Journal of the Serbian Society for Computational Mechanics / Special issue 2020/ pp 85-95
(10.24874/jsscm.2020.01.08)

Thermodynamic and Kinetic Investigation of Antiradical Potential of Cyanidin

Dejan Milenković[1*], Jelena Đorović[1], Edina Avdović[1], Žiko Milanović[2], Marko Antonijević[1]

[1] Department of Science, Institute for Information Technologies, University of Kragujevac, Jovana Cvijića bb, 34000 Kragujevac, Republic of Serbia
e-mail: deki82@kg.ac.rs@uni.kg.ac.rs
[2] University of Kragujevac, Faculty of Science, Department of Chemistry, 12 Radoja Domanovića Street, 34000 Kragujevac, Serbia.
e-mail: ziko.milanovic@pmf.kg.ac.rs

**corresponding author*

Abstract

In the present paper, M05-2X/6-311+G(d,p) level of theory was used to investigate antiradical activity of cyanidin towards highly damaging radical species (·OH, ·OCH_3, ·OOH and ·$OOCH_3$). The applied method successfully reproduces the values of reaction enthalpies (ΔH_{BDE}, ΔH_{IP}, and ΔH_{PA}). These parameters are important to determine which of the mechanisms are preferred. Reaction enthalpies related to the antioxidant mechanisms of the investigated species were calculated in water and DMSO. The enthalpies of reactions indicate the preferred radical scavenging mechanisms in polar (water) and polar aprotic (DMSO) solvents. Single- electron transfer followed by proton transfer (SET-PT) is not a favorable reaction pathway under any conditions. Both remaining mechanisms, HAT and SPLET, are suitable for the reaction of cyanidin with •OH and •OCH_3 in all solvents under investigation. On the other hand, in the reaction of cyanidin with •OOH and •$OOCH_3$, the SPLET mechanism is possible in both solvents. Simulation of the reaction of the cyanidin anion with the hydroxy radical confirmed that position 3` of Cy–O^- is the most suitable for reaction with •OH through electron transfer mechanism (ET) in both solvents.

Keywords: cyanidin, antiradical activity, DFT, HAT and SPLET mechanisms

1. Introduction

Many diseases in human organisms originate as result of radical reactions. Anthocyanidins and anthocyanins, as hydroxylated and glycosidic flavonoids compounds, are plant pigments responsible for the red, blue and purple hues of flowers and fruits in nature. (T. Swain, 1976.) These compounds are flavonoids that belong to the family of polyphenols. An important property of the anthocyanidins is that they are natural antioxidants. They are well known for their therapeutic effects in the treatment of diabetes, atherosclerosis and cardiovascular diseases. (M. de Lorgeril et al., 2006). Their protective role correlates well with their

antioxidant activity, which is manifested through different actions, like direct radical scavenging, transition metal chelation, inhibition of certain enzymes, or removing oxidatively changed and damaged molecules. (H. Wang et al., 1999; T. Yoshimoto et al., 1983).

Cyanidin ((Cy) (2-(3,4-dihydroxy phenyl) chromenylium-3,5,7-triol) (Fig. 1)) is one of the major water-soluble anthocyanidins. According to one theory, dietary intake of Cy may inhibit the development of obesity and diabetes as well as provide anti-inflammatory effects. Cy and its glycosides are very strong antioxidants and they are active at pharmacological concentrations. The antioxidant activity is stronger than that of vitamin E, vitamin C and resveratrol and similar to other commercial antioxidants. Cy quickly neutralizes reactive oxygen species such as hydrogen peroxide, reactive oxygen, and hydroxy radical. (R. Sasaki et al., 2007)

Scavenging properties of flavonoids were related to their ability to transfer a hydrogen atom to a free radical species (RO$^{\bullet}$). This transfer can be achieved via at least three mechanisms characteristic to all phenolic antioxidants generally: hydrogen atom transfer (HAT, Eq. 1), single electron transfer followed by proton transfer (SET-PT, Eq. 2 and Eq.3), and sequential proton loss electron transfer (SPLET, Eq. 4 Eq. 5) (G.A. DiLabio et al., 2005; G. A. DiLabio et al., 2007; J. M. Mayer et al., 2004; Z. S. Marković et al., 2010). These mechanisms may coexist, and they depend on solvent properties and radical characteristics.

