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In Silico Study of Bioactive Compounds from *Acalypha indica* L. Interacting with the COX-2 Receptor as Potential Anti-Inflammatory Candidates

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Abstract

Acalypha indica L. is a medicinal herb traditionally used across Asia for treating inflammation-related conditions. Although several studies report anti-inflammatory activity in its extracts, little is known about the molecular interaction of its individual phytochemicals with cyclooxygenase-2 (COX-2)—a validated therapeutic target for inflammatory diseases. This study fills this gap by performing a comprehensive in silico analysis of 20 major bioactive compounds of *A. indica* using molecular docking, binding interaction profiling, and ADMET predictions. Docking against the COX-2 receptor (PDB: 3LN1) using AutoDock Vina revealed that rutin (−10.4 kcal/mol), kaempferol-3-O-rutinoside (−10.1 kcal/mol), quercetin (−9.6 kcal/mol), and luteolin (−9.3 kcal/mol) demonstrated strong predicted affinity and stable interactions with key residues Arg120, Tyr355, and Tyr385, comparable to celecoxib (−10.8 kcal/mol). ADMET profiling showed that aglycone flavonoids possessed more favorable drug-likeness properties than glycosides. These results suggest that *A. indica* contains multiple promising lead compounds for future COX-2 inhibition studies and highlight the molecular mechanisms supporting its ethnomedicinal use as an anti-inflammatory agent.

Keywords: *Acalypha indic*; COX-2; molecular docking; anti-inflammatory; flavonoids.

Abbreviations: Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET), Blood–Brain Barrier (BBB), Cyclooxygenase-2 (COX-2), Cytochrome P450 (CYP); Discovery Studio Visualizer (DSV), Gastrointestinal (GI), Hydrogen Bond (H-bond), High-Performance Computing (HPC), Merck Molecular Force Field 94 (MMFF94), Molecular Mechanics Generalized Born Surface Area (MM-GBSA), Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), Protein Data Bank (PDB), Protein Data Bank, Partial Charges, Torsions (PDBQT), Pharmacokinetics (PK), Topological Polar Surface Area (PSA/TPSA), Python Molecular Graphics Tool (PyMOL), Research Collaboratory for Structural Bioinformatics (RCSB), Root Mean Square Deviation (RMSD), Quantitative Structure–Activity Relationship (QSAR), Structure Data File (SDF), Statistical Package for the Social Sciences (SPSS), AutoDock Vina Docking Software (Vina)

INTRODUCTION

Inflammation is a fundamental biological response that protects the body against harmful stimuli; however, persistent or dysregulated inflammation contributes to the development and progression of numerous chronic diseases, including rheumatoid arthritis, cardiovascular disease, neurodegenerative disorders, metabolic syndromes, and several types of cancer (Chen et al., 2018). One of the key molecular regulators of inflammatory pathways is cyclooxygenase-2 (COX-2), an inducible isoform of the cyclooxygenase enzyme family responsible for catalyzing the conversion of arachidonic acid to pro-inflammatory prostaglandins. COX-2 is upregulated in response to inflammatory signals and cellular stress, making it an important therapeutic target for anti-inflammatory drug development. Clinically, selective COX-2 inhibitors such as celecoxib have been widely prescribed because they provide effective anti-inflammatory activity with fewer gastrointestinal side effects compared to non-selective NSAIDs. However, long-term use of such inhibitors has been associated with increased cardiovascular and thrombotic risks, raising concerns regarding their safety profile and prompting the ongoing search for alternative compounds with improved therapeutic margins (Nissen et al., 2016). In this context, naturally derived phytochemicals offer a promising avenue for the discovery of safer COX-2 modulators with multi-target bioactivity.

