International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

INTERACTIONS OF *ORTHOSIPHON STAMINEUS* COMPOUNDS AGAINST COX-2 AS AN ANTI-INFLAMMATORY USING *IN SILICO* METHODS AND TOXICITY PREDICTION

MUCHTARIDI[1*], MICHELLE DARMAWAN[2], MARIA ELIZABETH[2], DELA NURZANAH[2], PRISKILA MARGARETHA[2], ANGELA ALYSIA ELAINE[2], NELI[1], NURHANIFAH PUSPITADEWI[2], LUTHFI UTAMI SETYAWATI[1], NUR KUSAIRA KHAIRUL IKRAM[3]

[1]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, 45363 Sumedang, Indonesia, [2]Faculty of Pharmacy, Universitas Padjadjaran, 45363 Sumedang, Indonesia, [3]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[*]Corresponding author: Muchtaridi; [*]Email: muchtaridi@unpad.ac.id

Received: 21 Jun 2023, Revised and Accepted: 02 Sep 2023

ABSTRACT

Objective: *Orthosiphon stamineus*, or cat's whiskers, are known to have several pharmacological activities, one of which is anti-inflammatory. An *in silico* study was conducted to determine the active compound with anti-inflammatory activity from *Orthosiphon stamineus* leaves while also assessing their toxicity.

Methods: AutoDock 4 was used to perform molecular docking, while LigandScout 4.4.3 Advanced was used to screen pharmacophores. The Swiss ADME and PreAdmet websites were used to screen the prediction of Lipinski's rules of 5 and toxicity.

Results: In this *in silico* study on the COX-2 enzyme (PDB ID: 3ln1) with a RMSD validation value of 1.00 Å, Tetramethyl Luteolin emerged as the most promising candidate, exhibiting the lowest binding energy of-9.90 kcal/mol and a KI value of 55.80 nM, indicating favorable interactions within the active site. The compound also satisfied the Lipinski Rules and demonstrated favorable absorption and distribution characteristics, with HIA at 98.440681% and CaCO2 permeability at 53.1689 nm/sec, along with a small BBB value of 0.0154021 and quite good %PPB of 87.388706. Furthermore, Tetramethyl Luteolin obtained a pharmacophore fit score of 32.42, indicating possession of key structural features essential for desired biological activity.

Conclusion: The flavonoid-derived compounds in cat's whisker leaf extract show promise as potential anti-inflammatory drug candidates, with Tetramethyl luteolin emerging as the best candidate among nine compounds, meeting Lipinski rules and exhibiting superior ADMET properties. These results highlight the potential of Tetramethyl Luteolin as a lead compound, necessitating additional research into its intended target or biological function.

Keywords: Anti-inflammatory, Molecular docking, Orthosiphon stamineus Benth, COX-2

DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.48663. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Inflammation is a response to injury or infection and initiates the wound-healing process. Steroid anti-inflammatory drugs (SAIDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are the two types of anti-inflammatory medications. NSAIDs are further classified into two types: non-selective NSAIDs that inhibit COX-1 and COX-2 enzymes and selective NSAIDs that work by selectively inhibiting COX-2 [1]. Pharmacists face a number of issues, but one of the most pressing is the creation of safer, more effective anti-inflammatory agents with fewer adverse effects [2]. Cyclooxygenase-2 (COX-2) is the main cyclooxygenase involved in the inflammatory response. When COX-2 is induced, there will be excessive production of PGE2 (Prostaglandin E2) and other prostaglandins, which can induce pain and diseases related to inflammation [3]. Clinical evidence suggests that while COX-2 enzymes are essential for injury repair, they can also contribute to pathological processes like carcinogenesis and cancer progression [4]. They are highly expressed in inflammatory processes and malignancies, such as IBD and colon cancer, while their expression in normal colon cells is minimal or undetectable [5]. This emphasizes the significance of COX-2 in the inflammatory response and its therapeutic potential. The development of anti-inflammatory medications that successfully reduce inflammation-related disorders while having minimal impact on healthy cells is a current area of intense research [6]. In the pursuit for new and improved anti-inflammatory agents, selective inhibition of COX-2 is a promising strategy. To date, no *in silico* study between COX-2 and compounds in cat's whiskers leaves has been conducted.

Orthosiphon stamineus (cat's whiskers) is a Southeast Asian medicinal herb that has traditionally been used to treat rheumatoid arthritis, gout, and other inflammatory disorders. Terpenoids, sterols, and polyphenols are among the active ingredients of the plant. Cat's whisker leaves are known to have several pharmacological activities, one of which is anti-inflammatory. Chloroform and ethanol extracts from the leaves as well as their flavonoid-rich fraction, were reported to have anti-inflammatory activity in carrageenan-induced foot inflammation in male Wistar rats [7, 8]. The therapeutic effect of the cat's whiskers plant mainly comes from its polyphenols (lipophilic flavonoids and phenolic acids), which are the most dominant constituents of the plant's leaves [9, 10].

Flavonoids in the cat's whiskers plant are hydrophilic (glycoside flavonoid) and lipophilic. The types of flavonoids contained in the plant include sinensetin [11-13], eupatorin [11-13], tetramethoxyflavone (TMF) [12, 13], salvigenin [12, 13], cirsimaritin [13], rhamnazin [12, 13], pilloin [12, 13], trimethyl apigenin [12, 13], and tetramethyl luteolin [12-14]. The presence of the benzopyrone ring in the structure of the flavonoid binds to the cyclooxygenase and lipoxygenase enzymes, causing anti-inflammatory activity [15]. Eupatorin, TMF (tetramethoxyflavone), and sinensetin are substances that can modulate COX-2-dependent prostanoid production and/or NO generation via the iNOS pathway [7].

In silico testing was carried out to determine the active compounds with anti-inflammatory activity in the leaves of the cat's whiskers. The series of computational tests or virtual screenings that were carried out consisted of Lipinski predictions, ADMETOX predictions, molecular docking, and structure-based pharmacophore screening. The concept of ligand-receptor recognition underlies this computational testing for both target structure-based drug

development and small molecule structure-based drug development [16]. This study aims to explore the anti-inflammatory potential of *Orthosiphon stamineus* compounds against COX-2 using *in silico* methods and integrated ADME/Toks studies, with potential implications for the development of new anti-inflammatory agents.

MATERIALS AND METHODS

Tools

The hardware used is a personal laptop with Intel(R) Core(TM) i7-10510U processor specifications @1.80GHz, 2.30 GHz, 8.00 GB of RAM with Windows 10 64-bit operating system. Software used for a series of computing methods are Autodock 4, Biovia Discovery Studio 2020, ChemDraw Ultra 12.0, Chem3D Pro 12.0, LigandScout 4.4.3 Advanced, RCSB PDB Web (https://www.rcsb.org/), PubChem Web (https://pubchem.ncbi.nlm.nih.gov/), Swiss ADME Web (http://www.swissadme.ch/index.php#), PreAdme/Tox Web (https://preadmet.bmdrc.kr/), and the DUD-E Web (http://dude.docking.org/targets).