$$ArOH \rightarrow ArO^{\cdot} + H \tag{1}$$

$$ArOH \rightarrow ArOH^{+\cdot} + e^{-} \tag{2}$$

$$ArOH^{+\cdot} \rightarrow ArO^{\cdot} + H^{+} \tag{3}$$

$$ArOH \rightarrow ArO^{-} + H^{+} \tag{4}$$

$$ArO^{-} \rightarrow ArO^{\cdot} + e^{-} \tag{5}$$

Many factors affect neutralization of a free radical, and that is a very complex process (Xie et al. 2014). One of them is the chemical nature of the scavenged free radicals, which have an important role in the scavenging processes (Rimarčik et al. 2010). Investigation of free radical scavenging potency of the investigated compounds was performed with radicals such as: $^{\bullet}$OH (hydroxy), $CH_3O^{\bullet}$ (methoxy), $^{\bullet}$OOH (hydroperoxy), and $CH_3OO^{\bullet}$ (methyl peroxy). Selected oxygen-derived free radicals have different characteristics. The $^{\bullet}$OH radical is the most reactive and electrophilic of the oxygen-centered radicals. Therefore, hydroxy radical can withdraw an electron or H-atom from almost any compound in its vicinity (Rose et al. 1993), and it is considered as the main source of biological damage in living organisms. The peroxy radicals, such as $^{\bullet}$OOH, $CH_3OO^{\bullet}$, are less reactive than hydroxy radical. The methyl peroxy here represents lipid peroxy radical.

The enthalpies of the reactants, products, and enthalpies of the reactions with described free radicals were calculated. It is well known that the values of the reaction enthalpies can significantly contribute to understanding of the investigated reaction mechanisms. The reaction with the free radical (RO$^{\bullet}$) can occur via three mentioned mechanisms. In the HAT mechanism, the reaction can be presented by Eq. 6:

$$Ar-OH \; +^{\cdot} OR \rightarrow Ar-O^{\cdot} + ROH \tag{6}$$

The SET-PT mechanism takes place in two steps as it is described above in Eqs. 2 and 3. In interaction with free radicals (RO$^{\bullet}$), it can be presented by Eq. 7 and Eq. 8:

$$Ar-OH \; +^{\cdot} OR \rightarrow Ar-OH^{+\cdot} +^{-} OR \tag{7}$$

$$Ar-OH^{+\cdot} +^{-} OR \rightarrow Ar-O^{\cdot} + ROH \tag{8}$$

The SPLET mechanism can be presented as follows:

$$Ar-OH \; + \bar{} OR \; \rightarrow \; Ar-O^{\bar{}} + \; ROH \tag{9}$$

$$Ar-O^{\bar{}} + \cdot OR \; \rightarrow \; Ar-O^{\cdot} + \bar{} \; OR \tag{10}$$

The reaction of the examined compound with the particular free radical is considered thermodynamically favourable if it is exothermic:

$$\Delta H = \left[H(product) - \; H(reac\tan ts) \right] < 0 \tag{11}$$

The present paper aims to provide quantitative tools to determine the antiradical mechanisms of Cyanidin (Cy) (Fig. 1) by calculating the energy requirements for the reactions of these molecules with $^{\bullet}OH$, $^{\bullet}OCH_3$, $^{\bullet}OOH$, and $^{\bullet}OOCH_3$ radicals in different media. The enthalpies of reaction may indicate which radical scavenging mechanism is thermodynamically preferred and point out active sites for radical inactivation.

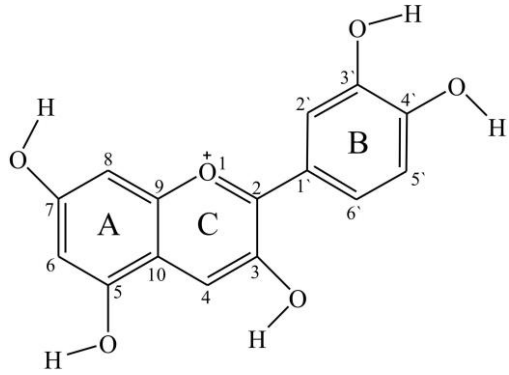

Figure 1. Chemical structure of cyanidin.

2. Methodology section

The majority of theoretical investigation of Cy is focused on aromatic rings where OH groups are located. Geometry optimizations for all species under investigation and frequency calculations have been carried out using a DFT method M05-2X (Y. Zhao et al., 2006) combined with the 6-311+G(d,p) basis set. The influence of water and dimethylsulfoxide (DMSO) as solvents was approximated using the SMD solvation model. (A.V. Marenich et al., 2009) Geometries were fully optimized without imposing any restrictions. Local minima were confirmed by the absence of imaginary frequencies. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. In the case of the transition states, it was verified that the imaginary frequency (the number of imaginary frequencies is one for transition state) corresponds to the expected motion along the reaction coordinate, by Intrinsic Coordinate Calculations (IRC). The optimization and energy calculation of all geometries were performed without any constraints. All the electronic calculations were performed with the Gaussian 09 program package. (M.J. Frisch et al., 2009).

