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Reliability of bond dissociation enthalpy calculated by the PM6 method and experimental TEAC values in antiradical QSAR of flavonoids

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ABSTRACT

The applicability of the newly developed RM1 and PM6 methods implemented in the semiempirical quantum chemistry MOPAC2009™ software package in modeling free radical scavenging activity of flavonoids was examined. Bond dissociation enthalpy (BDE) of OH groups could be calculated much faster than with DFT method but with similar quality. Despite the known shortcomings of the Trolox equivalent antioxidant capacity (TEAC) assay, we show that taking into account the hydrogen atom transfer (HAT) mechanism of free radical scavenging of flavonoids encoded by minimal BDE values (BDE_{min}) and the number of OH groups (*n*OH), as well as experimental data, reasonable QSAR models could be developed. For TEAC values of 38 flavonoids measured by the ABTS free radical, a model based on BDE_{min} and *n*OH was developed, having very good statistical parameters ($r = 0.983$, $r_{cv} = 0.976$). The applicability of this model to three different data sets of flavonoids and reliability of TEAC values measured in distinct laboratories were discussed. Finally, a reasonably good model of experimental vitamin C equivalent antioxidant capacity (VCEAC) of 36 flavonoids was obtained ($r = 0.954$, $r_{cv} = 0.947$), involving BDE_{min} and *n*OH as descriptors. Additionally, all presented models have comparable fit and cross-validated statistical parameters, as well as significant regression coefficients.

1. Introduction

Oxidative stress induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as the superoxide anion radical $O_2^{\cdot -}$, hydroxyl radical ·OH, lipid peroxyl radical LOO·, or peroxynitrite $ONOO^-$, can cause damage to cellular proteins, membrane lipids and nucleic acids, where this process has been implicated in the pathogenesis of various diseases, including coronary heart disease and some forms of cancer.[1] Flavonoids are natural polyphenolic multifunctional antioxidants capable of eliminating ROS and RNS by scavenging free radicals, chelating metal ions, inhibiting prooxidant enzymes and activating antioxidant and detoxifying enzymes. Due to their protective effects, flavonoids are recognized as potential drug candidates to be used in the treatment of diseases such as cancer, atherosclerosis, cardiovascular and coronary heart diseases, as well as neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and other age-related diseases.[2]

The scavenging of free radicals seems to play a notable part in the antioxidant activity of flavonoid compounds. Antiradical properties of flavonoids (FlO–H) are related to their ability to transfer their phenolic H-atom to a free radical (e.g., alkoxyl radical, RO·). The formal H-atom abstraction from flavonoids described by:

$$\mathrm{FlO{-}H} + \mathrm{RO^{\cdot}} \rightarrow \mathrm{Fl{-}O^{\cdot}} + \mathrm{ROH} \qquad \text{(i)}$$

is known to involve complex processes. It has been recognized that this reaction proceeds via at least four different mechanisms: single-step hydrogen atom transfer (HAT), sequential proton loss electron transfer (SPLET), proton coupled electron transfer (PCET), and stepwise electron transfer followed by proton transfer (ET-PT).[3,4] These mechanisms may co-exist, and depend on solvent and radical characteristics. The net result from all mechanisms is the same, that is, as it is given in reaction i.

According to the current knowledge of the radical scavenging processes of flavonoid antioxidants, the single-step HAT seems to be the simplest mechanism.[5] In the HAT mechanism, the hydrogen atom (proton together with the one of its two bonding electrons) is

Abbreviations: ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AM1, Austin Model 1; BDE, bond dissociation enthalpy; CV, cross-validation; DFT, density functional theory; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EF, eigenvector following; ET-PT, electron transfer proton transfer; HAT, hydrogen atom transfer; LOO, leave-one-out; PCET, proton coupled electron transfer; PM3, parametric method 3; PM6, parametric method 6; QSAR, quantitative structure–activity relationship; QSPR, quantitative structure–property relationship; RM1, Recife Model 1: a reparameterization of the AM1; RNS, reactive nitrogen species; ROS, reactive oxygen species; SPLET, sequential proton loss electron transfer; TEAC, Trolox equivalent antioxidant capacity; VCEAC, vitamin C equivalent antioxidant capacity.

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transferred to the free radical. This reaction, which prevails in nonpolar solvents, is depicted in Scheme 1.

The product of this reaction is a stable flavonoid phenoxyl radical (Fl−O·). The hydrogen atom donating ability of flavonoids to scavenge free radicals can be characterized by the bond dissociation enthalpy (BDE) of OH group.[3] In the HAT mechanism, the BDE value of the O–H bond is an important parameter in evaluating the antiradical action, because the weaker the O–H bond the faster the radical inactivation. Stability of generated flavonoid phenoxyl radical corresponds to a better efficiency of the flavonoid antioxidant. To be effective, Fl−O· must be a relatively stable free radical, so that it reacts slowly with a substrate but rapidly with RO·. Generally, intramolecular hydrogen bonds, expanded electron delocalization, and resonance stabilization contribute to the stability of Fl−O·.[6]

Numerous authors have investigated the antioxidant activity of flavonoids, and many attempts have been made to establish the relationship between flavonoid structure and their radical scavenging activity.[7] This activity depends mainly on the substitution pattern of the hydroxyl groups, that is, on the availability of phenolic hydrogens and on the possibility of stabilizing the resulting flavonoid phenoxyl radicals. The structural requirements considered essential for effective radical scavenging by flavonoids are the presence of 3′,4′-dihydroxy group in the B ring and/or the presence of the 3-OH group in the C ring. In addition, the 5-OH group in combination with a 4-oxo moiety and C2–C3 double bond may increase the radical scavenging activity.[6,8]

Published structure–antioxidant activity studies containing BDEs for OH groups of flavonoids, calculated by the DFT method, for data sets larger than 10 compounds, are very rare. In this study, we are dealing with six data sets, each containing between 23 and 40 flavonoids. Due to the exceeding computational times required for DFT calculations of BDEs of flavonoids, and due to very good accuracy of newly developed semiempirical methods implemented in the MOPAC2009™ program, we used the latter for building antiradical QSAR of flavonoids. In addition, we also analyzed the dependence of quality of the obtained model on different series of experimental flavonoid antiradical values, measured in different laboratories.

