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Molecular docking studies of phytochemicals from *Triumfetta cordifolia* and *Spondias mombin* against COX-1 and COX-2 enzymes

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Abstract. *Amos-Tautua BM, Ajoko IT, Bunu SJ. 2025. Molecular docking studies of phytochemicals from* Triumfetta cordifolia *and* Spondias mombin *against COX-1 and COX-2 enzymes. Asian J Trop Biotechnol 22: 30-40. Triumfetta cordifolia* A.Rich. and *Spondias mombin* Jacq. are plants with a long history of traditional use as anti-inflammatory agents are the subjects of this study. The study employs molecular docking to evaluate the inhibitory potential of phytochemicals identified by GC-MS from the leaf extracts of *T. cordifolia* and *S. mombin* against aspirin-acetylated cyclooxygenase-1 (PDB ID: 3N8Y) and celecoxib-bound COX-2 (PDB ID: 3LN1) using the Schrodinger suite. About six major phytochemical compounds including apigenin, catechin, quercetin, luteolin, kaempferol, and myricetin, were analyzed for their drug likeliness based on Lipinski's rule. All studied phytochemicals complied with Lipinski's Rule of Five, indicating favorable drug-likeness and potential oral bioavailability. Molecular docking results revealed strong binding affinities, with apigenin (-8.66 kcal/mol) and catechin (-8.64 kcal/mol) exhibiting the highest binding scores for COX-1, surpassing diclofenac (-7.20 kcal/mol). Similarly, quercetin (-8.06 kcal/mol) and luteolin (-8.14 kcal/mol) demonstrated strong interactions with COX-2, outperforming aspirin (-6.47 kcal/mol). Ligand-protein interaction analysis confirmed the presence of key hydrogen bonds and hydrophobic interactions, reinforcing the stability of these compounds within the active sites of COX enzymes. These findings underscore the strong inhibitory potential of the phytochemicals from *T. cordifolia* and *S. mombin* possess strong inhibitory potential against COX-1 and COX-2, highlighting their promise as natural anti-inflammatory agents.

Keywords: Anti-inflammatory, cyclooxygenase, *in silico* analysis, phytochemicals bindings, *Spondias mombin*, *Triumfetta cordifolia*

Abbreviations: cLogP: calculated LogP, HAC: Heavy Atom Count, HBA: Hydrogen Bond Acceptors, HBD: Hydrogen Bond Donors, LE: Ligand Efficiency, MW: Molecular Weight, STD: standard compounds

INTRODUCTION

Inflammation is a fundamental biological response to infection, injury, and harmful stimuli, involving complex biochemical and cellular processes. It is also a defense response of the body characterized by pain, redness, heat, swelling, and loss of function (Bandaru et al. 2021). Inflammation is orchestrated by immune cells, cytokines, and lipid mediators, particularly prostaglandins, which play a crucial role in pain and fever regulation (Maddipati 2020). Two forms of COX enzymes have recently been identified (Bandaru et al. 2021). In addition, the cyclooxygenase enzymes (COX-1 and COX-2) are central to prostaglandin synthesis, with COX-1 involved in physiological homeostasis and COX-2 predominantly expressed during inflammation (Ali et al. 2023). Overexpression of COX-2 has been implicated in various chronic diseases, including arthritis, cancer, and cardiovascular disorders (Chen et al. 2021).

The most common Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) used to inhibit COX enzymes and alleviate inflammation are ibuprofen, naproxen, diclofenac, indomethacin, and ketoprofen (Ghosh et al. 2015). These drugs inhibit the expression of cyclooxygenase 2 (COX-2) enzymes responsible for the biosynthesis of prostaglandin E2 (PGE2) (Jemal 2019). However, their long-term use is associated with significant risks, including gastrointestinal ulceration and renal toxicity. These adverse effects are primarily due to the non-selective inhibition of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes, leading to a decrease in protective prostaglandins in the gastrointestinal tract and kidneys (Tai and McAlindon 2021). In addition, these drugs are very expensive. This has driven interest in plant-derived bioactive compounds as safer alternatives with selective COX-2 inhibition, potentially reducing adverse effects. Plant-derived bioactive compounds have emerged as a promising avenue in this regard, offering the potential for selective COX-2 inhibition with fewer adverse effects (Nwankwo et al. 2021; Bunu et al. 2023). Many natural compounds, such as flavonoids, alkaloids, and polyphenols, have demonstrated anti-inflammatory properties by selectively targeting COX-2 while sparing COX-1 (Nasim et al. 2022).

Molecular docking is a widely used computational approach that predicts the binding interactions between small molecules and target proteins, aiding in drug discovery and lead optimization (Agu et al. 2023). The primary objective of ligand-protein docking is to identify

the most probable binding conformation between a ligand and a target protein (Saravanan et al. 2022). These predictions play a crucial role in aiding researchers to design potent drugs capable of effectively interacting with the target protein. Moreover, molecular docking can be used to optimize lead compounds by guiding the rational design of chemical modifications that enhance binding affinity, selectivity, and pharmacokinetic properties (Pinzi and Rastelli 2019; Baba and Bunu 2025). In-silico approaches facilitate the rapid screening of numerous therapeutic candidates within a virtual framework, thereby reducing the need for extensive animal experimentation and addressing associated ethical considerations. Additionally, these computational insights enable the identification of high-potential lead molecules for further confirmation through laboratory-based *in vitro* and *in vivo* investigations (Puspa et al. 2024).

Triumfetta cordifolia A.Rich. and *Spondias mombin* Jacq. are medicinal plants known for together diverse pharmacological properties, including antidiabetic, antioxidant, and anti-inflammatory (Ajaegbu et al. 2022; Ajoko et al. 2023). Phytochemical analysis of these plants has revealed the presence of flavonoids, alkaloids, tannins, phenolic compounds, and terpenoids (Ikponmwosa-Eweka and Omoregie 2024; Ajoko et al. 2020). Previous reports have indicated the possibility of anti-inflammatory properties in *T. cordifolia* and *S. mombin* (Ajoko et al. 2023; Amos-Tautua et al. 2025). However, until now, no detailed molecular docking analysis has been carried out to investigate the therapeutic potential of these plants.

Therefore, this study aims to address this gap by conducting a molecular docking analysis on *T. cordifolia* and *S. mombin* against cox-1 and cox-2 enzymes. To the best of our knowledge, this report represents the first attempt to conduct a molecular docking analysis of these plants with cox-1 and cox-2. The findings will provide valuable insights into their potential as lead compounds for drug development.

MATERIALS AND METHODS

Collection of plant materials

Spondias mombin and *T. cordifolia* leaves were collected from Yenagoa (4°55'36.30" N, 6°16'3.50" E) and Amassoma (4°58'13.15" N, 6°06'32.94" E), respectively, in Bayelsa State, Nigeria (Figure 1). Prof. K.K. Ajibesin identified both plants from the Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Nigeria.

