Jurnal Biolokus: Jurnal Penelitian Pendidikan Biologi dan Biologi
Vol. 8, Issue 2, December 2025
Pages : 214 – 233

p-ISSN: 2621-3702
e-ISSN: 2621-7538

Secondary metabolites from *Lansium domesticum* as potential anti-inflammatory iNOS inhibitors: An *in silico* study

Yadi Oktariansyah*, Laila Hanum, Muhammad Habiburrahman
Department of Biology, Faculty of Mathematics & Natural Sciences, Sriwijaya University
Jalan Raya Palembang–Prabumulih km 32, Indralaya, Indonesia.
*corresponding author: yadioktariansyah@mipa.unsri.ac.id

ABSTRACT

Inflammation is a crucial mechanism by which the immune system combats disease. However, when it becomes uncontrolled, it can result in acute inflammatory disorders. Although non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for treatment, their associated side effects have prompted the search for safer, natural alternatives. One of the enzymes central to the progression of acute inflammation is inducible nitric oxide synthase (iNOS), particularly its active site, plays a key role in the progression of acute inflammation. *Lansium domesticum* Corrêa, 1807 have been reported to contain secondary metabolites with anti-inflammatory potential, but their interaction with iNOS has not been systematically explored. This study is the first to comprehensively evaluate these secondary metabolites against iNOS using an *in silico* approach combing biological activity prediction, physicochemical and pharmacokinetic profiling, and molecular docking simulations. From 53 screened metabolites, nine compounds met drug-likeness and pharmacokinetic criteria and were further analyzed through molecular docking. Ellagic Acid, (+)-Spathulenol, α-Cadinene, and τ-Muurolol showed substantial binding affinities at the iNOS active site, which suggests that they could be good anti-inflammatory agents. These results establish a basis for subsequent *in vitro* and *in vivo* validation aimed at creating safe, natural iNOS inhibitors as herbal-based for the alternative anti-inflammatory medicines.

Keywords: Anti-inflammatory, bioinformatics, *in silico*, iNOS, *Lansium domesticum*

INTRODUCTION

Inflammation is a fundamental component of the immune response, protecting the body from infection, injury, and harmful stimuli through immune cell activation, pro-inflammatory cytokine release, and alterations in blood flow (Guzik et al., 2024; Sun et al., 2022). Acute inflammation is essential for initiating healing and contributes to wound healing progression as part of the local immune response (Wang et al., 2022). However, chronic inflammation can become maladaptive, contributing to the pathogenesis of various diseases, including arthritis (Rajendran et al., 2018), cardiovascular disorders (Cifuentes et al., 2025), and creating a microenvironment favorable for cancer development (Danforth, 2021).

Synthetic anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), alleviate inflammation and pain by inhibiting cyclooxygenase (COX) enzymes, which convert arachidonic acid into prostaglandins and prostacyclins (Tonk et al., 2020). However, prolonged NSAID use is associated with adverse effects, including gastrointestinal ulcers and bleeding (Domper Arnal et al., 2022), renal damage (Rudichenko & Sokolenko, 2023), and cardiovascular complications (Panchal & Prince Sabina, 2023). These risks have driven the search for safer alternatives, particularly bioactive compounds from natural sources with anti-inflammatory potential.

Enzymatic response plays a vital role in inflammatory activity. Enzymatic responses are key to inflammation, with inducible nitric oxide synthase (iNOS) producing nitric oxide (NO) as a mediator in local and systemic immune responses (Cinelli et al., 2020). While NO supports immune defense, chronic overproduction induces oxidative stress, contributing to diseases such as rheumatoid

arthritis, cardiovascular disorders, and neurodegeneration (Prata et al., 2024; Rana, 2022). Inhibiting iNOS with natural compounds may reduce excessive NO, alleviating inflammation-related conditions.

Lansium domesticum Corrêa, 1807 (*langsat/duku*) is a tropical fruit native to Southeast Asia, especially Malaysia and Indonesia (Techavuthiporn, 2018). Belonging to the Meliaceae family, it is valued in traditional medicine for secondary metabolites with antimicrobial and anti-inflammatory effects (Mayanti et al., 2022), and antioxidant (Abdallah et al., 2022). Flavonoids in *L. domesticum* can reduce IL-6, TNF-α, IL-1, IL-8, and NO production (Hestiningsih et al., 2022), highlighting its potential as a natural source for anti-inflammatory therapeutics. In South Sumatra, *L. domesticum* holds cultural and economic importance for local communities and is comparatively under-researched at the molecular and phytochemical levels in comparison to other tropical medicinal plants. Figuring out precisely what its bioactive chemicals are could help researchers come up with novel pharmaceuticals. We chose *L. domesticum* as the main species for this study because it has both ethnobotanical significance and research potential that hasn't been fully explored.

A novel approach is needed to evaluate the potential of *L. domesticum* secondary metabolites as anti-inflammatory agents. One promising strategy is the *insilico* method, a bioinformatic approach that simulates interactions between small molecules and biological targets such as inducible nitric oxide synthase (iNOS) (Shaker et al., 2021). This technique predicts biological activity, physicochemical characteristics profiling, pharmacokinetic properties, enzyme inhibition potential, safety profiles, and in vivo performance (Al-Mohaya et al., 2024; Gazzillo et al., 2024). It streamlines drug discovery, reduces time and cost, and supports personalized medicine through pharmacogenomics and artificial intelligence integration.

In silico screening typically involves evaluating absorption, distribution, metabolism, excretion, and toxicity (ADMET) to determine a compound's therapeutic viability (Sulistyowaty et al., 2021). Computational algorithms predict ligand–enzyme binding modes, affinities, and stability (Karim et al., 2024), enabling rapid molecular docking and virtual screening of large natural product libraries (Thangavel & Albratty, 2022). This approach, demonstrated in studies such as SARS-CoV-2 Mpro screening (Mazzini et al., 2020) is particularly valuable for identifying bioactive compounds in plants like *L. domesticum*, whose diverse metabolites may serve as starting points for anti-inflammatory drug development targeting iNOS.

Although *in silico* approaches have been applied to various plant-derived anti-inflammatory compounds, for example, *Physalis peruviana* (Fadzillah et al., 2024), and other medicinal plants (Diep et al., 2023; Maiolini et al., 2025), however these studies are often limited to a small number of metabolites or target other inflammatory pathways. To date, no comprehensive *in silico* screening has systematically explored the diverse secondary metabolites of *L. domesticum* against iNOS, despite ethnomedicinal evidence supporting its anti-inflammatory use. Therefore, this study addresses this gap by integrating activity prediction, ADMET profiling, and molecular docking to identify novel *L. domesticum* metabolites with strong binding affinities to iNOS.

Based on the background described, it is important to carry out research focused on selecting secondary metabolite compounds from *L. domesticum* that show potential anti-inflammatory activity through the inhibition of iNOS. This study aims to evaluate the predicted biological activity, physicochemical characteristics, pharmacokinetic behavior, and toxicity profiles of these compounds using *in silico* methods. Furthermore, the research also seeks to analyze the interaction between the selected secondary metabolites and the iNOS

enzyme receptor through molecular docking simulations, to assess their potential as effective anti-inflammatory agents.

METHOD

An *in silico* approach, based on the method described by Shaker et al. (2021), was employed to investigate the potential of *L. domesticum* secondary metabolites as anti-inflammatory agent agents targeting the inducible Nitric Oxide Synthase (iNOS) enzyme (3E7G). Computational techniques, including biological activity prediction, physicochemical property assessment, pharmacokinetic screening, and molecular docking, were applied to evaluate ligand–receptor interactions and binding affinities.

The study took place from October 2024 to February 2025 at the Biosystematics Laboratory within the Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Inderalaya, South Sumatra, Indonesia. The study utilized standard computational facilities and several key software tools, including ChemSketch, Discovery Studio, Open Babel, and PyRx. The primary databases utilized included KNApSAcK (http://www.knapsackfamily.com/knapsack_core/top.php), RCSB Protein Data Bank (https://www.rcsb.org), and PubChem (https://pubchem.ncbi.nlm.nih.gov/). Furthermore, the platforms ADMET (https://admetlab3.scbdd.com/), SwissADME (http://www.swissadme.ch/), and Way2Drug (https://www.way2drug.com/) were utilized for predictions related to pharmacokinetics and toxicity.

Sample collection and receptor preparation

The three-dimensional (3D) structure of inducible nitric oxide synthase (iNOS; PDB ID: 3E7G) was retrieved from the Protein Data Bank in .pdb format. Secondary metabolites of *Lansium domesticum* were compiled from the KNApSAcK and IMPPAT databases and relevant literature, with structural data obtained from PubChem. The complete metabolite list is presented in Tables S1–S3.