Acalypha indica L. (Euphorbiaceae), known locally in Indonesia as “Anting-Anting,” is a medicinal plant widely used in traditional healthcare systems across Asia

and Africa. Historically, it has been applied to treat inflammation-associated conditions such as bronchitis, wounds, rheumatism, and skin infections, suggesting the presence of bioactive secondary metabolites with anti-inflammatory effects (Sharma, 2024). Phytochemical investigations have confirmed that *A. indica* contains diverse classes of compounds, including flavonoids such as quercetin, kaempferol, rutin, and luteolin; phenolic acids; alkaloids; triterpenes such as lupeol; and phytosterols including β-sitosterol (Ghosh et al., 2023). Many of these compounds have shown anti-inflammatory or antioxidant potential in other plant species, particularly flavonoids which are known for their ability to modulate COX-2 expression, inhibit prostaglandin synthesis, and regulate cellular redox signaling (Lee et al., 2010; Mukherjee et al., 2022). Despite this biochemical richness and ethnopharmacological relevance, the molecular mechanism underlying the anti-inflammatory effects of *A. indica* remains poorly characterized, especially at the level of direct interactions between its phytochemicals and COX-2.

The urgency of conducting a molecular-level investigation lies in the fact that most previous studies on *A. indica* have relied primarily on crude extracts, which complicates interpretation of their specific mechanisms of action. Without isolating or characterizing individual compounds, it is difficult to determine which phytochemicals contribute meaningfully to COX-2 inhibition or whether their potential activity stems from synergistic interactions within the extract. Furthermore, no integrative research has systematically mapped the binding behavior of the major bioactive compounds of *A. indica* toward COX-2, despite strong ethnomedicinal claims and initial pharmacological evidence. This gap limits scientific understanding of the plant's therapeutic relevance and hinders rational drug development based on its bioactive metabolites. Computational approaches such as molecular docking offer a rapid, ethical, and cost-effective method for predicting interactions between phytochemicals and biological targets, allowing researchers to identify promising compounds prior to costly laboratory experiments.

Several clear research gaps need to be addressed. First, there is no comprehensive *in silico* screening of *A. indica* phytochemicals against COX-2, even though the plant contains multiple compounds structurally capable of interacting with enzyme active sites. Second, current literature lacks detailed structure–activity insights into how individual compounds bind within the COX-2 catalytic pocket, which is crucial for understanding their inhibitory potential. Third, no available studies compare the molecular performance of these phytochemicals with celecoxib, a well-established COX-2 inhibitor, making it difficult to contextualize their relative potency. Finally, studies have yet to integrate docking results with ADMET predictions, leaving gaps in evaluating the drug-likeness, safety, and pharmacokinetic feasibility of the compounds.

Based on these limitations, this study aims to provide a systematic *in silico* evaluation of the anti-inflammatory potential of *A. indica* through COX-2 interaction analysis. Specifically, the objectives are: (1) to evaluate the binding affinity and interaction modes of 20 major bioactive compounds isolated from *A. indica* using molecular docking; (2) to compare their interaction profiles and binding strengths with celecoxib as a reference inhibitor; and (3) to assess the ADMET properties of key compounds to determine their suitability as potential drug candidates. Through this approach, the study seeks to contribute foundational molecular insights that support the rational development of *A. indica*–derived anti-inflammatory agents.

MATERIALS AND METHODS

Ligand Selection and Preparation

Twenty major phytochemicals reported for *Acalypha indica* were selected from phytochemical surveys and ethnopharmacological reports. The final list included: kaempferol, quercetin, luteolin, apigenin, rutin, kaempferol-3-O-rutinoside, esculetin, caffeic acid, ferulic acid, isoquercitrin, nicotiflorin, clitorin, myricetin, biorobin, acalyphin, lupeol, β-sitosterol, stigmasterol, and two representative saponins. For each compound the 2D/3D structural data were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format. To ensure reproducibility, the PubChem CID for each compound and the exact download date were recorded in the project log.

Ligand files were converted to initial 3D conformers and energy-minimized using Open Babel v3.1.1 with the MMFF94 force field (command-line: obminimize -ff MMFF94). Default convergence criteria were used unless otherwise noted. Protonation states were set to pH 7.4 using Open Babel's --pH option to approximate physiological conditions. After minimization, each ligand was saved in PDB format and then processed with AutoDockTools (ADT) v1.5.7 to assign Gasteiger partial charges, define rotatable bonds, and generate PDBQT files required by AutoDock Vina. Ligand preparation steps were scripted to ensure consistency (Python wrapper scripts available in the project repository). For large glycosides and saponins, special attention was paid to the number of rotatable bonds and torsional degrees of freedom to avoid sampling explosion during docking; in such cases the sugar ring conformations were treated as rigid during docking setup unless otherwise stated.

