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Unraveling the Antioxidant Potential of Tectochrysin: A Quantum Chemical Approach to Radical Scavenging Mechanisms

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ABSTRACT
In this study, we conducted a comprehensive quantum chemical investigation to elucidate the antioxidant mechanisms of tectochrysin via hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) pathways. All calculations were performed at the BMK/6-311+G(d,p) level in both gas and aqueous phases, complemented by intrinsic reaction coordinate (IRC) and transition state analyses. The HAT mechanism with hydroxyl radicals was determined to be both thermodynamically ($\Delta G = -18.13$ kcal/mol) and kinetically ($\Delta G^{\#} = 14.93$ kcal/mol) favorable, with a reaction rate nearing diffusion control ($k = 6.17 \times 10^{12}$ dm³/mol·s). SPLET became dominant in polar media, supported by a significantly stabilized anionic form. Conversely, SET-PT and NO-radical pathways were found to be mechanistically irrelevant. Molecular docking simulations revealed a favorable binding affinity of tectochrysin to oxidative stress-related proteins, mediated by hydrogen bonding and hydrophobic interactions. This integrated study provides valuable mechanistic insights into tectochrysin's antioxidant potential and highlights its pharmacological promise as a multi-pathway ROS scavenger.

Keywords: Tectochrysin, Antioxidant mechanism, DFT, Hydrogen atom transfer, Molecular docking

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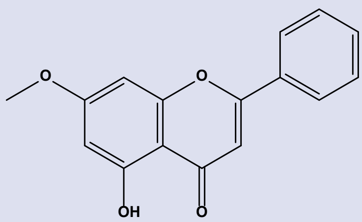
Introduction

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and the antioxidant defense system of the organism, represents a central pathogenic factor in the onset and progression of numerous chronic and degenerative diseases, including cardiovascular disorders, neurodegenerative syndromes, diabetes mellitus, and a variety of cancers [1-5]. These highly reactive oxygen-centered radicals can initiate uncontrolled chain reactions that damage critical biomolecules such as nucleic acids, proteins, and membrane lipids, thereby disrupting cellular integrity and functionality [6-7]. Consequently, mitigating oxidative stress through the use of endogenous or exogenous antioxidants is a pivotal strategy in preventive and therapeutic medicine. In this regard, flavonoids, ubiquitous polyphenolic compounds biosynthesized in plants, have garnered substantial interest due to their well-documented antioxidant potential, structural diversity, and broad pharmacological profiles [8, 9]. Within the flavonoid class, tectochrysin (5-hydroxy-7-methoxyflavone) (Scheme 1), a naturally occurring flavone found in the rhizomes of Alpinia oxyphylla and various types of propolis, has emerged as a bioactive molecule of considerable significance [10-13]. Accumulating evidence indicates that tectochrysin exhibits a wide range of biological activities, including anti-inflammatory [14], antimicrobial [15], hepatoprotective, anticancer [16, 17], and antioxidant effects [18]. However, despite these promising pharmacological attributes, the precise mechanisms through which tectochrysin exerts its antioxidant action remain poorly elucidated, particularly at the molecular level [19].

Understanding the antioxidant behavior of flavonoids such as tectochrysin requires a mechanistic exploration of their thermodynamic and kinetic profiles in the context of radical-scavenging reactions. The antioxidant efficacy of such molecules is determined mainly by their ability to donate hydrogen atoms or electrons, stabilize resulting radicals, and interact with various reactive species under physiological conditions. Therefore, unraveling the dominant antioxidant pathways of tectochrysin is essential not only for assessing its biological relevance but also for enabling structure-based optimization for therapeutic applications. To conclude, the present study employs a comprehensive computational strategy based on density functional theory (DFT) to systematically investigate the antioxidant capacity of tectochrysin through multiple mechanistic pathways. Specifically, we calculated a series of key physicochemical descriptors bond dissociation enthalpy (BDE), ionization energy (IE), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer energy (ETE) to evaluate the thermodynamic viability of three principal antioxidant mechanisms: hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET). The study also investigates the interaction of hydroxyl (OH) and nitric oxide (NO) radicals, which are reactive oxygen species (ROS) in biological systems, with tectochrysin via hydrogen abstraction [20-24].

Computational investigations were conducted in both gas-phase and aqueous environments to simulate conditions relevant to biological systems. The inclusion of solvent effects via the conductor-like polarizable continuum model (C-PCM) [25] enabled an accurate representation of solvation influences on reaction energetics, intermediate stability, and transition state formation. To validate the predicted reaction routes, intrinsic reaction coordinate (IRC) analyses were also performed to confirm the connectivity between reactants, transition states, and products, thereby ensuring the reliability of the proposed mechanistic pathways. Complementing the quantum chemical calculations, molecular docking simulations were employed to explore potential binding interactions between tectochrysin and key redox-related enzymes and proteins involved in oxidative stress response. These simulations aim to uncover whether tectochrysin not only neutralizes radicals through chemical means but also exerts biological modulation by interacting with catalytic or regulatory biomolecular targets.

By integrating thermodynamic modeling, elucidation of the radical scavenging pathway, solvation analysis, and biological docking insights, this study provides a comprehensive understanding of tectochrysin's antioxidant capabilities. Beyond theoretical validation, the results hold significant implications for the rational design of flavonoid-based antioxidants and for expanding the pharmacological applications of tectochrysin as a promising natural agent against oxidative damage-related pathologies.



Scheme 1. Chemical structure of Tectochrysin (5-hydroxy-7-methoxyflavanone)

Computational Methods

Accurately assessing antioxidant activity at the molecular level requires the use of reliable and high-precision quantum chemical methods. One of the most effective approaches is Density Functional Theory (DFT), which is powerful for elucidating electronic structures, predicting thermodynamic properties, and modeling radical scavenging mechanisms with high accuracy. In this study, all quantum mechanical calculations were conducted using the GAUSSIAN 09 [26] software suite, a widely accepted platform for performing ab initio and DFT-based molecular simulations. To ensure a robust description of the antioxidant behavior of tectochrysin, the BMK functional a τ-dependent hybrid functional developed by Boese and Martin was selected. This function has been demonstrated to provide improved performance in calculating thermochemical data for radical-involved reactions. It is particularly suitable for systems where hydrogen abstraction and electron transfer are mechanistically significant. The 6-311+G(d,p) basis set, chosen in combination with BMK, is well-established for its accuracy in describing valence and diffuse electron densities, thereby instilling confidence in our computational approach [27-29].

