ORIGINAL PAPER

CORRELATION OF POLYPHENOLIC COMPOUNDS STRUCTURAL PROPERTIES WITH THEIR ANTIOXIDANT CAPACITY

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Abstract. *In the group of polyphenolcarboxylic acids and flavonoids, over time, some molecules have been noted for their special properties. Among them we set out to analyze: caffeic acid, chlorogenic acid, apigenol, quercetol. In this paper we set out to test the antioxidant capacity of plant extracts using docking technique. The plant extracts will interact with the enzyme iNOS, a nitric oxide generator, a free radical with multiple implications in autoimmune diseases. From the values obtained from the study the most stable polyphenolic compound (high ΔE) is apigenol. The highest dipole moment value belongs to apigenol, which could anticipate stronger interaction with iNOS. The most stable iNOS-polyphnolic compound is for apigenol, having the free binding energy -6.36 kcal/mol, followed by the iNOS-quercetol complex (-4.44 kcal/mol).*

Keywords: *iNOS, molecular docking, polyphenolic compounds.*

1. INTRODUCTION

Herbal extracts are commonly used in modern therapy, as single therapy or in combination with a medication. Polyphenolcarboxylic acids and flavonoids are a group of secondary metabolites of plants, being frequently encountered in fruits, vegetables, medicinal plants, etc., having numerous therapeutic effects [1-10].

Nitrogen oxide synthases (NOS) catalyze the production of nitric oxide (NO) from L-arginine. The inducible form of nitrogen oxide synthase (iNOS) is observed in various human malignancies such as breast, lung, prostate, bladder, colorectal cancer and malignant melanoma. Also, an increased level of iNOS expression and activity has been found in gynecological, mammary, and malignant tumor cells of head and neck cancer. Due to its importance in the production of malignancies, the iNOS enzyme has become a new target in finding new inhibitors that can be used as anti-cancer agents.

Caffeic acid is an organic compound, being a hydroxylated derivate in the aromatic nucleus of the cinnamic acid. It is found in most plant species, being an important intermediary in lignin biosynthesis [11]. Caffeic acid has a variety of potential pharmacological effects in *in vitro* and in animal models studies, the inhibitory effect of

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caffeic acid on cancer cell proliferation has recently been established by an oxidative mechanism in the HT-1080 human fibrosarcoma cell line. Caffeic acid is an antioxidant *in vitro* and also *in vivo*, having also antibacterial effect [12, 13]. Caffeic acid also has immunomodulatory and anti-inflammatory activity. Recent studies show that oxidative stress that triggers or increases the production of aflatoxin (*Aspergillus flavus*) can be stopped by caffeic acid [14].

Chlorogenic acid is the ester of caffeic acid and (-) quinic acid, which functions as an intermediate in lignin biosynthesis. Taken as a dietary supplement or ingested as a coffee, chlorogenic acid slightly lowers blood pressure. It has also been tested for possible anti-inflammatory effects. Chlorogenic acid has been studied as a possible chemical sensitizer involved in respiratory allergy to certain plant allergenic components [15].

Apigenin (4',5,7-trihydroxyphlavone), found in many plants, is a natural product of the flavone class, which is the aglycone of several naturally occurring glycosides. There are studies that claim that apigenin stimulates adult neurogenesis *in vivo* and *in vitro* by promoting neuronal differentiation. Through its effects on cell signaling, inflammation, cell cycle and protease production, apigenin has demonstrated efficacy against a wide range of cancers, while showing no toxicity to normal cells [16].

Quercetol is a flavonol from the flavonoid group derived from polyphenols, found in many fruits, vegetables, leaves and cereals 17].

Quercetol supplements are promoted for the treatment of different forms of cancer, having antioxidant effect [18].

There are experimental reports certifying the ability to inhibit quercetol against the iNOS enzyme. Polyphenols and flavonoids also have notable antiviral effects (comparable to acyclovir and oseltamivir) or antifungal medicines [19].

The present work focuses on the interaction analysis of active compounds present in commonly consumed plant products against the iNOS enzyme.

