Food Chemistry 119 (2010) 1205–1210

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidative and vasodilatory effects of phenolic acids in wine

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ARTICLE INFO

Article history:
Received 2 April 2009
Received in revised form 14 July 2009
Accepted 27 August 2009

Keywords:
Phenolic acids
Vasodilation
Antioxidative activity
Wine
Quantitative structure–activity relationship

ABSTRACT

Phenolic acids represent important fraction of wine phenolics, but their biological effects have been scarcely investigated. We examined the interrelationship between antioxidative capacity and vasodilatory activity, two potentially beneficial biological effects, of nine phenolic acids from wine. The observed antioxidative and vasodilatory effects of the tested phenolic acids were further evaluated through quantitative structure–activity relationship (QSAR) analysis, by using molecular properties, "two-dimensional" (2D) and "three-dimensional" (3D) molecular descriptors. The antioxidative capacity of phenolic acids was measured by ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) methods, whereas their vasodilatory activity was determined in the precontracted rat aortic rings.

FRAP and TEAC values for antioxidative capacity positively correlated, but antioxidative capacity and maximal vasodilatory effect of the acids showed a negative correlation. This was best illustrated by poor vasodilatory activity of gallic acid, which is the strongest antioxidant among the tested phenolic acids. QSAR study described how antioxidative and vasodilatory effects of phenolic acids relate to the number of hydroxyl groups in the phenyl ring, degree of compactness and branching of molecules, and three-dimensional distributions of atomic polarisability of the tested molecules.

1. Introduction

Epidemiological studies have shown that moderate wine intake may be beneficial for human health (Renaud & De Lorgeril, 1992). Among many beneficial effects, wine inhibits low density lipoprotein (LDL) oxidation (Frankel, Kanner, German, Parks, & Kinsella, 1993), increases antioxidative capacity in humans (Maxwell, Cruickshank, & Thorpe, 1994; Modun et al., 2008) and modulates vascular function by inducing vasodilation through increased production of nitric oxide (NO) (Fitzpatrick, Hirschfield, & Coffey, 1993; Flesch, Schwarz, & Bohm, 1998). These effects are mainly attributed to the wine phenolics, especially flavonoids, as their intake is also inversely associated with the incidence of many diseases, including coronary heart disease (CHD) (Stoclet et al., 2004).

An important fraction of wine phenolics are phenolic acids (German & Walzem, 2000). Phenolic acids and stilbenes are important non-flavonoid compounds present in grapes and wine. Phenolic acids are present in their free form or as glycosylated derivatives and esters of tartaric, quinic and shikimic acid in both red and white wines (Monagas, Bartolome, & Gomez-Cordoves, 2005). As well as grapes and wine, phenolic acids are also present in other fruits and vegetables, and in tea (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). In spite of that, phenolic acids have been insufficiently investigated, especially for a possible interrelationship between their different biological effects, like antioxidative and vasodilatory activity. The structure-antioxidative activity relationship *in vitro* has been determined for several phenolic acids (Kim & Lee, 2004). Also, some wine phenolic acids have been shown *in vitro* to have vasodilatory activity (Andriambeloson et al., 1998). Ferulic acid lowers blood pressure in spontaneously hypertensive rats (Suzuki et al., 2002), and restores endothelium-dependent vasodilatation in aortas from spontaneously hypertensive rats, by increasing NO bioavailability due to its antioxidative activity (Suzuki et al., 2007).

