

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

Ivana Novak Jovanović and Ante Miličević

Institute for Medical Research and Occupational Health, Zagreb, Croatia

[Received in May 2017; Similarity Check in May 2017; Accepted in June 2017]

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_{p1} 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 ($\log \text{cL}_{50}$) 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 ($\geq 98\%$), morin hydrate, (\pm)-naringenin ($\sim 95\%$), galangin, apigenin ($\geq 95\%$), rutin hydrate ($\geq 94\%$), chrysin (97%), (-)-epigallocatechin gallate [(-)-EGCG] ($\geq 95\%$), (-)-epigallocatechin [(-)-EGC] ($\geq 95\%$), (-)-epicatechin gallate [(-)-ECG] ($\geq 95\%$), (-)-epicatechin [(-)-EC] ($\geq 95\%$) and gallic acid monohydrate [GA] ($\geq 99\%$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Myricetin, dihydromyricetin (both $\geq 95\%$) 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 $\geq 18 \text{ M}\Omega \text{ cm}$.

Correspondence to: Ante Miličević, Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10001 Zagreb, Croatia
E-mail: antem@imi.hr

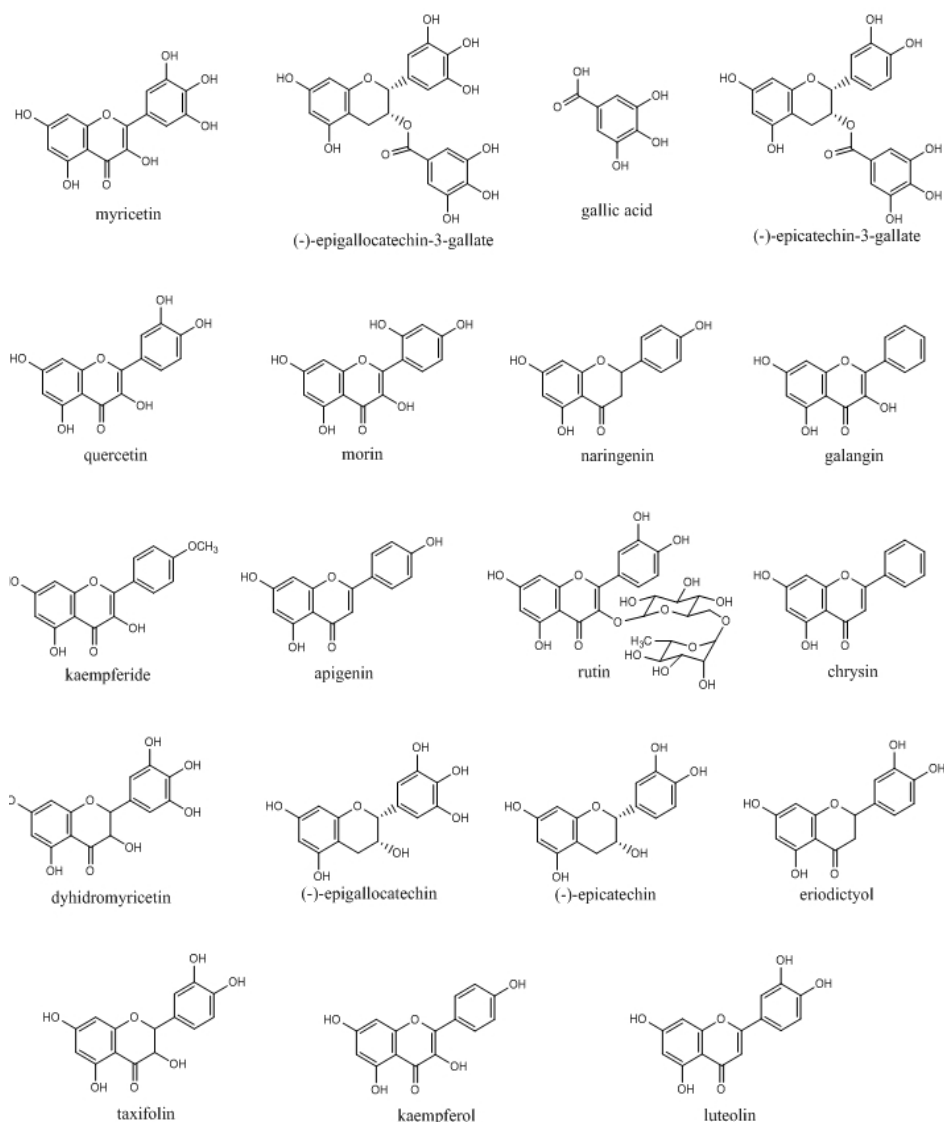


Figure 1 Structures of the 19 polyphenolic compounds included in this study

Stock standard solutions of polyphenols ($1.0 \times 10^{-2} \text{ mol L}^{-1}$) 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 μ Autolab (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 ($\varnothing=6 \text{ mm}$), an Ag/AgCl ($3 \text{ mol L}^{-1} \text{ NaCl}$)

