Journal of Theoretical and Computational Chemistry
Vol. 13, No. 1 (2014) 1450004 (24 pages)

DOI: 10.1142/S0219633614500047

Quantum-Chemical Study on Relationship Between Structure and Antioxidant Properties of Hepatoprotective Compounds Occurring in *Cynara Scolymus* and *Silybum Marianum*

Damian Mikulski*,†,‡, Krzysztof Eder† and Marcin Molski*

*Department of Theoretical Chemistry, Faculty of Chemistry
A. Mickiewicz University, ul. Grunwaldzka 6
60-780 Poznań, Poland

†Gen. Zamoyska and Helena Modrzejewska High School no. 2
ul. Matejki 8/10 60-766 Poznań, Poland
‡dmkwant@amu.edu.pl

Received 5 September 2013
Accepted 26 October 2013
Published 16 December 2013

Accurate quantum computations based on the density functional theory have been performed to study the relationship between the electronic geometry and antioxidant capacity of chlorogenic acid, silybin and all geometric stereoisomers of cynarine, isolated from plant extract of *Cynara scolymus* and *Silybum marianum*. To elucidate their antioxidant activity, the HOMO orbital distribution, adiabatic ionization potential (AIP), spin density in free radicals, homolytic dissociation enthalpies (BDE), and proton dissociation enthalpies (PDE) of the O–H bonds have been calculated. For minimum energy conformations, the antioxidative parameters were quantitatively analyzed at the B3LYP/6-311G(d,p) level of theory. The results have shown that the hydrogen transfer mechanism is more preferable in nonpolar medium than in water. From the results obtained we can conclude that SET-PT (single electron transfer followed by proton transfer) is the most preferred mechanism in water medium. The catechol moiety and planar geometry of *trans*-stilbene and *cis*-stilbene moieties in cynarine stereoisomers, chlorogenic acid and their phenoxy radicals strongly contribute to enhancement of the antioxidant activity of these compounds. *Trans,trans*-cynarine appears to be the best candidate for proton and hydrogen atom donor. It has been predicted that *cis,cis*, *trans,cis* and *cis,trans* stereoisomers of cynarine show antioxidant capacity. Our study shows that all of the investigated compounds reveal strong antioxidant activity.

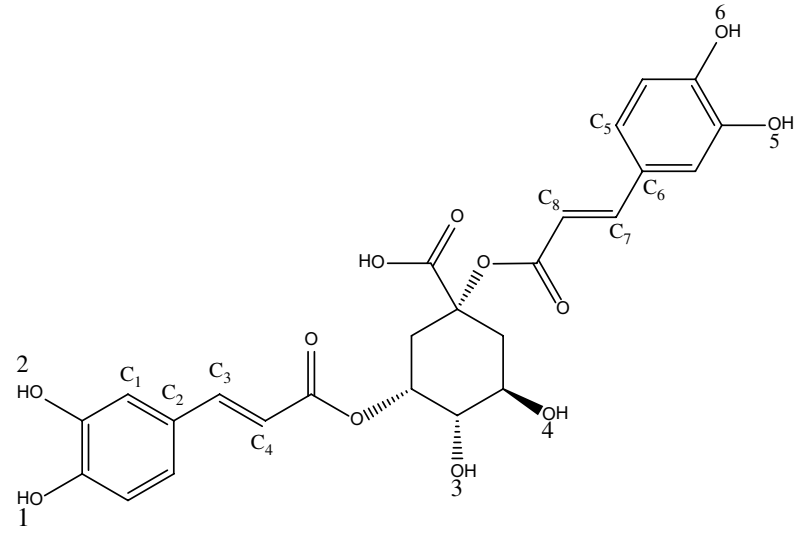
Keywords: DFT method; hepatoprotective compounds; silybin; cynarine; antioxidant.

1. Introduction

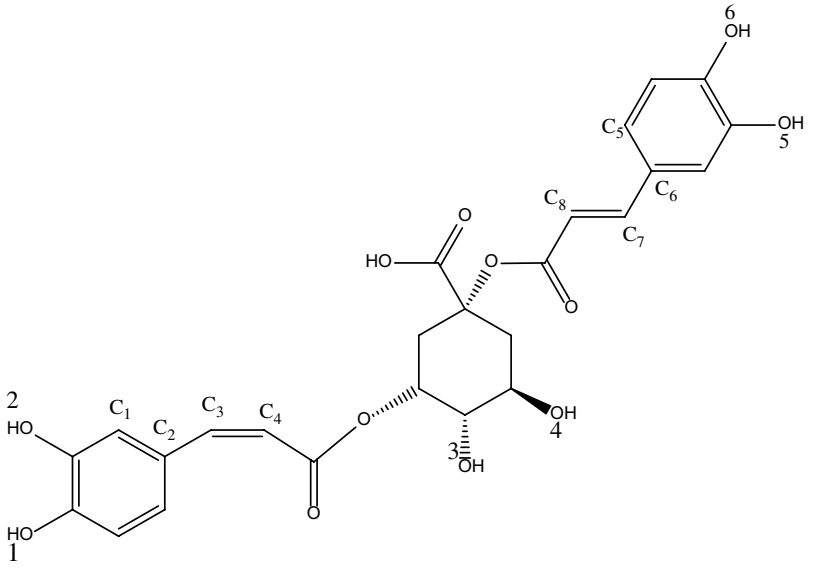
Within the past decade multiple beneficial effects of silymarin (extract obtained from the seeds of the milk thistle of *Silybum marianum*) for the prevention and treatment

‡Corresponding author.

of many liver diseases have been reported.[1,2] The major component of this plant extract is flavonolignan silybin (3,5,7-trihydroxy-2-[3-(R)-(4-hydroxy-3-methoxyphenyl)-2-(R)-(hydroxymethyl)-2,3-dihydro-1,4-benzodioxin-6-yl]chroman-4-one) (Fig. 1) which shows strong biological activity and is used as hepatoprotective drug.

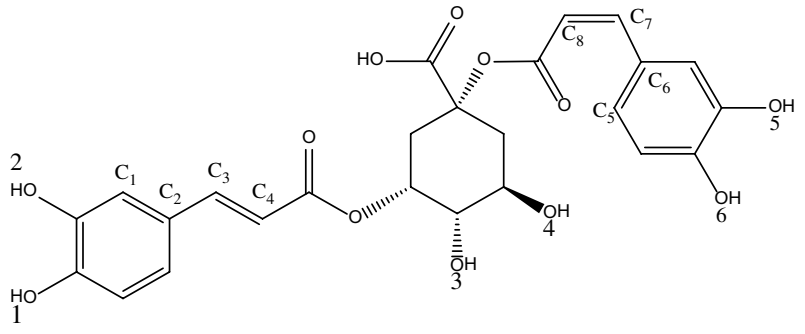


(a)

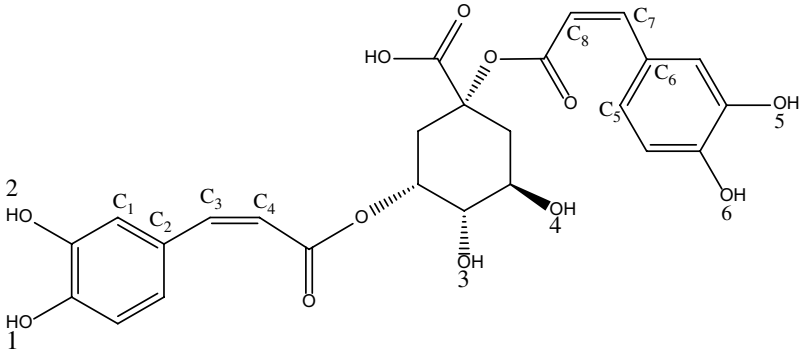


(b)

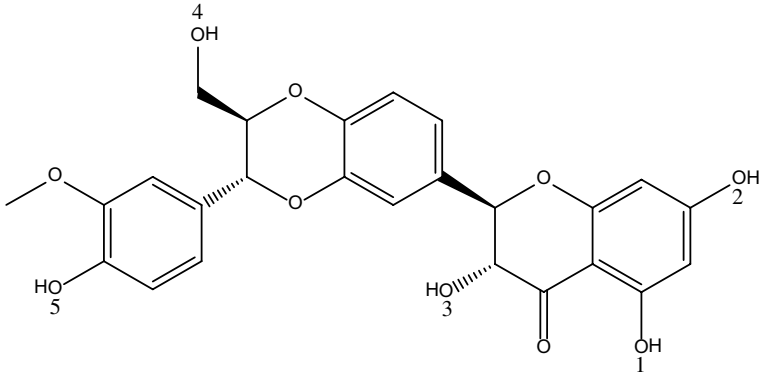
Fig. 1. The molecular structures of (a) *trans,trans*-cynarine, (b) *cis,trans*-cynarine, (c) *trans,cis*-cynarine, (d) *cis,cis*-cynarine, (e) silybin, and (f) chlorogenic acid.



