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Comparative Docking and Pharmacokinetic Insights into Flavonoid Modulation of Antioxidant Enzymes: A Molecular Basis for Nrf2 Activation and Oxidative Stress Mitigation

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ABSTRACT: Environmental toxicants like dibutyl phthalate (DBP) trigger oxidative stress by increasing reactive oxygen species (ROS), disrupting cellular redox balance and contributing to chronic diseases. Flavonoids are known antioxidants, but their molecular interactions with key antioxidant enzymes are not fully understood. This study used molecular docking to examine the binding of morin, myricetin, and quercetin to four major antioxidant targets: Nrf2-Keap1, superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), with epigallocatechin gallate (EGCG) as a reference Nrf2 activator. Docking results revealed that EGCG consistently exhibited the strongest binding affinity across all enzymes, while quercetin and morin demonstrated comparable or superior interactions in specific cases, particularly with CAT and GR. Key residues such as Ala556 in Nrf2-Keap1, Arg203 in CAT, and Lys66 in GR were consistently involved in ligand stabilization through hydrogen bonding, π–π stacking, and hydrophobic contacts. ADMET predictions indicated that morin and quercetin possess favorable pharmacokinetic and drug-likeness properties, including good gastrointestinal absorption and minimal structural alerts. In contrast, EGCG displayed limited absorption and bioavailability despite its high binding affinity. These findings underscore the therapeutic potential of selected flavonoids in oxidative stress-related disorders and support their further exploration as modulators of endogenous antioxidant defense mechanisms.

Keywords: Flavonoids, Molecular docking, Nrf2-Keap1, Antioxidant enzymes, Catalase, Glutathione reductase, Superoxide dismutase, EGCG, ADMET, Oxidative stress modulation.

INTRODUCTION

Oxidative stress has emerged as a central pathological mechanism linked to a range of chronic inflammatory conditions, including reproductive damage (Agarwal *et al*., 2012). It results from an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms that neutralize them. This imbalance can disrupt cellular homeostasis, damage vital biomolecules, and impair biological functions. Exposure to environmental toxicants such as di-n-butyl phthalate (DBP) is known to enhance ROS production by decreasing antioxidant enzyme activity and increasing lipid peroxidation, particularly affecting testicular function (Zhou *et al*., 2010).

Among the critical cellular defense mechanisms, the Keap1-Nrf2 signaling axis plays a pivotal role in sensing oxidative stress and initiating protective gene expression (Taguchi & Yamamoto 2021). Under basal conditions, Keap1 binds to Nrf2, retaining it in the cytoplasm and targeting it for degradation. Upon oxidative challenge, this complex dissociates, allowing Nrf2 to translocate into the nucleus, where it activates antioxidant response element (ARE)-driven genes involved in detoxification and redox balance (Ngo and Duennwald 2022; He *et al.,* 2020). This regulatory system is essential for maintaining redox homeostasis and protecting against cellular injury (Chakkittukandiyil *et al.,* 2022).

Natural products, especially flavonoids, have gained attention as potential modulators of the Keap1-Nrf2-ARE pathway. These compounds have shown promising antioxidant properties and therapeutic benefits in various disease models by upregulating cytoprotective genes such as HO-1 and NQO1 (Zhou *et al*., 2019). Flavonoids exert their effects through multiple signaling pathways, including PI3K/Akt, MAPK, and NF-κB, and may also influence Nrf2 activation via miRNA-mediated regulation (Adinew *et al*., 2021). However, their bioavailability and capacity to reach target sites remain significant challenges. Importantly, flavonoids also exhibit potential in mitigating oxidative stress induced by endocrine disruptors, which elevate intracellular ROS and contribute to DNA, lipid, and protein damage (Li *et al.,* 2023; Muscolo *et al.,* 2024). While flavonoids often enhance antioxidant enzyme activity, such as SOD,

CAT, and GR, some may exhibit pro-oxidant effects at high concentrations or under specific conditions (Procházková *et al.*, 2011; Pérez-Torres *et al*., 2017). These dual roles underscore the complexity of flavonoid-enzyme interactions and the need for precise characterization.

Mechanistically, flavonoids activate the Nrf2-ARE pathway, leading to increased expression of antioxidant enzymes and bolstered cellular defenses (Mendonca and Soliman 2020). The ARE, a cis-regulatory element in promoter regions of detoxifying genes, is essential for both basal and inducible antioxidant gene expression (Wild and Mulcahy 2020). Studies in Nrf2-deficient mice confirm the central role of Nrf2 in orchestrating ARE-mediated transcription and protecting against oxidative and chemical insults (Singh *et al.*, 2010).