2. MATERIALS AND METHODS

The Monte Carlo method is the method used by the chemical modeling programs, in which the configuration space of a compound is explored through individual, successive, movements of the position of an atom, and the energy of the ensemble is recalculated. The mutations leading to energy minimization are retained, but a random term is introduced for accepting the mutations that increase the energy of the system, in order to avoid "dropping" the system to a local minimum energy [20]. Currently, molecular modeling programs are used for the correct spatial representation of chemical structures. The programs calculate on the basis of quantum formulas the angles between the chemical bonds, the interatomic distances and the total energy of the molecule. All structures have been optimized with the help of Gaussian 09 software (Gauss View interface 16) using the DFT/B3LYP/6-31G method [21]. Calculation of molecular descriptors was performed using Gaussian 09 software.

AutoDock involves an automatic system for predicting the interaction of ligands with their macromolecular targets. An ideal process should be able to find the global minimum in the interaction energy between the substrate and the target protein, exploring all the degrees of freedom available to the system [22]. For the molecular docking method we added all the hydrogen atoms and selected Gasteiger charge. In the grid phase we set the grid box of 40X40X40, at a distance of 0.375 angstroms from the center of the enzyme. In the docking stage we chose Lamarckian Genetic Algorithm (LGA) with 30 runs [23].

The chemical structure of the polyphenolic enzyme-complex complexes are perfomed by PyMol software (Schrodinger) and Biovia Discovey Studio (Cambridge) [24, 25].

The X-ray crystal structure of $GABA_A$ receptor (with 4COF code and X-ray diffraction at 2.97 Å resolution) was taken from the Protein Data Bank and refined with Modrefiner program [26].

The free binding energy estimated is calculated as $\Delta G \sim bind \sim = \Delta H - T\Delta S$, where ΔH represents the enthalpy and $T\Delta S$ the entropic contribution (only the negative ΔG value is energetically favorable and the process is spontaneous).

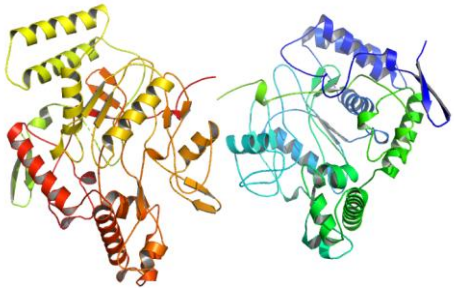
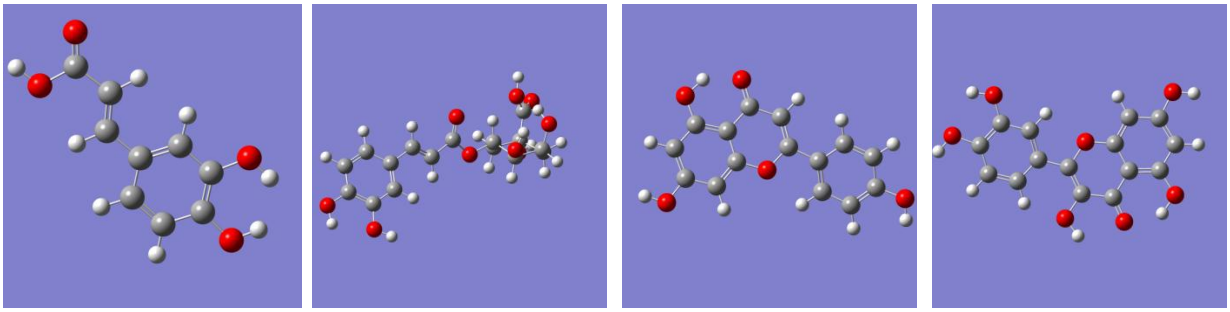


Figure 1. iNOS enzyme.

