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Quantitative structure-activity relationships of antioxidant phenolic compounds

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ABSTRACT

In the present work, we carried out a quantitative structure-activity relationship (QSAR) study, using 15 phenolic compounds with antioxidant activity. For each compound, two electronic properties, BDE-OH (OH bond homolytic dissociation enthalpy) and IP (ionization potential), and two lipophylic parameters, LogP (lipophilicity) and LogD (relative lipophilicity), were estimated. The best QSAR model obtained by multiple regression analysis, using the systematic approach for variable selection, corresponds to the equation: pIC_{50} = 6.68 – 0.023(BDE-OH) – 0.0036(IP), which showed a high statistical significance (N = 15, R = 0.941, R^2 = 0.885, Q^2 = 0.807, s = 0.057, F = 46.09, p = 0.05).

Keywords: QSAR; antioxidant activity; phenolic compounds; free radical.

INTRODUCTION

Interest in finding naturally occurring antioxidants for use in the medical management of a number of pathophysiological conditions, involving free radical damage, [1,2] is on the increase. Free radicals are highly reactive chemical species ever present in nature. They can be produced from exogenous sources (e.g. exposure to X-rays, ozone, cigarette smoke, air pollution, and industrial chemicals) [3] or endogenous sources [4] (e.g. related to enzymatic biological processes, such as those resulting from the action of peroxydases, cyclooxygenases, lipoxygenases and dehydrogenases). Moreover, inflammation mediators, such as the C-reactive protein (CRP) and the tumor necrosis factor-alpha (TNF-α), the catecholamines, and the oxidized low-density lipoprotein (ox-LDL), can increase the production of such reactive species [5,6].

The role of free radical mediated oxidative stress in the etiology and progression of several acute and chronic clinical disorders has led to the proposition that antioxidants can be beneficial to

health as prophylactic agents. Recently, Kaliora and co-workers reported the beneficial effects of several antioxidants in the treatment and prophylaxis of atherosclerosis [7]. Among those antioxidants, some are lipid-soluble, such as beta-carotene (a polyene derivative and the precursor of vitamin A), alpha-tocopherol (or vitamin E, a phenolic derivative), coenzyme Q10 (a 1,4-benzoquinone derivative) and polyphenols (e.g., the flavonoid quercetin), and some are hydrosoluble, such as vitamin C (Figure 1). Epidemiological studies have consistently shown an inverse association between the consumption of vegetables and fruits, and the risk of cardiovascular diseases [4], certain forms of cancer (e.g., lung cancer), retina injury, dementia syndrome, rheumatoid arthritis, and autoimmune diseases [8-10]. Although the protective effects have been primarily attributed to well-known antioxidants, such as vitamins C and E [11], phenolic compounds from plants may also play a significant role in this association [12].

Phenolic (including polyphenolic) compounds are a group of plant secondary metabolites, universally distributed and, therefore, they are an integral part of the human diet [13]. Although the dietary intake of phenolic compounds varies considerably over geographic regions, it is estimated that the daily ingestion ranges from about 20 mg to 1 g, which is higher than that of vitamin E [14]. Phenolic compounds exhibit a broad range of biological activities, including antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anti-carcinogenic [10]. Many of these biological actions have been attributed to their antioxidant profile.

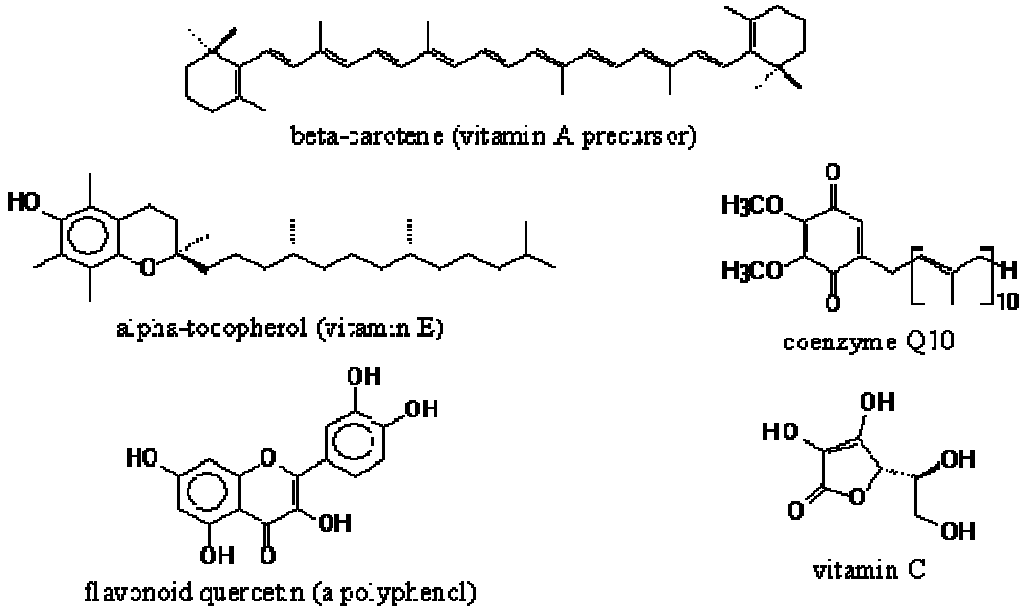


Figure 1. Chemical structures of some natural antioxidants

Cheng and co-workers have evaluated experimentally the antioxidant activity of 15 phenolic compounds in a linolenic acid micelle, in order to inactivate the lipid peroxidation (LPO) process

[15]. The chemistry of the initial steps of the LPO process, induced by a mixture of Fe(III)-ADP and ascorbic acid (vitamin C), has been described by Bondet and co-workers [16]. In this system, the initiation step (reaction 1, Figure 2), activated by reactive oxygen species (ROS), such as the hydroxyl radical ($HO^{\bullet}$), is too brief to be prevented. Once a free alkyl radical ($R^{\bullet}$) has been generated, a chain reaction takes place (reactions 2 and 3, Figure 2), and more lipid molecules (RH) are converted into lipid hydroperoxide species (ROOH), resulting in rancidity, due to fat oxidation. Therefore, according to the authors, the antioxidants could only break the propagation step (reactions 2 and 3, Figure.2), by scavenging radical products ($ROO^{\bullet}$ and $R^{\bullet}$).