In this study, we aim to investigate the molecular binding mechanisms of selected flavonoids with antioxidant enzymes, with a particular focus on the Keap1-Nrf2 axis. Through this exploration, we seek to identify promising flavonoid candidates for mitigating oxidative stress and countering the toxic effects of endocrine disruptors.

MATERIALS AND METHODS

A. Selection and preparation of flavonoids

To explore the molecular interactions between flavonoids and antioxidant enzymes, we selected a panel of structurally diverse flavonoids with well-documented antioxidant properties. Specifically, morin (CID: 5281670), myricetin (CID: 5281672), quercetin (CID: 5280343) and (–)-epigallocatechin-3-gallate (EGCG) (CID: 65064) were chosen for their reported ability to modulate key antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and glutathione reductase (GR). The 3D molecular structures of these flavonoids were retrieved from the PubChem database (Kim *et al*., 2016). Ligand geometries were optimized using the Universal Force Field (UFF) in Open Babel and converted into AutoDock-compatible PDBQT format for docking analysis (Kumar Konidala *et al.*, 2022). Epigallocatechin gallate (EGCG) was used as a reference template due to its well-established role as a natural Nrf2 activator and antioxidant modulator.

B. Protein structure preparation

The crystal structures of target proteins, Nrf2-Keap1 complex (PDB ID: 7OFE) (Narayanan *et al.*, 2022), CAT (PDB ID: 1DGF) (Putnam *et al.*, 2020), GR (PDB ID: 1GSN) (Becker *et al.*, 1998), and SOD (PDB ID: 5YTO) (Manjula *et al.*, 2018) were obtained from the RCSB Protein Data Bank. Structures with resolutions between 1.50–1.90 Å were selected to ensure high-quality docking results. Water molecules and co-crystallized ligands were removed, hydrogen atoms were added, and Gasteiger charges were assigned using UCSF Chimera v1.10.2. All prepared protein models were saved in PDBQT format using AutoDock tools (Bommu *et al*., 2017).

C. Molecular docking

Molecular docking was performed using AutoDockvinav4 implicated in PyRx virtual screening software v8.0 (Dallakyan & Olson (2014), a validated tool for predicting ligand-protein interactions and estimating binding affinities (Bommu *et al.*, 2017; Kumar Konidala *et al.*, 2022). Grid box parameters were defined to fully enclose the active sites of each enzyme: grid centers were set at X = 50 Å, Y = 50 Å, Z = 50 Å, with box dimensions of 25 × 25 × 25 Å and a spacing of 0.375 Å (Bommu *et al.*, 2017; Kumar Konidala *et al*., 2022). These configurations were selected to ensure accurate sampling of potential binding poses. The docking protocol included evaluation of ligand binding conformations, affinity scores, and comparison with reference binding sites to ensure reliability.

D. Visualization of protein–ligand interactions

Docked complexes were visualized and analyzed using BIOVIA Discovery Studio Visualizer v24.1.0.23298 to assess the spatial orientation of the ligands, identify interaction types, and determine key amino acid residues involved in binding stabilization.

E. ADME prediction

The pharmacokinetic profiles of all flavonoids were evaluated using the SwissADME web server (http://www.swissadme.ch/) (Daina *et al*., 2017). Evaluated parameters included: physicochemical properties (molecular weight, hydrogen bond donors/acceptors, TPSA); lipophilicity (XLOGP3); pharmacokinetics, including gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, P-glycoprotein (P-gp) substrate status, CYP450 inhibition (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4), and skin permeation (Log Kp); drug-likeness (Lipinski rule violations, bioavailability score); and medicinal chemistry filters, including PAINS, Brenk alerts, lead-likeness, and synthetic accessibility.

RESULTS AND DISCUSSION

This study employed molecular docking to investigate the binding interactions of three flavonoids, morin, myricetin, and quercetin with key antioxidant enzymes: Nrf2-Keap1, superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). To provide a comparative benchmark, the binding profiles of these flavonoids were evaluated against epigallocatechin gallate (EGCG), a well-characterized natural Nrf2 activator.

A. Binding mode of flavonoids with Nrf2-Keap1

Binding energies were calculated as –6.9 kcal/mol for morin, –6.7 kcal/mol for myricetin and quercetin, while EGCG exhibited a superior binding energy of –7.6 kcal/mol, serving as a reference Nrf2 activator (Table 1).

Morin showed a binding energy of –6.9 kcal/mol and established key hydrogen bonds with Ser363, Arg380, and Asn382, with interaction distances ranging from 2.47 to 3.92 Å. Notably, the Arg380 and Ala556 residues participated in Pi-alkyl interactions, contributing to the hydrophobic stabilization of the complex (Fig. 1a). These interactions suggest a moderately stable binding conformation within the Nrf2 pocket.