The aim of this study was to determine and correlate antioxidative and vasodilatory activities of nine phenolic acids from wine: *p*-hydroxybenzoic, protocatechuic, vanillic, gallic, and syringic acids, as derivatives of hydroxybenzoic acid, and *p*-coumaric, caffeic, ferulic, and sinapic acids, as derivatives of hydroxycinnamic acid (Fig. 1). The observed antioxidative capacities and vasodilatory activities of the tested phenolic acids were further evaluated

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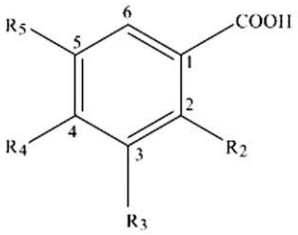
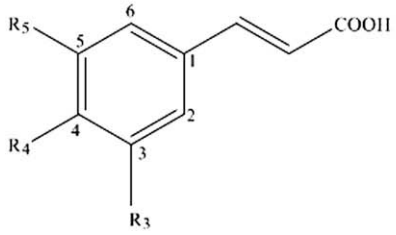
doi:10.1016/j.foodchem.2009.08.038



Hydroxybenzoic acid	R_2	R_3	R_4	R_5
p-Hydroxybenzoic	H	H	OH	H
Protocatechuic	H	OH	OH	H
Vanillic	H	OCH_3	OH	H
Gallic	H	OH	OH	OH
Syringic	H	OCH_3	OH	OCH_3



Hydroxycinnamic acid	R_3	R_4	R_5
p-Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	OCH_3	OH	H
Sinapic	OCH_3	OH	OCH_3

Fig. 1. Structures and classification of the tested phenolic acids.

through quantitative structure–activity relationship (QSAR) analysis, by using molecular properties, "two-dimensional" (2D) and "three-dimensional" (3D) molecular descriptors.

2. Materials and methods

2.1. Chemicals

Phenolic acids (*p*-hydroxybenzoic, *p*-coumaric, vanillic, ferulic, protocatechuic, caffeic, sinapic, syringic, and gallic acid) were purchased as pure compounds from Sigma–Aldrich Chemie (Steinheim, Germany). Ferric chloride ($FeCl_3$), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ), 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS), Trolox, potassium persulfate, noradrenaline (NA) and acetylcholine (Ach) were also obtained from Sigma–Aldrich Chemie. All solvents used were of HPLC grade or the highest purity available. All solutions and reagents were made with deionised (Milli Q) water.

2.2. Antioxidative capacity of phenolic acids

The antioxidative capacity of phenolic acids (1 mM) was measured by ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) methods.

In the FRAP assay, antioxidants are evaluated as reductants of Fe^{3+} to Fe^{2+}, which is chelated by TPTZ to form a Fe^{2+}–TPTZ complex absorbing at 593 nm (Benzie & Strain, 1996). TEAC assay is based on the inhibition of the absorbance of the radical cation of 2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonate) ($ABTS^{\cdot+}$), which has a characteristic long-wavelength absorption spectrum showing a maximum at 734 nm (Re et al., 1999), with the tested antioxidant. Absorbance was monitored by a UV–Vis spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany), equipped with a six-cell holder and a thermostatically controlled bath. All measurements were done in triplicate. Results were compared with a standard curve prepared daily with different concentrations of Trolox, a water-soluble analogue of vitamin E, and were expressed as milimolar Trolox equivalents.

