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A new, simplified model for the estimation of polyphenol oxidation potentials based on the number of OH groups

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We present a new and simpler regression model for the estimation of the first oxidation potentials (E_{p1}) of flavonoids based on the number of phenolic, alcoholic, and carboxylic OH groups. In the regression we included the E_{p1} of 12 polyphenols (mostly flavonols and catechins) that were measured in our laboratory at pH 3. The model yielded r=0.986 and SE=0.040. Later successive inclusions of previously reported E_p values into the regression model, 7 at pH 3, the model (N=19) yielded r=0.980, SE=0.046 and 19 at pH 7 the model (N=38), yielded r=0.985, SE=0.044.

KEY WORDS: *flavonoids; molecular modelling; QSAR/QSPR*

Since Renaud's paper and introduction of the French paradox (1), the interest in polyphenols (phenolic acids, flavonoids, tannins, etc.) has increased considerably. This diverse group of secondary plant metabolites has shown a number of beneficial effects on human health. Polyphenols can prevent oxidative stress-related diseases such as cardiovascular diseases (2), cancer (3-6), neurodegenerative diseases (7), diabetes (8), osteoporosis (9), and allergic diseases (10). These beneficial effects stem from their ability to scavenge free radicals (2, 3, 6-8, 11-17), which, in turn, depends on their electro-oxidation potential (E_p) and the O-H bond dissociation enthalpies (18, 19).

In fact, the free radical scavenging activity of a polyphenolic compound can be estimated from their electro-oxidation potential using the quantitative structure-activity/property relationship (QSAR/QSPR) models (20-23) because oxidation potential depends on the number and position of hydroxyl groups in a molecule required for the conjugation between the B and C ring. Polyphenols with lower electro-oxidation peak potentials have higher electron-donating ability, i.e. higher radical scavenging capacity. Indeed, a number of studies reported strong correlations between electro-oxidation peak potentials and spectrophotometrically determined radical scavenging activity of polyphenols (21, 24-26). A satisfactory correlation was also reported for polarographic oxidation half-peak potential ($E_{p/2}$) and water/octanol partition coefficient (log P) with flavonoid prooxidant toxicity (log cL_{50}) to HL-60 cells (r^2=0.915) (27). Perron et al. (28) reported that the first oxidation potential (E_{p1}) correlated with the pK_a of the most acidic phenolic hydrogen and with DNA damage inhibition under Fenton reaction conditions. We reported similar correlations and proposed models for the estimation of E_{p1} based on OH-related descriptors (29-31).

In this study we developed and tested an even simpler model based on the total number of hydroxyl groups (phenolic, alcoholic, and carboxylic) in polyphenol molecules (N_{OH}). The electro-oxidation potentials of 12 polyphenolic compounds (1-12) were measured in our laboratory at pH 3 using square-wave voltammetry (Table 1, Figure 1). The experimental values for some polyphenols (no. 1-4 and 13-15) at pH 3 and pH 7, were taken from our previous measurements (22, 32-34) and from Hotta et al. (23).

MATERIALS AND METHODS

Reagents

Figure 1 shows the structure of the 19 polyphenols included in this study. Quercetin dihydrate (≥98%), morin hydrate, (±)-naringenin (~95%), galangin, apigenin (≥95%), rutin hydrate (≥94%), chrysin (97%), (-)-epigallocatechin gallate [(-)-EGCG] (≥95%), (-)-epigallocatechin [(-)-EGC] (≥95%), (-)-epicatechin gallate [(-)-ECG] (≥95%), (-)-epicatechin [(-)-EC] (≥95%) and gallic acid monohydrate [GA] (≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Myricetin, dihydromyricetin (both ≥95%) and kaempferide were obtained from Extrasynthese (Genay, France). KNO_3 and absolute ethanol (both pro analysis) were purchased from Kemika (Zagreb, Croatia). Buffer solution pH 3 was obtained from Reagecon (Shannon, Co. Clare, Ireland). Water was deionised by the Millipore Milli-Q system to the resistivity ≥18 MΩ cm.