Two mechanistic proposals were introduced in order to explain the antioxidant preventive action of phenolic compounds (ArOH): (i) H-atom transfer (reaction 4, Figure 2) and (ii) electron transfer (reaction 5, Figure 2). According to Wright and co-workers [17], the reaction rate with respect to the peroxy-alkyl radical ($ROO^{\bullet}$), in the first pathway (reaction 4, Figure 2), depends on the energy barrier for an H-atom transfer from RH (reaction 3, LPO process) or ArOH (reaction 4, antioxidant action). The reaction between $ROO^{\bullet}$ and ArOH has a smaller energy barrier than that between $ROO^{\bullet}$ and RH. Therefore, the antioxidant reacts faster with peroxyalkyl radicals than with lipidic substrates, preventing the LPO process. In the electron transfer pathway (reaction 5, Figure 2) [17], the antioxidant molecule (ArOH) transfers one electron to the free alkyl radical ($R^{\bullet}$), forming a phenolic radical cation ($ArOH^{+\bullet}$). Thus, the generated radical cation must be sufficiently stable to prevent reaction with lipidic substrates (RH), and, consequently, to break the chain reaction.

Lipid Peroxidation				
Reaction 1	Initiation	$RH + HO^{\bullet}$	$\rightarrow$	$R^{\bullet} + H_2O$
Reaction 2	Propagation (O_2 addition)	$R^{\bullet} + O_2$	$\rightarrow$	$ROO^{\bullet}$
Reaction 3	Propagation ($H^{\bullet}$ transfer)	$ROO^{\bullet} + RH$	$\rightarrow$	$ROOH + R^{\bullet}$
Antioxidant Action				
Reaction 4	"Scavenger Step" ($H^{\bullet}$ transfer)	$ROO^{\bullet} + ArOH$	$\rightarrow$	$ROOH + ArO^{\bullet}$
Reaction 5	"Scavenger Step" (electron transfer)	$R^{\bullet} + ArOH$	$\rightarrow$	$R^{-} + ArOH^{+\bullet}$

Figure 2. Schematic lipid peroxidation (LPO) process (reactions 1 to 3) and antioxidant action by H-atom transfer (reaction 4) and electron transfer (reaction 5)

The antioxidant activity has been discussed in several works [15,17-20], and many authors have tried to construct mathematical models (e.g., Quantitative Structure-Activity Relationships, QSAR, equations) in order to correlate the antioxidant activity of phenolic compounds with calculated physico-chemical properties. However, these works present some drawbacks, such as the arbitrary use of theoretical methods to calculate molecular properties without a validation study, as well as a lack of statistical significance.

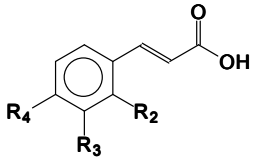
In the present work, the Density Functional Theory (DFT), using the B3LYP functional form, was used to calculate two electronic properties: OH bond homolytic dissociation enthalpy (BDE-OH), and ionization potential (IP). In addition, the ACD/LogP 9.5 program and the LogD calculator server were used to estimate two lipophilic properties, lipophilicity (LogP) and

relative lipophilicity (LogD), respectively. These electronic and lipophilic parameters were evaluated in order to construct QSAR models, using 15 phenolic compounds (Table 1) with known experimental antioxidant activity [15]. A stepwise linear regression analysis was performed in order to obtain a correlation between these putative descriptors and the experimental antioxidant activity of these 15 phenolic compounds. In this way, we hope to understand which molecular features are correlated with the antioxidant activity of phenolic compounds against free radical and associated diseases.

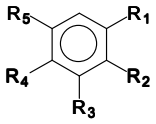
EXPERIMENTAL SECTION

The structure of each phenolic compound (ArOH) (Table 1) was firstly submitted to a systematic conformational analysis, considering the default torsion angles (ϕ) and using the MMFF94 force field [30] and, subsequently, the generated conformations were fully optimized by the semi-empirical method AM1 [31] in the Spartan'06 program [32]. All calculations were performed considering the isolated molecule in vacuum, and without any geometrical restriction.

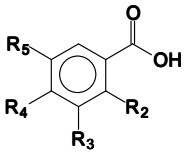
Table 1. Chemical structures and nomenclature of the 15 phenolic compounds, and the corresponding position (RP) and number of free radical formed (NR)



(1-4)



(5-10)



(11-15)

Comp. #	Nomenclature	R ≠ H	RP [m]	NR [n]
1	*o*-Coumaric acid [a]	R_2 = OH	R_2	1
2	*p*-Coumaric acid [b]	R_4 = OH	R_4	1
3	Ferulic acid [c]	$R_3 = OCH_3$, R_4 = OH	R_4	1
4	Caffeic acid [d]	$R_3 = R_4$ = OH	R_3 and R_4	2
5	Catechol [e]	$R_1 = R_2$ = OH	R_1 and R_2	2
6	Pyrogallol [f]	$R_1 = R_2 = R_3$ = OH	R_1, R_2 and R_3	3
7	Phloroglucinol [g]	$R_1 = R_3 = R_5$ = OH	R_1	1
8	Resorcinol [h]	$R_1 = R_3$ = OH	R_1 and R_3	2
9	Hydroquinone [i]	$R_1 = R_4$ = OH	R_1	1
10	*p*-Aminophenol	R_1 = OH, $R_4 = NH_2$	R_1	1
11	Protocatechuic acid [j]	$R_3 = R_4$ = OH	R_3 and R_4	2
12	Gallic acid [k]	$R_3 = R_4 = R_5$ = OH	R_3, R_4 and R_5	3
13	Salicylic acid [l]	R_2 = OH	R_2	1
14	*m*-Hydroxybenzoic acid	R_3 = OH	R_3	1
15	*p*-Hydroxybenzoic acid	R_4 = OH	R_4	1