(c)

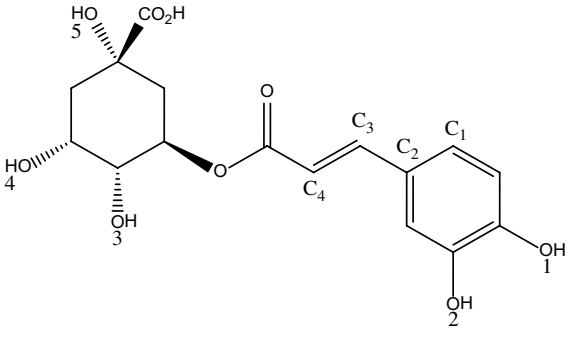


(d)



(e)

Fig. 1. (*Continued*)



(f)

Fig. 1. (*Continued*)

Antioxidant, metal-binding and DNA-damaging properties of flavonolignans have been studied by Vacek *et al.*[3] Also Zatloukalova *et al.*[4] have investigated the interactions of these compounds with DNA. In this paper the binding ability of flavonolignans to DNA in the presence of Cu (II) has been examined. Recently, it has become increasingly clear that cynarine (1,5-dicaffeoylquinic acid) and chlorogenic acid (3-(3,4-dihydroxycinnamoyl)quinic acid) (Fig. 1) are naturally occurring compounds which convey a number of health benefits to humans and may very well be antioxidants, anticancer and antihepatotoxic agents for several diseases states.[5–7] The biological properties of cynarine have been confirmed by Panizzi and Scarpati.[8] In this work the authors isolated from *Cynara scolymus* a crystalline substance which can also be found in *Cynara cardunculus*. They have shown that the substance, which they had originally named as "cynarine", stimulates biliary secretion and cholesterol metabolism. Wójcicki[9] studied the effect of 1,5-dicaffeylquinic acid (cynarine) on cholesterol levels in serum and liver of acute ethanol-treated rats. He reported that in rats receiving ethanol and cynarine simultaneously, a distinct reduction of the serum and hepatic cholesterol levels was observed. A study on enzymatic degradation of cynarine has been reported by Wölkart *et al.*[10] The authors have demonstrated that cynarine is highly susceptible to enzymatic degradation and oxidation in hydroalcoholic solutions. In this paper the authors show that polyphenol oxidase (PPO) is mainly responsible for the oxidative degradation of cynarine. Electrospray ionization-liquid chromatography-mass spectrometry (ESI-LC-MS) method was used to monitor this effect and to determine the kinetics of degradation. The common experimental recognition of the antioxidant activity of novel chlorogenic acid derivatives has been reported by Kweon *et al.*[11] Employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and superoxide anion radical in the xanthine oxidase assay system they measured antioxidant capacity of the derivatives of chlorogenic acid. This study has revealed that all of the compounds studied exhibit high scavenging activity. Additionally, the authors have shown that these compounds

have inhibitory effects on xanthine oxidase. The anti-inflammatory properties of chlorogenic acid have been investigated by Dong *et al.*[12] The purpose of this study was to investigate the effects of chlorogenic acid on liver inflammation and fibrosis induced by carbon tetrachloride. The authors have demonstrated that the hepatic mRNA expression and serum levels of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) were significantly increased in CCl_4-treated rats and attenuated by cotreatment with chlorogenic acid. Also in this important study the authors have proved that the chlorogenic acid may efficiently inhibit CCl_4-induced liver fibrosis in rats and the protective effect may be due to the inhibition of TLR4/MyD88/NF-κB signaling pathway. The antioxidant potency of chlorogenic acid has been investigated *in vitro* and *in vivo* by Sato *et al.*[13] They have used an intestinal ischemia-reperfusion model to evaluate antioxidant activities *in vivo*. They have found that the compound positively affected intestinal ischemia-reperfusion injury. They have also investigated caffeic acid, which can be obtained by hydrolyses of chlorogenic acid in the intestines. The results obtained show that caffeic acid has stronger antioxidant activity than chlorogenic acid. It is worth bearing in mind that also absorption and emission properties of various substituted cinnamic acids and cinnamates have been investigated by Promkatkaew *et al.*[14] The antibacterial potency and mechanism of chlorogenic acid activity against bacteria have been for the first time examined by Lou *et al.*[15] The data obtained revealed that at the minimum inhibitory concentration of this compound the extent of inhibition of the growth of all tested bacterial pathogens is significantly high. The results supported by experimental data show that this antioxidant is able to bind to the outer membrane and to disrupt it. Finally, during this process cytoplasm macromolecules were released which led to the cell death. The utility of silymarin in the treatment of the liver diseases has been investigated by Saller *et al.*[16] The studies have shown that silymarin has metabolic and cell-regulating effects at concentrations found in clinical conditions, namely carrier-mediated regulation of cell membrane permeability, scavenging of reactive oxygen species (ROS) of the R–OH type, action on DNA-expression, for example, via suppression of nuclear factor (NF)-kappa B and inhibition of the 5-lipoxygenase pathway. The data obtained from case record studies involving 452 patients with *Amanita phalloides* poisoning revealed a highly significant difference in mortality in favour of silybin (mortality 9.8% versus 18.3% with standard treatment; $p < 0.01$).

Trouillas *et al.*[17] have performed quantum calculations and experimental studies to elucidate the antioxidant activity of flavonolignans from silymarin. In this important work the antioxidant parameters for silybin and its derivatives (methylated at different positions) were computed adopting DFT method. The authors have proved that for these compounds the electron transfer mechanism is more preferred than the H-atom transfer mechanism. Furthermore, detailed conformational analysis and its relation to the antioxidant capacity have been analyzed. It should be stressed that in this work oxidation potentials were determined experimentally. Moreover, quantum-chemical calculations were performed to verify experimental results.

In view of the lack of knowledge about the mechanisms of action of cynarine isomers and chlorogenic acid as free radicals scavengers we have decided to study these mechanisms using quantum chemistry approach. Only Trouillas[17] reported theoretical and experimental investigation of the antioxidant activity of silybin and its derivatives. Also in our work we studied silybin in order to explain other mechanisms of free radicals scavenging realized by this compound. It should be stressed that the study by Trouillas has inspired us to investigate other compounds with strong hepatoprotective properties. The main purpose of the present study is to explain the role of different OH groups in the antioxidant activity of the compounds studied. We theoretically explain the mechanisms of the free radicals scavenging by the compounds presented. Moreover, a systematic conformational analysis has been performed to investigate the relation between antioxidant capacity and the geometric features of the most stable conformers. Taking into account that antioxidant processes mainly occur in living cells with water environment the solvent effects have been examined. Furthermore, we predict which of the mechanisms studied is the most preferred for each compound studied.

2. Computational Methods

All quantum computations were made using the Gaussian 09 W package.[18] Full optimization of the electronic geometry of the compounds investigated was carried out without symmetry constraints and with the use of Cartesian coordinates. For the species having more conformers, all conformers were investigated. The conformers with the lowest electronic energy were used in this work. For the optimization of geometries and energies in their ground state the Becke's three-parameter hybrid functional B3LYP with the gradient-corrected correlation functional by Lee *et al.*[19] and the 6-311G(d,p) functional basis set were employed. Its choice was motivated by the fact that this functional basis gives a quite reliable estimation of antioxidant parameters and a better description of electronic interaction far from the nucleus (electron delocalization) due to the presence of polarization functions. Because of the computational cost for the chemical systems studied, larger basis sets were not considered in calculations. The optimization procedure adopted to radicals was applied in two steps. At the first step the parent geometries of the compounds studied were optimized. In further calculations these geometries have been used to obtain energy-minimum structures of phenoxy radicals and cation radicals. As radicals and cation radicals are open-shell systems, for the full optimization of their structures the unrestricted B3LYP/6-311G(d,p) level of theory was applied. The computations for the phenoxy radicals and cation radicals were performed from the fully-optimized geometries of the neutral molecules, after H-atom abstraction from OH groups and electron abstraction from the neutral compounds. It should be stressed that during full optimization of geometry of the open-shell systems examined the spin contamination was monitored. Finally, for the fully optimized geometries, the harmonic frequencies were analytically computed to verify whether these

geometries correspond to true energy minima on the potential energy surface (PES). The positive values of these harmonic frequencies prove that these geometries are in local energy minima on the PES. When imaginary value of frequency is detected in the computations, the geometry corresponding to this frequency is in the saddle point on the PES. In all our computations positive values of the harmonic frequencies were obtained. Besides, we employed the harmonic frequencies calculations to extract the zero-point energy correction, enthalpy and thermal corrections to the enthalpies computed. The zero-point vibrational energies were scaled by a factor of 0.9805.[20] To investigate the influence of geometric stereoisomerism on the antioxidant activity three stereoisomers of cynarine were studied: *trans,trans*-cynarine, *trans,cis*-cynarine, *cis,trans*-cynarine and *cis,cis*-cynarine (Fig. 1).