Before engaging in production-level calculations, a benchmarking procedure was carried out to validate the selected method. Specifically, theoretical bond dissociation enthalpy (BDE) values were computed for structurally analogous flavonoids and compared against available experimental data. The strong correlation between calculated and empirical values confirmed the predictive reliability of the DFT protocol adopted in this study. Geometry optimizations of tectochrysin and its corresponding radical species were conducted without symmetry constraints. All optimized structures were verified through frequency calculations to confirm the absence of imaginary frequencies, thereby ensuring their characterization as true local minima.

To simulate realistic physiological conditions, solvent effects were incorporated using the conductor-like polarizable continuum model (C-PCM) at the same level of theory. All thermodynamic parameters, BDE, ionization energy (IE), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) were calculated in both gas and aqueous phases to assess how environmental polarity influences antioxidant activity. Furthermore, intrinsic reaction coordinate (IRC) calculations [30] were conducted for the hydrogen atom transfer (HAT) mechanism to verify the continuity of the reaction pathway and the identity of the transition states.

To deepen mechanistic understanding, three principal radical scavenging pathways were examined:

$$\mathrm{ArOH} + \mathrm{R}^{\cdot} \rightarrow \mathrm{ArO}^{\cdot} + \mathrm{RH} \quad (1)$$
$$\mathrm{ArOH} + \mathrm{R}^{\cdot} \rightarrow \mathrm{ArOH}^{+\cdot} + \mathrm{HO}^{-} \rightarrow \mathrm{RH} + \mathrm{ArO}^{\cdot} \quad (2)$$
$$\mathrm{ArOH} \rightarrow \mathrm{ArO}^{-} + \mathrm{H}^{+};\ \mathrm{ArO}^{-} + \mathrm{R}^{\cdot} \rightarrow \mathrm{ArO}^{\cdot} + \mathrm{R}^{-} \quad (3)$$

This study presents a detailed computational analysis that provides valuable insights into the antioxidant behavior of tectochrysin at the molecular level. It connects the known pharmacological properties of tectochrysin with the underlying mechanisms of its radical-scavenging activity. By systematically evaluating key thermodynamic parameters across the three main pathways, HAT (Reaction 1), SET-PT (Reaction 2), and SPLET (Reaction 3), this research reveals the energetic feasibility of each antioxidant route in the gas phase. Notably, tectochrysin demonstrates favorable reactivity across all three mechanisms, highlighting its versatility as a radical scavenger. Furthermore, the validation of transition states through IRC calculations, alongside supporting molecular docking simulations, reinforces the mechanistic plausibility of the theoretical predictions. These findings provide a strong foundation for further developing tectochrysin as a multifunctional antioxidant candidate in future pharmacological applications.

For the applicability of these mechanisms, thermodynamic parameters such as bond dissociation enthalpy (BDE), ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) of the phenolic compounds (PCs) should be calculated according to the following equations. In other words, the enthalpy changes associated with each mechanistic pathway serve as the basis for quantifying these key thermodynamic descriptors, thereby enabling a detailed energetic evaluation of the antioxidant activity. Specifically, BDE (Equation 4) reflects the energy required for homolytic cleavage of the O–H bond, serving as a critical indicator of hydrogen atom donation ability in the HAT mechanism. IP (Equation 5) and PDE (Equation 6) are fundamental for assessing the SET-PT mechanism, which involves the initial removal of an electron followed by the release of a proton. In contrast, the SPLET pathway is governed by PA (Equation 7), representing the tendency of a molecule to lose a proton, and ETE (Equation 8), which captures the feasibility of subsequent electron transfer from the conjugate base. Together, these equations provide a comprehensive thermodynamic framework for evaluating the antioxidant potential of tectochrysin under radical stress [31,32].

H_{BDE}=H(ArO$^{\bullet}$) + H(RH) –H(Ar-OH) – H(R$^{\bullet}$) (4)

ΔH_{IP}=H(ArOH$^{+\bullet}$) + H(R^{-}) –H(Ar-OH) – H(R$^{\bullet}$ (5)

ΔH_{PDE}=H(ArO$^{\bullet}$) + H(RH) –H(ArOH$^{+\bullet}$) – H(R^{-}) (6)

ΔH_{PA}=H(ArO^{-}) + H(RH) –H(ArOH) – H(R^{-}) (7)

ΔH_{ETE}=H(ArO$^{\cdot}$) + H(R^{-}) –H(ArO^{-}) – H(R$^{\cdot}$) (8)

Kinetic parameters were evaluated using classical transition state theory (TST) according to the Eyring-Polanyi equation [33].

The results for both kinetic and thermodynamic parameters of the studied reactions were obtained from vibrational frequency calculations at a temperature of 298 K The rate constant at a given temperature was calculated using the equation derived from classical transition state theory, expressed as follows:

Based on these frequency-derived parameters, the kinetic behavior of each reaction was quantitatively assessed. The rate constants k(T) for all reactions between tectochrysin and the OH and NO radicals were calculated using the transition state theory equation:

$$k(T) = \frac{K_B T}{h} e^{-\Delta G^{\neq}/RT} \tag{9}$$

where K_B, h, and R represent the Boltzmann constant, Planck constant, and universal gas constant, respectively; T is the absolute temperature, and $\Delta G^{\neq}$ is the activation free energy. The computed rate constants for all radical reactions are listed in Table 3.

To further investigate the antioxidant potential of tectochrysin, a molecular docking study was conducted. The optimized geometry of tectochrysin, obtained via DFT calculations, was used in docking simulations with Human Peroxiredoxin-5 (HP5) [34], a key antioxidant enzyme. This approach enabled the evaluation of potential binding affinity and interaction modes, offering complementary insight into the compound's radical-scavenging behavior at the molecular level.