The enthalpies of reaction related to the studied free radical scavenging mechanisms can be calculated by the following equations (Klein et al. 2007):

$$BDE \; = H(Ar-O^{\cdot}) \; + H\left(H^{\cdot}\right) \; - H(Ar-OH) \tag{12}$$

$$IP = H(Ar-OH^{\cdot+}) + H\left(e^{-}\right) - H(Ar-OH) \quad (13)$$

$$PDE = H(Ar-O^{\cdot}) + H\left(H^{+}\right) - H(Ar-OH^{\cdot+}) \quad (14)$$

$$PA = H(Ar-O^{\cdot-}) + H\left(H^{+}\right) - H(Ar-OH) \quad (15)$$

$$ETE = H(Ar-O^{\cdot}) + H\left(e^{-}\right) - H(Ar-O^{-}) \quad (16)$$

where H(Ar–OH), H(Ar –O$^{\bullet}$), H(Ar –OH$^{\bullet+}$), H(Ar –O^{-}), H(H$^{\bullet}$), H(e^{-}) and H(H^{+}) are the enthalpies of parent molecule, radical, radical cation, and anion of the examined compound, hydrogen atom, electron and proton, respectively.

In radical inactivation, the HAT mechanism (Eq. 6) is characterized by the H-atom transfer from the examined compounds to the free radical (RO$^{\bullet}$). $\Delta_r H_{BDE}$ can be calculated using the following equation (Dimitrić Marković et al. 2014):

$$\Delta H_{BDE} = H(Ar-O^{\cdot}) + H\left(ROH\right) - H(Ar-OH) - H\left(^{\cdot}OR\right) \quad (17)$$

The SET-PT mechanism is described by Eqs. 7 and 8. The first step of this mechanism is determined by $\Delta H_{IP,}$ while the second step is determined by ΔH_{PDE} (Eqs. 18 and 19, respectively):

$$\Delta H_{IP} = H(Ar-OH^{\cdot+}) + H\left(^{-}OR\right) - H(Ar-OH) - H\left(^{\cdot}OR\right) \quad (18)$$

$$\Delta H_{PDE} = H(A-O^{\cdot}) + H\left(ROH\right) - H(A-OH^{\cdot+}) - H\left(^{-}OR\right) \quad (19)$$

ΔH_{PA} and ΔH_{ETE} are the reaction enthalpies related to the SPLET mechanism (Eqs. 9 and 10), and they are calculated using Eqs. 20 and 21, respectively:

$$\Delta H_{PA} = H(Ar-O^{\cdot-}) + H\left(ROH\right) - H(Ar-OH) - H\left(^{-}OR\right) \quad (20)$$

$$\Delta H_{ETE} = H(Ar-O^{\cdot}) + H(^{-}OR) - H(Ar-O^{-}) - H(^{\cdot}OR) \quad (21)$$

Transition state theory (TST) represents one of the simplest theoretical approaches for estimating the rate constants (k), which requires only structural, energetic, and vibrational frequency information for reactants and transition states. (A. Galano et al., 2014). The main advantage of using conventional TST is that it requires very limited potential energy information (only on reactants and the transition states), which makes it practical for a wide range of chemical reactions. Despite its relative simplicity, this theory has been proven to be good enough to reproduce experimental rate constants of free radical scavenging reactions. (A. Galano et al., 2013)

The rate constant for the radicals-cyanidin reaction was calculated using TST, implemented in TheRate program (W.T. Duncan et al., 1998) and 1M standard state is calculated as follows:

$$k_{TST} = \sigma\kappa \frac{k_B T}{h} \exp\left(\frac{-\Delta G^{\neq}}{RT}\right) \quad (22)$$

where k_B and h stand for the Boltzmann and Planck constants, $\Delta G^{\neq}$ is the free states and reactants, σ represents degeneracy accounting for the number of equivalent reaction paths, and κ accounts for tunneling corrections (C. Eckart et al., 1930; R. A. Marcus et al., 1964).

3. Results and discussion

Antiradical mechanisms of cyanidin with different free radicals

Scavenging properties of Cy are related to their ability to transfer H atom to a free radical specie. In order to examine, the influence of radical species to an antiradical mechanism of Cy (Fig.1), the reactive particle RO$^{\bullet}$ were used. In the present paper, RO$^{\bullet}$ particle represents hydroxy, methoxy, hydroperoxy and methyl peroxy radicals. The preferred mechanism of the antiradical activity of the considered compound can be estimated from ΔH_{BDE}, ΔH_{IP}, and ΔH_{PA} values. Namely, the lowest of these values indicates which mechanism would be most favorable.

