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Quantum Chemical Insights into the Antioxidant Mechanisms of Luteolin and Isorhamnetin: Elucidating Structure-Reactivity Relationships, Pharmacokinetics, and Toxicity for Therapeutic Potential

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***Abstract**: This study presents a comprehensive computational evaluation of the antioxidant properties, physicochemical characteristics, pharmacokinetics, and toxicity profiles of two naturally occurring flavonoids—luteolin and isorhamnetin. Using quantum chemical descriptors and density functional theory (DFT) at the B3LYP/6-31G(d,p) level, we assessed the bond dissociation enthalpy (BDE), adiabatic ionization potential (AIP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron transfer enthalpy (ETE) of both compounds. Isorhamnetin demonstrated superior antioxidant potential, with lower BDE values (79 kcal/mol in vacuum and 71 kcal/mol in water) compared to luteolin (84 and 82 kcal/mol, respectively), suggesting enhanced hydrogen atom donation capacity via the HAT mechanism. Bond order analysis showed higher stability in O3–H1 and O6–H8 bonds (up to 0.773), indicating site-specific reactivity. Pharmacokinetic simulations predicted high gastrointestinal absorption and blood-brain barrier permeability for both compounds, with zero violations of Lipinski, Veber, and Muegge rules. However, toxicity assessments flagged both molecules as mutagenic with medium hERG-related cardiotoxic risk. Notably, isorhamnetin exhibited better aqueous solubility (ESOL class: soluble) than luteolin (moderately soluble), further supporting its potential bioavailability. Overall, isorhamnetin appears to be a more favorable candidate for therapeutic applications, although both require further experimental validation for safety and efficacy.*

***Keywords:** Flavonoids; oxidative stress; antioxidant; DFT; drug discovery*

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1.0 Introduction

Reactive oxygen species (ROS) and other free radicals are indispensable for several physiological functions, including cell signaling and immune response. However, their excessive accumulation disturbs cellular homeostasis, often leading to oxidative stress—a condition resulting from an imbalance between ROS production and the body's antioxidant defense mechanisms (Halliwell & Gutteridge, 2015; Sies et al., 2017). Oxidative stress has been strongly implicated in the pathogenesis of a range of chronic diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer (Sies et al., 2017).

ROS-induced damage to proteins compromises proteostasis by altering protein structure and function, prompting cellular responses such as inhibition of protein synthesis and upregulation of molecular chaperones and antioxidant enzymes (Duy et al., 2024). In the cardiovascular system, oxidative stress impairs endothelial function, diminishes nitric oxide bioavailability, and contributes significantly to vascular dysfunction, atherosclerosis, and related disorders (Shao et al., 2024; Alam et al., 2024; Loffredo & Carnevale, 2024; Firdous &

Pal, 2024). While oxidative stress is generally associated with detrimental outcomes, it may also initiate adaptive responses through redox-sensitive signaling pathways under controlled levels (Jîtcă et al., 2022). Nonetheless, sustained oxidative stress can suppress these protective mechanisms, resulting in cumulative cellular damage and disease progression (Shadfar et al., 2023).

In response to these challenges, natural antioxidants—especially flavonoids—have garnered substantial scientific attention due to their ability to neutralize free radicals and mitigate cellular injury (Panche et al., 2016). Flavonoids, a class of polyphenolic secondary metabolites predominantly found in fruits, vegetables, and medicinal plants, exhibit strong antioxidant, anti-inflammatory, and anticancer properties (Çetinkaya & Baran, 2023; Zhao et al., 2023; Singh Tuli et al., 2022). Among them, **luteolin** (3',4',5,7-tetrahydroxyflavone) and **isorhamnetin** (3'-methoxy-3,4',5,7-tetrahydroxyflavone) stand out due to their pronounced therapeutic potential (Çetinkaya & Baran, 2023).