Materials

The materials used for the molecular docking test were cyclooxygenase-2 receptors (PDB ID: 3ln1) along with 9 test compounds (sinensetin, tetramethoxyflavone, eupatorin, salvigenin, cirsimaritin, pilloin, rhamnazin, trimethyl apigenin, and tetramethyl luteolin). The materials used for pharmacophore modeling are cyclooxygenase-2 receptors with the code PGH2/3ln1 along with 9 test compounds.

Lipinski's prediction

The analysis was carried out through the SwissADME website by entering the SMILES isomeric structure of each compound obtained from the PubChem website [17].

ADME/TOX predictions

The analysis was carried out via the PreAdmet website by entering .mol file of the 2D structure of the compound that had been created using ChemDraw [18-21].

Molecular docking

The COX-2 receptor structure (PDB ID: 3ln1) was downloaded from the PDB website, and receptor preparation was performed using the Biovia Discovery Studio application, separating it from its natural ligand. Test ligands were prepared by importing the. sdf file and minimizing their energy structures in Chem3D. The receptor files and test ligand compounds were then prepared using AutoDock 4, considering charges and hydrogen atoms, and saved in the. pdbqt format. Molecular docking simulation was conducted with specific GridBox and docking parameters, incorporating Lamarckian Parameters and setting the Number of GA Runs to 100 repetitions, resulting in. gpf and. dpf files. Finally, docking was run using the command prompt, and the molecular docking results were analyzed using the BIOVIA application [21, 22].

Pharmacophore modeling

The analysis involved the creation of a database with active compounds and decoys from code 3ln1 on the website http://dude.docking.org/targets. Using the Biovia Discovery Studio application, 100 active compounds and 400 decoy compounds were obtained and saved in'.ldb' format. A separate test compound database was created with 9 test compounds, minimized for energy, and saved in'.pdb' format. Pharmacophore modeling was conducted using LigandScout, resulting in 1-10 pharmacophore models saved in'.pmz' format. The pharmacophore models were validated by calculating the AUC values, and the model with the best ROC was selected for further analysis [19, 23].

RESULTS

Lipinski's prediction

According to the Lipinski parameter test results (table 1), the nine test compounds had no more than 5 donor hydrogen bonds and 10 acceptor hydrogen bonds, their molecular weight was less than 500 Da, and the Log P value obtained was less than 514, indicating that they met the requirements to be used as oral preparations.

Table 1: Lipinski test results

No.	Compound name	Molecular weight (<500 Da)	Log P (<5)	Hydrogen bonds		Interpretation
				Donor (<5)	Acceptor (<10)	
1.	3'-hydroxy-5,6,7,4'-Tetramethoxyflavone (TMF)	358.34 g/mol	2.75	1	7	Qualify
2.	Sinensetin (5,6,7,3',4'-pentamethoxyflavone)	372.37 g/mol	3.10	0	7	Qualify
3.	Cirsimaritin (4',5-Dihydroxy-6,7-dimethoxyflavone)	314.29 g/mol	2.52	2	6	Qualify
4.	Eupatorin (5,3'-dihydroxy-6,7,4'-trimethoxyflavone)	344.32 g/mol	2.53	2	7	Qualify
5.	Trimetil Apigenin (4',5,7-Apigenin trimethyl ether; 4',5,7-Trimethoxyflavone)	312.32 g/mol	3.10	0	5	Qualify
6.	Pilloin (3',5-Dihydroxy-4',7-dimethoxyflavone)	314.29 g/mol	2.57	2	6	Qualify
7.	Salvigenin (5-Hydroxy-6,7,4'-trimethoxyflavone)	328.32 g/mol	2.88	1	6	Qualify
8.	Rhamnazin (3',7-Dimethylquercetin)	330.29 g/mol	2.02	3	7	Qualify
9.	Tetramethyl luteolin (3',4',5,7-Tetramethoxyflavone)	342.34 g/mol	3.10	0	6	Qualify

Table 2: **ADME-Toxic test results on** 9 **active compounds of *Orthosiphon stamineus***

No.	Compound name	Absorption		Distribution		Toxicity	
		HIA (%)	$CaCO_2$ (nm/sec)	PPB (%)	BBB	Mutagenic	Carcinogenic
1.	3'-hydroxy-5,6,7,4'-Tetramethoxyflavone (TMF)	96.806364	30.4826	85.826279	0.0140884	Mutagenic	Mouse: -Rat: +
2.	Sinensetin	98.886176	51.2255	86.241520	0.0236938	Mutagenic	Mouse: -Rat: +
3.	Cirsimaritin	93.377855	8.36789	88.058295	0.0573971	Mutagenic	Mouse: +Rat: +
4.	Eupatorin	93.449297	7.14362	85.458905	0.0313627	Mutagenic	Mouse: -Rat: +
5.	Trimethyl Apigenin	97.924880	55.3919	89.230735	1.0053	Mutagenic	Mouse: -Rat: +
6.	Pilloin	93.377749	9.7712	87.195822	0.0616361	Mutagenic	Mouse: -Rat: +
7.	Salvigenin	96.486354	33.065	87.411271	0.0197439	Mutagenic	Mouse: -Rat: +
8.	Rhamnazin	87.828569	5.09497	81.216853	0.0565755	Mutagenic	Mouse: -Rat: +
9.	Tetramethyl luteolin	98.440681	53.1689	87.388706	0.0154021	Mutagenic	Mouse: -Rat: +

Pre-ADMET test

Pharmacokinetic and toxicity studies showed that the absorption parameters of all compounds met the HIA criteria, which was 70-100%, whereas the $CaCO_2$ criteria was met by the trimethyl apigenin compound with a value of 55.3919 nm/sec (table 2). Regarding the distribution parameters, the rhamnazin compound shows the lowest %PBB criterion of 81.22%, whereas the TMF

compound obtained the lowest percentage of BBB of 0,014. Toxicity studies found a compound carcinogenic in mouse is-cirsimaritin.

Molecular docking

The RMSD value of 1.00 Å was obtained from the best-performing docking pose, while all other running RMSD values were ≤2 Å across 100 runs during the validation process. These findings indicate that the method's results meet the requirements (RMSD<2 Å for 70% of RMSD results) [24, 25], supporting its accuracy and reliability. Consequently, the study can proceed to the next stage using the Grid box values X = 30.092, Y =-22.559, and Z =-15.758.