reference electrode, and a platinum wire counter electrode. Before each run the working electrode was polished with diamond spray ($6 \mu\text{m}$) and rinsed with ethanol and deionised water.

The working solutions of polyphenols ($1 \times 10^{-4} \text{ mol L}^{-1}$) were obtained by diluting the stock solutions with a supporting electrolyte ($0.1 \text{ mol L}^{-1} \text{ KNO}_3$ 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_{pl}) of 19 polyphenolic compounds at pH 3 and pH 7

No.	Polyphenol	E_{pl} (V) at pH 3		E_{pl} (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 ^c	4	0
4	Epicatechin-3-gallate	0.477 ^a	0.409 ^d	0.162 ^d		7	1
5	Quercetin	0.435 ^a		0.178 ^c		5	0
6	Morin	0.458 ^a		0.203 ^c		5	0
7	Naringenin	0.929 ^a		0.688 ^c		3	1
8	Galangin	0.655 ^a				3	0
9	Kaempferide	0.584 ^a				3	0
10	Apigenin	0.928 ^a		0.658 ^c		3	1
11	Rutin	0.504 ^a		0.360 ^c		4	0
12	Chrysin	1.162 ^a		0.794 ^c		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 ^c	5	0
16	Eriodictyol			0.240 ^c		4	0
17	Taxifolin			0.248 ^c		5	0
18	Kaempferol			0.242 ^c		4	0
19	Luteolin			0.306 ^c		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).

CROMRsel program (35). The standard error (SE) of the cross-validation (cv) estimate was calculated as follows:

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

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

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

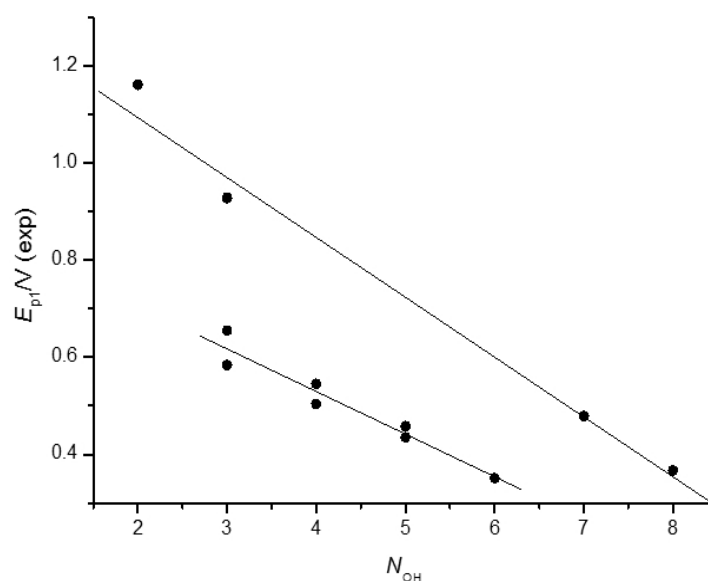


Figure 2 Correlations between N_{OH} and E_{pl} measured in this study at pH 3 (polyphenols no. 1-12, Table 1)

