Research Article

# *In Silico* Screening of *Syzygium cumini* Flavonoids against *Eimeria tenella* EtSAG19

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**Abstract** | This study aimed to analyze the *in silico* binding potential of flavonoid compounds from *Syzygium cumini*, including quercetin, myricetin, and apigenin, binding to the Surface Antigen Glycoprotein (EtSAG19) through *in silico* molecular docking combined with pharmacokinetic, biological activity prediction, and toxicity assessments. The molecular docking method was performed using Gnina software. The ligands consisted of quercetin, myricetin, and apigenin, while the receptor used was the EtSAG19 protein with PDB ID 6ZZB. Evaluations were conducted on binding free energy (ΔG) values, interaction types, and predictions for pharmacokinetics, toxicity, and biological activity using SwissADME, ProTox, and PASS Online. Pharmacokinetic analysis indicated that myricetin violated a single criterion of Lipinski's Rule of Five regarding hydrogen bond donors but still showed acceptable absorption properties. Toxicity prediction classified apigenin as relatively non-toxic, while quercetin and myricetin exhibited moderate toxicity potential. Biological activity prediction showed that all flavonoids had a higher probability of activity (Pa) than inactivity (Pi), with Pa values of 0.257, 0.252, and 0.352 for quercetin, myricetin, and apigenin, respectively, confirming their predicted biological potential. The compounds had Pi values of 0.101, 0.107, and 0.035 for quercetin, myricetin, and apigenin. Molecular docking simulations revealed that all flavonoids interacted favorably with the EtSAG19 protein, exhibiting negative binding free energy (ΔG) values. Myricetin and quercetin displayed comparable binding affinities, with nominal ΔG values of -7.98 kcal/mol and -7.61 kcal/mol, respectively, within the binding region. These findings characterize these phytochemicals as preliminary structural binders to the target glycoprotein, serving as baseline models for further evaluation.

**Keywords** | *Eimeria tenella*, Flavonoids, *In silico*, Molecular docking, Surface antigen glycoprotein, *Syzygium cumini*

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## INTRODUCTION

The use of synthetic drugs in coccidiosis therapy for poultry has led to widespread resistance (Abbas *et al.*, 2011). Resistance has been documented against ionophores like monensin (Chapman and Hacker, 1994) and synthetic compounds like toltrazuril (Flores *et al.*, 2022) across multiple countries. Additionally, the presence

of chemical residues in poultry products poses a significant global challenge. Compared to synthetic anticoccidials, natural compounds are generally associated with fewer chemical residues in poultry products, provided they are used under regulated conditions (Elbasuni *et al*., 2024). Therefore, the utilization of medicinal plants serves as a viable solution, particularly those containing bioactive compounds such as flavonoids. *Syzygium cumini* is known to contain natural flavonoids with antioxidant activity that help mitigate parasite-induced oxidative stress in poultry. The presence of specific flavonoids, including quercetin, myricetin, and apigenin in *Syzygium cumini* extracts, has been well documented in previous phytochemical profiling studies (Chagas *et al*., 2018; El-Safy *et al*., 2023). Generally, flavonoids have been shown to target the asexual phase of protozoa, reducing both intestinal invasion and fecal oocyst shedding during *Eimeria* infections (Hascoët *et al*., 2025). Consequently, this study utilizes molecular docking to investigate the binding potential of these specific compounds against EtSAG19, a key surface protein directly involved in that parasite invasion process. The crystal structure of EtSAG19 provides a unified framework for the divergent *Eimeria* SAG family (Ramly *et al*., 2021). Although its role in cell adhesion is not fully understood, this structure enables computational evaluation as a potential target.

## MATERIALS AND METHODS

### Preparation of receptors and test compounds

The *Eimeria tenella* protein EtSAG19 was retrieved from the Protein Data Bank (PDB) with PDB ID 6ZZB. This structure has a high resolution of 1.32 Å. The structural file was downloaded in .pdb format and subsequently prepared using AutoDock Tools version 1.5.7, which involved removing water molecules and non-amino acid ligands to obtain the receptor in its pure state. The test compounds, consisting of the flavonoids quercetin, myricetin, and apigenin, were obtained from the PubChem database. These specific compounds were selected because they are documented as the active flavonoids found in *Syzygium cumini* extracts (Chagas *et al*., 2018; El-Safy *et al*., 2023).