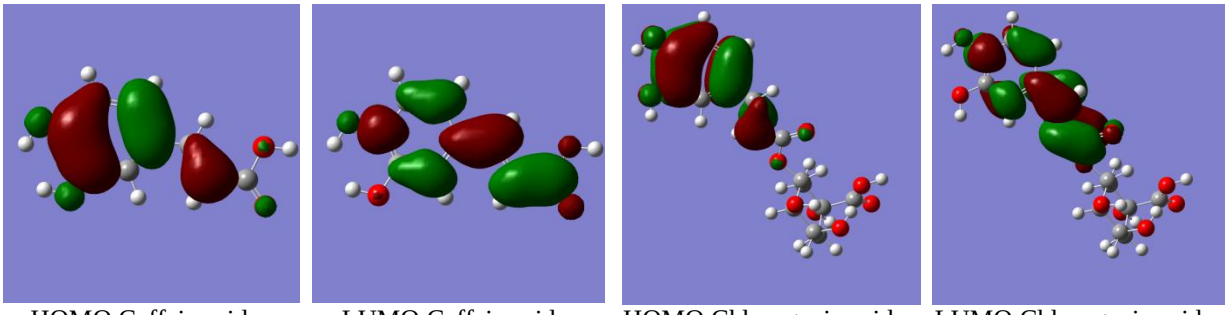
3. RESULTS AND DISCUSSION

3.1. RESULTS

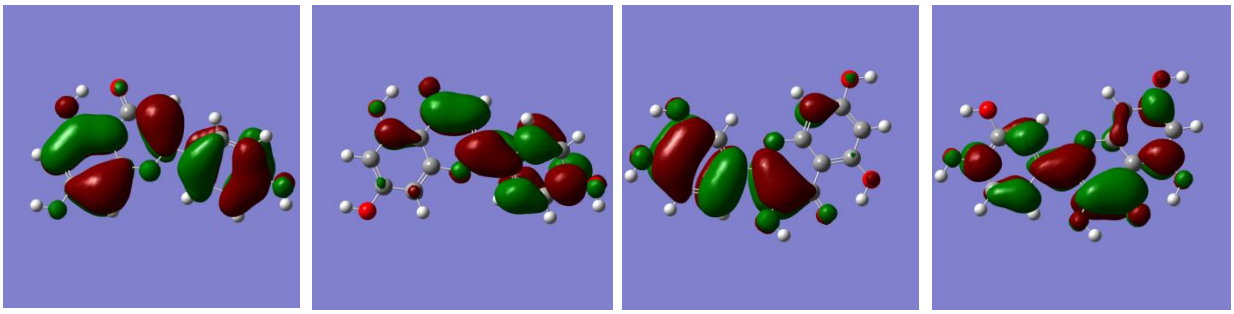


Caffeic acid Chlorogenic acid Apigenol Quercetol

Figure 2. Structures of polyphenolic compounds DFT optimized



HOMO Caffeic acid LUMO Caffeic acid HOMO Chlorogenic acid LUMO Chlorogenic acid



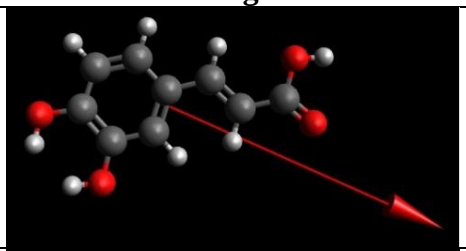
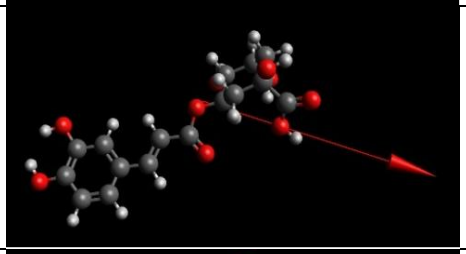
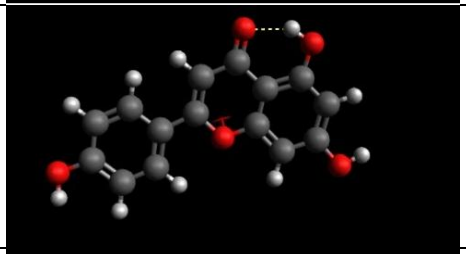
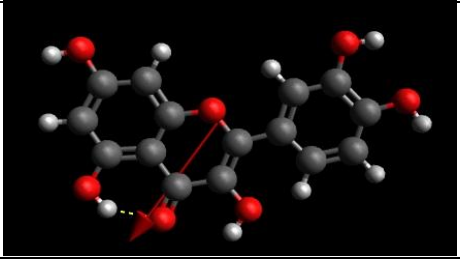
HOMO Apigenol LUMO Apigenol HOMO Quercetol LUMO Quercetol