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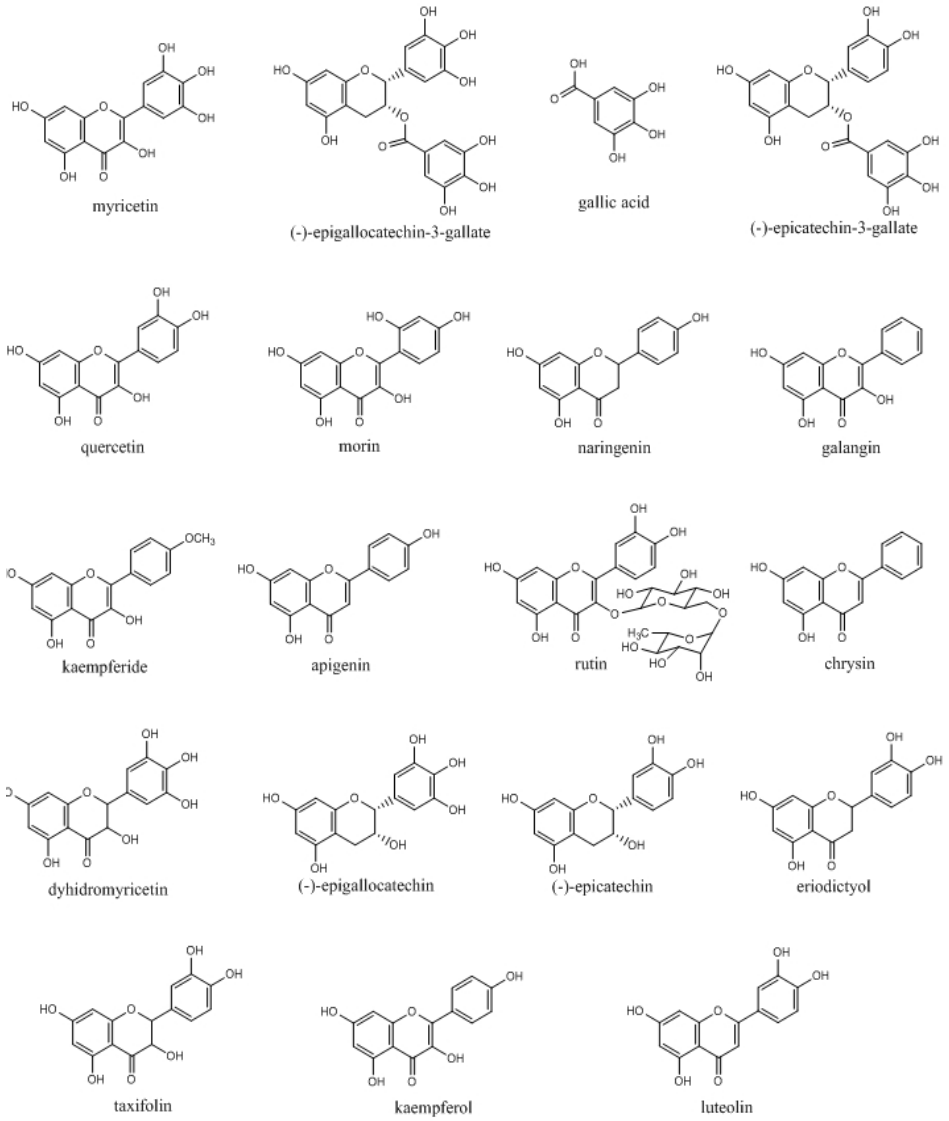

Figure 1 *Structures of the 19 polyphenolic compounds included in this study*

Stock standard solutions of polyphenols ($1.0x10^{-2}$ mol L^{-1}) were prepared from the dry pure substances. The stock solutions of catechins (EGCG, EGC, ECG, and EC) and gallic acid were prepared in deionised water obtained from a Millipore Milli-Q purification system. All other flavonoid stock standard solutions were prepared in absolute ethanol. The stock solutions were protected from light with aluminium foil and kept in a refrigerator.

