

## Theoretical study of the antioxidant mechanism of chrysin and its metabolites – a computational approach

Anamika Basu\*

Department of Biochemistry, Gurudas College, Kolkata, India

**Abstract.** Flavonoid chrysin, obtained from different plants, has different pharmacological property, due to its antioxidative effects. But the poor bioavailability of chrysin limits its therapeutics application. In systemic circulation, chrysin can be transported by binding with serum albumin protein. Furthermore, in hepatic circulation, two chrysin metabolites e.g. chrysin-7-O- glucuronide (C7G) and chrysin-7-O-sulphate (C7S) are formed by biotransformation process in liver. By using computational methods like density functional theory (DFT) study, the antioxidant potential of these four compounds has been investigated. In this study, both the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) have been identified by using Gaussian software. The energies of these orbitals, as well as the energy gap between HOMO and LUMO, have been calculated. Using these calculations, it can be concluded that chrysin in its bound form with human serum albumin, is the best antioxidant among these four compounds. Since, it has the lowest energy gap between the calculated HOMO and LUMO. In this bound form, the cinnamoyl group present in chrysin molecule participates in the charge transfer event. The antioxidant activity by donating electrons to scavenge the free radicles, has been explained for chrysin and its derivatives. Global descriptive parameters, which describe the reactivity and stability of molecules, are calculated to highlight the electron or proton donation/acceptance of these four compounds. Antioxidant efficiency correlates strongly with electron-donating ability and stability of the resulting radical. The albumin bound chrysin is considered as best antioxidant among these four compounds for the three types of antioxidation reaction mechanisms. This finding will help us to overcome the problem regarding poor bioavailability of chrysin during its performance as antioxidant.

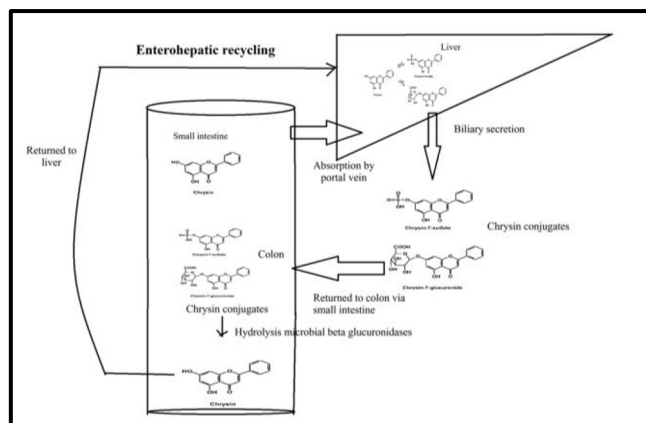
\*e-mail: [anamika.biochem@gurudas.education](mailto:anamika.biochem@gurudas.education)

## 1. Introduction

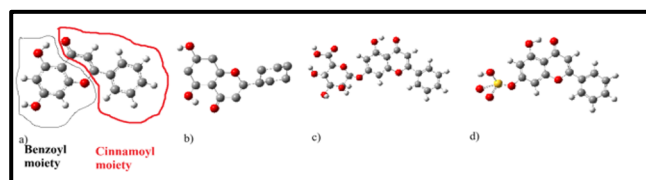
Different medicinal plants, fruits e.g. mango, doum palms, bitter melon etc and honey, propolis contain chrysin [1], a flavonoid with intriguing pharmacological qualities that include antioxidant, anticancer [2], anti-angiogenic, anti-inflammatory, and neuroprotective actions. Its capacity to trigger apoptosis, scavenge free radicals, reduce oxidative stress, and alter inflammatory pathways makes it potentially medicinal [3]. Antioxidant activity of 5,7 dihydroxy flavone (chrysin) present in mango pulp has been demonstrated by FRAP assay [4].

Chrysin's weak water solubility and substantial presystemic clearance in enterocytes and hepatocytes result in a limited oral bioavailability [5]. Its biotransformation results in the creation of conjugated metabolites. In humans, chrysin-7-O-glucuronide (C7G) and chrysin-7-O-sulfate (C7S) are the two main products, which are produced in liver by hepatic enzymes uridine-5'-diphosphoglucuronyl transferase and sulfotransferase respectively [6]. Poor bioavailability [7], however, limits the practical use of chrysin, and further human clinical research is required to validate its advantages in the treatment of illnesses including cancer, neurological disorders, and liver diseases considering its bio transformed products in human body system. From nutrients after absorption through gastrointestinal tract, chrysin binds with human serum albumin (most abundant plasma protein) during transportation through circulatory system. However, based on current knowledge, the antioxidant activity of chrysin in free form (C), in albumin bound form (CA), as well as two chrysin biometabolites (C7G, C7S), have not been reported till now, though all these four compounds are predominant in human circulation (Figure 1) [8]. Structures

of chrysin, its bound form and two metabolites are shown in Figure 2.