### Pharmacokinetic and toxicity tests

Pharmacokinetic properties were evaluated using the SwissADME web tool. The pharmacokinetic assessment in this analysis utilized Lipinski's Rule of Five as the primary reference to determine the drug-likeness of the candidate compounds. This rule defines the molecular characteristics required for a compound to exhibit favorable pharmacokinetics within the human body. The criteria include a molecular weight ≤ 500g/mol (Dalton), a log P value ≤5, a maximum number of H donors of 5, a maximum number of hydrogen acceptors of 10, and a molar refraction value in the range of 40-130 (Ibrahim *et al*., 2021; Lohit *et al*., 2024). A lower molecular weight, ideally below 500 g/mol, facilitates membrane penetration, thereby enhancing the compound's systemic effectiveness (Lipinski *et al*., 2012). Furthermore, the log P value indicates the level of lipophilicity of a compound (ability to dissolve in fat). Since the target EtSAG19 is a surface antigen that points outwards from the plasma membrane and is readily solvent-accessible, internal parasite membrane penetration is not required for ligand binding (Ramly *et al*., 2021). Toxicity prediction was conducted using the ProTox 3.0 platform, which evaluates compounds based on chemical similarity to established toxicological datasets. The evaluation followed the principle that lower numerical LD50 values represent a lower threshold dose required to induce lethality, thereby indicating a higher acute toxic potential, while higher values suggest a wider safety margin for development as a new drug candidate (Amorim *et al*., 2024).

### Biological activity test

Biological activity was predicted using the PASS Online server. This assessment is based on the concept of Structure-Activity Relationship (SAR), which explores the correlation between the chemical structure of a compound and its specific biological activities. In this test, the probability of activity (Pa) and the probability of inactivity (Pi) serve as the primary references. The results were reported as Pa and Pi values, where a Pa > Pi ratio was considered indicative of potential biological activity. The determination of these values specifically focused on the predicted antiprotozoal (coccidial) activity. The Pa value represents the extent of a compound's potential to exhibit the targeted biological activity, while the Pi value indicates the potential for inactivity. Consequently, a compound possessing a Pa > Pi value suggests that its biological activity is significant and aligns with the expected outcomes (Filimonov *et al*., 2014).

### Molecular docking and visualization

Molecular docking was performed to screen the interaction between ligands and the target protein. The binding free energy (ΔG) value is the main indicator in this assay. By assessing the affinity of the ligand and target protein interaction, a lower ΔG value indicates a higher binding affinity. Thermodynamically, this suggests that the lower the binding free energy (ΔG) during the formation of the ligand-target protein complex, the more stable and favorable the complex becomes (Sandrawati *et al*., 2023). The interaction between ligands and the target protein was analyzed through specific docking using the Gnina 1.0 platform, utilizing its default built-in empirical and Convolutional Neural Network (CNN) scoring function via an NVIDIA Tesla T4 GPU hardware accelerator (Sunseri and Koes, 2021). The configuration parameters were executed via the exact command line: -r 6ZZB_

pure.pdb -l [ligand].pdb --center_x 35.939 --center_y 41.42 --center_z 0.985 --size_x 52 --size_y 48 --size_z 50 --seed 0. Subsequently, the generated conformations were automatically ranked by the platform based on their CNN Pose score, and the specific poses exhibiting the lowest binding free energy (ΔG) values were selected for downstream comparison.

The selected ligands with their predicted target proteins were subsequently visualized using Biovia Discovery Studio software. 2D molecular interaction networks were generated to examine the binding site interactions and specific close contacts in detail. Further visualization analysis aimed to identify the amino acid residues involved in bond formation. These residues play a critical role in establishing contacts between ligands and target proteins, which contributes to inhibitory activity (Zou *et al*., 2023). Through this analysis, differences in structure, test compounds, and their respective biological activities were revealed. The types of interactions identified in the binding region included hydrogen bonds (with a maximum distance cutoff of 3.5 Å), hydrophobic interactions (with a maximum distance cutoff of 5.0 Å), and electrostatic forces, alongside the measurement of bond distances.