Molecular docking was performed after the validation results of the 3ln1 receptor-ligand were obtained. Table 3 shows the simulated results of molecular docking of the 3ln1 receptors with natural ligands, comparator chemicals, and compounds sustained in the cat's whiskers leaf extract *(Orthosiphon stamineus*). Eupatorin, Pilloin, and Tetramethyl luteolin exhibit hydrogen bonding similarities with natural ligands (Celecoxib) in their hydrogen bonding patterns, while Sinensetin, Savigenin, and Rhamnazin share similarities with hydrogen bonding patterns found in standard compound (Etoricoxib) (fig. 1). Based on the docking data, Tetramethyl luteolin emerged as the most promising molecule, displaying the lowest binding energy of-9.90 kcal/mol and a KI value of 55.80 nM.

Table 3: Results of compound molecular anchoring in *Orthosiphon stamineus* leaves with natural ligands and comparison compounds

No.	Compounds/ ligand test	ΔG (kcal/mol)	Inhibition constant KI (nM)	Amino acid residue	
				Hydrogen bond	Other bonds
Natural Ligand					
	Celecoxib	-10.50	20.01 nM	ILE A: 503; PHE A: 504; GLN A: 178; SER A: 339; ARG A: 499	HIS A: 75 (Unfavorable Donor-Donor); VAL A: 509; VAL A: 335; TRP A: 373; LEU A: 370; MET A: 508; LEU A: 338; ALA A: 513; LEU A: 345; TYR A: 341
Standard					
	Etoricoxib	-10.39	24.32 nM	TYR A: 341	SER A: 339; SER A: 516; ALA A: 502; HIS A: 75; VAL A: 509; LEU A: 338 ALA A: 513; VAL A: 335; LEU A: 517; PHE A: 367; TRP A: 373
Active compounds of *Orthosiphon stamineus* leaves					
1.	TMF	-9.71	76.82 nM	-	ALA A: 513; MET A: 508; GLN A: 178; SER A: 339; LEU A: 370; PHE A: 367; TYR A: 371; VAL A: 335; VAL A: 509; ALA A: 502; HIS A: 75; TRP A: 373; PHE A: 504
2.	Sinensetin	-9.53	103.69 nM	TYR A: 341	MET A: 508; VAL A: 335; ALA A: 513; GLY A: 512; LEU A: 370; TRP A: 373; PHE A: 367; PHE A: 504; TYR A: 371; VAL A: 509; LEU A: 517; LEU A: 345; VAL A: 102
3.	Cirsimaritin	-3.81	1600 nM	VAL A: 102	LEU A: 345; TYR A: 341; VAL A: 335; LEU A: 517; PHE A: 504; TRP A: 373; VAL A: 509; ALA A: 513
4.	Eupatorin	-9.01	250.33 nM	GLN A: 178	LEU A: 338; SER A: 339; ALA A: 513; VAL A: 509; ALA A: 502; HIS A: 75; LEU A: 370; TRP A: 373; PHE A: 367; TYR A: 371; ILE A503 (Unfavorable Donor-Donor)
5.	Trimethyl Apigenin	-8.67	438.88 nM	-	GLN A: 178; SER A: 339; LEU A: 338; ALA A: 513; VAL A: 509; TYR A: 371; ALA A: 502; VAL A: 335; TRP A: 373; PHE A: 367; LEU A: 370
6.	Pilloin	-9.34	141.67 nM	SER A: 516; GLN A: 178; PHE A: 504	SER A: 339; HIS A: 75; ALA A: 502; LEU A: 370; TYR A: 371; PHE A: 367; TRP A: 373; VAL A: 509; LEU A: 338
7.	Salvigenin	-8.95	275.28 nM	ARG A: 106; TYR A: 341	TYR A: 371; GLY A: 512; ALA A: 513; MET A: 99; LEU A: 517; VAL A: 335; LEU A: 345; VAL A: 102
8.	Rhamnazin	-8.85	326.68 nM	TYR A: 341; TYR A: 371	HIS A: 75;PHE A: 504; ARG A: 499; ALA A: 502; VAL A: 335; TRP A: 373; LEU A: 370; SER A: 339; VAL A: 509; LEU A: 338
9	Tetramethyl Luteolin	-9.90	55.80 nM	LEU A: 338; PHE A: 504; GLN A: 178; HIS A: 75; ARG A: 499	GLY A: 512; VAL A: 509; TRP A: 373; MET A: 508; VAL A: 335; TYR A: 371; PHE A: 367; TYR A: 334

Table 4: Results of pharmacophore-fit score active compounds *Orthosiphon stamineus* leaves

No.	Compound	Matching features	Pharmacophore-fit score
1.	Eupatorin		40.01
2.	TMF (3'-hydroxy-5,6,7,4'-Tetramethoxyflavone)		39.92
3.	Sinensetin (5,6,7,3',4'-pentamethoxyflavone)		33.65
4.	Cirsimaritin (4',5-Dihydroxy-6,7-dimethoxyflavone)		33.58
5.	Pilloin (3',5-Dihydroxy-4',7-dimethoxyflavone)		32.46
6.	Tetramethyl luteolin (3',4',5,7-Tetramethoxyflavone)		32.42
7.	Trimethylapigenin		32.22
8.	Rhamnazin (3',7-Dimethylquercetin)		30.65

Pharmacophore screening results

The AUC-ROC value of a decent pharmacophore model had to be larger than 0.4-0.5 [26]. Based on the results, model 1 exhibited the highest AUC value of the ten models, at 0.73 (fig. 2). Structure-based pharmacophore features and ligand-based ones have been integrated into a single entity with the findings indicated that Eupatorin and TMF are the best pharmacophore models which

including four features: two hydrophobic features represented by yellow squares and two hydrogen bond acceptor features represented by red squares (table 4, fig. 2). Following the pharmacophore screening, it was discovered that only 8 compounds were suitable for screening, with the Eupatorin compound obtaining the highest Pharmacophore-Fit Score of 40.01 (table 4).