2.3. Vasodilatory properties of phenolic acids

All animal experiments were conducted in accordance with international ethical guidelines. The study was approved by the Ethics Committee of the University of Split School of Medicine. Wistar rats, 3 months old and 330 ± 20 g of body weight were used for this study. The animals received an intraperitoneal injection of urethane (1.2 g/kg). After becoming unresponsive to noxious stimuli, they were decapitated. The descending thoracic aorta was dissected free from the connective tissue and placed in modified Krebs–Henseleit solution. The aorta was carefully cleaned of the adhering fat and cut into four rings of 3–4 mm in length. After wash-out and stabilisation in modified Krebs–Henseleit solution, each ring was precontracted with the test dose of noradrenaline (NA, 10^{-7} M). When the contraction reached the plateau phase, endothelium-dependent relaxation was induced by acetylcholine (Ach, 10^{-6} M). The functionality of endothelium was confirmed if 10^{-6} M Ach induced more than 70% relaxation of precontracted rings. The relaxation was expressed as the percent decrease of vasoconstriction induced by NA. The rings that relaxed less than 70% were excluded from the study. After triple wash-out and tension stabilisation, the aortic rings were again precontracted with NA (10^{-7} M). After the stable plateau was reached, the rings ($n = 12$ per acid) were randomly exposed to cumulative concentrations of the tested phenolic acid (10^{-6}–10^{-3} g/l) corresponding to 7.25×10^{-9} to 7.25×10^{-6} M of *p*-hydroxybenzoic acid, to 6.09×10^{-9} to 6.09×10^{-6} M of *p*-coumaric acid, to 5.95×10^{-9} to 5.95×10^{-6} M vanillic acid, to 5.15×10^{-9} to 5.15×10^{-6} M ferulic acid, to 6.49×10^{-9} to 6.49×10^{-6} M protocatechuic acid, to 5.55×10^{-9} to 5.55×10^{-6} M caffeic acid, to 5.05×10^{-9} to 5.05×10^{-6} M syringic acid, to 4.46×10^{-9} to 4.46×10^{-6} M sinapic acid, and to 5.88×10^{-9} to 5.88×10^{-6} M gallic acid.

2.4. QSAR study

2.4.1. Generation of physicochemical properties and molecular descriptors

The geometry optimisation of the nine phenolic acids was performed using the Austin Model 1 (AM1) semi-empirical method (Dewar, Zoebisch, Healy, & Stewart, 1985) applying the HyperChem 8.0 Evaluation software package (Hypercube Inc., Gainesville, USA). Some of the molecular properties (e.g., surface area, volume of the molecule, hydration energy, refractivity, polarisability) were calculated by HyperChem. 2D topological indices (e.g., Wiener index (W), mean distance degree deviation (ΔD)), 3D molecular descriptors, and some molecular properties (lipophilicity, Topological Polar Surface Area) were calculated applying the online software Parameter Client (PCLIENT, an online version of the Dragon software, Milano, Italy). Five groups of 3D descriptors were used to generate QSAR models: geometrical, Molecule

Representation of Structures based on Electron diffraction (3D-MoRSE), Randic molecular profiles, GETAWAY (Geometry, Topology, and Atom Weights AssemblY) and RDF (Radial Distribution Function) (Tetko et al., 2005).

QSAR study also includes the number of hydroxyl and methoxy groups on the phenyl ring (n_{OH}, n_{OCH3}) and indicator variables as descriptors. Indicator variables were defined on the basis of former SAR studies (Nenadis & Tsimidou, 2002; Rice-Evans, Miller, & Paganga, 1996) as the presence of 3,4-OH groups ($I_{3,4\text{-OH}} = 1$) or their absence ($I = 0$).

2.4.2. Selection of descriptors and statistical analysis

The statistical analysis was performed using STATISTICA 6.0 (StatSoft, Inc., Tulsa, OK). The relationship between 2D, 3D descriptors, physicochemical properties, indicator variables and antioxidative/vasodilatory activities of phenolic acids were determined by simple linear and multiple regression analyses. The selection of predictor variables for regression was performed by best-subset method in which a regression equation was fitted to every subset of independent variables. The criterion used to determine "best" is based on the r^2 values of analysed models. To test the quality and accuracy of derived models, the following statistical parameters were used: squared correlation coefficient (r^2), standard deviation of regression (s) and Fisher ration values (F).

The best possible QSAR models, which are presented in this report, were selected on the basis of the highest correlation coefficients and F-test, and the lowest standard deviations. The selected models were additionally validated by the calculation of quality factor (Q) and significance level of the model p. Quality factor Q is defined as a ratio of correlation coefficient (r) and standard deviation of regression ($Q = r/s$) and is used for accounting predictive power of the model (Pogliani, 1996).