[a] (*E*)-3-(2-hydroxyphenyl)-2-propenoic acid; [b] (*E*)-3-(4-hydroxyphenyl)-2-propenoic acid; [c] (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid; [d] (*E*)-3-(3,4-dihydroxyphenyl)-2-propenoic acid; [e] 1,2-dihydroxybenzene (pyrocatechol); [f] 1,2,3-trihydroxybenzene; [g] 1,3,5-trihydroxybenzene; [h] 1,3-dihydroxybenzene; [i] 1,4-dihydroxybenzene; [j] 3,4-dihydroxibenzoic acid; [k] 3,4,5-trihydroxibenzoic acid; [l] 2-hydroxybenzoic acid; [m] RP = Position of the generated radical; [n] NR = number of generated radicals

It is to be expected that the minimum energy conformation in vacuum be similar to the one found in a hydrophobic environment, such as inside a linolenic acid micelle system [15], due to the

maximization of the intramolecular interactions. Finally, the AM1 minimum energy conformation of each phenolic compound was fully optimized using DFT [33], employing the B3LYP functional [34,35] and a 6-311++G(d,p) basis set, available in the Gaussian 98 package program [36].

The structure of each radical ($ArO^{\bullet}$) and radical cation ($ArOH^{+\bullet}$) (Table 1) was obtained using the DFT geometry optimization in which the starting coordinates were taken from the respective neutral phenolic compound. In this case, the B3LYP restricted open-shell formalism (RO-B3LYP) [37] with 6-311++G(d,p) basis set was used.

Calculation of BDE-OH

The BDE-OH parameter (i.e., the enthalpy per mole required to break homolytically the O-H bond of some specific molecule) [38] is related to the H-atom transfer pathway (reaction 4, Figure 2), in which the weaker the OH bond is, the faster will be the scavenging reaction of the free radicals. To be effective, a radical produced from a phenolic compound ($ArO^{\bullet}$) must be relatively stable; i.e., it must react slowly with RH.

According to Jursic [39], DFT calculations overestimate the total energy of the hydrogen atom ($H^{\bullet}$). An alternative approach is to calculate the energy required for homolytic O-H bond dissociation (BDE-OH) by the use of the Equation I, where phenol is used as reference, and the errors associated with the hydrogen atom are cancelled.

$$BDE-OH = [\Delta H_f(PhOH) + \Delta H_f(ArO^{\bullet})] - [\Delta H_f(PhO^{\bullet}) + \Delta H_f(ArOH)] + BDE-OH_{Exp}(PhOH) \quad \textbf{(I)}$$

In Equation I, ΔH_f(PhOH), ΔH_f($PhO^{\bullet}$), ΔH_f(ArOH), and ΔH_f($ArO^{\bullet}$) are the enthalpy of formation calculated for phenol (PhOH), phenoxyl radical ($PhO^{\bullet}$), the phenolic compound (ArOH), and the corresponding radical ($ArO^{\bullet}$); and $BDE\text{-}OH_{Exp}$(PhOH) is the experimental enthalpy of formation of phenol (i.e., 86.5 kcal/mol) [21].

Calculation of IP

The Ionization Potential (IP) is defined as the minimum energy required to remove an electron from an isolated molecule (or atom) in its ground state to form an ion in the gas phase [38]. The IP parameter can be related to the electron transfer pathway (reaction 5, Figure 2), in which low values of IP favor the electron transfer process in a molecule. Many authors correlate the IP of a compound to its antioxidant activity [15,17,24,25,40]. The IP values were calculated from the Equation II.

$$IP = \Delta H_f(ArOH^{+\bullet}) - \Delta H_f(ArOH) \quad \textbf{(II)}$$

In Equation II, ΔH_f(ArOH) and ΔH_f($ArOH^{+\bullet}$) are the enthalpy of formation calculated for the phenolic compound (ArOH), and the corresponding radical cation ($ArOH^{+\bullet}$).

Calculation of LogP and LogD

Additionally, to quench the LPO process in linolenic micelles, the antioxidant must penetrate the lipidic phase [15]. Thus, it is reasonable to try to associate lipophilic properties, such as LogP (lipophilicity) and LogD (relative lipophilicity), to the antioxidant activity of phenolic compounds [41,42]. Furthermore, the lipophilicity is described, as usual, in terms of the partition coefficient P (LogP), defined as the ratio of the concentrations of a compound in equilibrium between octanol and water [41].

Since an organic compound can be seen as a collection of functional groups and supposing ideal local additive properties, i.e., that each block has a defined lipophilicity, one can estimate the partition coefficient of a compound. For that, one may use many approaches, such as the Hansch method [43]. The LogP (lipophilicity) of the phenolic compounds was estimated using the ACD/LogP 9.5 program [44]. The prediction algorithm is expressed as $LogP = \sum fn + \sum Fm$, where "f" corresponds to the atomic or fragmental increments and "F" corresponds to the correction factors.

In addition, considering that the LPO assay [15] was performed at pH 7.4, it is also important to evaluate the relation between LogD and the antioxidant activity of the phenolic compounds. Differently from LogP, LogD is defined as the ratio between the equilibrium concentrations of all species (unionized and ionized) present in octanol and in water, at a given temperature, normally 25°C [42]. The LogD of the phenolic compounds was estimated using the LogD calculator server [45], where the prediction algorithm is expressed as LogD = f(LogP, *p*Ka).