The iNOS structure was prepared by removing water molecules and the co-crystallized ligand using Discovery Studio, yielding a purified receptor in .pdb format. This step prevents interference with ligand–receptor interactions and ensures accurate inhibitory activity assessment (Gunarti et al., 2024).

Screening for anti–inflammatory activity

Ligands obtained from the database are evaluated to determine the compound's biological activity. Secondary metabolites from *L. domesticum* are screened using the Way2Drug website's PASS tool, which uses SMILES from the compounds gathered to determine the compound's Pa (probability to be active) and Pi (probability to be inactive) values.

Predicting chemical characteristics

The chemical properties of candidate compounds were initially predicted using Lipinski's Rule of Five to evaluate their physicochemical suitability (Lipinski et al., 2001). The SMILES notation of compounds with potential anti-inflammatory activity was input into the SwissADME platform to determine key parameters, including molecular weight, lipophilicity (log P), the number of hydrogen bond donors and acceptors, and molar refractivity. Compounds meeting the Rule of Five criteria were then assessed for pharmacokinetic properties using the ADMETLab 3.0 platform. This step provided detailed ADMET parameters covering absorption (e.g., human intestinal absorption [HIA]), distribution (e.g., steady-state volume of distribution [VDss], blood–brain barrier permeability [BBB]), metabolism (e.g., CYP2D6 and CYP3A4 enzyme inhibition), and excretion (e.g., plasma clearance [CLplasma]). Finally, toxicity predictions were carried out to evaluate AMES mutagenicity, hepatotoxicity, and rat oral acute toxicity.

Ligand preparation for docking process

The three-dimensional (3D) structure of

the ligand, either constructed using ChemSketch or retrieved from a relevant database, is initially processed in Discovery Studio software to remove any water molecules. The resulting structure is then saved in Protein Data Bank (PDB) format and subsequently imported into PyRx through the Open Babel module. In PyRx, the "Minimize All" function is applied to reduce the ligand's energy, thereby enhancing molecular stability. This energy minimization process also optimizes the spatial arrangement of ligand atoms, which may improve the efficiency of interactions with the target molecule (Hanif et al., 2020).

Molecular docking and visualization

The prepared inducible nitric oxide synthase (3E7G) receptor and ligands were imported into PyRx via Vina Wizard, and docking was performed to obtain binding affinities and RMSD values (Agu et al., 2023). Ligands were ranked by predicted interaction energies, and receptor–ligand interactions were visualized in Discovery Studio, highlighting hydrogen bonds, van der Waals forces, hydrophobic contacts, and involved amino acid residues (Sari et al., 2020).

Data Analysis

Anti–inflammatory activity

Activity predictions were evaluated based on the Pa (probability of being active) and Pi (probability of being inactive) values for anti–inflammatory biological activity. A Pa value greater than 0.5 is considered indicative of potential biological activity and is therefore preferred. According to Riyadi et al. (2021), a Pa value below 0.5 suggests that the compound is unlikely to exhibit significant biological activity.

Physicochemical characteristics

The physicochemical properties of the secondary metabolites were analyzed based on Lipinski's Rule of Five using the SwissADME web tool. This rule outlines criteria that predict a compound's potential as an orally active drug, including a molecular weight of less than 500 g/mol, a lipophilicity value (logP) below 5, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a molar refractivity ranging between 40 and 130 (Lipinski et al., 2001).

Pharmacokinetic properties

Pharmacokinetic properties were predicted using the ADMETLab 3.0 platform (Fu et al., 2024) by evaluating key absorption, distribution, metabolism, and excretion parameters. Absorption was assessed via Human Intestinal Absorption (HIA), while distribution was analyzed based on the Volume of Distribution at steady state (VDss) and Blood–Brain Barrier (BBB) permeability. Metabolic potential was evaluated through the compound's ability to inhibit cytochrome P450 enzymes, specifically CYP2D6 and CYP3A4. Excretion characteristics were determined from plasma clearance (CLplasma) values.

Toxicity characteristic

Toxicity profiles were predicted using ADMETLab 3.0 platform, assessing AMES mutagenicity, human hepatotoxicity, and rat oral acute toxicity. The AMES test indicated mutagenic potential, hepatotoxicity predictions estimated liver toxicity risk, and acute toxicity values reflected potential effects after oral exposure in mammals (Fadzillah et al., 2024).

Binding affinity

Binding affinity from molecular docking is inversely related to interaction strength—more negative values indicate stronger ligand–receptor binding, while higher or positive values indicate weaker interactions (Uzzaman et al., 2023).

Root Mean Square Deviation (RMSD)

Docking results are considered reliable when the Root Mean Square Deviation (RMSD) is ≤ 2 Å (Liu & Kokubo, 2020), with values up to 3 Å still acceptable (Castro-Alvarez et al., 2017). RMSD values ≤ 2 Å indicate stable and accurate

docking poses (Ramírez & Caballero, 2018).

Observation variables

Observed variables included binding affinity and RMSD values to validate docking results. Binding affinities and ligand–receptor amino acid interactions will be presented in tables with 2D and 3D structural diagrams. Potential anti-inflammatory candidate compounds will also be summarized in a table.

RESULTS AND DISCUSSION

Anti–inflammatory activity

Based on literature reviews and database searches, 106 secondary metabolites were identified from *L. domesticum*. Their anti-inflammatory potential was predicted using the PASS (Prediction of Activity Spectra for Substances) tool on the Way2Drug platform, with SMILES data as input. Results showed that 53 compounds from *L. domesticum*, along with two synthetic anti-inflammatory drug controls, had Pa values > 0.5 (Table 1), indicating ≥50% probability of anti-inflammatory activity. Higher Pa values correspond to greater likelihood of significant biological effects. Compounds with Pa < 0.5 are considered to have low potential as alternative medicines (Riyadi et al., 2021).

Table 1. Anti–Inflammatory activity

Chemical Compounds	Pa	Pi
Ibuprofen (Control)	0.660	0.021
Aspirin (Control)	0.762	0.009
Honghelin	0.500	0.057
6–Acetoxymexicanolide	0.503	0.056
δ–Selinene	0.505	0.055
(+)–Spathulenol	0.521	0.051
Kokosanolide B	0.541	0.045
Stigmasterol	0.542	0.045
Catechin	0.548	0.044
Gallic acid	0.548	0.044
Lamesticumins B	0.557	0.041
Methyl lansiolate	0.557	0.041
Lansiolic acid	0.559	0.041
τ–Muurolol	0.566	0.039
Kokosanolide C	0.567	0.036
Lansionic Acid	0.574	0.037
Ethyl lansiolate	0.585	0.035
Lansioside B	0.592	0.034
Chlorogenic acid	0.598	0.032
Kokosanolide A	0.599	0.032
Lamesticumins D	0.604	0.031
Lansioside C	0.613	0.029
Lansioside D	0.613	0.029
Hexadecanoic acid	0.614	0.029
Oleic acid	0.614	0.029
(+)–α–muurolene	0.619	0.028
α–cadinene	0.619	0.028
Scopoletin	0.629	0.029
Domesticulide D	0.637	0.025
γ–muurolene	0.640	0.024
P–Coumaric acid	0.641	0.024
Azadiradion	0.644	0.024
Lamesticumins E	0.656	0.022
Bicycloelemene	0.665	0.02
Ethyl oleate	0.672	0.019
Lamesticumin C	0.677	0.019
Lamesticumin G	0.681	0.018
Quercetin	0.689	0.017
7,14(27)–Onoceradiene–3,21–dione	0.690	0.017
Methyl lansioside	0.691	0.017
α–Calacorene	0.698	0.016
21 α –Hydroxyonocera–8(26),14–dien–3–one	0.705	0.015
3β–Hydroxyonocera–8(26),14–dien–21–one	0.705	0.015
Octadecanoic acid	0.727	0.002
Palmitic acid	0.727	0.002
Rutin	0.728	0.013
Humulene	0.741	0.011
Ellagic acid	0.749	0.010
Lamesticumins F	0.770	0.009
3–Hydroxycycloart–24–en–21–oic acid	0.811	0.006
β–Elemene	0.819	0.005
3–Oxo–24–cycloarten–21–oic acid	0.825	0.050
α–Cubebene	0.888	0.005
β–Cubebene	0.923	0.004

Abbreviation: Pa: probability of being active,
Pi : probability of being inactive.