The number of descriptors in multiple regression analysis was limited to two, in accordance with the rule that the number of compounds in the data set should be three to six times greater than the number of parameters in the equation (Agrawal, Srivastava, & Khadikar, 2004). Terminal selection of the models was based on an inter-correlation study between variables included in the equation. Two-parameter models with highly collinear descriptors ($|r| \geqslant 0.7$) were not considered.

3. Results

3.1. Antioxidative capacity

Antioxidative capacity, measured by FRAP method, was 0, 0.12, 0.37, 0.99, 1.02, 1.11, 1.26, 1.47, and 2.32 mM Trolox equivalents and the values obtained by TEAC method were 0.06, 1.09, 0.87, 1.80, 1.22, 1.42, 1.96, 1.36, and 2.79 mM Trolox equivalents, for *p*-hydroxybenzoic, *p*-coumaric, vanillic, ferulic, protocatechuic, caffeic, sinapic, syringic, and gallic acid, respectively (Fig. 2). There was a positive correlation between FRAP and TEAC values for antioxidative capacity of phenolic acids ($r = 0.8859$, $p = 0.0015$).

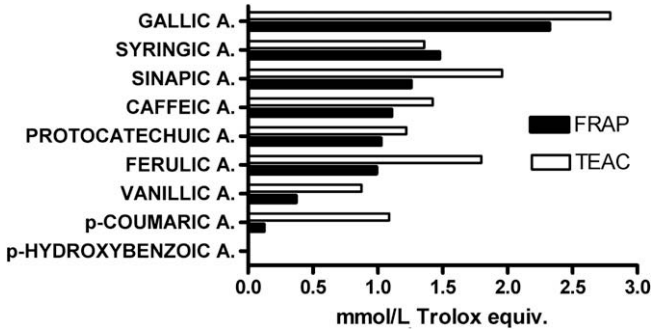


Fig. 2. Antioxidative capacity of nine phenolic acids from wine, determined by FRAP and TEAC methods, and expressed as mmol/L of Trolox equivalents.

The best di-parametric model for expression of FRAP values was obtained using topological polar surface area with polar contributions of nitrogen and oxygen atoms ($TPSA_{No}$) and topological descriptor, mean distance degree deviation (ΔD):

$$\mathrm{FRAP} = -2.973(\pm 0.38) + 0.032(\pm 0.001)\Delta D + 0.051(\pm 0.005)TPSA_{NO} \quad (1)$$

$$n = 9,\ r^2 = 0.950,\ s = 0.148,\ F = 60.93,\ Q = 6.59,\ p = 0.0001.$$

The best relation between antioxidative capacity expressed by TEAC values and structure of molecules was demonstrated by QSAR model that included two GETAWAY descriptors, *R7p* and *HATS 3e*, which were related to atomic polarisabilities and electronegativities, respectively:

$$\mathrm{TEAC} = -17.558(\pm 2.857) - 0.011(\pm 0.001)R7p + 0.162(\pm 0.024)\ HATS\ 3e \quad (2)$$

$$n = 9,\ r^2 = 0.955,\ s = 0.151,\ F = 64.00,\ Q = 6.47,\ p = 0.00009.$$

A scatter plot of the observed ($FRAP_{obs.}$) *versus* the calculated (using Eq. (1)) FRAP values ($FRAP_{calc.}$), together with the observed ($TEAC_{obs.}$) *versus* the calculated (using Eq. (2)) TEAC values ($TEAC_{calc.}$) is shown in Fig. 3.

3.2. Vasodilatory activity

Basal tension of the rat aortic rings ($n = 108$) after exposure to NA was 14.4 ± 0.1 mN. The tested phenolic acids caused different concentration-dependent vasodilatory response in the NA-precontracted vascular rings (Fig. 4).