Electrochemical measurements

For this study we determined the electrochemical oxidation potentials of 12 polyphenolic compounds using square-wave voltammetry (SWV) (Table 1). Voltammetric measurements were carried out using the computer-controlled electrochemical system μAutolab (Eco-Chemie, Utrecht, Netherlands) equipped with GPES software. Voltammetric curves were recorded using a three-electrode system (BioLogic, Claix, France) with a glassy-carbon (GC) working electrode (Ø=6 mm), an Ag/AgCl (3 mol L^{-1} NaCl) reference electrode, and a platinum wire counter electrode. Before each run the working electrode was polished with diamond spray (6 μm) and rinsed with ethanol and deionised water.

The working solutions of polyphenols ($1x10^{-4}$ mol L^{-1}) were obtained by diluting the stock solutions with a supporting electrolyte (0.1 mol L^{-1} KNO_3 buffered to pH 3) directly in the electrochemical cell. The solutions were degassed with a high-purity nitrogen prior to the electrochemical measurements and a nitrogen blanket was maintained thereafter. Square-wave voltammograms were recorded as soon as the working electrode was immersed into the solution to minimise polyphenol adsorption on the GC electrode surface. The SWV conditions were as follows: frequency - 100 Hz; square-wave amplitude - 25 mV; step potential - 2 mV. All experiments were performed at room temperature. The oxidation potentials of the remaining seven polyphenols and some overlapping polyphenols

Table 1 *First oxidation potentials (E_{p1}) of 19 polyphenolic compounds at pH 3 and pH 7*

No.	Polyphenol	E_{p1} (V) at pH 3		E_{p1} (V) at pH 7		N_{OH}	*In*
1	Myricetin	0.351[a]	0.357[b]	0.089[b]		6	0
2	Epigallocatechin-3-galate	0.367[a]	0.318[c]	0.051[c]		8	1
3	Gallic acid	0.545[a]	0.449[c]	0.267[c]	0.233[e]	4	0
4	Epicatechin-3-gallate	0.477[a]	0.409[d]	0.162[d]		7	1
5	Quercetin	0.435[a]		0.178[e]		5	0
6	Morin	0.458[a]		0.203[e]		5	0
7	Naringenin	0.929[a]		0.688[e]		3	1
8	Galangin	0.655[a]				3	0
9	Kaempferide	0.584[a]				3	0
10	Apigenin	0.928[a]		0.658[e]		3	1
11	Rutin	0.504[a]		0.360[e]		4	0
12	Chrysin	1.162[a]		0.794[e]		2	1
13	Dihydromyricetin	0.354[b]		0.098[b]		6	0
14	Epigallocatechin	0.307[c]		0.028[c]		6	0
15	Epicatechin	0.390[d]		0.150[d]	0.215[e]	5	0
16	Eriodictyol			0.240[e]		4	0
17	Taxifolin			0.248[e]		5	0
18	Kaempferol			0.242[e]		4	0
19	Luteolin			0.306[e]		4	0

N_{OH} - *number of OH groups;* In - *indicator variable;* [a] *measured in this study;* [b, c, d, e] *taken from references 32-34, and 23, respectively*

(Table 1) were taken from measurements reported elsewhere (22, 23, 32-34).

Theoretical methods

The variables used for the modelling of the polyphenol oxidation potentials included the number of OH groups (N_{OH}) and the indicator variable (*In*=0 or 1).

Regression calculations, including the leave-one-out procedure (LOO) of cross validation were done using the CROMRsel program (35). The standard error (SE) of the cross-validation (cv) estimate was calculated as follows:

$$SE_{cv} = \sqrt{\sum_i \frac{\Delta X_i^2}{N-1}} \qquad \text{[eq. 1]}$$

where ΔX and N denote cv residuals and the number of reference points, respectively.