RESULTS AND DISCUSSION

Analysis of BDE-OH

The accurate estimation of BDE-OH from theoretical calculations is still a challenging task, since high levels of theory are necessary for taking into account the effect of the electron correlation [21]. High level *ab initio* calculations are prohibitive for longer size of substituted phenols. Recently, Cheng and co-workers [15] used the AM1 semi-empirical method to calculate the BDE-OH of phenolic compounds, but they were not able to find any correlation between the theoretical results and the experimental values in the gas phase [22]. In addition, Brinck and co-workers [23] showed that the MP2 and MP4 methods overestimate the absolute BDE-OH value. On the other hand, Chandra and Uchimaru [21] found that the use of B3LYP-DFT lead to excellent agreement with the experimental gas phase values, thus, supporting the use of this method in the current study.

According to our results (Table 2), the B3LYP/6-311++G(d,p) level of theory, as in Klein and Lukes studies [24], gave calculated BDE-OH values in excellent agreement with the experimental ones for resorcinol (8) ($BDE\text{-}OH_{Exp}$ = 86.7 kcal/mol), hydroquinone (9) ($BDE\text{-}OH_{Exp}$ = 81.5 kcal/mol), and *p*-aminophenol (10) ($BDE\text{-}OH_{Exp}$ = 77.3 kcal/mol) [24].

Although BDE-OH experimental values are not available for all phenolic compounds, we found that the cinnamic acid derivatives (1-4) show similar values (1, $BDE\text{-}OH_{Calc}$ = 84.4 kcal/mol; 2, $BDE\text{-}OH_{Calc}$ = 84.9 kcal/mol; 3, $BDE\text{-}OH_{Calc}$ = 84.5 kcal/mol, Table 2) (Figure S1, Supplementary Information), except for compound 4 ($BDE\text{-}OH_{Calc}$ = 74.9 kcal/mol). The

simultaneous presence of OH groups in the *meta* and *para* positions of compound 4 decreases the calculated BDE-OH value by 10 kcal/mol, probably due to the presence of an intramolecular hydrogen bond, which could stabilize the respective radical.

In the dihydroxyl phenolic series (5, 8, and 9), the *ortho* isomer (5) has a lower calculated BDE-OH value than the *meta* (8) and *para* (9) isomers. As in the case of compound 4, only the radical from the *ortho* isomer is stabilized by an intramolecular hydrogen bond. Additionally, there is a considerable difference between 8 and 9 (ΔBDE-OH = 5.5 kcal/mol), probably due to different electronic effects of the OH groups. In the *para* isomer, the OH group acts as an electron donor (σ_p = –0.37), stabilizing the corresponding radical. Differently, in the *meta* isomer, the OH group acts as an electron withdrawing group (σ_m = 0.12), providing a destabilizing effect. Compound 10 also has an electron donor group (NH_2, σ_p = – 0.66) at the *para* position, which also stabilizes the corresponding radical, but in a higher degree.

The comparison between the cinnamic (1-4) and the benzoic acids (11-15) series shows that the presence of a carboxyl group destabilizes the corresponding radicals (except for compounds 4, 12, and 13 that present intramolecular hydrogen bonds). This effect is more pronounced when the carboxyl group is directly attached to the aromatic ring, as in *ortho*- (13), *meta*- (14), and *para*-hydroxybenzoic (15) acids.

Analysis of IP

DFT methods have been used to calculate the ionization potentials of phenolic compounds, such as *p*-aminophenol (10) (IP_{Calc} = 163.0 kcal/mol) [17], and for caffeic acid (4) (IP_{Calc} = 181.1 kcal/mol) and catechol (5) (IP_{Calc} = 184.4 kcal/mol) [3], which are in a very good agreement with our results (Table 2). The IP value of *p*-coumaric acid (2) (IP_{Calc} = 194.9 kcal/mol) calculated by Chen and co-workers [3] is higher than our calculated value (Table 2) (Figure S2, Supplementary Information), which can be attributed to different methodologies and a different choice of conformation of the ground state. The calculated IP value for caffeic acid (4) (IP_{Calc} = 181.2 kcal/mol) [3,25] agrees with our results, but their value for gallic acid (12) does not (IP_{Calc} = 189.09 kcal/mol) (Table 2). This discrepancy may also be due to different calculation methodologies.

Moreover, the direct comparison between the calculated and the experimental IP values was only possible for resorcinol (8) (IP_{Exp} = 199.0 kcal/mol) and hydroquinone (9) (IP_{Exp} = 194.6 kcal/mol). Although these experimental values are higher than those that we found, the energy difference between the theoretical (ΔIP_{Calc} = 8.5 kcal/mol) and experimental values (ΔIP_{Exp} = 5.4 kcal/mol) of 8 and 9 is very small (Table 2). The presence of electron donating groups directly attached to the phenolic ring decreases the energy difference between the ground state and the respective radical cations, leading to calculated IP values lower than the IP calculated for phenol (IP_{Calc} = 193.2 kcal/mol) (Table 2).

In the cinnamic acid group (1-4), the IP difference between *ortho* (1) and *para* (2) isomers is approximately 4 kcal/mol. This difference is lower for the *para* isomer than for the *ortho* isomer (Table 2). The presence of additional electron donating groups attached to the aromatic ring also decreases the theoretical IP values 42 (Table 2, compounds 3 and 4). Again, compound 4, which has an intramolecular hydrogen bond, has a higher calculated IP.

The relative position between the di- (5, 8, and 9) and trihydroxylated (6 and 7) phenols also alters the calculated IP. The relative radical cation stability is *meta* (7 and 8) < *ortho* (5 and 6) < *para* (9). This result suggests that the radical cation stabilization is due, exclusively, to the electronic effects of the OH group.

In the benzoic acid group (11-15), the calculated IP values are higher than that of phenol. As before, compounds 11 and 12, which have more than one OH group, show higher calculated IP values as compared to the mono-hydroxy substituted compounds (Table 2).

Analysis of LogP and LogD

Table 2 shows the calculated values of lipophilicity ($LogP_{Calc}$) (Figure S3, Supplementary Information) and the relative lipophilicity ($LogD_{Calc}$) (Figure S5, Supplementary Information) for the 15 phenolic compounds, as well as the experimental LogP values ($LogP_{Exp}$) compiled from the literature for compounds 2-15.