Maximal vasodilation induced by derivatives of hydroxybenzoic acid was 26.0 ± 3.3, 12.0 ± 4.1, 38.0 ± 3.3, 21.7 ± 3.3, and 5.9 ± 2.1 % for *p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic acid, respectively. Maximal vasodilation induced by derivatives of hydroxycinnamic acid was 25.6 ± 4.6, 27.0 ± 1.4, 22.0 ± 6.1, and 21.7 ± 5.4%, for *p*-coumaric, caffeic, ferulic, and sinapic acid, respectively.

There was a significant negative correlation between antioxidative capacity (measured by FRAP method) and maximal vasodilatory effect of the phenolic acids ($r = -0.7348$, $p = 0.0241$).

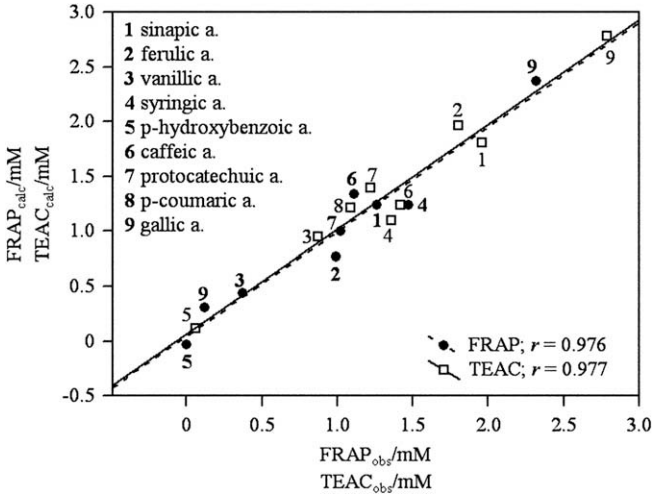


Fig. 3. Relationship between observed ($FRAP_{obs.}$) and calculated FRAP values ($FRAP_{calc.}$), with relationship between observed ($TEAC_{obs.}$) and calculated TEAC values ($TEAC_{calc.}$), for nine fenolic acids from wine.

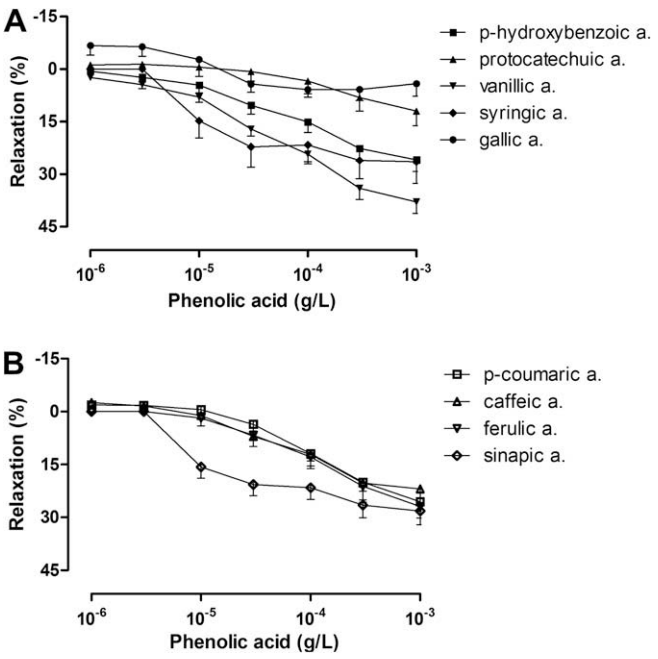


Fig. 4. Dose–response vasodilatory effect of nine phenolic acids (derivatives of hydroxybenzoic acid in panel A; derivatives of hydroxycinnamic acid in panel B) in NA (10^{-7} M)-precontracted rat aortic rings ($n = 12$ per acid). Data are expressed as mean ± SEM (standard error of the mean). Phenolic acids doses are expressed in g/l.