## RESULTS AND DISCUSSIONS

### Preparation of receptors

Receptor preparation involved downloading, cleaning, and defining the binding area (grid box) on the target protein. Water molecules and non-amino acid ligands were removed using AutoDock Tools to obtain the receptor in its pure state, as shown in Figure 1a. The binding area was determined by using the EtSAG19 protein structure as a reference to observe the interactions between the test compounds and the active site. This process established a specific grid box area, centered at coordinates x = 35.939, y = 41.42, and z = 0.985, with dimensions of 52 × 48 × 50 Å units (Figure 1b). The grid box parameters were configured to encompass the entire structural framework of the protein, ensuring that all potential interaction surfaces on the target glycoprotein were completely accessible for ligand binding analysis.

### Preparation of test compounds

The flavonoids tested in this study include quercetin, myricetin, and apigenin. The compound structures were downloaded from PubChem in .sdf format, then converted to .pdb format using Discovery Studio Visualizer (Table 1). This conversion was necessary to ensure compatibility with the Gnina platform for the molecular docking process.

### Pharmacokinetic test

The approach used in this analysis is the application of Lipinski's rules/references, which serve as guidelines in assessing the feasibility of compounds based on molecular characteristics as new drug candidates. Parameters analysed include molecular weight, log P, number of hydrogen bond donors and acceptors, and molar refraction values. Based on these results, the three compounds (quercetin, myricetin, and apigenin) fulfilled most of the pharmacokinetic parameters based on Lipinski's rule, but myricetin violated one of Lipinski's rules, namely in the hydrogen bond donor part more than 5 (Table 2).

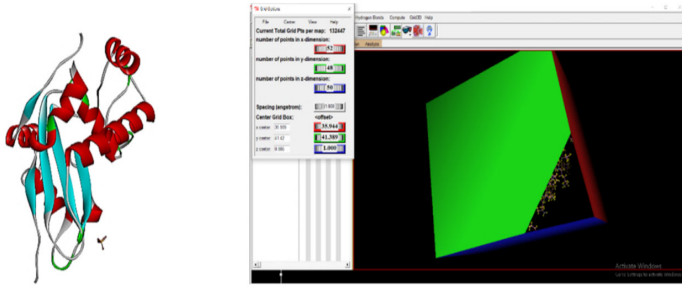



**Figure 1:** (a) Ribbon structure of EtSAG protein, (b) Grid box area for molecular docking.

**Table 1:** Structure of test compounds quercetin, myricetin, and apigenin.

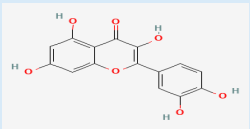
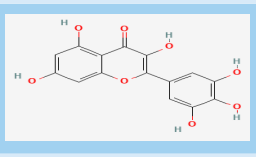
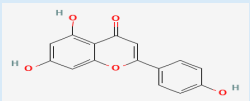
| Test compound | Pubchem code | 2D structure | Reference |
|---|---|---|---|
| Quercetin | 5280343 |  | (National Center for Biotechnology Information, 2025) |
| Myricetin | 5281672 |  | (National Center for Biotechnology Information, 2025) |
| Apigenin | 5280443 |  | (National Center for Biotechnology Information, 2025) |

**Table 2:** Pharmacokinetic test results on quercetin, myricetin, and apigenin compounds.

| Test compound | Molecule weight (g/mol) | Log P | Hydrogen bond donor | Hydrogen bond acceptor | Molar refraction |
|---|---|---|---|---|---|
| Quercetin | 302,24 | -0,56 | 5 | 7 | 78,03 |
| Myricetin | 318,24 | -1,08 | 6 | 8 | 80,06 |
| Apigenin | 270,24 | 0,52 | 3 | 5 | 73,99 |

Pharmacokinetic assessment using the SwissADME platform was conducted to evaluate the drug-likeness of the tested compounds based on Absorption, Distribution, Metabolism, and Excretion (ADME) parameters (Daina

*et al*., 2017). The evaluation referred to Lipinski's Rule of Five, which defines ideal oral drug candidates as compounds with a molecular weight not exceeding 500 g/mol, a logarithmic partition coefficient (log P) of ≤5, no more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, and a molar refractivity ranging from 40 to 130.