Considering that there are no experimental LogP values for all compounds in this study, and in order to validate the calculated LogP values, we correlated them with the experimental LogP values (Table 2), using a regression analysis. The excellent correlation (R = 0.949, R^2 = 0.901, s = 0.211, F = 109.01) between the theoretical and experimental values (Figure S4, Supplementary Information) justifies the use of theoretical values in the QSAR study.

Table 2. DFT calculated values of BDE-OH (kcal/mol) and IP (kcal/mol) (B3LYP/6-311++G(d,p)), calculated LogP ($LogP_{Calc}$) and LogD (pH 7.4), and experimental values of LogP ($LogP_{Exp}$) of the 15 phenolic compounds

Comp. #	BDE-OH [a]	IP [b]	$LogP_{Calc}$	LogD	$LogP_{Exp}$
1	84.4	188.8	2.43	-1.06	nd [c]
2	84.9	184.9	1.88	-1.51	1.46
3	84.5	177.6	1.64	-1.78	1.51
4	74.9	181.6	1.42	-2.01	1.15
5	76.4	184.5	0.88	0.88	0.88
6	77.7	183.2	0.29	0.29	0.68
7	87.7	188.2	0.06	0.06	0.16
8	86.1	186.9	0.76	0.76	0.80
9	80.6	178.4	0.64	0.64	0.59
10	76.8	163.2	0.04	-0.29	0.04
11	79.6	228.2	1.16	-1.73	1.15
12	79.8	228.6	0.91	-2.08	0.70
13	93.0	196.6	2.06	-1.09	2.26
14	88.9	199.0	1.50	-1.47	1.50
15	89.2	200.3	1.42	-1.33	1.58

[a] BDE-OH (O-H bond dissociation enthalpy, kcal/mol) (experimental BDE-OH of phenol in gas phase = 86.5 kcal/mol); [b] IP (ionization potential, kcal/mol) (calculated IP of phenol = 193.2 kcal/mol); [c] Calculated by DFT (B3LYP/6-311++G(d,p)); [d] Not determined.

Analysis of the QSAR Models

The selection process of independent variables must consider at least five or six compounds (observations) for each included variable in the final QSAR model, preventing model overfitting [26,27]. Among the methods used for variable selection, the systematic search method is the best choice, when possible. This method consists of a combination of *m* available variables

(parameters or properties) used to build and to analyze all possible regression equations with *k* variables, in order to select the best equation (model) containing the most important variables (descriptors). The systematic search is the only selection method in which the best combination will be really found (see, for instance, Ferreira and coworkers) [28].

We have used the systematic search and performed 14 regression analyses with the antioxidant activity of 15 phenolic compounds, as dependent variables (Table 5) (Log $1/IC_{50Exp}$ = − Log IC_{50Exp} = pIC_{50Exp}), and the calculated electronic (BDE-OH and IP) and lipophilic (LogP and LogD) properties, as independent variables. The calculated properties, used as parameters to build up the QSAR models, selected in each equation and their respective statistic parameters (*R*, correlation coefficient; R^2, coefficient of determination; R^2_{Adjus}, adjusted coefficient of determination; *s*, standard error; *F*-test and *p*-value) are shown in Table 3.

Comparing the best equations of the three groups containing one (Equation 1 to Equation 4), two (Equation 5 to Equation 10) and three (Equation 11 to Equation 14) parameters, i.e., Equation 1 (pIC_{50} = 6.151 – 0.24(BDE-OH), R^2_{Adjus} = 0.704), Equation 5 (pIC_{50} = 6.682 – 0.023(BDE-OH) – 0.0036(IP), R^2_{Adjus} = 0.866), and Equation 12 (pIC_{50} = 6.611 – 0.022(BDE-OH) – 0.003(IP) + 0.012(LogD), R^2_{Adjus} = 0.860), respectively, the BDE-OH parameter is present in all of them. However, Equation 1 could be excluded because it has the lowest R^2_{Adjus} value (used to compare equations containing a different number of parameters). Therefore, considering only Equation 5 and Equation 12, we can observe that the inclusion of LogD parameter in Equation 12 does not alter, significantly, the R^2_{Adjus} value, which makes these two equations equivalents (Table 3). Nevertheless, since the parsimony principle advises the choice of the simplest model, Equation 5 was used to calculate the antioxidant activity for the 15 phenolic compounds (Table 5), using as descriptors the BDE-OH and IP parameters (Table 2).

Table 3. QSAR equations obtained from the systematic combination of the four theoretical parameters (BDE-OH, IP, $LogP_{Calc}$, and LogD)

Eq. # [a]	BDE-OH	IP	$LogP_{Calc}$	LogD	*R*	R^2	R^2_{Adjus}	*s*	*F*
1	•				*0.852*	*0.725*	*0.704*	*0.085*	*34.33*
2		•			0.531	0.282	0.227	0.137	5.11
3			•		0.396	0.157	0.092	0.149	2.01
4				•	0.336	0.134	0.067	0.151	2.42
5	•	•			*0.941*	*0.885*	*0.866*	*0.057*	*46.09*
6	•		•		0.853	0.728	0.682	0.088	16.03
7	•			•	0.888	0.789	0.754	0.077	22.48
8		•	•		0.616	0.379	0.276	0.133	3.66
9		•		•	0.546	0.298	0.181	0.141	2.55
10			•	•	0.429	0.184	0.048	0.152	1.35
11	•	•	•		0.941	0.885	0.853	0.060	28.17
12	•	•		•	*0.944*	*0.890*	*0.860*	*0.058*	*29.76*
13	•		•	•	0.897	0.805	0.752	0.078	15.18
14		•	•	•	0.620	0.384	0.216	0.138	2.29

[a] *Equations with one (Eq.1 to Eq.4), two (Eq.5 to Eq.10) and three (Eq.11 to Eq.14) parameters. The best equation of each group is in italic. For all equations N = 15 and p = 0.05. R = correlation coefficient. R^2 = coefficient of determination. R^2_{adjust} = adjusted coefficient of determination. s = standard error. F = Fischer test value.*

Furthermore, it is also important to verify the degree of correlation between the descriptors used in the same equation, because two or more variables highly correlated can generate linear dependence problems in the data set and a numeric imprecision in the model construction [29]. The analysis of the cross-correlation matrix of the calculated parameters (BDE-OH, IP, LogP, and LogD) (Table 4) shows that there are no parameters highly correlated.

