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In Silico Study of Torch Ginger Flower (*Etlingera elatior* (Jack) R.M.Sm. flos) Bioactive Compounds Targeting TGF-β Receptor Type I (TGF-βR1) as Potential Tumor Suppressor Agents

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Abstract

Cancer remains one of the leading causes of death worldwide, with an estimated 16.3 million cancer-related deaths projected by 2040. Current cancer therapies still face various challenges such as resistance, toxicity, and high costs, highlighting the need for more targeted approaches in the discovery of new therapies. Torch ginger flower (*Etlingera elatior* (Jack) R.M.Sm. flos) has been reported to contain bioactive compounds with potential tumor-suppressive activities through modulation of cancer-related pathways. However, *in silico* evidence evaluating its active compounds as potential inhibitors of transforming growth factor-beta receptor type I (TGF-βR1), a protein involved in tumor proliferation and metastasis remains limited. This study aimed to predict and evaluate the potential of *Etlingera elatior* compounds as tumor-suppressing agents targeting TGF-βR1 using computational approaches. Lipinski's Rule of Five and ADME-Tox predictions were performed to assess drug-likeness and pharmacokinetic properties, while pharmacophore screening and molecular docking were conducted to identify hit compounds and predict their binding affinities. Among the tested compounds, kaempferol and quercetin showed the highest pharmacophore fit scores (47.21% and 47.07%, respectively) and the best binding affinities to TGF-βR1 (-7.98 kcal/mol; Ki 1.42 μM for kaempferol and -7.87 kcal/mol; Ki 1.72 μM for quercetin), and although their binding poses were not the most similar to the reference inhibitor LY3200882 (-8.39 kcal/mol; Ki 0.71 μM), the consistent alignment of favorable pharmacophore fit and binding energy still reinforces their potential. These findings indicate that kaempferol and quercetin have promising potential as candidate natural tumor-suppressive agents targeting TGF-βR1.

Keywords: *cancer, TGF-βR1, torch ginger flower, molecular docking.*

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INTRODUCTION

Cancer represents one of the most pressing worldwide health challenges; in 2020, cancer resulted in nearly 10 million deaths throughout the world, a number projected to exceed 16.3 million annually by 2040 as populations increase and age (Cancer Research UK, 2023; World Health Organization, 2025). Many cancers, including breast, colorectal and hepatocellular cancers still present major challenges due to resistance against drugs, drug toxicities or high costs. This underscores the need for new therapeutic strategies with better results and a lower side-effect profile such as natural-based treatments.

Transforming growth factor-beta (TGF-β) is one of the signaling pathways which have a pivotal role in tumor initiation and progression. TGF-β type I receptor (TGF-βR1) has recently been discovered to modulate the proliferation, migration and epithelial-mesenchymal transition in different cancers (Wang, *et al*., 2021). In normal conditions, TGF-β maintains a state of tissue homeostasis and acts as an apoptosis regulator. Some excess TGF-β is believed to be associated with tumor progression, in particular, it plays a role in promoting the abnormal proliferation of malignant cells. Thus, TGF-β is an interesting therapeutically target for a new generation of anticancer compounds.

Torch ginger flower (*Etlingera elatior* (Jack) R.M.Sm. flos) is a plant from the Zingiberaceae family that is commonly consumed as food and traditional medicine in Indonesia which has been known to possess various bioactive compounds, such as flavonoids, terpenoids, saponins and tannins. Among the identified flavonoids are kaempferol and quercetin, both known for their potent biological activities (Farida and Maruzy, 2016). Despite the extensive pharmacological investigations on *Etlingera elatior*, most studies have focused on its antioxidant, antidiabetic, and nephroprotective properties rather than on its potential modulation of the TGF-β signaling pathway. However, no prior studies have specifically explored *Etlingera elatior* derived flavonoids as direct inhibitors of TGF-βR1 in cancer-related contexts. Previous studies have explored the anticancer potential of *Etlingera elatior* through both *in vitro* and *in silico* approaches targeting other signaling pathways, including ERK, AKR, and progesterone receptor inhibition (Krajarng, *et al*., 2017; Afladhanti, *et al*., 2023). Previous work on other flavonoids, such as kaempferol-3-O-gentiobioside, demonstrated TGF-βRI inhibition activity (Zhang, *et al*., 2021) and several computational studies have examined natural flavonoids as potential TGF-βR1 inhibitors in general (Shah, *et al*., 2024). Yet, none have integrated *in silico* pharmacophore modeling, ADME/Tox prediction, and docking analysis focused on *Etlingera elatior* phytoconstituents.