Table 1. Energy values of ion and radical structures of tectochrysin calculated at BMK\ 6-311+G(d,p) calculation level (units are given in Hartree)

Tectochrysin	$E_{neutral}$	$E_{radical}$	E_{anion}	E_{cation}
Gas phase	-917.508985	-916.837867	-916.941703	-917.214579
Water	-917.522251	-916.861726	-917.040345	-917.285765

Table 2. BMK/6-311+G (d, p) ΔH_{BDE}, ΔH_{IP}, ΔH_{PA}, $\Delta H_{PDE,}$ and ΔH_{ETE} values of the studied for the reaction of tectochrysin with NO and OH radicals at BMK/6-311+G(d,p) level in the gas phase and in water (Units are given as kcal/mol)

Reactions	HAT		SET-PT				SPLET			
	ΔH_{BDE}		ΔH_{IP}		ΔH_{PDE}		ΔH_{PA}		ΔH_{ETE}	
	Gas	Water	Gas	Water	Gas	Water	Gas	Water	Gas	Water
Tectochrysin+•OH	-14.97	-23.02	146.21	30.53	-161.18	-53.55	-43.47	-18.21	28.50	-4.81
Tectochrysin+•NO	95.03	45.99	207.23	101.75	-112.20	-55.75	5.50	-20.41	89.53	66.41

Abbreviations: BDE, bond dissociation enthalpy; ETE, electron transfer enthalpy; HAT, hydrogen atom transfer; IP, ionization potential; PA, proton affinity; PDE, proton dissociation enthalpy; SET-PT, single electron transfer–proton transfer; SPLET, sequential proton loss electron transfer.

Table 3. Enthalpy values of the reaction of tectochrysin with OH and NO radicals in the gas phase at BMK calculation level (Units are given as Hartree)

HAT mechanism	H(Reactant)	H(Transition state)	H(Product)
Tectochrysin+•OH	-992.966977	-992.940541	-992.988675
Tectochrysin +•NO	-1047.100239	-1047.025772	-1047.024595

Table 4. Calculated Gibbs free energy, reaction enthalpy, Gibbs free energy of the reactant complex, Gibbs activation energy barrier, and calculated reaction rate constant for the reaction of Tectochrysin with OH and NO radicals in the gas phase

HAT mechanism	ΔG (kcal/mol)	ΔH (kcal/mol)	G(reactant complex)/Hartree	$\Delta G^{\neq}$ (kcal/mol)	$K(dm^3/mol.s)$
Tectochrysin +•OH	-18.13	-13.62	-992.966977	14.93	$6.172.10^{12}$
Tectochrysin +•NO	49.80	47.47	-1047.175815	51.34	$6.082.10^{12}$

Results and Discussion

The comprehensive quantum chemical analysis presented in this study offers valuable insights into the radical-scavenging behavior of tectochrysin at the molecular level, effectively bridging its known pharmacological benefits with the underlying thermodynamic and mechanistic pathways of antioxidant action. By systematically evaluating the hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) mechanisms in the gas phase, this research delineates the energetic feasibility of each pathway under physiological-like conditions. The findings demonstrate that tectochrysin exhibits a thermodynamically favorable HAT pathway, corroborating earlier reports that emphasize the role of phenolic O–H bonds and resonance stabilization in antioxidant efficacy. Furthermore, the observed reactivity trends are consistent with previous DFT-based studies on hydroxycinnamate-ferrocene hybrids and flavonoid systems, where the presence of conjugated systems and electron-donating substituents significantly lowers BDE and enhances radical stabilization.

By restricting our analysis to the gas phase, we ensure high accuracy in the intrinsic reactivity profile of tectochrysin, laying a solid foundation for future solvation and protein-interaction studies. The confirmation of transition state structures via IRC calculations further reinforces the mechanistic validity of our predictions. These theoretical insights not only reaffirm tectochrysin's versatility as a multi-route antioxidant agent but also expand the structure–activity relationship framework necessary for rational drug design. Collectively, this work advances our understanding of natural flavonoid antioxidants and supports the development of tectochrysin-derived molecules as potential therapeutic candidates against oxidative stress-related diseases.

A detailed comparison of the electronic energies of tectochrysin and its radical, anionic, and cationic forms, both in the gas phase and in an aqueous medium, is presented in Table 1, which serves as the thermodynamic foundation for interpreting the antioxidant activity of this molecule. These total energy values, computed at the BMK/6-311+G(d,p) level of theory, are crucial for deriving the reaction enthalpies associated with hydrogen atom transfer (HAT), single-electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET) mechanisms [35, 36].

As shown in Table 1, the neutral tectochrysin molecule in the gas phase has an energy of –917.508985 Hartree, while the corresponding radical and anionic forms are energetically higher at –916.837867 and –916.941703 Hartree, respectively. The cationic form is the least stable species, exhibiting an energy of –917.214579 Hartree. These relative energy differences directly affect the magnitudes of bond dissociation enthalpy (BDE), ionization potential (IP), and electron transfer enthalpy (ETE), reflecting the thermodynamic cost of each mechanistic pathway in the gas phase.

In the aqueous phase, as also reflected in Table 1, solvation introduces a notable stabilization effect. The neutral molecule becomes more stabilized (–917.522251 Hartree), and a substantial decrease in energy is observed for the anion (–917.040345 Hartree) and cation (–917.285765 Hartree), indicating that polar media significantly promote charge separation and thus favor mechanisms like SPLET over SET-PT, which remains thermodynamically unfavorable due to its high ionization cost. These trends align closely with previous findings on polyphenolic antioxidants, including flavonoids such as naringenin and anthocyanins. Taken together, the data presented in Table 1 not only demonstrate the energetic hierarchy of tectochrysin's reactive species but also provide a robust computational basis for evaluating its antioxidant behavior across different environmental conditions. These findings highlight the molecule's structural versatility and its ability to adapt mechanistically in a phase-dependent manner, thereby reinforcing its potential as a multi-pathway antioxidant agent.

