# Theoretical Studies of Molecular Mechanics on Polyphenolic Compounds Separated from Phytopharmaceutical Products from *Caprifoliaceae* Family

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*The studied substances are eight carboxylic acids and two polyphenolic compounds separated by HPLC from phytopharmaceutical products mainly from Caprifoliaceae family. Polyphenols are natural antioxidants that along with antioxidant enzyme system of the body help to the prevention of diseases mediated by free radicals. Due to the importance of these compounds we considered necessary their structural characterization by molecular mechanics studies. We also conducted a QSPR study by regression correlation of octanol/ water partition coefficient with molecular parameters describing the structures of the analysed compounds in comparison with their retention times in the column of liquid chromatography.*

*Keywords: polyphenols, antioxidants, log P, QSPR*

Phytopharmaceutical products are a good source of natural antioxidants that can be taken with any medication, without any side reactions or contraindications.

During normal cellular metabolism, approximately 98% of all molecular oxygen is reduced to water. The remaining 2% produces reactive oxygen species, directly affecting tissues, by oxidation of cellular protein, lipids and nucleic acids. In normal physiological conditions, an equilibrium exists between free radicals formation and their removal by antioxidant defense systems of the body. In pathological conditions, the production of free radicals increases, along with an increase in consumption and a decrease in the production of such antioxidant defense molecules leading to cellular damage or death.

Polyphenols are natural antioxidants that help along with antioxidant enzyme system of the body to the prevention of diseases mediated by free radicals.

Plants contain a variety of substances that neutralize the excess of reactive oxygen species: polyphenols, flavonoids, terpenoids. This substances are named natural antioxidants and their effects in the prevention and treatment of diseases caused by ROS are extensively studied [1].

It was found that a diet rich in vegetals with high polyphenols content has more intense protective action than taking dietary supplements, most likely due to the synergistic effect of the components from natural material [2,3].

Due to the importance of these compounds we considered necessary their structural characterization by molecular mechanics studies.

We also performed regressional correlation of the octanol/water partition coefficient with the molecular parameters that describe the structures of the analyzed compounds.

## Experimental part

*HPLC analysis*

The studied substances are eight carboxilic acids and two polyphenolic compounds separated by HPLC from many indigenuous or medicinal plants (*Viburnum* [4], *Lonicera*, *Sambucus, Artemisia, Scilla* [5] *or Geranium* [6] species).

HPLC analyzes were performed on-Thermo Surveyor HPLC equipped with a DAD detector, and HP Agilent 1200 Series with quaternary pump, DAD G1315D gradient.

In all HPLC analysis of these compounds, silica gel-18 columns with 15 cm length, 4.6 mm internal diameter, particle size 5μm have been used. Regardless of the used mobile phase, the elution order of the components remain the same, retention times changing slightly. The flow rate of the mobile phase in all assays was 1 mL/min.

Chromatographic characteristics obtained for these polyphenols are shown in table 1.

For these chemicals the following molecular properties were chosen for analysis: The partition coefficient octanol / water, water-solubility and the energy of stabilization of the molecule, in correspondence with the retention time; the values of these parameters are presented in table 2.

The first two properties presented were taken from literature [8] and the stabilization energy of the molecules was calculated using the program HyperChem [9]. To calculate these parameters, modeling and optimization of chemical structures were performed with the software HyperChem 8 using the method Molecular Mechanics (MM+) and the procedure semiempirical PM 3 (Parametrization Model 3 / SCF).

