0.69	78.06	13.84
29	94.8	46.342	2.632	6.107	74.18	20.62	0.77	0.69	79.53	15.27

using GAUSSIAN 03 program [44]. The geometries of $R^{\bullet}$, RH and $FO^{\bullet}$ are optimized using the same method. The optimized geometries are characterized by harmonic-vibrational frequencies, which confirmed that the structures obtained are minimum on the potential energy surface. Various global and local reactivity descriptors are calculated from the Gaussian03 output file. The chemical hardness (η) has been calculated using Eq. 4. The group philicities (ω_k^q) have been calculated using Eq. 11 by summing the local electrophilicities of the constituent atoms of the aromatic rings A & B and the group frontier electron densities (F_g^E,F_g^N) have been calculated using Eq. 14 and Eq. 15 by summing the frontier electron densities of the constituent atoms of the aromatic rings A & B.

Results and discussion

QSAR analysis and model validation

QSAR models were derived by multiple linear regression (MLR) using observed free radical scavenging activities as the dependent variables and various combinations of the chosen descriptors as the independent variables. The quality of the model was considered as statistically satisfactory on the basis of number of data points (n), square of correlation coefficient (r^2), standard deviation (σ), population (P), F-statistics (F) and t-statistics (t).

The models obtained were validated by calculating the cross-validated squared correlation coefficient (q^2), which are calculated from "leave-one-out" (LOO) test [45, 46]. A data point is removed from the data set and the regression recalculated; the predicted value for that point is then compared to its actual value. This is repeated until each datum has been omitted once; the sum of squares of these deletion residuals can then be used to calculate q^2, an equivalent statistic to r^2. The q^2 values can be considered as a measure of the predictive power of a regression equation; whereas r^2 can always be increased artificially by adding more parameters (descriptors), q^2 decreases if a model is over-parameterized [47] and is therefore a more meaningful summary statistic for QSAR models. Many authors consider higher $q^2 > 0.5$ as an indicator that the model is highly predictive [48, 49].

To ascertain the relationship between chemical structures of selected flavonoid derivatives and RSA, we have generated various equations through different combinations of DFT based local and global reactivity descriptors (supplementary materials) keeping in mind that the number of descriptors should be as small as possible and have maximum correlation coefficient with RSA. Out of these generated equations, the significant one, developed from the descriptors: hardness (η), electrophilic group frontier electron density at the aromatic ring A (F_A^E) and group-philicity index (ω_B^+) at the aromatic ring B, having highest predictive power is given below:

$$\mathrm{RSA}(\%) = 444.59 - 7.69\eta - 0.078F_A^E - 2.25\omega_B^+$$
$$\mathrm{n} = 29, \mathrm{r}^2 = 0.768, \mathrm{q}^2 = 0.699, \mathrm{P} = 0.000,\ \mathrm{F} = 27.96, \sigma = 20.18 \quad (16)$$

and other statistical parameters are listed in Table 2. The prediction and deviations of regression are listed in Table 3.

In general, a regression model is significant at $P<0.001$ and $q^2 \geq 0.5$, so the above QSAR model is statistically significant. Further, the statistical result of Eq. 16 reveals