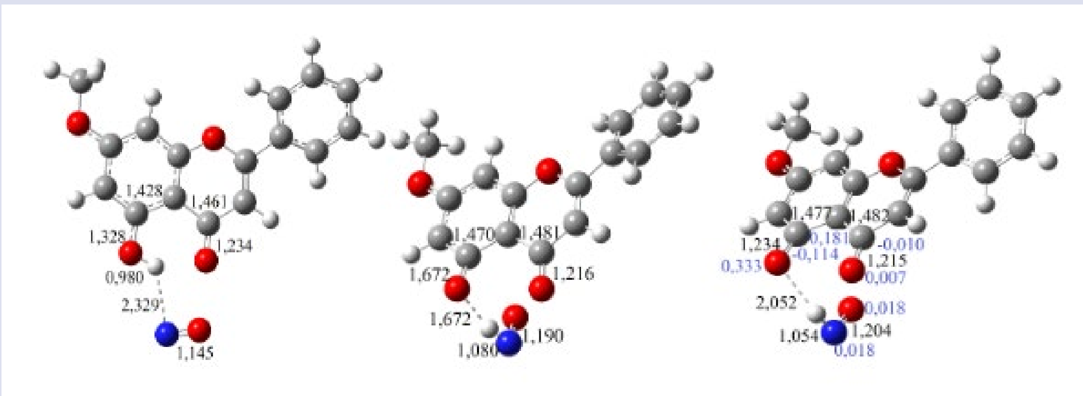
The antioxidant activity of tectochrysin is strongly influenced by the presence of functional groups and conjugated multiple bonds within its structure. The hydroxyl group at the C-5 position acts as the primary hydrogen donor site, enabling hydrogen atom transfer and contributing to radical stabilization through resonance delocalization of the unpaired electron across the aromatic ring. The methoxy group at C-7 further modulates electron density, enhancing the electron-donating ability of the hydroxyl group and lowering the bond dissociation enthalpy (BDE). Additionally, the extended conjugated π-system of the flavone backbone facilitates the efficient delocalization of radical intermediates, thereby enhancing thermodynamic stability. These structural features collectively explain the high reactivity of tectochrysin toward highly electrophilic radicals such as •OH and the environment-dependent preference for different antioxidant mechanisms.

The thermodynamic parameters presented in Table 2 provide valuable insights into the antioxidant mechanism preferred by tectochrysin. Among the parameters evaluated, the bond dissociation enthalpy (BDE) in the gas phase was relatively low. This indicates that hydrogen atom transfer (HAT) is a thermodynamically favorable pathway for neutralizing radicals in nonpolar environments. In contrast, the ionization potential (IP) and proton dissociation enthalpy (PDE) values were significantly higher, making the single-electron transfer followed by proton transfer (SET-PT) mechanism energetically unfavorable. These findings align with previous studies, suggesting the inefficiency of SET-PT in flavonoids that lack extensive conjugation or strong electron-donating groups [35-37].

In contrast, the proton affinity (PA) and electron transfer enthalpy (ETE) values, particularly in polar environments such as water, exhibited markedly lower values compared to the corresponding IP and PDE. This thermodynamic profile indicates that the sequential proton loss electron transfer (SPLET) mechanism becomes more feasible under aqueous conditions. The enhanced stability of the anionic form in water, as previously shown in Table 1, further supports the dominance of SPLET in biologically relevant environments. Taken together, these thermodynamic indicators establish a clear polarity-dependent shift in antioxidant mechanism, governed by the relative energetic favorability of each pathway. These results underscore the environment-sensitive mechanistic flexibility of tectochrysin. In contrast, the hydrogen atom transfer (HAT) pathway is thermodynamically favored in the gas phase or nonpolar media; the sequential proton loss electron transfer (SPLET) mechanism predominates under aqueous conditions, in agreement with trends observed for structurally related flavonoids such as naringenin and anthocyanins [35-37].

Following the initial thermodynamic assessments, further mechanistic insights were obtained by evaluating the enthalpy changes and activation parameters associated with the interaction of tectochrysin with OH and NO radicals. As shown in Table 3, the relative electronic energies of the reactants, transition states, and products were calculated at the BMK/6-311+G(d,p) level of theory in the gas phase. These values enabled the direct computation of reaction enthalpies and served as the foundational dataset for the kinetic analysis. To complement this analysis, Gibbs free energy changes (ΔG), reaction enthalpies (ΔH), activation barriers ($\Delta G^{\#}$), and rate constants (k) were derived through vibrational frequency calculations at 298 K, as summarized in Table 4. The HAT mechanism involving the hydroxyl radical was found to be highly exergonic (ΔG = –18.13 kcal/mol), with a modest reaction enthalpy (ΔH = –13.62 kcal/mol) and a relatively low activation barrier ($\Delta G^{\#}$ = 14.93 kcal/mol). This thermodynamic profile is indicative of a spontaneous and kinetically feasible pathway, consistent with a calculated rate constant of 6.17×10^{12} dm^3/mol·s, which approaches the diffusion-controlled limit. In contrast, the HAT reaction between tectochrysin and the nitric oxide radical (NO) proved to be both thermodynamically and kinetically disfavored, as reflected by the large positive ΔG (49.80 kcal/mol), high enthalpy change (47.47 kcal/mol), and substantial activation energy ($\Delta G^{\#}$ = 51.34 kcal/mol). Despite having a numerically high rate constant (6.08×10^{12} dm^3/mol·s), the steep energetic barrier renders this reaction unlikely under physiological or gas-phase conditions, suggesting a clear preference for •OH scavenging.