Physicochemical properties

Physicochemical profiles of the compounds were assessed using Lipinski's Rule of Five, which evaluates molecular weight, lipophilicity (log P), hydrogen bond donors, hydrogen bond acceptors, and molar refractivity (Lipinski et al., 2001). This framework provides a predictive basis for drug-likeness, reflecting a compound's potential for adequate absorption and metabolism (Fadzillah et al., 2024). While Lipinski proposed log P ≤ 5 as a general guideline

for oral bioavailability, subsequent analyses of approved drugs reveal that many successful agents, particularly anti-inflammatory compounds, cluster around log P values close to 4.7 (Pirie et al., 2024). Experimental determinations of NSAIDs further support this range, with reported values typically between 3 and 4.2—for instance, ketoprofen (3.13), phenylbutazone (3.16), flurbiprofen (4.00), and indomethacin (4.18) (Pyka-Pająk et al., 2019). Since excessive lipophilicity is associated with reduced solubility, nonspecific tissue binding, and higher toxicity, a stricter threshold of log P < 4.15 was therefore adopted. This cutoff was chosen to prioritize secondary metabolites with physicochemical properties most consistent with orally active anti-inflammatory drugs while minimizing risks linked to overly lipophilic compounds.

Of the 53 *L. domesticum* metabolites with predicted anti-inflammatory activity, 26 satisfied Lipinski's criteria (Table 2), indicating favorable drug–likeness and suitability for further predictive analysis. A single violation is generally tolerable; however, exceeding one parameter often signals poor bioavailability and reduced pharmacokinetic performance (Kumar & Kim, 2021).

Table 2. Predicted Physicochemical Profiles

Chemical Compounds	MW (<500 g/mol)	Log P (<4.15)	HBA (<10)	HBD (<5)	Molar refractivity (40–130)
Ibuprofen (Control)	206.28	3.13	2	1	62.18
Aspirin (Control)	180.16	1.51	4	1	44.90
δ–Selinene	204.35	4.63	0	0	68.78
(+)–Spathulenol	220.35	3.67	1	1	68.34
Catechin	290.27	0.24	6	5	74.33
Gallic acid	170.12	–0.16	5	4	39.47
τ–Muurolol	222.37	3.67	1	1	70.72
Kokosanolide C	486.55	2.06	8	1	123.84
Kokosanolide A	500.54	1.2	9	1	124.04
Hexadecanoic acid	254.41	4.09	2	1	80.32
Oleic acid	282.46	4.57	2	1	89.94
(+)–α–muurolene	204.35	4.63	0	0	69.04
α–cadinene	204.35	4.63	0	0	69.04
Scopoletin	192.17	0.76	4	1	51.00
γ–muurolene	204.35	4.63	0	0	69.04
P–Coumaric acid	164.16	1.28	3	2	45.13
Azadiradion	450.57	3.28	5	0	125.48
Bicycloelemene	204.35	4.63	0	0	68.52
Ethyl oleate	310.51	5.03	2	0	99.06
Quercetin	302.24	–0.56	7	5	78.03
α–Calacorene	200.32	5.36	0	0	68.39
Octadecanoic acid	284.48	4.67	2	1	90.41
Palmitic acid	256.42	4.19	2	1	80.8
Humulene	204.35	4.53	0	0	70.42
Ellagic acid	302.19	0.14	8	4	75.31
β–Elemene	204.35	4.53	0	0	70.42
α–Cubebene	204.35	5.65	0	0	67.14
β–Cubebene	204.35	5.65	0	0	67.14

Abbreviations : MW refers to Molecular Weight; LogP denotes the lipophilicity of a compound, representing its solubility in octanol and water; HBD stands for Hydrogen Bond Donor; HBA represents Hydrogen Bond Acceptor; MR indicates Molar Refractivity. A red tag signifies that the compound does not comply with Lipinski's Rule of Five.

Several compounds exhibited single violations of Lipinski's Rule of Five. For example, Kokosanolide A exceeded the molecular weight threshold (>500 g/mol), which is associated with reduced absorption and limited suitability for oral administration (Asano et al., 2023).

Fifteen compounds—δ–Selinene, Oleic acid, (+)–α–muurolene, α–cadinene, γ–muurolene, Bicycloelemene, Ethyl oleate, α–Calacorene, Octadecanoic acid, Palmitic acid, Humulene, β–Elemene, α–Cubebene, and β–Cubebene—had logP values above 4.15, indicating a likelihood of poor solubility, absorption, and suboptimal distribution (Adachi et al., 2024; Sun et al., 2022b). Moderate logP values (2–4) are generally considered optimal for balancing solubility and permeability.

All 26 Lipinski-compliant compounds met the hydrogen bond acceptor (HBA) criterion (<10), but Catechin and Quercetin exceeded the hydrogen bond donor (HBD) limit, with values of 5, potentially reducing oral absorption (Rai et al., 2023).

Among the analyzed compounds, Gallic Acid is the only one that does not meet the molar refractivity criteria, with a value of 39.47. This suggests that Gallic Acid may have limited potential for oral absorption and systemic distribution. In contrast, the remaining compounds fulfill the molar refractivity parameter, indicating a more favorable potential for oral bioavailability. As noted by Ibrahim et al. (2021), an optimal molar refractivity value ranges from 40 to 130, which is essential for ensuring adequate absorption and oral bioavailability.

Pharmacokinetic properties profiling

Pharmacokinetic profiling assessed absorption (HIA), distribution (VDss, BBB), metabolism (CYP2D6, CYP3A4 inhibition), and excretion (CLPlasma). Based on these pharmacokinetic thresholds, nine compounds were identified that met all criteria and were therefore considered to have strong potential for further anti-inflammatory evaluation (Table 3).

Table 3. Predicted Pharmacokinetic Profiles

Chemical compound	Absorption	Distribution		Metabolism		Excretion
	HIA (%) (59–100%)	VDss (L / kg) (0.04–20)	BBB	CYP2D6	CYP3A4	CLplasma (0–15)
Aspirin (Control)	76.938	0.148	–0.332	No	No	2.766
Ibuprofen (Control)	94.064	0.160	0.310	No	No	1.038
(+)–Spathulenol	93.906	1.154	0.617	No	No	10.770
τ–Muurolol	94.296	0.109	0.596	No	No	11.579
Hexadecanoic acid	92.510	0.217	–0.084	No	No	3.371
Oleic acid	91.823	0.259	–0.168	No	No	3.500
(+)–α–muurolene	94.640	3.060	0.785	No	No	11.520
α–cadinene	94.640	3.214	0.785	No	No	12.115
Scopoletin	95.277	0.376	–0.299	No	No	3.224
γ–muurolene	96.475	3.715	0.809	No	No	10.575
Ellagic acid	86.684	0.444	–1.272	No	No	14.743

Abbreviations: HIA refers to Human Intestinal Absorption (accepted range; 59 – 100%, Fadzillah et al. (2024); VDss denotes Volume of Distribution; BBB represents the Blood–Brain Barrier; CYP2D6 and CYP3A4 indicate cytochrome P450 enzymes involved in drug metabolism; OCT2 refers to the Renal Organic Cation Transporter; CLTOT stands for Total Clearance (Distribution, Metabolism and Excretion's accepted range was refered to ADMETlab 3).

Pharmacokinetic evaluation of the nine selected compounds revealed HIA values between 86–96%, indicating efficient gastrointestinal absorption and suitability for oral administration (Fadzillah et al., 2024). VDss values ranged from 0.109–3.715, within the acceptable 0.04–20 range (Fu et al., 2024), suggesting effective systemic distribution. Based on experimental classification (Smith et al., 2015; Waters & Lombardo, 2010), τ–Muurolol, Hexadecanoic acid, Oleic acid, Ellagic acid, and Scopoletin showed low VDss (<0.6 L/kg), (+)–Spathulenol had moderate VDss (0.6–3 L/kg), and α–cadinene and γ–muurolene exhibited high VDss (>3–5 L/kg), potentially increasing tissue accumulation risk.

For BBB permeability, five compounds—(+)–Spathulenol, τ–Muurolol, (+)–α–muurolene,

α–cadinene, and γ–muurolene—had values >0.1, indicating potential brain penetration. Since anti–inflammatory agents typically target peripheral tissues, further optimization may be required to reduce CNS exposure (Fengler et al., 2021; Zhao et al., 2025).

Metabolic profiling showed none of the compounds inhibited CYP2D6 or CYP3A4, minimizing risk of impaired metabolism and hepatic toxicity (Fadzillah et al., 2024). Plasma clearance (CLplasma) values ranged from 0–15; Hexadecanoic acid, Oleic acid, and Scopoletin (0–5) indicated prolonged circulation, while the remaining six compounds (5–15) were acceptable but may require dosing adjustments.

Toxicity predictions (Table 4) indicated no AMES mutagenicity, human hepatotoxicity, or acute oral toxicity in rats, supporting the compounds' favorable safety profiles.