The best QSAR model for vasodilatory activity of phenolic acids (expressed as log of % maximal vasodilation, log *MV*) was obtained by multiple regression with Topological Polar Surface Area ($TPSA_{No}$) and GETAWAY descriptor $R^{+}_{3}(u)$:

$$logMV = 7.685(\pm 1.345) - 0.052(\pm 0.013)R^{+}_{3}(u) - 0.014(\pm 0.003)TPSA_{NO} \quad (3)$$

$$n = 9,\ r^2 = 0.916,\ s = 0.065,\ F = 32.763,\ Q = 14.72,\ p = 0.00059.$$

A scatter plot of the experimental data ($\log MV_{obs.}$) *versus* the calculated (using Eq. (3)) log *MV* values ($\log MV_{calc.}$) is shown in Fig. 5.

Significant correlation was also obtained by simple linear regression between the maximal vasodilatory activity and the number of hydroxyl groups (n_{OH}):

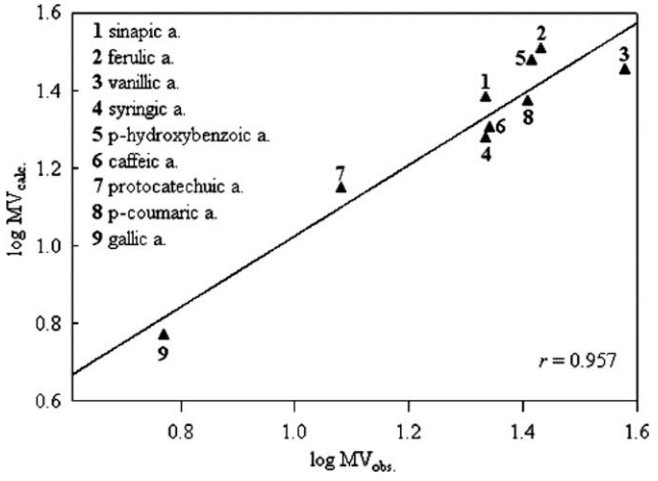


Fig. 5. Relationship between observed maximal vasodilation (*MV*) (expressed as log of % $MV_{obs.}$) and calculated log *MV* values ($\log MV_{calc.}$) for nine phenolic acids from wine.

$$logMV = 1.722(\pm 0.889) - 0.293(\pm 0.056)n_{OH} \quad (4)$$

$$n = 9,\ r^2 = 0.798,\ s = 0.100,\ F = 27.69,\ Q = 8.93,\ p = 0.0011.$$

4. Discussion

In this study, we examined interrelationship between antioxidative capacity and vasodilatory activity, two potentially beneficial biological effects, of nine phenolic acids from wine. The key finding of this study is the negative correlation between *in vitro* antioxidative and vasodilatory activity of the tested phenolic acids. This is best illustrated by poor vasodilatory activity of gallic acid, the strongest antioxidant among the tested phenolic acids. Generally, phenolic acids appear to be weaker vasodilators than antioxidants.

Considering that antioxidative action of phenolic compounds arises from scavenging the free radicals by the donation of a hydrogen atom to radicals, antioxidative activities of these compounds greatly depend on the number and position of hydroxyl groups in the aromatic ring (Rice-Evans et al., 1996). In the present study, QSAR models for the prediction of antioxidative activities (TEAC and FRAP) of phenolic acids also demonstrated the relevance of electronegative atoms, precisely, oxygen atoms from hydroxyl groups. Namely, positive coefficients of $TPSA_{No}$ (an area of surface that arises from oxygen or nitrogen atoms or hydrogen atoms attached to oxygen atom) in Eq. (1), and *HATS 3e* in Eq. (2), indicate that molecules with more hydroxyl groups in the phenyl ring have greater antioxidative activity.