PES was built by changing the dihedral angles α (C_5–C_6–C_7–C_8) and θ (C_1–C_2–C_3–C_4) in chlorogenic acid and geometric isomers of cynarine. The conformational energy map was obtained through discrete rotation of these selected torsional angles, in 10° increments from 0° to 180°. At each point, the total energy was computed at the HF/6-31+G(d) level of theory. Since the relative hydrogen orientation in OH groups is also the most important geometrical parameter, for the most stable geometries obtained from previous full optimizations, the PESs describing the relation between these orientations and total energy were built. These surfaces were generated at the HF/6-31+G(d) level of theory by changing the corresponding dihedral angles in 60° increments with constraint on all other geometrical parameters. In the calculations we used the angle increment by 60° in order to find out whether in the most stable conformations the hydrogen atoms from the OH groups were in the plane of the molecule or out of the plane. Afterwards, the most stable structures obtained from the scan were fully optimized at the B3LYP/6-311G(d,p) level without any geometrical and symmetry constraints around each potential minimum.

The homolytic bond dissociation enthalpies (BDE) were computed as the differences between enthalpies of reagents (phenoxy radicals, hydrogen atom and neutral molecule of antioxidant) under standard conditions (1 atm. and 298.15 K). To calculate this antioxidant parameter the following mathematical formula was used:

$$\mathrm{BDE} = H_{\mathrm{radical}} + H_{\mathrm{H}} - H_{\mathrm{neutral}}, \tag{1}$$

where H_{radical} refers to the total enthalpy of phenoxy radical, H_{H} is the total enthalpy of the hydrogen atom, while H_{neutral} denotes the total enthalpy of the neutral compound. In this paper the total enthalpies of the species were computed as the sum of total electronic energy, zero-point energy and the translational, rotational and vibrational contributions to the total enthalpy. Finally, RT term was added to convert the energy to enthalpy. The correlation between experimental values of the BDE and computed ones was discussed. The adiabatic ionization potential (AIP) was estimated according to the following formula:

$$\mathrm{AIP} = H_{\mathrm{cation\ radical}} + H_{\mathrm{electron}} - H_{\mathrm{radical}}, \tag{2}$$

in which $H_{\text{cation radical}}$ is the total enthalpy of a cation radical, whereas H_{electron} denotes the total enthalpy of the electron. The proton dissociation enthalpy (PDE) was computed according to the expression below:

$$\text{PDE} = H_{\text{radical}} + H_{\text{H}^+} - H_{\text{cation radical}}, \tag{3}$$

in which H_{H^+} refers to the total enthalpy of the proton.

In the calculations, the following enthalpy values were used: $H(\text{H}\cdot)_{\text{vacuum}} = -0.49764$ Ha (i.e. hartree) $H(\text{H}^+) = 0.00236$ Ha, gas-phase enthalpy of electron $H(\text{e}^-) = 0,20977$ Ha,[21] $\Delta H(\text{H})_{\text{solv}} = -0.00152$ Ha. Because H^+ and e^- solvation enthalpies in water are not available and their values depend on the quantum method used, they were determined using CPCM B3LYP/6-311G(d,p) calculations as the enthalpy changes in the processes:

$$\text{H}_2\text{O}_{(\text{H}_2\text{O})} + \text{H}^+_{(\text{vacuum})} \rightarrow \text{H}_3\text{O}^+_{(\text{H}_2\text{O})},$$
$$\text{H}_2\text{O}_{(\text{H}_2\text{O})} + \text{e}^-_{(\text{vacuum})} \rightarrow \text{H}_2\text{O}^-_{(\text{H}_2\text{O})}.$$

Adopting this theoretical model we obtained the following values: $\Delta H(\text{e}^-)_{\text{solv}} = -0.20043$ Ha, $\Delta H(\text{H}^+)_{\text{solv}} = -0.39838$ Ha. To perform these calculations we adopted the method proposed by Rimarčík *et al.*[21]

The HOMO orbital distribution in parent molecules was determined for their fully optimized geometries of parent molecules. However, the three-dimensional spin density distribution and spin density on carbon and oxygen atoms were computed for the most energetically stable geometries of the phenoxy radicals. For these calculations unrestricted B3LYP/6-311G(d,p) level of theory has been employed.

The solvent effects were considered by using the continuum polarizable solvation model (CPCM)[22] implemented in Gaussian 09 W package. In this simple model water is assumed to be a continuum medium with a characteristic dielectric constant. The UAO model of solvation cavity was used in our calculations. The CPCM computations were performed with tesserae of 0.2 Å average size. The polarizable dielectric medium is described by the dielectric constant of the solvent (78.39 for water). The solvent effects on the values of antioxidant parameters were estimated for the equilibrium geometries obtained in vacuum. It should be pointed out that we investigated the influence of polar medium on AIP values because of the presence of charged species (cation radical are able to interact strongly with polar environment). The calculation results obtained in vacuum can be related to antioxidative behavior of the compounds studied in nonpolar environment of the lipid-peptide membranes of living cells. The above methods and parameters selected were proven to be rational and efficacious by our previous published works[23–25] and many other theoretical studies.

3. Results and Discussion

3.1. *Geometrical analysis*

At the first step we performed conformation analysis to investigate the relation between geometry of the compounds studied and their antioxidant activity. The

geometry is the most important factor which has a great influence on the electron delocalization which is the major electronic property determining the antioxidant potency. Figure 2 presents the equilibrium geometries of the compounds studied. The geometrical parameters (dihedral angles, bond lengths) computed at the B3LYP level of theory in vacuum and water medium are presented in the Tables 1 and 2. It should be stressed that all geometries computed in this study are in global minima on the PES. This finding is supported by the fact that all values of computed harmonic

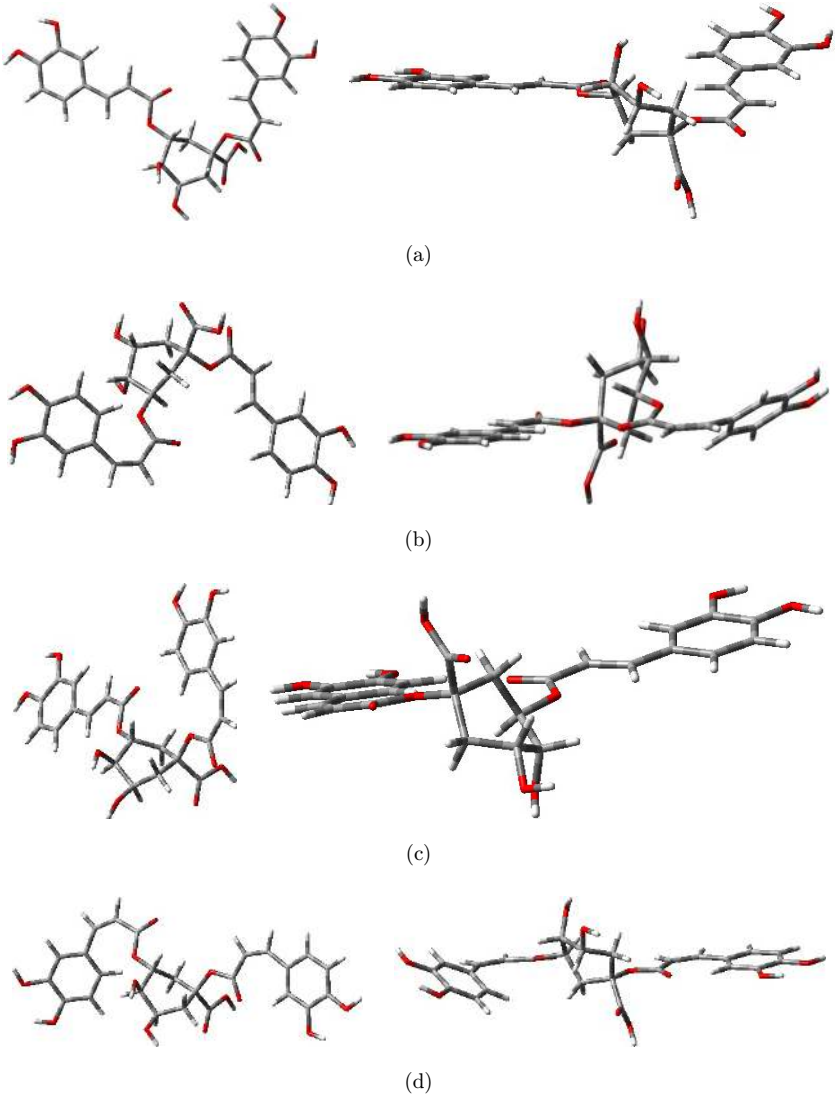
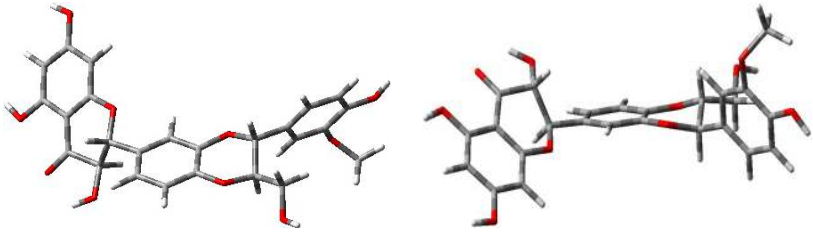
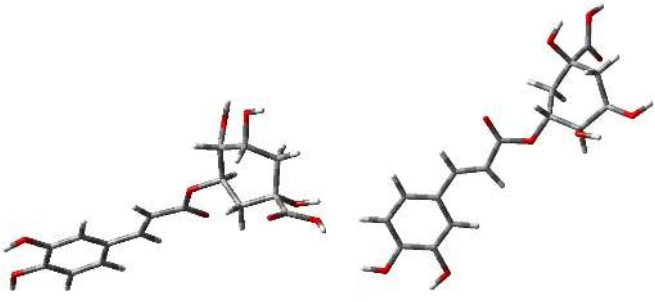