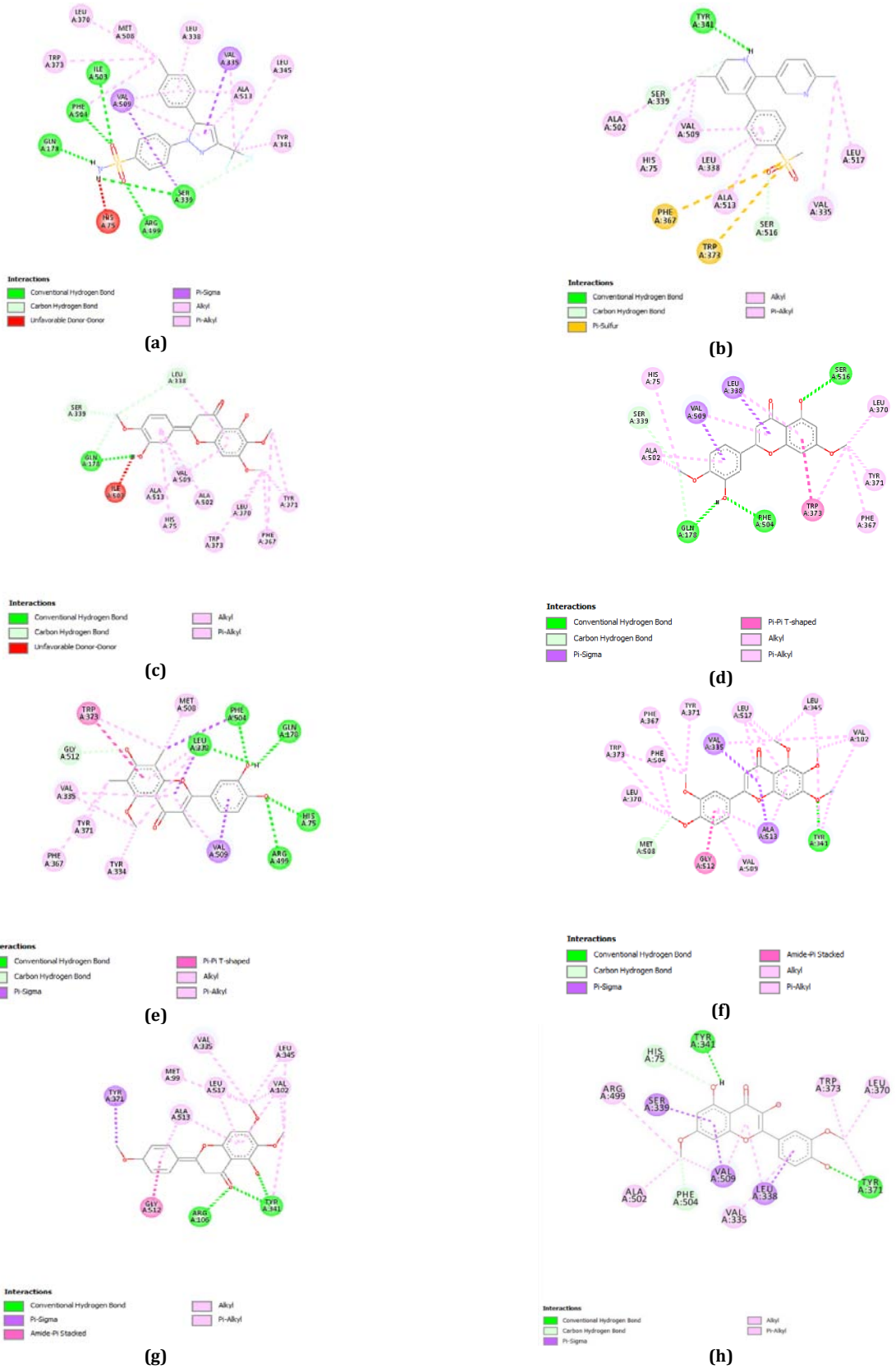

Fig. 1: Visualization of the interaction of Celecoxib (a), Etoricoxib (b), Eupatorin (c), Pilloin (d), Tetramethyl luteolin (e), Sinensetin (f), Salvigenin (g), Rhamnazin (h) with COX-2 receptors (PDB ID: 3ln1)