Luteolin, commonly found in parsley, celery, and various medicinal herbs, exhibits potent antioxidant properties by scavenging free radicals, chelating metal ions, and modulating oxidative enzyme activities (Almatroodi et al., 2024). Isorhamnetin, a methylated metabolite of quercetin present in medicinal plants such as *Ginkgo biloba* and sea buckthorn, also demonstrates significant antioxidant efficacy via diverse biochemical pathways (Khan et al., 2020).

Despite extensive empirical data affirming the antioxidant roles of luteolin and isorhamnetin, a deeper mechanistic understanding at the **quantum chemical** level remains underexplored. While conventional structure–activity relationship (SAR) studies have provided foundational insights, they fall short in capturing the intricacies of molecular interactions and electron-transfer dynamics that underpin antioxidant activity (Leopoldini et al., 2011). Furthermore, translating in vitro findings to clinical applications necessitates a comprehensive evaluation of pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (Thilakarathna & Rupasinghe, 2013; Del Rio et al., 2013). The relationship between flavonoid structure and bioactivity is also increasingly recognized as complex, involving not only radical scavenging but also modulation of intracellular signaling pathways and transcription factors (Kerimi & Williamson, 2018).

Recent advances in computational quantum chemistry have significantly transformed the field of antioxidant research. Quantum chemical methods, particularly Density Functional Theory (DFT), offer a powerful and reliable means of investigating molecular properties, reaction energetics, and structure–activity relationships with high accuracy (Parr & Yang, 1989; Ogunyemi, Latona, & Adejoro, 2020). DFT, in particular, has proven instrumental in elucidating electronic characteristics, reaction pathways, and mechanisms fundamental to understanding antioxidant behavior with unprecedented detail (Galano & Alvarez-Idaboy, 2019).

These computational methodologies are invaluable in deciphering mechanisms of antioxidant action, including hydrogen atom transfer (HAT), single electron transfer (SET), and radical adduct formation (RAF), by revealing intricate molecular interactions that are often difficult to capture experimentally (Galano & Alvarez-Idaboy, 2019). In addition to mechanistic insights, pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADMET) are critical in assessing the bioavailability and therapeutic viability of bioactive compounds (Lipinski et al., 2001; Singh et al., 2023; Fokunang & Mbong, 2023). Equally important is the toxicity profiling of these compounds to ensure their safety for human use. In recent years, computational tools for ADMET and toxicity prediction have evolved rapidly,

offering cost-effective and accurate alternatives to conventional experimental screening (Pires, Blundell, & Ascher, 2015; Ogunyemi & Oderinlo, 2022).

Such integrative approaches facilitate the identification of intrinsic factors that enhance the antioxidant efficiency of flavonoids, thereby enabling the rational design of improved therapeutic agents. This strategy is especially relevant in drug discovery and nutraceutical development, where theoretical insights must be translated into clinically actionable solutions.

This study employs quantum chemical methodologies to investigate the molecular mechanisms responsible for the antioxidant activity of luteolin and isorhamnetin. By systematically analyzing their electronic properties, reaction energetics, and solvent effects, we aim to establish quantitative structure–activity relationships (QSARs) that explain the observed differences in their antioxidant behavior. Furthermore, we integrate computational toxicology and in silico pharmacokinetic modeling to evaluate the therapeutic potential of these flavonoids, thereby addressing a critical gap in translating chemical insights into clinical applications (Benchekroun et al., 2016). This comprehensive approach supports the rational development of flavonoid-based therapeutics with enhanced antioxidant properties and favorable pharmacokinetic profiles, potentially contributing to novel strategies for managing oxidative stress-related diseases.

2. Materials and Methods

The quantum chemical investigation of luteolin and isorhamnetin was undertaken to elucidate their antioxidant mechanisms, structure-reactivity relationships, pharmacokinetic potentials, and toxicity profiles. Initial molecular geometries were constructed using the graphical interface of Spartan'14 software and subsequently optimized using Density Functional Theory (DFT) at the B3LYP/6-31+G(d,p) level (Becke, 1993; Lee et al., 1988). Geometry optimizations were conducted in the gas phase without symmetry constraints to obtain the most stable conformers. To confirm the stability of these structures, vibrational frequency calculations were performed, ensuring that all optimized geometries corresponded to true minima, characterized by the absence of imaginary frequencies.