Myricetin demonstrated a comparable binding energy of –6.7 kcal/mol and engaged in multiple hydrogen bonding interactions with Ser363, Ser555, and Asn382, spanning a range of hydrogen bond lengths (2.42–3.91 Å). Additionally, Pi-alkyl interactions involving Arg380 and Ala556 supported further stabilization (Fig. 1b). The presence of multiple polar contacts indicates a higher degree of specificity in the myricetin binding mode compared to morin.

Quercetin also bound with a docking score of –6.7 kcal/mol. It formed hydrogen bonds with Arg415, Ser555, and Ser602, as well as prominent electrostatic and aromatic interactions, including Pi-Pi T-shaped (Tyr525), Pi-cation (Arg415), and Pi-alkyl (Ala556) (Fig. 1c). These interactions suggest a diverse binding mechanism that may influence the conformational flexibility of the Nrf2 domain.

The reference compound EGCG displayed the most favorable binding energy of –7.6 kcal/mol. It engaged in a hydrogen bond with Ser602 and showed extensive aromatic interactions with Tyr334, Tyr525, Ala556, and Tyr572, including Pi-Pi stacking and T-shaped orientations, consistent with high-affinity binding and Nrf2 activation potential (Fig. 1d). Across all flavonoid complexes, the Ala556 residue emerged as a recurrent site for hydrophobic (Pi-alkyl or Pi-Pi) interactions, suggesting its importance in ligand recognition. Residues such as Arg380, Ser363, and Tyr525 played key roles in forming stabilizing hydrogen and electrostatic contacts. Although all three flavonoids demonstrated moderate affinity toward Nrf2, EGCG's higher binding energy and more diverse interaction profile underline its superior activation capability.

B. Binding mode of flavonoids with catalase (CAT)

Molecular docking analyses revealed favorable binding affinities of the selected flavonoids with catalase (PDB ID: 1DGF). The binding energies were calculated as –8.3 kcal/mol for morin, –8.6 kcal/mol for myricetin, and –8.7 kcal/mol for quercetin, while the reference compound EGCG exhibited the most stable interaction with a binding energy of –8.9 kcal/mol (Table 2).

Morin showed significant binding to CAT through multiple hydrophobic and aromatic interactions. Notable Pi-alkyl contacts were observed with Pro151 and Arg203, while Pi–Pi stacking and T-shaped interactions occurred with Phe198 and Phe446, indicating aromatic ring engagement within the enzyme's active site. A Pi-cation interaction with Arg203:NH2 (4.29 Å) further stabilized the complex (Fig. 1e). No classical hydrogen bonding was observed, suggesting a predominantly hydrophobic mode of binding.

Myricetin demonstrated enhanced binding affinity with one hydrogen bond involving Arg203 (3.06 Å; 111.01°). This interaction was complemented by extensive aromatic stacking and alkyl contacts, including Pi–Pi stacking with Phe198 and Phe446, Pi-T-shaped interactions, and Pi-cation bonding with Arg203:NH2. Additional Pi-alkyl interactions with Pro151, Arg203, Val302, and His305 contributed to the overall stabilization of the complex (Fig. 1f).

Quercetin showed the strongest binding among the three flavonoids (–8.7 kcal/mol), stabilized by three conventional hydrogen bonds with Ser201 and Tyr215, with bond lengths between 2.02 and 2.61 Å. These polar contacts were reinforced by Pi-stacking and Pi-cation interactions with Phe198, Arg203, Phe446, and His305 (Fig. 1g). The hydrophobic and electrostatic environment contributed significantly to the complex stability and orientation within the catalytic pocket.

EGCG, displayed the most favorable binding energy (–8.9 kcal/mol). It formed a network of six hydrogen bonds with Phe198, Ser201, Arg203, Tyr215, and His305, indicating strong polar interactions. These were complemented by Pi-cation and Pi-alkyl interactions with Arg203 and Phe198 (Fig. 1h), establishing a robust and stable binding conformation.

Overall, Phe198 and Arg203 consistently participated in aromatic and cationic interactions, highlighting their critical role in ligand anchoring within the CAT binding pocket. The presence of Pi–Pi stacking, Pi-cation, and Pi-alkyl contacts, particularly with flavonoid aromatic rings, underscores the significance of hydrophobic interactions in catalase modulation (Vernon *et al.*, 2018). Notably, quercetin and EGCG displayed stronger and more diverse interaction profiles, suggesting potential superiority in enhancing CAT activity or stabilization.