Table 4. Cross-correlation matrix among the theoretical parameters (BDE-OH, IP, $LogP_{Calc}$ and LogD)

	BDE-OH	**IP**	**$LogP_{Calc}$**	**LogD**
BDE-OH	1.000	0.161	0.414	0.136
IP		1.000	0.168	0.481
$LogP_{Calc}$			1.000	0.592
LogD				1.000

Table 5 shows the calculated pIC_{50} values (pIC_{50Calc}), the residuals ($pIC_{50Exp} - pIC_{50Calc}$), and the percentage errors for the 15 phenolic compounds, using the best equation (Equation 5). The predicted activity values show percentile errors ranging from 0 to 3.09%, which is lower than 5%, characterizing the absence of any outlier. The plot of the experimental (pIC_{50Exp}) versus the calculated (pIC_{50Calc}) values and the plot of the residual values ($pIC_{50Exp} - pIC_{50Calc}$) for the 15 phenolic compounds are shown in Figure 3 and Figure S6 (Supplementary Information), respectively.

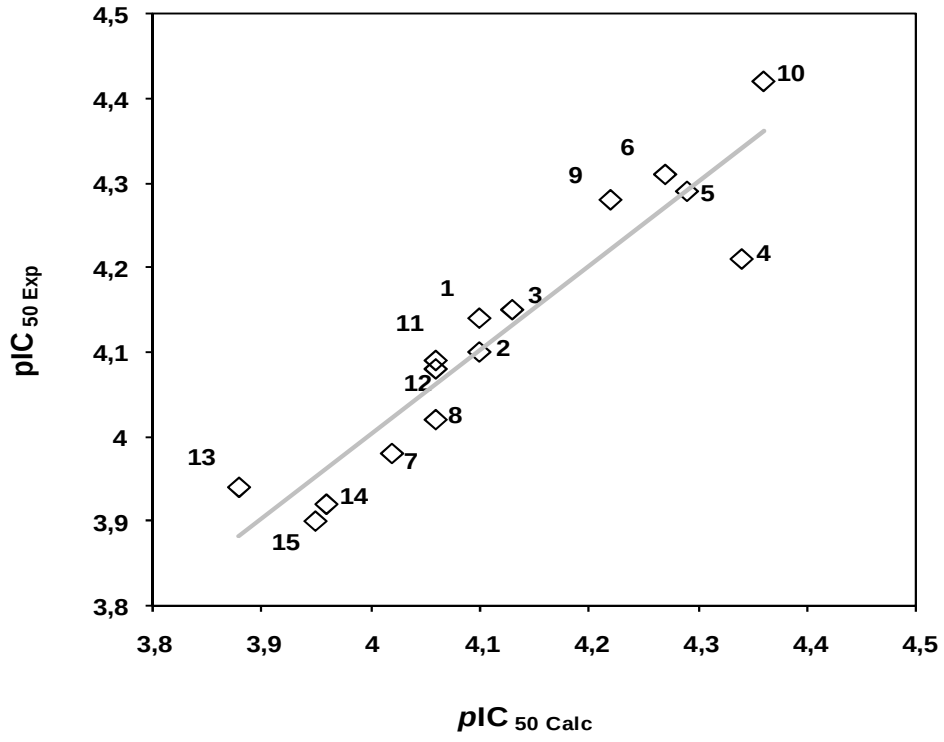


Figure 3. Correlation between the experimental (pIC_{50Exp}) and the theoretical antioxidant activity values (pIC_{50Calc}) of the 15 phenolic compounds

Table 5. Values of $pIC_{50\ Exp}$, $pIC_{50\ Calc}$, residues ($pIC_{50Exp} - pIC_{50Calc}$) and percentile error

Comp. #	pIC_{50Exp}	pIC_{50Calc} [a]	Residue	% Error
1	4.14	4.10	0.04	0.98
2	4.10	4.10	0.00	0.00
3	4.15	4.13	0.02	0.48
4	4.21	4.34	-0.13	3.09
5	4.29	4.29	0.00	0.00
6	4.31	4.27	0.04	0.94
7	3.98	4.02	-0.04	1.00
8	4.02	4.06	-0.05	1.00
9	4.28	4.22	0.06	1.42
10	4.42	4.36	0.06	1.38
11	4.09	4.06	0.02	0.74
12	4.08	4.06	0.02	0.49
13	3.94	3.88	0.06	1.55
14	3.92	3.96	-0.03	1.02
15	3.90	3.95	-0.05	1.28

[a] *(Eq.7) $pIC_{50} = 6.68 - 0.023\ (BDE\text{-}OH) - 0.0036(IP)$ ($N = 15$, $R = 0.941$, $R^2 = 0.885$, $s = 0.057$, $F = 46.09$, $p = 0.05$)*

CONCLUSION

In this work, for a series of 15 phenolic compounds, we calculated two electronic (BDE-OH and IP) and two lipophilic (LogP and LogD) properties, and used them as parameters to construct and evaluate a QSAR (Quantitative Structure-Activity Relationship) model. The best QSAR model of the 15 phenolic compounds with antioxidant activity corresponds to Equation 5, $pIC_{50} = 6.68 - 0.023(BDE\text{-}OH) - 0.0036(IP)$, which shows a high statistical significance ($N = 15$, $R = 0.941$, $R^2 = 0.885$, $Q^2 = 0.807$, $s = 0.057$, $F = 46.09$, $p = 0.05$), and does not show any outliers. The most significant descriptors associated with the antioxidant activity of these compounds were the dissociation energy of the homolytic cleavage of the OH bond (BDE-OH), and the ionization potential (IP). The model predicts as the best antioxidants those compounds that contain electron donor groups directly attached to the aromatic ring. Moreover, the small number of molecular descriptors allows a good interpretation of the data in terms of the substituent electronic effect. The best model also lacks the presence of any lipophilic descriptors (LogP and LogD), and this suggests that the difference in the solubility between the aqueous and hydrophobic phases does not play a crucial role on the antioxidant activity of these series of phenolic compounds.