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

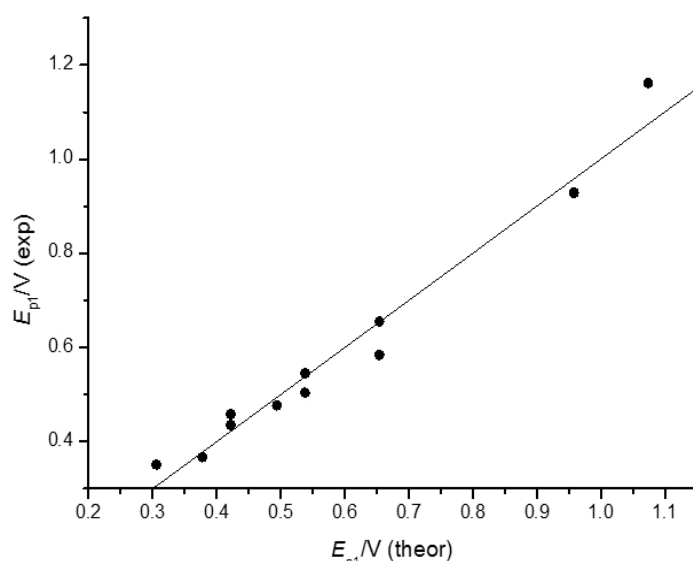


Figure 3 Experimental (measured in this work) vs. theoretical E_{p1} values at pH 3; $r=0.986$, $SE=0.040$ ($N=12$, Table 2)

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

pH	N	Slope (SE)			Intercept (SE)	r	SE	SE _{cv}
		Independent variable						
		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

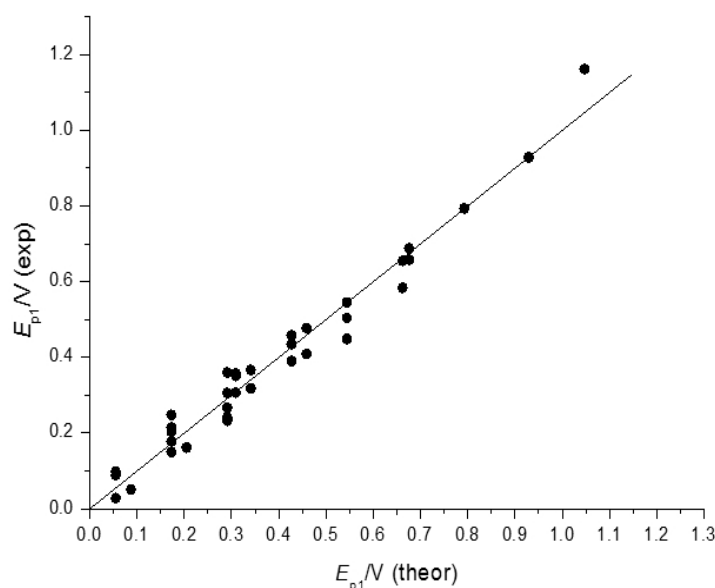


Figure 4 Experimental vs. theoretical E_{p1} values at pH 3 and 7; $r=0.985$, $SE=0.044$ ($N=38$, Table 2)

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

Acknowledgment

This work was supported by the Croatian Ministry of Science, Technology, Education and Sport, and the Croatia-Serbia Bilateral Agreement 2016-2017.