Table 4. Toxicological Profile Prediction

Chemical compounds	AMES Toxicity	Hepatoxicity	Rat Oral Acute Toxicity
Aspirin (Control)	0.256	0.407	0.346
Ibuprofen (Control)	0.046	0.740	0.307
(+)–Spathulenol	0.454	0.363	0.651
τ–Muurolol	0.323	0.606	0.333
Hexadecanoic acid	0.095	0.232	0.083
Oleic acid	0.088	0.256	0.088
(+)–α –murolene	0.127	0.520	0.203
α–cadinene	0.203	0.162	0.162
Scopoletin	0.587	0.425	0.494
γ–murolene	0.197	0.610	0.198
Ellagic acid	0.670	0.616	0.509

Description: 0.000–0.300: Safe; 0.301–0.700: Moderate; >0.701: Toxic (ADMETLab3)

The predicted toxicity probabilities for the nine compounds ranged from 0.083 to 0.651, placing them in the safe to moderate range according to ADMETLab3 thresholds. Among the tested compounds, (+)–Spathulenol, τ–Muurolol, and Scopoletin exhibit moderate toxicity across all three assessed parameters, suggesting careful dosage regulation is needed to mitigate potential toxic effects. Meanwhile, Hexadecanoic acid, Oleic acid, and α–Cadinene fall within the safe range, indicating low toxicity risk. However, it should be noted that these values are computational predictions rather than direct experimental data, and thus only provide a preliminary indication of potential toxicity. Experimental validation is required to confirm these findings and determine safe dosage levels *in vitro* and *in vivo*.

Docking results of *L. domesticum* compounds with the iNOS receptor

Following the identification of potential biological activity and the evaluation of hysicochemical and pharmacokinetic profiles of *L. domesticum* secondary metabolites, molecular docking analysis was performed using the inducible nitric oxide synthase (iNOS) receptor. Docking visualizations and binding affinity value are summarized in Table 5 and Figure 1.

For the natural ligand N-acetylglucosamine (NAG) bound to iNOS (PDB ID: 3E7G), hydrogen bond interactions revealed key active site residues: GLU377, ARG381, ASP382, and ARG388. Compounds interacting with these residues are hypothesized to exhibit low binding affinity values. The donor–acceptor hydrogen bond distance was observed to be a critical determinant of interaction strength; shorter distances generally indicate stronger binding and lower binding affinity values. In NAG, bonds with GLU377 and ARG388 displayed relatively long distances (3.26 Å and 3.32 Å, respectively), suggesting weaker interactions. By contrast, bonds with GLU377, ARG381, and ASP382 exhibited moderate distances of 3.11 Å, 2.93 Å, and 2.70 Å, respectively.

According to Fadzillah et al. (2024), hydrogen bonds are classified as strong (2.2–2.5 Å), moderate (2.5–3.2 Å), or weak (>3.2 Å). Thus, compounds capable of forming multiple strong or moderate hydrogen bonds with iNOS active site residues are more likely to yield stable complexes and potent anti-inflammatory activity.

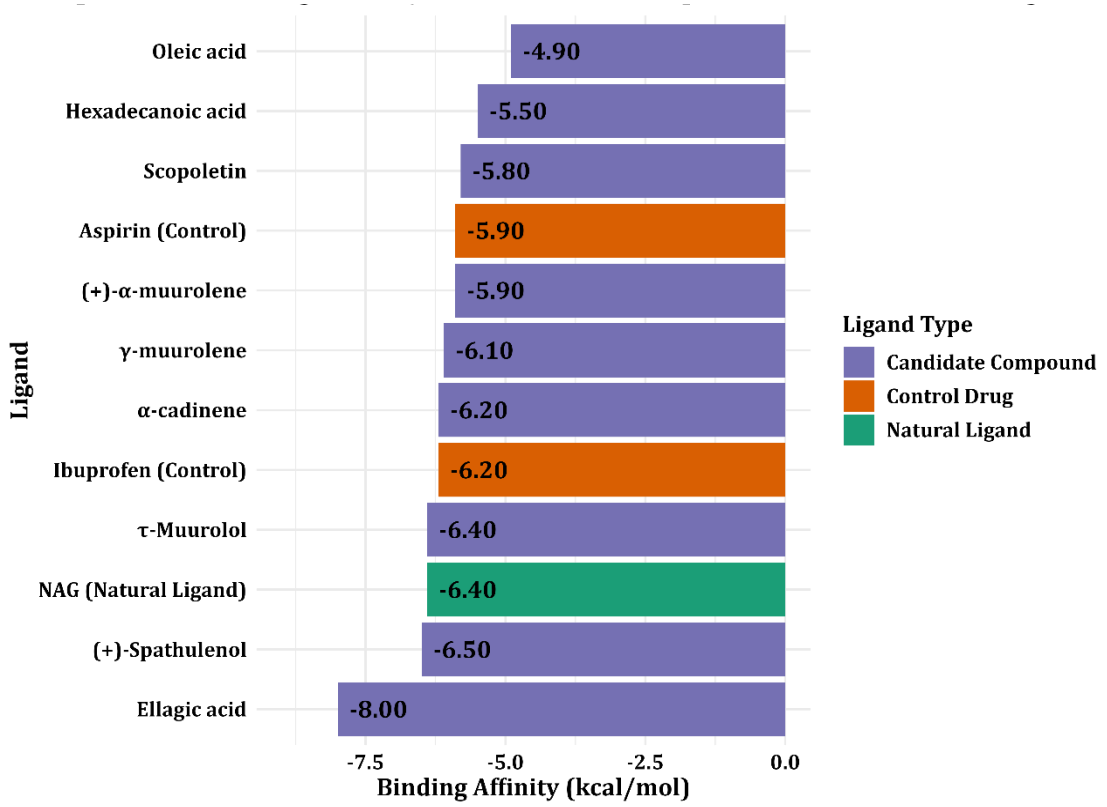
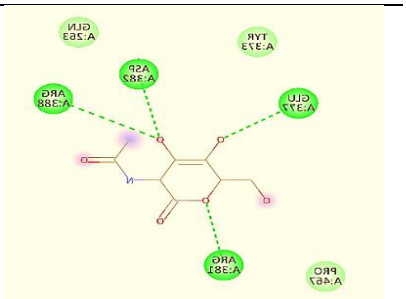
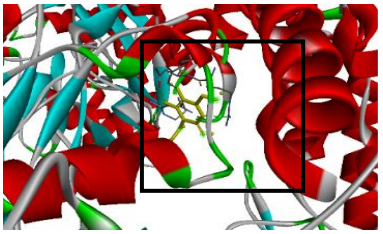
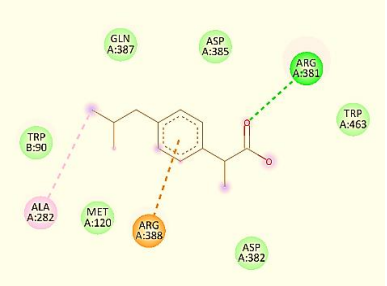
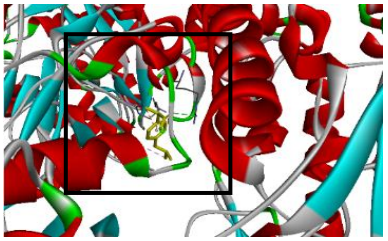
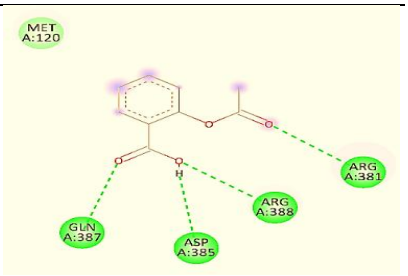
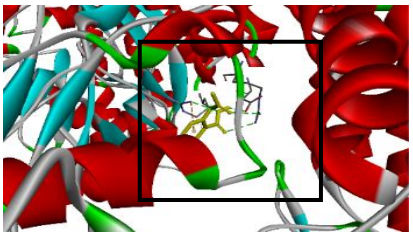
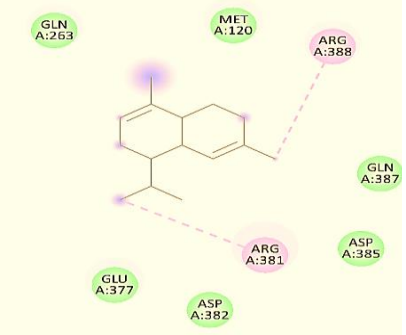
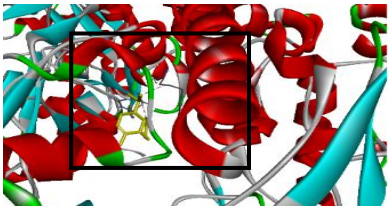
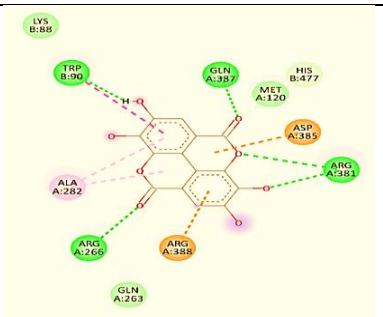
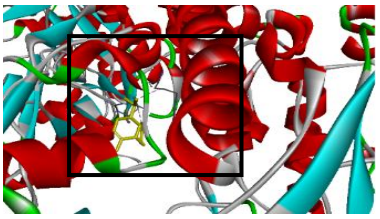
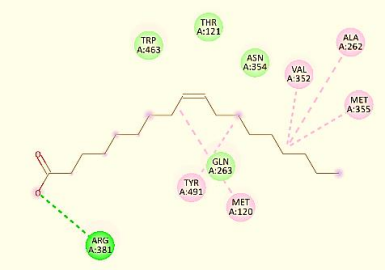
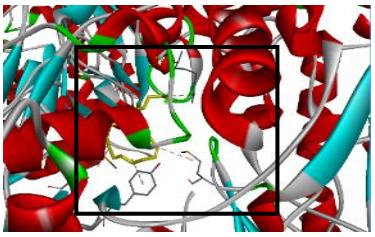
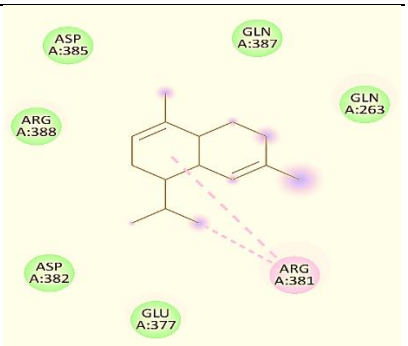
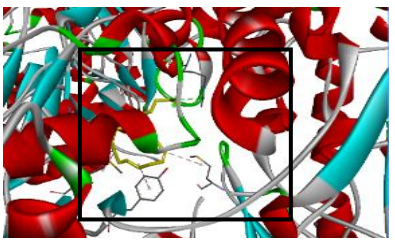