Compounds with lower molecular weight are generally more capable of penetrating biological membranes, which enhances their pharmacological effectiveness (Tang *et al*., 2023). As presented in Table 2, quercetin (302.24 g/mol), myricetin (318.24 g/mol), and apigenin (270.24 g/mol) all exhibit comparable molecular weights well within Lipinski's acceptable baseline threshold, suggesting a shared structural potential for favorable membrane permeability rather than a definitive experimental rate.

The evaluated compounds showed negative log P values for quercetin at -0.56 and myricetin at -1.08, indicating a hydrophilic character, while apigenin showed a log P of 0.52. Since the target EtSAG19 is an external surface glycoprotein ectodomain that points outwards from the plasma membrane and is readily solvent-accessible, internal parasite membrane penetration is not required for ligand binding (Ramly *et al*., 2021). This hydrophilic nature is favorable as it ensures solubility within the aqueous environment of the chicken intestinal lumen, enabling direct contact with the exposed target framework without needing to cross the internal parasite pellicle.

Hydrogen bond donors and acceptors are critical determinants of molecular interactions with biological targets and solvents. Apigenin complies with Lipinski's criteria for hydrogen bond donors with three donors, while quercetin meets the exact maximum threshold of five donors. Conversely, myricetin exceeds the parameter limit with six donors. However, all compounds satisfy the hydrogen bond acceptor requirement, with quercetin, myricetin, and apigenin having seven, eight, and five acceptors, respectively.

Molar refractivity, which represents molecular polarizability, also fell within the acceptable range for all compounds. Quercetin (78.03), myricetin (80.06), and apigenin (73.99) met the criteria specified by Lipinski's rule. Overall, although myricetin exhibits a single parameter violation (hydrogen bond donor > 5), it still strictly complies with Lipinski's screening criteria, which explicitly permits a maximum of one violation for drug-likeness selection (Lipinski *et al*., 2012). Therefore, all three compounds can be classified as candidates with favorable pharmacokinetic characteristics.

## Toxicity test

The toxicity prediction of these compounds is very important in the development of new drug designs. The three compounds, namely quercetin, myricetin, and apigenin, were tested for toxicity and then analyzed. Based on the ProTox results presented in Table 3, quercetin and myricetin exhibited an $LD_{50}$ value of 159 mg/kg, placing them in Toxicity Class 3 (toxic if swallowed). In contrast, apigenin showed the highest $LD_{50}$ value at 2500 mg/kg and is predicted to be categorized under Toxicity Class 5 (may be harmful if swallowed).

**Table 3:** Predicted value of toxicity properties in quercetin, myricetin, and apigenin compounds.

| Test compound | $LD_{50}$ (mg/kg) | Toxicity level |
|---|---|---|
| Quercetin | 159 | 3 |
| Myricetin | 159 | 3 |
| Apigenin | 2500 | 5 |

*In silico* toxicity evaluation was performed using the ProTox web server. According to the applied principle, compounds with lower predicted $LD_{50}$ values are considered more toxic, whereas higher $LD_{50}$ values indicate lower toxicity and improved safety profiles (Nursanti *et al*., 2022). ProTox also categorizes compounds based on the Globally Harmonized System (GHS) for chemical classification.

As shown in Table 3, quercetin and myricetin were classified under Class 3 (toxic if swallowed), each with a predicted $LD_{50}$ value of 159 mg/kg body weight. These results suggest that careful consideration is required when determining their dosage and formulation during drug development. In contrast, apigenin was categorized under Class 5 (may be harmful if swallowed) with a substantially higher predicted $LD_{50}$ value of 2500 mg/kg, indicating a wider safety margin.

Since ProTox results are predictive and based strictly on computational models, further validation through in vitro and in vivo toxicity studies is essential before definitive safety conclusions can be established. Within this predictive framework, apigenin demonstrated the lowest toxicity potential, making it computationally safer than quercetin and myricetin. Given these profiles, all three compounds serve as potential candidates for subsequent experimental evaluation and structural optimization to mitigate toxicity risks.