Further validation of this mechanistic pathway was achieved through Intrinsic Reaction Coordinate (IRC) analysis, which confirmed the continuity of the HAT mechanism by tracing the minimum energy path from reactants to products. As presented in Figure 4, the IRC energy profile for the tectochrysin •OH reaction displayed a smooth and symmetric curvature, characteristic of a concerted, single-step hydrogen abstraction process. This finding was further corroborated by Figures 1 and 3, which illustrate optimized geometries and bond length evolution along the reaction coordinate, particularly the O–H bond elongation at the transition state, a hallmark of hydrogen atom transfer. Collectively, the data from Tables 3 and 4, reinforced by IRC and geometric analyses, provide strong computational evidence that the interaction of tectochrysin with •OH follows a thermodynamically favorable, kinetically accessible HAT mechanism. The nitric oxide pathway, by comparison, lacks such feasibility, underscoring the radical selectivity and mechanistic specificity of tectochrysin.



Reactant complex | Transition state | Product complex

Figure 1. The optimized geometries of the reactant complex, transition state, and product complex at BMK/6-311+G** level, for the reactions of tectochrysin with •NO. Representative bond lengths (Å) and spin density are reported in the gas phase. The color codes red, gray, and white represent the oxygen, carbon, and hydrogen atoms, respectively. The color code numbers black and blue represent the bond lengths (Å) and spin density, respectively.

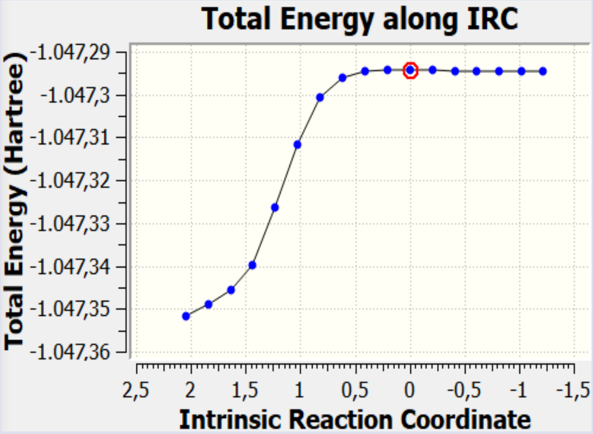
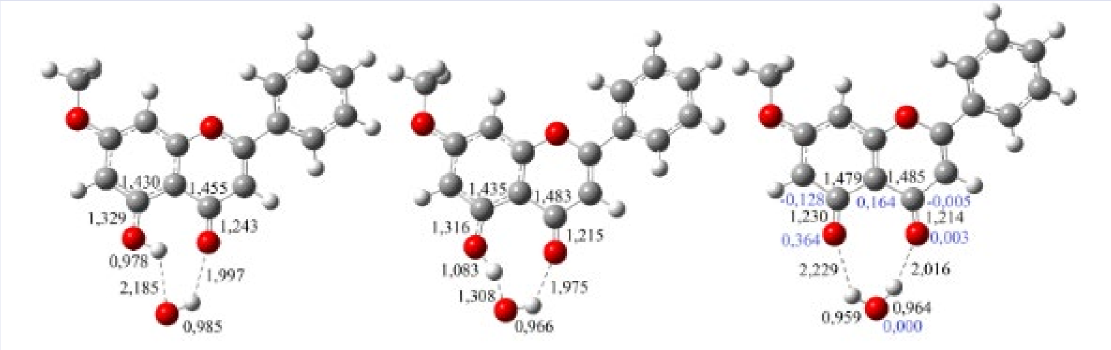


Figure 2. IRC energy profile for the HAT mechanism between tectochrysin and •NO radical in the gas phase at the BMK/6-311+G(d,p) level.

This smooth energy pathway reinforces the kinetic accessibility and mechanistic clarity of the HAT process, especially for the interaction between tectochrysin and the hydroxyl radical. In contrast, the interaction with the nitric oxide radical (•NO) is thermodynamically and kinetically unfavorable (ΔG = +49.80 kcal/mol; ΔG# = 51.34 kcal/mol), indicating that •OH scavenging is the dominant and energetically preferred pathway under gas-phase conditions. This mechanistic preference is further corroborated by the optimized geometries of the reactant complex, transition state, and product shown in Figure 1 (tectochrysin + NO) and Figure 3 (tectochrysin + OH). The geometric progression between these states for the OH-mediated pathway reveals a smoothly evolving reaction coordinate, characterized by systematic shortening of the O–H bond in the transition state, suggesting a concerted hydrogen atom abstraction. In contrast, the NO system exhibits distorted geometries and less favorable electron delocalization, consistent with its high-energy profile.



Reactant complex Transition state Product complex

Figure 3. The optimized geometries of the reactant complex, transition state, and product complex at the BMK/6-311+G** level for the reactions of tectochrysin with •OH. Representative bond lengths (Å) and spin density are reported in the gas phase. The color codes red, gray, and white represent the oxygen, carbon, and hydrogen atoms, respectively. The color code numbers black and blue represent the bond lengths (Å) and spin density, respectively.

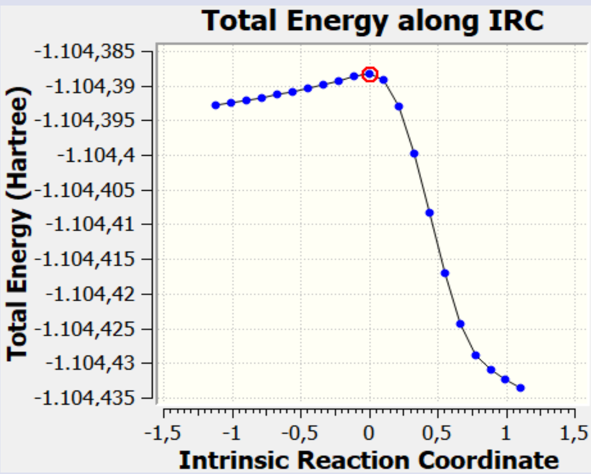


Figure 4. Intrinsic reaction coordinate (IRC) energy profile of the HAT mechanism between tectochrysin and hydroxyl radical in the gas phase, computed at the BMK/6-311+G(d,p) level.