C. Binding mode of flavonoids with glutathione reductase (GR)

Molecular docking studies revealed that the flavonoids Morin, Myricetin, and Quercetin exhibited binding energies of –7.1, –7.2, and –7.2 kcal/mol, respectively, with Glutathione Reductase (PDB ID: 1GSN), while the reference compound EGCG showed a slightly stronger binding energy of –7.4 kcal/mol (Table 3). The interaction profiles of these compounds suggest distinct yet overlapping binding mechanisms.

Morin formed two hydrogen bonds, one with Lys66 (1.97 Å, 154.25°) and another with Val370 (2.88 Å, 140.94°). Additionally, it engaged in hydrophobic interactions, including a π-sulfur contact with Cys63 and π-alkyl interactions with Lys67 and Pro340. A π–π T-shaped interaction with Phe372 further stabilized the complex (Fig. 1i), indicating that Morin binds effectively through a combination of hydrogen bonding and aromatic interactions.

Myricetin formed three hydrogen bonds, involving Cys63 and Lys66 through both its carbon and terminal amino groups, with bond distances ranging from 1.95 to 3.18 Å. It also engaged in π-alkyl interactions with Lys67 and Pro340 and a π–π T-shaped interaction with Phe372 (Fig. 1j). These interactions suggest that Myricetin is stabilized predominantly through polar contacts and non-polar stacking, particularly involving Lys66.

Similarly, Quercetin showed a strong hydrogen bond with Lys66 (1.98 Å, 154.44°), and shared interaction patterns with Morin, including π-sulfur bonding with Cys63 and π-alkyl and π–π T-shaped contacts with Lys67, Pro340, and Phe372 (Fig. 1k). The structural similarity in binding between Myricetin and Quercetin

suggests they may share comparable inhibitory potential.

EGCG displayed the most complex binding pattern among all compounds, forming five hydrogen bonds with Ser30, Arg37 (three interactions), and Tyr106, with distances between 2.01 and 2.92 Å. In addition, EGCG exhibited π-cation and π-alkyl interactions with Arg203 and Val64, and π–π stacking with Tyr114 (Fig. 1l). These multiple interactions are likely responsible for its stronger binding affinity.

Overall, key residues such as Cys63, Lys66, Lys67, Pro340, and Phe372 were consistently involved in π-sulfur, π-alkyl, and π–π interactions across all complexes. The diversity and complementarity of hydrogen bonding and hydrophobic interactions indicate a robust and flexible binding cavity within GR that accommodates these flavonoids (Patil *et al*., 2010). These findings suggest that the antioxidant activity of these compounds may, in part, be attributed to their capacity to modulate GR function by stabilizing its catalytic domain and potentially interfering with glutathione metabolism.

D. Binding mode of flavonoids with superoxide dismutase (SOD)

Molecular docking studies demonstrated that Morin, Myricetin, and Quercetin bind to Superoxide Dismutase (SOD; PDB ID: 5YTO) with comparable binding energies of –7.2, –7.1, and –7.2 kcal/mol, respectively, while the reference compound EGCG exhibited a stronger binding energy of –7.7 kcal/mol. The interaction profiles reveal that these flavonoids engage the active site of SOD through a combination of hydrogen bonds, π-interactions, and hydrophobic contacts, primarily involving residues such as Glu21, Lys23, and Lys30.

Morin established a conventional hydrogen bond with Lys23 (2.74 Å, 107.57°), and hydrophobic interactions dominated its binding profile. These included π-anion and amide–π stacking with Glu21 and Lys23, respectively, and alkyl interactions with Lys23 and Lys30 (Fig. 1m). These multiple interactions highlight the role of cationic and acidic residues in anchoring the ligand through aromatic ring stacking and electrostatic interactions.

Myricetin formed three hydrogen bonds with Thr2 (2.47–2.71 Å) and Glu100 (2.69 Å), indicating strong polar contacts with both N-terminal and loop-region residues. Its hydrophobic interactions included π-alkyl and π-anion contacts with Lys3 and Glu21, along with π-sigma interactions with Lys23 and additional alkyl stacking with Lys30 (Fig. 1n). This suggests that Myricetin engages in a more dispersed interaction network, stabilizing its binding conformation through both edge-on and face-on stacking geometries.

Quercetin mirrored Morin's binding profile with hydrogen bonds formed via Lys23 and Glu100, and several π-interactions including π-anion with Glu21 and amide–π stacking and alkyl contacts with Lys23 and Lys30 (Fig. 1o). These overlapping interaction modes between Morin and Quercetin indicate potential redundancy or synergy in their antioxidant action via SOD modulation.