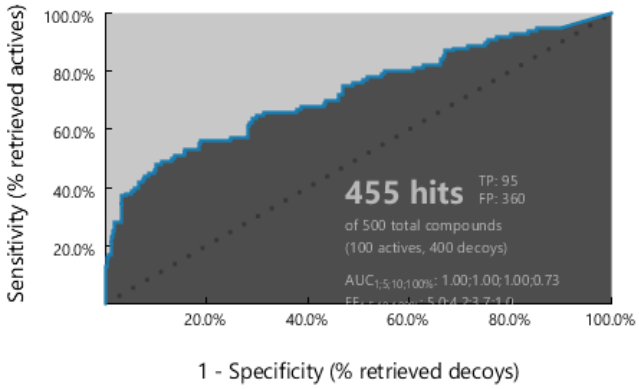

Fig. 2: ROC curve

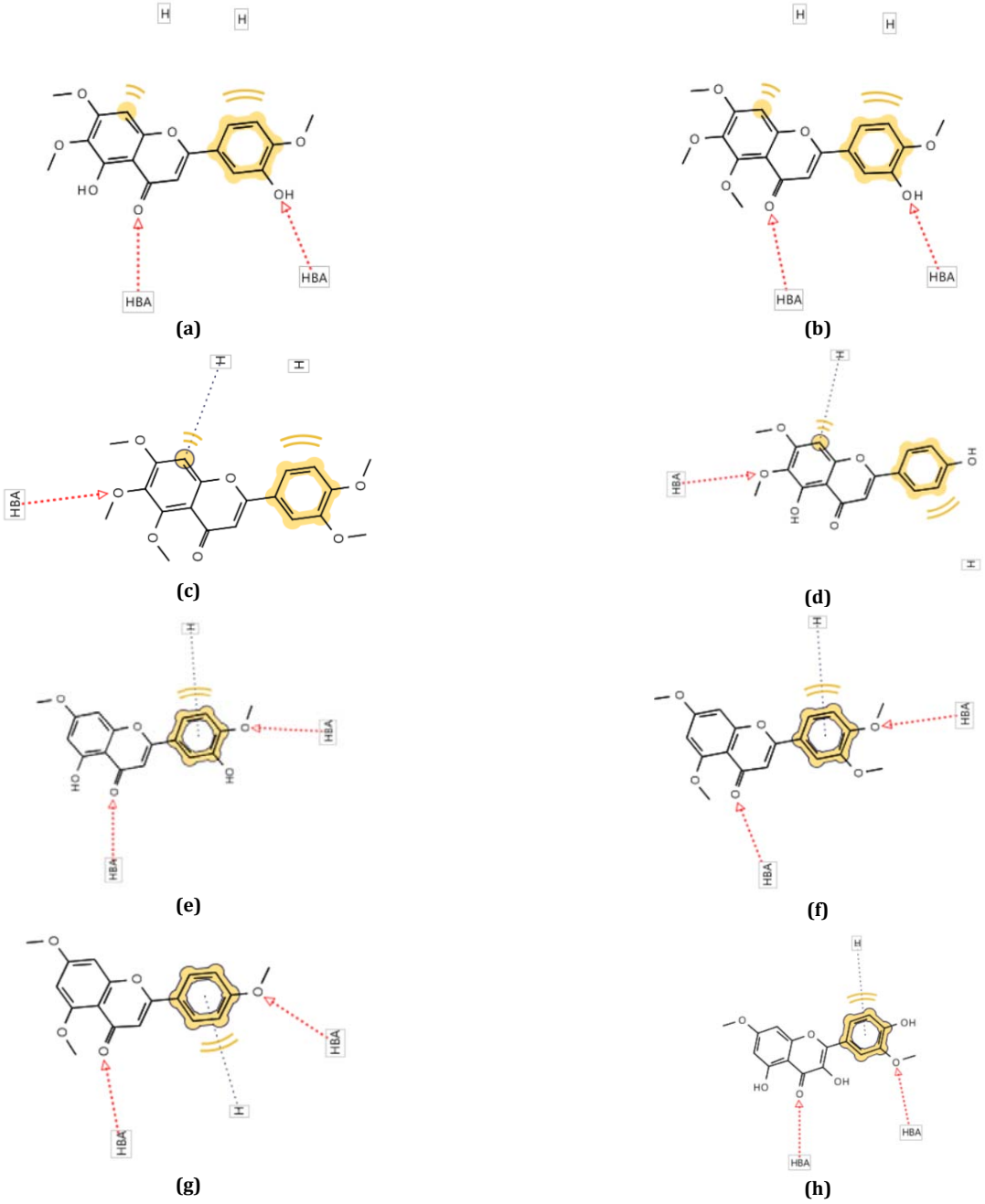

Fig. 3: Mapping of active training compounds: Eupatorin (a); TMF (b); Sinensetin (c); Cirsimaritin (d); Pilloin (e); Tetramethyl luteolin (f); Rhamnazin (g) on the model 1 in 2D

Table 5: Molecular docking results and pharmacophore screening scores

No.	Compounds/ligand test	ΔG (kc al/mol)	Inhibition constant KI (nM)	Pharmacophore fit score
1.	TMF	-9.71	76.82 nM	39.92
2.	Sinensetin	-9.53	103.69 nM	33.65
3.	Cirsimaritin	-3.81	1600 nM	33.58
4.	Eupatorin	-9.01	250.33 nM	40.01
5.	Trimethyl Apigenin	-8.67	438.88 nM	32.22
6.	Pilloin	-9.34	141.67 nM	32.46
7.	Salvigenin	-8.95	275.28 nM	-
8.	Rhamnazin	-8.85	326.68 nM	30.65
9.	Tetramethyl Luteolin	-9.90	55.80 nM	32.42

Compatibility of molecular docking and pharmacophore screening results

A comparison of *Orthosiphon stamineus* flavonoid compounds based on their molecular docking results, including Gibbs free energy and inhibition constant values, as well as pharmacophore fit scores, shown in table 5. The lower Gibbs free energy and inhibition constant values indicate stronger binding affinity, while higher pharmacophore fit scores suggest better alignment with the pharmacophore model, providing valuable insights into the potential binding and activity of the compounds.

DISCUSSION

Herbal plants are frequently utilized as alternative medicine because they contain substances useful for treating illnesses with few adverse effects and are easily obtained from our surroundings. *Orthosiphon stamineus* leaves are known to have several pharmacological activities, one of which is anti-inflammatory. The lack of studies regarding docking compounds in *Orthosiphon stamineus* leaves that were specifically directed to COX-2 led to the selection of this receptor as the target protein. The benzopyrone ring in flavonoids binds to cyclooxygenase and lipoxygenase enzymes, resulting in anti-inflammatory activity [15]. In the pursuit of identifying COX-2-specific anti-inflammatory candidates from the abundance of flavonoids in *Orthosiphon stamineus*, we selected flavonoids with known anti-inflammatory activity and performed *in silico* screening and toxicity prediction. This comprehensive approach allowed us to focus on promising compounds with potential therapeutic value, providing a foundation for further experimental investigations and potential drug development in the field of anti-inflammatory research. In this study, 9 active flavonoid compounds were tested in *Orthosiphon stamineus* leaves, such as TMF; Sinensetin; Cirsimaritin; Eupatorin; Trimethyl Apigenin; Pilloin; Salvigenins; Rhamnazin; and Tetramethyl Luteolin.

The physicochemical properties of the test compounds were predicted using Lipinski's Rule of Five. Parameters considered are molecular weight (MW), Hydrogen Bond Acceptors (HBA), Hydrogen Bond Donors (HBD), and partition coefficient (LogP). Nine compounds in the cat's whiskers' leaves met the Lipinski rule requirements, indicating that the compounds have the potential to be used as an oral preparation. Lipinski's Rule of Five states that the compound should have no more than 5 HBD, and 10 HBA, a molecular weight less than 500 Da, and a Log P value less than 5 [27]. ADMET Prediction was conducted to predict the absorption, distribution, and toxicity of substances that occur in the human body. The research was conducted *in silico* on several different parameters, such as Human Intestinal Absorption (HIA), $CaCO_2$, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB), and Potential Mutagen/Toxicity. HIA and $CaCO_2$ are used in predicting drug absorption in the body. HIA is the total bioavailability of absorption as determined by the ratio of excretion via urine, bile, and feces. The HIA parameter aims to predict the absorption process in the intestine. The HIA percentage score criteria are divided into three categories: 70-100% (high absorption class); 20-70% (medium absorption class); and 0-20% (low absorption class). Meanwhile, $CaCO_2$ cells are a parameter of permeability ability used to determine drug transport through intestinal epithelial cells derived from human colon adenocarcinoma using several transport pathways in an *in vitro* model. The permeability value of $CaCO_2$ cells can be categorized into high permeability (>70 nm/s), moderate permeability (4–70 nm/s), and low permeability (<4 nm/s) [28]. PreADMET test results show that all compounds have a good level of absorption in the digestive tract.

The parameters used to predict drug distribution are Plasma Protein Binding (PPB) and Blood-Brain Barrier (BBB). PPB refers to how much of a drug is bound to proteins in the plasma. The efficiency of the drug in penetrating or diffusing through the cell membrane is seen from the less binding of a drug, where the drug bound to plasma proteins is inactive, and only the drug in a free and unbound state can produce a biological response because it can act on the target until it finally enters the elimination process. Molecules with %PPB values exceeding 90% are considered tightly bound to plasma proteins, whereas values below 90% indicate weak binding [28]. Based on the results, all test compounds have a %PPB below 90%, which means that they are not strongly bound to plasma proteins and are likely to produce biological effects as they can readily interact with target molecules in the body. Meanwhile, the BBB penetration indicates drug concentration in the brain and blood to avoid CNS side effects [29]. Classification of BBB penetration based on Pre-ADMET, namely a BBB value>2.0 (high absorption into the central nervous system); a BBB value between 0.1-2.0 (moderate absorption to the central nervous system); and a BBB value<0.1 (low absorption to the central nervous system) [28]. Based on the results obtained from the nine test compounds, there was one test compound that had a BBB value of 1.0053, namely trimethyl apigenin, which indicates that it has a moderate ability to cross the blood-brain barrier, while other compounds have a low ability to cross the blood-brain barrier (BBB<0.1). Overall, sinensetin and tetramethyl luteolin were identified as the two compounds with the most favorable test values for absorption and distribution because they have the highest HIA and CaCO2 permeability values and a small BBB value because this drug candidate was shown to be anti-inflammatory.