A clear trade-off is observed between binding affinity and predicted safety profiles among the compounds. Apigenin exhibits the widest safety margin categorized under Class 5 with an LD50 of 2500 mg/kg, but demonstrates the weakest binding affinity at -7.12 kcal/mol. Conversely, myricetin and quercetin exhibit stronger thermodynamic

affinities (-7.98 kcal/mol and -7.61 kcal/mol, respectively) but carry moderate toxicity profiles under Class 3 with an LD50 of 159 mg/kg. In early stage design cascades, sufficient receptor interaction is prioritized as the primary selection criterion because low-affinity compounds are therapeutically non-viable, while the moderate toxicity risks of Class 3 candidates can be managed through precise dosage optimization in subsequent validation phases.

## Biological activity test

The results of biological activity prediction using PASSonline are presented in the form of probability of activity (Pa) and inactivity (Pi) values against the Antiprotozoal (Coccidial) activity category. The biological activity test results show that all test compounds, namely quercetin, myricetin, and apigenin, have higher Pa values than Pi, as shown in Table 4.

**Table 4:** Predicted biological activity values of the test compounds quercetin, myricetin, and apigenin.

| Test compound | Pa | Pi |
|---|---|---|
| Quercetin | 0,257 | 0,101 |
| Myricetin | 0,252 | 0,107 |
| Apigenin | 0,352 | 0,035 |

Biological activity prediction was conducted using a Structure-Activity Relationship (SAR) approach, which correlates chemical structure with potential biological effects. The analysis aimed to estimate the anticoccidial activity of the compounds based on their molecular features.

PASS online analysis provides probability values of activity (Pa) and inactivity (Pi). A compound is considered to possess potential biological activity if its Pa value exceeds its Pi value (Filimonov *et al*., 2014). The Pa value represents the likelihood that a compound exhibits a particular biological activity, whereas Pi reflects the probability of inactivity against the same target.

As summarized in Table 4, all tested compounds, namely quercetin, myricetin, and apigenin, showed Pa values greater than their corresponding Pi values. The Pa values were 0.257 for quercetin, 0.252 for myricetin, and 0.352 for apigenin, while the Pi values were 0.101, 0.107, and 0.035, respectively. These findings indicate that all compounds possess predicted biological activity, with apigenin exhibiting the highest probability of activity, suggesting strong anticoccidial potential.

## Molecular docking

The molecular docking results against EtSAG19 are summarized in Tables 5, 6, and 7. Quercetin exhibited a minimum binding free energy (ΔG) of -7.61 kcal/mol at pose 5, with the remaining poses spanning a narrow energy range, indicating a well-defined binding pocket. For myricetin, the lowest ΔG was observed at pose 9 (-7.98 kcal/mol). This selection prioritized the semi-empirical ΔG score as the primary thermodynamic metric, while deep-learning CNN affinity scores served as secondary structural validation. Apigenin demonstrated the weakest binding affinity among the three compounds, with a minimum ΔG of -7.12 kcal/mol at pose 6.

**Table 5:** Result of molecular docking of quercetin compound on grid box area.

| Pose | Affinity (kcal/mol) | Intramol (kkal/mol) | CNN Pose score | CNN affinity |
|---|---|---|---|---|
| 1 | -6.64 | -0.29 | 0.7606 | 4.460 |
| 2 | -6.85 | -0.15 | 0.6865 | 4.321 |
| 3 | -6.48 | -0.31 | 0.6741 | 4.274 |
| 4 | -6.59 | -0.22 | 0.6278 | 4.249 |
| 5 | -7.61 | -0.20 | 0.6267 | 4.303 |
| 6 | -6.08 | -0.29 | 0.5475 | 3.501 |
| 7 | -6.01 | -0.31 | 0.5417 | 4.229 |
| 8 | -5.97 | -0.25 | 0.5263 | 3.954 |
| 9 | -7.05 | -0.52 | 0.5191 | 3.786 |