**Fig. 1.** Chrysin and its biologically important derivatives



**Fig. 2.** Structure of a) chrysin b) chrysin bound with albumin c) chrysin-7-O-glucuronide (C7G) d) chrysin-7-O-sulfate (C7S)

## 2. Methodology

Analysis of chrysin and its derivatives for their antioxidant activity has been executed as shown in Figure 2.



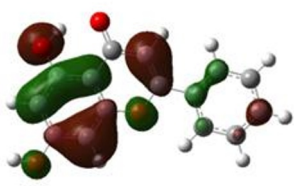
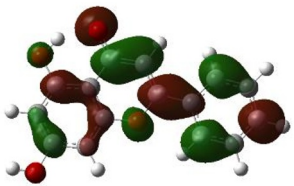
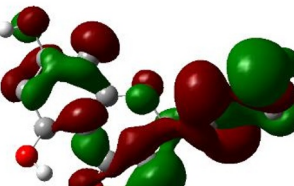
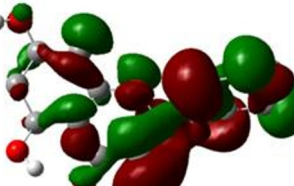
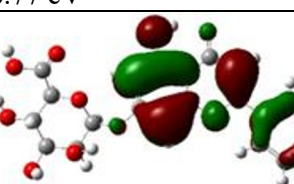
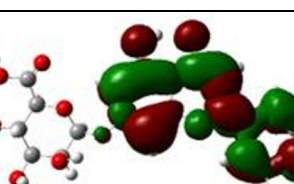
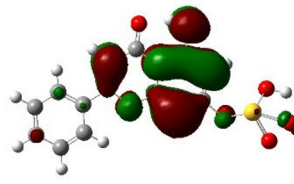
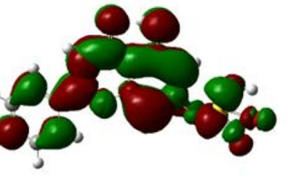
**Fig. 1.** Workflow for analysis of antioxidant activity

Computational methods like density functional theory

(DFT) can be used as a powerful tool to investigate the antioxidant potential of any chemical compound. A theoretical study is conducted to explore the antioxidant activity and as well as its mechanism, for chrysin in its free and bound form, along with its two derivatives, chrysin-7-O-glucuronide (C7G) and chrysin-7-O-sulfate (C7S). Moreover, that DFT can be used to calculate different molecular level properties such as energy gap between HOMO-LUMO orbitals, which can highlight the chemical properties like stability and reactivity of these antioxidant compounds. DFT (Density Functional Theory) also allows to compute global reactivity descriptors, the quantities that describe the overall tendency of any molecule to gain/loss electrons, accept/donate charge, or resist deformation of its electron cloud.

The presence of hydroxyl groups and conjugated bonds in chrysin chemical structure recommends its antioxidant potential through various mechanisms like hydrogen atom transfer (HAT), sequential proton loss electron transfer (SPLET) and single electron transfer – proton transfer (SETPT).

Table 1. HOMO and LUMO orbitals of all four compounds

Compound	HOMO	LUMO	$\Delta E$ (in eV)
Chrysin	 -6.47 eV	 -2.41 eV	-0.406
Chrysin_albumin	 -6.77 eV	 -6.11 eV	-0.66
Chrysin-7-O-glucuronide	 -8.77 eV	 1.47 eV	-10.24
Chrysin-7-O-sulphate	 -6.19 eV	 -2.28 eV	-3.91

The investigation about the antioxidant character of these molecules follows a comprehensive in-silico approach as shown in Figure 3.

The structure files for chrysin (CID 5281607), chrysin-7-glucuronide (CID 1435335) and chrysin -7-sulphate (CID 69503899) containing the X, Y, Z coordinates, obtained from PubChem, freely accessible database [9]. From our previous work, the best docking posture for the human serum albumin protein and chrysin binding, is used. The three dimensional conformation of the chrysin molecule in the docking structure, is considered as the 3D structure of bound form of chrysin with albumin in the current study.