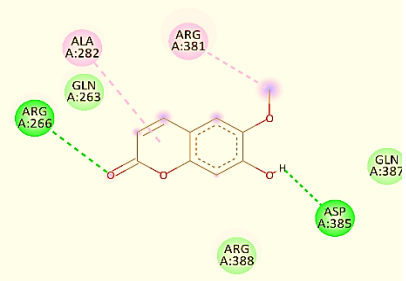
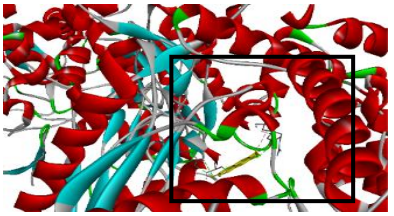
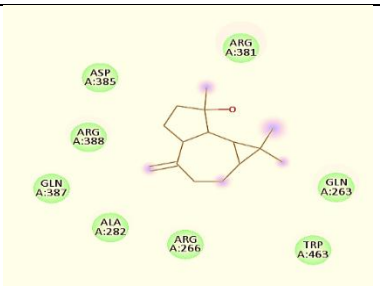
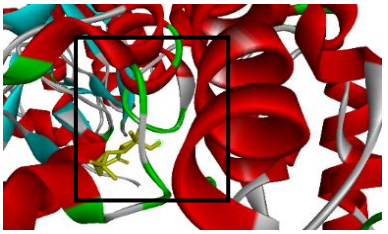
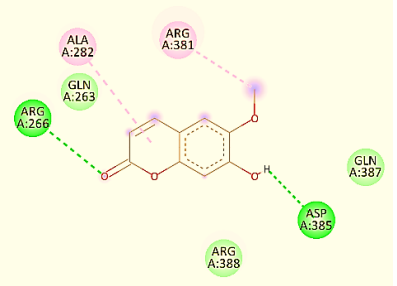
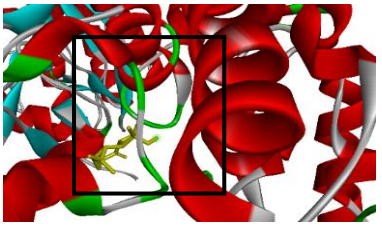
Figure 1. Comparative binding affinity of candidate compounds and control drugs

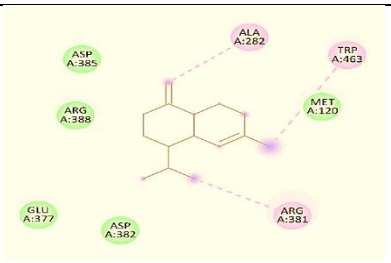
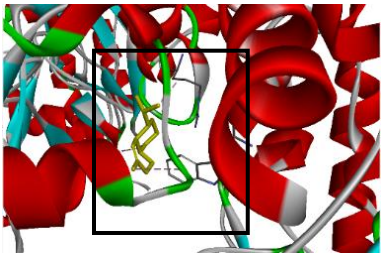
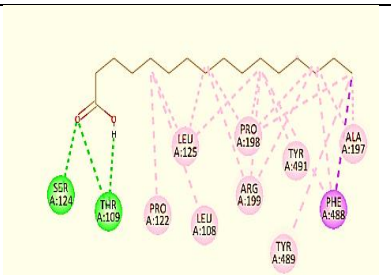
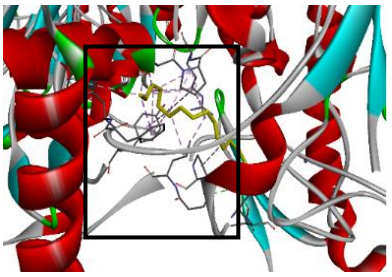
Table 5. The molecular docking results the iNOS receptor

Ligand	Binding affinity value (kcal/mol)	Hydrogen Bond Type	Hydrogen Bond Distance (Å)	Visualization on Type	Visualization
NAG (Natural ligand)	-6.4	GLU377 ARG381 ASP382 ARG288	3.11;3.26 2.93 2.7 3.32	2D	
				3D	

Ligand	Binding affinity value (kcal/mol)	Hydrogen Bond Type	Hydrogen Bond Distance (Å)	Visualization on Type	Visualization
Ibuprofen (Control)	-6.2	ARG381	2.85	2D	
				3D	
Aspirin (Control)	-5.9	ARG381 ASP385 GLN387 ARG388	3.09 2.82 3.18 2.85	2D	
				3D	
(+)-α-muurolene	-5.9	-	-	2D	
				3D	

Ligand	Binding affinity value (kcal/mol)	Hydrogen Bond Type	Hydrogen Bond Distance (Å)	Visualization on Type	Visualization
Ellagic acid	-8	TRP90 ARG266 ARG381 GLN387	2.92 3.30 2.83; 3.14 3.20	2D	
				3D	
Oleic acid	-4.9	ARG381	3.24	2D	
				3D	
α-cadinene	-6.2	-	-	2D	
				3D	

Ligand	Binding affinity value (kcal/mol)	Hydrogen Bond Type	Hydrogen Bond Distance (Å)	Visualization on Type	Visualization
Scopoletin	-5.8	ARG266 ASP385	3.10; 3.13 2.23	2D	
				3D	
(+)-Spathulenol	-6.5	-	-	2D	
				3D	
τ-Muurolol	-6.4	ARG388	3.2	2D	
				3D	

Ligand	Binding affinity value (kcal/mol)	Hydrogen Bond Type	Hydrogen Bond Distance (Å)	Visualization on Type	Visualization
γ-muurolene	-6.1	-	-	2D	
				3D	
Hexadecanoic Acid	-5.5	THR109 SER124	2.23; 2.86; 3.07 2.97	2D	
				3D	

Ibuprofen (–6.2 kcal/mol) formed a moderate hydrogen bond with ARG381 (2.85 Å) and additional van der Waals and hydrophobic contacts, consistent with stable active-site binding (Ossowicz-Rupniewska et al., 2022). Aspirin (–5.9 kcal/mol) engaged four hydrogen bonds with ARG381, ASP385, GLN387, and ARG388, together with a van der Waals contact to MET120, further supporting its anchoring within the iNOS catalytic channel (Takahashi et al., 2022).