**Table 6:** Result of molecular docking of myricetin compound on grid box area.

| Pose | Affinity (kcal/mol) | Intramol (kkal/mol) | CNN Pose score | CNN affinity |
|---|---|---|---|---|
| 1 | -6.43 | -0.05 | 0.7731 | 4.047 |
| 2 | -6.38 | -0.14 | 0.6666 | 4.140 |
| 3 | -7.62 | -0.32 | 0.6217 | 4.423 |
| 4 | -6.74 | -0.29 | 0.6201 | 3.710 |
| 5 | -5.94 | -0.34 | 0.5962 | 3.817 |
| 6 | -5.83 | -0.33 | 0.5673 | 4.505 |
| 7 | -6.04 | -0.35 | 0.5609 | 4.434 |
| 8 | -6.36 | -0.43 | 0.5538 | 4.048 |
| 9 | -7.98 | -0.32 | 0.5301 | 4.025 |

**Table 7:** Result of molecular docking of apigenin compound on grid box area.

| Pose | Affinity (kcal/mol) | Intramol (kkal/mol) | CNN Pose score | CNN affinity |
|---|---|---|---|---|
| 1 | -5.70 | -0.31 | 0.7786 | 4.518 |
| 2 | -5.77 | -0.27 | 0.7561 | 4.264 |
| 3 | -5.99 | -0.27 | 0.7561 | 4.308 |
| 4 | -5.48 | -0.32 | 0.7342 | 4.633 |
| 5 | -6.69 | -0.30 | 0.7105 | 4.913 |
| 6 | -7.12 | -0.30 | 0.6706 | 4.545 |
| 7 | -5.60 | -0.29 | 0.6016 | 3.979 |
| 8 | -7.08 | -0.27 | 0.5886 | 4.253 |
| 9 | -6.17 | -0.31 | 0.5822 | 3.706 |

Molecular docking studies demonstrated that all ligands successfully interacted with the Surface Antigen Glycoprotein (EtSAG19) receptor within the defined grid box, producing nine binding conformations for each compound. The binding affinity of each ligand–receptor complex was evaluated based on the binding free energy (ΔG), where more negative values indicate stronger and more stable interactions.

Binding energy reflects the cumulative contribution of hydrogen bonding, hydrophobic interactions, and electrostatic forces. Ligand conformations that optimally fit the receptor's binding site typically result in lower ΔG values, indicating enhanced stability of the complex (Tallei *et al*., 2024).

The docking results revealed that quercetin achieved its best binding affinity at pose 5 with a ΔG value of −7.61 kcal/mol. Myricetin exhibited the strongest interaction at pose 9, with a ΔG value of −7.98 kcal/mol. Apigenin showed its most favorable binding at pose 6, with a ΔG value of −7.12 kcal/mol.

## Lowest binding free energy (ΔG) value

Analysis of the results of molecular docking between ligand and receptor is based on the binding free energy (ΔG) value obtained from the docking process using Gnina. In this study, the test and comparison ligands produced various conformations, which were then ranked based on the ΔG value. Based on Table 8, myricetin and quercetin exhibited comparable binding free energies, with myricetin presenting a nominal value of -7.98 kcal/mol and quercetin at -7.61 kcal/mol . Given the standard error margins inherent to computational scoring functions, this nominal difference of 0.37 kcal/mol indicates comparable thermodynamic affinity profiles rather than a definitive biological differentiation.

**Table 8:** The lowest free binding energy (ΔG) Value of the ligand in the grid box area.

| Ligand | Free binding energy value (ΔG) value (kkal/mol) |
|---|---|
| Quercetin | -7.61 |
| Myricetin | -7.98 |
| Apigenin | -7.12 |

Comparative analysis of the docking results indicated that all ligands produced negative ΔG values, suggesting predicted thermodynamic feasibility with the EtSAG19 receptor (Prasetiawati *et al*., 2021). Among the tested compounds, myricetin displayed a nominal binding free energy of −7.98 kcal/mol, suggesting a favorable predicted affinity within this simulation framework. Quercetin followed with a ΔG value of −7.61 kcal/mol, while apigenin exhibited a value of −7.12 kcal/mol.

Based on molecular docking performed using Gnina, myricetin demonstrated a stable ligand–receptor complex within the grid box, indicating its predicted binding potential to the EtSAG19 protein.

## Visualization

Visualization of molecularly tethered interactions using Discovery Studio Visualizer enables mapping of bond positions in two-dimensional form. This approach visually displays interactions such as hydrogen, hydrophobic, and electrostatic bonds.