For the QSPR study we browse the following steps:

- molecular modeling of chemical structures for the 10 studied substances (table 1);
- quantum molecular calculations of the molecular geometries;

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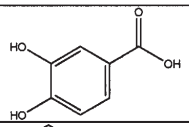
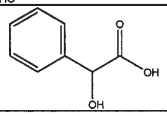
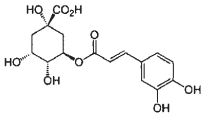
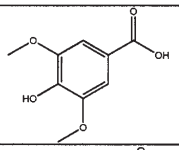
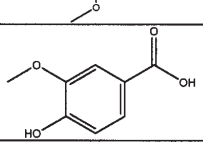
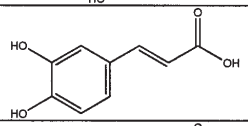
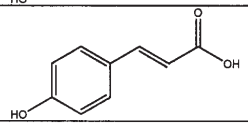
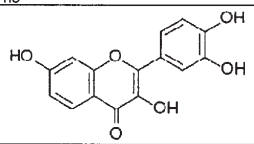
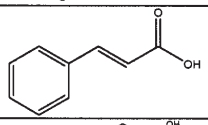
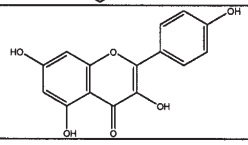
| No. | Polyphenol | Structure | $t_R$, min. |
|---|---|---|---|
| 1 | 3,4-dihydroxybenzoic acid |  | 5.24 |
| 2 | mandelic acid |  | 5.81 |
| 3 | chlorogenic acid |  | 6.2 |
| 4 | syringic acid |  | 7.16 |
| 5 | vanillic acid |  | 7.4 |
| 6 | caffeic acid |  | 7.95 |
| 7 | coumaric acid |  | 10.693 |
| 8 | fisetin |  | 11.38 |
| 9 | Trans-cinnamic acid |  | 14.44 |
| 10 | kaempferol |  | 21.5 |

**Table 1**
THE ELUTION ORDER ON THE COLUMN AND THE RETENTION TIMES OF THE STUDIED COMPOUNDS [7]

- estimation of molecular descriptors and structure – activity correlation.

The first step of the chemical structure modeling was performed using the HyperChem program 8 using the method Molecular Mechanics (MM+) (Semiempirical Metod PM3) [10]. The obtained geometries were used as input data in the software package MOPAC 7.0. Output data include levels of molecular electronics, electronic population, net tasks atoms bond orders and free valences, dipole moments, moment of inertia, polarizability and different energy partition by type of interactions and chemical bonds.

This set of structural data representing each molecule was correlated with the partition coefficient log P. Biological properties were used for topological descriptors, the shape descriptors and electrostatic fingerprint [11]. For this purpose was used MOPAC output data, the resulting descriptors are selected and filtered by the process of crossvalidation (R2 = 0.8987) and then correlated by multilinear regression with partition molecular properties (log P) and the retention time on the column.

## Results and discussions

The stabilisation energy of the molecule describes a molecular reactivity, by default a tendency of a substance to manifest character acid through the disposal of protons from the donor groups (the phenolic groups, the carboxylic groups or the alcoholic groups). Therefore, the substance with the most donor groups (substance 3) has the lowest stability, that is forming the most hydrogen bonds with the mobile phase (it has the character hydrophilic) and, for this reason, it has a low retention time. The partition coefficient (P) is the ratio between the concentration of the organic solvent (C1) and the concentration of the same substance in water (C2), expressing measure differentiated solubility of a substance between two immiscible liquids.

$$P = C_1 / C_2.$$

Among the systems investigated for pharmaceutical substances, the system octanol - water has proved the corresponding appropriate modeling biological characteristics. In practice, is used the logarithmic form of the partition coefficient, log P. It should be noted that a substance is considered hydrophobic if P is greater than one (log P> 0), if not, the substance is considered to be hydrophilic. The partition coefficient is a descriptor of hydrophobicity (lipophilicity) substances. Therefore, the partition coefficient increases with decreasing water