In the HAT mechanism, the hydrogen atom is transferred from Cy to the free radicals RO$^{\bullet}$:

$$Cy-OH \; + {}^{\cdot}OR \; \rightarrow \; Cy-O^{\cdot} + \; ROH \tag{23}$$

$\Delta \boldsymbol{H}_{\mathrm{BDE}}$ for the HAT mechanism can be calculated using the following equations:

$$\Delta H_{BDE} = H(Cy-O^{\cdot}) \; + H\left(ROH\right) \; - H(Cy-OH) \; - H\left({}^{\cdot}OR\right) \tag{24}$$

The first step in the SET-PT mechanism is transfer of an electron from Cy to free radical species, yielding the Cy radical cation Cy–OH$^{\bullet+}$ and the corresponding anion.

$$Cy-OH \; + {}^{\cdot}OR \; \rightarrow \; Cy-OH^{+\cdot} + {}^{-}OR \tag{25}$$

$\Delta \boldsymbol{H}_{\mathrm{IP}}$ for the first step of the SET-PT mechanism can be calculated as follows:

$$\Delta H_{IP} = H(Cy-OH^{+\cdot}) \; + H\left({}^{-}OR\right) \; - H(Cy-OH) \; - H\left({}^{\cdot}OR\right) \tag{26}$$

The second step of this mechanism is deprotonation of Cy–OH$^{\bullet+}$ by RO^{-}:

$$Cy-OH^{+\cdot} + {}^{-}OR \; \rightarrow \; Cy-O^{\cdot} + \; ROH \tag{27}$$

$\Delta \boldsymbol{H}_{\mathrm{PDE}}$ can be calculated using the following equations:

$$\Delta H_{PDE} = H(Cy-O^{\cdot}) \; + H\left(ROH\right) \; - H(Cy-OH^{+\cdot}) \; - H\left({}^{-}OR\right) \tag{28}$$

The first step in the SPLET mechanism is deprotonation of a Cy by RO^{-}. The outcome of this reaction is the formation of the Cy anion, Cy–O^{-}:

$$Cy-OH \; + {}^{-}OR \; \rightarrow \; Cy-O^{-} + \; ROH \tag{29}$$

$\Delta \boldsymbol{H}_{\mathrm{PA}}$ can be calculated as follows:

$$\Delta H_{PA} = H(Cy-O^{-}) \; + H\left(ROH\right) \; - H(Cy-OH) \; - H\left({}^{-}OR\right) \tag{30}$$

In the next step electron transfer from Cy–O^{-} to RO$^{\bullet}$ take place:

$$Cy-O^{-} + {}^{\cdot}OR \; \rightarrow \; Cy-O^{\cdot} + {}^{-}OR \tag{31}$$

$\Delta \boldsymbol{H}_{\mathrm{ETE}}$ can be determined by the equations:

$$\Delta H_{ETE} = H(Cy-O^{\bullet}) \; + \; \left(RO^{-}\right) \; - \; (Cy-O^{-}) \; - \; \left(RO^{\bullet}\right) \tag{32}$$

The reaction enthalpies for the reaction of Cy with selected radicals were calculated using M05-2X/6-311+G(d,p) model. Calculations were performed in water and dimethyl sulfoxide

(DMSO) as solvents. Which of the mechanisms is preferred can be estimated from the lowest value of calculated thermodynamic parameters ($\Delta \boldsymbol{H}_{\mathrm{BDE}}$, $\Delta \boldsymbol{H}_{\mathrm{IP}}$, and $\Delta \boldsymbol{H}_{\mathrm{PA}}$ values). The calculated reactions enthalpies are presented in Table 1.

M05-2X/6-311+G(d,p)										
cyanidin	Water ε=78.35					DMSO ε=46.83				
	HAT	SET-PT		SPLET		HAT	SET-PT		SPLET	
	$\Delta \boldsymbol{H}_{\mathrm{BDE}}$	$\Delta \boldsymbol{H}_{\mathrm{IP}}$	$\Delta \boldsymbol{H}_{\mathrm{PDE}}$	$\Delta \boldsymbol{H}_{\mathrm{PA}}$	$\Delta \boldsymbol{H}_{\mathrm{ETE}}$	$\Delta \boldsymbol{H}_{\mathrm{BDE}}$	$\Delta \boldsymbol{H}_{\mathrm{IP}}$	$\Delta \boldsymbol{H}_{\mathrm{PDE}}$	$\Delta \boldsymbol{H}_{\mathrm{PA}}$	$\Delta \boldsymbol{H}_{\mathrm{ETE}}$
		100					124			
CyOH-3 + ·OH	-131		-231	-145	14	-136		-260	-143	7
CyOH-3`+·OH	-136		-236	-130	-7	-143		-267	-122	-20
CyOH-4`+·OH	-140		-240	-156	16	-147		-271	-163	16
CyOH-5+·OH	-119		-219	-151	32	-122		-246	-151	29
CyOH-7+·OH	-114		-214	-151	37	-114		-238	-152	37
		124					228			
CyOH-3+·OCH_3	-74		-198	-112	38	-68		-296	-179	111
CyOH-3`+·OCH_3	-79		-203	-96	17	-75		-303	-159	84
CyOH-4`+·OCH_3	-83		-207	-123	40	-79		-307	-199	120
CyOH-5+·OCH_3	-62		-185	-117	56	-54		-282	-187	133
CyOH-7+·OCH_3	-57		-180	-118	61	-47		-275	-188	141
		151					223			
CyOH-3+·OOH	1		-150	-64	65	2		-222	-104	106
CyOH-3`+·OOH	-4		-155	-49	45	-5		-228	-84	79
CyOH-4`+·OOH	-8		-159	-75	67	-9		-232	-125	115
CyOH-5+·OOH	13		-138	-70	83	16		-207	-112	128
CyOH-7+·OOH	18		-133	-70	89	23		-200	-113	136
		158					229			
CyOH-3+·$OOCH_3$	7		-151	-66	72	10		-220	-102	112
CyOH-3`+·$OOCH_3$	2		-156	-50	51	3		-226	-82	85
CyOH-4`+·$OOCH_3$	-2		-160	-77	74	-1		-230	-123	122
CyOH-5+·$OOCH_3$	19		-139	-71	90	24		-205	-110	134
CyOH-7+·$OOCH_3$	24		-134	-71	95	31		-198	-112	143