Hydrogen bonds in the visualization are depicted as dashed lines (Figure 2). These bonds show specific interactions between the hydrogen atoms of the ligand and the target protein. The hydrogen bridges resulting from these bonds help stabilize molecular conformation and enhance binding specificity. Hydrogen bonding is an important factor in the formation of stable molecular complexes due to its specificity and relative stability among non-covalent interactions, despite its non-covalent nature.

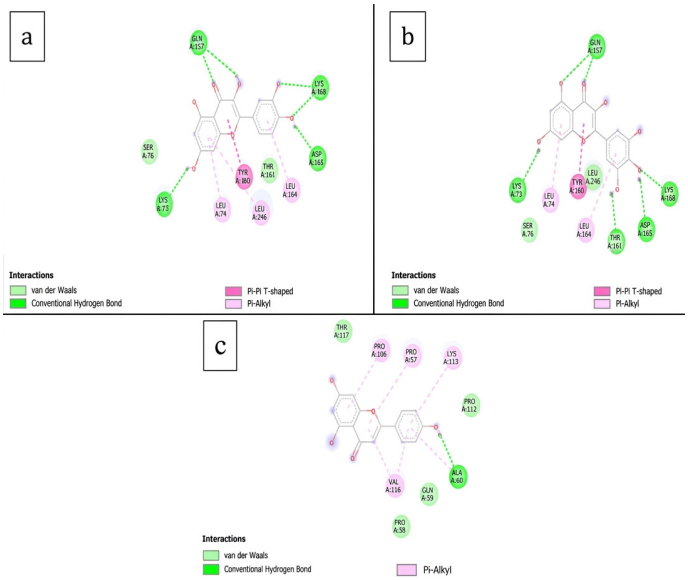



**Figure 2:** 2D molecular interaction networks of (a) quercetin, (b) myricetin, and (c) apigenin within the EtSAG19 binding pocket.

Visualization analysis was conducted to identify amino acid residues involved in ligand–receptor interactions. These interactions, including hydrogen bonding, hydrophobic contacts, and electrostatic forces, are critical contributors to binding stability and inhibitory potential (de Freitas and Schapira, 2017).

Hydrogen bonds, depicted as dashed lines, are observed within the simulated complexes, representing structural components of the binding orientation via 2D molecular

interaction networks (Figure 2). These non-covalent interactions contribute to the calculated thermodynamic profile, alongside enthalpy-driven electrostatic attractions and entropy-driven hydrophobic contacts within the nonpolar regions of the receptor (Arwansyah *et al*., 2014). Given that the scoring function provides a cumulative binding free energy (ΔG), these distinct interactions collectively characterize the predicted alignment within the binding pocket.

The docking visualization revealed distinct interaction patterns for each ligand, explaining differences in binding energy and affinity toward the EtSAG19 receptor.

**Table 9:** 2D visualization analysis of interactions between ligands and the receptor within the grid box area.

| Ligand | ΔG value (kkal/mol) | Hydrogen bond | | Hydrophobic and weak contacts | Electrostatic interaction |
|---|---|---|---|---|---|
| | | AA | Distance (Å) | | |
| Quercetin | -7.61 | Asp165<br>Gln157<br>Lys73<br>Lys168 | 1.98<br>2.46<br>2.32<br>2.25 | Tyr160, Ser76, Thr161 | - |
| Myricetin | -7.98 | Asp165<br>Gln157<br>Lys73<br>Lys168<br>Thr161 | 2.02<br>2.74<br>2.30<br>2.03<br>2.40 | Ser76, Leu246, Tyr160 | - |
| Apigenin | -7.12 | Ala60 | 2.63 | Thr117, Pro58, Pro112, Gln59 | - |