Molecular docking

Six major bioactive compounds (Figure 2) identified by HPLC analysis from *T. cordifolia* and *S. mombin* leaf extracts as earlier reported in our studies (Ajoko et al. 2023; Amos-Tautua et al. 2025) were selected and subjected to molecular docking (*in-silico*) analysis with the X-ray crystal structures of aspirin-acetylated COX-1 (PDB ID: 3N8Y) and celecoxib-bound COX-2 active site (PDB ID: 3LN1) enzymes (Sidhu et al. 2010; Wang et al. 2010), retrieved from the Protein Data Bank (PDB) (https://www.rcsb.org/). Aspirin and diclofenac (anti-inflammatory) were used as standard controls. The ligands and the target protein were prepared using the Maestro Molecular Modeling Suite 2020. The canonical smiles of the bioactive compounds were retrieved from the PubChem database and were converted to a 3-D structure using the LigPrep wizard of Schrödinger's Maestro.

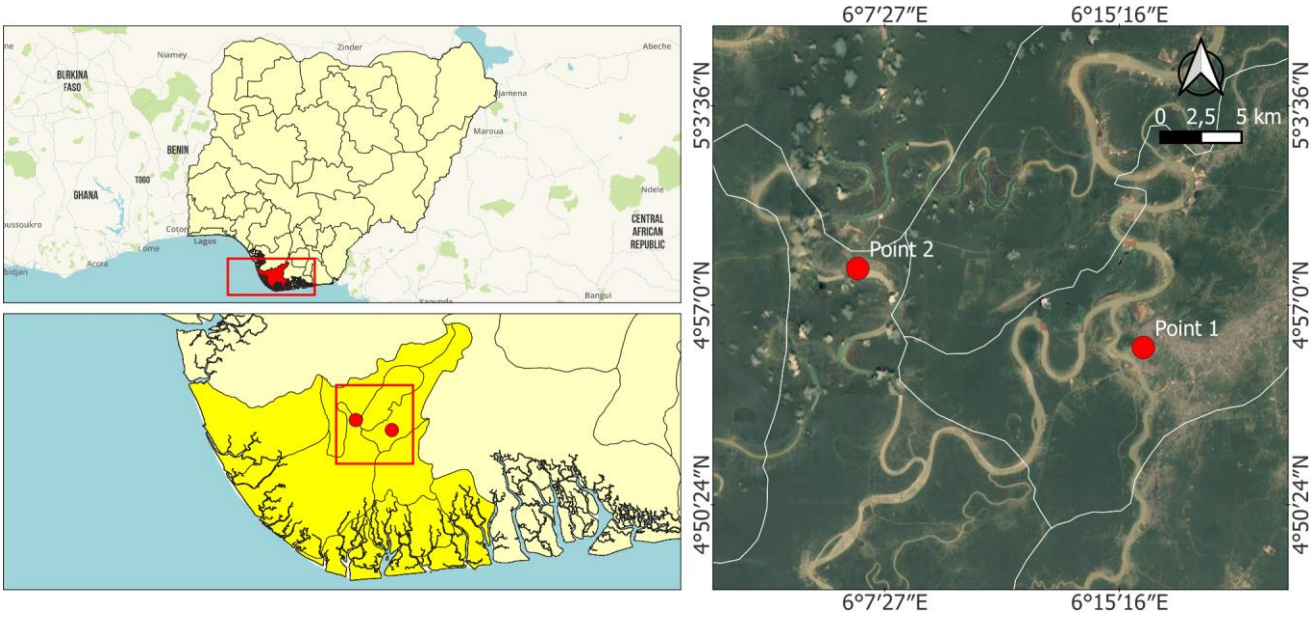

Figure 1. Location of study area in Bayelsa State, Nigeria, i.e., Yenagoa (point 1) indicating the collection site of *Spondias mombin*, and Amassoma (point 2) indicating the collection site of *Triumfetta cordifolia*

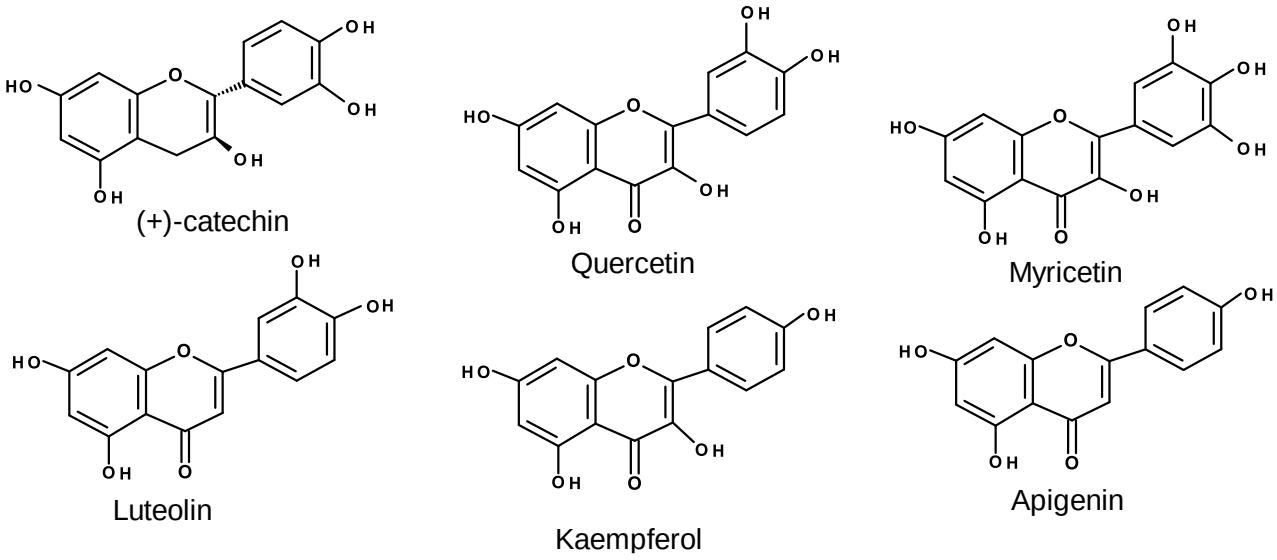

Figure 2. Bioactive compounds identified by HPLC analysis from *Triumfetta cordifolia* and *Spondias mombin* leaf extracts

Preparation of the proteins' crystallographic structures was accomplished using the protein preparation module of Schrödinger's Maestro molecular modeling suite. In this management, bond orders were assigned, water molecules were deleted beyond 5 Å from het groups, and het state was left in default pH (7.0 ± 2.0) using Epik. Lastly, grid generation was done, and the bounding box was set, which can cover the whole target site for docking simulation. After the completion of the pre-requisite steps, the docking simulations were carried out in the SP glide of Schrödinger's Maestro Suite (Maestro 2020). The phytocompounds were selected based on Lipinski's rule of five parameters, such as molecular weight, LogP, number of hydrogen bond donors, number of hydrogen bond acceptors, and HAC (Table 1).