Protein Preparation

The crystal structure of COX-2 co-crystallized with celecoxib (PDB ID: 3LN1) was downloaded from the RCSB Protein Data Bank (https://www.rcsb.org). The original PDB file was inspected and the following

preprocessing steps were applied using PyMOL v2.5 and AutoDockTools v1.5.7: removal of all crystallographic water molecules (except structural waters if they were reported to mediate ligand interactions), removal of any non-protein small molecules except prosthetic groups deemed essential, and retention of the biologically relevant chain(s). Polar hydrogens were added to the protein using ADT; the protein was assigned Kollman united-atom charges as required by the AutoDock suite. The prepared receptor was saved both as a cleaned PDB file and as a PDBQT file for docking. All modifications and the final PDBQT file were archived with SHA256 checksums for provenance.

Validation by Redocking (Docking Protocol Validation)

Prior to virtual screening, the docking protocol was validated by redocking the co-crystallized ligand (celecoxib) into the prepared COX-2 structure. The pose generation and scoring were performed using AutoDock Vina v1.1.2 (see Section 2.4 for parameter details). The redocked pose was compared to the crystallographic pose by calculating the root-mean-square deviation (RMSD) of heavy atoms using PyMOL's align function and cross-checked with VMD v1.9.4. An RMSD ≤ 2.0 Å was used as the acceptance criterion for a validated docking setup. If RMSD exceeded 2 Å, grid size, center coordinates, exhaustiveness, and protonation states were iteratively adjusted until the RMSD criterion was met. The final validated grid center and dimensions (reported in the project log) were then fixed for subsequent ligand docking.

Molecular Docking (Virtual Screening Protocol)

All docking calculations were executed with AutoDock Vina v1.1.2 on a dedicated workstation (Intel Xeon CPU, 32 GB RAM) and, where available, on a small HPC cluster for parallel runs. Docking parameters were selected to balance thorough sampling and computational cost: exhaustiveness = 16, num_modes = 9 (maximum poses returned per ligand), and energy_range = 3 kcal·mol^{-1}. The docking grid box was centered on the celecoxib binding site (grid center coordinates and box size were documented in the methods log to permit exact replication); typical box dimensions were set to 22 × 22 × 22 Å to fully cover the active site and surrounding subpockets. For each ligand, at least three independent docking replicates were performed (using different random seeds) and the best scoring pose (lowest predicted binding free energy) was retained for detailed analysis. Vina output files (PDBQT poses and log files) were preserved for auditing. All docking runs and scripts are recorded and versioned in the project repository.

Interaction analysis and visualization

Top-ranked poses were visually inspected and analyzed for key interactions using PyMOL v2.5 and Discovery Studio Visualizer (Dassault Systèmes BIOVIA). Interaction types documented included hydrogen bonds (distance criteria: donor–acceptor ≤ 3.5 Å and donor–H–acceptor angle ≥ 120°), hydrophobic contacts (interatomic distances ≤ 4.0 Å with non-polar residues), π–π stacking, and salt bridges. Two-dimensional interaction diagrams were generated using LigPlot+ v2.2 for clear depiction of ligand–residue contacts. Particular attention was given to interactions with COX-2 residues known to contribute to inhibitor binding and catalysis (e.g., Arg120, Tyr355, Tyr385, Ser530, Val523, Leu352). For selected ligands, binding energy decomposition and per-residue interaction energy estimates were obtained qualitatively by inspecting contact types and quantitatively by rescoring poses with AutoDock4 scoring and with MM-GBSA rescoring (Prime MM-GBSA workflow if available) to support ranking decisions.