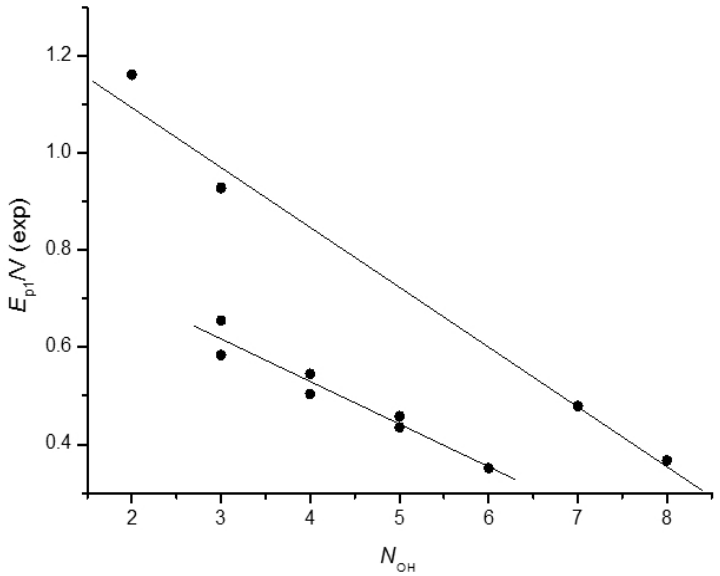

Figure 2 *Correlations between N_{OH} and E_{p1} measured in this study at pH 3 (polyphenols no. 1-12, Table 1)*

RESULTS AND DISCUSSION

The correlation of N_{OH} with the E_{p1} of the 12 polyphenols we measured at pH 3 (polyphenols no. 1-12, Table 1) yielded two polyphenol subsets. Both show the linear dependence of E_{p1} on N_{OH} and similar slopes [-0.1231(98) and -0.088(27)]. This is why we introduced into the regression the indicator variable (*In*) with values 0 and 1 for the two subsets: *In*=0 for polyphenols (no. 1, 3, 5, 6, 8, 9, and 11; Table 1) with N_{OH}=3-6 and at least two neighbouring OH groups or an OH group and carbonyl oxygen (because of the tautomerism involving neighbouring OH group and carbonyl oxygen) (36) and *In*=1 for other polyphenols (no. 2, 4, 7, 10, and 12; Table 1).

The model obtained in this way yielded r=0.986, SE=0.040, and SE_{cv}=0.057 (Table 2, Figure 3; N=12), which seems very good, considering that the standard deviations of the measurements reach 0.019, and the range of the experimental potentials is 0.811 V (*i.e.*, SE is only 5.4 % of the range).

To test the stability of our model, we added to the regression previously reported measurements (32-34) for polyphenols no. 1-4 and 13-15 (Table 1). This is why the regression includes two E_{p1} values (pH 3, N=19) for polyphenols no. 1 through 4. Even though the E_{p1} values for polyphenols no. 2, 3, and 4 reported earlier are much lower than the current measurements (differences ranging from 0.049 to 0.096 V), the model yielded only slightly lower statistics: r=0.980, SE=0.046 and SE_{cv}=0.059, which confirms its stability.

We also tested the model on the previously reported potentials of the 19 polyphenols measured at pH 7 (23, 32-34). Regardless of the differences between certain measurements (see polyphenols no. 3 and 15 in Table 1), again the model produced the statistics and slopes similar to the regressions for the measurements at pH 3 (r=0.983, SE=0.038, and SE_{cv}=0.043; Table 2). When we combined all the E_p values at both pH (N=38) and included pH as an additional variable into the regression, the model yielded r=0.985, SE=0.044, and SE_{cv}=0.049 (Table 2, Figure 4; N=38), which is just 3.9 % of the range of all E_p values.

CONCLUSION

The new model we have developed for the estimation of oxidation potentials of flavonoids based on the number of OH groups is simpler than our previous models (29-31) and yielded better statistics than the models reported in references 29 and 30. In addition, the model has turned out