RESULTS AND DISCUSSION

Drug-likeness analysis of compounds

Drug-likeness characteristics play a crucial role in assessing the quality of emerging anti-inflammatory compounds. Lipinski's Rule of Five is a widely accepted criterion used to assess the drug-likeness of compounds. It evaluates Molecular Weight (MW), Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and calculated LogP (cLogP) to predict oral bioavailability (Haritha 2024). According to Lipinski's 5 rules (Lipinski 2004), an oral drug should have a Molecular Weight (MW) not exceeding 500 mg/mol, hydrogen bond acceptors not exceeding 10, hydrogen bond donors not exceeding 5, and a LogP value less than 5. Generally, a molecule is considered non-oral active if two or more of these rules are broken (Afroz Shoily et al. 2025). Interestingly, in our study, all six compounds from *T. cordifolia and S. mombin* have good drug-likeness parameters since they follow Lipinski's RO5 as indicated in Table 1. This compliance suggests that these compounds possess good pharmacokinetic properties, which is promising for their development as orally active anti-inflammatory agents.

Binding affinity analysis

The binding affinity of phytochemicals to COX-1 (3N8Y) and COX-2 (3LN1) was evaluated using molecular docking scores. The docking results were represented in the form of e-negative values (Table 2). In the docking studies, higher negative e-values represent high binding affinity between the receptor and ligand molecules, indicating the higher efficiency of the bioactive compounds (Jemal 2019). As shown in Table 2, all the phytochemicals except myricetin displayed stronger binding than the standards diclofenac (-7.20 kcal/mol) and aspirin (-5.71 kcal/mol for COX-1 enzyme. Similarly, for 3LN1, all the phytochemicals, including myricetin, outperformed aspirin (-6.47 kcal/mol).

These results suggest that certain phytochemicals could act as potential COX inhibitors, potentially outperforming conventional NSAIDs. These results highlight that phytochemicals, particularly apigenin, catechin, quercetin, and luteolin, exhibit superior binding affinity, suggesting their potential use as natural anti-inflammatory agents. The interaction with COX-2, a preferred target for selective inhibition to reduce gastrointestinal side effects, also suggests that luteolin and quercetin may have favorable pharmacological properties (Rouzer and Marnett 2020). The high binding affinity of these compounds suggests that they may interfere with the enzymatic activity of COX-1 and COX-2, thereby reducing inflammation through prostaglandin inhibition (Verma et al. 2020). Furthermore, the interaction profiles of these phytochemicals indicate their potential to serve as selective COX-2 inhibitors, minimizing the adverse effects commonly associated with non-selective NSAIDs. The docking results of bioactive compounds are shown in Figures 3, 5, 7, and 9.

Table 1. Lipinski's properties of the selected phytocompounds from extracts of *Triumfetta cordifolia* and *Spondias mombin*

Ligand	PubChem ID	MW	HBA	HBD	cLogP	HAC
Aspirin	2244	286.2	4	1	0.52	13
Diclofenac	3033	296.10	3	2	3.67	19
Apigenin	5280443	270.24	5	3	3.07	20
Catechin	9064	290.27	6	5	1.92	21
Kaempferol	5280863	286.20	6	4	2.80	21
Luteolin	5280445	286.20	6	4	2.80	21
Myricetin	5281672	318.20	8	6	2.26	23
Quercetin	5280343	302.20	7	5	2.53	22

Table 2. Docking scores and ligand efficiency of selected phytochemicals and standards on COX-1 and COX-2

Ligand	COX-1(3N8Y)		COX-2 (3LN1)	
	Docking Score (kcal/mol)	Ligand Efficiency	Docking Score (kcal/mol)	Ligand Efficiency
Aspirin	-5.71	-0.44	-6.47	-0.50
Diclofenac	-7.20	-0.38	-8.20	-0.43
Apigenin	-8.66	-0.43	-7.40	-0.37
Catechin	-8.64	-0.41	-7.75	-0.37
Kaempferol	-8.24	-0.39	-7.38	-0.35
Luteolin	-7.33	-0.35	-8.14	-0.39
Myricetin	-6.28	-0.27	-6.54	-0.28
Quercetin	-7.63	-0.35	-8.06	-0.37

Ligand efficiency of standards and phytochemicals

Ligand efficiency is a measurement of the binding energy per atom of a ligand to its binding partner, such as a receptor or enzyme (Hopkins et al. 2014). A "good" ligand efficiency is normally counted to be about -0.3 kcal/mol per heavy atom. This underscores that a molecule with a higher LE value achieves a substantial level of binding affinity relative to its molecular size, making it a more needed lead compound in drug discovery. The ligand efficiency values for the studied phytochemicals and standard drugs are presented in Table 3. The LE values were calculated using the Equation 1:

$$\text{LE - Ligand Efficiency (kcal/atom)} = \frac{\text{Docking Score (kcal/mol)}}{\text{HAC}}$$

The docking scores and ligand efficiency values that reveal important trends in the binding interactions between selected phytochemicals and standard drugs (aspirin and diclofenac) with COX-1 (3N8Y) and COX-2 (3LN1) enzymes are presented in Table 2. Aspirin demonstrates moderate binding affinity (-5.71 for COX-1 and -6.47 for COX-2), which aligns with its established mechanism of reversible COX-1 inhibition and irreversible COX-2 acetylation. In contrast, diclofenac shows significantly stronger binding (-7.20 for COX-1 and -8.20 for COX-2), consistent with its well-documented potency as a COX-2-preferential anti-inflammatory agent (Na'imah 2019; Bunu et al. 2023). Among the phytochemicals, apigenin and catechin exhibit particularly high docking scores for COX-1 (-8.66 and -8.64, respectively), indicating robust binding interactions. These computational findings are supported by experimental studies demonstrating the COX-1 inhibitory activity of such flavonoids (Ribeiro et al. 2015). Similarly, luteolin and quercetin display notable binding affinity for COX-2 (-8.14 and -8.06, respectively), reinforcing their potential as COX-2 inhibitors, as evidenced by previous research on their anti-inflammatory properties (Li et al. 2016).

The ligand efficiency metric, which accounts for molecular size, offers valuable insights into drug-like characteristics. While diclofenac shows stronger absolute binding, its ligand efficiency (-0.38 for COX-1, -0.43 for COX-2) is lower than that of aspirin (-0.44, -0.50), reflecting its larger molecular structure. Apigenin demonstrates well-balanced ligand efficiency (-0.43 for COX-1, -0.37 for COX-2), suggesting it as a promising lead compound. In contrast, myricetin's relatively low ligand efficiency (-0.27 to -0.28) may be attributed to its higher molecular weight and polarity, factors known to limit bioavailability potentially. The data reveal distinct selectivity patterns: most phytochemicals, including apigenin and catechin, preferentially bind COX-1, while luteolin and quercetin show greater affinity for COX-2. This variability in selectivity among flavonoids has been previously documented (Yang et al. 2023) and mirrors the COX-2 preference observed with diclofenac, a clinically used COX-2 preferential NSAID. Additional support comes from studies linking apigenin COX-1 inhibition to antiplatelet effects (Zaragozá et al. 2022) and quercetin's COX-2 binding to macrophage suppression (Xiao et al. 2011), both validating the current docking results.