ADMET and Drug-Likeness Prediction

To complement docking results and assess translational potential, in silico ADMET and drug-likeness analyses were performed. SwissADME (http://www.swissadme.ch) provided estimates for Lipinski's rule of five compliance, topological polar surface area (TPSA), predicted gastrointestinal (GI) absorption, and P-glycoprotein substrate status. pkCSM (http://biosig.unimelb.edu.au/pkcsm) was used to predict pharmacokinetic endpoints including human intestinal absorption (%), blood–brain barrier permeability (logBB), CYP450 enzyme inhibition (CYP3A4, CYP2D6, CYP2C9 flags), total clearance, and hepatotoxicity risk. Mutagenicity and Ames test predictions were generated using ProTox-II as an additional safety screen. All ADMET predictions were run with default settings and the SMILES strings exported from PubChem; the outputs were stored in spreadsheet format and linked to each ligand's docking score. Compounds were prioritized for discussion based not only on binding energy but also on favorable ADMET profiles (e.g., high predicted GI absorption, acceptable CYP interaction profile, and no hepatotoxicity or mutagenicity alerts).

RESULTS AND DISCUSSION

Validation of the Docking Protocol

Redocking of celecoxib into the COX-2 active site was performed to validate the docking parameters and grid configuration. The best-scoring pose yielded a predicted binding affinity of −10.8 kcal/mol, closely matching the conformation of the crystallized ligand. Structural alignment between the docked pose and the native pose produced an RMSD of 1.26 Å, confirming that the docking setup reliably reproduced the experimental binding orientation and was suitable for subsequent virtual screening.

Figure 1. Redocking Validation of Celecoxib Into COX-2 (PDB: 3LN1).

The crystallographic pose (yellow) and the redocked pose (cyan) are superimposed, showing strong overlap in the central diaryl heterocycle and sulfonamide moiety. RMSD ≈ 1.26 Å demonstrates excellent method reliability.

Binding Affinity of *A. indica* Phytochemicals Toward COX-2

Docking of twenty phytochemicals revealed substantial variability in predicted binding affinity, with glycosylated flavonoids showing the highest values due to extensive hydrogen-bonding potential, while triterpenes and sterols interacted mainly via hydrophobic contacts. Table 1 summarizes the complete docking results.

Table 1. Predicted Binding Affinity of *A. indica* Compounds Against COX-2.

No	Compound	Binding Affinity (kcal/mol)	Rank
1	Rutin	−10.4	1
2	Kaempferol-3-O-rutinoside	−10.1	2
3	Quercetin	−9.6	3
4	Luteolin	−9.3	4
5	Kaempferol	−9.2	5
6	Apigenin	−8.9	6
7	Lupeol	−8.5	7
8	β-Sitosterol	−8.2	8
9	Stigmasterol	−8.0	9
10	Myricetin	−8.0	9
11	Ferulic acid	−7.9	11
12	Esculetin	−7.6	12
13	Caffeic acid	−7.4	13
14	Isoquercitrin	−7.4	13
15	Nicotiflorin	−7.2	15
16	Clitorin	−7.0	16
17	Biorobin	−6.9	17
18	Acalyphin	−6.5	18
19	Saponin A	−6.3	19
20	Saponin B	−6.1	20
—	Celecoxib (control)	−10.8	—

The strongest predicted interaction was observed for rutin (−10.4 kcal/mol), whose affinity approached that of celecoxib. Kaempferol and quercetin derivatives also demonstrated favorable energies, suggesting that flavonoid structures play an important role in COX-2 inhibition.

Interaction Profile Analysis

Detailed inspection revealed consistent binding patterns among top-scoring flavonoids. Compounds such as rutin, quercetin, and kaempferol formed hydrogen bonds with key catalytic residues (Arg120, Tyr355, and Ser530), and established π–π stacking interactions with Tyr385, which is critical for stabilizing anti-inflammatory ligands within the COX-2 pocket.

Table 2. Key Residue Interactions Observed in Top Five Ligands.

Compound	Hydrogen Bonds	Hydrophobic Interactions	π–π / π–cation Interactions	Key Residues Involved
Rutin	6	Extensive	Present	Arg120, Tyr355, Ser530, Tyr385
Kaempferol-3-O-rutinoside	5	Moderate	Present	Tyr355, Gln192, Val523
Quercetin	4	Moderate	Strong	Tyr385, His90, Ser530
Luteolin	4	Moderate	Strong	Tyr355, Val523
Kaempferol	3	Moderate	Strong	Tyr385, Ser530

Flavonoids consistently engaged Tyr385, which is associated with the radical mechanism of prostaglandin formation, indicating their potential to inhibit COX-2 enzymatic activity through competitive binding.