EGCG showed the most complex interaction pattern. It formed five hydrogen bonds with Glu21, Gln22, and Pro28 (distances ranging from 1.95–2.42 Å), establishing a robust hydrogen bond network. Furthermore, EGCG exhibited multiple hydrophobic and π-based interactions involving Lys23, Pro28, Lys30, and Glu100 (Fig. 1p). Notably, π-anion and amide–π stacking with Glu100 and Lys23 reinforced the binding strength, correlating with its highest docking score.

Overall, residues such as Glu21, Lys23, Lys30, and Glu100 recurrently appeared across all flavonoid complexes, playing essential roles in hydrogen bonding, π-interactions, and hydrophobic contacts. The interplay of these dynamic interactions suggests that flavonoid binding induces a conformational flexibility in SOD, potentially stabilizing its structure and modulating its enzymatic function (Kamel *et al*., 2025). This structural stabilization may hinder the formation or regulation of reactive oxygen species (ROS), underlining the antioxidant potential of these phytochemicals through SOD inhibition or modulation.

E. ADMET and medicinal chemistry profile analysis

The pharmacokinetic and drug-likeness profiles of Morin, Myricetin, Quercetin, and EGCG were assessed using the SwissADME tool (http://www.swissadme.ch/index.php) to evaluate their potential as bioavailable antioxidants.

Morin and Quercetin showed optimal molecular weight (302.24 g/mol), moderate TPSA (131.36 Å²), and favorable hydrogen bonding profiles, indicating good membrane permeability. EGCG, with the highest MW (458.37 g/mol), TPSA (197.37 Å²), and H-bond donors/acceptors, exhibited poor absorption potential. LogP values for all compounds were in the moderate range (1.17–1.54), supporting balanced lipophilicity (Table 5).

GI absorption was high for Morin and Quercetin but low for Myricetin and EGCG. None of the compounds were BBB permeant or P-gp substrates, minimizing CNS toxicity and efflux concerns. EGCG showed the least skin permeability due to its large polar surface (Table 5).

CYP inhibition analysis revealed that Morin and Quercetin inhibited CYP1A2, CYP2D6, and CYP3A4, suggesting drug interaction risks. Myricetin inhibited two CYPs, while EGCG showed no CYP inhibition, indicating metabolic safety despite low absorption (Table 5).

All compounds largely met Lipinski's rules, except EGCG, which had two violations and the lowest bioavailability score (0.17). Morin and Quercetin were most favorable, with no rule violations and better oral drug-likeness. Synthetic accessibility was acceptable for all, though EGCG was slightly more complex. PAINS and Brenk alerts were absent in Morin, but present in the others, pointing to fewer structural liabilities for Morin (Table 5).

Overall, Morin and Quercetin emerged as the most promising candidates for further development based on their favorable ADMET and medicinal chemistry profiles.