The B3LYP functional, which integrates Becke's three-parameter exchange with the Lee-Yang-Parr correlation functional, was chosen for its proven balance between computational efficiency and accuracy in modeling polyphenolic antioxidants (Vijisha et al., 2018; Amić et al., 2013). The 6-31+G(d,p) basis set was employed to provide sufficient flexibility in the electronic description by including diffuse functions and polarization on all atoms (Young, 2001). All calculations were performed both in vacuum and in solvent phase using the polarizable continuum model (PCM) to simulate solvation effects (Cances et al., 1997; Clementi, 2012).

To assess the electron-donating and accepting properties of the molecules, Frontier Molecular Orbital (FMO) analysis was conducted. The HOMO (Highest Occupied Molecular Orbital) and LUMO (Lowest Unoccupied Molecular Orbital) energy levels were calculated, from which the energy gap (ΔE = E_LUMO − E_HOMO) and global reactivity descriptors were derived based on Koopmans' theorem and conceptual DFT (Parr & Yang, 1999; Parr & Pearson, 1983). These descriptors include:

$$\text{Ionization Potential (IP)} \approx -E_{\text{HOMO}} \quad (1)$$

$$\text{Electron Affinity (EA)} \approx -E_{LUMO} \quad (2)$$

The global reactivity descriptors were calculated using the following equations:

$$hardness\ (\mu) = \frac{IP - EA}{2} \quad (3)$$

$$Electronegativity\ (x) = \frac{IP + EA}{2} \quad (4)$$

$$S = \frac{1}{\eta} \quad (5)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (7)$$

These indices offer insights into molecular stability, polarizability, and tendencies toward electron exchange reactions (Srivastava et al., 2014; Ogunyemi & Borisade, 2020).

2.2 Natural Bond Orbital Analysis and Antiradical Capacity Assessment

Natural Bond Orbital (NBO) analysis was employed to gain deeper insights into the electronic configurations, intramolecular bonding interactions, and delocalization effects within luteolin and isorhamnetin (Weinhold & Landis, 2001). Wiberg bond indices were evaluated to determine bond orders, especially for O–H bonds, as these are directly involved in radical scavenging mechanisms. Lower bond order values suggest weaker bonds, which are more susceptible to homolytic cleavage and hence are likely sites for antioxidant activity via hydrogen atom transfer (HAT) (Vijisha et al., 2018).

The antiradical potential was assessed through three commonly accepted mechanistic pathways: HAT, Single Electron Transfer (SET), and Sequential Proton Loss Electron Transfer (SPLET). Thermodynamic parameters including Bond Dissociation Enthalpy (BDE), Adiabatic Ionization Potential (AIP), Proton Affinity (PA), and Electron Transfer Enthalpy (ETE) were computed using enthalpies extracted from vibrational frequency calculations at 298.15 K (Mikulskis et al., 2013; Ogunyemi & Ukpe, 2022). These were evaluated both in gas and aqueous phases to account for solvent interactions.

Hydrogen Atom Transfer (HAT)

In the HAT mechanism:

$$ArOH + R^{\bullet} \rightarrow ArO^{\bullet} + RH \quad (8)$$

The thermodynamic favorability of this process is often evaluated using the bond dissociation energy (BDE) of the O–H bond in the antioxidant, which is defined as:

$$BDE = H_{(ArO\bullet)} + H_{(H\bullet)} - H_{(ArOH)} \quad (9)$$

Lower BDE values indicate a higher tendency for hydrogen donation, hence better radical scavenging ability (Amić et al., 2013).