| *No.* | *log P* | *S, mg/l* | *$E_{st}$, kcal/mol* | *No. proton donor groups in the molecule* |
|---|---|---|---|---|
| 1 | 1.01 | $5.098 \cdot 10^4$ | -1902.34 | 3 |
| 2 | 0.55 | $1.763 \cdot 10^5$ | -2071.84 | 2 |
| 3 | -0.356 | $4.047 \cdot 10^5$ | *-4563.46* | *6* |
| 4 | 1.283 | $5.783 \cdot 10^3$ | -2537.44 | 2 |
| 5 | 1.304 | $3.770 \cdot 10^3$ | -2170.21 | 2 |
| 6 | 1.424 | $5.407 \cdot 10^4$ | -2335.74 | 3 |
| 7 | 1.876 | $1.830 \cdot 10^4$ | -2232.49 | 2 |
| 8 | 2.412 | $5.489 \cdot 10^3$ | -3619.02 | 4 |
| 9 | 1.97 | $2.911 \cdot 10^3$ | -2126.51 | 1 |
| 10 | 2.685 | $1.191 \cdot 10^3$ | -3623.69 | 4 |

log P – partition coefficient; S – water-solubility; $E_{st}$ – the energy of stabilization

**Table 2**
CHARACTERISTIC MOLECULAR PROPERTIES OF THE STUDIED COMPOUNDS

solubility of the chemical substances, the solubility is directly related to the availability of the polar groups present in the molecules.

In liquid chromatography, the stationary phase is considered to be hydrophobic and the mobile phase (containing usually mixture of water with polar organic solvent, such as acetonitrile, methanol or ethanol) is considered to be polar. The nature of the two phases supposes, therefore, that the analytes separated by the chromatographic technique are hydrophobic or at least to have a lower polarity than that of mobile phase.

In liquid chromatography, the main retention mechanism is represented by the hydrophobic interaction between the analyte molecule and the stationary phase (based on van der Waals forces). Thus, the hydrophobicity of the analyte is greater interaction with the stationary phase will be stronger and chromatography retention will be higher. This is easily understood because the stationary phase is less polar (more hydrophobic) than the mobile phase.

As can be seen in table 2, the variation increasing of the partition coefficient follows the order:

logP (3) < logP (2) < logP (1) < logP (4) < logP (5) < logP (6) < logP (7) < logP (9) < logP (8) < logP (10).

This indicates that the most lipophilic compound consisting in the series of substances studied is kaempferol and the least lipophilic compound (the hydrophilic compound) is chlorogenic acid, which explains the high retention of kaempferol compared to the retention times of the other compounds. In the case of chlorogenic acid, the only hydrophilic substance in the set of compounds studied, the retention time has the smallest value, the corresponding 3,4-dihydroxybenzoic acid. The explanation may be given by the higher molecular weight of the chlorogenic acid (354.31) compared to that of the 3,4-dihydroxybenzoic acid (154.12), which makes it difficult the diffusion in the column.

Also, the solubility of the compound (numbered 3) is the largest, which is explained by the high number of OH groups of the proton donor (1 carboxyl group 3 and 2 alcoholic hydroxyl groups, phenolic hydroxyl groups).

The ascending order of solubility in water: S (10)< S (9) < S (5) < S (8) < S (4) < S (7) < S (6) < S (2 ) < S (1 ) < S (3) placed kaempferol on the lower position, which explains the high retention time corresponding to an increased solubility in organic solvents.

With respect to stabilization energy, the numbered compounds 8 and 10 have the comparable energies (approx. -3600 kcal / mol), because they have the same molecular weight (286.24). The same compounds have a high lipophilic character, which determines a delayed elution compared to others polyphenols. The retention times are dependent on the interaction between the hydrophobic stationary phase (C18), the molecules of the analyte and the solvents used.

The most stable of the molecule under study, having a high stabilization energy (-1902.34 kcal / mol) is 3,4-dihydroxybenzoic acid, which has the lowest retention time in the column chromatography, that is the first component eluted. Hydrophobicity has a fundamental role in liquid chromatography. The differentiated retention of components of the mixture along the the stationary phase is based on their hydrophobicity.