The varying strengths of interaction between tectochrysin and different radicals can be rationalized by considering the intrinsic electronic and structural properties of the radicals. The hydroxyl radical (•OH), being highly electrophilic and possessing a localized spin density, readily abstracts a hydrogen atom from the phenolic –OH group of tectochrysin. This process is further facilitated by the resonance stabilization of the resulting phenoxyl radical, as well as the possibility of hydrogen bonding interactions that lower the activation barrier. In contrast, the nitric oxide radical (NO) exhibits a more delocalized spin density and comparatively lower electrophilicity, which reduces its ability to form strong interactions with tectochrysin. Consequently, the weaker overlap of frontier molecular orbitals and the lack of significant hydrogen bonding contributions render the reaction with •NO thermodynamically and kinetically unfavorable. These differences highlight that the radical's electrophilicity, spin density distribution, and hydrogen-bonding capacity, combined with tectochrysin's structural features such as its hydroxyl and methoxy groups, govern the radical selectivity observed in our study.

To definitively validate the mechanistic continuity of the HAT process, Intrinsic Reaction Coordinate (IRC) analysis was conducted (see IRC plot), tracing the minimum energy path from the transition state to both the reactant and product wells. The smooth, symmetric curvature observed in the IRC plot for the tectochrysin + OH reaction (see Fig. 4) confirms the existence of an actual transition state, connecting well-defined reactant and product complexes without any intermediate discontinuities or energy plateaus. The minimal curvature near the transition apex also suggests a low-energy reaction coordinate with no significant energy traps, reinforcing the feasibility and single-step concerted nature of the HAT mechanism.

Taken together, the data from Tables 2-4, supported by Figures 2, 4, and IRC analysis, provide compelling evidence that the reaction of tectochrysin with •OH proceeds via a thermodynamically spontaneous and kinetically accessible HAT pathway.

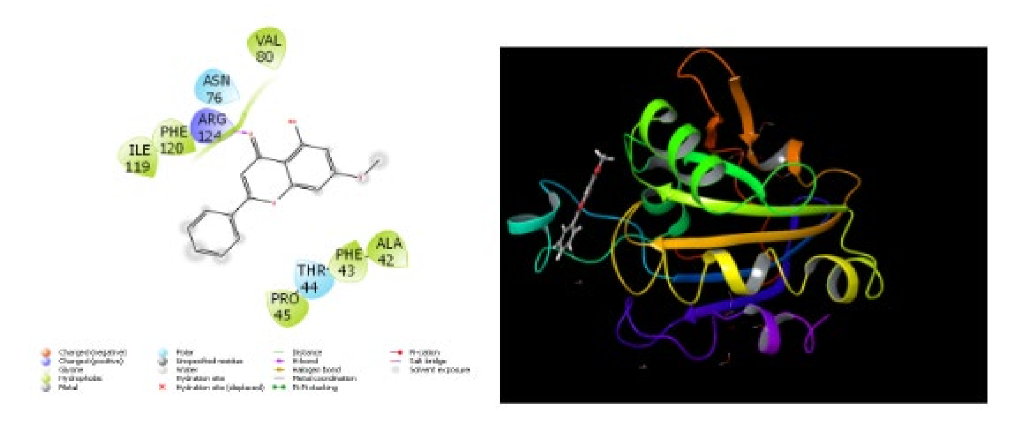


Figure 5. 2D and 3D demonstration of interactions between tectochrysin with HP 5 proteins

Table 5. Numerical values of the docking parameters were obtained from the interaction of the studied molecule."

	Docking score	glide ligand efficiency	glide hbond	glide rewards	glide evdw	glide emodel	glide energy	glide internal	glide posenum
Tectochrysin	-4,01	-0,20	-0,17	-17,70	-5,57	-27,15	-23,27	5,69	64

Molecular Docking Analysis

Molecular docking is a widely employed computational technique designed to simulate the interaction between bioactive ligands and macromolecular targets. By predicting the most energetically favorable binding conformations, docking serves as a critical tool in rational drug design and lead optimization. It not only reveals the spatial orientation and interaction profiles of ligands within target proteins but also enables the quantitative assessment of binding affinity through energy-based scoring functions. These predictions significantly reduce experimental workload by narrowing down potential candidates for synthesis and biological testing [37-39]. In this context, a molecular docking study was conducted to explore the antioxidant potential of tectochrysin through its binding interaction with the HP-5 protein, a model for an enzyme associated with oxidative stress. The primary aim was to evaluate the structural compatibility, non-covalent interaction patterns, and binding energetics of tectochrysin within the receptor's active site, complementing its previously established thermodynamic and mechanistic antioxidant properties.

As illustrated in Figure 5, tectochrysin is well accommodated within the active binding cavity of HP-5. The 2D interaction diagram reveals that tectochrysin establishes strong hydrophobic and polar interactions with key residues, including Phe43, Ala42, Thr44, Pro45, Asn76, Arg124, and Ile119. Notably, residues such as ARG124 and ASN76 are involved in potential hydrogen bonding interactions, which further stabilize the ligand within the pocket. Meanwhile, hydrophobic contacts with PHE and ILE residues significantly contribute to van der Waals stabilization, thereby enhancing binding specificity. The corresponding 3D conformation confirms an optimal orientation of tectochrysin, surrounded by the helical and β-sheet structures of the protein backbone, validating its spatial complementarity. The quantitative evaluation of docking parameters, presented in Table 5, further substantiates these findings. Tectochrysin achieved a docking score of –4.01, which, although moderate, aligns well with its favorable van der Waals energy (glide evdw = –5.57 kcal/mol) and high binding stability as indicated by a low emodel energy (–27.15 kcal/mol). Its internal energy (–23.27 kcal/mol) and glide reward (–17.70) reflect strong and energetically supported binding. The hydrogen bonding contribution (glide hbond = –0.17), though modest, is consistent with polar contacts observed in the 2D diagram. The glide pose energy (5.69) and pose enumeration (6) demonstrate that tectochrysin can adopt multiple energetically feasible binding conformations, suggesting conformational flexibility without compromising binding strength.