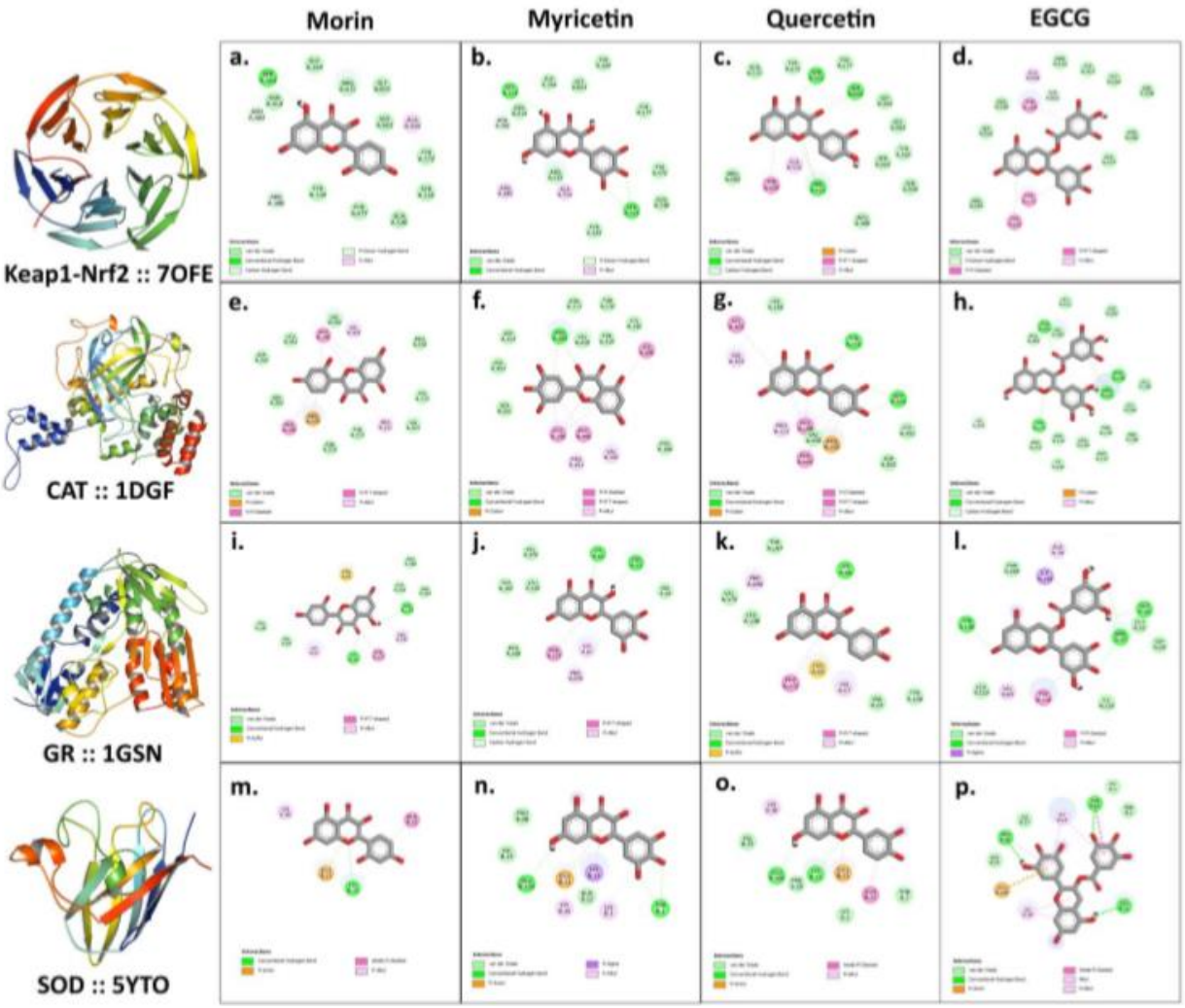

Fig. 1. Molecular interaction profiles of selected flavonoids (morin, myricetin, quercetin, and EGCG) with antioxidant targets. Visualizations illustrate binding orientations, key hydrogen bonds, and hydrophobic or π-based interactions within the active sites of Nrf2-Keap1, CAT, GR, and SOD.

Table 1: Binding energy and key molecular interactions of flavonoids and EGCG with the Nrf2 receptor.

Ligand	Binding energy (kcal/mol)	Hydrogen bond interactions			Hydrophobic, electrostatic and other interactions			
		Interactions (Protein-----Ligand)	Distance (Å)	Angle (°)	Interactions (Residues)	Type	Distance(Å)	Angle (°)
5281670 (Morin)	**-6.9**	Ser363:OG-----H Arg380:CD-----O Asn382:ND2^{Pi-H}	2.47 3.67 3.92	96.54 46.76 36.53	Arg380 Ala556	Pi-Alkyl Pi-Alkyl	5.46 4.06	-
5281672 (Myricetin)	**-6.7**	Ser363:O - H Ser363:OG - H Asn382:ND2^{Pi-H} Ser555:HG - O Ser555:HG - O	2.85 2.56 3.91 2.42 2.55	101.32 90.14 35.85 119.08 109.09	Arg380 Ala556	Pi-Alkyl Pi-Alkyl	5.40 4.18	-
5280343 (Quercetin)	**-6.7**	Arg415:CD - O Arg415:NH2 - O Ser555:HG - O Ser602:HG - O	3.76 3.34 2.48 3.02	42.14 45.14 95.73 97.23	Tyr525 Arg415 Ala556	Pi-Pi T-shaped Pi-Cation Pi-Alkyl	5.15 4.96 4.50	25.06 24.16 -
65064 (EGCG)	**-7.6**	Ser602:HG^{Pi-H}	3.42	36.06	Tyr334 Tyr525 Ala556 Tyr572 Tyr572	Pi-Pi Stacked Pi-Pi T-shaped Pi-Alkyl Pi-Pi T-shaped Pi-Alkyl	4.47 4.91 5.33 5.40 4.86	27.33 13.57 - 10.98 -

Table 2: Molecular docking-derived binding energies and key interactions of flavonoids with catalase (CAT).