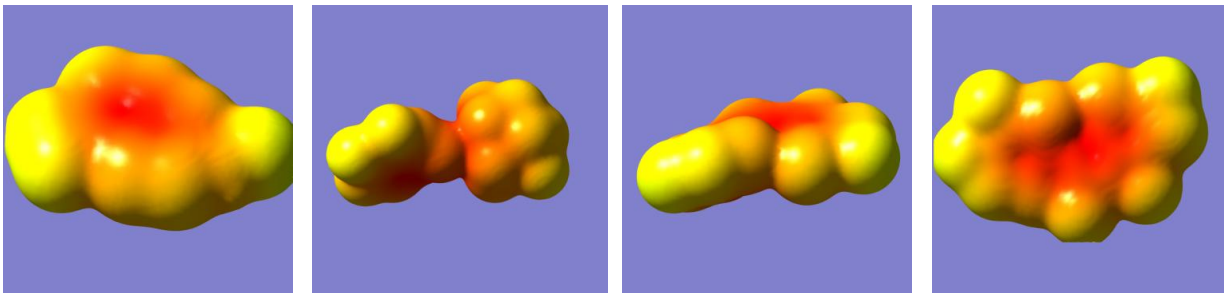
Figure 3. HOMO/LUMO polyphenolic compounds orbitals.

Table 1. Polyphenolic compounds stability.

Polyphenolic compound	**ΔE**
Caffeic acid	8.097
Chlorogenic acid	8.079
Apigenol	8.214
Quercetol	7.590

Table 2. Polyphenolic compounds dipole moment.

Substance	**Image**	**Dipole moment**
Caffeic acid		3.742
Chlorogenic acid		3.318
Apigenol		4.705
Quercetol		1.713

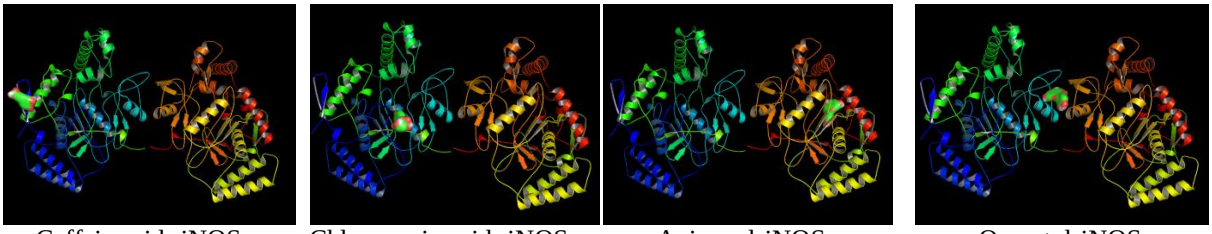


Caffeic acid Chlorogenic acid Apigenol Quercetol

Figure 4. The surface of the molecular electrostatic potential for polyphenolic compounds.

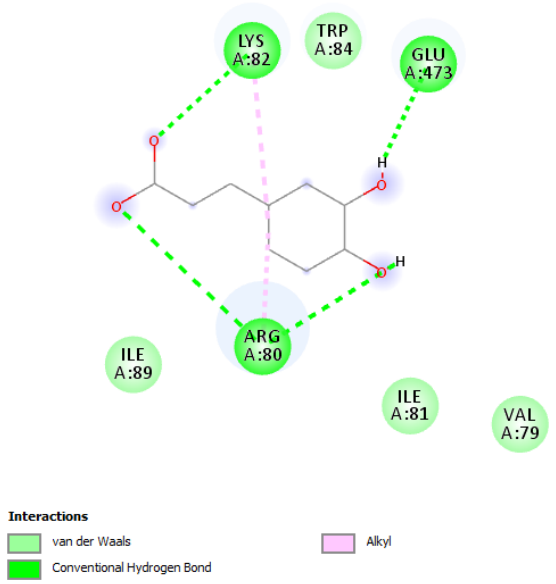
Table 3. The values of the binding energies of polyphenolic compound-iNOS.