These results, when interpreted in conjunction with the DFT-based findings on HAT, SET-PT, and SPLET pathways, reveal a coherent antioxidant profile for tectochrysin. While the quantum chemical results emphasized HAT as the dominant mechanism in radical neutralization, the docking analysis illustrates that tectochrysin also demonstrates specific and stable interactions with oxidative stress-related protein targets, thereby potentially exerting both radical-scavenging and enzyme-modulatory antioxidant effects.

Conclusion

These findings reveal both the thermodynamic and kinetic preferences of tectochrysin's antioxidant mechanisms, providing strong evidence for its selectivity and effectiveness in neutralizing biologically relevant radicals. The superior reactivity toward hydroxyl radicals, confirmed through quantitative and structural analyses, highlights tectochrysin as a potent antioxidant with phase-specific advantages. Molecular docking results further support this dual role by demonstrating stable non-covalent interactions with redox-related enzymes, suggesting that tectochrysin may function through both direct radical scavenging and modulation of oxidative pathways.

Tectochrysin exhibits a highly favorable HAT mechanism with •OH radical in the gas phase, characterized by a negative Gibbs free energy (ΔG = –18.13 kcal/mol), low activation barrier ($\Delta G^{\#}$ = 14.93 kcal/mol), and an exceptionally high reaction rate constant ($k = 6.17 \times 10^{12}$ $dm^3/mol{\cdot}s$), indicating a spontaneous and kinetically accessible radical scavenging process.

The NO radical reaction pathway is thermodynamically and kinetically unfavorable, with a large positive ΔG (49.80 kcal/mol) and high activation energy (51.34 kcal/mol), thereby confirming its mechanistic irrelevance in the antioxidant profile of tectochrysin.

IRC analysis unequivocally confirms the concerted nature of the HAT mechanism, revealing a smooth and continuous energy path from reactants to products, devoid of any intermediates, thereby providing robust computational validation of the elementary step.

Geometrical evolution along the reaction coordinate (Figures 1 & 3) supports an efficient hydrogen transfer process, with significant bond length changes observed only in the OH reaction pathway, further emphasizing the selectivity and structural suitability of tectochrysin for •OH scavenging.

Collectively, the data position tectochrysin as a structurally and kinetically optimized flavonoid antioxidant, capable of neutralizing highly reactive oxygen species through a single-step hydrogen atom transfer (HAT) process, making it a strong candidate for targeted antioxidant drug design.

Molecular docking results validate the dual antioxidant potential of tectochrysin, confirming its ability to both neutralize free radicals and engage in stabilizing interactions with oxidative stress-related enzymes.

This study provides a comprehensive integration of quantum chemical descriptors, kinetic modeling, and IRC validation to elucidate the mechanistic basis of tectochrysin's antioxidant potential. By bridging intrinsic reactivity with protein-level interactions, it provides a novel insight into structure-reactivity relationships that are underrepresented in the current antioxidant literature.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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References

[1] Reddy V.P., Oxidative stress in health and disease, *Biomedicines*, 11 (11) (2023) 2925.

[2] Chandimali N., Bak S.G., Park E.H., Lim H.J., Won Y.S., Kim E.K., Lee S.J., Free radicals and their impact on health and antioxidant defenses: a review, *Cell Death Discovery*, 11 (1) (2025) 19.

[3] Pooja G., Shweta S., Patel P., Oxidative stress and free radicals in disease pathogenesis: a review, *Discover Medicine*, 2 (1) (2025) 104.

[4] Mandal M., Sarkar M., Khan A., Biswas M., Masi A., Rakwal R., Sarkar A., Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) in plants–maintenance of structural individuality and functional blend, *Advances in Redox Research*, 5 (2022) 100039.

[5] Krishnamurthy H.K., Pereira M., Rajavelu I., Jayaraman V., Krishna K., Wang T., Rajasekaran J.J., Oxidative stress: fundamentals and advances in quantification techniques, *Frontiers in Chemistry*, 12 (2024) 1470458.

[6] Tumilaar S.G., Hardianto A., Dohi H., Kurnia D., A comprehensive review of free radicals, oxidative stress, and antioxidants: Overview, clinical applications, global perspectives, future directions, and mechanisms of antioxidant activity of flavonoid compounds, *Journal of Chemistry*, 2024 (1) (2024) 5594386.

[7] Balakrishnan M., Kenworthy A.K., Lipid Peroxidation Drives Liquid–Liquid Phase Separation and Disrupts Raft Protein Partitioning in Biological Membranes, *Journal of the American Chemical Society*, 146 (2) (2024) 1374-1387.

[8] Jomova K., Alomar S.Y., Valko R., Liska J., Nepovimova E., Kuca K., Valko M., Flavonoids and their role in oxidative stress,