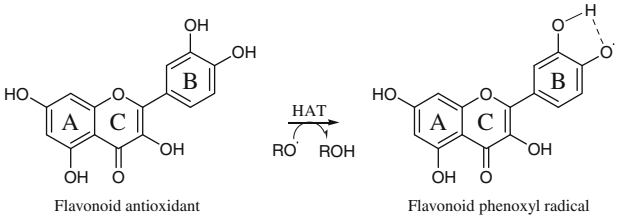
2. Materials and methods

2.1. Bond dissociation enthalpy

The ability of flavonoid antioxidants to donate a hydrogen atom is mainly governed by the O–H BDE value. The O–H BDE can be calculated by the following equation:[3]

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

where $\Delta_f H(\mathrm{Fl{-}O^{\cdot}})$ is the enthalpy of formation of the flavonoid phenoxyl radical generated after H· abstraction, $\Delta_f H(\mathrm{H})$ is the enthalpy of formation of the hydrogen atom, and $\Delta_f H(\mathrm{Fl{-}OH})$ is the enthalpy of formation of the parent flavonoid molecule. A lower BDE value is usually attributed to a greater ability to donate a hydrogen atom from the hydroxyl group and resulting in an easier free radical scavenging reaction. Wright et al.[3] have suggested that BDE is an excellent primary descriptor of the antioxidant activity. The catechol moiety in the B ring of flavonoids has the advantage for scavenging free radicals because of a relatively low BDE value for O–H.[9]



Scheme 1.

2.2. Quantum chemical calculations

Structural optimization was performed by the RM1 and PM6 methods implemented in the semiempirical quantum chemistry MOPAC2009™ software package.[10] The RM1 method is a reparameterization of AM1, that is, all RM1 parameters are optimized.[11] As a result, RM1 is a significant improvement and is much more precise than the previously used AM1 and PM3 methods.[10] A new and more accurate parameterization using experimental and *ab initio* data has been implemented in the PM6 method.[12] The PM6 method is able to predict geometries and heat of formation consistent with DFT results and experimental observations, as is shown on a large set of molecules.[10] Generally, semiempirical methods are several orders of magnitude faster than more accurate DFT methods. The latter are more time consuming, especially in the case of glycosylated flavonoids. The speed of MOPAC2009™ and improved accuracy of RM1 and PM6 are particularly valuable for generating electronic descriptors for quantitative structure–activity relationships (QSAR) and quantitative structure–property relationships (QSPR). Recently, Puzyn et al.[13] recommended the use of the semiempirical PM6 and RM1 methods in QSAR/QSPR studies instead of the much more expensive DFT method. They found that QSPR models based on PM6 and RM1 descriptors are of similar quality to DFT-based models but can be built in a significantly shorter time. In the present work, we examined applicability of the newly developed RM1 and PM6 methods in modeling the free radical scavenging activity of flavonoids.

The RM1 and PM6 geometry optimizations of flavonoids (minimal energy conformation) were carried out using the eigenvector following (EF) optimization procedure with a final gradient norm of the energy gradient less than 0.05 kcal/mol (PRECISE). The enthalpy of formation of the flavonoid phenoxyl radical generated after H· abstraction from selected O–H group and enthalpy of formation of the parent flavonoid molecule were calculated using the PM6 method.

2.3. Statistical parameters and validation procedure

Regression coefficients and the corresponding errors of regression coefficients were computed using the least-square fit procedure. We expressed the statistical performance of fit, and of leave-one-out (LOO) cross-validation (CV) for each developed model by the correlation coefficient of fit (r) and cross-validation (r_{cv}). We also calculated the standard error of fit (s) and of LOO CV (s_{cv}), both by using N in the denominator (N is the total number of compounds used in data set). The Fisher F-value is also reported for each model.

3. Results and discussion

As a starting point in our study, we used quercetin, the most investigated flavonoid by quantum chemical calculations. Among the descriptors useful in modeling the antiradical activity of flavonoids, the BDE of OH groups is of particular relevance. Radical scavenging potency of flavonoids is mostly related to the presence of O–H groups at specific positions on the flavonoid core. The minimal value of the BDE of O–H bonds ($\mathrm{BDE_{min}}$) indicates which O–H group on the flavonoid core posses the most abstractable hydrogen, that is, which O–H group is targeted for radical attack. The O–

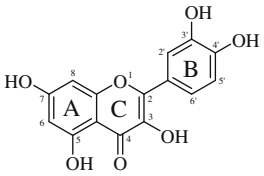
H BDEs of quercetin has been calculated by several research groups using different quantum chemical methods.[14–22] We have summarized previous results in Table 1, together with results obtained in this study.

As can be seen from Table 1, the DFT calculations identified the 4′-OH group of quercetin as the group with BDE_{min}. BDE values in Table 1 corresponding to BDE_{min} are presented in bold. The average sequence of BDEs for the OH groups is 4′-OH < 3′-OH < 3-OH < 7-OH < 5-OH. This indicates that vicinal OH groups on the B-ring and 3-OH group of the C-ring are preferred sites for hydrogen abstraction. The PM6 method gives results consistent with DFT. Exceptions are DFT results of Anbazhagan et al.[18] and Sadeghipour et al.[20] as well as the results obtained using the less accurate AM1 and PM3 methods.