Among the metabolites, (+)-α-muurolene (–6.1 kcal/mol) showed moderate affinity through hydrophobic and van der Waals interactions with residues including MET120, GLN263, GLU377, and ARG388, suggesting steric stabilization even in the absence of hydrogen bonding (Maiolini et al., 2025). Ellagic acid displayed the strongest binding (–8.0 kcal/mol), forming five hydrogen bonds—three with active-site residues—plus multiple van der Waals contacts, indicating strong anchoring and potential direct blockade of L-arginine access (Gil et al., 2021; Murphy et al., 2020). In other hand, another study by Lin et al. (2020) revealed that ellagic acid's effects as anti–inflammatory

agent are linked to suppression of NF-κB signaling and antioxidant activity, which together reduce iNOS expression and NO overproduction

Scopoletin (−5.8 kcal/mol) and oleic acid (−4.9 kcal/mol) exhibited weaker binding, with limited hydrogen bonding to ARG266 and ARG381, respectively, which may limit active-site stabilization (Kumar & Kim, 2021). In contrast, α-cadinene achieved an affinity comparable to ibuprofen (−6.2 kcal/mol) through extensive hydrophobic contacts, implying steric hindrance of the substrate channel. τ-Muurolol (−6.4 kcal/mol) combined a moderate hydrogen bond with ARG388 (3.20 Å) and multiple noncovalent interactions, supporting literature reports of cytokine suppression (Yang et al., 2019).

Hexadecanoic acid (−5.5 kcal/mol) displayed limited activity, with interactions located outside the catalytic residues, suggesting weak or noncompetitive binding. Conversely, (+)-spathulenol (−6.5 kcal/mol) engaged multiple van der Waals contacts, including with ARG381 and ARG388, consistent with steric occupation of the catalytic pocket and experimental evidence of nitric oxide inhibition (Diep et al., 2023; Souza et al., 2020). γ-Muurolene (−6.1 kcal/mol) also showed moderate affinity through van der Waals and hydrophobic interactions with GLU377, ASP382, and ARG381.

Taken together, these results highlight ellagic acid, (+)-spathulenol, α-cadinene, and τ-muurolol as the most promising iNOS inhibitors. Their binding profiles suggest that inhibition may occur via anchoring within the catalytic pocket through hydrogen bonding, which stabilizes the ligand and prevents L-arginine orientation, combined with steric hindrance from hydrophobic interactions, which physically obstruct substrate diffusion into the active site.

Study limitations and future prospects

This study was conducted exclusively through *in silico* approaches, which, although powerful for preliminary screening, cannot fully capture the complexity of biological systems. The predictive models used for activity, pharmacokinetics, and toxicity rely on computational algorithms and databases (Fadzillah et al., 2024) and therefore the results may not always translate directly to experimental or clinical settings. Binding affinities from docking simulations provide valuable insights into possible ligand–iNOS interactions, but they do not account for dynamic conformational changes, protein flexibility, or cellular context that may lead to inaccurate predictions of binding modes and affinities, as real proteins undergo significant movement that affects ligand binding (Sheng et al., 2015; Vidal-Limon et al., 2022). Additionally, toxicity outcomes represent probability-based predictions rather than actual toxicological data, highlighting the need for empirical validation (Limbu et al., 2024).

Future research should therefore extend these findings into *in vitro* and *in vivo* studies to confirm the inhibitory effects of the identified compounds—particularly ellagic acid, (+)-spathulenol, α-cadinene, and τ-muurolol—on iNOS activity and inflammatory pathways. Molecular dynamics simulations could further refine docking predictions by assessing the stability of ligand–enzyme complexes over time (Salo-Ahen et al., 2020). Moreover, structure–activity relationship (SAR) studies may aid in optimizing these metabolites for enhanced potency and selectivity, while pharmacological assays will be critical to evaluate efficacy, dosage safety, and potential off-target effects (Brueckner et al., 2024; Lazim et al., 2020). Ultimately, integrating computational predictions with laboratory validation will accelerate the development of *L. domesticum*–derived metabolites as candidates for safer anti-inflammatory therapeutics development of herbal-based.

CONCLUSION

This study identified four lead secondary

metabolites from *L. domesticum* including ellagic acid, (+)-spathulenol, α-cadinene, and τ-muurolol as the most promising iNOS inhibitors with potential anti-inflammatory effects. These findings demonstrate the utility of *in silico* screening for prioritizing natural compounds with favorable physicochemical, pharmacokinetic, and safety profiles. The results provide a foundation for the development of *L. domesticum*–derived anti-inflammatory agents, but the predictions remain preliminary. Therefore, further validation through *in vitro* and *in vivo* experiments is essential to confirm efficacy, safety, and mechanism of action in order to develop herbal-based for the anti-inflammatory agents.

REFERENCES

Abdallah, H. M., Mohamed, G. A., & Ibrahim, S. R. M. (2022). Lansium domesticum—A Fruit with Multi-Benefits: Traditional Uses, Phytochemicals, Nutritional Value, and Bioactivities. *Nutrients*, *14*(7), 1531. https://doi.org/10.3390/nu14071531

Adachi, K., Sasaki, T., Arai, A., Shimizu, M., & Yamazaki, H. (2024). Impact of variability of *in silico* and *in vitro* octanol/water partition coefficients partition coefficients of compounds on the input parameters and results of simplified human physiologically based pharmacokinetic models after virtual oral administrations. *The Journal of Toxicological Sciences*, *49*(10), 459–466. https://doi.org/10.2131/jts.49.459

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., & Aja, P. M. (2023). *Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management*. *Scientific Reports*, *13*(1), 13398. https://doi.org/10.1038/s41598-023-40160-2

Al-Mohaya, M., Mesut, B., Kurt, A., & Çelik, Y. S. (2024). In silico approaches which are used in pharmacy. *Journal of Applied Pharmaceutical Science*. https://doi.org/10.7324/JAPS.2024.154854

Asano, D., Takakusa, H., & Nakai, D. (2023). Oral absorption of middle-to-large molecules and its improvement, with a focus on new modality drugs. *Pharmaceutics*, *16*(1), 47. https://doi.org/10.3390/pharmaceutics16010047

Brueckner, A. C., Shields, B., Kirubakaran, P., Suponya, A., Panda, M., Posy, S. L., Johnson, S., & Lakkaraju, S. K. (2024). MDFit: automated molecular simulations workflow enables high throughput assessment of ligands-protein dynamics. *Journal of Computer-Aided Molecular Design*, *38*(1), 24. https://doi.org/10.1007/s10822-024-00564-2

Castro-Alvarez, A., Costa, A., & Vilarrasa, J. (2017). The Performance of Several Docking Programs at Reproducing Protein–Macrolide-Like Crystal Structures. *Molecules*, *22*(1), 136. https://doi.org/10.3390/molecules22010136

Cifuentes, M., Verdejo, H. E., Castro, P. F., Corvalan, A. H., Ferreccio, C., Quest, A. F. G., Kogan, M. J., & Lavandero, S. (2025). Low-Grade Chronic Inflammation: a Shared Mechanism for Chronic Diseases. *Physiology*, *40*(1), 4–25. https://doi.org/10.1152/physiol.00021.2024

Cinelli, M. A., Do, H. T., Miley, G. P., & Silverman, R. B. (2020). Inducible nitric oxide synthase: Regulation, structure, and inhibition. *Medicinal Research Reviews*, *40*(1), 158–189. https://doi.org/10.1002/med.21599

Danforth, D. N. (2021). The Role of Chronic Inflammation in the Development of Breast Cancer. *Cancers*, *13*(15), 3918. https://doi.org/10.3390/cancers13153918

Diep, T. T., Van Dung, L., Van Trung, P., Hoai, N. T., Thao, D. T., Uyen, N. T. T., Linh, T. T. H., Truc, H. T., & Tran, L. T. T. (2023). Essential oils from *Acronychia pedunculata* (L.) Miq. in Vietnam: chemical composition, antimicrobial, cytotoxicity and nitric oxide inhibition activities. *Journal of Essential Oil*

Bearing Plants, *26*(6), 1460–1472. https://doi.org/10.1080/0972060X.2023.2287595

Domper Arnal, M.-J., Hijos-Mallada, G., & Lanas, A. (2022). Gastrointestinal and cardiovascular adverse events associated with NSAIDs. *Expert Opinion on Drug Safety*, *21*(3), 373–384. https://doi.org/10.1080/14740338.2021.1965988

Fadzillah, S. N., Hernawati, D., & Putra, R. R. (2024). Analisis *In Silico* : Aktivitas Antiinflamasi dan Prediksi Toksisitas Senyawa Metabolit Sekunder Daun Ciplukan (*Physalis peruviana* L.). *Bioscientist : Jurnal Ilmiah Biologi*, *12*(1), 517. https://doi.org/10.33394/bioscientist.v12i1.9592