On the other hand, kaempferol and luteolin displayed moderate ligand efficiency, suggesting they could serve as promising drug candidates with further structural optimization. Higher ligand efficiency values indicate that a compound binds strongly with minimal structural complexity, making it a preferable candidate for further drug development. Apigenin and catechin, which showed relatively high ligand efficiency, could be explored for structural modifications to enhance their specificity and potency against COX-2, reducing off-target effects typically associated with NSAIDs (Listyani et al. 2024).

Ligand-protein amino acid interactions with 3N8Y and 3LN1

The affinity between proteins and ligands is fundamental to many biological processes, as it dictates molecular recognition through specific physical and chemical interactions (Akhtar et al. 2024). The results of the molecular interactions of ligands (*T. cordifolia* and *S. mombin* leaf extract compounds) with COX-1 and COX-2 are presented in Tables 3 and 4. The binding interactions include hydrogen bonding, hydrophobic interactions, polar contacts, and π-π stacking, all of which contribute to ligand stability within these enzymes' active sites.

Table 3 shows the ligand-protein amino acid interactions with COX-1. The 2D structures of Figures 3 and 5 show all forms of interactions, while the 3D interactions (Figures 4 and 6) show the H-bonding interactions with respective receptor (protein) residues. This can be compared with the standard, along with binding affinity (docking scores). The COX-1 standard NSAIDs, aspirin and diclofenac, did not

exhibit hydrogen bonding interactions but relied predominantly on hydrophobic interactions, particularly with residues such as VAL-116, LEU-352, and ALA-537. The strong hydrophobic interactions observed with the phytochemicals, especially apigenin and catechin, indicate their ability to fit into the COX-1 active site and possibly act as competitive inhibitors (Shahwan et al. 2023). Furthermore, π–π stacking interactions were observed in catechin and luteolin with TYR-385 and TYR-355. Catechin, luteolin, and myricetin exhibit hydrogen bond, hydrophobic bond, polar, and π-π stacking interactions, whereas apigenin, kaempferol, and quercetin exhibit only hydrogen bond, hydrophobic bond, and polar interactions. Apigenin and kaempferol formed hydrogen bonds with MET-522 and TYR-385, both of which are crucial for the catalytic function of COX-1. Similarly, myricetin exhibited the highest number of hydrogen bonds, interacting with TYR-385, TYR-355, and ARG-120, suggesting enhanced stability within the enzyme's active site. Catechin formed a hydrogen bond with MET-522, and quercetin also formed hydrogen bonds with MET-522, TYR-355, reinforcing their potential to disrupt the enzyme's activity.

Table 3. Ligand-protein amino acid interactions with COX-1 (3N8Y)

Ligand	Type of interaction & protein residues			
	H-bonding	Hydrophobic	Polar	π–π stacking
Aspirin	-	VAL-116, LEU-352, LEU-359, ALA-537, LEU534, TRP-387	SER-353, SER-530	-
Diclofenac	-	VAL-116, LEU-534, ALA-527, MET-522, LEU-531, TYR-355	SER-353, SER-530	-
Apigenin	MET-522, TYR-385	ILE-523, LEU-359, VAL-116, ALA-527	SER-353, SER-530	-
Catechin	MET-522	MET-113, LEU-531, ALA-527, TYR-355	SER-353, SER-530	TYR-385
Kaempferol	TYR-385, MET-522	ALA-527, ILE-532, VAL-116, LEU-531	SER-353, SER-530	-
Luteolin	TYR-385	LEU-359, VAL-349, VAL-116, PHE-516	SER-353, SER-530	TYR-355
Myricetin	TYR-385, TYR-355, ARG-120	LEU-352, VAL-349, LEU-534, PHE-205, ALA-527, ILE-523	SER-353, SER-530	TYR-355
Quercetin	TYR-385, MET-522	VAL-116, LEU-531, ALA-527, PHE-518	SER-353, SER-530	-

Table 4. Ligand-protein amino acid interactions with COX-2 (3LN1)

Ligand	Type of interaction and protein residues				
	H-bonding	Hydrophobic	Polar	π–π Stacking	π– Cation
Aspirin	-	LEU-338, VAL-335, MET-508, VAL-509, ALA-513, LEU-517	SER-339, SER-516	-	-
Diclofenac	TYR-371	PHE-504, MET-508, VAL-509, ALA-513, LEU-517, VAL-335	SER-339, SER-516	TRP-373	-
Apigenin	ARG-106	LEU-517, ALA-513, VAL-335, ALA-502	SER-339, SER-516, HIE-75, GLN-178	-	-
Catechin	LEU-338	TYR-341, VAL-102, LEU-345, VAL-335	SER-339, SER-516, HIE-75, GLN-178	-	ARG-106
Kaempferol	-	VAL-509, ALA-502, LEU-517, VAL-335	SER-339, SER-516, HIE-75, GLN-178	-	ARG-106
Myricetin	LEU-338	TYR-341, VAL-335, ALA-513, LEU-517	SER-516, GLN-178, SER-339, -HIE-75	-	-
Luteolin	LEU-338	TYR-341, VAL-335, ALA-513, LEU-517	SER-339, SER-516, HIE-75, GLN-178	-	ARG-106
Quercetin	LEU-338	TYR-341, ILE-503, VAL-335, LEU-517	SER-339, SER-516, HIE-75, GLN-178	-	ARG-106

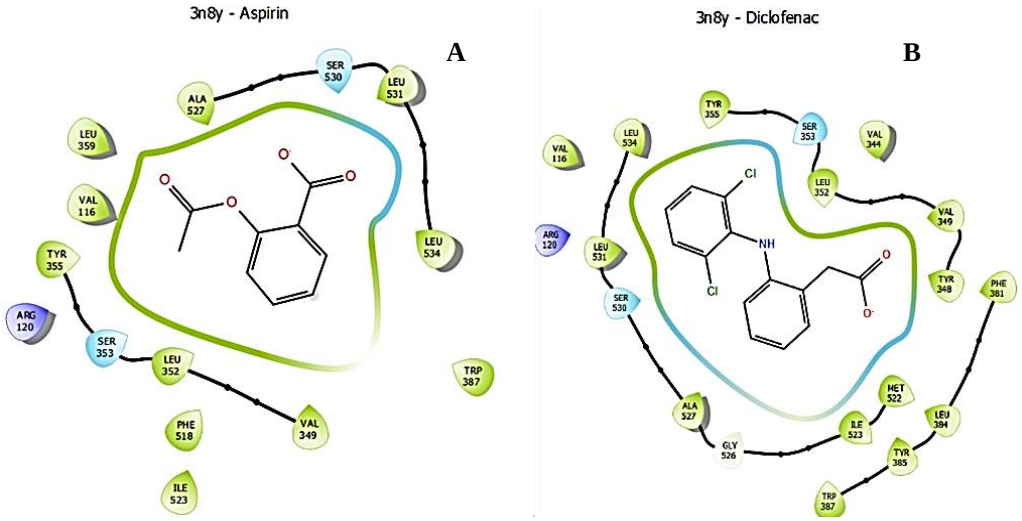

Figure 3. 2D interactions of standard compounds with 3N8Y. A. Aspirin, B. Diclofenac