Visualization results (Table 9) showed that myricetin a nominal ΔG value of −7.98 kcal/mol within the simulated pocket framework. This thermodynamic profile was structurally characterized by five independent hydrogen bonds involving Asp165 (2.02 Å), Gln157 (2.74 Å), Lys73 (2.30 Å), Lys168 (2.03 Å), and Thr161 (2.40 Å) acting as distinct directional contacts, alongside hydrophobic interactions with Ser76, Leu246, and Tyr160. Quercetin displayed a comparable nominal binding affinity (ΔG = −7.61 kcal/mol), establishing hydrogen bonds with Asp165, Gln157, Lys73, and Lys168, along with hydrophobic interactions involving Tyr160, Ser76, and Thr161. In contrast, apigenin demonstrated a nominal ΔG value of −7.12 kcal/mol, forming hydrophobic interactions with Thr117, Pro58, Pro112, and Gln59, and a single hydrogen bond with Ala60 (2.63 Å). This weaker binding is directly attributed to the scarcity of directional, high-affinity hydrogen bonds compared to myricetin and quercetin, resulting in lower enthalpic stabilization. Within the high-affinity complexes, the hydrogen bonds formed with residues Asp165 and Lys168 consistently exhibit the shortest geometric distances (1.98–2.25 Å for quercetin and 2.02–2.03 Å for myricetin), indicating stronger electrostatic stabilization and lower susceptibility to thermal disruption at physiological temperatures (Steiner, 2002; Alexandrescu and Dregni, 2024).

Overall, all compounds interacted with residues surrounding the EtSAG19 binding site. Targeting this surface antigen is highly relevant as it represents a major surface glycoprotein expressed during the invasive stages of the parasite (Ramly *et al*., 2021). Inhibiting this protein offers an *in silico* potential to disrupt parasite-host cell interactions. These findings support the potential of *Syzygium cumini* flavonoids as anticoccidial candidates.

Several technical limitations in this screening framework must be acknowledged. While Lipinski's rules and SwissADME metrics serve as conservative physicochemical filters, they are optimized for human parameters rather than avian physiology, necessitating future in vivo poultry validation. Mechanistically, the protocol omitted manual protonation at physiological pH levels and relied on a single deterministic docking run per compound without independent triplicate executions; thus, the outputs function as comparative nominal descriptors rather than statistically distinct indicators. Consequently, these models do not simulate rapid metabolic degradation by avian gut microbiota or host liver enzymes, nor do they establish exact in vivo concentration thresholds. However, because the target EtSAG19 is an extracellular surface protein directly exposed within the intestinal lumen, the predicted low systemic bioavailability of myricetin sustains a local concentration in the gut environment required for physical contact with this external parasite framework. Downstream formulation designs, such as protective nano-encapsulation, remain required to address rapid metabolic clearance and manage predicted Class 3 toxicity risks during subsequent experimental phases.

## CONCLUSIONS

In conclusion, *in silico* profiling indicates that the evaluated flavonoids from *Syzygium cumini* exhibit distinct pharmacokinetic and toxicity profiles, with myricetin displaying a minor violation of Lipinski's Rule of Five and Class 3 predicted toxicity. Computational docking demonstrates that all three compounds possess structural compatibility with the EtSAG19 protein binding pocket, where myricetin presents a nominal binding free energy (ΔG) of -7.98 kcal/mol. These findings characterize these phytochemicals as preliminary structural binders to the target glycoprotein, accurately describing their moderate toxicity profile while highlighting the necessity for strict dosage optimization and safety trials in future formulations to validate their actual anticoccidial efficacy.

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## NOVELTY STATEMENT

This study explores the targeted molecular interactions of *Syzygium cumini* flavonoids with the structure of the EtSAG19 surface antigen glycoprotein of *Eimeria tenella* (PDB ID: 6ZZB) to map potential parasite-host disruption mechanisms. The findings demonstrate the calculated thermodynamic binding affinities, pharmacokinetic profiles, and predicted safety parameters of these natural flavonoids, identifying them as potential non-synthetic structural binding models for future veterinary therapeutics and sustainable poultry management.

## AUTHORS CONTRIBUTION

Lilik Maslachah designed the study, interpreted the data, and drafted the manuscript. Lilik Maslachah and Bayu Apriliadi were involved in the collection of data and also contributed to manuscript preparation. Rimayanti, Lucia Tri Suwanti, Hartanto Mulyo Raharjo, and Ratna Damayanti took part in preparing and critical checking of this manuscript.

### Generative AI and AI assisted technology statement

The authors used artificial intelligence-based tools solely for language polishing and grammar improvement. No AI tools were used in data analysis, interpretation, or the creation of scientific content. All results and interpretations were conducted and verified by the authors.

### Conflict of interest

The authors have declared no conflict of interest.

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