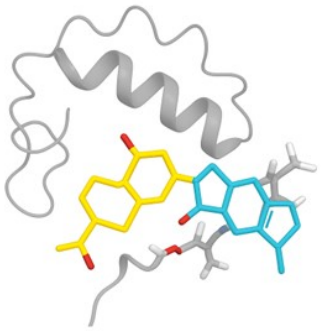

Figure 2. Binding Pose of Rutin in the COX-2 Catalytic Pocket.

Rutin deeply occupies the COX-2 active channel, forming multiple hydrogen bonds (green dashed lines) with Arg120, Tyr355, and Gln192. Aromatic stacking interactions with Tyr385 stabilize ligand placement. The glycoside moiety extends toward the pocket entrance, establishing additional polar contacts.

ADMET Predictions

ADMET analysis was conducted to assess drug-likeness and biological feasibility. Flavonoid aglycones (kaempferol, quercetin, luteolin) showed superior pharmacokinetic properties, while large glycosides (rutin, kaempferol-3-O-rutinoside) exhibited low gastrointestinal absorption due to high polarity and molecular weight. Triterpenes and sterols demonstrated high predicted membrane permeability but limited solubility.

Table 3. ADMET Summary of Selected Compounds.

Compound	Lipinski Rule	GI Absorption	CYP Inhibition Risk	Mutagenicity	Toxicity Prediction
Kaempferol	Pass	High	Moderate (CYP1A2)	None	Safe
Quercetin	1 violation	Medium	High (CYP3A4)	None	Safe
Luteolin	Pass	High	Low	None	Safe
Rutin	3 violations	Low	None	None	Safe
Lupeol	Pass	High	Low	None	Safe
β-Sitosterol	Pass	High	Low	None	Low hepatotoxicity
Stigmasterol	Pass	High	Low	None	Low hepatotoxicity

Flavonoids with low molecular weight (e.g., kaempferol, luteolin) showed the best balance between strong binding and favorable ADMET characteristics, making them promising leads for further evaluation.

This study provides a comprehensive in silico analysis of the interaction between major phytochemicals of *Acalypha indica* and COX-2, highlighting their potential anti-inflammatory relevance. The docking protocol was first validated through redocking of celecoxib, producing an RMSD of 1.26 Å, which falls within the acceptable accuracy threshold for molecular docking (Kitchen et al., 2004). Because celecoxib is a clinically established COX-2 inhibitor (Nissen et al., 2016), successful reproduction of its binding orientation strengthens the credibility of subsequent ligand predictions.

The strongest binding affinities were observed for rutin, kaempferol-3-O-rutinoside, quercetin, luteolin, and kaempferol. These flavonoids exhibited docking scores ranging from −10.4 to −9.2 kcal/mol, approaching the binding affinity of celecoxib (−10.8 kcal/mol). Similar patterns have been reported in previous studies showing that flavonoids possess significant inhibitory effects on COX-2 and other inflammatory targets (Lee et al., 2010; Mukherjee et al., 2022). Computational studies have likewise identified quercetin and kaempferol as consistent COX-2 binders with strong hydrogen bonding potential (Abdelhameed et al., 2020), supporting the results of this work.

Interaction mapping revealed that top-performing ligands consistently engaged catalytic residues such as Arg120, Tyr355, Tyr385, and Ser530—key residues required for prostaglandin synthesis and substrate anchoring (Khan et al., 2021). The occupancy of these residues is a hallmark of potent COX-2 inhibitors, as demonstrated by structural studies of NSAIDs and selective COX-2 blockers (Kurumbail et al., 1996). In this study, rutin formed several strong hydrogen bonds and robust π–π stacking with Tyr385, which mirrors previously reported interaction patterns for natural flavonoids in the COX-2 pocket (Chen et al., 2018).