Ligand	Binding energy (kcal/mol)	Hydrogen bond interactions			Hydrophobic and Electrostatic interactions			
		Interactions (Protein-----Ligand)	Distance (Å)	Angle (º)	Interactions (Residues)	Type	Distance(Å)	Angle (º)
5281670 (Morin)	**-8.3**	-	-	-	Pro151 Phe198 Phe198 Phe198 Arg203 Arg203:NH2 Val302 Phe446	Pi-Alkyl Pi-Pi Stacked Pi-Pi Stacked Pi-Pi T-shaped Pi-Alkyl Pi-Cation Pi-Alkyl Pi-Pi T-shaped	5.13 4.56 5.54 4.95 4.58 4.29 4.43 4.76	- 48.50 70.85 68.15 - 24.51 - 78.62
5281672 (Myricetin)	**-8.6**	Arg203:H-----O	3.06	111.01	Pro151 Phe198 Phe198 Phe198 Arg203:NH2 Arg203 Val302 His305 Phe446	Pi-Alkyl Pi-Pi Stacked Pi-Pi Stacked Pi-Pi T-shaped Pi-Cation Pi-Alkyl Pi-Alkyl Pi-Pi Stacked Pi-Pi T-shaped	5.08 4.60 5.68 4.85 4.28 4.65 4.40 5.69 4.76	- 50.23 71.38 66.29 22.53 - - 40.74 77.26
5280343 (Quercetin)	**-8.7**	Ser201:HG-----O Ser201:HG-----O Tyr215:HN-----O	2.02 2.22 2.61	130.17 119.81 99.67	Pro151 Phe198 Phe198 Phe198 Arg203:NH2 Arg203 Val302 His305 Phe446	Pi-Alkyl Pi-Pi Stacked Pi-Pi Stacked Pi-Pi T-shaped Pi-Cation Pi-Alkyl Pi-Alkyl Pi-Pi Stacked Pi-Pi T-shaped	5.08 4.60 5.70 4.83 4.29 4.67 4.39 5.69 4.74	- 50.418 71.445 65.295 21.253 - - 40.622 77.174
65064 EGCG	**-8.9**	Phe198:O-----N Ser201:HG-----OC Arg203:HH-----OC Arg203:O-----N Tyr215:HH-----O His305:CE1-----O	2.68 2.42 2.69 2.01 2.71 3.36	112.21 158.90 101.39 165.15 103.38 20.51	Arg203 Phe198 Arg203 Arg203	Pi-Cation Pi-Alkyl Pi-Alkyl Pi-Alkyl	4.02 4.72 5.39 3.99	-

Table 3: Docking-based interaction profiles of flavonoids with glutathione reductase (GR).

Ligand	Binding energy (kcal/mol)	Hydrogen bond interactions			Hydrophobic, electrostatic and other interactions			
		Interactions (Protein-----Ligand)	Distance (Å)	Angle (º)	Interactions (Residues)	Type	Distance(Å)	Angle (º)
5281670 (Morin)	**-7.1**	Lys66:HZ1-----O Val370:OC-----N	1.97 2.88	154.25 140.94	Cso63:SG Lys67 Pro340 Phe372	Pi-Sulfur Pi-Alkyl Pi-Alkyl Pi-Pi T-shaped	4.30 3.93 5.09 5.17	22.44 - - 74.36
5281672 (Myricetin)	**-7.2**	Cso63:OC-----N Lys66:CE-----O Lys66:HZ1-----O	2.31 3.18 1.95	145.16 52.41 159.97	Lys67 Pro340 Phe372	Pi-Alkyl Pi-Alkyl Pi-Pi T-shaped	3.96 5.02 5.20	-
5280343 (Quercetin)	**-7.2**	Lys66:HZ1-----O	1.98	154.44	Cso63:SG Lys67 Pro340 Phe372	Pi-Sulfur Pi-Alkyl Pi-Alkyl Pi-Pi T-shaped	4.32 3.93 5.09 5.16	22.78 - - 74.32
65064 (EGCG)	**-7.4**	Ser30:CO-----N Arg37:HH-----O Arg37:HH-----O Arg37:HH-----O Tyr106:HH-----O	2.67 2.92 2.16 2.84 2.46	163.86 91.40 141.10 131.77 101.66	Ala34 Ile343 Val64 Tyr114	Pi-Alkyl Pi-Sigma Pi-Alkyl Pi-Pi Stacked	5.20 3.96 5.45 5.42	- 21.484 - 59.86

Table 4: Molecular docking interaction summary of flavonoids with superoxide dismutase (SOD).