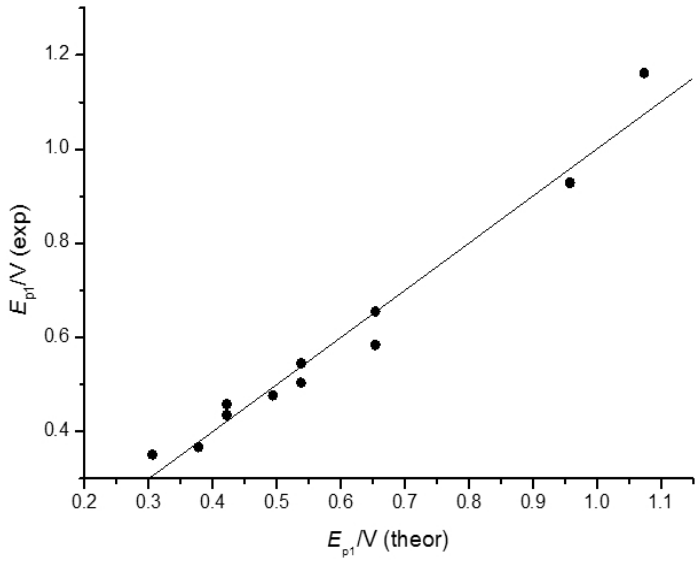

Figure 3 *Experimental (measured in this work)* vs. *theoretical* E_{p1} *values at pH 3; r=0.986, SE=0.040 (N=12, Table 2)*

Table 2 *Linear models for the estimation of* E_{p1}

pH	*N*	Slope (SE) Independent variable			Intercept (SE)	r	SE	SE_{cv}
		N_{OH}	*In*	pH				
3	12	-0.1161 (77)	0.305 (27)	-	1.002 (37)	0.986	0.040	0.057
3	19	-0.1179 (68)	0.287 (24)	-	1.009 (35)	0.980	0.046	0.059
7	19	-0.1191 (68)	0.244 (22)	-	0.774 (35)	0.983	0.038	0.043
3 and 7	38	-0.1177 (47)	0.267 (16)	-0.0635 (38)	1.205 (32)	0.985	0.044	0.049

r - *regression coefficient; SE - standard error;* SE_{cv} *- standard error of cross-validation*

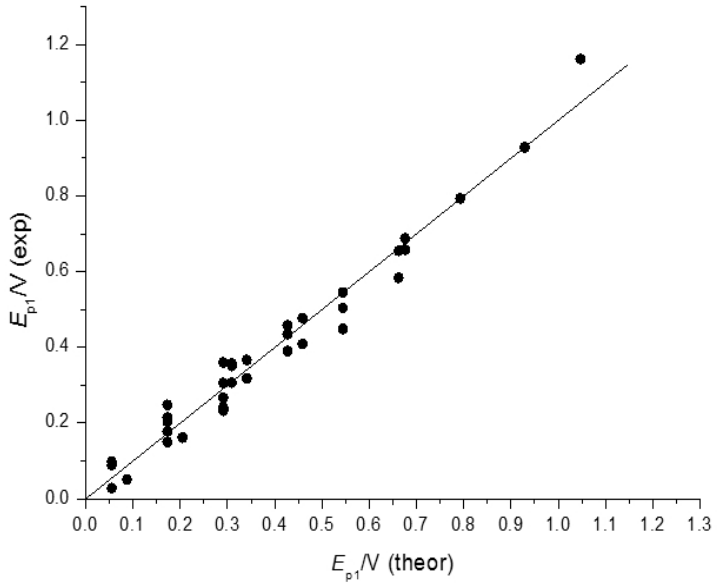

Figure 4 *Experimental* vs. *theoretical* E_{p1} *values at pH 3 and 7;* r=*0.985, SE=0.044 (N=38, Table 2)*

to be very stable, even though the inter-laboratory differences between experimental potentials were high (up to 0.096 V).

Acknowledgment

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Model za procjenu vrijednosti oksidacijskih potencijala polifenola temeljen na broju OH skupina

U radu je predstavljen novi i jednostavniji model za procjenu vrijednosti prvog oksidacijskog potencijala, E_{p1}, flavonoida, koji se temelji na broju fenolnih, alkoholnih i karboksilnih OH skupina. U regresiju smo uključili prve oksidacijske potencijale 12 polifenola (uglavnom flavonola i katehina) mjerene u našem laboratoriju pri pH 3. Model je dao r=0,986 i SE=0,040. Nakon uključivanja sedam ranije objavljenih E_{p1} vrijednosti, mjerenih pri pH 3, u regresiju, model daje r=0,980 i SE=0,046 (N=19), a nakon uključivanja njih još 19, mjerenih pri pH 7 (N=38), r=0,985, SE=0,044.

KLJUČNE RIJEČI: *flavonoidi; molekulsko modeliranje; QSAR/QSPR*