Accordingly, gallic acid had highest FRAP value (2.32 mM Trolox equiv.) due to three hydroxyl groups attached to the phenyl ring, hydroxybenzoic acid with two hydroxyl groups (protocatechuic acid) had FRAP of 1.02 mM Trolox equiv., while *p*-hydroxybenzoic acid, as the representative of hydroxybenzoic acids with only one hydroxyl group, demonstrated no activity. These results are consistent with data reported by Sroka and Cisowski (2003) who also showed a positive correlation between the number of hydroxyl groups bonded to the aromatic ring and the ability to scavenge free radicals by phenolic acids.

The degree of branching and shape of molecules also considerably affect FRAP values. namely, positive coefficient of mean distance degree deviation (ΔD) in Eq. (1) implies that more compact and less branched molecules have a lower antioxidative capacity (Konstantinova, 1996). This is in agreement with the fact that hydroxycinnamic acids, which are larger more branched molecules, are generally more effective than smaller and less branched hydroxybenzoic acids. For example, in spite of the identical substitutions in the aromatic ring, sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) has a higher TEAC value (1.96 mM Trolox equiv.) than syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) (1.36 mM Trolox equiv.).

Presence of descriptor *R7p* in Eq. (2) indicates that the three-dimensional distribution of atomic polarisability also plays a role in the antioxidative activity of the phenolic acids. Because of a negative coefficient of that descriptor in Eq. (2), it would be expected that phenolic acids with more pairs of distant atoms with elevated polarisabilities have a less efficient antioxidative activity. That explains why the substitution of the 3- and 5-hydroxy groups with methoxy groups in syringic acid result in decreased antioxidative activity (FRAP 1.47 and TEAC 1.36 mM Trolox equiv.) in comparison to the trihydroxybenzoic acid, gallic acid (FRAP 2.32 and TEAC 2.79 mM Trolox equiv.).

The most important variables in models for prediction of vasodilatory activity are related to the lipophilicity and local atom distribution. previous QSAR studies have shown that beside lipophilicity and molar refractivity, the polar surface area also correlated well with the drug transport properties, such as intestinal and oral

absorption, as well as with blood–brain barrier penetration (Clark, 1999; Kelder, Grootenhuis, Bayada, Delbressine, & Ploemen, 1999).

The negative correlation between antioxidative activity and vasodilatory effect of the phenolic acids are related to negative coefficients of variables $TPSA_{No}$ in Eq. (3), and n_{OH} in Eq. (4), indicating that the increase in the number of hydroxyl groups in the phenyl ring is unfavourable for vasodilatory effect. Hence, monohydroxybenzoic acids, vanillic, p-hydroxybenzoic, and syringic acid, with low corresponding $TPSA_{No}$ values (37.97 Å^2; 57.53 Å^2; 75.99 Å^2, respectively), induce greater vasodilation than the dihydroxybenzoic acid, protocatechuic acid, with $TPSA_{No}$ value of 77.76 Å^2. Accordingly, trihydroxybenzoic acid, gallic acid, with the largest $TPSA_{No}$ value of 97.99 Å^2 showed the smallest vasodilatory potential. Considering that n_{OH} and $TPSA_{No}$ have negative influence on vasodilatory activity, we may conclude that polar atoms cause steric hindrance over the process of vasodilation.

Taken together, QSAR models presented in this study, revealed that the number of hydroxyl groups in the phenyl ring, degree of compactness and branching, and the three-dimensional distributions of atomic polarisability are important molecular properties of the phenolic acids, which contribute to their antioxidative activity. The increased number of hydroxyl groups in the aromatic ring is related to good antioxidative but poor vasodilatory activity of phenolic acids. Rare QSAR studies of other workers on activities of phenolics acids (Cheng, Ren, Li, Chang, & Chen, 2002), cannot be compared with our study, as they include different descriptors. Because GETAWAY, RDF, 3D-MoRSE and Randic molecular profile descriptors were developed recently, QSAR studies of antioxidative activity of phenolic acids using these descriptors are lacking. Moreover, there is no literature evidence about QSAR study for vasodilatory activity.