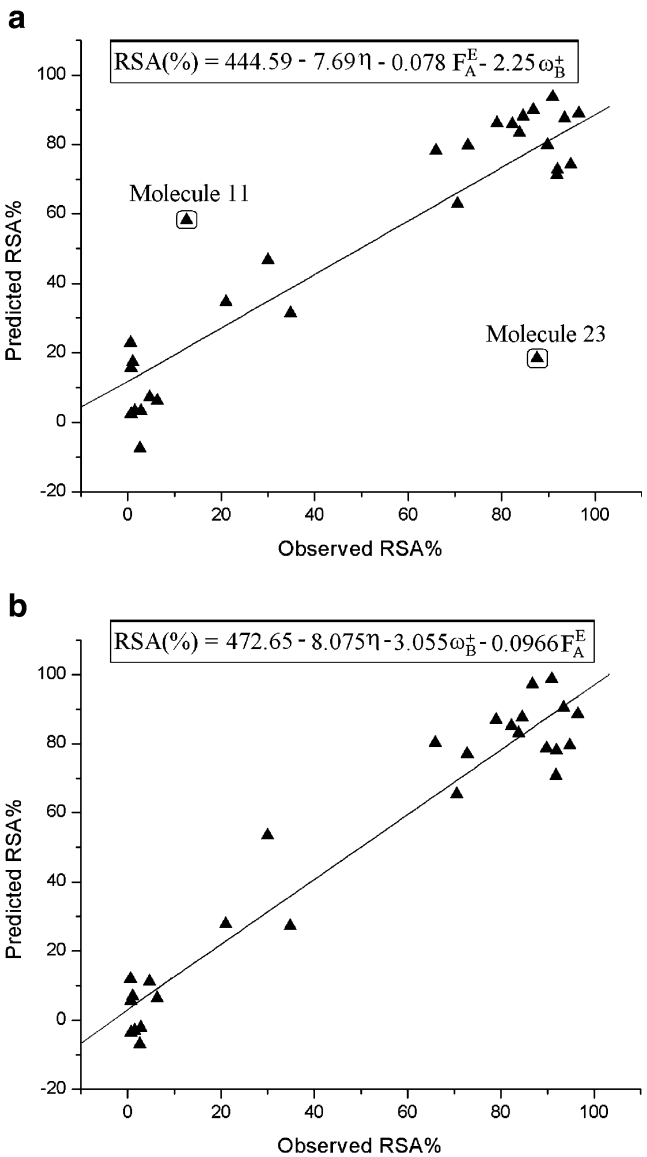

Fig. 3 (**a**) Plot of observed versus calculated RSA % as given in Eq. 16. Molecules 11 and 23 are two outliers (**b**) Plot of observed versus calculated RSA % as given in Eq. 17. Here two outlier molecules have been left out of the regression model

that the square correlation coefficient has borderline value and the standard error (σ= 20.18) is rather high. In order to obtain better QSAR we examine Table 3. Clearly, the residual prediction error for molecule 11 (3,5,7,3',4',5' hexamethoxy flavones) & 23 (Luteolin 7-O-glucoside) are 45.58 and 69.12, respectively, which are larger than two times of standard error (σ). Further, by inspecting the 'leave one out' regression and cross validation data (Table 3) also, we see that the square of correlation and cross-validation coefficients (r_j^2 *and* q_j^2) of molecules 11 and 23 are comparatively higher than the other r^2 and q^2 values, respectively. So compounds 11 and 23 may be considered as two outliers. Outliners are the molecules that have unexpected biological activity and are unable to fit in the QSAR model. Omitting molecules 11 and 23 from the regression model we have

$$\mathrm{RSA}(\%) = 472.65 - 8.075\eta - 3.055\omega_B^+ - 0.0966F_A^E \quad \mathrm{n} = 27,\ r^2 = 0.937,\ q^2 = 0.916,\ \mathrm{P} = 0.000,\ \mathrm{F} = 114.70,\ \sigma = 10.49 \tag{17}$$

and other statistical parameters are listed in Table 2.

We have seen that after removing the outliers r^2, q^2, F-test values increase from 0.768, 0.699, 27.96 to 0.937, 0.916, 114.70, respectively and the standard error (σ) decreases from 20.18 to 10.49. In addition, Table 2 shows the improvement of t-test and P values of the descriptors in Eq. 17. So the descriptors are statistically significant. The plot of the observed versus predicted RSA(%) values in Eq. 16 and Eq. 17 of flavonoid compounds are depicted in Fig. 3a and b.

The obtained QSAR based on the DFT shows that the lower the global hardness (i.e., a small energy gap between the HOMO and the LUMO) the higher the activity. Moreover, the negative coefficients of the F_A^E and ω_B^+ terms indicated that the higher activities are related to the lower electrophilic group frontier electron density of aromatic ring A and group electrophilicity of ring B.

The choice of the electrophilic group frontier density (F_A^E) as one of the descriptors is based on our experience during the development of DFT based QSAR. We observed that the frontier electrophilic electron density of every atom of ring A had some correlations with RSA. To make fewer number of descriptors, we introduce group frontier electron density descriptor which lead to a significant improvement in the correlation and its negative coefficient shows that the lower the electrophilic frontier electron density of ring A, the higher the activity, which shows that the ability of the $F_A^E$to have lower HOMO electron density or the decrement of electron donating capacity of ring A has an important effect on the antioxidant activity. To the best of our knowledge this is the first time that group frontier electron density is used as a descriptor. The physical significance of the descriptor is to elucidate the cooperativity of a planar ring as a whole not by a single atom. Here the decrement of electron donating capacity of ring A means the increment of π-type electron of the ring which favors the formation of the π-system in the singly occupied molecular orbital (SOMO) of flavonoid radicals, which stabilizes the radical through resonance. The quantum chemical descriptor helps to explain the contradictory results regarding the antioxidant activities of flavonoids. This shows that the activity depend on "group frontier electron density" descriptor not by any individual substituents on the rings, but the effect of the substituents as a whole on the planar ring via the F_A^E descriptor. Thus the new descriptor unequivocally explains