Polyphenolic compounds	Ligand/iNOS
Caffeic acid	-3.82
Chlorogenic acid	-3.53
Apigenol	-6.36
Quercetol	-4.44

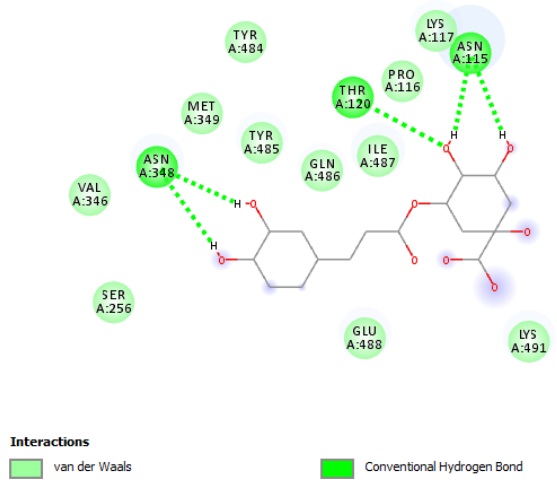


Caffeic acid -iNOS Chlorogenic acid -iNOS Apigenol-iNOS Queretol-iNOS

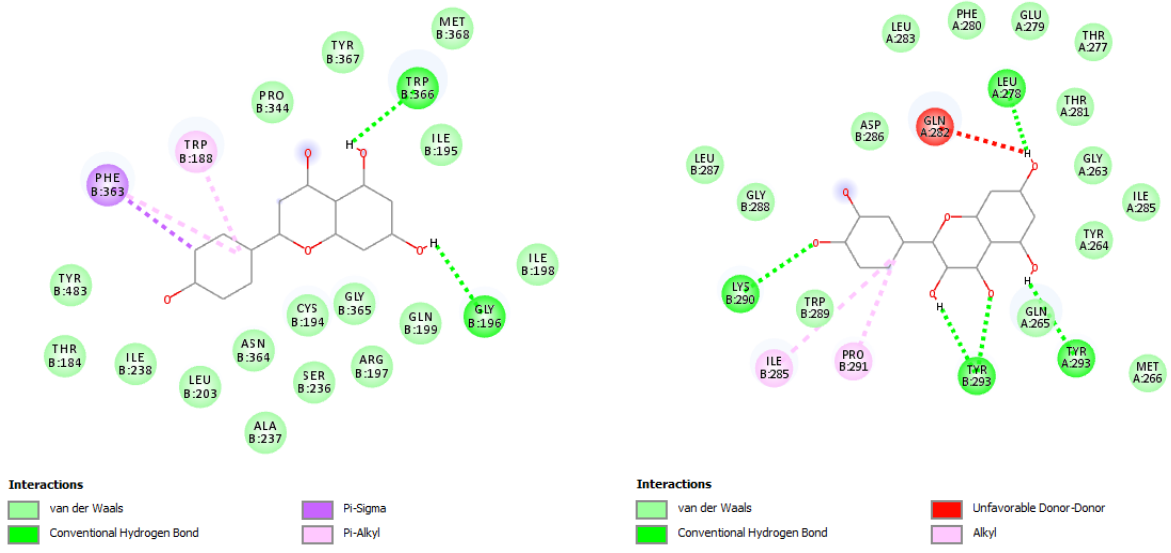
Figure 5. 3D Ligand/iNOS intreaction (ligands are represented with surface and target with cartoon).



Caffeic acid -iNOS



Chlorogenic acid -iNOS



Apigenol-iNOS Queretol-iNOS

Figure 6. 2D map interaction ligand/iNOS.

3.2. DISCUSSION

In the present study different structural descriptors have been calculated which may contribute to the biological activity manifested by the studied polyphenolic compounds.