Table 1. Calculated reaction enthalpies (kJ/mol) for the reactions of Cy with ·OH, ·OCH_3, ·OOH, and ·$OOCH_3$ radicals.

On the basis of thermodynamic values in Table 1, it is clear that only HAT and SPLET are operative radical scavenging mechanisms of Cy in both solvents. On the basis of the obtained values for $\Delta \boldsymbol{H}_{\mathrm{BDE}}$, it is clear that 4`−OH group should be the most reactive OH group of Cy. The 4`−OH group has the lowest $\Delta \boldsymbol{H}_{\mathrm{BDE}}$ value in all solvents. Values of $\Delta \boldsymbol{H}_{\mathrm{PA}}$ for all present OH groups of Cy are indicating that proton transfer from C4` group is more favorable in comparison to the other OH groups. In all solvents, $\Delta \boldsymbol{H}_{\mathrm{PA}}$ values are significantly lower than corresponding $\Delta \boldsymbol{H}_{\mathrm{BDE}}$ values, with the exception of OH radical inactivation. This indicates that SPLET mechanism thermodynamically represents the more probable reaction pathway regarding scavenging HOO·, CH_3O· and CH_3OO· radicals. The $\Delta \boldsymbol{H}_{\mathrm{BDE}}$ and $\Delta \boldsymbol{H}_{\mathrm{PA}}$ values show that the 4`-OH group is the most reactive OH group of Cy. Regarding the hydroxy radical, the

HAT and SPLET mechanisms are in competition in both solvents; however, it is interesting to notice that SPLET is dominant in all cases except when the inactivation is initiated by the group in position 3` of Cy. It is also important to notice that the $\Delta \boldsymbol{H}_{\mathrm{ETE}}$ values for this position is slightly negative, which makes it important for kinetic analysis. The SET-PT mechanism proves to be thermodynamically unfavorable for all four radical species in both solvents.

Mechanistic approaches of reaction of Cy with hydroxyl radical

One of the viable mechanisms to scavenge free radicals is electron transfer (ET), the second step in the SPLET mechanism (Burton & Ingold, 1984):

$$\mathrm{Cy{-}O^{-}} + \mathrm{HO^{\bullet}} \rightarrow \mathrm{Cy{-}O^{\bullet}} + \mathrm{HO^{-}} \tag{33}$$

Cyanidin anion acts as the free radical scavenger in reaction Eq. 33. Transition states are necessary for calculating the $\Delta G^{\neq}$ term in Eq. (22) for HAT reactions. However, for electron transfer reaction, transition state cannot be located using electronic structure methods, as it is not possible to describe mechanistic pathway of electron motion. To estimate the reaction barrier (the $\Delta G^{\neq}$ term) in such cases it is necessary to use the Marcus theory. (Marcus et al., 1997). Within this transition-state formalism, the SPLET activation barrier ($\Delta G^{\neq}_{\mathrm{SPLET}}$) is defined in terms of the free energy of reaction ($\Delta G^{0}_{\mathrm{SPLET}}$) and the nuclear reorganization energy (λ):

$$\Delta G^{\neq}_{\mathrm{SPLET}} = \frac{\lambda}{4}\left(1 + \frac{\Delta G^{0}_{\mathrm{SPLET}}}{\lambda}\right)^{2} \tag{34}$$

