PRELIMINARY COMMUNICATION

CROATICA CHEMICA ACTA

Croat. Chem. Acta **2016**, *89*(4), 511–515
Published online: December 29, 2016
DOI: 10.5562/cca2996

HKD 1926

Modelling of Protective Mechanism of Iron(II)-polyphenol Binding with OH-related Molecular Descriptors

Ante Miličević,* Nenad Raos

Institute for Medical Research and Occupational Health, Ksaverska c. 2, P.O. Box 291, HR-10001 Zagreb, Croatia
* Corresponding author's e-mail address: antem@imi.hr

RECEIVED: September 19, 2016 ✶ REVISED: November 21, 2016 ✶ ACCEPTED: November 21, 2016

THIS PAPER IS DEDICATED TO PROF. NENAD TRINAJSTIĆ ON THE OCCASION OF HIS 80TH BIRTHDAY

Abstract: The linear models for the calculation of pIC_{50}, pK_{a1} and Ep_a for 12 polyphenolic compounds (catechins, flavonols, catechol and gallol derivatives) were developed. As descriptors we used the number of vicinal (N_v) and non-vicinal (N_{nv}) OH groups, as well as the number of OH vicinal pairs as possible Fe^{2+} chelate sites (N_{ch}). The models gave $r > 0.9$ and standard errors of 0.13, 0.26 and 0.04 for pIC_{50}, pK_{a1} and Ep_a, respectively. For modelling of pIC_{50}, N_{ch} is better variable than N_v, and *vice versa* for modelling of pK_{a1} and Ep_a. This result, along with good correlations between pIC_{50}, pK_{a1} and Ep_a, suggests two effects for antioxidative activity of polyphenols; their reaction(s) with OH and prevention of Fenton reaction by Fe^{2+} chelation.

Keywords: antioxidative activity, Fenton reaction, DNA damage, QSAR.

INTRODUCTION

THE protective action of polyphenols is well known,[1–5] but the mode of their action has not yet been sufficiently explained. There are general reaction mechanisms for scavenging of radicals by polyphenols, such as single-step hydrogen atoms transfer (HAT), single electron transfer followed by proton transfer (SET-PT) and sequential proton loss electron transfer (SPLET).[6,7,8] However, this paper is concerned with the mechanism involving polyphenol interaction with iron(II),[9] preventing in this way Fenton reaction:[10,11]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + \cdot OH \qquad (1)$$

The last mechanism is also relevant to DNA damage because hydroxyl radical is the primary cause of cell death under oxidative stress conditions.[12,13] The protective role of polyphenols is thus viewed as being iron(II) chelators, preventing the reduction of Fe^{2+} with H_2O_2. The theory is supported by direct experimental evidence,[14,15] as well as the study of polyphenol binding to iron(II) and other heavy metals.[16–21] Analogous mechanism was also proposed for copper(I) / copper(II) system, but it proved less efficient than the already mentioned.[22,23]

There is yet no general regression model (QSAR or QSPR) for the activity and physicochemical properties of polyphenols, despite many models with various molecular descriptors were tried. The binding of flavonoids to P-glycoprotein was modelled by sophisticated CoMFA and CoMSIA methods[24] and MIFs and VolSurf descriptors,[25] but also using a simple linear model based on zero-order valence molecular connectivity index.[26] The flavonoid toxicity (log cL_{50}) to HL-60 and lamb embrio kidney fibroblast (FLK) cells were correlated to polarographic oxidation half-peak potential ($E_{2/p}$) and water / octanol partition coefficient (log *P*) yielding in two-variable linear regression satisfactory correlation for HL-60 ($r^2 = 0.915$), but not for FLK cells ($r^2 = 0.674$).[27] Filipović and co-workers correlated VCEAC (antioxidant capacity equivalent to vitamin C concentration) values for 21 polyphenols with a

number of descriptors, but BDE (bond dissociation enthalpy), PA (proton affinity), ETE (electron-transfer enthalpy) and nOH_{vic} (number of vicinal OH groups) proved best.[28] By combining two (BDE, nOH_{vic}) and three descriptors (PA, ETE, nOH_{vic}) they obtained r = 0.957 and r = 0.962 for the first and the second model, respectively. The similar model was used by Amić *et al.* on the set of 29 flavonoids (r = 0.974).[29] Perron and coworkers used pK_a of the most acidic phenolic hydrogen as a sole descriptor for the modeling of inhibition of DNA damage under Fenton reaction conditions, but found the same regression cannot be successfully used for all the investigated polyphenols.[15] However, pK_a proved significantly better descriptor than the reduction or oxidation potential.