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Supplementary Information Available
Supplementary information associated with this article can be found at the end.

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Supplementary Information

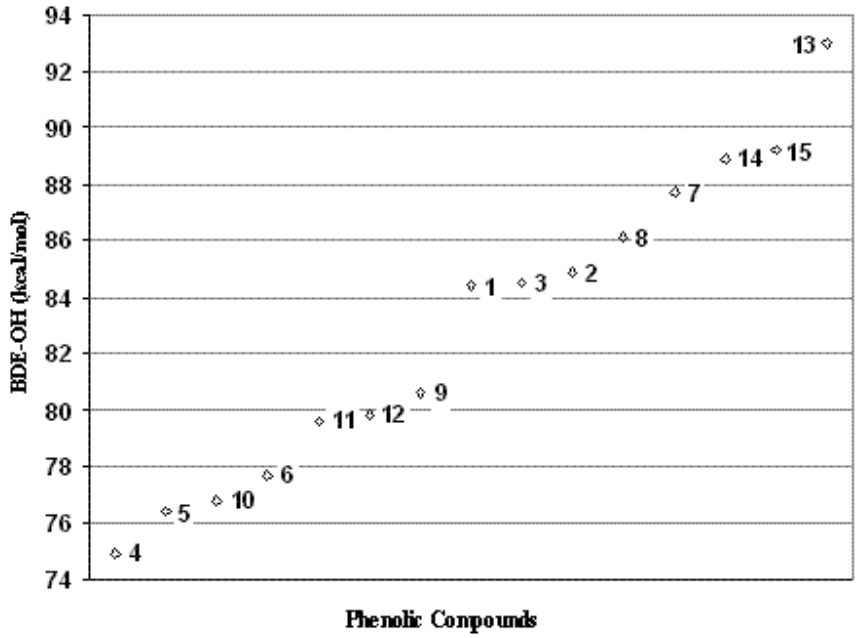

Figure S1. BDE-OH values of the phenolic compounds

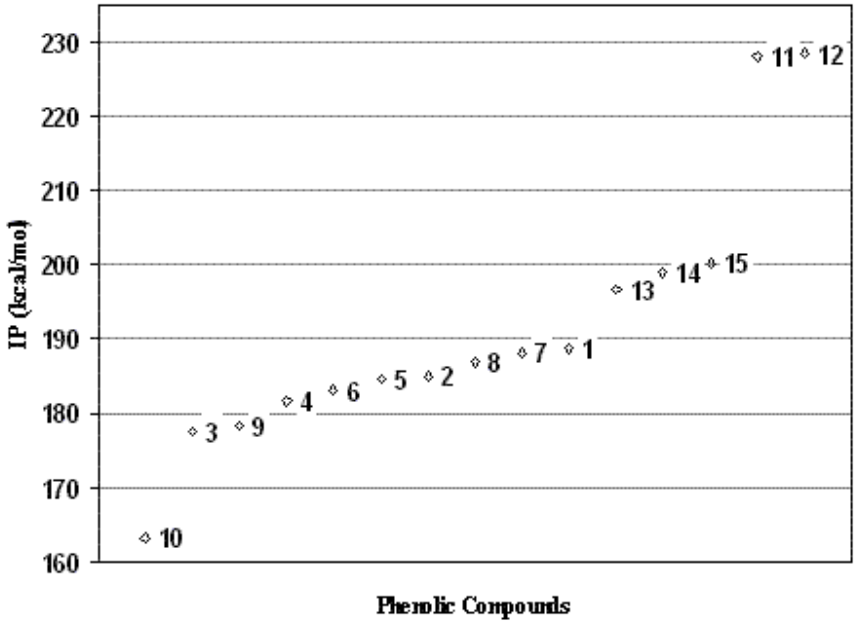

Figure S2. IP values of the phenolic compounds

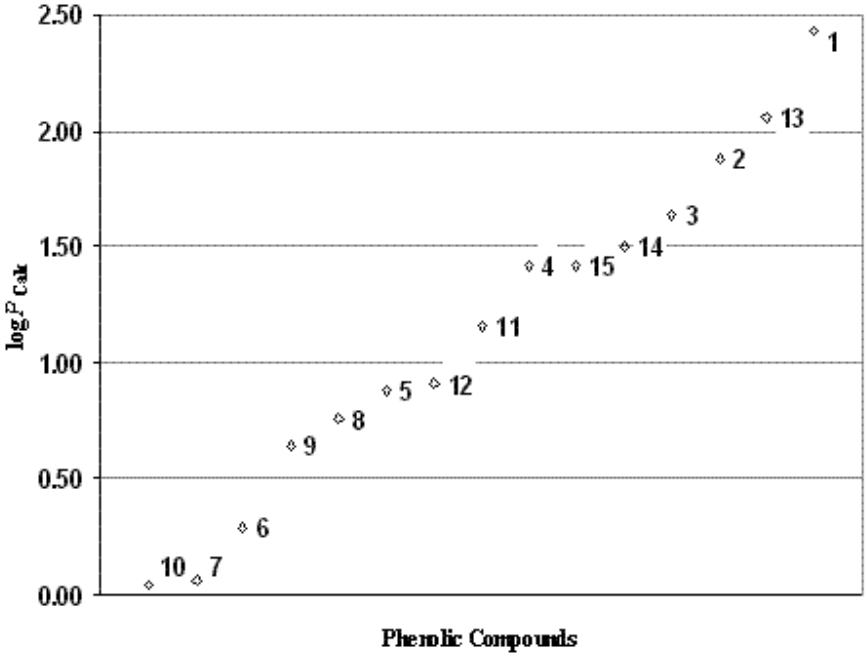