This is reason we found necessary to conduct a study QSPR for the class of compounds analyzed, with the aim of discovering the structural parameters (the molecular descriptors) that contribute the most to the hydrophobicity, expressed as log P. Such a study QSPR (Quantitative Structure - Property Relationship) can be used to predict the optimal partition coefficient for a new compound, untested experimentally.

As can be seen in tables 1 and 2, along with the increase of the values of the partition coefficient, which facilitate the elution through the chromatographic column, it increases their retention time. It is expected that molecular descriptors which contribute to the hydrophobicity, to describe also the retention of the substances by the chromatographic column.

Through QSPR type methods, the structure of a chemical substance is represented by different descriptors (variables) which may be physico-chemical parameters (partition constants, polarizability, dissociation constant, heat of formation etc.) or structural, topological, electrostatic, electronics etc. descriptors calculated by different cuantomoleculare methods semiempirical or ab initio. These descriptors can be correlated with the biological activity that follows to be optimized through the chemical modulation, namely by inserting a different chemical groups with the aim of modifying a physico-chemical or biological properties [12-14].

| *No. descriptors (i)* | *R2* | *F* | *Descriptors* |
|---|---|---|---|
| 2 | 0.9526 | 70.28 | D1 - Min valency of a C atom<br>D2 - Min electroph. react. index for a O atom |
| 2 | 0.9159 | 38.11 | D3 - Avg valency of a O atom<br>D4 - Final heat of formation |
| 1 | 0.8279 | 38.48 | D1 - Min valency of a C atom |
| 1 | 0.6453 | 14.55 | D5 - Avg electroph. react. index for a O atom |
| 1 | 0.5870 | 11.37 | D3 - Avg valency of a O atom |
| 1 | 0.3371 | 4.068 | D6 - Max valency of a O atom |

**Table 3**
HANSCH CORRELATION, log P= $a_0 + \Sigma a_i D_i$, WHERE $D_i$ = DESCRIPTORS INVOLVED IN THE REGRESSION

| i | $a_i$ | Δa | T test |
|---|---|---|---|
| 0 | -172.64 | 16.997 | -10.1574 |
| 1 | 45.263 | 4.4301 | 10.2172 |
| 2 | 708.78 | 165.25 | 4.2891 |

**Table 4**
REGRESSION PARAMETERS R2=0.9526 F=70.28 s2=0.0781

**Tabel 5**
PARTITION COEFFICIENT VALUES

| *Structure* | *Calculated log P* | *Experimental log P* | *Difference* |
|---|---|---|---|
| 1 | 1.0947 | 1.0100 | 0.0847 |
| 2 | 0.4121 | 0.5500 | -0.1379 |
| 3 | 0.0507 | -0.3560 | 0.4067 |
| 4 | 1.1698 | 1.2830 | -0.1132 |
| 5 | 1.0757 | 1.3040 | -0.2283 |
| 6 | 1.1792 | 1.4240 | -0.2448 |
| 7 | 1.9079 | 1.8760 | 0.0319 |
| 8 | 2.5105 | 2.4120 | 0.0985 |
| 9 | 1.9525 | 1.9700 | -0.0175 |
| 10 | 2.8049 | 2.6850 | 0.1199 |

The results obtained are shown in the table 3.

As can be seen in table 3, in the optimal regression with two descriptors to the hydrophobicity of the studied compounds contributes mainly descriptors Extreme (minimum, maximum) or average value of free valency for C and O atoms, Extreme (minimum, maximum) or average electrophilic reactivity index - Fukui (minimum, maximum and average) for O atoms but also Heat of formation of the molecule [14].

With these descriptors we can obtain a Hansch equation for the best two descriptors correlation with good predictive power for regression ($R^2$ = 0.9526 / F= 70.28 / s2 = 0.0488 / crossvalidated R2=0.8987):

$$\log P = a_0 + a_1 D_1 + a_2 D_2,$$
$$\log P = -172.64 + 45.263 \cdot D_1 + 708.78 \quad D_2 \qquad (1)$$

in which D1 is Minimum value of free valency for C atoms and D2 - Minimum electrophilic reactivity index for O atoms.