The aim of this contribution is to apply on the mentioned DNA system[15] a simpler, but possibly more successful set of molecular descriptors. For that purpose we chose descriptors based on number and position of phenolic and other hydroxyl group (alcoholic, carboxylic) in the molecules of polyphenols.

METHODS

For the given set of 12 polyphenols (catechins, flavonols, catechol and gallol derivatives, Figure 1) we tried to estimate their antioxidative activity by modelling pIC_{50} (obtained from the percentage of DNA damage inhibition) and Ep_a (oxidation potential), as well as pK_{a1} values of the

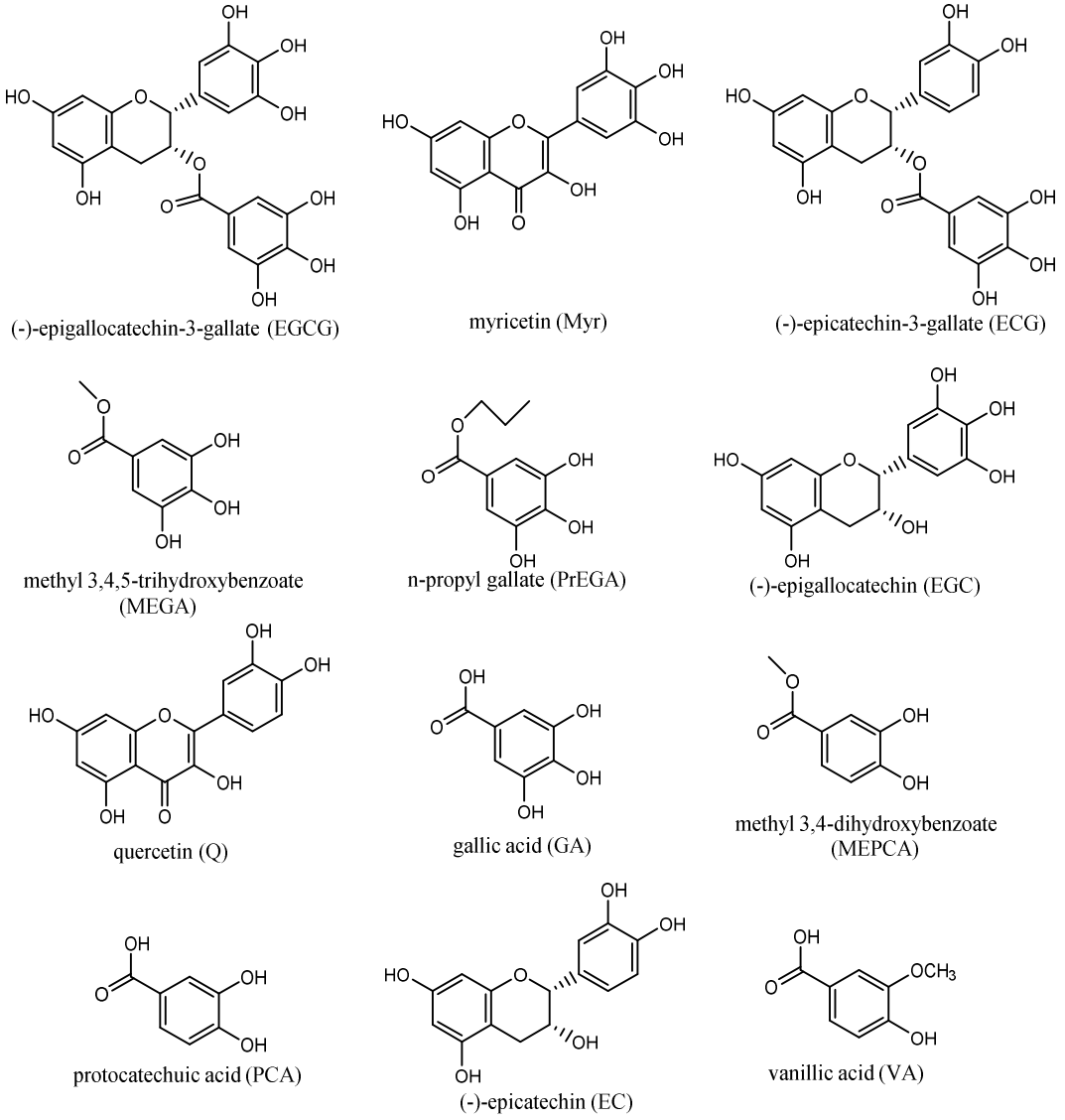

Figure 1. Structures of polyphenolic compounds and their abbreviations.

most acidic (phenolic) hydrogen compiled from six different sources.[15]

The variables used for modelling pIC_{50} of polyphenols are related to the number and position of OH groups in molecule *viz.* the number of vicinal (N_v) and of non-vicinal (N_{nv}) OH groups, and the number of OH pairs which may form a stable five-membered chelate rings with Fe^{2+} (N_{ch}). As carbonyl oxygen of flavonols participates in Fe^{2+} chelation,[30] it is treated in the same way as OH group.