Fig. 2. The optimized molecular geometries of (a) *trans,trans*-cynarine, (b) *cis,trans*-cynarine, (c) *trans,cis*-cynarine, (d) *cis,cis*-cynarine, (e) silybin, and (f) chlorogenic acid obtained with a use of the B3LYP/6-311G(d,p) level of theory.



(e)



(f)

Fig. 2. (*Continued*)

frequencies are positive. A close look into the results obtained allows to conclude that *trans*-stilbene and *cis*-stilbene moieties in stereoisomers of cynarine, chlorogenic acid, their radicals and cation radicals are planar both in vacuum and water medium (Fig. 2). Only in *cis,cis*-cynarine and *cis,trans*-cynarine can we observe a deviation of one *cis*-stilbene moiety from planarity (Table 2). From the results obtained we can conclude that planar conformations of the flat compounds enhance their antioxidant activity. The planar geometry of the stilbene fragments provides more energetically favorable electron density delocalization over the phenyl rings. The equilibrium bond lengths are similar in each compound studied. The analysis of these values in the stereoisomers investigated reveals that they are comparable (Table 1). It should be

Table 1. The computed values of particular O–H bond lengths [Å] in optimized molecules, obtained at the B3LYP/6-311G(d,p) level of theory in vacuum and (water medium).

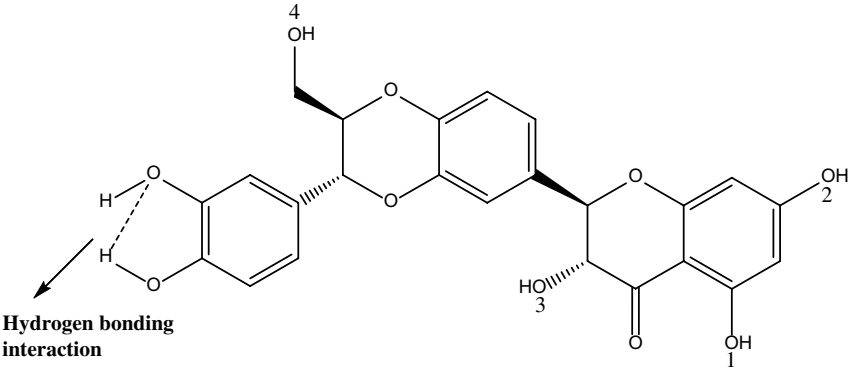
Bond	*Cis,trans*-cynarine	*Trans,cis*-cynarine	*Cis,cis*-cynarine	*Trans,trans*-cynarine	Chlorogenic acid	Silybin
O_2–H_2	0.96 (0.96)	0.96 (0.97)	0.96 (0.96)	0.96 (0.96)	0.96 (0.96)	0.96 (0.97)
O_3–H_3	0.96 (0.96)	0.96 (0.96)	0.97 (0.96)	0.96 (0.96)	0.97 (0.97)	0.96 (0.96)
O_5–H_5	0.96 (0.97)	0.96 (0.96)	0.96 (0.97)	0.96 (0.96)	0.96 (0.96)	0.96 (0.96)

Table 2. The equilibrium magnitudes of dihedral angles [°] computed at the B3LYP/6-311G(d,p) level of theory in vacuum.

Torsion angle	*Cis,trans*-cynarine	*Trans,cis*-cynarine	*Cis,cis*-cynarine	*Trans,trans*-cynarine	Chlorogenic acid
C_1–C_2–C_3–C_4	153.22	−178.61	152.23	−178.46	−179.49
C_5–C_6–C_7–C_8	−178.03	−176.26	−179.75	−178.99	—

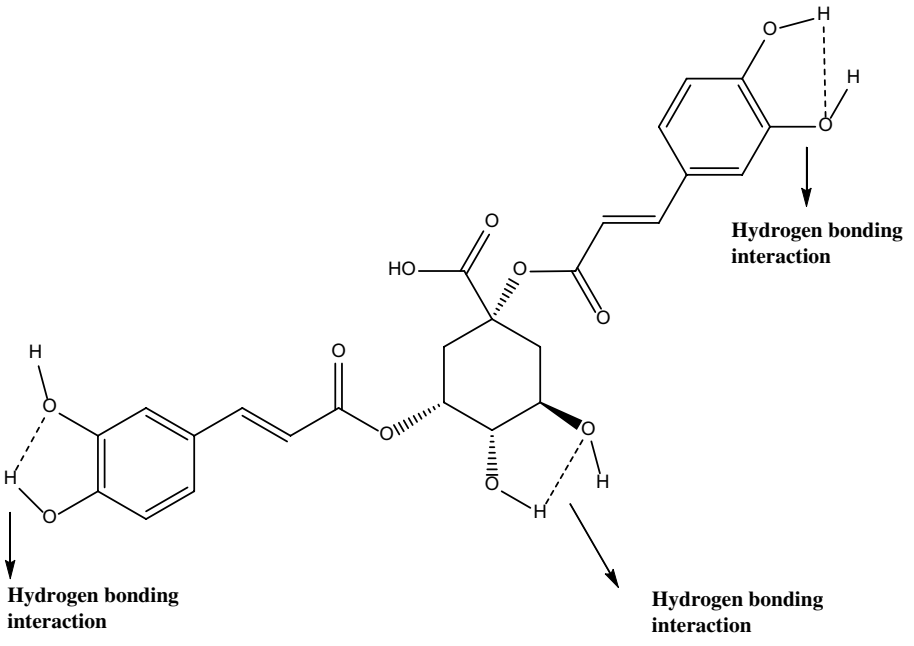
pointed out that the hydrogen atoms from the hydroxyl groups are in positions co-planar with phenyl rings. This orientation of hydrogen atoms may provide the most energetically preferable interaction with free radicals in living cells. From the findings obtained we can claim that stilbene skeleton contributes to enhancement of the stability of ester bonds in chlorogenic acid and cynarine geometrical isomers. The α, β-unsaturated systems in cynarine stereoisomers and chlorogenic acid provide significant stability of ester bond. The vinyl bond connecting phenyl ring and the second fragment of geometric stereoisomers studied has double-bond characteristics with typical optimized length 1.35 Å. This suggests that the presence of a π-delocalization system in chlorogenic acid and geometric stereoisomers of cynarine contributes to conjugative stabilization effect. Fully optimized geometries of these open-shell systems have semi-quinone geometry, in which unpaired electron is delocalized over *trans*-stilbene moiety and the vinyl bond.