Usually, previously published studies dealing with the BDEs of flavonoids using the DFT approach investigated a limited number of flavonoid aglycones.[23–26] Those dealing with less accurate semi-empirical methods focused on larger numbers of aglycones and glycosides.[27,28] Constraints on computational resources have restricted the accurate calculations of BDE of OH groups to a pool of several flavonoids. Recently Kozlowski et al.[15] published O–H BDEs for a set of 11 flavonoids predicted by DFT, and Li et al.[17] published ONIOM results for set of 8 flavonoids. Their results are presented in Table 2 along with our PM6 results. Bold values indicate BDE_{min}. It can be safely seen from Table 2, as well as from Figures 1 and 2, that MOPAC2009™ PM6 results are consistent with ONIOM, as well as with DFT results. As a rule, ONIOM calculations overestimate results obtained using DFT while the PM6 method underestimates them.

BDE_{min} could serve as a theoretical measure for ranking flavonoids as antioxidants because most active flavonoids possess lower values of BDE_{min}. The TEAC (Trolox equivalent antioxidant capacity) assay is frequently used for assessing antioxidant activity and developing structure–activity relationships (SARs) of flavonoids.[8,29–33] The TEAC assay is based on the scavenging of the ABTS radical and converting it into a colorless product. The TEAC is defined as the millimolar concentration of a Trolox solution with the same antioxidant activity as a 1.0 mM concentration of the

Table 1
Comparison of BDE (kcal/mol) values for all OH positions in quercetin (gas phase) calculated by different quantum chemical methods



Method	Ref.	BDE				
		3-OH	5-OH	7-OH	3′-OH	4′-OH
DFT	14	83.7	99.3	88.6	77.0	**74.6**
DFT	14	79.7	94.7	84.4	73.6	**71.1**
DFT	15	83.7	99.5	88.2	77.0	**74.6**
DFT	16	77.28	89.41	88.10	81.41	**76.63**
ONIOM	17	85.5	99.9	93.2	81.8	**78.7**
DFT	18	**73.12**	86.66	87.27	74.88	77.39
DFT	19	83.36	97.40	89.45	86.47	**75.20**
DFT	20	95.59	110.4	98.06	**88.13**	94.87
AM1	21[a]	**83.27**	99.07	97.77	85.59	85.37
AM1	22[a]	**83.10**	98.77	98.87	83.96	83.90
PM6	This study	74.84	87.14	89.89	77.49	**70.09**
PM3	This study	**67.76**	78.45	80.36	74.35	70.60

[a] BDEs were originally expressed without enthalpy correction for hydrogen atom.

Table 2
Comparison of BDE (kcal/mol) values for all OH positions of 13 flavonoids (gas phase) calculated by different quantum chemical methods

	Compound		BDE[a] kcal/mol						
			3	5	6	7	2′	3′	4′
1	Quercetin	DFT	83.7	99.6		88.3		77.0	**74.6**
		ONIOM	85.5	99.9		93.2		81.8	**78.7**
		PM6	74.84	87.14		89.89		77.49	**70.09**
2	Taxifolin	DFT	108.1	99.8		92.2		76.1	**76.1**
		PM6	104.81	96.15		94.24		78.11	**73.07**
3	Eriodictyol	DFT		102.0		91.1		76.5	**76.3**
		PM6		97.98		92.87		78.66	**73.59**
4	Luteolin	DFT		103.9		89.1		77.9	**76.4**
		ONIOM		105.6		94.3		97.9	**80.8**
		PM6		88.04		89.04		79.16	**73.51**
5	Catechin	DFT	103.8	83.1		85.2		77.3	**76.6**
		PM6	101.75	81.47		81.49		77.61	**72.28**
6	Morin	DFT	**83.2**	99.7		88.6	87.0		86.1
		ONIOM	**85.1**	100.4		93.5	90.6		89.7
		PM6	**76.87**	87.31		89.79	78.56		77.86
7	Kaempferol	DFT	**83.7**	99.0		88.4			83.8
		ONIOM	**85.8**	99.9		93.4			86.5
		PM6	**76.14**	87.97		90.39			76.39
8	Galangin	DFT	**84.8**	99.0		89.2			
		PM6	**76.02**	87.76		90.34			
9	Apigenin	DFT		103.7		89.4			**86.1**
		ONIOM		105.4		94.3			**88.8**
		PM6		88.11		88.91			**81.13**
10	Naringenin	DFT		101.9		104.7			**86.1**
		PM6		88.06		90.49			**80.43**
11	Chrysin	DFT		103.6		**89.8**			
		PM6		99.48		**91.85**			
12	Myricetin	ONIOM	85.0	99.6		93.5	—	**72.5**	73.8
		PM6	74.02	87.18		89.78	72.37	**71.08**	73.08
13	Baicalein	ONIOM		96.5	**80.9**	102.5			
		PM6		73.52	**72.30**	75.64			

[a] DFT values were taken from Ref. 15; ONIOM values were taken from Ref. 17.