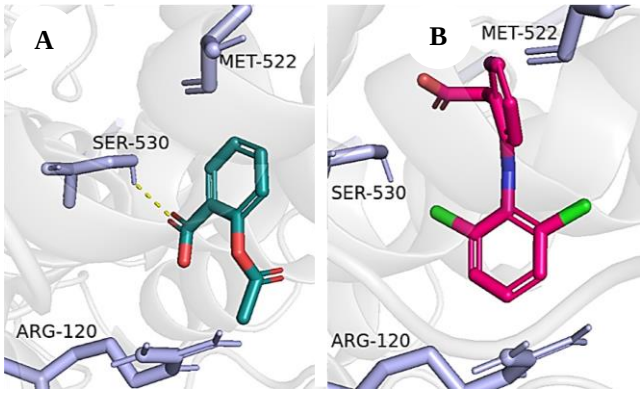

Figure 4. 3D interactions with COX-1(3N8Y). Standard compounds: A. Aspirin; B. Diclofenac

Table 4 shows the ligand-protein amino acid interactions with COX-2 (3LN1). The 2D structures of Figures 7 and 9 show all forms of interactions, while the 3D interactions (Figures 8 and 10) show the H-bonding interactions with respective receptor (protein) residues. Aspirin exhibited strong hydrophobic interactions with amino residues such as LEU-338, VAL-335 and MET-508 but lacked hydrogen bonding, which may explain its relatively weaker binding affinity. Diclofenac, in contrast, formed a hydrogen bonding with TYR-371 and a π-π stacking interaction with TRP-373, contributing to its enhanced stability within the active site. Among the phytochemicals, apigenin interacted with ARG-106 via hydrogen bonding, as well as hydrophobic interactions with LEU-517, ALA-513, and VAL-335. Similarly, catechin engaged in multiple hydrophobic interactions with TYR-341, VAL-102, and LEU-345, alongside a unique π-cation interaction with ARG-106, enhancing its binding stability.

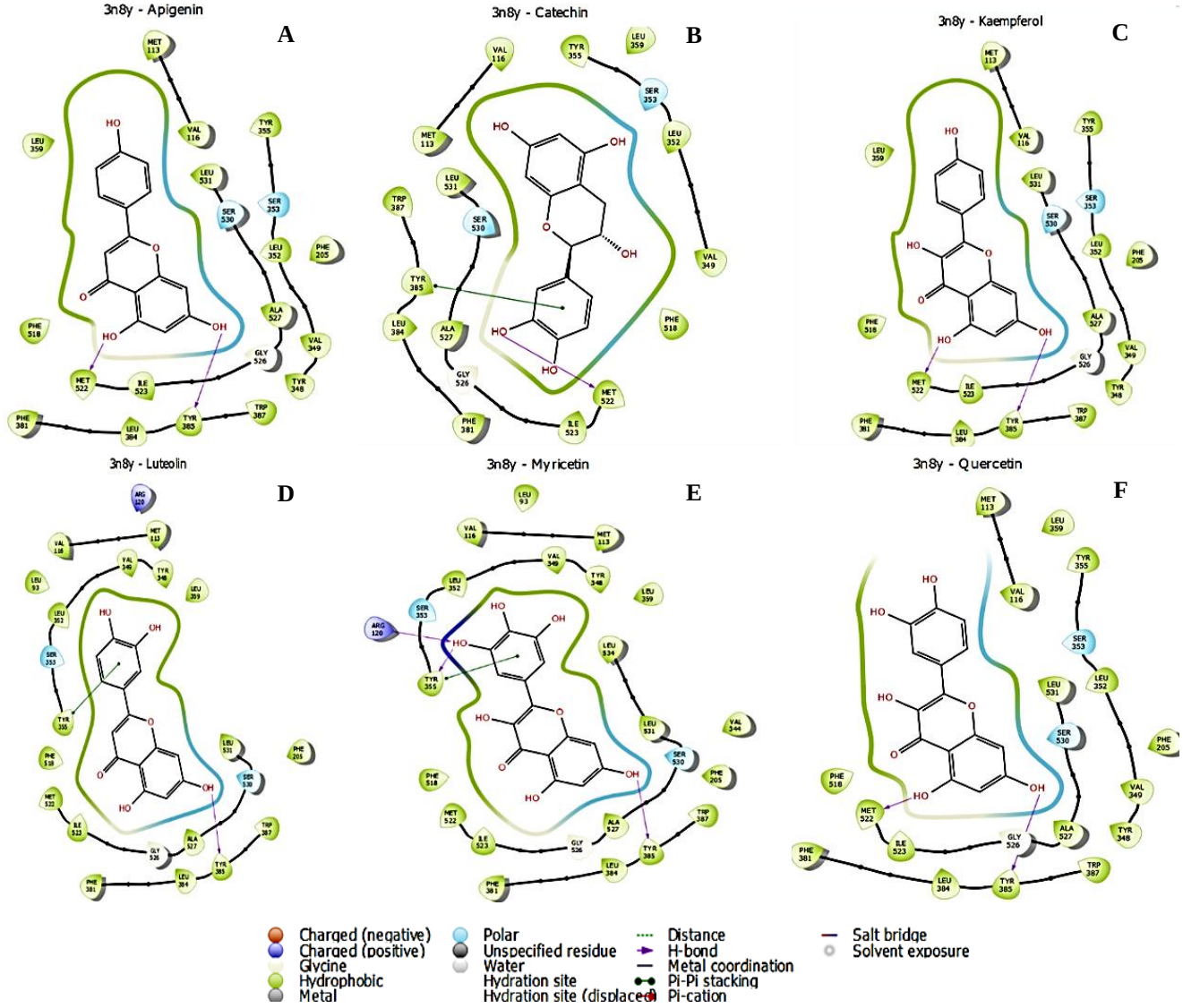

Figure 5. 2D interactions of phytochemicals present in both plants with COX-1(3N8Y). A. (apigenin, catechin, kaempferol, luteolin, myricetin, quercetin)