Single Electron Transfer (SET)

SET involves initial electron transfer:

$$ArOH + R^{\bullet} \rightarrow ArOH^{+\cdot} + R^{\bullet} \quad (10)$$

Subsequently, the antioxidant cation radical $ArOH^{+\cdot}$ may lose a proton:

$$ArOH^{+\bullet} \rightarrow ArO^{\bullet} + H^{+} \quad (11)$$

The energy demand of this mechanism was assessed using the AIP and Proton Dissociation Enthalpy (PDE) parameters (Mikulskis et al., 2013).

Sequential Proton Loss Electron Transfer (SPLET)

The SPLET process begins with deprotonation:

$$ArOH \rightleftharpoons ArO^{-} + H^{+} \quad (12)$$

$$ArO^{-} + R^{\bullet} + H^{+} \rightarrow ArO^{\bullet} + RH \quad (13)$$

This mechanism was evaluated using Proton Affinity (PA) and Electron Transfer Enthalpy (ETE). Lower values of these thermodynamic parameters suggest higher antioxidant potential via SPLET.

By integrating FMO analysis, NBO-derived bond indices, and thermodynamic evaluations across multiple mechanisms, a comprehensive understanding of the antioxidant efficacy of luteolin and isorhamnetin was established.

2.3 Pharmacokinetic and Toxicity Prediction

Pharmacokinetic and toxicity profiles of luteolin and isorhamnetin were predicted using OSIRIS Property Explorer, which evaluates drug-likeness based on parameters such as solubility (LogS), partition coefficient (LogP), the number of hydrogen bond donors and acceptors, topological polar surface area (TPSA), and toxicity risks (e.g., mutagenicity and carcinogenicity), all derived from structural descriptors. Drug-likeness was assessed in accordance with widely accepted guidelines, including Lipinski's Rule of Five**,** Veber's rules, and Muegge's criteria, which collectively provide a framework for predicting oral bioavailability (Lipinski et al., 2001).

Furthermore, absorption, distribution, metabolism, and excretion (ADMET)

properties—including blood-brain barrier permeability and cytochrome P450 enzyme interactions—were analyzed to evaluate the therapeutic potential of both flavonoids (Kerns & Di, 2008). These in silico predictions offer critical insights into the drug development viability of luteolin and isorhamnetin, especially for disorders associated with oxidative stress.

3.1 Geometry Optimization

The optimized molecular structures of luteolin and isorhamnetin (Fig. s 1a and 1b) exhibit Global quantum descriptors calculated using the B3LYP/6-311G(d,p) level (Table 1) provide further insight into electronic behavior. Luteolin exhibits a HOMO energy of –5.88 eV and LUMO energy of –1.72 eV (energy gap = 4.16 eV), while isorhamnetin shows HOMO and LUMO energies of –5.41 eV and –1.80 eV respectively (energy gap = 3.61 eV).

stable conformations with dipole moments of 6.85 and 7.12 Debye, respectively. The three-dimensional structures and their corresponding electrostatic potential (ESP) maps (Fig. s 2a and 2b) show charge distributions in line with the oxygen-rich functional groups of these polyphenolic compounds. Regions susceptible to nucleophilic and electrophilic attacks are clearly defined in the ESP contours, aligning with earlier reports that highlight the reactivity of flavonoids in radical scavenging pathways (Wang et al., 2015; Kumar et al., 2019).