The geometric analysis gives also insight into the importance of H-bonding in the compounds studied. We observe a strong stabilizing effect by this bond between two OH groups in phenyl rings and between H-atom of OH group and O-atom of methoxy group in silybin (Fig. 3). From these results we can conclude that these bonds may have strong effect on stability of the O–H bond. What is more, hydrogen bonding facilitates the proton and hydrogen atom transfer from the OH group in the

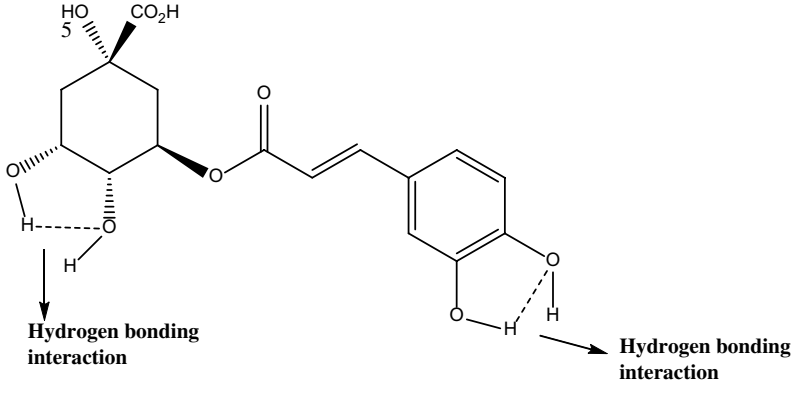


(a)

Fig. 3. The hydrogen bonding interactions in silybin, (a) *trans,trans*-cynarine (b) and chlorogenic acid (c).



(b)



(c)

Fig. 3. (*Continued*)

media studied. Also, in geometric stereoisomers of cynarine there are strong hydrogen bonding interactions between OH groups in catechol moieties (Fig. 3). The average length of these bonds is 2.1 Å, which suggests that the hydrogen bonding interactions in these compounds are considerable. Furthermore, in cyclohexane moiety of geometric isomers of cynarine and chlorogenic acid there are strong hydrogen bonding interactions between OH groups (Fig. 3).

3.2. *Antioxidant parameters (BDE, PDE, AIP)*

There are two mechanisms by which antioxidants can scavenge harmful free radicals in living cells. The first mechanism — hydrogen transfer mechanism (HAT) is based on direct hydrogen abstraction from the antioxidant, while the second — single electron transfer followed by proton transfer (SET-PT) involves the proton and electron transfer process. Generally, in the biological systems the first mechanism dominates in nonpolar environments in the cell regions, whereas the second mechanism mainly occurs in polar environments. BDE values are associated with the HAT mechanism, while AIP and PDE values correspond to the SET-PT mechanism. Analysis of AIP values in water medium reveals significant influence of this medium on the antioxidant activity of the compounds studied. As seen from Table 3 all the compounds studied have dramatically lower value of AIP in water medium than in vacuum. This result confirms the fact that in polar medium the compounds studied are more susceptible to donate electron. Probably the charged species of cation radicals strongly interact with polar environment. From among antioxidants investigated silybin is the most sensitive to electron donation in the media examined. It is likely that the practically planar geometry of silybin favors this property. Planar geometry implies that delocalization of free electron in phenoxy radicals is more energetically preferred. It should be stressed that the cynarine stereoisomers studied are characterized by similar values of AIP in the media studied, which means that these geometrical isomers have comparable ability to donate electron in the biological systems. From among the steroisomers studied *trans,cis*-cynarine has highest ability to donate electron in the media considered.

The direct comparison between the theoretical and experimental AIP (obtained in nonpolar environment) values (resorcinol $AIP_{exp} = 199.0$ kcal/mol, hydroquinone $AIP_{exp} = 194.6$ kcal/mol) shows that the experimental data values are higher than those that we found in vacuum. Probably, the presence of electron donating groups directly attached to the phenyl rings in the compounds considered decreases the energy difference between neutral molecule and its cation radical, leading to the lower calculated AIP values than experimental ones.

Analysis of the BDE values (Tables 4 and 5) reveals that the bond O_1–H_1 in *trans,trans*-cynarine is the most susceptible to homolytic dissociation in water

Table 3. The computed values of the AIP [kcal mol^{-1}] obtained in vacuum and water medium at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	AIP (vacuum)	AIP (water medium)
Cis,trans-cynarine	175.355	20.981
Trans,cis-cynarine	172.665	21.678
Cis,cis-cynarine	174.012	20.563
Trans,trans-cynarine	175.848	23.002
Chlorogenic acid	184.943	22.238
Silybin	171.394	20.33

Table 4. The computed values of BDE [kcal mol^{-1}] obtained in vacuum at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	O_1–H_1	O_2–H_2	O_3–H_3	O_4–H_4	O_5–H_5	O_6–H_6
Cis,trans-cynarine	74.994	82.442	105.980	100.891	85.028	73.073
Trans,cis-cynarine	73.324	85.486	105.949	100.213	74.805	82.699
Cis,cis-cynarine	82.223	74.866	105.776	101.022	85.334	73.400
Trans,trans-cynarine	73.158	85.272	105.873	100.804	84.943	73.039
Chlorogenic acid	72.841	85.065	105.095	99.812	102.171	—
Silybin	85.467	87.745	104.531	99.015	76.594	—

medium, whereas the O_6–H_6 bond can easily break with the abstraction of hydrogen atom in vacuum. These facts can be explained by the presence of *trans*-stilbene moiety. Furthermore, we can conclude that the weakest bond in each compound is the one in *para*-position relatively to the *trans*-stilbene moiety. Analyzing the computed values of BDE in the stereoisomers studied we can conclude that O_1–H_1 and O_6–H_6 bonds are very sensitive to homolytic break in the media studied. Moreover, *cis,cis*-cynarine and *trans,trans*-cynarine have comparable ability to donate hydrogen atom. Note that O_3H_3, O_4H_4 hydroxyl groups in geometrical stereoisomers have similar values of the BDE, which means that these groups possess similar abilities to donate hydrogen atom. It should be pointed out that the weakest bond appears in chlorogenic acid (O_1–H_1 bond) and is mainly responsible for its antioxidant activity. On the basis of the results obtained we can claim that the presence of catechol moiety in cynarine stereoisomers and chlorogenic acid is the main structural feature associated with the antioxidant activity. This moiety confers high stability to the phenoxy radicals formed after free radicals capture. In the silybin molecule the lowest value of the BDE is obtained for its O_5–H_5 bond. This result is in an excellent agreement with the calculations performed by Trouillas *et al.*[17] It is probably due to the stabilizing effect of hydrogen bonding between the oxygen atom of methoxy group and the hydrogen atom from O_5H_5 group.

The computed BDE values indicate that O_3–H_3 and O_4–H_4 bonds stand out from the other bonds as those of the highest stability in the media studied. Comparing the magnitudes of the BDE in vacuum and water medium we find out that the stability of all O_3–H_3 bonds decreases, while that of the O_4–H_4 bonds increases in water

Table 5. The computed values of BDE [kcal mol^{-1}] obtained in water medium at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	O_1–H_1	O_2–H_2	O_3–H_3	O_4–H_4	O_5–H_5	O_6–H_6
Cis,trans-cynarine	76.048	79.423	104.676	101.192	82.412	74.887
Trans,cis-cynarine	74.425	82.038	105.157	101.026	79.583	76.205
Cis,cis-cynarine	79.060	75.687	104.316	100.913	81.302	74.877
Trans,trans-cynarine	74.348	81.961	104.206	100.825	82.082	74.557
Chlorogenic acid	74.289	81.988	103.681	100.284	101.416	—
Silybin	84.798	88.657	102.320	99.512	77.646	—

medium. The O–H bonds in *para*-position to the vinyl bond in *trans*-stilbene moiety become stronger in water environment. Hence, we can conclude that the polar medium strongly contributes to stability of these bonds. In other words, the water medium makes a homolytic dissociation of these bonds difficult.

As the compounds investigated are polyphenolic derivatives we can compare the computed values of BDE with the experimental data. We do not possess the experimental data for the compounds studied, hence using the data for phenol, resorcinol, hydroquinone, and p-aminophenol is expedient. According to our results we state that this data is in a good agreement with the BDE estimated for phenol, resorcinol, hydroquinone, and *p*-aminophenol. The most recent experimental values of the BDE for phenol are 88.3 ± 0.8 kcal/mol[26] and 88.7 ± 0.5 kcal/mol,[27] where the latter is retained as the more reliable one. However, the experimentally estimated values of the BDE for resorcinol, hydroquinone and *p*-aminophenol are 86.7, 81.5 and 77.3 kcal/mol, respectively.[28] Note that deviations between the remaining computed and experimental BDE values lie within 5 kcal/mol. This fact strongly indicates that our calculation method provides the reliable BDE results for the antioxidants studied.