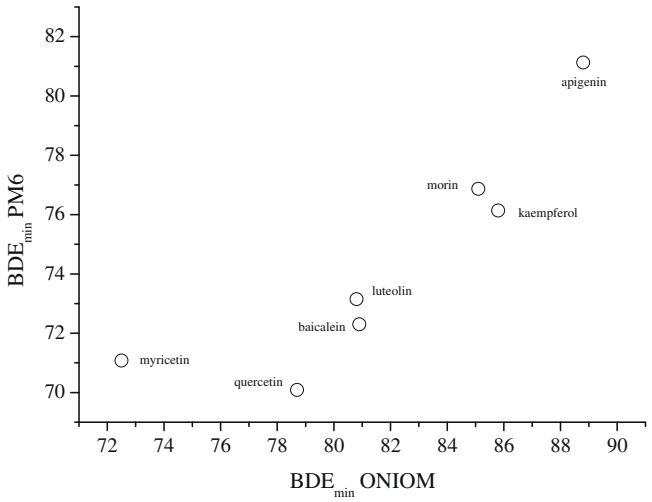


Figure 1. Scatter plot of BDE_{min} values calculated by the PM6 method versus BDE_{min} calculated by the ONIOM method (Ref. 17).

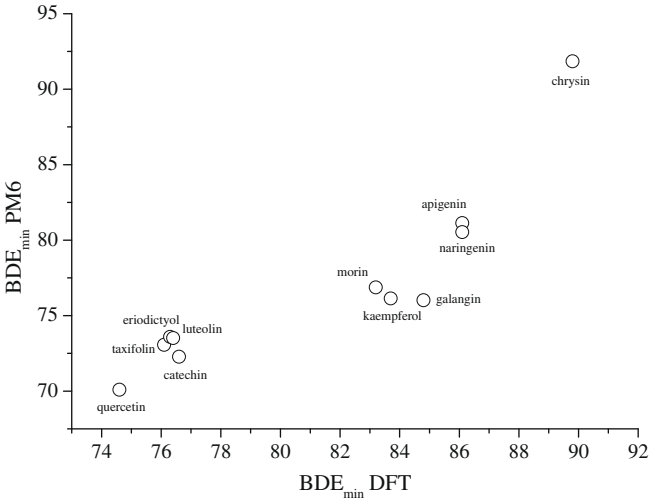


Figure 2. Scatter plot of BDE_{min} values calculated by the PM6 method versus BDE_{min} calculated by the DFT method (Ref. 15).

substance under investigation. It was reported by van den Berg et al.[32] that TEAC values of the series of flavonoids correlate well with the number of aromatic hydroxyl groups, *n*OH. Recently, however, it has been found that reaction products might contribute to the TEAC value of a compound, limiting the applicability of this assay in constructing SAR models.[34,35]

The development of QSAR models is a procedure based on some generally accepted principles related to the correct selection and partition of data, selection of model parameters, testing the model complexity and robustness, model validation, and model accuracy estimation.[36,37] In order to be applicable, model simplicity and proper validation are eligible, and its parameters should possess clear physicochemical interpretation. Models presented in this study are simple linear regression models including one or two parameters with clear physicochemical meaning.

It is generally agreed upon that the major role in free radical scavenging is that of aromatic hydroxyl groups attached to flavonoid nuclei. It seems that OH groups of attached sugars, as well as hydrogens attached to flavonoid core, do not contribute to scavenging potency. Some conflicting results have been published regarding the possibility of allylic hydrogen abstraction.[24] The number of hydroxyl groups and, especially, their position could be essential for radical scavenging. The increasing *n*OH could be related to the increasing ability of H atom abstraction and enhanced scavenging of free radicals. Weakly reactive flavonoid phenoxyl radicals formed by the H atom abstraction are stabilized by hydrogen bonds: thus, a favorable position of the OH groups, like the 3′,4′-diOH moiety in the B ring or 3-OH in conjunction with the C-4 keto group, could be a prerequisite for the stability of flavonoid phenoxyl radicals. In addition, stabilization of flavonoid phenoxyl radicals to semiquinone structures is achieved by suitable arrangement of OH groups.[7]

The TEAC values determined by the ABTS radical used for development and validation of QSAR models are collected from results of four different research groups: Cai et al. (values obtained also by DPPH radical),[38] Rice-Evans et al.,[8,29] Soobrattee et al.,[39] and Ishige et al.[40] Experimental conditions used in these studies were not identical, and full description of methods can be found in corresponding articles.[8,29,38–40] Numerical TEAC values are shown in Table 3 along with BDE_{min} calculated by MOPAC2009™ software package, as well as *n*OH.

We started QSAR modeling using data set of 100 phenolic compounds by Cai et al.[38] The authors performed TEAC measurements based on ABTS and DPPH free radicals (carried out in triplicate for both assays) and obtained high correlation between these two assays ($r = 0.995$). Though, this reported correlation coefficient should be taken with caution because distributions of ABTS and DPPH TEAC values deviate significantly from normal distributions.[37] From the data set of 100 polyphenols, we extracted all flavonoids. This set of 40 flavonoids taken from Ref. 38 was used in the modeling (Table 3). It includes diverse classes of flavonoids, that is, flavones, flavonols, isoflavones, flavanones, catechins, procyanidins and chalcones. Among these, 13 compounds are glycosides. It should be noted that calculations using MOPAC2009™ are very fast; for example, the computational time required for calculating the final heat of formation of flavonoid diglycoside is less than 60 s. More accurate DFT calculations for such molecules are more complex and time consuming.

A scatter plot between the experimental TEAC values for ABTS radical and calculated BDE_{min} for set of 40 flavonoids from Ref. 38 is shown in Figure 3. From this set of TEAC data given in Table 3 values of four compounds identified as outliers were omitted (chrysin and three procyanidins). A modest linear relationship ($r = -0.850$) indicates a general trend that higher TEAC is associated with lower BDE_{min}. In this report, all BDEs were calculated in the gas phase. For more accurate results solvent effects, anions derived from proton dissociation (i.e., acidity of hydroxyl groups), as well as intramolecular hydrogen bonding should be taken into account.[41,42]

We also performed a test of significance of the obtained correlation coefficient between TEAC and BDE_{min} in order to be sure that the obtained r value is not due to high chance correlation. One thousand random descriptors, each time ordered in a different random order and having 38 values each, were generated by the MS Excel program and each of them was correlated with the experimental TEAC values. Among 1000 calculated correlation coefficients, all values were between −0.45 and +0.45. This simple test clearly shows that descriptor BDE_{min} correlates with the TEAC significantly above the level of random correlation for this number of cases (i.e., 38 molecules). Because Eqs. 1–5 contain between 36 and 38 compounds, the described test and conclusions regarding random correlation are valid for all models given in this study.