Therefore, the present study fills this research gap by systematically applying a comprehensive computational pipeline Lipinski's Rule of Five, pharmacophore screening, ADMET profiling, and molecular docking to evaluate *Etlingera elatior* flavonoids against TGF-βR1. The findings highlight kaempferol and quercetin as the most promising lead candidates, providing the first *in silico* evidence linking *Etlingera elatior* bioactives to direct TGF-βR1 inhibition. This novelty lies in both the compound source and the mechanistic target explored, offering an early hypothesis framework that may guide subsequent biochemical and cellular validation studies toward rational TGF-β-targeted drug discovery.

METHODS

Materials and Tools

The hardware used was a laptop equipped with Intel® Core™ i5-7200U CPU @ 2.50GHz and 8.00 GB RAM. Software tools included: LigandScout for pharmacophore modeling, Chem3D Pro 12.0.2 for 3D ligand geometry optimization, AutoDockTools 1.5.7 and AutoDock v.4.2.6 for ligand preparation and molecular

docking, and BIOVIA Discovery Studio 2025 for visualization of docking results. Web-based tools were also utilized, including Pubchem (https://pubchem.ncbi.nlm.nih.gov/) for compound data sources, MCule (https://mcule.com/apps/property-calculator/) for evaluating the physicochemical properties of compounds, PreADMET (https://preadmet.webservice.bmdrc.org/) for ADME-Tox prediction, and Database of Useful Decoys-Enhanced or DUDE-E (https://dude.docking.org/targets) as a source of decoy compounds. Materials used were TGF receptor with PDB ID: 3HMM, obtained from DUDE-E, LY3200882 as a positive control which has successfully induced lasting tumor regression, and ten bioactive compounds from *Etlingera elatior*, were selected through a research journal titled "Kecombrang (*Etlingera elatior*): Sebuah Tinjauan Penggunaan Secara Tradisional, Fitokimia dan Aktivitas Farmakologinya" by Farida and Maruzy (2016) for analysis, which included tannin (CID: 16165470), kaempferol (CID: 5280863), quercetin (CID: 5280343), decanal (CID: 8175), saponin (CID: 198016), dodecanal (CID: 8194), dodecyl ester (CID: 545395), lauric acid (CID: 3893), lauryl alcohol (CID: 8193), and 1-tetradecene (CID: 14260).

Procedure

Lipinski's Rule of Five Prediction

Lipinski's Rule of Five prediction is done by finding bioactive compounds from torch ginger flowers and predicting its physicochemical properties utilizing the web-based tool Mcule (https://mcule.com/apps/property-calculator). Lipinski's Rule of Five defines key physicochemical parameters predictive of oral bioavailability, stating that a compound should possess a molecular weight under 500 Da, a partition coefficient (log*P*) not exceeding 5, no more than five hydrogen bond donors, and fewer than ten hydrogen bond acceptors. A compound that violates more than one of these criteria is typically deemed unsuitable for oral administration (Lipinski, 2004). Before making predictions, the compounds utilized in this study were derived from *Etlingera elatior,* predominantly identified in its flowers (Farida and Maruzy, 2016).

ADME-Tox Prediction

Pharmacokinetics and toxicity prediction is done utilizing web-based tool PreADMET (https://preadmet.webservice.bmdrc.org/), by selecting one of the prediction features between ADME prediction (Absorption, Distribution, Metabolism, and Excretion) and toxicity prediction. Results appear as parameters of absorption, distribution and toxicity (Luhung, *et al*., 2024). Parameters included Human Intestinal Absorption (HIA), Caco-2 permeability, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB) penetration, Ames mutagenicity, and mouse carcinogenicity. The purpose of conducting ADME-Tox prediction is to evaluate the pharmacokinetic behavior and potential toxic effects of candidate compounds early in the drug development process, thereby simplifying the development of safe and effective therapeutic agents.