The initial step for analysis involves the optimization and frequency calculation for all four compounds using DFT technique. These calculations are executed using Gaussian 09 programming package [10]. Here, Becke's [B] exchange functional formula combining with correlation function of Lee, Yang and Parr [LYP] is used. The basis set of 6-311G of People is selected to optimize the chrysin compound and its derivatives. After optimization, considering Parr and Pearson theory of DFT and Koopmans theorem, the energies of both frontier molecular orbitals such as HOMOs and LUMOs have been calculated. Furthermore, some global descriptive parameters, also known as

Quantum chemical descriptors, calculated according to the Koopmans' theorem. These descriptors, which are used to identify the reactivity of a molecule, named as electronic affinity (A), ionization potential (I), softness ( $\sigma$ ), hardness ( $\eta$ ), chemical potential ( $\mu$ ), electronegativity ( $\chi$ ) and electrophilicity ( $\omega$ ). These parameters are calculated by using the following formula:

$$\Delta E = E_{LUMO} - E_{HOMO} \quad (1)$$

$$A = -E_{LUMO} \quad (2)$$

$$I = -E_{HOMO} \quad (3)$$

$$\eta = (I - A) / 2 \quad (4)$$

$$\chi = (I + A) / 2 \quad (5)$$

$$\sigma = 1/\eta = 1/(I - A) \quad (6)$$

$$\omega = \chi^2 / 2\eta \quad (7)$$

$$\mu = -\chi \quad (8)$$

### 3. Result

#### 3.1 Calculation of Frontier Molecular Orbitals (FMOs)

Here, Frontier molecular orbitals (FMOs) are applied to

identify the most reactive segments in molecule chrysin along with its derivatives to describe their reaction mechanism as antioxidant. These Frontier molecular orbitals (FMO) characteristics, for example, HOMO and LUMO orbitals, are highly valuable parameters to highlight the chemical behavior of the molecules. The HOMO is considered as an electron rich orbital which has a property to transfer the electrons to unoccupied molecular orbitals. Energy of HOMO,  $E_{HOMO}$  represents the electron donating capacity of a molecule in a thermodynamically favourable reaction. On the contrary, LUMO is marked as an orbital which has deficiencies of electrons and it can easily accept electrons from any other occupied molecular orbitals. As a result,  $E_{LUMO}$  associates with the ability of the molecule to accept electrons. Lower value of  $E_{HOMO}$  means reduced electron-donating capacity, and the position of the HOMO reveals the probable site for free radicle interaction. Considering the fact, the molecule of interest chrysin is composed of benzoyl and cinnamoyl group, both molecular orbitals are identified. The Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) molecular orbitals along with their corresponding energies of each four compounds are depicted in Table 1.

In HOMO/LUMO orbitals, red and green coloration signify the (+) ve and (-) ve phases of the molecular orbital in form of wave functions respectively. The energy gap between HOMO & LUMO will help to predict the reactivity, stability of the compound as well as the colour of the compound. The bound form of chrysin more likely absorb light in the visible region of light, thus, it appears as colored compound. This finding has been confirmed with the electronic UV-Visible absorption spectra of chrysin when bound with human serum albumin (Figure 4).

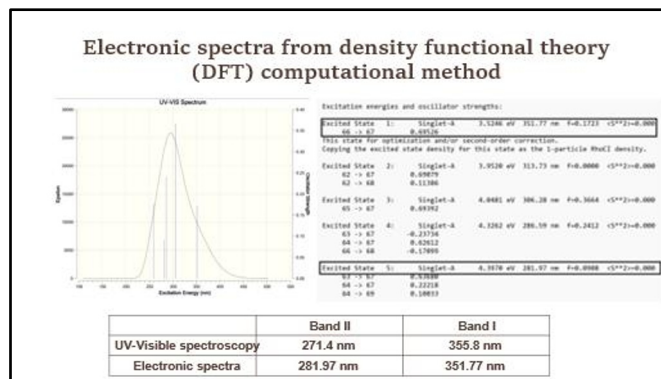


Fig. 4. Electronic spectra of chrysin

The HOMO is found primarily on the cinnamoyl moiety of all molecules except chrysin, where it is located on benzoyl ring structure. Similarly, the LUMO orbitals are located in all four compounds on the cinnamoyl group. Hence, the HOMO and LUMO analysis exposed the probable sites for charge transfer through the molecules. This study with chrysin molecule and its derivatives, indicates that among all these four molecules, this unbound form of chrysin is most reactive, least stable. Conversely, the largest energy gap in between HOMO and LUMO orbital energy levels confirms the highest stability and least reactivity of compound chrysin-7-O-glucuronide.