The number of phenolic OH groups (*n*OH) attached to the flavonoid core seems to be essential for radical scavenging.[32] By using *n*OH as the second molecular descriptor and excluding two outliers (myricetin and chrysin) we obtained the two-descriptor model given by Eq. 1:

Table 3
Experimental TEAC values, calculated O–H bond dissociation enthalpies (kcal/mol), and number of OH groups

	Compound	TEAC[a]	TEAC[b]	TEAC[c]	TEAC[d]	DPPH[e]	BDE_{min}[f]	*n*OH[g]
1	Apigetrin	0.083				0.050	81.18	2
2	Glycitein	0.097				0.020	78.03	2
3	Naringin	0.098				0.077	80.52	2
4	Formononetin		0.11				85.96	1
5	Astralagin	0.138				0.151	80.26	3
6	Isovitexin			0.15			82.35	3
7	Vitexin	0.216				0.205	82.24	3
8	Flavonol	0.707			1.06	0.484	76.08	1
9	Narirutin		0.76				81.72	2
10	Luteolin-3′,7-diglucoside		0.79				79.38	2
11	Myricitrin			0.89			73.49	5
12	6-Hydroxyflavone				0.95		78.65	1
13	Orientin			1.05			74.09	4
14	Hesperidin	0.104	1.08			0.075	76.23	2
15	Daidzin	0.072	1.15			0.035	78.36	1
16	Biochanin A		1.16				88.07	2
17	Genistin	0.077	1.24			0.026	78.25	2
18	Daidzein	0.101	1.25			0.033	78.34	2
19	Rhamnetin			1.27			71.88	4
20	Pelargonidin		1.30				70.28	3
21	Kaempferol	1.59	1.34	1.03	1.45	1.32	76.14	4
22	Hesperetin	0.403	1.37			0.268	77.35	3
23	Dihydrokaempferol		1.39				80.04	4
24	Chrysin	0.081	1.43		2.52	0.053	91.85	2
25	Apigenin	0.086	1.45	2.01	2.80	0.041	81.13	3
26	Luteolin-7-glucoside	1.47				1.39	73.57	3
27	6-Hydroxyflavanone				1.47		76.90	1
28	Galangin	1.12	1.49		2.08	0.705	76.02	3
29	Naringenin	0.217	1.53	0.13	2.48	0.136	80.43	3
30	Baicalin	1.55				1.79	73.92	2
31	Quercetin-3-glucoside-7-rhamnoside	1.56				1.63	72.18	3
32	3,7-Dihydroxyflavone				1.65		76.13	2
33	Luteolin-4′-glucoside		1.74				74.03	3
34	Oenin		1.78				72.83	2
35	Phloretin	1.79				1.24	78.36	4
36	Eriodictyol		1.80				73.59	4
37	Taxifolin		1.90		3.09		73.07	5
38	Sappanchalcone	1.93				1.82	72.17	3
39	Malvidin		2.06				70.09	3
40	3,6-Dihydroxyflavone				2.06		75.71	2
41	Luteolin	2.18	2.10	2.23	2.48	2.24	73.51	4
42	Quercitrin	2.18		1.75		2.57	72.34	4
43	Peonidin		2.22				69.03	3
44	Hyperoside			2.33			70.86	4
45	Apigenidin		2.35				74.57	2
46	Quercetin-3-glucoside	2.39		2.72		2.16	72.66	4
47	Catechin	3.04	2.40	3.16	3.42	2.95	72.28	5
48	Rutin	2.02	2.40		2.67	2.33	73.09	4
49	Butein	2.42				2.27	71.56	4
50	Epicatechin	3.08	2.50	3.58	3.16	3.18	72.75	5
51	Morin	2.68	2.55		2.60	2.75	76.87	5
52	Baicalein	2.56			1.22	2.74	72.30	3
53	Isorhamnetin			2.69			71.73	4
54	Fisetin				2.80		70.32	4
55	Ideain		2.90				70.70	3
56	Genistein	0.123	2.90	0.32	2.96	0.095	77.99	3
57	Myricetin	1.31	3.10	3.07	3.08	1.38	71.08	6
58	Keracyanin		3.25				67.39	3
59	Epigallocatechin	3.71	3.80	3.86		3.56	69.41	6
60	Cyanidin		4.40	0.96	3.63		69.57	4
61	Delphinidin		4.44				67.23	5
62	Quercetin	4.42	4.70	3.68	4.84	4.60	70.09	5
63	Epigallocatechin gallate	5.95	4.80	4.39		6.08	69.01	8
64	Epicatechin gallate	5.29	4.90	4.23		5.26	69.04	7
65	Catechin-3-gallate	5.25			5.12	5.56	69.09	7
66	Procyanidin B-1	6.14		6.55		5.94	72.21	10
67	Procyanidin B-2			7.58			72.35	10
68	Procyanidin C-1	8.29				7.93	71.62	15
69	Procyanidin B-2 digallate	9.18				8.79	72.85	14

[a] Data set 1: TEAC values were taken from Ref. 38—ABTS assay.
[b] Data set 2: TEAC values were taken from Refs. 8,29.
[c] Data set 3: TEAC values were taken from Ref. 39.
[d] Data set 4: TEAC values were taken from Ref. 40.
[e] Values were taken from Ref. 38—DPPH assay.
[f] BDE_{min} (kcal/mol) is the minimal O–H bond dissociation enthalpy for a flavonoid.
[g] *n*OH is the total number of phenolic hydroxyl groups in a molecule.