The prediction of toxicity was performed using the Ames Test and Carcinogenicity parameters. The toxicity test is one of the most important steps in drug development [30]. Based on the results of the Ames test, all the tested compounds are predicted to exhibit mutagenic properties against *Salmonella typhimurium* strains. Additionally, the compounds are also predicted to be carcinogenic in rats [31].

Molecular docking is a technique used to help discover new lead compounds and promote drug repositioning; hence, it is promising for expediting new drug discovery [32]. It is an *in silico* method used to analyze interactions between two molecules, in which one molecule will act as a test compound or ligand, while the other will act as a target protein known as a receptor [33]. Molecular docking attempts to predict non-covalent interactions between ligands and their target proteins. Typical docking procedures incorporate two important components, namely, the prediction of binding pose and the estimation of binding affinity [32]. In this study, the COX-2 enzyme was collected from the Protein Data Bank (PDB) that was used in *Spodoptera frugiperda* and has been tested on Mus musculus as a receptor with the protein code 3ln1. This receptor is complexed with Celecoxib and has no mutations, as determined by X-ray diffraction with a resolution of 2.40 Å. Before docking the test compounds, re-docking of the complex compound, namely Celecoxib, was carried out for validation purposes. The results of the validation obtained RMSD values of 2Å in 100 runs (100%). In

addition, grid boxes and grid coordinates were also obtained, which were used when docking the test compounds. The grid box used is 40 x 40 x 40, with a center X = 30.092, Y =-22.559, and Z =-15.758 with a spacing of 0.375. The purpose of determining the grid box is to find out the reactive groups in macromolecules so that they can interact with the ligands [32]. This is an important parameter for identifying the low energy binding potential of drug candidates.

The two primary parameters of the docking results, such as the docking score and the interacting amino acid residues, are often analyzed independently when observing molecular docking data. Both characteristics are equally essential in docking result analysis. If the docking score is frequently related to the ligand's affinity for the receptor, the receptor-ligand interaction is an indicator of whether the resulting interaction can generate activity or not when compared to the reference ligand or cocrystal [33]. Energy binding describes the ability of two or more molecules to interact with each other. The lower the energy binding, the less energy is needed by the compound to bind to the target protein [34]. Meanwhile, the Ki value is the concentration of the inhibitor, which can reduce half of the enzyme activity [35]. If the Ki value is getting smaller, the inhibitory ability of the compound is also getting stronger. Based on the data obtained, Tetramethyl Luteolin (test compound 9) exhibited the highest affinity for the COX-2 receptor compared to the other tested compounds. It demonstrated the lowest binding energy, with a value of-9.90 kcal/mol, indicating a strong interaction between the compound and the target protein. Additionally, the inhibition constant (Ki) value for Tetramethyl Luteolin was 55.80 nm, suggesting potent inhibitory activity against the enzyme.

The molecular design of synthetic COX-2 inhibitors is based on modulating hydrophobic and hydrophilic interactions with key amino acid residues within the active site channel, including Arg120, Arg513, Leu503, Val523, Val434, Tyr385, Ser530, Tyr255, and Ala513 [36]. These interactions influence the potency (IC50) and selectivity (SI) of COX-2 inhibition. Studies on various derivatives and hybrid structures have revealed that aryl sulfonyl groups (–SO2NH2 and–SO2Me) at the para position with Leu338, Ser339, Arg499, Phe504, Arg513, and Arg106 residues as well as π–π interactions mostly with Tyr341 and Val509 residues are involved in crucial H-bond interactions with specific residues and π–π interactions with others, contributing to COX-2 inhibitory activity [36]. Additionally, amino acids such as Arg513, Gln192, Ser353, Ser530, Arg499, His90, Tyr341, and Val102 primarily participate in hydrophobic interactions with COX-2 inhibitors [36]. Additionally, previous studies have revealed that the PHE A: 504 residue is involved in the formation of hydrogen bonds in a variety of COX-2 selective inhibitor drugs. The existence of H-bonds at GLN A: 178 and ARG A: 499 was similarly linked to improved COX-2 inhibitory action [37]. Low energy in the COX-2 docking process, the interaction and similarity of amino acid residues with the original ligand show its efficacy as a selective COX-2 inhibitor.

In Celecoxib, a pyrazole-based COX-2 inhibitor, significant hydrogen bond interactions with key residues, including Arg513, Ser339, and Tyr341, within the active site channel have been identified [36]. Substitutions like para-halogen, alkoxy, and methylsulfonyl groups on the phenyl ring of COX-2 inhibitors contribute to strong interactions with amino acids within the active site channel [36]. Various hydrophobic interactions with amino acids like Val102, Leu338, Leu352, Phe381, Ala513, Val349, Arg120, and Arg513 enhance the inhibitory activity of COX-2 inhibitors [36].

In this study, we investigate the molecular interactions between celecoxib, a natural ligand, and its target protein, focusing on hydrogen bonding amino acid interactions. Celecoxib forms five hydrogen bonding interactions with specific amino acids within the protein structure: ILE503, PHE504, GLN178, SER339, and ARG499. Our analysis reveals intriguing similarities between Eupatorin, Pilloin, and Tetramethyl Luteolin and known natural ligands based on hydrogen bonding interactions. Tetramethyl Luteolin exhibits three similar hydrogen bonding amino acid interactions (PHE504, GLN178, and ARG499) to the Celecoxib complex ligand, which could potentially indicate a similar mechanism of action and pharmacological activity in inhibiting enzyme function [33]. These findings align well with the ADME results, where Tetramethyl Luteolin emerges as one of the best compounds.

Pharmacophore modeling, an intriguing advance in modern drug design, provides a technique to qualitatively describe drug-receptor interactions to provide imprecise guides for selecting promising drug candidates. The steric and electronic properties required to promote effective interaction between ligands and protein targets with specific biological targets to produce their biological response are referred to as pharmacophores. Pharmacophore modeling takes many active and inactive (decoy) compound observations and seeks to extract statistically significant patterns that predict pharmacological activity [38, 19]. The Feature Mapping module is utilized to identify all potential pharmacophore feature mappings that correspond to the desired chemical groups. Subsequently, a standardized protocol for generating a common feature pharmacophore is executed. This protocol encompasses features such as ring aromatic (RA), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), positively charged group (P), negatively charged group (N), and hydrophobic (HY). The selection of the most accurately mapped compound is determined by evaluating the fit values and aligning the pharmacophoric features [39]. The purpose of this procedure was to demonstrate the value of refined pharmacophoric characteristics in the detection of active substances.