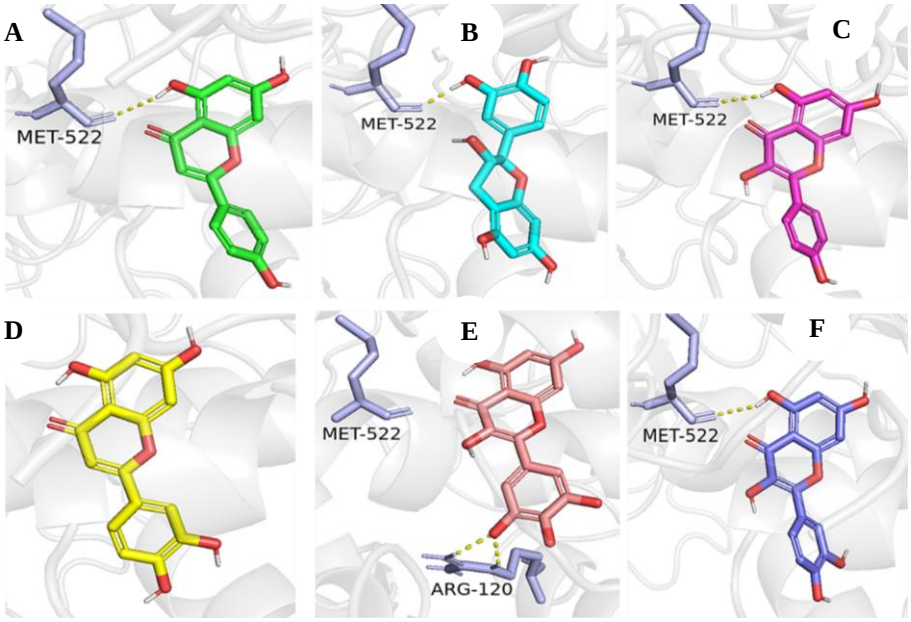

Figure 6. 3D interactions of phytochemicals present in both plants with COX-1 (3N8Y). A. Apigenin; B. Catechin; C. Kaempferol; D. Luteolin; E. Myricetin; F. Quercetin

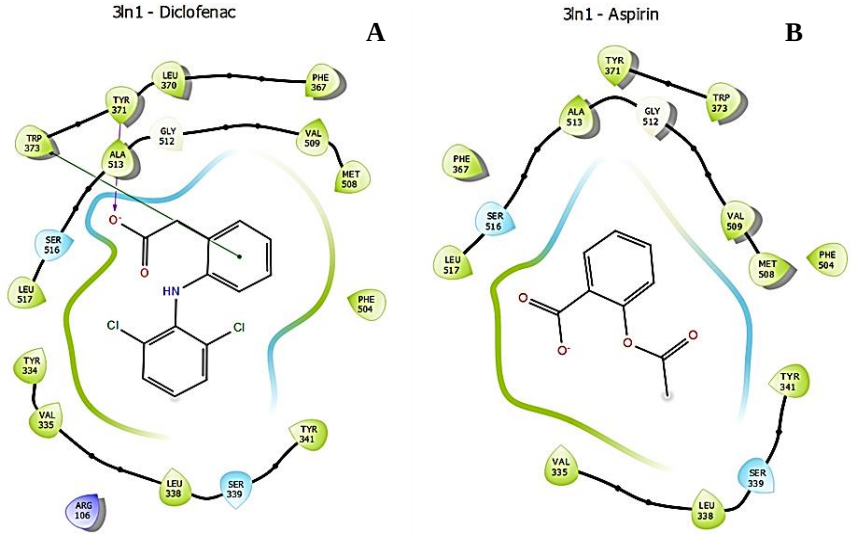

Figure 7. 2D interactions of standard compounds with COX-2 (3LN1)

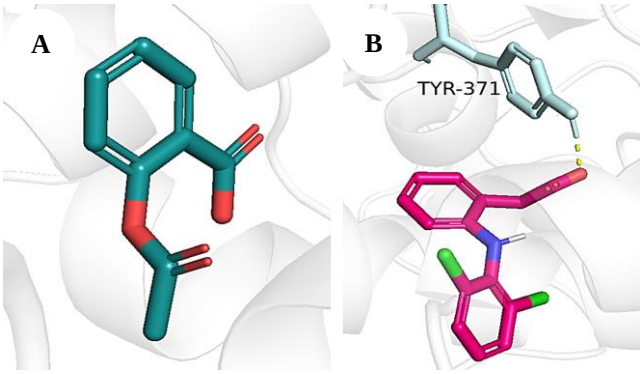

Figure 8. 3D interactions with COX-2 (3LN1). Standard compounds: A. Aspirin; B. Diclofenac

Hydrophobic synergy is the main aspect of the firmness of proteins. Hydrogen bonding further maintains protein firmness, yet to a minimized degree than hydrophobic synergy (Bandaru et al. 2021). The presence of strong hydrophobic interactions and hydrogen bonding in both COX-1 and COX-2 binding studies suggests that these phytochemicals have high affinity for their target enzymes. In addition, analysis of the binding interactions of these phytochemicals and standard NSAIDs suggests that myricetin demonstrates the highest potential for COX inhibition due to its extensive hydrogen bonding and hydrophobic interactions. Apigenin and catechin also exhibited strong interactions with key COX-1 and COX-2 residues, suggesting their dual-inhibition potential. The ability of these compounds to engage in multiple interaction types, including π–π stacking, hydrophobic contacts, and hydrogen bonding, reinforces their suitability as alternative anti-inflammatory agents (Deogratias et al. 2022; Prohens et al. 2025).