λ is the energy associated with the nuclear rearrangement involved in the formation of products in an electron transfer reaction, which implies not only the nuclei of the reacting species but also those of the surrounding solvent. For λ calculation, a very simple approximation was used:

$$\lambda \approx \Delta E - \Delta G^{0}_{\mathrm{SPLET}} \tag{35}$$

ΔE is the non-adiabatic energy difference between reactants and vertical products, that is, $\mathrm{Cy{-}O^{\bullet}}$ and $\mathrm{HO^{-}}$ in geometries of $\mathrm{Cy{-}O^{-}}$ and $\mathrm{HO^{\bullet}}$:

$$\Delta E_{\mathrm{SPLET}} = E(\mathrm{Cy{-}O^{\bullet}}) + E(\mathrm{HO^{-}}) \; - \; E(\mathrm{Cy{-}O^{-}}) - E(\mathrm{HO^{\bullet}}) \tag{36}$$

The adiabatic Gibbs free energies of reaction were calculated as:

$$\Delta G^{0}_{\mathrm{SPLET}} = [G(\mathrm{Cy{-}O^{\bullet}}) + G(\mathrm{HO^{-}})\,] - [G(\mathrm{Cy{-}O^{-}}) + G(\mathrm{HO^{\bullet}})] \tag{37}$$

This approach is similar to the one used by Nelsen and co-workers (1987) for a large set of self-exchange reactions. The theory of diffusion-controlled reaction was originally utilized by R.A. Alberty, Gordon Hammes and Manfred Eigen to estimate the upper limit of enzyme-substrate reaction. (Z. Marković et al., 2012) According to their estimation, the upper limit of enzyme-substrate reaction was $10^{9}\ \mathrm{M^{-1}\,s^{-1}}$. This fact has influence on final value of Steady-state Smoluchowski rate constant, k_d. (Z. Marković et al., 2013; M. Leopoldini et al., 2004). If calculated rate constant is close to the diffusion limit, appropriate corrections are considered (the Collins–Kimball theory) as proposed by Galano and Alvarez-Idaboy. (A. Galano et al., 2013, C. Iuga et al., 2012). The apparent rate constant (k_{app}) cannot be directly obtained from TST calculations. The Collins–Kimball (Collins et al., 1949) theory is used to correct the rate constant, and k_{app} is calculated as:

$$k_{app} = \frac{k_d k}{k_d + k} \tag{38}$$

where k is the thermal rate constant, obtained from TST calculations. This constant, k_d, for an irreversible bimolecular diffusion-controlled reaction, can be calculated with following equation:

$$k_d = 4\pi R D_{AB} N_A \tag{39}$$

where R denotes the reaction distance, NA is the Avogadro number, and DAB is the mutual diffusion coefficient of the reactants A (free radical (HO•) and B (Cy–O$^-$)). DAB were calculated from DA and DB according to Truhlar. (Schwenke et al., 1985) DA and DB were estimated from the Stokes–Einstein approach (1903) (Stroke, 1901):

$$D = \frac{k_B T}{6\pi\eta\alpha} \tag{40}$$

where η denotes the viscosity of the solvents, in our case water (η=8.9x10^{-4} Pa s) and DMSO (η=2.0x10^{-3} Pa s) and α is the radius of the solute. On the basis of the obtained ΔH_{ETE} values (Table 1), the reaction between HO• and Cy–O$^-$ were studied only in positions C3, C3` and C4`. The kinetic parameters of the reactions were analyzed in terms of their Gibbs free energies (Table 2).

	M052X/6-311+G(d,p)									
cyanidin	$\Delta G^{\neq}_{SPLET}$ (kJ/mol)	ΔG^{0}_{SPLET} (kJ/mol)	λ (kJ/mol)	k_d ($M^{-1}s^{-1}$)	k_{app} ($M^{-1}s^{-1}$)	$\Delta G^{\neq}_{SPLET}$ (kJ/mol)	ΔG^{0}_{SPLET} (kJ/mol)	λ (kJ/mol)	k_d ($M^{-1}s^{-1}$)	k_{app} (M^{-1} s^{-1})
	Water η=8.9x10^{-4}					DMSO η=2.0x10^{-3}				
3+•OH	21.1	15.0	50.0	4.2x10^9	9.5x10^8	15.9	5.9	51.3	1.84x10^9	1.56x10^9
3`+•OH	9.8	-5.7	49.8	8.3x10^9	7.8x10^9	3.3	-21.2	45.8	3.7x10^9	3.7x10^9
4`+•OH	24.0	18.3	52.9	8.4x10^9	1.9x10^9	22.6	19.7	41.7	3.7x10^9	5.7x10^8

Table 2. DFT calculations of rate constants related to second step of SPLET mechanisms

As can be seen from Table 2, the 3`–OH group of Cy–O$^-$ has the lowest values of activation energy (corresponding values of rate constants are higher and possible reaction is faster) in reaction with hydroxy radical in both solvents, implying that it is most favorable position for the reaction via electron transfer mechanism (ET). These results are in accordance with the results presented in Table 1.