A close look into the BDE and AIP values for all compounds investigated shows that AIP values obtained in vacuum are significantly higher than those of BDE. In contrast, in water medium we can observe exactly opposite tendency. Summarizing, the results obtained lead to a suggestion that in nonpolar environments the compounds studied can be more susceptible to hydrogen atom donation than in water medium, whereas in polar medium these compounds show higher ability to donate electron than in vacuum.

PDE is a useful physical parameter to characterize the antioxidant activity of the compounds studied and describes the ability of these compounds to donate proton in the environments investigated. The antioxidants with lower values of PDE are more susceptible to proton abstraction. It should be pointed out that SET-PT mechanism of antioxidant action is mainly determined by the values of this parameter. The calculated values of PDE in vacuum in water medium are presented in Tables 6 and 7. Analysis of the PDE indicates that the most prone to the proton donation are the OH groups, localized in *para*-position to *trans*-stilbene skeleton. It is interesting to note that the O_3H_3 and O_4H_4 groups have the lowest ability to donate proton in

Table 6. The computed values of PDE [kcal mol^{-1}] obtained in vacuum at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	O_1–H_1	O_2–H_2	O_3–H_3	O_4–H_4	O_5–H_5	O_6–H_6
Cis,trans-cynarine	226.556	234.005	257.542	252.453	236.590	224.636
Trans,cis-cynarine	227.464	239.727	260.197	254.461	229.054	236.948
Cis,cis-cynarine	235.129	227.772	258.681	253.928	238.240	226.306
Trans,trans-cynarine	224.227	236.341	256.943	251.874	236.013	224.109
Chlorogenic acid	214.822	227.033	247.069	241.786	244.151	—
Silybin	240.993	243.265	260.054	254.535	232.118	—

Table 7. The computed values of PDE [kcal mol^{-1}] obtained in water medium at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	O_1–H_1	O_2–H_2	O_3-H_3	O_4-H_4	O_5–H_5	O_6–H_6
Cis,trans-cynarine	7.180	10.555	35.808	32.324	13.544	6.019
Trans,cis-cynarine	4.861	12.473	35.592	31.461	10.018	6.640
Cis,cis-cynarine	10.609	7.236	35.866	32.463	12.851	6.427
Trans,trans-cynarine	3.459	11.072	33.318	29.936	11.193	3.668
Chlorogenic acid	4.164	11.863	33.555	30.159	31.291	—
Silybin	16.574	20.434	34.096	31.288	9.423	—

the media studied, whereas the O_1–H_1 and O_6–H_6 bonds are the most susceptible to proton donation in the media studied. From all the stereoisomers studied in *trans,trans*-cynarine the O_1H_1 group has the highest ability to donate proton in water medium, while the O_6H_6 group reveals this ability in vacuum. As follows from our results, chlorogenic acid has the highest ability to donate proton in vacuum (nonpolar medium). The high acidity of this compound can be associated with the presence of *trans*-stilbene moiety and ester bond in this molecule. Moreover, the strong intramolecular hydrogen bonding between two hydroxyl groups in phenyl ring (optimized O_1–H_2 bond length is 2.17 Å, Fig. 3) contributes to the enhancement of the acidity of chlorogenic acid. Additionally, the presence of catechol moiety in chlorogenic acid determines the importance of the O_1H_1 and O_2H_2 groups as proton donors. The significantly high values of the PDE in vacuum suggest that the compounds investigated show rather poor ability to donate proton in nonpolar media.

When the solvent is included in the quantum calculations, the PDE values are significantly lower than in vacuum. This result reveals that the compounds studied are able to donate more proton in polar environment than in nonpolar medium. Besides, the results obtained suggest that the SET-PT mechanism of free radicals scavenging can dominate in water medium. In water medium very low values of PDE are strongly correlated with high acidity of the compounds studied in polar environment. Probably, polar medium enables the abstraction of proton from the hydroxyl groups. The results obtained prove that antioxidant properties of the compounds investigated depend strongly on the presence of polar solvents. Further quantum calculations are needed to determine the thermodynamical parameter such as the free Gibbs enthalpy which can enable assessment of the thermodynamic spontaneity of the reaction of the compounds studied with water molecules. On the basis of these calculations we will investigate the energetic stability of the ionic forms of antioxidants examined. Also, accurate kinetics analysis is needed to compute the energy of activation of the process of ions and radicals formation and to determine the rate constants of the reactions studied. Also, sequential proton loss electron transfer (SPLET) mechanism for free radicals scavenging will be studied in our future work.

The spin density is the most important parameter correlated with the antioxidant activity of the compounds studied. This parameter was computed at unrestricted

B3LYP/6-311G(d,p) level in vacuum and water medium. Figure 4 presents the values of this density on particular atoms in chlorogenic acid in the media studied. Spin density characterizes the distribution of electron spin in the phenoxy radicals and is responsible for the energetic stability of these radicals. The high spin density

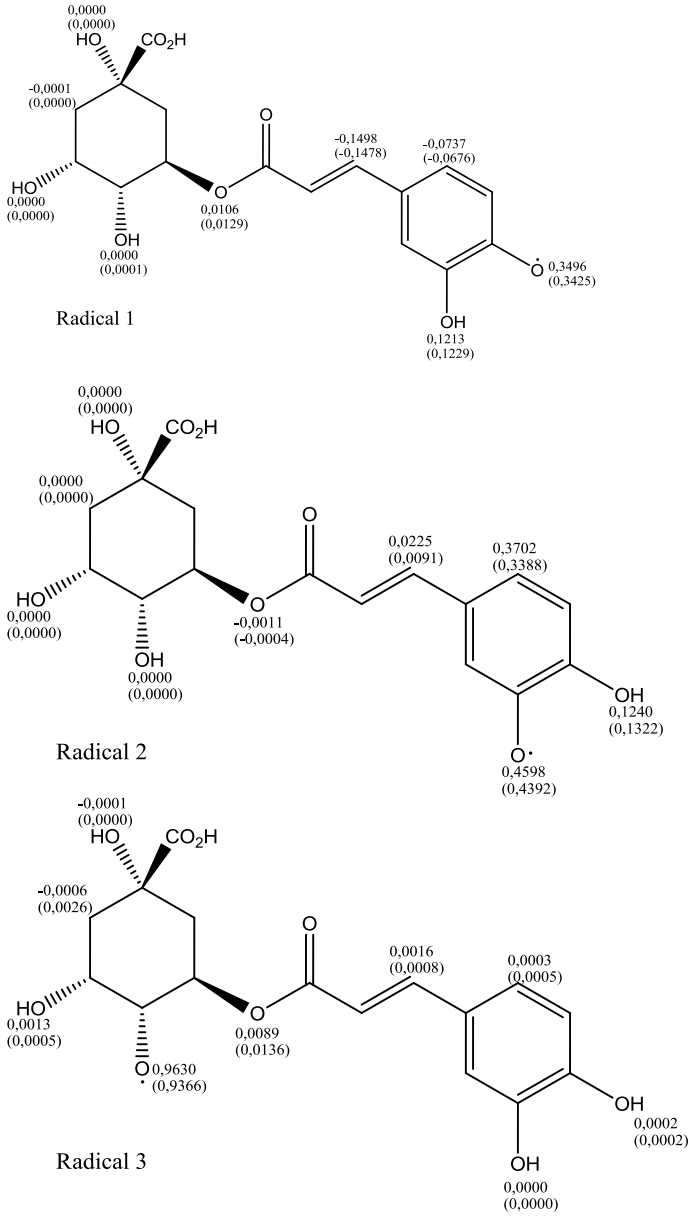


Fig. 4. The spin density on particular atoms in phenoxy radicals of chlorogenic acid computed with a use of the B3LYP/6-311G(d,p) level of theory.