Fengler, S., Kurkowsky, B., Kaushalya, S. K., Roth, W., Fava, E., & Denner, P. (2021). *Human iPSC-derived brain endothelial microvessels in a multi-well format enable permeability screens of anti-inflammatory drugs*. https://doi.org/10.1101/2021.05.03.442133

Fu, L., Shi, S., Yi, J., Wang, N., He, Y., Wu, Z., Peng, J., Deng, Y., Wang, W., Wu, C., Lyu, A., Zeng, X., Zhao, W., Hou, T., & Cao, D. (2024). ADMETlab 3.0: an updated comprehensive online ADMET prediction platform enhanced with broader coverage, improved performance, API functionality and decision support. *Nucleic Acids Research*, *52*(W1), W422–W431. https://doi.org/10.1093/nar/gkae236

Gazzillo, E., Colarusso, E., Giordano, A., Chini, M. G., Potenza, M., Hofstetter, R. K., Iorizzi, M., Werz, O., Lauro, G., & Bifulco, G. (2024). Repositioning of Small Molecules through the Inverse Virtual Screening in silico Tool: Case of Benzothiazole-Based Inhibitors of Soluble Epoxide Hydrolase (sEH). *ChemPlusChem*. https://doi.org/10.1002/cplu.202400234

Gil, T.-Y., Hong, C.-H., & An, H.-J. (2021). Anti-Inflammatory Effects of Ellagic Acid on Keratinocytes via MAPK and STAT Pathways. *International Journal of Molecular Sciences*, *22*(3), 1277. https://doi.org/10.3390/ijms22031277

Gunarti, N. S., Ruswanto, R., Angelica, E. O., & Hidayah, H. (2024). Bioinformatics Studies of Flavonoid Derivatives Compound from Saga Rambat Leaves as an Antipyretic Candidate. *Jurnal Kimia Sains Dan Aplikasi*, *26*(12), 466–476. https://doi.org/10.14710/jksa.26.12.466-476

Guzik, T. J., Nosalski, R., Maffia, P., & Drummond, G. R. (2024). Immune and inflammatory mechanisms in hypertension. *Nature Reviews Cardiology*, *21*(6), 396–416. https://doi.org/10.1038/s41569-023-00964-1

Hanif, A. U., Lukis, P. A., & Fadlan, A. (2020). Pengaruh Minimisasi Energi MMFF94 dengan MarvinSketch dan Open Babel PyRx pada Penambatan Molekular Turunan Oksindola Tersubstitusi. *ALCHEMY*, *8*(2), 33–40. https://doi.org/10.18860/al.v8i2.10481

Hestiningsih, T., Gassani, F. F., & K, T. (2022). Antiinflammation Activity of Duku Seed Extract Gel in Carragenan Induced Wistar White Rats. *Sriwijaya Journal of Dentistry*, *3*(1), 6–14. https://doi.org/10.32539/sjd.v3i1.20

Ibrahim, Z. Y., Uzairu, A., Shallangwa, G. A., & Abechi, S. E. (2021). Pharmacokinetic predictions and docking studies of substituted aryl amine-based triazolopyrimidine designed inhibitors of Plasmodium falciparum dihydroorotate dehydrogenase (PfDHODH). *Future Journal of Pharmaceutical Sciences*, *7*(1), 133. https://doi.org/10.1186/s43094-021-00288-2

Karim, B. K., Tsamarah, D. F., Zahira, A., Rosandi, N. F., Swarga, K. F., Aulifa, D. L., Elaine, A. A., & Sitinjak, B. D. P. (2024). Studi In-Silico Senyawa Aktif Daun Jambu Biji (Psidium guajava L.) sebagai Kandidat Obat Antikanker Payudara. *Indonesian Journal of Biological Pharmacy*, *3*(3), 194. https://doi.org/10.24198/ijbp.v3i3.52060

Kumar, S., & Kim, M. (2021). SMPLIP-Score: predicting ligand binding affinity from simple and interpretable on-the-fly interaction fingerprint pattern descriptors. *Journal of Cheminformatics*, *13*(1), 28. https://doi.org/10.1186/s13321-021-00507-1

Lazim, R., Suh, D., & Choi, S. (2020). Advances in Molecular Dynamics Simulations and Enhanced Sampling Methods for the Study of Protein Systems. *International Journal of Molecular Sciences*, *21*(17), 6339. https://doi.org/10.3390/ijms21176339

Limbu, S., Glasgow, E., Block, T., & Dakshanamurthy, S. (2024). A Machine-Learning-Driven Pathophysiology-Based New Approach Method for the Dose-Dependent Assessment of Hazardous Chemical Mixtures and Experimental Validations. *Toxics*, *12*(7), 481. https://doi.org/10.3390/toxics12070481

Lin, Z., Lin, C., Fu, C., Lu, H., Jin, H., Chen, Q., & Pan, J. (2020). The protective effect of Ellagic acid (EA) in osteoarthritis: An in vitro and in vivo study. *Biomedicine & Pharmacotherapy*, *125*, 109845. https://doi.org/10.1016/j.biopha.2020.109845

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings 1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3–25. 1. *Advanced Drug Delivery Reviews*, *46*(1–3), 3–26. https://doi.org/10.1016/S0169-409X(00)00129-0

Liu, K., & Kokubo, H. (2020). Prediction of ligand binding mode among multiple cross-docking poses by molecular dynamics simulations. *Journal of Computer-Aided Molecular Design, 34*, 1195–1205. https://doi.org/10.1007/s10822-020-00340-y

Maiolini, T. C. S., Nicácio, K. de J., Rosa, W., Miranda, D. de O., Santos, M. F. C., Bueno, P. C. P., Lago, J. H. G., Sartorelli, P., Dias, D. F., Chagas de Paula, D. A., & Soares, M. G. (2025). Potential anti-inflammatory biomarkers from Myrtaceae essential oils revealed by untargeted metabolomics. *Natural Product Research*, *39*(4), 985–992. https://doi.org/10.1080/14786419.2023.2283758

Mayanti, T., Sinaga, S. E., & Supratman, U. (2022). Phytochemistry and biological activity of *Lansium domesticum* Corr. species: a review. *Journal of Pharmacy and Pharmacology*, *74*(11), 1568–1587. https://doi.org/10.1093/jpp/rgac057

Mazzini, S., Musso, L., Dallavalle, S., & Artali, R. (2020). Putative SARS-CoV-2 Mpro Inhibitors from an In-House Library of Natural and Nature-Inspired Products: A Virtual Screening and Molecular Docking Study. *Molecules*, *25*(16), 3745. https://doi.org/10.3390/molecules25163745

Murphy, M. T., Qin, X., Kaul, S., Barrientos, G., Zou, Z., Mathias, C. B., Thomas, D., & Bose, D. D. (2020). The polyphenol ellagic acid exerts anti-inflammatory actions via disruption of store-operated calcium entry (SOCE) pathway activators and coupling mediators. *European Journal of Pharmacology*, *875*, 173036. https://doi.org/10.1016/j.ejphar.2020.173036

Ossowicz-Rupniewska, P., Klebeko, J., Świątek, E., Szachnowska, J., Janus, E., Rangelov, M., Todorova, N., Taneva, S. G., Krachmarova, E., & Guncheva, M. (2022). Binding behavior of ibuprofen-based ionic liquids with bovine serum albumin: Thermodynamic and molecular modeling studies. *Journal of Molecular Liquids*, *360*, 119367. https://doi.org/10.1016/j.molliq.2022.119367

Panchal, N. K., & Prince Sabina, E. (2023). Non-steroidal anti-inflammatory drugs (NSAIDs): A current insight into its molecular mechanism eliciting organ

toxicities. *Food and Chemical Toxicology*, *172*, 113598. https://doi.org/10.1016/j.fct.2022.113598

Pirie, R., Stanway-Gordon, H. A., Stewart, H. L., Wilson, K. L., Patton, S., Tyerman, J., Cole, D. J., Fowler, K., & Waring, M. J. (2024). An analysis of the physicochemical properties of oral drugs from 2000 to 2022. *RSC Medicinal Chemistry*, *15*(9), 3125–3132. https://doi.org/10.1039/D4MD00160E

Prata, C., Angeloni, C., & Maraldi, T. (2024). Strategies to Counteract Oxidative Stress and Inflammation in Chronic-Degenerative Diseases 2.0. *International Journal of Molecular Sciences*, *25*(9), 5026. https://doi.org/10.3390/ijms25095026

Pyka-Pająk, A., Parys, W., & Dołowy, M. (2019). Comparison of the Utility of RP-TLC Technique and Different Computational Methods to Assess the Lipophilicity of Selected Antiparasitic, Antihypertensive, and Anti-inflammatory Drugs. *Molecules*, *24*(17), 3187. https://doi.org/10.3390/molecules24173187