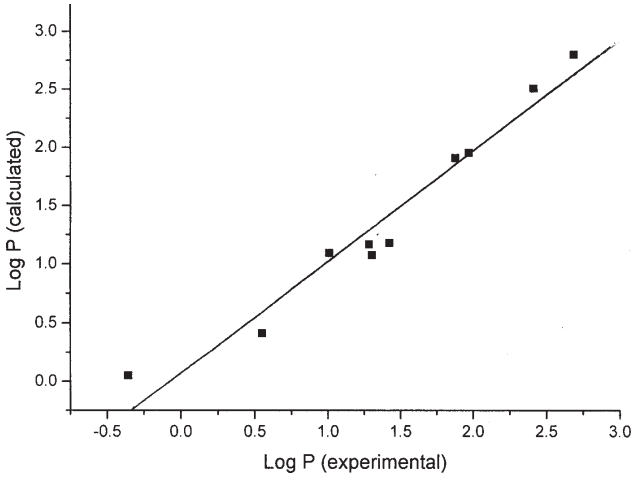



Fig. 1. The degree of confidence for Hansch equation /log P (1)

The predicted values by the Hansch equation (1) previously established for the partition coefficient at the 10 structures are preented in table 5.

The degree of confidence between the observed values and the predicted values with this equation is shown in figure 1.

In the case of the retention time, the regression correlation indicates the participation of the same type of descriptors (molecular shape descriptors and electrical charges), as in the case of the partition coefficient (Table 6).

For the best correlation (R2 = 0.8957, crossvalidated R2=0.7224), Hansch equation is:

$$t_R = -1.6942 - 47.714 \; . \; D_7 \; + 4811.8 \; . \; D_2,$$

in which D7 is Minimum values of the net charges on the atoms of a C atoms (in Mulliken approach) and D2 - Minimum electrophilic reactivity index for O atoms [11].

## Conclusions

One of the two molecular descriptors (D2) which best contributes to the linear regression of the hydrophobicity

| *No. descriptors (i)* | *R2* | *F* | *Descriptors* |
|---|---|---|---|
| 2 | 0.8957 | 30.07 | D7 - Min net atomic charge for a C atom<br>D2 - Min electroph. react. index for a O atom |
| 2 | 0.8740 | 24.29 | D6- Max valency of a O atom<br>D2 - Min electroph. react. index for a O atom |
| 1 | 0.7241 | 20.99 | D7 - Min net atomic charge for a C atom |
| 1 | 0.4810 | 7.4142 | D1 - Min valency of a C atom |

**Table 6**
HANSCH CORRELATION, $t_R$ (RETENTION TIME) = $a_0 + \Sigma a_i D_i$

of the eluents, log P, is also involved in the optimal regression with two descriptors of the retention time of the same substances by the chromatographic column.

Therefore:

Min electroph. react. index for a O atom ($D_2$) <*versus*> Min electroph. react. index for a O atom ($D_2$)

Min valency of a C atom ($D_1$) <*versus*> Min net atomic charge for a C atom (D7).

The difference in the QSPR description of the partition coefficient compared to the QSPR description of the retention time in the column for the ten chemical structures occurs in the load distribution descriptor on the C atoms.

In conclusion, the arrangement of atoms and the load distribution on the C and O atoms seem to play an essential role in the hydrophobicity of substances studied.

It is known that the shape of the molecule is essential in the ligand (drug) - biological receptor interaction, conforming to the model "key-lock" /13/ and it is also important in the elution of on chromatographic column.

Carbon and oxigen atoms and the load distribution on them describe not only the mechanism of partition and transport from the aqueous phase (mobile) in the lipid phase (stationary), but may be responsible for the chemical interaction between the substances and lipid phase represented by chromatographic column. This is very interesting and deserves to be systematically studied in other chemicals used as solvents or those substances that need to be separated by liquid chromatography.

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