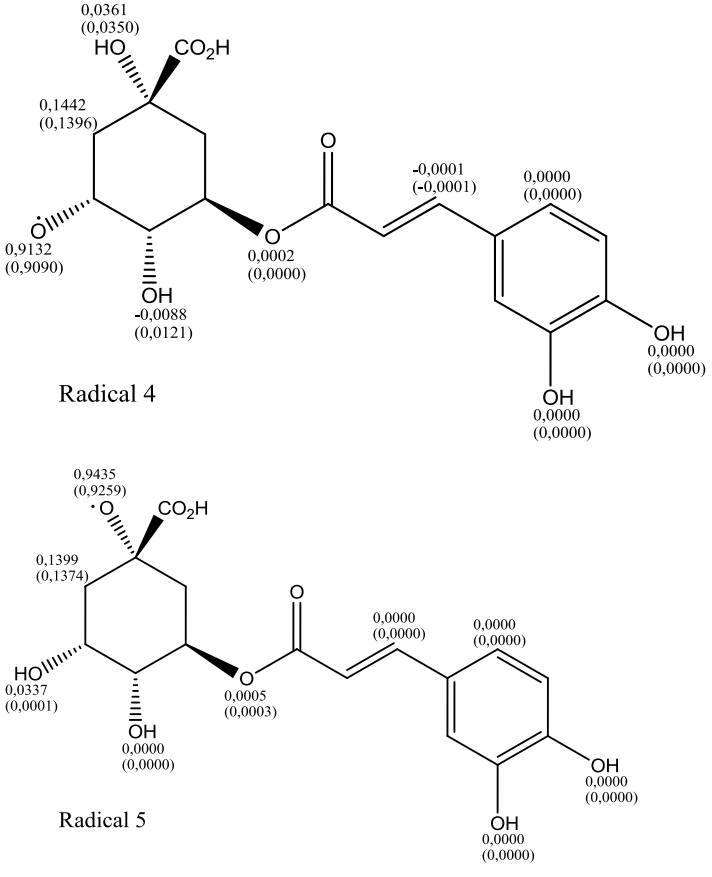
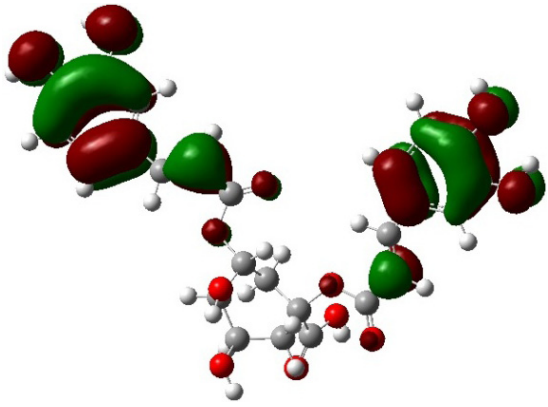


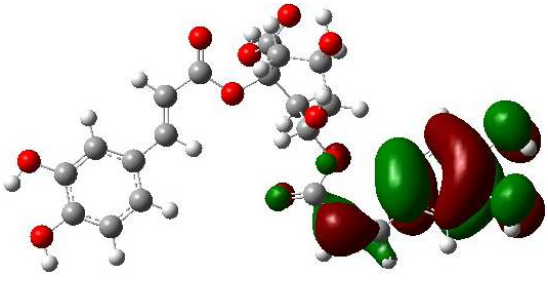
Fig. 4. (*Continued*)

at any site of the radicals is strongly associated with the reactivity of these sites. Analysis of the computed spin densities reveals their significantly high concentration on oxygen atoms in *trans*-stilbene moiety (Fig. 4). Other compounds examined show similar distribution of spin on these atoms. Furthermore, we observe that all atoms of the *trans*-stilbene moiety in chlorogenic acid and cynarine geometric stereoisomers are characterized by nonzero values of spin density. The presence of vinyl bond is responsible for the considerable spin delocalization. High contribution to the global spin density from the atoms of vinyl bond leads to a conclusion that this fragment of the molecules studied is sensitive to the attack of free radicals. Our results show that water medium decreases the spin density. Probably, this finding is correlated with a strong interaction of polar medium with phenoxy radicals. Taking into account the fact that, the lower the values of spin density, the higher the energetic stability of the phenoxy radicals, we can conclude that in polar medium the phenoxy radicals studied are more stable than in vacuum.

The antioxidative behavior of many antioxidants can be elucidated by the distribution of the HOMO molecular orbitals and their eigenvalues computed with the use of the quantum chemistry methods. It should be noted that the antioxidants with lower energy of this orbital have poorer ability to donate electron during interaction with free radicals. The molecular-electron donating ability is strictly correlated with the highest occupied molecular orbital (HOMO) eigenvalue, while active redox sites in antioxidants are determined by high values of energy of HOMO orbitals. Moreover, the high electronic density of distribution of HOMO orbital determines in antioxidants the most probable sites which can be easily attacked by free radicals. The three-dimensional shapes (Fig. 5) of the HOMO orbital distribution in the chlorogenic acid and *trans,trans*-cynarine show that the high density of this distribution is mainly localized over *trans*-stilbene moiety. We find that in the rest of the cynarine stereoisomers the shape of HOMO orbital is mainly delocalized over *cis*-stilbene moiety. The torsion angle in this moiety minimizes the steric hindrances and allows this delocalization. Besides, the significantly high density of

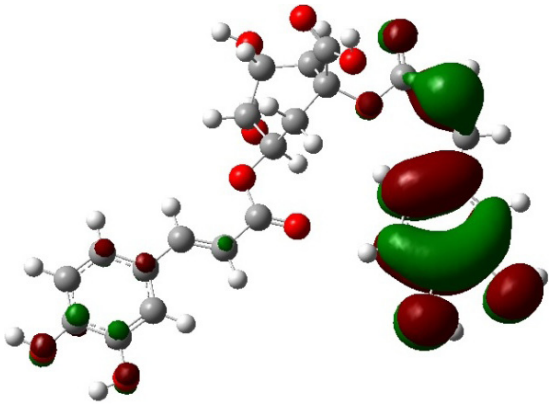


(a)

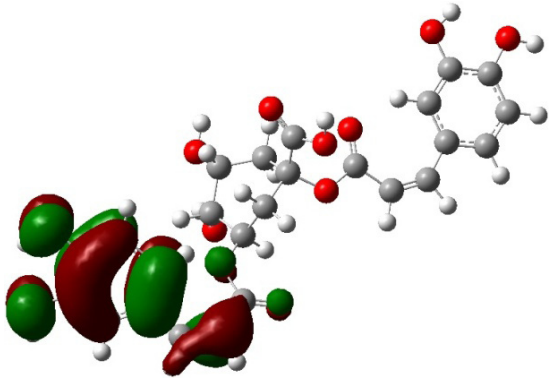


(b)

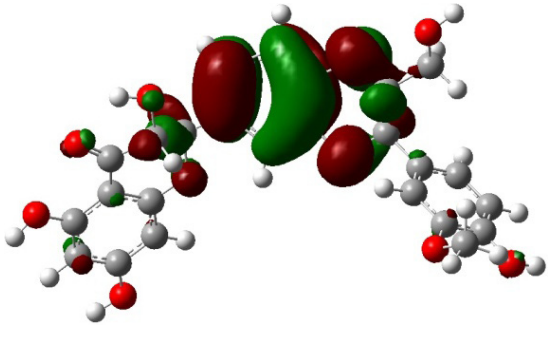
Fig. 5. The three-dimensional distribution of HOMO orbital for (a) *trans,trans*-cynarine, (b) *cis,trans*-cynarine, (c) *trans,cis*-cynarine, (d) *cis,cis*-cynarine, (e) silybin, and (f) chlorogenic acid computed with a use of the B3LYP/6-311G(d,p) level of theory.



(c)

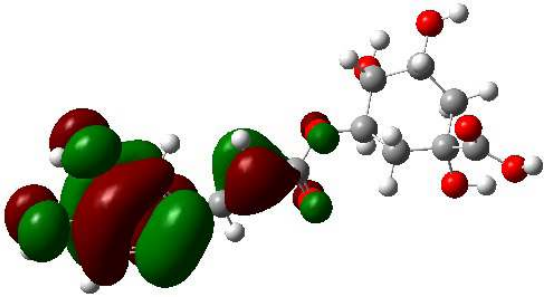


(d)



(e)

Fig. 5. (*Continued*)



(f)

Fig. 5. (*Continued*)

Table 8. The computed HOMO orbital [eV] eigenvalues obtained in vacuum and water medium at the B3LYP/6-311G(d,p) level of theory.

Chemical compound	HOMO (vacuum)	HOMO (water medium)
Cis,trans-cynarine	−6.897	−6.745
Trans,cis-cynarine	−6.465	−6.331
Cis,cis-cynarine	−6.655	−6.568
Trans,trans-cynarine	−6.903	−6.817
Chlorogenic acid	−7.124	−7.041
Silybin	−6.134	−6.088

HOMO orbital at the *trans*-stilbene fragment indicates that this region is characterized by many active redox sites. As follows from the findings obtained, the HOMO eigenvalues in gas phase is lower than in water medium for all the substances studied (Table 8). According to this result we can conclude that all the antioxidants studied are more active in water medium, since they are more susceptible to ionization. This result is in a good agreement with the computed values of AIP in water medium and proves that in water medium the compounds studied have higher ability to scavenge free radicals via SET mechanism. Comparing the computed values of energies of HOMO orbital we claim that silybin is characterized by the highest value of this parameter in the media studied. Therefore, this compound has a significant ability to interact with free radicals and to scavenge them by electron donation process.