The COX-2 receptor, PGH2/3ln1, available on the website dude. docking. org, was utilized for pharmacophore modeling in this study. Validation of the eight tested pharmacophore models showed that model 1 had the highest AUC value of 0.73. Subsequently, pharmacophore screening using model 1 was performed on the eight test compounds from *Orthosiphon stamineu*s leaf extract, and Eupatorin exhibited the highest pharmacophore fit score of 40.01. Furthermore, a comparison of pharmacophore matching features identified Eupatorin and TMF as the best pharmacophore models, both comprising four features: two hydrophobic features (yellow squares) and two hydrogen bond acceptor features (red squares). The best mapping for the pharmacophore model is represented by the highest pharmacophore fit score, resulting in the identification of highly active hit compounds [40].

CONCLUSION

Based on the results, Tetramethyl Luteolin emerges as the most promising anti-inflammatory drug among the test compounds. It exhibits strong binding affinity with COX-2, evident from its lowest binding energy (-9.90 kcal/mol) and KI value (55.80 nM). The pharmacophore-fit score of 32.42 confirms its ability to interact effectively with the COX-2 enzyme. Furthermore, pre-ADMET testing reveals favorable absorption and distribution characteristics (%HIA: 98.440681, CaCO2 permeability: 53.1689%), indicating its potential to interact with target molecules in the body because of its high absorption and moderate penetration in the human intestine. %PPB value of 87.388706 and %BBB value of 0.0154021 indicating that it is likely to produce biological effects because it can easily interact with target molecules in the body and has low absorption in the central nervous system, reducing the risk of unwanted neurological effects. Despite its promise, toxicity predictions indicate potential mutagenic and carcinogenic properties. Thus, Tetramethyl Luteolin could be a promising lead candidate for anti-inflammatory drug development, but further experimental and *in vivo* studies are necessary to validate its therapeutic efficacy and safety.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Conceptualization; MD, ME, DN, PM, and MM; methodology, MD, ME, DN, PM; formal analysis, MD, ME, DN, PM; investigation, MD, ME, DN, PM, NL, and NP; writing—original draft preparation, MD, ME, DN, PM; writing—review and editing, MM, LUS, NK and AAE; visualization, MD, ME, DN, PM; supervision, NL, NP, MM, LUS. and NK. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest in this work.