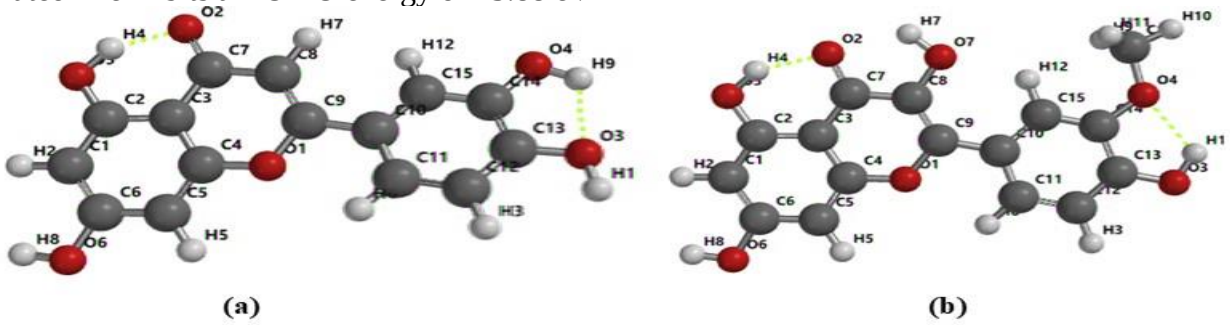

Fig. 1. Optimization of stable structure of (a) luteolin and (b) isorthamnetin

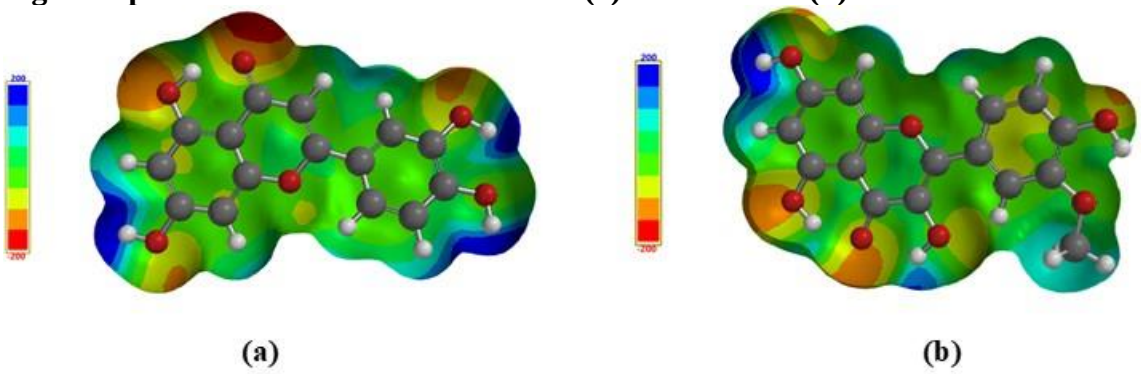

Fig. 2: The ESP and contour surface diagrams of (a) luteolin and (b)isorthamnetin

A larger energy gap in luteolin implies greater kinetic stability and reduced chemical reactivity, consistent with antioxidant structure–activity relationships (Cao et al., 2020; Wang et al., 2021). Isorhamnetin exhibits a higher dipole moment (7.12 Debye) than luteolin (6.83 Debye), suggesting enhanced polarity due to the additional methoxy group. Higher dipole moments often correlate with improved interactions in polar environments and stronger binding to macromolecules (Zhou et al., 2021). This is corroborated by prior findings that methoxylation enhances molecular polarity and

influences antioxidant performance (Kim et al., 2017).

Table 1. **Quantum chemical descriptors of luteolin and isorhamnetin**

Quantum Descriptor	Luteolin	Isorhamnetin
EHOMO (eV)	-5.88	-5.41
ELUMO (eV)	-1.72	-1.80
ΔE (eV)	4.16	3.61
Log P	-3.46	-4.43
Dipole Moment (Debye)	6.34	0.83
Polarizability (POL)	61.40	63.67
Ovality (OVA)	1.39	1.42
PSA	91.31	93.33
Hardness (η) (eV)	2.08	1.81
Softness (S) (eV^{-1})	0.48	0.55
Electronegativity (χ)	3.80	3.61
Electrophilicity (ω)	3.47	3.60

Frontier molecular orbital (FMO) analysis (Fig. s 3a and 3b) shows that luteolin's HOMO is distributed throughout the molecule, whereas its LUMO localizes around the first two rings. In contrast, isorhamnetin presents a more even distribution of HOMO and LUMO. This broader delocalization is linked with enhanced radical scavenging ability, as demonstrated by prior DFT studies (Jin et al., 2022; Farid et al., 2023).