REFERENCES

1. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 1992;339:1523-6. doi: 10.1016/0140-6736(92)91277-F
2. Quiñones M, Miguel M, Alexandre A. Beneficial effects of polyphenols on cardiovascular disease. *Pharmacol Res* 2013;68:125-31. doi: 10.1016/j.phrs.2012.10.018
3. Di Domenico F, Foppoli C, Coccia R, Perluigi M. Antioxidants in cervical cancer: Chemopreventive and chemotherapeutic effects of polyphenols. *Biochim Biophys Acta* 2012;1822:737-47. doi: 10.1016/j.bbdis.2011.10.005
4. Kim M-J, Kim Y-J, Park H-J, Chung J-H, Leem K-H, Kim H-K. Apoptotic effect of red wine polyphenols on human colon cancer SNU-C4 cells. *Food Chem Toxicol* 2006;44:898-902. doi: 10.1016/j.fct.2005.08.031
5. Yamauchi R, Sasaki K, Yoshida K. Identification of epigallocatechin-3-gallate in green tea polyphenols as a potent inducer of p53-dependent apoptosis in the human lung cancer cell line A549. *Toxicol in Vitro* 2009;23:834-9. doi: 10.1016/j.tiv.2009.04.011
6. Stagos D, Amoutzias GD, Matakos A, Spyrou A, Tsatsakis AM, Kouretas D. Chemoprevention of liver cancer by plant polyphenols. *Food Chem Toxicol* 2012;50:2155-70. doi: 10.1016/j.fct.2012.04.002
7. Aquilano K, Baldelli S, Rotilio G, Ciriolo MR. Role of nitric oxide synthases in Parkinson's disease: A review on the antioxidant and anti-inflammatory activity of polyphenols. *Neurochem Res* 2008;33:2416-26. doi: 10.1007/s11064-008-9697-6
8. Plaza M, Batista ÂG, Cazarin CBB, Sandahl M, Turner C, Östman E, Maróstica Júnior MR. Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. *Food Chem* 2016;211:185-97. doi: 10.1016/j.foodchem.2016.04.142
9. Đudarić L, Fužinac-Smojver A, Muhvić D, Giacometti J. The role of polyphenols on bone metabolism in osteoporosis. *Food Res Int* 2015;77:290-8. doi: 10.1016/j.foodres.2015.10.017
10. Singh A, Holvoet S, Mercenier A. Dietary polyphenols in the prevention and treatment of allergic diseases. *Clin Exp Allergy* 2011;41:1346-59. doi: 10.1111/j.1365-2222.2011.03773.x
11. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2009;2:270-8. doi: 10.4161/oxim.2.5.9498
12. Guo Q, Zhao B, Shen S, Hou J, Hu J, Xin W. ESR study on the structure-antioxidant activity relationship of tea catechins and their epimers. *Biochim Biophys Acta* 1999;1427:13-23. doi: 10.1016/S0304-4165(98)00168-8
13. Nakagawa T, Yokozawa T. Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem Toxicol* 2002;40:1745-50. doi: 10.1016/S0278-6915(02)00169-2
14. Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Rad Biol Med* 1994;16:845-50. doi: 10.1016/0891-5849(94)90202-X
15. Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, Pieters L, Vlietnick AJ, Vanden Berghe D. Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J Nat Prod* 1998;61:71-6. doi: 10.1021/np970237h
16. Miller NJ, Castelluccio C, Tijburg L, Rice-Evans C. The antioxidant properties of theaflavins and their gallate esters - radical scavengers or metal chelators? *FEBS Lett* 1996;392:40-4. doi: 10.1016/0014-5793(96)00780-6