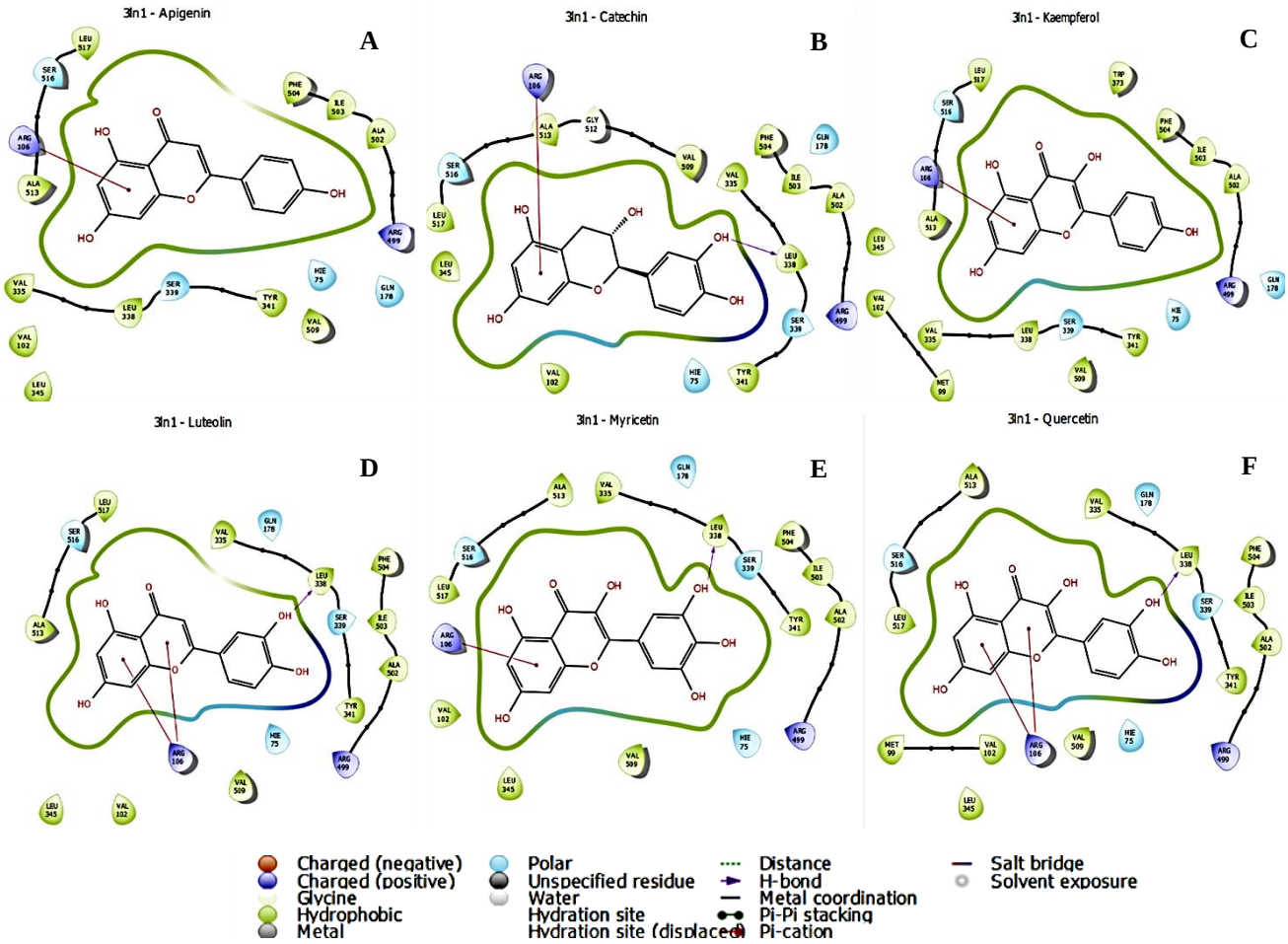

Figure 9. 2D interactions of phytochemicals present in both plants with COX-2 (3LN1). A. Apigenin, B. Catechin, C. Kaempferol, D. Luteolin, E. Myricetin, F. Quercetin

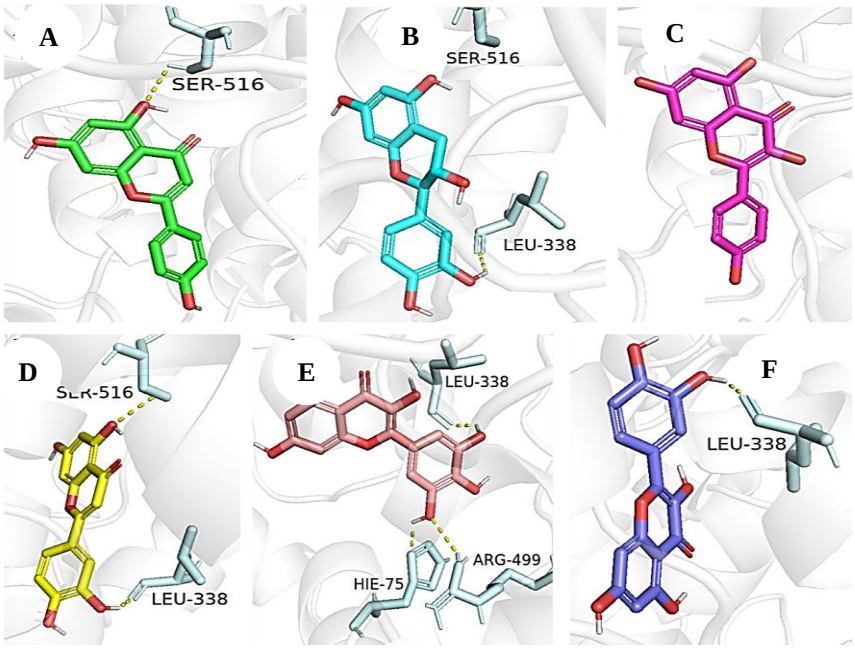

Figure 10. 3D interactions of phytochemicals present in both plants with COX-2 (3LN1). A. Apigenin; B. Catechin; C. Kaempferol; D. Luteolin; E. Myricetin; F. Quercetin

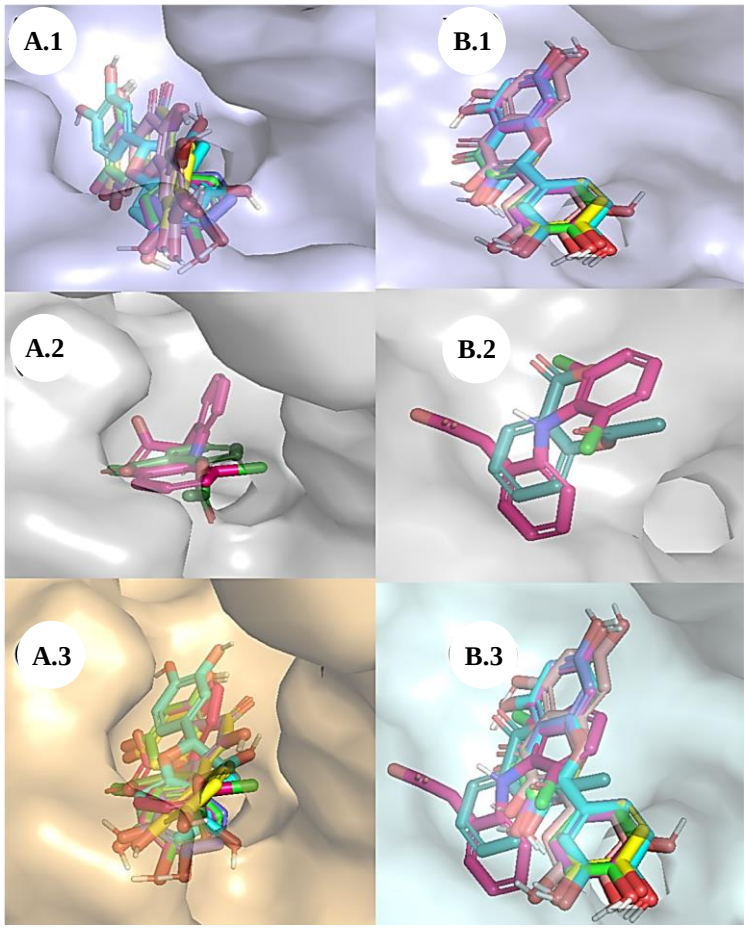

Figure 11. Binding modes with COX-1 (3N8Y). A.1. Phytochemicals: apigenin, catechin, kaempferol, luteolin, myricetin, quercetin; A.2. Standard compounds (aspirin, and diclofenac); A.3. Combined binding pose. Binding modes with COX-2 (3LN1), B.1. Phytochemicals; apigenin, catechin, kaempferol, luteolin, myricetin, quercetin; B.2. Standard compounds: aspirin and diclofenac; B.3. Combined binding poses