### 3.2 Calculation of global descriptive parameters

Table 2. The calculated molecular orbital energies and the global descriptive parameters for all four compounds

Compounds	HOMO (eV)	LUMO (eV)	IP (eV)	EA (eV)	$\chi$ (eV)	$\eta$ (eV)	$\mu$ (eV)	$\sigma$ (eV)	$\omega$ (eV)
Chrysin (C)	-6.47	-2.41	6.47	2.41	4.44	2.03	-4.44	0.49	4.85
Chrysin bound with albumin (CA)	-6.77	-6.11	6.77	6.11	4.59	0.33	-4.59	3.03	31.92
Chrysin-7-O-glucuronide (C7G)	-8.77	1.47	8.77	-1.47	3.65	5.12	-3.65	0.19	1.30
Chrysin-7-O-sulfate (C7S)	-6.19	-2.28	6.19	2.28	4.23	1.95	-4.23	0.51	4.58

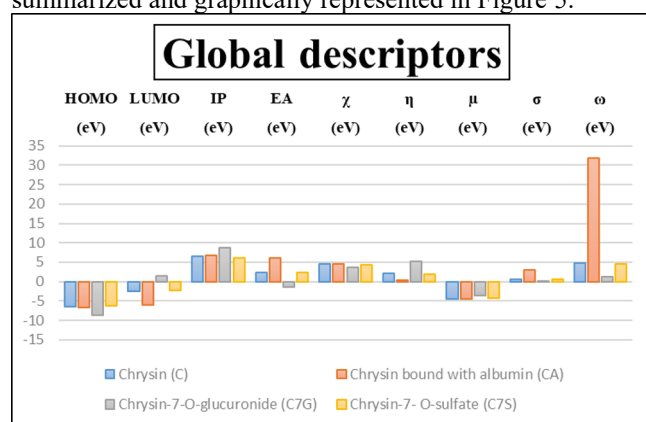
Global descriptive parameters describe the *reactivity* and *stability* of molecules in a global (system-wide) sense, which comes from Conceptual Density Functional Theory (CDFT). Ionization potential (IP) value of any compound well-defined as the required energy value to donate one electron from the ground state of the compound. Similarly, the energy value for electron affinity (EA) is similar with the energy value for accepting an electron in ground state of the molecule. The highest IP value, 8.77 eV for chrysin-

7-glucuronide (C7G), indicates that it has highest chemical stability among the four compounds. On the contrary, the highest electron affinity value, 6.11 eV for albumin bound chrysin (CA), reveals that it has highest electron acceptance capacity than other three compounds.

Furthermore, according to Table 2, comparing the values for hardness ( $\eta$ ) and softness ( $\sigma$ ), C7G molecule shows its highest value of resistance for changing its electron

distribution during chemical reaction, whereas albumin bound chrysin has lowest resistance during changing its electronic distribution in participated reaction. Another descriptor, chemical potential ( $\mu$ ) of a compound, is related with the probability of participating any chemical reaction. The least negative value of  $\mu$  i.e. -3.65 eV for C7G, indicates that it can easily donate electrons. Similarly, more negative (-4.59 eV) value of  $\mu$  for CA reveals that it can easily accept electrons, compared to the other molecules. The parameters electrophilicity ( $\omega$ ) is considered as a predictor which relates with the electrophilic nature of the compound. The highest value i.e. 31.92 eV of electrophilicity of albumin bound chrysin (CA), characterizes the good electrophilic nature or the highest propensity to accept an electron among all four strong electrophilic compounds.

Comparative values of different global descriptors are summarized and graphically represented in Figure 5.



**Fig. 5.** The values of global descriptors along with energies of HOMO and LUMO

## 4. Discussion

HOMO and LUMO orbitals identification study reveals that the probable site for free radicle interaction is localized on cinnamoyl residue present in CA, C7G and C7S. But for chrysin molecule, the benzoyl group contains its HOMO orbital.

Antioxidant activity of these four compounds, involves the electron or proton donation/acceptance, which can be characterized by the values of the electronic reactivity descriptors of them. Antioxidant efficiency generally correlates strongly with electron-donating ability and stability of the resulting radical.

The smallest energy gap between HOMO and LUMO orbitals for CA, confirms the highest antioxidant activity of the molecule due to its highest value of chemical softness and as well as chemical potential. The compound is predicated as the best free radicle scavenger among all compounds due to its highest electronegativity value.