REFERENCES

1. Hamzah N, Najib A, Thahir N, Misqawati I. Studi farmakofor reseptor COX-2 sebagai anti inflamasi. Jf Fik Uinam. 2015 Jan;2(3):99-107.
2. Dinarello CA. Anti-inflammatory agents: present and future. Cell. 2010 Mar 19;140(6):935-50. doi: 10.1016/j.cell.2010.02.043, PMID 20303881.
3. Ali A, Jalil J, Husain K, Ahmad W. Raging the war against inflammation with natural products. Front Pharmacol. 2018 Sep 7;9(976):1-27.
4. Barone M, Pannuzzo G, Santagati A, Catalfo A, De Guidi GD, Cardile V. Molecular docking and fluorescence characterization of benzothieno[3,2-d]pyrimidin-4-one sulphonamide thio-derivatives, a novel class of selective cyclooxygenase-2 inhibitors. Molecules. 2014 May 14;19(5):6106-22. doi: 10.3390/molecules19056106, PMID 24830713.
5. Moossavi S, Bishehsari F. Inflammation in sporadic colorectal cancer. Arch Iran Med. 2012 Mar;15(3):166-70. PMID 22369306.
6. Miladiyah I, Jumina J, Haryana SM, Mustofa M. *In silico* molecular docking of xanthone derivatives as cyclooxygenase-2 inhibitor agents. Int J Pharm Pharm Sci. 2017 Feb 3;9(3):98-104. doi: 10.22159/ijpps.2017v9i3.15382.
7. Laavola M, Nieminen R, Yam M MF, Sadikun S, Asmawi MZ, Basir R, Welling J, Vapaatalo H, Korhonen R, Moilanen E. Flavonoids eupatorin and sinensetin present in orthosiphon stamineus leaves inhibit inflammatory gene expression and STAT1 activation. Planta Med. 2012 Apr 19;78(8):779-86.
8. Surahmaida S, Umarudin U, Junairiah J. Senyawa bioaktif daun kumis kucing (Orthosiphon stamineus). J Kimia Riset. 2019 Jun;4(1):81-8. doi: 10.20473/jkr.v4i1.13176.
9. Hossain MA, Mizanur Rahman SM. Isolation and characterisation of flavonoids from the leaves of medicinal plant Orthosiphon stamineus. Arab J Chem. 2015 Mar;8(2):218-21. doi: 10.1016/j.arabjc.2011.06.016.
10. Faramayuda F, Julian S, Windyaswari AS, Mariani TS, Elfahmi E, Sukrasno S. Review: flavonoid pada tanaman kumis kucing (Orthosiphon stamineus Benth.): review: flavonoid compounds in orthosiphon stamineus. Proceeding Mulawarman Pharm Conf. 2021 Aug 2;13(1):281-7.
11. Alshehade SA, Al Zarzour RH, Murugaiyah V, Lim SYM, El-Refae HG, Alshawsh MA. Mechanism of action of orthosiphon stamineus against non-alcoholic fatty liver disease: insights from systems pharmacology and molecular docking approaches. Saudi Pharm J. 2022;30(11):1572-88. doi: 10.1016/j.jsps.2022.09.001, PMID 36465851.
12. Li Z, Qu B, Zhou L, Chen H, Wang J, Zhang W. A new strategy to investigate the efficacy markers underlying the medicinal potentials of Orthosiphon stamineus Benth. Front Pharmacol. 2021 Sep 24;12:748684. doi: 10.3389/fphar.2021.748684, PMID 34630118.
13. Zhang B, Hu XT, Gu J, Yang YS, Duan YT, Zhu HL. Discovery of novel sulfonamide-containing aminophosphonate derivatives as selective COX-2 inhibitors and anti-tumor candidates. Bioorg Chem. 2020 Dec;105:104390. doi: 10.1016/j.bioorg.2020.104390, PMID 33137555.
14. Veitch NC, Smith M, Barnes J, Anderson LA, Phillipson JD. Herbal medicines. 4th ed. London, UK: Pharmaceutical Press; 2013. p. 813.
15. Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr. 2001 Oct;74(4):418-25. doi: 10.1093/ajcn/74.4.418, PMID 11566638.
16. Shen J, Xu X, Cheng F, Liu H, Luo X, Shen J. Virtual screening on natural products for discovering active compounds and target information. Curr Med Chem. 2003 Nov;10(21):2327-42. doi: 10.2174/0929867033456729, PMID 14529345.
17. Daina A, Michielin O, Zoete V. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017 Mar 3;7(1):42717. doi: 10.1038/srep42717, PMID 28256516.
18. Lee SK, Lee IH, Kim HJ, Chang GS, Chung JE, No KT. The PreADME approach: web-based program for rapid prediction of physico-chemical, drug absorption and drug-like properties. Euro QSAR 2002-Designing Drugs and Crop Protectants: Processes Problems and Solution. 2002 Jan 1;2003:418-20.
19. Setyawati LU, Parlan FIHB, Ikram NKK, Yusuf, Muchtaridi M. Molecular dynamic simulation and 3rd pharmacophore modeling of alpha mangostin and its derivatives against estrogen alpha receptor. Lett Drug Des Discov. 2023 Mar 9;20(20):1-17.
20. Bhowmik R, Roy S, Sengupta S, Sharma S. Biocomputational and pharmacological analysis of phytochemicals from Zingiber officinale (Ginger), allium sativum (garlic), and murrayakoenigii (curry leaf) in contrast to type 2-diabetes. Int J App Pharm. 2021 Jul 3;13(5):280-6. doi: 10.22159/ijap.2021v13i5.42294.
21. Lestari D, Sari RP, Musfiroh I, Megantara S, Praceka MS, Ikram NKK. Interactions of xantone compounds from the mangosteen (Garcinia Magostana L.) pericarps against INOS, COX-1, and COX-2 enzyme receptors as anti-inflammatory. Int J Appl Pharm. 2023 Jan;15(1):186-94.
22. Hermanto F, Subarnas A, Bambang Sutjiatmo AB, Berbudi A. Molecular docking study and pharmacophore modelling of ursolic acid as an antimalarial using structure-based drug design method. Int J App Pharm. 2023 Jan 7;15(1):206-11. doi: 10.22159/ijap.2023v15i1.46298.
23. Mysinger MM, Carchia M, Irwin JJ, Shoichet BK. Directory of useful decoys, enhanced (DUD-E): better ligands and decoys for better benchmarking. J Med Chem. 2012 Jun 20;55(14):6582-94. doi: 10.1021/jm300687e, PMID 22716043.
24. Holik HA, Rifasta MI, Murdaya N, Sagitasa S. *In silico* study of yodium leaf (Jatropha multifida Linn) active compound as antibiotic for diabetic wounds. Int J App Pharm. 2022 Nov;14(4):111-5. doi: 10.22159/ijap.2022.v14s4.PP22.
25. Sari IW, Junaidin J, Pratiwi D. Studi molecular docking senyawa flavonoid herba kumis kucing (Orthosiphon stamineus B.) pada reseptor α-glukosidase sebagai antidiabetes tipe 2. J Farmagazine. 2020 Aug 28;7(2):54-60. doi: 10.47653/farm.v7i2.194.
26. Arba M, Arfan TA, Trisnawati A, Kurniawati D. Pemodelan farmakofor untuk identifikasi inhibitor heat shock proteins-90 (HSP-90): pharmacophore modeling to identify heat shock proteins-9 (HSP-90) inhibitors. J Farmasi Galenika. 2020 Sep 30;6(2):229-36. doi: 10.22487/j24428744.2020.v6.i2.15036.
27. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the rule of 5 and drug ability. Adv Drug Deliv Rev. 2016 Jun 1;101:89-98. doi: 10.1016/j.addr.2016.05.007, PMID 27182629.
28. Cheng F, Li W, Liu G, Tang Y. *In silico* ADMET prediction: recent advances, current challenges and future trends. Curr Top Med Chem. 2013;13(11):1273-89. doi: 10.2174/15680266113139990033, PMID 23675935.
29. Fauzi M, Fadillah A, Rahman F, Ramadhani J, Erlianti K, Hasniah SYB. Activity screening and structure modification of artocarpin against ACE2 and main protease through *in silico* method. Int J App Pharm. 2021 Nov 7;13(6):192-8. doi: 10.22159/ijap.2021v13i6.42571.
30. Setyawati LU, Nurhidayah W, Khairul Ikram NK, Mohd Fuad WE, Muchtaridi M. General toxicity studies of alpha mangostin from Garcinia mangostana: a systematic review. Heliyon. 2023 May 8;9(5):e16045. doi: 10.1016/j.heliyon.2023.e16045, PMID 37215800.
31. Ames BN, Gurney EG, Miller JA, Bartsch H. Carcinogens as frameshift mutagens: metabolites and derivatives of 2-acetylaminofluorene and other aromatic amine carcinogens. Proc Natl Acad Sci USA. 1972 Nov 1;69(11):3128-32. doi: 10.1073/pnas.69.11.3128, PMID 4564203.
32. Feinstein WP, Brylinski M. Calculating an optimal box size for ligand docking and virtual screening against experimental and predicted binding pockets. J Cheminform. 2015 May 15;7(18):18. doi: 10.1186/s13321-015-0067-5, PMID 26082804.
33. Pratama MRF, Poerwono H, Siswodihardjo S. Introducing a two-dimensional graph of docking score difference vs. similarity of ligand-receptor interactions. Indones J Biotechnol. 2021 Mar 30;26(1):54-60. doi: 10.22146/ijbiotech.62194.
34. Jia CS, Wang YT, Wei LS, Wang CW, Peng XL, Zhang LH. Predictions of entropy and Gibbs energy for carbonyl sulfide. ACS Omega. 2019

Nov 14;4(22):20000-4. doi: 10.1021/acsomega.9b02950, PMID 31788634, PMCID PMC6882136.

35. Shityakov S, Forster C. *In silico* structure-based screening of versatile P-glycoprotein inhibitors using polynomial empirical scoring functions. Adv Appl Bioinform Chem. 2014;7:1-9. doi: 10.2147/AABC.S56046, PMID 24711707.
36. Ahmadi M, Bekeschus S, Weltmann KD, von Woedtke T, Wende K. Non-steroidal anti-inflammatory drugs: recent advances in the use of synthetic COX-2 inhibitors. RSC Med Chem. 2022 Feb 14;13(5):471-96. doi: 10.1039/d1md00280e, PMID 35685617, PMCID PMC9132194.
37. Adriani. Prediksi senyawa bioaktif dari tanaman sanrego (Lunasia amara Blanco) sebagai inhibitor enzim siklooksigenase (COX-2) melalui pendekatan molecular docking. J Ilmiah Pena. 2018;1(1):6-11.
38. Proekt A, Hemmings HC. Mechanisms of drug action. In: Hemmings HC, Egan TD, editors. Pharmacology and physiology for anesthesia. 2nd ed. Amsterdam: Elsevier; 2019. p. 2-19.
39. Vadlakonda R, Enaganti S, Nerella R. Insilico discovery of human aurora b kinase inhibitors by molecular docking, pharmacophore validation and admet studies. Asian J Pharm Clin Res. 2017 Feb 1;10(2):165-74. doi: 10.22159/ajpcr.2017.v10i2.14974.
40. Chandrasekaran B, Agrawal N, Kaushik S. Pharmacophore development. In: Elsevier P, editor. Encyclopedia of Bioinformatics and Computational Biology. Vol. 2; 2019. p. 1162.