Regression calculations, including the leave-one-out procedure (LOO) of cross validation were done using the CROMRsel program.[31] The standard error of the cross-validation estimate was defined as:

$$S.E._{cv} = \sqrt{\sum_i \frac{\Delta X_i^2}{N-1}} \quad (2)$$

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

RESULTS AND DISCUSSION

From Table 2 it can be seen that Model 2 (Figure 2) is consistently better than Model 1. Variable N_{ch}, if taken alone, is better descriptor (r = 0.946) than N_v (r = 0.922) when correlated to pIC_{50}. This speaks in favour of the assumption[9,15] that Fe^{2+} chelation is the dominant effect for antioxidative activity, for N_{ch} is a measure of chelating capacity of the studied compounds.

As shown previously,[15] pIC_{50} shows also a good correlation with pK_{a1} (r = –0.897, Figure 3), but our models proved better and they don't use experimental values as descriptors. Correlation of pK_{a1} to pIC_{50} also points to the ability of iron bonding, but pK_{a1} is not directly related to chelate but rather to inductive effect (electron affinity). This suggests that both effects participate in the antioxidative activity of polyphenols. Comparison of Models 3 and 4 leads to the same conclusion. Model 3 is better than Model 4, and pK_{a1} correlates better with N_v (r = –0.867) than with N_{ch} (r = –0.818). Namely, N_v is not related directly to the number of chelation sites but to the number of neighbouring OH groups which mutually affect acidity.

We also correlated oxidation potential, Ep_a, to pIC_{50} (r = –0.770) and to pK_{a1} (r = 0.824) (Figures 4 and 5), which is comparable with the previously published results of Ep_a *vs.* IC_{50} for six cateholate compound (r = –0.889).[15] The correlations were substantially improved after removal of EGCG (the highest N_{ch} and the highest pIC_{50}) and other catechins (ECG, EGC, and EC); we obtained r = –0.953 and 0.969 for Ep_a, *vs.* pIC_{50} and Ep_a, *vs.* pK_{a1}, respectively. Also, for the estimation of Ep_a, Model 5 proved better than model 6, and N_v gives higher correlation (r = –0.933) than N_{ch} (r = –0.911) with Ep_a.

Table 1. pIC_{50}, pK_{a1} and Ep_a values[15] for 12 polyphenolic compounds and number of vicinal, N_v, and nonvicinal, N_{nv}, OH groups and OH pairs as chelation sites, N_{ch}.

polyphenol	pIC_{50}	pK_{a1}	Ep_a / V	N_v	N_{nv}	N_{ch}
EGCG	5.9586	7.55	0.293	6	2	4
Myr[(a)]	5.6990	6.89	0.169	5	2	3
ECG	5.6383	7.6	0.316	5	2	3
MEGA	5.3979	7.90	0.293	3	0	2
PrEGA	5.2924	7.77	0.288	3	0	2
EGC	5.0088	8.51	0.255	3	3	2
Q[(a)]	4.9706	7.65	0.250	4	2	2
GA	4.8539	8.45	0.433	3	1	2
MEPCA	4.8069	8.12	0.380	2	0	1
PCA	4.4634	8.64	0.538	2	1	1
EC	4.2284	8.76	0.356	2	3	1
VA	3.8539	9.39	0.771	0	2	0

[(a)] Carbonyl oxygen is treated as OH group.

Table 2. Linear models for the estimation of pIC_{50}, pK_{a1} and Ep_a.