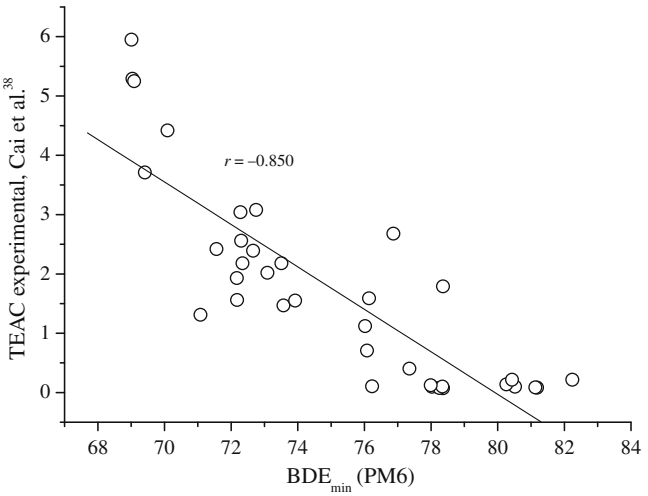


Figure 3. Scatter plot of experimental TEAC values obtained by the ABTS radical versus calculated BDE_{min} for data set of 40 flavonoids from Ref. 38 (four outliers were omitted).

$$\mathrm{TEAC} = 14.598(\pm 1.734) + 0.588(\pm 0.028)n\mathrm{OH} - 0.199(\pm 0.022)\mathrm{BDE}_{\min} \quad N = 38,\ r = 0.983,\ r_{cv} = 0.976,\ s = 0.424,\ s_{cv} = 0.507,\ F = 504.63 \tag{1}$$

In Figure 4, we give the plot of experimental TEAC for ABTS radical from Ref. 38 versus TEAC values calculated by Eq. 1.

One can see that each regression coefficient is eight times (or more for descriptor *n*OH) higher than the corresponding error of regression coefficient (given in brackets), confirming significance of the related descriptor. This is also a prerequisite for model stability. Differences between fitted and cross-validated statistical parameters are relatively small, confirming that the model given by Eq. 1 is of good quality.

Descriptors used in the construction of the QSAR model (1) possess a clear physicochemical meaning and reflect some driving forces related to antiradical activity. An increased number of OH groups could be related to the increased ability of H atom abstraction and increased scavenging of free radicals. Flavonoid phenoxyl radicals formed by abstraction of the H atom are stabilized by hydrogen bonding. Increasing the number of OH groups offers more possibilities for hydrogen bonding. Because of the negative sign of the regression coefficient, increase of BDE_{min} values results in a decrease of TEAC values. It should be noted that negative correlation exists between BDE_{min} and *n*OH, that is, lower BDE_{min} values are associated with larger number of OH groups on flavonoid core. A negative correlation between BDE_{min} and *n*OH can be explained by the electron donating effect of the other OH groups, resulting in a weakening of the O–H bond studied.[43]

Using TEAC values measured by the DPPH assay,[38] we obtained Eq. 2 of similar quality to Eq. 1:

$$\mathrm{TEAC} = 16.527(\pm 1.931) + 0.555(\pm 0.031)n\mathrm{OH} - 0.223(\pm 0.025)\mathrm{BDE}_{\min} \quad N = 38,\ r = 0.979,\ r_{cv} = 0.971,\ s = 0.473,\ s_{cv} = 0.554,\ F = 398.58 \tag{2}$$

This is an expected result because of the high correlation between ABTS and DPPH assays ($r = 0.995$ on the set of 100 polyphenols, and $r = 0.995$ on the set of 38 flavonoids used in Eqs. 1 and 2).[38]

To verify the QSAR model (Eq. 1), we used TEAC data sets of another authors obtained by the ABTS free radical. Using several identical compounds from two (or more) data sets with corresponding activities determined by the same method in different laboratories can serve not only to validate a QSAR model, but also for validation of experimental techniques used for the measurement of activities for series of molecules.[28,44]

The initial set we used for this kind of model validation, an experimental data set containing TEAC values of 38 flavonoids obtained by the ABTS free radical (Table 3, the second column containing TEAC values), was taken from Rice-Evans et al.[8,29] Twenty-one of those 38 flavonoids are the same compounds as in the data set of 40 flavonoids. As can be seen from Table 3, experimental TEAC values for the same compounds in some cases differ significantly. This illustrates the fact that a relatively small difference in methodology can cause a huge difference in results.[45,46] Correlation of TEAC values of 21 identical flavonoids from the first[38] and the second data set[8,29] is presented in Figure 5 ($r = 0.887$). It should be noted that the root mean squared difference between 21 identical compounds of these two TEAC assays is relatively high (1.085), comparing with the range of TEAC values in Table 3.