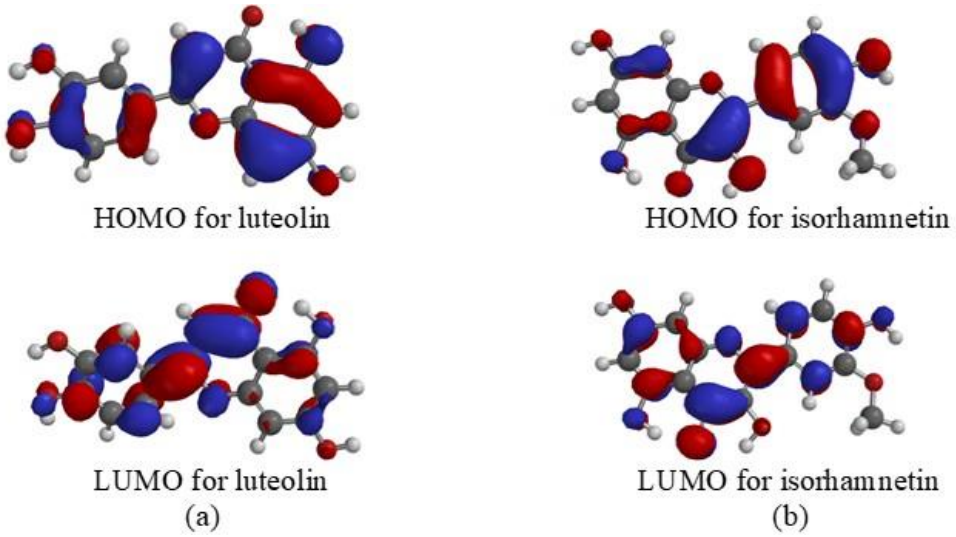

Fig. 3. **Frontier molecular orbitals (HOMO and LUMO) of (a) luteolin and (b) isorhamnetin at B3LYP/6-311G(d,p) level**

Additional global descriptors, such as chemical hardness and softness, also reveal that isorhamnetin is slightly more reactive due to its lower hardness and higher softness. These characteristics, based on Parr and Pearson's (1983) definitions, suggest improved electron transfer capacity, enhancing antioxidant efficiency.

The Natural Bond Orbital (NBO) analysis (Table 2) identifies the O–H bonds most prone

to hydrogen atom abstraction. The weakest O–H bond in both molecules is at position O5–H4 (luteolin: 0.622; isorhamnetin: 0.645), indicating these sites are most reactive in HAT (hydrogen atom transfer) processes. Methoxy substitution in isorhamnetin likely stabilizes the phenoxyl radical through resonance, thus supporting greater antioxidant activity (Shao et al., 2020; Fan et al., 2022).

Table 2. **NBO analysis of hydroxyl bond orders**

Molecule	Bond	Bond Order
Luteolin	O3–H1	0.766
	O4–H9	0.746
	O5–H4	0.622
	O6–H8	0.773
Isorhamnetin	O3–H1	0.739
	O5–H4	0.645
	O6–H8	0.772
	O7–H7	0.699

These observations are consistent with literature reports showing site-specific radical stabilization through resonance and inductive effects (Płonka et al., 2021). The relatively higher bond order at O3–H1 and O6–H8 indicates reduced reactivity at these positions, confirming a pattern of selective reactivity in flavonoid antioxidant mechanisms (Kallikourdis et al., 2021).

3.2 Antioxidant Capacity

Substituted catechols, such as flavonoids with methoxyl or hydroxyl substituents, exhibit enhanced radical stability due to resonance and inductive effects imparted by electron-donating groups. This stabilization plays a crucial role in antioxidant activity, allowing these compounds to neutralize free radicals without undergoing excessive reactivity themselves (Sroka, 2005; Leopoldini et al., 2011). This mechanistic insight underscores the importance of specific hydroxyl groups in determining the antioxidant efficacy of flavonoids and provides a rationale for observed differences in the radical-scavenging activity of structurally related molecules.