4. Conclusions

The quantum computations performed within the DFT method showed that the studied biologically active components of *Cynara scolymus* and *Silybum marianum* are very efficient antioxidants. It has been found that in water medium these compounds can mainly scavenge the free radicals by the SET mechanism. As follows from the results obtained, the HAT mechanism is more preferred in nonpolar media than in water medium. Hence, we can conclude that this mechanism may play a considerable

role in nonpolar lipid-peptide membranes of living cells. The findings demonstrate that the compounds studied are able to donate more electron and proton in water medium than in gas phase. The stereoisomers studied reveal comparable ability to donate electron, proton and hydrogen atom. In *trans,trans*-cynarine O_1H_1 and O_6H_6 groups are the most effective donors of proton and hydrogen atom. However, on the basis of the computed AIP values the most active system of electron transfer seems to be silybin. The most energetically stable conformations of chlorogenic acid and geometric stereoisomers of cynarine have practically strictly planar skeleton of *trans*-stilbene moiety in the environments studied. The strictly planar structure of this fragment provides energetically preferable delocalization of the HOMO orbital over this skeleton. Moreover, strictly planar geometry of *trans*-stilbene moiety in phenoxy radicals and cation radicals determines energetically favorable delocalization of unpaired electron. However, the catechol moiety is responsible for strong antioxidant properties of the compounds studied. The results obtained indicate that the therapeutic action of these substance in the human liver can be realized by the effective scavenging of harmful free radicals. To explain this pharmaceutical property further experiments are needed. Additionally, the synthesis and biological evaluation of new analogues of the compounds studied towards their antioxidant capacity should be performed.

Acknowledgments

This study is supported by the PCSS grant in Poznań No. 131 "Theoretical investigation of antioxidant activity of green tea".

References

1. Morazzoni P, Bombardelli E, Silybum marianum (Carduus marianus), *Fitoterapia* **66**:3–42, 1995.
2. Saller R, Meier R, Brignoli R, The use of silymarin in the treatment of liver diseases, *Drugs* **61**:2035–2063, 2001.
3. Vacek J, Zatloukalova M, Desmier T, Nezhodova V, Hrbac J, Kubala M, Kren V, Ulrichova J, Trouillas P, Antioxidant, metal-binding and DNA-damaging properties of flavonolignans: A joint experimental and computational highlight based on 7-O-galloylsilybin, *Chem-Biol Inter* **205**:173–180, 2013.
4. Zatlaukalova M, Kren V, Gazak R, Kubala M, Trouillas P, Ulrichova J, Vacek J, Electrochemical investigation of flavonolignans and study of their interactions with DNA in the presence of Cu (II), *Bioelectrochemistry* **82**:117–124, 2011.
5. Huntimer E-D, Halaweish F-T, Chase C-C, Proliferative activity of Echinacea angustifolia root extracts on cancer cells: Interference with doxorubicin cytotoxicity, *Chem Biodivers* **3**:695–703, 2006.
6. Angelini A, Di Pietro R, Centurione L, Castellani M-L, Conti P, Porreca E, F. Cuccurullo F, Inhibition of P-glycoprotein-mediated transport by S-adenosylmethionine and cynarin in multidrug-resistant human uterine sarcoma MES-SA/Dx5 cells, *J Biol Regul Homeost Agents* **26**:495–504, 2012.

7. Jung H-A, Park J-C, Chung H-Y, J. Kim J, Choi J-S, Antioxidant flavonoids and chlorogenic acid from the leaves of Eriobotrya japonica, *Arch Pharm Res* **22**:213–218, 1999.
8. Panizzi L, Scarpati M-L, Constitution of cynarine, the active principle of the artichoke, *Nature* **174**:1062–1067, 1954.
9. Wójcicki J, Effect of 1,5-dicaffeylquinic acid (Cynarine) on cholesterol levels in serum and liver of acute ethanol-treated rats, *Drug Alcohol Depend* **3**:143–145, 1978.
10. Wölkart K, Gangemi D, Turner R, Bauer R, Enzymatic degradation of echinacoside and cynarine in *Echinacea angustifolia* root preparations, *Pharm Biol* **42**:443–448, 2004.
11. Kweon M-H, Hwang H-J, Sung H-C, Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*), *J Agric Food Chem* **49**:4646–4655, 2001.
12. Shi H, Dong L, Jiang J, Zhao J, Zhao G, Dang X, Lu X, Jia J, Chlorogenic acid reduces liver inflammation and fibrosis through inhibition of toll-like receptor 4 signaling pathway, *Toxicology* **303**:107–114, 2013.
13. Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, Sugawara M, Iseki K, *In vitro* and *in vivo* antioxidant properties of chlorogenic acid and caffeic acid, *Int J Pharmacol* **403**:136–138, 2011.
14. Promkatkaew M, Suramitr S, Karpkird T, Ehara M, Hannongbua S, Absorption and emission properties of various substituted cinnamic acids and cinnamates, based in TDDFT investigation, *Int J Quant Chem* **113**:542–554, 2013.
15. Lou Z, Wang H, Zhu S, Ma C, Wang Z, Antibacterial activity and mechanism of action of chlorogenic acid, *J Food Sci* **76**:398–403, 2011.
16. Saller R, Meier R, Brignoli R, The use of silymarin in the treatment of liver diseases, *Drugs* **61**:2035–2063, 2001.
17. Trouillas P, Marsal P, Svobodová A, Vostálová J, Gažák R, Hrbáč J, Sedmera P, Křen V, Lazzaroni R, Duroux J-L, Walterová D, Mechanism of the antioxidant action of silybin and 2,3-dehydrosilybin flavonolignans: A joint experimental and theoretical study, *J Phys Chem* **112**:1054–1063, 2008.
18. Frisch MJ, Trucks GW, Schlegel HB *et al.*, Gaussian 09, Revision A.02, Gaussian Inc., Wallingford, CT 2009.
19. Lee C, Yang W, Parr R-G, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, *Phys Rev B: Conden Matter* **37**:785, 1988.
20. Scott A-P, Radom L, Harmonic vibrational frequencies: An evaluation of Hartree−Fock, Møller−Plesset, quadratic configuration interaction, density functional theory, and semiempirical scale factors, *J Phys Chem* **100**:16502–16513, 1996.
21. Rimarčík J, Lukeš V, Klein E, Ilčin M, Study of the solvent effect on the enthalpies of homolytic and heterolytic N–H bond cleavage in p-phenylenediamine and tetracyano-p-phenylenediamine, *J Mol Struct (THEOCHEM)* **952**:25–30, 2010.
22. Klamt A, Schürmann G, A new approach to dielectric screening in solvents with explicit expression for screening energy and its gradient, *J Chem Soc Perkin Trans* **2**:799–811, 1993.
23. Mikulski D, Górniak R, Molski M, A theoretical study of the structure-radical scavenging activity of *trans*-resveratrol analogues and *cis*-resveratrol in gas phase and water environment, *Eur J Med Chem* **45**:1015–1027, 2010.
24. Mikulski D, Molski M, Quantitative structure-antioxidant activity relationship of *trans*-resveratrol oligomers, *trans*-4,4′-dihydroxystilbene dimer, trans-resveratrol-3-O-glucuronide, glucosides: *trans*-piceid, *cis*-piceid, *trans*-astringin and *trans*-resveratrol-4′-O-beta-D-glucopyranoside, *Eur J Med Chem* **45**:2366–2380, 2010.

25. Mikulski D, Szelag M, Molski M, Górniak R, Quantum-chemical study on the antioxidations mechanisms of *trans*-resveratrol reactions with free radicals in the gas phase, water and ethanol medium, *J Mol Struct (THEOCHEM)* **951**:37–48, 2010.
26. Pedulli G-F, Lucarini M, Pedrielli P, *Free Radicals in Biology and Environment*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1997.
27. Dos Santos R-M-B, Simoes J-A-M, Energetics of the O–H bond in phenol and substituted phenols: A critical evaluation of literature data, *J Phys Chem Ref Data* **27**:707–740, 1998.
28. Klein E, Lukeš V, Study of gas-phase O–H bond dissociation enthalpies and ionization potentials of substituted phenols — Applicability of *ab initio* and DFT/B3LYP methods, *Chem Phys* **330**:515–525, 2006.