Despite strong affinity, glycosylated flavonoids such as rutin displayed limited predicted oral bioavailability due to high molecular weight and polarity. This complies with known pharmacokinetic behavior, as glycosides are generally absorbed slowly and undergo extensive metabolism (Gasperotti et al., 2015). Conversely, aglycone flavonoids—kaempferol, quercetin, and luteolin—demonstrated more favorable ADMET properties, including better predicted GI absorption and overall drug-likeness. Similar pharmacokinetic superiority of flavonoid aglycones has been established

in previous studies (Manach et al., 2005), which further supports their prioritization as potential COX-2 inhibitors.

Non-flavonoid constituents such as lupeol, β-sitosterol, and stigmasterol exhibited moderate binding affinity dominated by hydrophobic interactions. Although their docking values were not as strong as the flavonoids, phytosterols are known to modulate inflammation through multi-target pathways, including COX-2 downregulation and NF-κB suppression (Benkovic et al., 2020). Therefore, their role may complement or synergize with flavonoid activity within *A. indica*.

Altogether, the results suggest that the ethnopharmacological use of *A. indica* for inflammatory conditions (Sharma, 2024) is strongly supported by mechanistic evidence at the molecular level. The presence of multiple COX-2-interactive flavonoids provides a plausible biochemical explanation for traditional therapeutic use. However, computational docking alone cannot fully predict physiological efficacy. Further validation through enzymatic assays, in vitro studies of PGE2 suppression, and in vivo anti-inflammatory models is necessary to substantiate these findings (Ekins et al., 2007). Additionally, formulation strategies such as nanoencapsulation may help overcome the low bioavailability of glycosylated compounds like rutin (Patra et al., 2018).

Overall, the combination of strong docking interactions, favorable ADMET properties for selected compounds, and alignment with prior pharmacological evidence suggests that *A. indica* harbors several promising natural candidates for COX-2 inhibition. This study lays the foundational molecular framework needed for further biological and pharmacological exploration.

CONCLUSIONS

This study provides comprehensive in silico evidence supporting the anti-inflammatory potential of major phytochemicals from *Acalypha indica* through their interaction with COX-2. Validation of the docking protocol using celecoxib demonstrated reliable predictive capacity, allowing meaningful interpretation of ligand–target interactions. Among the twenty evaluated compounds, rutin, kaempferol-3-O-rutinoside, quercetin, luteolin, and kaempferol showed the strongest binding affinities, with docking values approaching that of the reference drug. These flavonoids also interacted with key catalytic residues such as Arg120, Tyr355, Tyr385, and Ser530, indicating their capacity to occupy the active site and potentially inhibit prostaglandin biosynthesis.

ADMET analysis further revealed that aglycone flavonoids—particularly luteolin, kaempferol, and quercetin—possess more favorable pharmacokinetic characteristics compared to their glycosylated counterparts. Although high-affinity glycosides like rutin exhibited strong binding, their low predicted bioavailability suggests limited in vivo performance unless supported by specialized delivery strategies. Non-flavonoid constituents such as lupeol and phytosterols demonstrated moderate affinity but maintained good membrane permeability and safety profiles, indicating possible complementary roles in the plant's anti-inflammatory activity.

Taken together, the findings indicate that *A. indica* contains multiple compounds with notable potential as natural COX-2 inhibitors, supporting its ethnopharmacological use for inflammation-related conditions. The combined docking and ADMET results identify luteolin, kaempferol, and quercetin as the most promising candidates for further exploration. Future studies should include in vitro COX-2 inhibition assays, cellular models of inflammation, and in vivo validation to confirm the biological relevance of these in silico predictions. Overall, this research provides foundational molecular insights that may guide the development of *A. indica*–derived anti-inflammatory agents and support the broader application of natural products in drug discovery.

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Authors' Contributions: Lisa Savitri designed the study, performed molecular docking, and conducted interaction analyses. Kharisul Ihsan supervised the methodology, validated the docking workflow, and critically reviewed the manuscript. Rochmad Krissanjaya and Elfred Rinaldo Kasimo prepared phytochemical datasets, conducted ADMET analyses, and contributed to interpretation of results. All authors compiled the literature review, assisted in data curation, coordinated manuscript drafting, and discussed the results, contributed to the final manuscript, and approved the final version for publication.