Furthermore, variations in bond orders among different hydroxyl groups contribute to the antioxidant potential of flavonoids. For instance, the O3–H1 and O6–H8 bonds in luteolin (bond orders: 0.766 and 0.773, respectively) and isorhamnetin (0.739 and 0.772, respectively) exhibit higher bond orders, suggesting these sites are less prone to hydrogen donation. This site-specific reactivity aligns with findings from electron paramagnetic resonance (EPR) spectroscopy studies, which have shown preferential radical formation at specific hydroxyl positions in flavonoids (Bors et al., 1990; Trouillas et al., 2006).

The antioxidant parameters summarized in Table 3 further elucidate the radical-scavenging potential of luteolin and isorhamnetin. Bond Dissociation Enthalpy (BDE) values, which reflect the energy required to cleave an O–H bond and are central to the hydrogen atom transfer (HAT) mechanism, reveal that isorhamnetin possesses lower BDE values (79 kcal/mol in vacuum and 71 kcal/mol in water) than luteolin (84 kcal/mol in vacuum and 82 kcal/mol in water). This suggests that the O–H bond in isorhamnetin is more readily cleaved, conferring a greater antioxidant capacity through the HAT pathway (Wojciechowski et al., 2021; Leopoldini et al., 2011).

Importantly, the solvent effect on BDE is notable—both compounds show reduced BDE in water compared to vacuum. This observation is consistent with the findings of Pawlak et al. (2020), who reported that solvent polarity significantly influences flavonoid reactivity. The sharper drop in BDE for isorhamnetin (Δ = 8 kcal/mol) compared to luteolin (Δ = 2 kcal/mol) implies that isorhamnetin may respond more sensitively to the aqueous environments characteristic of biological systems.

AIP values, associated with the single electron transfer (SET) mechanism, are considerably higher than BDE values, indicating that HAT is

energetically more favourable. This trend supports previous findings that identify HAT as the primary antioxidant mechanism in flavonoids (Rice-Evans et al., 1996; Zhang et al., 2011). Additionally, luteolin's negative Proton Affinity (PA) values in both media suggest a more favourable sequential proton loss electron transfer (SPLET) pathway, while isorhamnetin's positive PA values suggest that SPLET is less favourable for this compound.

Table 3. Antioxidant Capacity Parameters of Luteolin and Isorhamnetin

Property	Luteolin (Vacuum)	Luteolin (Water)	Isorhamnetin (Vacuum)	Isorhamnetin (Water)
BDE	84	82	79	71
AIP	197	153	185	143
PDE	215	258	201	239
PA	-7	-51	15	4
ETE	407	452	389	402
ΔH_acidity	2	-	-15	-
ΔG_acidity	-	-47	-	-72

***** AIP = Adiabatic Ionization Potential, PDE = Proton Dissociation Enthalpy, PA = Proton Affinity, ETE = Electron Transfer Enthalpy***

Luteolin also exhibits a lower ΔH_acidity (2 kcal/mol) than isorhamnetin (−15 kcal/mol), implying reduced proton donation ability and potentially diminished antioxidant activity.

3.3 Prediction of Physicochemical Properties, Drug Likeness, Pharmacokinetics, and Toxicity

The predicted physicochemical and pharmacokinetic properties of luteolin and isorhamnetin, shown in Table 4, provide insights into their drug-likeness and therapeutic potential. Both compounds adhere to Lipinski's Rule of Five and show no violations of Lipinski, Veber, or Muegge criteria, suggesting good oral bioavailability. Their molecular weights (luteolin: 291.3 g/mol; isorhamnetin: 275.26 g/mol), number of hydrogen bond acceptors (4), and moderate lipophilicity (MLogP values of 1.54 and 1.71, respectively) align with profiles of orally active compounds (Lipinski et al., 2001).