Rai, M., Singh, A. V., Paudel, N., Kanase, A., Falletta, E., Kerkar, P., Heyda, J., Barghash, R. F., Pratap Singh, S., & Soos, M. (2023). Herbal concoction Unveiled: A computational analysis of phytochemicals' pharmacokinetic and toxicological profiles using novel approach methodologies (NAMs). *Current Research in Toxicology*, *5*, 100118. https://doi.org/10.1016/j.crtox.2023.100118

Rajendran, P., Chen, Y., Chen, Y., Chung, L., Tamilselvi, S., Shen, C., Day, C. H., Chen, R., Viswanadha, V. P., Kuo, W., & Huang, C. (2018). The multifaceted link between inflammation and human diseases. *Journal of Cellular Physiology*, *233*(9), 6458–6471. https://doi.org/10.1002/jcp.26479

Ramírez, D., & Caballero, J. (2018). Is It Reliable to Take the Molecular Docking Top Scoring Position as the Best Solution without Considering Available Structural Data? *Molecules*, *23*(5), 1038. https://doi.org/10.3390/molecules23051038

Rana, T. (2022). Influence and Implications of the Molecular Paradigm of Nitric Oxide Underlying Inflammatory Reactions of the Gastrointestinal Tract of Dog: A Major Hallmark of Inflammatory Bowel Disease. *Inflammatory Bowel Diseases*, *28*(8), 1280–1288. https://doi.org/10.1093/ibd/izac017

Riyadi, P., Romadhon, Anggo, A., Suharto, S., Tanod, W., & Aryani, A. (2021). Anti-Inflammatory Potential from Tilapia (*Oreochromis niloticus*) Viscera Hydrolysate with Bioinformatics Analysis (Prediction of Activity Spectra for Substances – PASS). *IOP Conference Series: Earth and Environmental Science*, *750*(1), 012044. https://doi.org/10.1088/1755-1315/750/1/012044

Rudichenko, V., & Sokolenko, A. (2023). Importance of Prophylactic Strategies in Prevention of Gastrointestinal Injuries by Nonsteroidal Antiinflammatory Drugs (Clinical case). *Family Medicine. European Practices*, *4*, 83–88. https://doi.org/10.30841/2786-720X.4.2023.297048

Salo-Ahen, O. M. H., Alanko, I., Bhadane, R., Bonvin, A. M. J. J., Honorato, R. V., Hossain, S., Juffer, A. H., Kabedev, A., Lahtela-Kakkonen, M., Larsen, A. S., Lescrinier, E., Marimuthu, P., Mirza, M. U., Mustafa, G., Nunes-Alves, A., Pantsar, T., Saadabadi, A., Singaravelu, K., & Vanmeert, M. (2020). Molecular Dynamics Simulations in Drug Discovery and Pharmaceutical Development. *Processes*, *9*(1), 71. https://doi.org/10.3390/pr9010071

Sari, I. W., Junaidin, J., & Pratiwi, D. (2020). Studi Molecular Docking Senyawa Flavonoid dari Herba Kumis Kucing (*Orthosiphon stamineus* B.) pada Reseptor α-Glukosidase sebagai Antidiabetes Tipe 2. *Jurnal Farmagazine*, *7*(2), 54. https://doi.org/10.47653/farm.v7i2.194

Shaker, B., Ahmad, S., Lee, J., Jung, C., & Na, D. (2021). In silico methods and tools for drug discovery. *Computers in Biology and Medicine*, *137*, 104851. https://doi.org/10.1016/j.compbiomed.2021.104851

Sheng, Y., Zhong, L., Guo, D., Lau, G., & Feng, C. (2015). Insight into structural rearrangements and interdomain interactions related to electron transfer between flavin mononucleotide and heme in nitric oxide synthase: A molecular dynamics study. *Journal of Inorganic Biochemistry*, *153*, 186–196. https://doi.org/10.1016/j.jinorgbio.2015.08.006

Smith, D. A., Beaumont, K., Maurer, T. S., & Di, L. (2015). Volume of Distribution in Drug Design. *Journal of Medicinal Chemistry*, *58*(15), 5691–5698. https://doi.org/10.1021/acs.jmedchem.5b00201

Souza, M. A., Guzatti, J. G. G., Martello, R. H., Schindler, M. S. Z., Calisto, J. F. F., Morgan, L. V., Aguiar, G. P. S., Locateli, G., Scapinello, J., Müller, L. G., Oliveira, J. V., & Dal Magro, J. (2020). Supercritical CO2 extraction of Aloysia gratissima leaves and evaluation of anti-inflammatory activity. *The Journal of Supercritical Fluids*, *159*, 104753. https://doi.org/10.1016/j.supflu.2020.104753

Sulistyowaty, M. I., Widyowati, R., Putra, G. S., Budiati, T., & Matsunami, K. (2021). Synthesis, ADMET predictions, molecular docking studies, and *in-vitro* anticancer activity of some benzoxazines against A549 human lung cancer cells. *Journal of Basic and Clinical Physiology and Pharmacology*, *32*(4), 385–392. https://doi.org/10.1515/jbcpp-2020-0433

Sun, D., Gao, W., Hu, H., & Zhou, S. (2022a). Why 90% of clinical drug development fails and how to improve it? *Acta Pharmaceutica Sinica B*, *12*(7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

Sun, D., Gao, W., Hu, H., & Zhou, S. (2022b). Why 90% of clinical drug development fails and how to improve it? *Acta Pharmaceutica Sinica B*, *12*(7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

Takahashi, M., Chen, S., Matsui, H., Morimoto, N., & Ikemoto, Y. (2022). Van der Waals interactions regulating the hydration of 2-methacryloyloxyethyl phosphorylcholine, the constructing monomer of biocompatible polymers. *Scientific Reports*, *12*(1), 20393. https://doi.org/10.1038/s41598-022-24841-y

Techavuthiporn, C. (2018). Langsat— Lansium domesticum. In *Exotic Fruits* (pp. 279–283). Elsevier. https://doi.org/10.1016/B978-0-12-803138-4.00036-8

Thangavel, N., & Albratty, M. (2022). Pharmacophore model-aided virtual screening combined with comparative molecular docking and molecular dynamics for identification of marine natural products as SARS-CoV-2 papain-like protease inhibitors. *Arabian Journal of Chemistry*, *15*(12), 104334. https://doi.org/10.1016/j.arabjc.2022.104334

Tonk, R., Tewatia, S., Majeed, S., & Dagar, M. (2020). Non-steroidal Anti-inflammatory Drugs (NSAIDS): Chemistry, Mechanism and their Adverse events. *American Journal of PharmTech Research*, *8*(5), 43–49. https://doi.org/10.46624/ajphr.2020.v8.i5.004

Uzzaman, M., Fatema, K., Akhter, S., Hossain, Md. R., Akter, N., Tasnuva, S., Jahan, S., Hasan, Md. K., Shimu, Mst. S. S., Hossain, Md. K., & Afrin, Mst. F. (2023). Medicinal and toxicological investigation of some common NSAIDs; A computer-aided drug design approach. *Journal of Molecular Structure*, *1292*, 136124. https://doi.org/10.1016/j.molstruc.2023.136124

Vidal-Limon, A., Aguilar-Toalá, J. E., & Liceaga, A. M. (2022). Integration of Molecular Docking Analysis and Molecular Dynamics Simulations for Studying Food Proteins and Bioactive Peptides. *Journal of Agricultural and Food Chemistry*, *70*(4), 934–943. https://doi.org/10.1021/acs.jafc.1c06110

Wang, Z., Qi, F., Luo, H., Xu, G., & Wang, D. (2022). Inflammatory Microenvironment of Skin Wounds. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.789274

Waters, N. J., & Lombardo, F. (2010). Use of the Øie-Tozer Model in Understanding Mechanisms and Determinants of Drug Distribution. *Drug Metabolism and Disposition*, *38*(7), 1159–1165. https://doi.org/10.1124/dmd.110.032458

Yang, J., Choi, W.-S., Kim, J.-W., Lee, S.-S., & Park, M.-J. (2019). Anti-Inflammatory Effect of Essential Oils Extracted from Wood of Four Coniferous Tree Species. *Journal of the Korean Wood Science and Technology*, *47*(6), 674–691. https://doi.org/10.5658/WOOD.2019.47.6.674

Zhao, M., Lin, Y., Zeng, Y., Lv, Z., Liang, J., Tang, P., Zhen, X., & Han, L. (2025). Biomimetic membrane-coated nanoparticles specially permeate the inflammatory blood-brain barrier to deliver plasmin therapy for brain metastases. *Journal of Controlled Release*, *378*, 763–775. https://doi.org/10.1016/j.jconrel.2024.12.066