Divergence of the experimental results for the same compounds obtained by different authors but by the same method could be one of the reasons why QSAR often disappoints, that is, why a

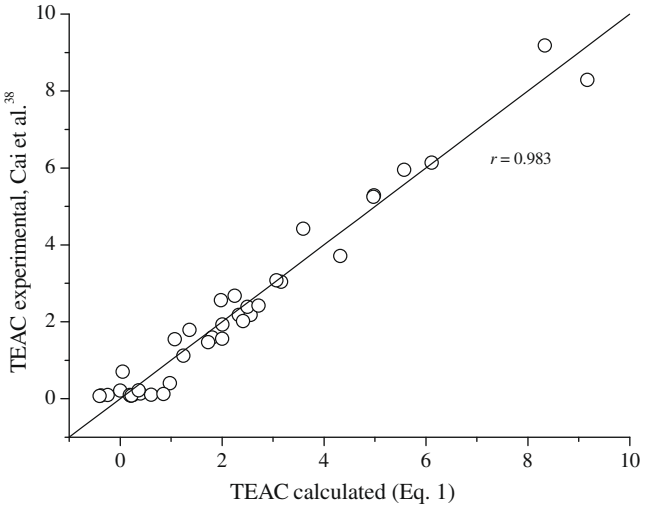


Figure 4. Scatter plot of experimental TEAC values obtained by the ABTS radical versus TEAC values calculated by Eq. 1.

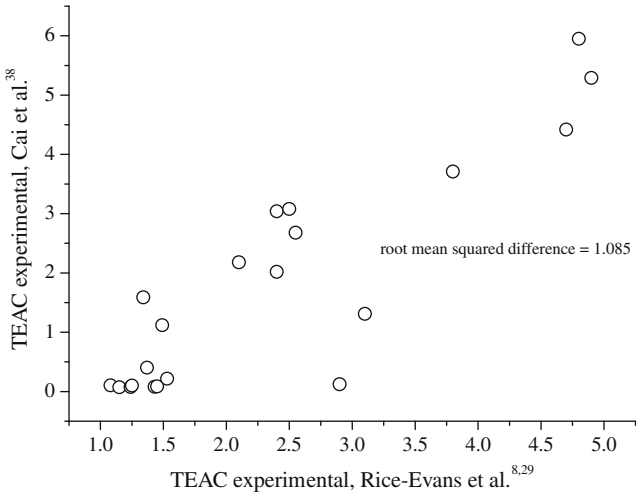


Figure 5. Correlation of TEAC values obtained by the ABTS radical for the same 21 compounds from the first (Ref. 38) and the second (Refs. 8,29) data sets.

reliable QSAR model developed using one set of experimental data might not be transferable to another data set. This is illustrated by Eq. 3 developed by using 38 flavonoids from the second data set:[8,29]

$$\mathrm{TEAC} = 6.733(\pm 2.081) + 0.421(\pm 0.089)n\mathrm{OH} - 0.080(\pm 0.025)\mathrm{BDE}_{\min} \quad N = 38,\ r = 0.831,\ r_{cv} = 0.792,\ s = 0.672,\ s_{cv} = 0.739,\ F = 38.92 \tag{3}$$

Eq. 3 possesses all of the features of the model described by Eq. 1. However, regarding statistical parameters, Eq. 3 is inferior to Eq. 1.The applicability of the model described by Eq. 1 to the third[39] and fourth[40] data sets is even poorer. The quality of experimental results in the first data set of flavonoids is confirmed by the high correlation coefficient (r = 0.995 on the set containing 100 polyphenols)[38] between measurements performed by two methods, that is, ABTS and DPPH assays.

The inconsistency of results obtained using different methods of measuring antioxidant potency was recognized previously as the antioxidant controversy. Even small differences in protocol for the same method (for example in TEAC assay, see Table 3) may produce inconsistent results. According to van den Berg et al.,[47] evaluation of antioxidant capacity using TEAC assay can be troublesome or even impossible, but it can be applicable for ranking of antioxidants. There is an obvious need for standardized methods for the determination of antioxidant capacity of phenolic compounds. Any single method is insufficient to achieve this goal.[45,48,49] Despite certain limitations of the ABTS assay, it is currently one of the methods considered for determination of standardized antioxidant capacity of molecules having antioxidant potential.[48] Reliable experimental data are of outmost importance in applying the QSAR procedure.

In the TEAC assay, the antioxidant potential of compounds is based on the activity of Trolox (a water-soluble synthetic vitamin E analogue) in molar units. Trolox is not a natural compound found in foods. Kim and Lee[50] introduced vitamin C (natural nutrient and antioxidant) as a standard in the ABTS assay, on a weight basis (mg/L). Vitamin C equivalent antioxidant capacity (VCEAC) is defined as the antioxidant capacity equivalent to vitamin C concentration (mg/L). The antioxidant capacity of vitamin C was designated at a value of 100 mg/L and VCEAC values for compounds from Ref. 50 were also evaluated at this concentration. The higher the VCEAC value of the test compound, the more effective the antioxidant. A VCEAC value greater than 100 indicates that the corresponding compound is a more effective antioxidant than vitamin C. It was found a very good linear correlation (r^2 = 0.914, i.e., r = 0.956) between VCEAC of 39 flavonoid aglycones and the number of OH groups on the flavonoid core.[50]

From this set of 39 flavonoid aglycones, we excluded three compounds without OH groups. First, we developed one-descriptor model using the number of OH groups:

$$\mathrm{VCEAC} = -28.98(\pm 11.71) + 48.74(\pm 3.388)n\mathrm{OH} \quad N = 36,\ r = 0.927,\ r_{cv} = 0.920,\ s = 29.74,\ s_{cv} = 31.12,\ F = 206.95 \tag{4}$$

It should be noted that descriptor nOH has its own limitations, because the assumption of equal contributions of OH groups at different positions to free radical scavenging is questionable. Obviously, an equivalent value of nOH does not necessarily mean an equivalent value of VCEAC. Two criteria for the effective radical scavenging activity were pointed out:[50] (i) vicinal OH groups in the B ring; (ii) the 3-OH group in the C ring. These criteria had previously been introduced[6] and used in QSAR modeling of the free radical scavenging activity of flavonoids.[51]

To verify the developed model described by Eq. 1 we also used the above set of flavonoid aglycones.[50] This data set contains 19 flavonoids identical to flavonoids of the first data set[38] and 17 different flavonoids. VCEAC and $\mathrm{BDE}_{\min}$ values, and the number of OH groups for the 36 flavonoid aglycones are shown in Table 4.