Pharmacophore Validation and Screening

Pharmacophore modelling begins with database preparation, including active, decoy, and test compound datasets. Ligand-Based Perspective in LigandScout was used to generate pharmacophore models. Ten pharmacophore models were obtained and validated using the active and decoy databases to produce ROC curves, yielding an AUC of 0.94 and an EF of 3.6, indicating strong discriminatory ability. The model with the best ROC values was used to screen ten bioactive compound samples. The test compound database is then loaded via the Screening Perspective, the best pharmacophore model is transferred to the Screening Perspective, and screening is executed. The resulting hit compounds are identified upon completion (Fariha, *et al.*, 2024).

Preparation of Receptors, Natural Ligands, and Test Ligands

The TGF-β receptor with PDB ID 3HMM was downloaded from DUDE-E (https://dude.docking.org/targets) and processed using BIOVIA Discovery Studio by removing water and the native ligand. The receptor was refined by adding polar hydrogens and Kollman charges using AutoDockTools, then saved as a .pdbqt file. The native ligand was prepared similarly with added hydrogens, Gasteiger charges, and torsion parameters. Test ligands were obtained from PubChem, converted to 3D and energy-minimized in Chem3D Pro 12.0.2, then prepared in AutoDockTools 1.5.7 following the same steps as the native ligand (Afladhanti, *et al*., 2023).

Molecular Docking Validation

Validation of the molecular docking was performed by re-docking the natural ligand with the target receptor using the AutoDock v4.2.6 application. This validation step is crucial to ensure the accuracy and reliability of the docking protocol used in the research. The result of acceptable validation is the Root Mean Square Deviation (RMSD) value of ≤2.0 Å, which indicates that the predicted binding pose is very close to the experimentally determined position (Siswanto, *et al*., 2019).

Molecular Docking Simulation

Molecular docking was performed using AutoDock v4.2.6 software. Docking settings involve defining Grid Box that constrain the search space for ligands within the target receptor. And set the Grid Address are set with Grid Box values X: 40, Y: 40 and Z: 40, as well as Grid Coordinate values X: 14.421, Y: 66.575 and Z: 5.147. Docking Parameters for GA Runs were set to 100 and for a distance of 0.375 Å. This parameter selection optimizes the reliability and accuracy of docking predictions by thoroughly exploring the binding modes of ligands in biologically relevant sites. (Fariha, *et al*., 2024).

Results Visualization

Visualization of test results was performed in both 2D and 3D formats to comprehensively analyze the molecular interactions between ligands and receptors. The software used, namely AutoDock v4.2.6 which provides initial docking poses and interaction maps, and BIOVIA Discovery Studio v21.1.0.20298 which offers advanced visualization capabilities including detailed interaction diagrams. The visualization results obtained are stored in .png format. This step is critical for understanding the binding mode, identifying key interacting residues, and supporting the interpretation of docking scores, ultimately contributing to the validation and discussion of potential drug candidates (Baroroh, *et al*., 2023).

RESULTS

Lipinski's Rule of Five Prediction

Based on Table 1, two compounds do not meet the Lipinski's Rule of Five requirements, namely: tannin with a molecular weight of more than 500 Da and a Log *P* value that exceeds 5 and saponin with a molecular weight of more than 500 Da, the number of donor hydrogen bonds is more than 5 and number of acceptor hydrogen bonds is more than 10. This value indicates that neither compound is suitable for oral administration as a drug.

As summarized in Table 1, compounds that fulfilled most of Lipinski's criteria were further analyzed using ADME-Tox prediction to assess their pharmacokinetic and toxicity characteristics prior to molecular docking. This step aimed to ensure forward for binding analysis

ADME-Tox Prediction

The ADME-Tox prediction of compounds from *Etlingera elatior* is summarized in Table 2. The analysis covers parameters related to absorption, distribution, and toxicity. In terms of absorption, Human Intestinal Absorption (HIA) and Caco-2 permeability were used as indicators.