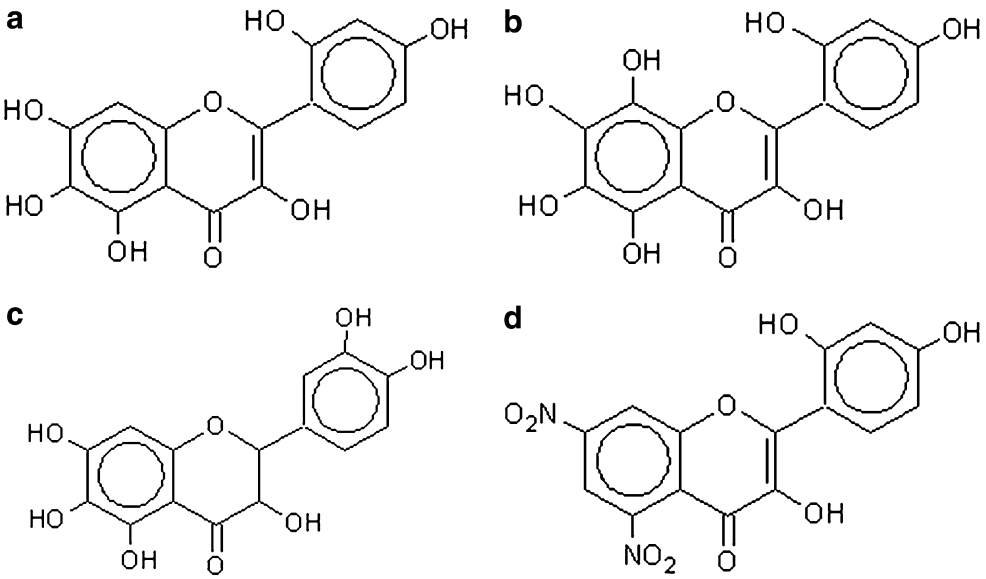

Fig. 4 Structural diagrams of four new designed antioxidants

Table 4 Computational results for four designed compounds

Compound	η	F_A^E	ω_B^+	RSA(%)
a	41.133	78.701	8.045	109.18
b	39.313	235.952	9.376	105.34
c	40.443	41.885	10.871	109.23
d	29.838	41.930	4.227	217.11

the controversies over the scavenging mechanism of flavonoid antioxidant in the sense that when one takes into account the contribution of a group of atoms in a ring then there exists no discrepancy.

Design of new compounds with higher activities

Based on the above discussion, we have seen that the lower values of three parameters η, $F_A^E$and ω_B^+ enhance the scavenging activities of flavonoids. On the basis of that fact, we have theoretically modeled several molecules and the best four are shown in Fig. 4. The parameters, calculated at the previous level of theory, are given in Table 4. The RSA (%) values predicted according to Eq. 17 show that the radical scavenging activities of four compounds are much greater than the flavinoids used in the study. Such results further indicate that our model established via the QSAR studies is significant and predictive, and that the consideration on the molecular design is also reasonable.

Explanation for the antioxidant activity of flavonoids

According to Eq. 1, antioxidants are oxidized by free radicals. Since the reaction is a dynamical process, the reaction kinetics can be written as

$$RH \rightleftharpoons R^{\bullet} + H^{\bullet} \tag{18}$$

$$FOH \rightleftharpoons FO^{\bullet} + H^{\bullet} \tag{19}$$

Now, according to front orbital theory in DFT [50], the higher the HOMO energy of a molecule, the easier they lose electrons and the reaction is faster for higher HOMO energy. Table 5 shows the HOMO energies of all FOH are greater than that of RH. This means reaction involving Eq. 19 is faster than Eq. 18. So, the forward reaction is faster than the backward reaction in Eq. 1.

On the other hand, the lower the LUMO energy of a radical, the easier it is to accept electrons. Table 5 shows that the LUMO energies of $FO^{\bullet}$ are all higher than that of $R^{\bullet}$. So the backward reaction of Eq. 18 with respect to the backward reaction of Eq. 19 is more favorable, which leads the forward reaction of Eq. 1 to be favorable.

Thus, higher HOMO of FOH and LUMO of $FO^{\bullet}$ makes the forward reaction of Eq. 1 highly favorable and FOH must show anti-oxidant activity.