Obtained Eq. 5 possesses better statistical characteristics than Eq. 4.

Table 4
Experimental VCEAC values,[50] calculated O–H bond dissociation enthalpies (kcal/mol), and number of OH groups

	Compound	VCEAC	$\mathrm{BDE}_{\min}$	nOH
1	7-Hydroxyflavone	5.3 ± 3.7	85.51	1
2	5-Hydroxyflavone	11.6 ± 2.9	83.83	1
3	4′-hydroxyflavanone	21.5 ± 1.5	80.53	1
4	6-Hydroxyflavone	22.4 ± 3.6	78.65	1
5	Chrysin	24.9 ± 1.0	91.85	2
6	Biochanin A	25.6 ± 3.0	88.07	2
7	2′-Hydroxyflavanone	29.4 ± 2.2	79.64	1
8	6-Hydroxyflavanone	31.8 ± 3.4	76.90	1
9	Flavonol	34.7 ± 2.4	76.08	1
10	7-Flavonol	45.1 ± 3.0	76.13	2
11	Daidzein	71.8 ± 5.1	78.34	2
12	Apigenin	89.8 ± 5.6	81.13	3
13	Galangin	91.9 ± 6.2	76.02	3
14	6-Flavonol	99.9 ± 4.9	75.71	2
15	Hesperetin	101.1 ± 2.6	77.35	3
16	7,8-Dihydroxyflavone	108.0 ± 8.6	73.22	2
17	Kaempferol	114.6 ± 3.3	76.14	4
18	Fisetin	118.1 ± 8.9	70.32	4
19	Baicalein	118.2 ± 5.8	72.30	3
20	Butein	119.5 ± 10.1	71.56	4
21	Genistein	128.0 ± 7.1	77.99	3
22	Peonidin	133.5 ± 2.9	69.03	3
23	Naringenin	135.1 ± 1.8	80.43	3
24	Phloretin	146.6 ± 7.4	78.36	4
25	Malvidin	155.8 ± 5.4	70.09	3
26	Pelargonidin	157.7 ± 3.4	70.28	3
27	Morin	165.3 ± 3.7	76.87	5
28	Luteolin	178.3 ± 2.3	73.51	4
29	Taxifolin	213.5 ± 5.1	73.07	5
30	Catechin	215.7 ± 6.6	72.28	5
31	Quercetin	229.4 ± 5.1	70.09	5
32	Cyanidin	240.0 ± 6.1	69.57	4
33	Epicatechin	245.5 ± 6.2	72.75	5
34	Delphinidin	260.2 ± 3.0	67.23	5
35	Myricetin	261.8 ± 2.9	71.08	6
36	Epigallocatechin	264.4 ± 3.8	69.41	6

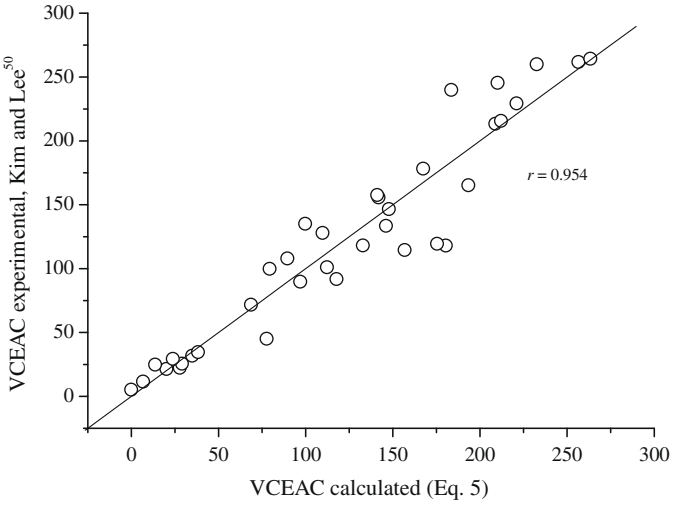


Figure 6. Scatter plot between experimental VCEAC values obtained by the ABTS radical and those calculated by Eq. 5.

$$\mathrm{VCEAC} = 308.30(\pm 79.04) + 39.58(\pm 3.48)n\mathrm{OH} - 4.07(\pm 0.95)\mathrm{BDE}_{\min} \quad N = 36,\ r = 0.954,\ r_{cv} = 0.947,\ s = 23.81,\ s_{cv} = 25.39,\ F = 165.89 \tag{5}$$

Eq. 5 is good confirmation of quality of model described by Eq. 1: an increased number of OH groups and lower BDE_{min} values contribute to higher VCEAC values. In Figure 6, we show the scatter plot of experimental VCEAC versus VCEAC values calculated by Eq. 5.

4. Conclusions

The newly developed RM1 and PM6 methods implemented in the semiempirical quantum chemistry MOPAC2009™ software package could be a useful tool in QSAR modeling of the radical scavenging activity of flavonoids. Calculations of the minimal energy conformations, as well as the heats of formation, are much faster than with the DFT method but with similar quality. The mechanisms of antiradical activity of flavonoids are still unclear and seem to be very complex. H-atom donation could be one of the dominant mechanisms, and may be modeled with BDE. The lack of standardized antioxidant assays and consequently reliable experimental data sets makes the successful use of the QSAR procedure doubtful. However, we showed that by taking into account some driving forces of the free radical scavenging mechanism and associated descriptors (*n*OH and BDE_{min}), and particular assays based on the ABTS radical (TEAC and VCEAC), a reasonable QSAR models could be obtained.

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