Table 1. Lipinski's rule of five prediction results.

No	Compound	Molecular Weight (<500 Da)	Log *P* (<5)	Hydrogen Bond		Druglikeness
				Donor (<5)	Acceptor (<10)	
1	LY3200882	435.52	4.74	2	8	Acceptable
2	Tannin	1441.26	7.14	24	30	Not Acceptable
3	Kaempferol	286.24	2.28	4	6	Acceptable
4	Quercetin	302.23	1.99	5	7	Acceptable
5	Decanal	156.26	3.33	0	1	Acceptable
6	Saponin	1223.35	-4.07	15	27	Not Acceptable
7	Dodecanal	184.32	4.11	0	1	Acceptable
8	Dodecyl ester	270.45	5.64	0	2	Acceptable
9	Lauric acid	184.32	4.11	0	1	Acceptable
10	Lauryl alcohol	186.33	3.90	1	1	Acceptable
11	l-tetradecene	196.37	5.48	0	0	Acceptable

Parameters in ADME-Tox prediction include HIA, Caco-2, BBB, PPB, mutagenicity, and carcinogenicity. These pharmacokinetic evaluations serve as early screening phase to identify compounds with favorable bioavailability and safety profiles. Compounds with acceptable HIA, moderate permeability, and low predicted toxicity were prioritized for further pharmacophore and docking analysis. HIA classification divides compounds into three categories: low absorption (0–20%), moderate absorption (20–70%), and high absorption (70–100%). The Caco-2 cell classification is divided into three categories: low permeability (values less than 4 nm/s), moderate permeability (4–70 nm/s), and high permeability (more than 70 nm/s). A BBB value above 2 indicates the compound's ability to cross the blood-brain barrier. A PPB value above 90% indicates that the compound can bind strongly to receptors. Meanwhile, toxicity predictions that include mutagenicity and toxicity describe the mutagenic and carcinogenic properties of the tested ligands (Wulandari, *et al*., 2023). Two

Table 2. ADME-Tox prediction results.

No	Compound	Absorption		Distribution		Toxicity	
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen (Mouse)
1	LY3200882	94.76	22.19	86.30	0.041	Non-Mutagen	Negative
2	Tannin	0.00	15.38	100.00	0.03	Non-Mutagen	Negative
3	Kaempferol	79.44	9.58	89.61	0.29	Non-Mutagen	Positive
4	Quercetin	63.49	3.41	93.24	0.17	Mutagen	Negative
5	Decanal	100.00	42.59	100.00	2.57	Non-Mutagen	Negative
6	Saponin	0.00	19.72	15.90	0.03	Non-Mutagen	Negative
7	Dodecanal	100.00	45.77	100.00	12.08	Non-Mutagen	Negative
8	Dodecyl ester	100.00	56.70	100.00	13.84	Non-Mutagen	Positive
9	Lauric acid	97.01	22.28	100.00	2.30	Mutagen	Negative
10	Lauryl alcohol	100.00	37.35	100.00	14.27	Non-Mutagen	Positive
11	l -tetradecene	100.00	23.01	100.00	23.91	Non-Mutagen	Negative

compounds, including tannin and saponin (both 0%), showed poor HIA, while kaempferol and quercetin had relatively lower absorption (<80%) than others. Most compounds had moderate Caco-2 permeability (4–70), except quercetin, which was low (<4). Although kaempferol is close to the threshold, when compared to saponin, which has a PPB value of 15%, kaempferol still has a better value. Thus, saponin is the compound with the worst PPB value among the other compounds in torch ginger flower. Four compounds, namely tannin, kaempferol, quercetin, and saponin also had poor BBB penetration (value <2), while the others showed higher permeability. Two compounds were predicted as mutagens, and three as carcinogenic in mice. Based on ADME-Tox screening, dodecanal has the best results when compared with other compounds tested. The reference compound LY3200882 showed moderate Caco-2 permeability, PPB of 86.30%, and a low BBB value (0.0406), consistent with its peripheral target profile.