Figure 11 illustrates the interaction patterns of selected phytochemicals and reference compounds with the two COX enzyme structures (3N8Y and 3LN1). For COX-1 (3N8Y), phytochemicals such as apigenin, catechin, kaempferol, luteolin, myricetin, and quercetin are predicted to bind at the enzyme's active site, forming hydrogen bonds with key residues (Arg120, Tyr355, Ser530) essential for COX-1 inhibition. Notably, quercetin and myricetin interact with the catalytic Tyr385, interfering with arachidonic acid metabolism (Bai and Zhu 2010). Meanwhile, apigenin and kaempferol bind near the hydrophobic region (Val523, Leu352), resembling the mechanism of selective COX-1 inhibitors like celecoxib (Mandery et al. 2010). Research supports that these flavonoids act as competitive inhibitors, with quercetin exhibiting stronger binding due to its additional hydroxyl groups (Wang et al. 2020). In contrast, aspirin inhibits COX-1 irreversibly by acetylating Ser530, targeting the enzyme's channel entrance (Roth et al. 2020), while diclofenac binds to Arg120/Tyr355, mimicking arachidonic acid (Giménez-Bastida et al. 2019), with its carboxylate group playing a key role in ionic interactions. The combined binding pose suggests a potential synergistic effect, where phytochemicals like quercetin and diclofenac may simultaneously block catalytic and allosteric sites, enhancing inhibition.

For COX-2 (3LN1), a mutated form of the enzyme, binding affinities may vary. For example, luteolin exhibits stronger interactions due to increased conformational flexibility (Song et al. 2022), while catechin (being less polar than myricetin) favors hydrophobic subsites like Leu384. Aspirin shows reduced efficacy in 3LN1 because of Ser530 mutations (Giménez-Bastida et al. 2019), and diclofenac maintains binding but with diminished affinity due to disrupted salt bridges in the altered active site (Sharma et al. 2012). The combined binding strategy suggests that pairing kaempferol with aspirin could help overcome mutation-induced resistance. This analysis highlights the distinct binding mechanisms of phytochemicals and standard drugs, emphasizing their potential for synergistic COX-2 inhibition and drug resistance mitigation.

Interactions of flavonoids with COX-1 and COX-2

Flavonoids are a diverse class of polyphenolic compounds present in fruits, vegetables, and medicinal plants, and have gained considerable attention for their anti-inflammatory properties. Their effects are primarily attributed to interactions with cyclooxygenase enzymes, COX-1 and COX-2, which play a crucial role in

prostaglandin synthesis and the regulation of inflammation. Studies have demonstrated that specific flavonoids inhibit both COX-1 and COX-2 enzymes, leading to a decrease in pro-inflammatory prostaglandin production. For instance, Ribeiro et al. (2015) reported that flavonoids possessing a catechol moiety effectively inhibit COX enzymes and modulate cytokine production in human whole blood. Their findings indicated that flavonoids containing a catechol group in the B ring were particularly effective in simultaneously inhibiting the production of inflammatory prostaglandin E_2 (PGE_2) and pro-inflammatory cytokines, emphasizing the importance of specific structural features in their anti-inflammatory efficacy. This suggests that specific structural features, such as the catechol moiety, are critical determinants of their anti-inflammatory efficacy. Further studies have highlighted that the presence of a 4-oxo group in the C-ring, along with a 3′,4′-dihydroxy (catechol) structure in the B-ring, enhances the suppression of COX-2 transcriptional activity. These structural attributes contribute to the flavonoids' ability to modulate inflammatory responses effectively (Shamsudin et al. 2022). Beyond direct enzymatic inhibition, flavonoids such as nobiletin, amentoflavone, and apigenin have been observed to modulate COX-2 gene expression in various cellular systems. This dual ability to suppress COX-2 expression and inhibit its enzymatic activity underscores the multifaceted role of flavonoids in modulating inflammatory responses. The modulation of cyclooxygenase-2 (COX-2) expression by flavonoids is indeed context-dependent, varying with experimental conditions. A study by Chagas et al. (2022) investigated the effects of nine flavonoids on COX-2 expression in intestinal epithelial cells (IEC18). Under basal conditions, certain flavonoids increased COX-2 expression and activated NF-κB-dependent gene transcription. However, upon lipopolysaccharide (LPS) stimulation, the impact of flavonoids on COX-2 levels varied: some increased, others decreased, and some had no effect. This variability underscores the complex interactions between flavonoids and inflammatory pathways, influenced by the specific flavonoid structure and the cellular environment (Chagas et al. 2022). The structural characteristics of flavonoids significantly influence their interaction with cyclooxygenase (COX) enzymes, particularly in their anti-inflammatory activities. However, it's important to note that not all flavonoids uniformly inhibit COX enzymes. Some, such as myricetin and quercetin, have been reported to stimulate COX activity under certain conditions, indicating that the interaction between flavonoids and COX enzymes can be complex and context-dependent (D'Ambrosio et al. 2021). Hence, flavonoids' anti-inflammatory efficacy is closely linked to their structural characteristics, particularly the presence of catechol groups and other functional moieties that influence their interaction with COX enzymes.

In conclusion, the findings from this study highlight the potential of phytochemicals from *T. cordifolia* and *S. mombin* as promising COX-1 and COX-2 inhibitors. All tested compounds complied with Lipinski's Rule of Five, indicating their suitability for oral administration with favorable pharmacokinetic properties. Molecular docking results revealed that apigenin, catechin, quercetin, and luteolin exhibited strong binding affinities to COX-1 and COX-2, in some cases outperforming the standard NSAIDs, as aspirin and diclofenac. The ligand efficiency analysis further suggested that apigenin and catechin could serve as lead compounds due to their optimal balance between molecular size and binding affinity. These phytochemicals interacted with key residues via hydrogen bonding, hydrophobic forces, and π-π stacking, enhancing their stability within the enzyme active sites. These findings suggest that phytochemicals from *T. cordifolia* and *S. mombin* possess strong inhibitory potential against COX-1 and COX-2, highlighting their promise as natural anti-inflammatory agents. The need for further *in vitro* and *in vivo* studies is recommended to confirm their pharmacological efficacy and therapeutic potential.

While docking scores provide valuable insights into molecular interactions, they cannot fully predict in vivo effectiveness because they do not account for critical factors such as metabolic stability and cellular uptake. To verify these computational results, further experimental studies, such as enzymatic inhibition assays, are essential. These findings indicate that phytochemicals, particularly apigenin and quercetin, exhibit strong potential as COX inhibitors, with binding efficiencies rivaling conventional drugs. Further research into their selectivity could pave the way for more precise anti-inflammatory treatments.

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