4. Conclusion

Antiradical activity of the Cy was examined by analysing the thermodynamic parameters of the parent molecule, the corresponding radicals, radical cations and anions. The M05-2X/6-311+G(d,p) model was applied to examine antiradical activity of cyanidin towards highly damaging radical species (•OH, •OCH_3, •OOH and •$OOCH_3$). To estimate the effects of water and DMSO, the SMD solvation model was used. On the basis of the obtained results, it can be concluded that single electron transfer followed by proton transfer is not a favorable reaction pathway under any conditions. The HAT and SPLET are preferable mechanisms for reactioFn cyanidin with •OH and •OCH_3 in all analysed solvents. The SPLET mechanism is possible in

both solvents for the reaction of cyanidin with $^{\bullet}OOH$ and $^{\bullet}OOCH_3$. In the reaction of the cyanidin anion with the hydroxy radical it was confirmed that position 3` of $Cy–O^-$ is the most suitable for reaction with $^{\bullet}OH$ through electron transfer mechanism (ET) in both solvents.

Acknowledgements: This work was supported by the Serbian Ministry of Education, Science and Technological Development (Agreement Nos. 451-03-68/2020-14/200378 and 451-03-68/2020-14/200122).

References:

Burton, G. W., & Ingold, K. U. (1984). Beta-carotene: an unusual type of lipid antioxidant. *Science*, *224*(4649), 569-573.

Carpenter, J. E., & Weinhold, F. (1988). Analysis of the geometry of the hydroxymethyl radical by the "different hybrids for different spins" natural bond orbital procedure. *Journal of Molecular Structure: THEOCHEM*, *169*, 41-62.

Collins, F. C., & Kimball, G. E. (1949). Diffusion-controlled reaction rates. *Journal of colloid science*, *4*(4), 425-437.

de Lorgeril, M., & Salen, P. (2006). The Mediterranean-style diet for the prevention of cardiovascular diseases. *Public health nutrition*, *9*(1a), 118-123.

DiLabio, G. A., & Ingold, K. U. (2005). A theoretical study of the iminoxy/oxime self-exchange reaction. A five-center, cyclic proton-coupled electron transfer. *Journal of the American Chemical Society*, *127*(18), 6693-6699.

DiLabio, G. A., & Johnson, E. R. (2007). Lone pair− π and π− π interactions play an important role in proton-coupled electron transfer reactions. *Journal of the American Chemical Society*, *129*(19), 6199-6203.

Duncan, W. T., Bell, R. L., & Truong, T. N. (1998). TheRate: Program for ab initio direct dynamics calculations of thermal and vibrational-state-selected rate constants. *Journal of Computational Chemistry*, *19*(9), 1039-1052.

Eckart, C. (1930). The penetration of a potential barrier by electrons. *Arysical Review*, *35*(11), 1303.

Frisch M. J., Trucks G. W., Schlegel H. B., Scuseria G. E., Robb M. A., Cheeseman J. R., Zakrzewski V. G., Montgomery J. J., Stratmann R. E., Burant J. C., Dapprich S., Millam J. M., Daniels A. D., Kudin K. N., Strain M. C., Farkas O., Tomasi J., Barone V., Cossi M., Cammi R., Mennucci B., Pomelli C., Adamo C., Clifford S., Ochterski J., Petersson G. A., Ayala P. Y., Cui Q., Morokuma K., Malick A. D., Rabuck K. D., Raghavachari K., Foresman J. B., Cioslowski J., Ortiz J. V., Baboul A. G., Stefanov B. B., Liu G., Liashenko A., Piskorz P., Komaromi I., Gomperts R., Martin R. L., Fox D. J., Keith T., Al-Laham M. A., Peng C. Y., Nanayakkara A., Challacombe M., Gill P. M. W., Johnson B., Chen W., Wong M. W., Andres J. L., Gonzalez C., Head-Gordon M., Replogle E. S. and Pople J. A. (2009) Gaussian 09, Revision B.01, Gaussian Inc, Wallingford CT.

Galano, A., & Alvarez-Idaboy, J. R. (2013). A computational methodology for accurate predictions of rate constants in solution: Application to the assessment of primary antioxidant activity. *Journal of computational chemistry*, *34*(28), 2430-2445.

Galano, A., Francisco Marquez, M., & Pérez-González, A. (2014). Ellagic acid: an unusually versatile protector against oxidative stress. *Chemical research in toxicology*, *27*(5), 904-918.

Glendening, E. D., Badenhoop, J. K., Reed, A. E., Carpenter, J. E., Bohmann, J. A., Morales, C. M., & Weinhold, F. (2009). NBO 5.9. *Theoretical Chemistry Institute, University of Wisconsin, Madison*.