Model No.	N	Dependent variable	Slope (S.E.) Independent variable			Intercept (S.E.)	r	S.E.	$S.E._{cv}$
			N_{ch}	N_v	N_{nv}				
1	12	pIC_{50}	–	0.368(35)	–0.161(53)	4.09(14)	0.963	0.16	0.21
2	12	pIC_{50}	0.563(41)	–	–0.141(41)	4.15(10)	0.977	0.13	0.17
3	12	pK_{a1}	–	–0.376(57)	0.180(86)	9.02(22)	–0.913	0.26	0.34
4	12	pK_{a1}	–0.53(11)	–	0.15(11)	8.89(27)	–0.854	0.34	0.44
5	8	Ep_a	–	–0.124(13)	0.059(21)	0.672(43)	–0.975	0.04	0.07
6	8	Ep_a	–0.191(34)	–	0.038(34)	0.662(72)	–0.930	0.07	0.10

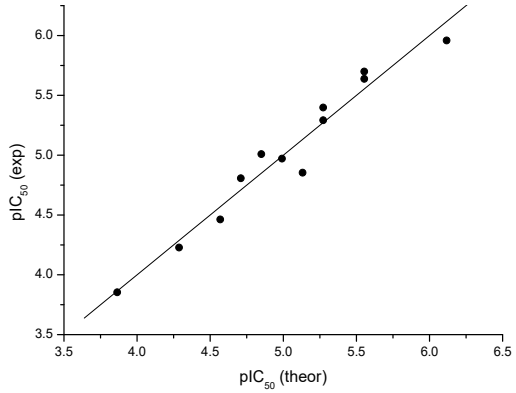

Figure 2. Experimental *vs.* theoretical pIC_{50} values for 12 polyphenoles (Model 2, Table 2).

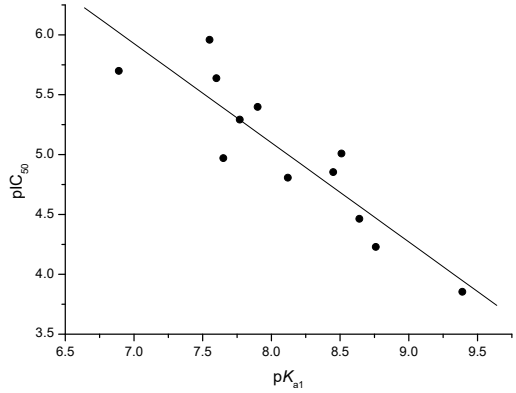

Figure 3. Correlation of experimental pK_{a1} to pIC_{50} values for 12 polyphenoles; r = –0.897, S.E. = 0.27, $S.E._{cv}$ = 0.32.

As the oxidation potential is a measure of ability for releasing of electrons, it is related to the antioxidative mechanisms involving reaction of polyphenols with ·OH. We would therefore conclude that the influence of two effects is different for catechins than for other polyphenols in the set. Although EGCG shows the highest activity, its Ep_a is higher than Ep_a of Myr, implying that EGCG activity is caused more by chelate effect than the activity of Myr.

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

REFERENCES

[1] A. Scalbert, I. T. Johnson, M. Saltmarsh, *Am. J. Clin. Nutr.* **2005**, *81*, 2015S.

[2] C. A. Rice-Evans, N. J. Miller, G. Paganga, *Free Rad. Biol. Med.* **1996**, *20*, 933.

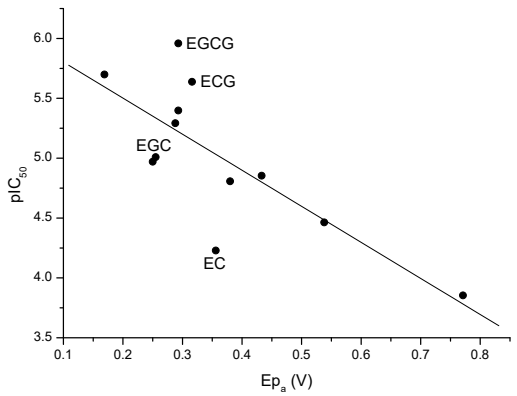

Figure 4. Correlation of experimental Ep_a to pIC_{50} values for 12 polyphenoles; r = –0.770, S.E. = 0.38, $S.E._{cv}$ = 0.43. (Catechins are marked with the letter code.)

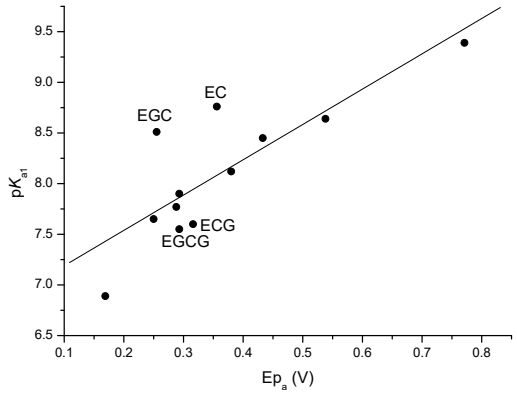

Figure 5. Correlation of experimental Ep_a to pK_{a1} values for 12 polyphenoles; r = 0.824, S.E. = 0.37, $S.E._{cv}$ = 0.44. (Catechins are marked with the letter code.)