Among them are boundary orbitals, HOMO (the highest energy level in the electron-occupied molecule) and LUMO (the lowest electron-occupied molecular level), which express how a molecule will interact with a biological receptor through transfer processes of electrons involving the OMO layer (Occupied Molecular Orbitals) or through electron acceptance processes involving the UMO (Unoccupieed Molecular Orbitals) layer.

The energy difference between HOMO and LUMO levels ($\Delta E = E_{LUMO} - E_{HOMO}$) is also a chemically important molecular descriptor, which explains the stability of the molecule, a low value indicating that the molecule is highly reactive [27]. From the values presented in the table 1, the most reactive compound is quercetol.

Another molecular parameter resulting from quantum chemical calculations is the electric dipole moment (μ), which reflects the partial separation of the electrical charge in the molecule. This molecular descriptor is also a predictor of the chemical reactivity of molecules. This parameter is a measure of the polarization of the molecular system. Table 2 shows the dipole moment values for the studied molecules [28]. As noted, the highest dipole moment value belongs to apigenol, which could anticipate stronger interaction with iNOS.

The interaction of drug substance - biological receptor, interaction that gives rise to an appropriate biological response [29], is possible only for a certain form of the molecule and a certain distribution of electron density in the molecule that generates regions rich in electrons.

Based on the molecular property called electrostatic potential, the three-dimensional map of the electron density was obtained, the different values of the electrostatic potential are marked by different colors (red for polar region) (Fig. 4). It can be used for quantomolecular appreciation of lipophilicity, because it characterizes the polarity of a particular region on the van der Waals surface of the molecule. High potential regions will strongly attract water molecules, and low value regions will not attract them, as they may be considered hydrophobic.

In the field of molecular modeling, molecular docking is an important method that predicts the preferred binding orientation of one molecule over another molecule, to form a stable complex. Knowing the preferred orientation can be used to predict the binding force or binding affinity between two molecules.

The most stable iNOS-polyphnolic compound is for apigenol, having the free binding energy -6.36 kcal / mol, followed by the iNOS-quercetol complex (-4.44 kcal / mol).

Caffeic acid forms three hydrogen bonds, respectively with ARG 80, LYS 82, GLU 473 and van der Waals interaction with VAL 79, ILE 81, TRP 84, ILE 89. An alkyl-type interaction is also present between caffeic acid and ARG 80, LYS 82.

Chlorogenic acid forms hydrogen bonds with ASN 115, THR 120, ASN 348. Van der Waals interactions are established with PRO 116, LYS 117, SER 256, VAL 346, MET 349, TYR 484, TYR 485, ILE 487, GLU 488, LYS 491.

Apigenol forms hydrogen bonds with GLY 196 and TRP 366. Pi-sigma interactions are established with PHE 363, Pi-alkyl interactions with TRP 188 and PHE 363. Apigenol forms van der Waals interactions with THR 184, CYS 194, ARG 197, ILE 198, GLN 199, LEU 203, ILE 238, PRO 344, GLY 365, TYR 367, MET 368, TYR 483.

Quercetol forms hydrogen bonds with LEU 278, LYS 290, TYR 293. Alkyl-alkyl interactions with ILE 285, PRO 291. Van der Waals interactions occur with GLY 263, TYR 264, GLN 265, MET 266, THR 277, GLU 279, PHE 280, THR 281, LEU 283, LEU 287, GLY 288, TRP 289.

4. CONCLUSIONS

Molecular docking research focuses on the computational simulation of the molecular recognition process. Its objective is to achieve optimized conformation for both protein and ligand and the relative orientation between the protein and ligand so that the free energy of the overall system is minimized.

Herbal medicine has proven effective in treating many conditions, since ancient times people have used whole plants or their plant parts rich in active principles, fresh or processed for their healing properties.

Scientists use theoretical chemical-physical data in studies to explain experimental results. At the basis of many discoveries in the pharmaceutical field, research involving quantum chemical calculations is the starting point.

The energy difference ΔE is a chemically important molecular descriptor, which explains the stability of the molecule, a low value indicating that the molecule is highly reactive. Of the values obtained from the study the most stable polyphenolic compound is apigenol.

The highest dipole moment value belongs to apigenol, which could anticipate stronger interaction with iNOS.

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