Ligand	Binding energy (kcal/mol)	Hydrogen bond interactions			Hydrophobic, electrostatic and other interactions			
		Interactions (Protein-----Ligand)	Distance (Å)	Angle (°)	Interactions (Residues)	Type	Distance(Å)	Angle (°)
5281670 (Morin)	**-7.2**	Lys23:HN-----O	2.74	107.57	Glu21 Lys23 Lys23 Lys23 Lys30	Pi-Anion Amide-Pi Stacked Alkyl Alkyl Alkyl	3.71 4.12 5.02 5.13 4.09	25.26 24.77 - - -
5281672 (Myricetin)	**-7.1**	Thr2:HG1-----O Thr2:HG1-----O Glu100:OE2-----H	2.71 2.47 2.69	109.19 127.09 115.61	Lys3 Glu21 Lys23 Lys23 Lys30	Pi-Alkyl Pi-Anion Pi-Sigma Pi-Alkyl Pi-Alkyl	5.30 4.65 3.41 4.89 5.06	- 34.30 4.01 - -
5280343 (Quercetin)	**-7.2**	Lys23:HN-----O Glu100:OE2-----H	2.76 2.18	107.38 147.41	Glu21 Lys23 Lys23 Lys23 Lys30	Pi-Anion Amide-Pi Stacked Pi-Alkyl Pi-Alkyl Pi-Alkyl	3.72 4.11 5.01 5.09 4.05	22.80 25.39 - - -
***65064 (EGCG)**	**-7.7**	Glu21:OE1-----H Gln22:HN-----O Pro28:O-----H	1.95 2.42 2.30	159.55 116.82 131.36	Lys23 Lys23 Lys23 Pro28 Lys30 Lys30 Glu100	Amide-Pi Stacked Pi-Alkyl Pi-Alkyl Pi-Alkyl Alkyl Pi-Alkyl Pi-Anion	4.20 4.44 3.85 4.26 4.10 4.17 4.79	30.667 - - - - - 27.674

Table 5: Predicted physicochemical properties, pharmacokinetics (ADME), and drug-likeness profiles of morin, myricetin, quercetin, and EGCG.

Category	Property	Morin	Myricetin	Quercetin	EGCG
Physicochemical	Molecular Weight (MW)	302.24	318.24	302.24	458.37
	Fraction Csp^3	0	0	0	0.14
	Rotatable Bonds	1	1	1	4
	H-bond Acceptors	7	8	7	11
	H-bond Donors	5	6	5	8
	Molar Refractivity (MR)	78.03	80.06	78.03	112.06
	Topological Polar Surface Area (TPSA)	131.36	151.59	131.36	197.37
	XLOGP3 (logP)	1.54	1.18	1.54	1.17
Absorption	GI Absorption	High	Low	High	Low
Distribution	BBB Permeant	No	No	No	No
	P-gp Substrate	No	No	No	No
Metabolism	CYP1A2 Inhibitor	Yes	Yes	Yes	No
	CYP2C19 Inhibitor	No	No	No	No
	CYP2C9 Inhibitor	No	No	No	No
	CYP2D6 Inhibitor	Yes	No	Yes	No
	CYP3A4 Inhibitor	Yes	Yes	Yes	No
Excretion*(inferred)*	Log Kp (skin permeability, cm/s)	–7.05	–7.4	–7.05	–8.27
Medicinal Chemistry	Lipinski Rule Violations (#)	0	1	0	2
	Bioavailability Score	0.55	0.55	0.55	0.17
	PAINS Alerts (#)	0	1	1	1
	Brenk Alerts (#)	0	1	1	1
	Leadlikeness Violations (#)	0	0	0	1
	Synthetic Accessibility (0–10)	3.25	3.27	3.23	4.20

CONCLUSIONS

This study provides a detailed comparative analysis of the molecular interactions between selected flavonoids and key antioxidant enzymes involved in cellular redox homeostasis. Docking results identified morin, myricetin, and quercetin as effective ligands with moderate to strong binding affinities toward Nrf2-Keap1, CAT, GR, and SOD, comparable to or approaching the performance of the reference antioxidant EGCG. Key active-site residues, including Ala556 (Nrf2), Arg203 (CAT), Lys66 (GR), and Glu21/Lys23 (SOD), were critical for ligand anchoring and stabilization. ADMET evaluations revealed that morin and quercetin possess better oral bioavailability and fewer pharmacological liabilities than EGCG, suggesting a more favorable profile for therapeutic development. Refining the understanding of these interactions not only underscores the mechanistic role of flavonoids in mitigating oxidative stress but also addresses the broader issue of countering cellular damage caused by environmental endocrine disruptors. This highlights the therapeutic relevance of targeting the Keap1-Nrf2-ARE axis and related antioxidant enzymes in oxidative stress-linked reproductive dysfunctions. Further in vitro and in vivo validation studies are warranted to confirm the bioactivity of these

flavonoids under physiological conditions. Additionally, structural modifications or nanoformulation approaches could be explored to enhance the bioavailability and tissue-targeting efficiency of these compounds. Long-term studies may also investigate their potential as preventive agents or adjuvants in the treatment of oxidative stress-induced diseases, particularly in reproductive toxicology and chronic inflammatory disorders.

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