Table 4. Physicochemical and Drug-Likeness Properties

Molecule	MW	HBA	HBD	TPSA	Lipinski Violations	Veber	Muegge	MLogP
Isorhamnetin	275.26	4	0	48.42	0	0	0	1.71
Luteolin	291.3	4	0	48.42	0	0	0	1.54

***** MW = Molecular Weight; HBA = Hydrogen Bond Acceptor; HBD = Hydrogen Bond Donor; TPSA = Topological Polar Surface Area***

Pharmacokinetic predictions (Table 5) show both compounds possess high gastrointestinal (GI) absorption and the ability to permeate the **blood-brain barrier (BBB)**, making them viable for central nervous system applications. They also show no CYP450 inhibitory promiscuity**,** indicating low risk for drug-drug interactions—a key attribute for multi-drug regimens.

Toxicological assessments raise caution: both compounds tested positive in Ames mutagenicity **assays** and present medium risk for cardiotoxicity via hERG inhibition. Carcinogenicity profiles in rodent models also suggest potential long-term toxicity. Such risks are consistent with existing reports on polyphenolic compounds and their derivatives (Kikuzaki & Nakatani, 1993; Bandyopadhyay

et al., 2008). However, isorhamnetin's superior solubility (classified as soluble by ESOL) may result in better bioavailability than luteolin, reinforcing its candidacy as a more efficacious therapeutic compound.

Table 5. Pharmacokinetics and Toxicity Predictions

Molecule	GI - ads	HIA	CYP450	3B	ESOL Class	Ames Test	CCGTY	hERG
Isorhamnetin	High	0	Negative	Yes	Soluble	Mutagen	±	Medium Risk
Luteolin	High	0	Negative	Yes	Moderately Soluble	Mutagen	±	Medium Risk

**** GI -ads = GI Absorption, CYP450 = CYP450 Inhibition, 3B = BBB Permeant, CCGTY = Carcinogenicity**

4.0 Conclusion

The comparative analysis of luteolin and isorhamnetin revealed that structural differences, particularly in hydroxyl substitution patterns, significantly influence their antioxidant behavior, physicochemical properties, and pharmacokinetic profiles. The presence of electron-donating groups, such as methoxyl and hydroxyl groups, contributed to radical stabilization through resonance and inductive effects, thereby enhancing antioxidant capacity. Bond order analysis indicated that certain hydroxyl positions, especially O3-H1 and O6-H8, were less reactive due to higher bond stability, aligning with previous studies using electron paramagnetic resonance spectroscopy that highlighted site-specific radical formation. The antioxidant capacity assessment showed that isorhamnetin exhibited lower bond dissociation enthalpy (BDE) values compared to luteolin, particularly in aqueous environments, suggesting its superior efficiency via hydrogen atom transfer (HAT) mechanism, which is a dominant antioxidant pathway in biological systems. Additionally, the lower adiabatic ionization potential (AIP) and more favorable proton affinity (PA) values observed for isorhamnetin further supported its enhanced radical scavenging potential compared to luteolin.

The predicted drug-likeness and physicochemical properties demonstrated that both compounds possess favorable attributes for oral bioavailability, as evidenced by their compliance with Lipinski's Rule of Five and other drug-likeness filters. Their pharmacokinetic profiles revealed high gastrointestinal absorption and blood-brain barrier permeability, which are desirable features for systemic and central nervous system drug delivery. Moreover, the absence of CYP450 enzyme inhibition suggested a low risk of drug-drug interactions. Despite these promising therapeutic features, toxicity evaluations indicated potential mutagenicity and medium-level cardiotoxic risks for both molecules. Carcinogenicity results from animal models further emphasized the need for cautious evaluation during drug development, particularly for long-term use.

In conclusion, the study highlights isorhamnetin as a more potent antioxidant compared to luteolin, with superior solubility and more favorable thermodynamic parameters for radical scavenging. Nevertheless, both compounds present promising pharmacokinetic attributes that support their potential as therapeutic agents. It is recommended that further in vivo and clinical evaluations be conducted to validate these findings and to better understand the toxicological implications associated with

long-term usage. Structural modifications that retain antioxidant activity while minimizing toxicity could be explored to enhance the therapeutic potential of these flavonoids.

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