Table 5 shows that the HOMO energies of four newly designed molecules (a, b, c, d) are all higher than that of

Table 5 HOMO & LUMO energies of flavonoids and their radicals in kcal mol^{-1}

Sl No	FOH		$FO^{\bullet}$		Sl No	FOH		$FO^{\bullet}$	
	HOMO	LUMO	HOMO	LUMO		HOMO	LUMO	HOMO	LUMO
1	−127.573	−41.7921	−15.7003	44.7226	18	−138.366	−44.8042	−20.5258	43.3170
2	−132.467	−45.0552	−17.1059	40.4493	19	−143.951	−40.0979	11.9039	55.5597
3	−126.192	−41.4784	−15.1795	44.6096	20	−135.793	−40.8509	−6.8148	39.4954
4	−133.660	−40.4116	−14.2256	41.4596	21	−130.585	−39.0938	−14.4264	30.1832
5	−123.745	−39.8469	−13.4664	33.9608	22	−136.421	−41.2901	−10.8371	37.6255
6	−124.059	−39.0311	−15.9827	33.8604	23	−135.228	−40.7881	−10.3351	38.1463
7	−133.660	−45.2434	−10.8810	46.0090	24	−146.210	−35.5798	6.9465	69.8042
8	−124.372	−41.3529	−15.6124	44.5344	25	−138.805	−32.8815	−5.6727	48.7136
9	−129.079	−44.6787	−18.8943	38.6107	26	−132.655	−21.0843	−6.5135	49.7992
10	−125.941	−42.2314	−16.0078	44.1516	27	−133.660	−31.4382	−4.7189	53.8654
11	−128.388	−35.2033	−16.0078	44.1516	28	−129.455	−36.3328	7.5176	21.7056
12	−132.279	−42.7962	−18.7123	37.0733	29	−131.526	−38.8428	−13.9433	56.5512
13	−129.267	−42.7334	−17.8401	38.5165	Designed Molecules				
14	−127.384	−44.4277	−20.5258	43.3170	a	−126.506	−44.2394	−17.6330	41.7984
15	−146.335	−42.6707	−29.9636	35.3853	b	−124.749	−46.1219	−14.0311	39.4390
16	−138.178	−46.7495	−8.1890	34.0926	c	−125.376	−44.4904	−15.0665	56.0993
17	−145.331	−40.7881	−14.4264	30.1832	d	−141.315	−81.6390	−35.9312	−34.9774

*RH: HOMO=−153.9155; LUMO=73.3057; $R^{\bullet}$: HOMO=−38.7110, LUMO=16.6353

RH and LUMO energies of the radicals of a, b, c and d are all higher than that of R$^{\bullet}$. These statistics confirm that the newly designed four molecules can neutralize the free radical according to Eq. 1.

Conclusions

The QSAR studies of a series of flavonoids have been carried out by using the conceptual density functional theory (DFT). Out of many equations generated, an optimal QSAR equation with three descriptors η, F_A^E, and ω_B^+ have been found to be main independent factors determining the antioxidant activity. The most significant outcome of the work is the introduction of a new descriptor namely, "group frontier electron density descriptor". The QSAR equation indicates that the group frontier electron density is a useful descriptor in determining the antioxidant radical scavenging activity.

The current study has shown that the use of DFT-based quantum chemical descriptors indeed led to a better molecular insight. From the analysis of the QSAR equation, it can be concluded that in order to get better radical scavenging activity, the hardness, group electrophilic frontier electron density of ring A, and group electrophilicity of ring B should be decreased. In other words, electron-donating capacity of ring A and electron-accepting capacity of ring B should be reduced. This descriptor explains the contradictory results regarding the antioxidant activities of flavonoids. It depends not on any individual substituents but the effect of the substituents as a whole on the planar ring via the F_A^E descriptor.

Based on the QSAR equation we have designed four new antioxidants which have a very high radical scavenging ability. So it can be expected that our result should not only help in design of new antioxidants which will prevent free radicals from acting but also facilitate the QSAR study of π-system stabilization in radical form.

In conclusion, the excellent QSAR results for flavonoids were obtained using important quantum chemical descriptors based on DFT method. The reason for the highest activity of compound 14 can also be explained rationally. The cross validation using the LOO method shows the QSARs model is reliable. Moreover, based on the QSAR equation, four new antioxidants were designed with radical scavenging activity. Therefore, DFT based QSARs could be expected to help to facilitate the future design of additional substituted flavonoids with good antioxidant activity.

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