## 5. Conclusion

Three different mechanisms such as hydrogen atom transfer (HAT), single electron transfer – proton transfer (SETPT) and sequential proton loss electron transfer (SPLET), of

antioxidant activity of chrysin and its derivatives, can be mechanistically interpreted with the values of DFT global descriptors. Firstly, HAT mechanism for antioxidant activity of albumin bound chrysin molecule can be related with its high value for HOMO along with the lowest value for the hardness descriptor. Hence, it can be predicted that this compound can easily cleave the -OH bond homolytically in hydrogen atom transfer reaction. Similarly, lower value of IP and least value of hardness, depicts the same molecule as the best free radical scavenger with single electron transfer – proton transfer mechanism. Lastly, this specific molecule, with high softness, low chemical hardness and negative chemical potential, can donate electron effectively following the sequential proton loss electron transfer (SPLET) mechanism for antioxidant activity. It can be concluded that the albumin bound form of chrysin, is considered as best antioxidant molecule in human circulatory system, among these four compounds considering these three above mentioned mechanisms for antioxidation reaction. Since, human serum albumin is the most abundant protein in human plasma, hence this finding will help us to overcome the problem regarding poor bioavailability of chrysin during its performance as antioxidant.

## ACKNOWLEDGEMENTS

Author acknowledges the financial support from Gurudas College for conducting this research.

## REFERENCES

1. M. Stompor-Gorący, A. Bajek-Bil, and M. Machaczka, "Chrysin: Perspectives on Contemporary Status and Future Possibilities as Pro-Health Agent," *Nutrients*, vol. 13, no. 6, p. 2038, Jun. 2021, doi: <https://doi.org/10.3390/nu13062038>.
2. P. Chandra, R. Pathak, and N. Sachan, "Chrysin: Chemistry, Occurrence, Pharmacokinetics, Toxicity, Molecular Targets, and Medicinal Properties of a Naturally Occurring Flavone," *Current Bioactive Compounds*, vol. 21, Dec. 2024, doi: <https://doi.org/10.2174/0115734072341901241121185020>.
3. R. Raina, R. Bhatt, and A. Hussain, "Chrysin targets aberrant molecular signatures and pathways in carcinogenesis (Review)," *World Academy of Sciences Journal*, vol. 6, no. 5, Jun. 2024, doi: <https://doi.org/10.3892/wasj.2024.260>.
4. R. Sinha, S. Poddar, S. Das, C. Mitra, and A. Basu. "Assessment of Total Flavonoid Content and Antioxidant Activity of Mango Pulp Extracts." In *Harmonizing Chemical and Biological Sciences for Sustainable Development*, H. S. Biswas, S. Poddar, D. K. Maiti and A. Bhaumik, Eds, Lincoln University College, Malaysia, 2025, pp.142-149, <https://books.lucp.net/wp-content/uploads/13.-Assessment-of-Total-Flavonoid-Content-and.pdf>
5. T. Walle, Y. Otake, J. A. Brubaker, U. K. Walle, and P. V. Halushka, "Disposition and metabolism of the flavonoid chrysin in normal volunteers," *British Journal of Clinical Pharmacology*, vol. 51, no. 2, pp. 143–146, Feb. 2001, doi: <https://doi.org/10.1111/j.1365-2125.2001.01317.x>.
6. A. Galijatovic, Y. Otake, U. K. Walle, and T. Walle, "Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells," *Xenobiotica*, vol. 29, no. 12, pp.

1241–1256, Jan. 1999, doi:  
<https://doi.org/10.1080/004982599237912>.

7. S. Gao, N. Siddiqui, I. Etim, T. Du, Y. Zhang, and D. Liang, “Developing nutritional component chrysin as a therapeutic agent: Bioavailability and pharmacokinetics consideration, and ADME mechanisms,” *Biomedicine & Pharmacotherapy*, vol. 142, p. 112080, Oct. 2021, doi:  
<https://doi.org/10.1016/j.biopha.2021.112080>.

8. V. Mohos *et al.*, “Interaction of Chrysin and Its Main Conjugated Metabolites Chrysin-7-Sulfate and Chrysin-7-Glucuronide with Serum Albumin,” *International Journal of Molecular Sciences*, vol. 19, no. 12, p. 4073, Dec. 2018, doi:  
<https://doi.org/10.3390/ijms19124073>.

9. PubChem, “PubChem,” *Nih.gov*, 2004.  
<https://pubchem.ncbi.nlm.nih.gov/>

10. A. F. Jalbout, F. Nazari, and L. Turker, “Gaussian-based computations in molecular science,” *Journal of Molecular Structure: THEOCHEM*, vol. 671, no. 1, pp. 1–21, Feb. 2004, doi: [https://doi.org/10.1016/S0166-1280\(03\)00347-6](https://doi.org/10.1016/S0166-1280(03)00347-6).