17. Na HK, Kim EH, Jung JH, Lee HH, Hyun JW, Surh YJ. (-)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. *Arch Biochem Biophys* 2008;476:171-7. doi: 10.1016/j.abb.2008.04.003
18. Estévez L, Mosquera RA. Molecular structure and antioxidant properties of delphinidin. *J Phys Chem A* 2008;112:10614-23. doi: 10.1021/jp8043237
19. Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* 2011;125:288-306. doi: 10.1016/j.foodchem.2010.08.012
20. Yang B, Kotani A, Aria K, Kusu F. Estimation of antioxidant activities of flavonoids from their oxidation potentials. *Anal Sci* 2001;17:599-604. PMID: 11708139
21. van Acker SABE, van den Berg DJ, Tromp MNJL, Griffioen DH, van Bennekom WP, van der Vijgh WJF, Bast A. Structural aspects of antioxidant activity of flavonoids. *Free Rad Biol Med* 1996;20:331-42. doi: 10.1016/0891-5849(95)02047-0
22. Komorsky-Lovrić Š, Novak Jovanović I. Abrasive stripping square wave voltammetry of some natural antioxidants. *Int J Electrochem Sci* 2016;11:836-42.
23. Hotta H, Nagano S, Ueda M, Tsujino Y, Koyama J, Osakai T. Higher radical scavenging activities of polyphenolic antioxidants can be ascribed to chemical reactions following their oxidation. *Biochim Biophys Acta* 2002;1572:123-32. doi: 10.1016/S0304-4165(02)00285-4
24. Firuzi O, Lacanna A, Petrucci R, Morrosu G, Saso L. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochim Biophys Acta* 2005;1721:174-84. doi: 10.1016/j.bbagen.2004.11.001
25. de Lima AA, Sussuchi LEM, de Giovanni WF. Electrochemical and antioxidant properties of anthocyanins and anthocyanidins. *Croat Chem Acta* 2007;80:29-34.
26. Aertega JF, Ruiz-Montoya M, Palma A, Alonso-Garrido G, Pintado S, Rodríguez-Mellado JM. Comparison of the simple cyclic voltammetry (CV) and DPPH assays for the determination of antioxidant capacity of active principles. *Molecules* 2012;12:5126-38. doi: 10.3390/molecules17055126
27. Dićkancaitė E, Nemeikaitė A, Kalvelytė A, Čėnas N. Prooxidant character of flavonoid cytotoxicity: Structure-activity relationship. *Biochem Mol Biol Int* 1998;45:923-30. doi: 10.1016/S0014-5793(99)01561-6
28. Perron NR, Hodges JN, Jenkins M, Brumaghim JL. Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. *Inorg Chem* 2008;47:6153-61. doi: 10.1021/ic7022727
29. Miličević A, Raos N. Modelling of protective mechanism of iron(II)-polyphenol binding with OH-related molecular descriptors. *Croat Chem Acta* 2016;89:1-5. doi: 10.5562/cca2996
30. Raos N, Miličević A. QSAR analysis of antioxidant properties of polyphenols by OH-related molecular descriptors. In: *Proceedings of the 4th South-East European Conference on Computational Mechanics, Kragujevac 2017* [in press].
31. I. Novak Jovanović I, Miličević A. A model for the estimation of oxidation potentials of polyphenols. *J Mol Liquids* 2017;241:255-9. doi: 10.1016/j.molliq.2017.06.017
32. Komorsky-Lovrić Š, Novak I. Abrasive stripping voltammetry of myricetin and dihydromyricetin. *Electrochim Acta* 2013;98:153-6. doi: 10.1016/j.electacta.2013.03.062
33. Novak I, Šeruga M, Komorsky-Lovrić Š. Electrochemical characterization of epigallocatechin gallate using square-wave voltammetry. *Electroanalysis* 2009;21:1019-25. doi: 10.1002/elan.200804509
34. Novak I, Šeruga M, Komorsky-Lovrić Š. Square-wave voltammetry of epicatechin gallate on glassy carbon electrode. *J Electroanal Chem* 2009;631:71-5. doi: 10.1016/j.jelechem.2009.03.005
35. Lučić B, Trinajstić N. Multivariate regression outperforms several robust architectures of neural networks in QSAR modeling. *J Chem Inf Comput Sci* 1999;39:121-32. doi: 10.1021/ci980090f
36. Chakraborty S, Basu S, Basak S. Effect of β -cyclodextrin on the molecular properties of myricetin upon nano-encapsulation: Insight from optical spectroscopy and quantum chemical studies. *Carbohydr Polym* 2014;99:116-25. doi: 10.1016/j.carbpol.2013.08.008

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; molekulska modeliranje; QSAR/QSPR