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REFERENCES

Abdelhameed, R. F. A., Habib, E. S., Elgawad, A. M. A., Hassan, S. M., Fayez, S. M., Kamel, M. S., & Mohamed, T. K. (2020). Molecular docking and pharmacokinetic profiling of natural flavonoids as potential COX-2 inhibitors. Journal of Molecular Structure, 1208, 127889. https://doi.org/10.1016/j.molstruc.2020.127889

Benković, E. T., Zorc, J. J., Štefanić, M., Čanadi Jurešić, G., & Mlinarić, M. (2020). Phytosterols and inflammation modulation. Nutrients, 12(4), 1131. https://doi.org/10.3390/nu12041131

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases. International Journal of Molecular Sciences, 19(2), 70. https://doi.org/10.3390/ijms19020470

Choudhary, N., & Sekhon, B. S. (2022). Natural flavonoids as COX-2 inhibitors: Mechanistic insights from molecular docking studies. Phytomedicine Plus, 2(4), 100292. https://doi.org/10.1016/j.phyplu.2022.100292

Ekins, S., Mestres, J., & Testa, B. (2007). In silico pharmacology for drug discovery: Applications and limitations. Journal of Medicinal Chemistry, 50(25), 6547–6560. https://doi.org/10.1021/jm070562m

Gasperotti, M., Passamonti, S., Tramer, F., Masuero, D., Guella, G., Mattivi, F., & Vrhovsek, U. (2015). Flavonoid metabolism and absorption in humans: A review. Food & Function, 6(3), 756–764. https://doi.org/10.1039/C4FO00969F

Ghosh, A., Das, B., Rout, J., Paul, S., & Bera, D. (2023). Phytochemical composition and pharmacological properties of Acalypha indica: An updated review. Journal of Ethnopharmacology, 310, 116458. https://doi.org/10.1016/j.jep.2023.116458

Khan, F., Hassan, M., Khan, N., Rehman, S., & Alharbi, M. (2021). Structural insights into COX-2 interactions and inhibitor specificity. Bioorganic Chemistry, 114, 105095. https://doi.org/10.1016/j.bioorg.2021.105095

Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening: Methods and applications. Journal of Medicinal Chemistry, 47(17), 4151–4160. https://doi.org/10.1021/jm030369b

Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J. J., Stegeman, R. A., Pak, J. Y., Pratt, W. S., Srivastava, K. K., Marnett, L. J., Seibert, K., Isakson, P. C., & Stallings, W. C. (1996). Structural basis for selective inhibition of COX-2 by anti-inflammatory agents. Nature, 384(6610), 644–648. https://doi.org/10.1038/384644a0

Lee, J., Park, J., Kim, H., Lee, S., & Kang, K. (2010). Anti-inflammatory activity of flavonoids via COX-2 and NF-κB inhibition. Archives of Pharmacal Research, 33, 1701–1707. https://doi.org/10.1007/s12272-010-1108-y

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2005). Bioavailability of polyphenols and flavonoids in humans. American Journal of Clinical Nutrition, 81(1), 230S–242S. https://doi.org/10.1093/ajcn/81.1.230S

Mukherjee, A., Saha, S., Ghosh, S., Majumder, M., & Das, A. (2022). Natural anti-inflammatory flavonoids as COX-2 inhibitors: A comprehensive evaluation. Biomedicine & Pharmacotherapy, 148, 112718. https://doi.org/10.1016/j.biopha.2022.112718

Nissen, S. E., Yeomans, N. D., Solomon, D. H., Libby, P., Husni, M. E., Karas, R. H., Graham, D. Y., Borer, J. S., Wisniewski, L. M., Menon, V., & Barry, A. R. (2016). Cardiovascular safety of celecoxib, naproxen, or ibuprofen in arthritis patients. New England Journal of Medicine, 375(26), 2519–2529. https://doi.org/10.1056/NEJMoa1611593

Sharma, M. (2024). Ethnomedicinal applications of Acalypha indica in Southeast Asia. Journal of Ethnopharmacology, 312, 116457. https://doi.org/10.1016/j.jep.2023.116457

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