inflammation, and human diseases, *Chemico-Biological Interactions*, (2025) 111489.
[9] Hassanpour S.H., Doroudi A., Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants, *Avicenna journal of phytomedicine*, 13 (4) (2023) 354.
[10] Patel D.K., Ethnopharmacological uses, pharmacological activities, and therapeutic applications of tectochrysin in medicine: An important class of dietary flavonoid, *Cardiovascular & Haematological Disorders-Drug Targets*, 23 (1) (2023) 11-20.
[11] Ijaz M.U., Alvi K., Hamza A., Anwar H., Al-Ghanim K.A., Riaz M.N., Curative effects of tectochrysin on paraquat-instigated testicular toxicity in rats: A biochemical and histopathological based study, *Heliyon*, 10 (3) (2024).
[12] He B., Xu F., Yan T., Xiao F., Wu B., Wang Y., Jia Y., Tectochrysin from Alpinia oxyphylla miq. Alleviates Aβ1–42 induced learning and memory impairments in mice, *European Journal of Pharmacology*, 842 (2019) 365-372.
[13] Lee S., Kim K.S., Park Y., Shin K.H., Kim B.K., In vivo antioxidant activities of tectochrysin, *Archives of pharmacal research*, 26 (2003) 43-46.
[14] Fang L., Yan Y., Xu Z., He Z., Zhou S., Jiang X., Yu D., Tectochrysin ameliorates murine allergic airway inflammation by suppressing Th2 response and oxidative stress, *European Journal of Pharmacology*, 902 (2021) 174100.
[15] Hernández Tasco A.J., Ramírez Rueda R.Y., Alvarez C.J., Sartori F.T., Sacilotto A.C.B., Ito I.Y., Salvador M.J., Antibacterial and antifungal properties of crude extracts and isolated compounds from Lychnophora markgravii, *Natural product research*, 34 (6) (2020) 863-867.
[16] Park M.H., Hong J.E., Park E.S., Yoon H.S., Seo D.W., Hyun B.K., Hong J.T., Anticancer effect of tectochrysin in colon cancer cell via suppression of NF-kappaB activity and enhancement of death receptor expression, *Molecular cancer*, 14 (2015) 1-12.
[17] Park M.H., Hong J.E., Park E.S., Yoon H.S., Seo D.W., Hyun B.K., Hong J.T., Anticancer effect of tectochrysin in colon cancer cell via suppression of NF-kappaB activity and enhancement of death receptor expression, *Molecular cancer*, 14 (2015) 1-12.
[18] Paraschiv C., Gosav S., Burlacu C.M., Praisler M., Exploring the Inhibitory Efficacy of Resokaempferol and Tectochrysin on PI3Kα Protein by Combining DFT and Molecular Docking against Wild-Type and H1047R Mutant Forms, *Inventions*, 9 (5) (2024) 96.
[19] Wu I.T., Kuo C.Y., Su C.H., Lan Y.H., Hung C.C., Pinostrobin and tectochrysin conquer multidrug-resistant cancer cells via inhibiting P-glycoprotein ATPase, *Pharmaceuticals,* 16 (2) (2023) 205.
[20] Kamel E.M., Bin-Ammar A., El-Bassuony A.A., Alanazi M.M., Altharawi A., Ahmeda A.F., Mahmoud A.M., Molecular modeling and DFT studies on the antioxidant activity of Centaurea scoparia flavonoids and molecular dynamics simulation of their interaction with β-lactoglobulin, *RSC advances*, 13 (18) (2023) 12361-12374.
[21] Rimarcík 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 (2010) 25.
[22] Özbakır Işın D., Theoretical study on the investigation of antioxidant properties of some hydroxyanthraquinones, *Mol. Phys*., 114 (2016) 3578.
[23] Wright J.S., Johnson E.R., DiLabio G.A., Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants, *J. Am. Chem. Soc*., 123 (2001) 1173.
[24] Koç E., Üngördü A., Candan F., Antioxidant properties of methanolic extract of 'Veronica multifida' and DFT and HF analyses of its major flavonoid component, *J. Mol. Struct*., 1197 (2019) 436.
[25] Cances E., Mennucci B., Tomasi J., A new integral equation formalism for the polarizable continuum model: theoretical background and applications to isotropic and anisotropic dielectrics, J*. Chem. Phys*., 107 (1997) 3032-3041.
[26] Frisch M.J., Gaussian 09W Reference, Wallingford, USA, 25p (2009) 470.
[27] Boese A.D., Martin J.M.L., Development of Density Functionals for Thermochemical Kinetics*, J. Chem. Phys*., 121 (2004) 3405-3416.
[28] Dao D.Q., Is Vitamin A an Antioxidant or a Pro-oxidant?, *J. Phys. Chem. B*, 121 (40) (2017) 9348-9357.
[29] Galano A., Alvarez-Idaboy J.R., Kinetics of radical-molecule reactions in aqueous solution: A benchmark study of the performance of density functional methods, *J. Comput. Chem*., 35 (28) (2014) 2019-2026.
[30] Fukui K., The path of chemical reactions-the IRC approach, *Acc. Chem. Res*., 14 (12) (1981) 363-368.
[31] Szeląg M., Mikulski D., Molski M., Quantum-chemical investigation of the structure and the antioxidant properties of α-lipoic acid and its metabolites, *J. Mol. Model*., 18 (2012) 2907-2916.
[32] Wright J.S., Johnson E.R., DiLabio G.A., Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants, *J. Am. Chem. Soc*., 123 (2001) 1173-1183.
[33] Glasstone K., Laidler K., Eyring H., The theory of rate processes, McGraw-Hill, New York (1941).
[34] Declercq J.P., Evrard C., Clippe A., Vander Stricht D., Bernard A., Knoops B., Crystal structure of human peroxiredoxin 5, a novel type of mammalian peroxiredoxin at 1.5 Å resolution, *J. Mol. Biol*., 311 (4) (2001) 751-759.
[35] Erdoğan Ş., Özbakır Işın D., Molecular docking and DFT study on the antiradical activity of naringenin and hesperetin with nitric oxide, peroxy, and methoxy radicals, *J. Phys. Org. Chem*., 36 (4) (2023) e4479.
[36] Erdoğan, Ş., & Özbakır Işın, D., A DFT study on OH radical scavenging activities of eriodictyol, Isosakuranetin and pinocembrin, *Journal of Biomolecular Structure and Dynamics*, *40*(21) (2022) 10802-10811.
[37] de Souza Farias S.A., da Costa K.S., Martins J.B., Analysis of conformational, structural, magnetic, and electronic properties related to antioxidant activity: revisiting flavan, anthocyanidin, flavanone, flavonol, isoflavone, flavone, and flavan-3-ol, *ACS Omega*, 6 (13) (2021) 8908-8918.
[38] Karrouchi K., Fettach S., Tüzün B., Radi S., Alharthi A.I., Ghabbour H.A., Garcia Y., Synthesis, crystal structure, DFT, α-glucosidase and α-amylase inhibition and molecular docking studies of (E)-N'-(4-chlorobenzylidene)-5-phenyl-1H-pyrazole-3-carbohydrazide, *J. Mol. Struct*., 1245 (2021) 131067.
[39] Poustforoosh A., Hashemipour H., Tüzün B., Pardakhty A., Mehrabani M., Nematollahi M.H., Evaluation of potential anti-RNA-dependent RNA polymerase (RdRP) drugs against the newly emerged model of COVID-19 RdRP using computational methods, *Biophys. Chem*., 272 (2021) 106564.