Figure S3. Log*P* $_{Calc}$ values of the phenolic compounds

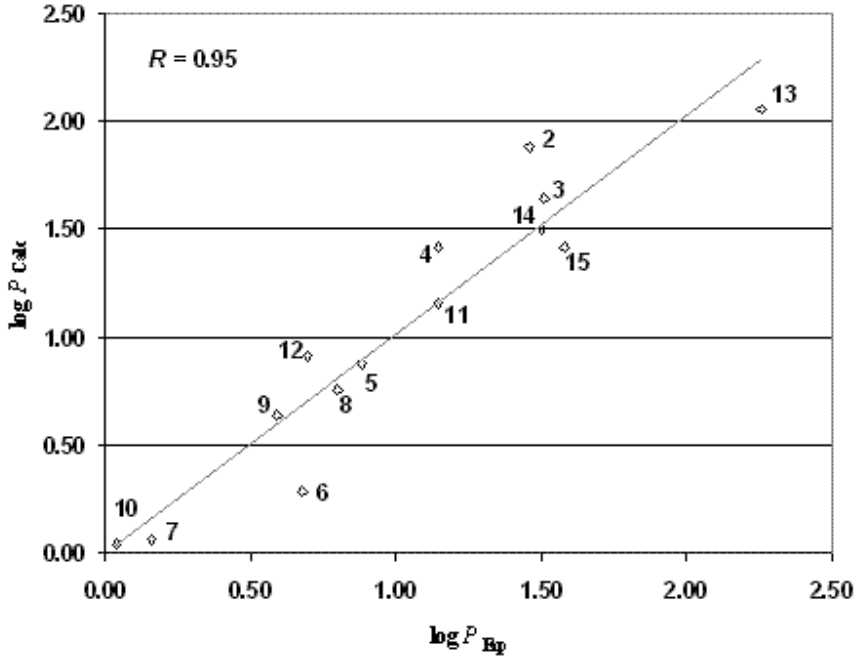

Figure S4. Correlation between the experimental and the calculated lipophilicities

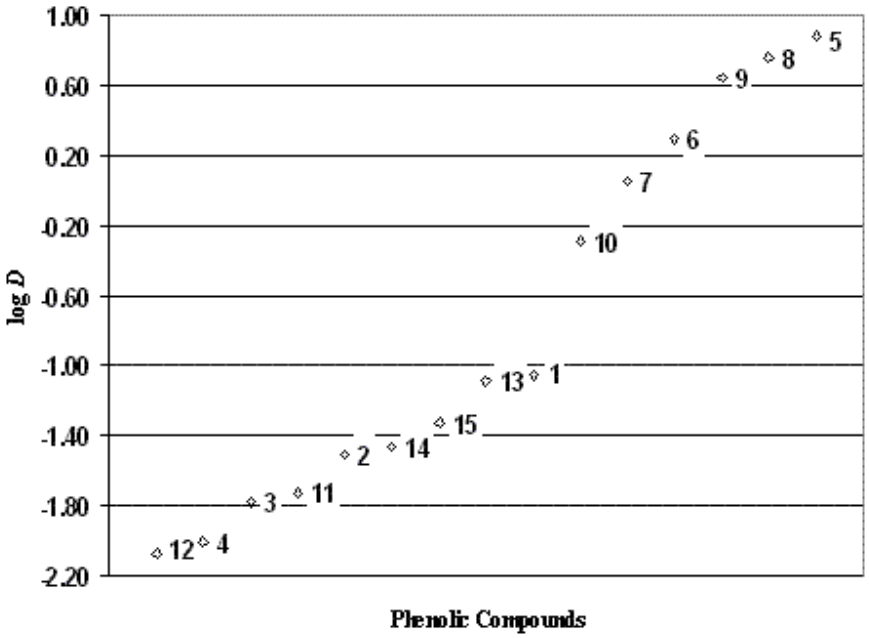

Figure S5. Log*D* values of the phenolic compounds

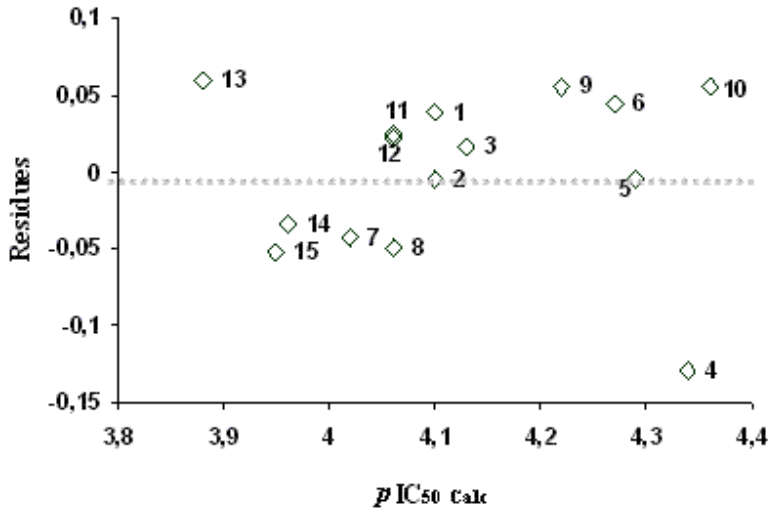

Figure S6. Distribution of the residual values ($pIC_{50\ Exp}$ - $pIC_{50\ Calc}$) for the 15 phenolic compounds