[3] I. S. Young, J. V. Woodside, *J. Clin. Pathol.* **2001**, *54*, 176.

[4] B. Halliwell, *Free Radic. Biol. Med.* **2009**, *46*, 531.

[5] B. Badhani, N. Sharma, R. Kakkar, *Roy. Soc. Chem. Adv.* **2015**, *5*, 27540.

[6] H.-Y. Zhang, H.-G. Ji, *New J. Chem.* **2006**, *30*, 503.

[7] G. Litwinienko, K. U. Ingold, *Acc. Chem Res.* **2007**, *40*, 222.

[8] J. S. Wright, E. R. Johnson, G. A. DiLabio, *J. Am. Chem. Soc.* **2001**, *123*, 1173.

[9] N. R. Perron, J. L. Brumaghin, *Cell. Biochem. Biophys.* **2009**, *53*, 75.

[10] J. A. Imlay, S. Linn, *Science* **1988**, *240*, 1302.

[11] E. S. Henle, Z. Han, N. Tang, P. Rai, Y. Luo, S. Linn, *J. Biol. Chem.* **1999**, *274*, 962.

[12] A. C. Mello-Filho, R. Meneghini, *Mutat. Res.* **1991**, 251, 109.

[13] L. Macomber, C. Rensing, J. A. Imlay, *J. Bacteriol.* **2007**, *189*, 1616.
[14] C. Zhao, G. Dodin, C. Yuan, H. Chen, R. Zheng, Z. Jia, B.-T. Fan, *Biochim. Biophys. Acta* **2005**, *1723*, 114.
[15] N. R. Perron, J. N. Hodges, M. Jenkins, J. L. Brumaghim, *Inorg. Chem.* **2008**, *47*, 6153.
[16] G. Erdogan, R. Karadag, E. Dolen, *Revs. Anal. Chem*. **2005**, *24*, 247.
[17] L. B. Escudero, C. M. Fusari, J. C. Altamirano, A. B. Camargo, R. G. Wuilloud, *J. Food Sci.* **2014**, *79*, C1933.
[18] G. Le Nest, O. Caille, M. Woudstra, S. Rodche, B. Burlat, V. Belle, B. Guigliarelli, D. Lexa, *Inorg. Chim. Acta* **2004**, 357, 2027.
[19] M. Andjelković, J. Van Camp, B. De Meulenaer, G. Depaemelaere, C. Socaciu, M. Verloo, R. Verhe, *Food Chem*. **2006**, *98*, 23.
[20] S. Teixeira, C. Siquet, C. Alves, I. Boal, M. P. Marques, F. Borges, J. L. F. C. Lima, S. Reis, *Free Rad. Biol. Med*. **2005**, *39*, 1099.
[21] R. C. Hider, Z. D. Liu, H. H. Khodr in *Methods in Enzymology*; *Vol. 335* (Ed.: L. Packer) Academic Press, San Diego, **2001**; pp 190–203.
[22] J. E. Brown, H. Khodr, R. C. Hider, C. A. Evans, *Biochem. J*. **1998**, *330*, 1173.
[23] N. R. Perron, C. R. García, J. R. Pinzón, M. N. Chaur, J. L. Brumaghim, *J. Inorg. Biochem*. **2011**, *105*, 745.
[24] G. Kothandan, C. G. Gadhe, T. Madhavan, C. H. Choi, S. J. Cho, *Eur. J. Med. Chem.* **2011**, 46, 4078.
[25] J. Boccard, F. Bajot, A. DiPietro, S. Rudaz, A. Boumendjel, E. Nicolle, P. A. Carrupt, *Eur. J. Pharm. Sci.* **2009**, 36, 254.
[26] A. Miličević, N. Raos, *Arh. Hig. Rada Toksikol.* **2016**, *67*, 55.
[27] E. Dičkancaité, A. Nemeikaité, A. Kalvelyté, N. Čénas, *Biochem. Mol. Biol. Intern*. **1988**, *45*, 923.
[28] M. Filipović, Z. Marković, J. Đorović, J. Dimitrić Marković, B. Lučić, D. Amić, *C. R. Chemie* **2015**, *18*, 492.
[29] D. Amić, D. Davidović-Amić, D. Bešlo, N. Trinajstić, *Croat. Chem. Acta* **2003**, *76*, 55.
[30] R. F. V. Souza, E. M. Sussuchi, W. F. Giovani, *Synt. React. Inorg. Met.-Org. Chem.* **2003**, *33*, 1125.
[31] B. Lučić, N. Trinajstić, *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 121.