Although, the results of the present study do not allow conclusions on possible *in vivo* vasodilatory effects of the tested phenolic acids, it should be noticed that maximal vasodilation was always achieved at the highest concentrations, which are not expected to be reached under normal *in vivo* conditions following intake of wine or other foods rich in phenolics.

In order to make our study comparable with the results of similar *in vitro* studies, and at the same time, to roughly "cover" concentrations that are feasible with the *in vivo* conditions, concentrations of the tested phenolic acids used in our study were chosen according to the studies by Andriambeloson et al. (1998) and Caccetta, Croft, Beilin, and Puddey (2000).

Andriambeloson et al. used vanillic, gallic, caffeic and coumaric acids over the concentration range of 10^{-5} to 3×10^{-1}g/l, which approximately corresponds to 50 nM to 2 mM. By using 10 times lower concentrations than the Andriambeloson's group, we were able to reasonably cover concentrations attainable in human plasma after wine consumption, as shown for 4-O-methylated form of gallic acid (176 nM) and caffeic acid (84 nM) by Caccetta et al. (2000).

At comparable concentrations, only syringic and sinapic acids showed vasodilatory effect in our study. However, negligible vasodilatory effect of other phenolic acids at these concentrations does not necessarily reflect their ineffectiveness under *in vivo* conditions. Namely, there is not a single phenolic compound from wine, which would be of predictive, or principal importance for its biological effects. For example, *in vitro* application of catechin failed to induce vasodilatation in rat aorta (Andriambeloson et al., 1997) and modulation of endothelial function in humans after wine consumption could not be predicted on the basis of plasma catechin concentration (Boban et al., 2006). In contrast, a maximal induction of endothelial NO synthase in human endothelial cells was achieved by synergistic effect of a blend of different wine phenolics, including some phenolic acids (Wallerath, Li, Godtel-Ambrust, Schwarz, & Forstermann, 2005). Similar synergistic effect was also found for the antioxidative activity of wine phenolics (Pignatelli et al., 2006). Therefore, determining levels and related biological effects of a single phenolic compound *in vitro* could be misleading in terms of its expected biological effects *in vivo*.

In addition, the concentrations of different phenolic acids in human plasma after consumption of wine, or other foods rich in phenolics, are mostly unknown and are difficult to predict. The tested acids are present in wine in different concentrations and in different conjugated forms. Phenolic acids occur in foods mainly in esterified forms with organic acids, sugars and lipids, which may affect their bioavailability and metabolism. Phenolic acids undergo conjugation reactions *in vivo* with sulfate, glucuronate, S-adenosylmethionine or their combination (Manach et al., 2004). Formation of the conjugated forms can significantly influence biological properties of the parent compounds.

Polyphenols that are not absorbed in the small intestine reach the colon where colonic microflora hydrolyse glycosides into aglycones and metabolise aglycones into various aromatic acids, which are further metabolised to derivatives of benzoic acid that also may be absorbed in blood. Hence, final plasma concentration of phenolic acids is the result of complex metabolic pathways followed by not only phenolic acids, but also other polyphenols (Manach et al., 2004).

Future studies are needed to determine possible additive or synergistic effects of phenolic acids and/or their metabolites.

Taken together, results of the present QSAR study show that recently developed molecular profile descriptors, GETAWAY, RDF, 3D-MoRSE and Randic can be successfully used to model possible vasodilatory activity of phenolic acids. QSAR techniques used in our study could also be suitable for investigation of other biological activities of phenolic acids and/or other wine phenolics.

Furthermore, our results imply that determining antioxidative capacity of phenolic compounds *in vitro* has no relevance for prediction of their other biological effects.

In conclusion, our results point out the relevance of wine phenolic acids as potent biologically active compounds that deserve more thorough research effort.

Acknowledgments

This work was supported in part by Grants 216-2160547-0537, 011-2160547-2226, and 006-006-1117-1237 from the Ministry of Science, Education and Sports of the Republic of Croatia.

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