Goodwin, T. W. (1976). Chemistry and biochemistry of plant pigments. *Academic Press*.

Harper, E. T., & Rose, G. D. (1993). Helix stop signals in proteins and peptides: the capping box. *Biochemistry*, *32*(30), 7605-7609.

Iuga, C., Alvarez-Idaboy, J. R., & Russo, N. (2012). Antioxidant activity of trans-resveratrol toward hydroxy and hydroperoxy radicals: a quantum chemical and computational kinetics study. *The Journal of organic chemistry*, *77*(8), 3868-3877.

Klein E, Lukeš V, Ilčin M (2007). DFT/B3LYP study of tocopherols and chromans antioxidant action energetics, *Chemical Physics*, 336, 51-57.

Leopoldini, M., Russo, N., & Toscano, M. (2006). Gas and liquid Arase acidity of natural antioxidants. *Journal of agricultural and food chemistry*, *54*(8), 3078-3085.

Marcus, R. A. (1964). Chemical and electrochemical electron-transfer theory. *Annual review of Arysical chemistry*, *15*(1), 155-196.

Marcus, R. A. (1997). Electron transfer reactions in chemistry. Theory and experiment. *Pure and Applied Chemistry*, *69*(1), 13-29.

Marenich, A. V., Cramer, C. J., & Truhlar, D. G. (2009). Performance of SM6, SM8, and SMD on the SAMPL1 test set for the prediction of small-molecule solvation free energies. *The Journal of Arysical Chemistry B*, *113*(14), 4538-4543.

Marković, J. M. D., Milenković, D., Amić, D., Popović-Bijelić, A., Mojović, M., Pašti, I. A., & Marković, Z. S. (2014). Energy requirements of the reactions of kaempferol and selected radical species in different media: towards the prediction of the possible radical scavenging mechanisms. *Structural chemistry*, *25*(6), 1795-1804.

Marković, Z. S., Marković, J. M. D., & Doličanin, Ć. B. (2010). Mechanistic pathways for the reaction of quercetin with hydroperoxy radical. *Theoretical Chemistry Accounts*, *127*(1-2), 69-80.

Marković, Z., Amić, D., Milenković, D., Dimitrić-Marković, J. M., & Marković, S. (2013). Examination of the chemical behavior of the quercetin radical cation towards some bases. *Arysical Chemistry Chemical Arysics*, *15*(19), 7370-7378.

Marković, Z., Milenković, D., Đorović, J., Marković, J. M. D., Stepanić, V., Lučić, B., & Amić, D. (2012). PM6 and DFT study of free radical scavenging activity of morin. *Food chemistry*, *134*(4), 1754-1760.

Mayer, J. M. (2004). Proton-coupled electron transfer: a reaction chemist's view. *Annu. Rev. Arys. Chem.*, *55*, 363-390.

Rimarčik D.,, Kurach, E., Djurado, J., Kornet, A., Wlostowski, M., Lukeš, V., ... & Pron, A. (2011). Effect of substituents on redox, spectroscopic and structural properties of conjugated diaryltetrazines—a combined experimental and theoretical study. *Physical Chemistry Chemical Physics*, *13*(7), 2690-2700.

Sasaki, R., Nishimura, N., Hoshino, H., Isa, Y., Kadowaki, M., Ichi, T., ... & Horio, F. (2007). Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice. *Biochemical Ararmacology*, *74*(11), 1619-1627.

Schwenke, D. W., & Truhlar, D. G. (1985). Systematic study of basis set superposition errors in the calculated interaction energy of two HF molecules. *The Journal of chemical physics*, *82*(5), 2418-2426.

Stokes, S. G. G. (1901). *Mathematical and physical papers.*

Surh, Y. J. (Ed.). (2005). *Oxidative stress, inflammation, and health*. CRC press.

Swain, T. (1976). Nature and properties of flavonoids. *Chemistry and Biochemistry of Plant Pigments. TW Goodwin*

Wang, H., Nair, M. G., Strasburg, G. M., Chang, Y. C., Booren, A. M., Gray, J. I., & DeWitt, D. L. (1999). Antioxidant and antiinflammatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries. *Journal of natural products*, *62*(2), 294-296.

Yoshimoto, T., Furukawa, M., Yamamoto, S., Horie, T., & Watanabe-Kohno, S. (1983). Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochemical and bioArysical research communications*, *116*(2), 612-618.

Zhao, Y., Schultz, N. E., & Truhlar, D. G. (2006). Design of density functionals by combining the method of constraint satisfaction with parametrization for thermochemistry, thermochemical kinetics, and noncovalent interactions. *Journal of Chemical Theory and Computation*, *2*(2), 364-382.