Among the compounds that were screened, kaempferol and quercetin showed acceptable toxicity and moderate absorption, while also preserving a favorable PPB ratio and limited BBB penetration. These characteristics indicate potential suitability as safe, peripherally acting agents. To further evaluate their target interaction capabilities, pharmacophore modeling, and molecular docking analyses were performed.

Pharmacophore Validation and Screening

Pharmacophore validation produces a pharmacophore model with an AUC value of 0.94 and an EF value of 3.6 by using 100 actives and 400 decoys (Figure 1), indicating sufficient predictive value for use in screening test compounds. Of the 10 plant compounds tested, only two compounds that meet the hits compound standard in ligand scout, namely kaempferol by 47.21% and quercetin by 47.07%. The pharmacophore screening results are consistent with the ADME-Tox findings, as kaempferol and quercetin reappear as the most pharmacologically relevant compounds. Their consistent performance in the initial screening phase strengthens their candidacy for further structural interaction analysis through molecular docking.

Of the 10 plant compounds tested, only two compounds that meet the hits compound standard in ligand scout, namely kaempferol by 47.21% and quercetin by 47.07% as shown in Table 3.

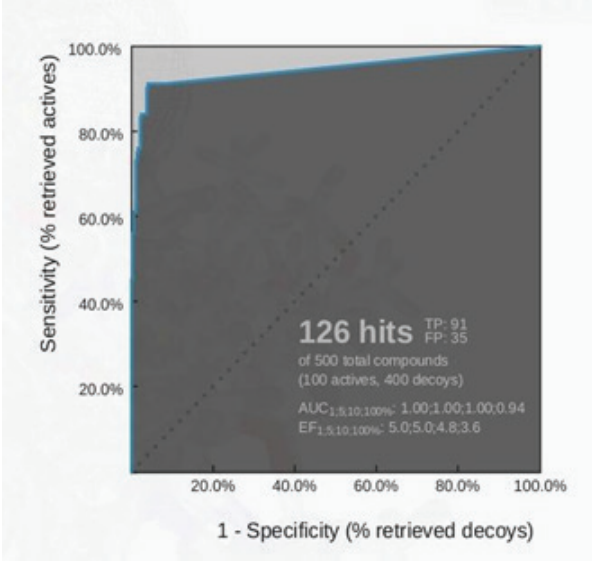


Figure 1. ROC curve of the pharmacophore model.

Table 3. Pharmacophore screening fit score results.

Compound	Fit Score (%)
Kaempferol	47.21
Quercetin	47.07

Validation of Molecular Docking

The success of the docking method can be seen from the similarity between the conformation and position of the ligand before and after docking, especially in terms of how well the two structures overlap within their binding sites (Table 4). The docking results produced a root mean square deviation (RMSD) value of 0.96 Å for the ligand. Since this value is well below the commonly accepted threshold of 2 Å, it indicates that the docking protocol is able to accurately reproduce

Table 4. Validation of molecular docking results and parameters.

Native Ligand	Binding Energy (kcal/mol)	Ki (μM)	RMSD (Å)	Gridbox	Gridbox size
GW855857	-8.5	0.59	0.96	X = 14.421 Y = 66.575 Z = 5.147	40×40×40

the experimentally relevant environmental poses. This low RMSD value indicates that the interaction between the ligand and the target protein is well maintained during the docking process. Therefore, the docking parameters used in this study can be considered appropriate and robust. These results provide a strong basis for further interpretation of interactions and subsequent computational analysis.

Molecular Docking

Molecular docking results obtained (Table 5) show that tannin and saponin compounds have a very large positive binding energy value, so the Ki or inhibition constant cannot be calculated. This indicates that the structure of tannin and saponin compounds is so complex that it cannot be explored further in this study.

Table 5. Molecular docking results.

Compound	Binding Energy (kcal/mol)	Ki (μM)	Amino Acid Interactions	
			Hydrogen Bond	Others
LY3200882	-8.39	0.71167	Lys A: 137	**Alkyl:** Val A: 19; Ala A: 30; Ala A: 150; Lys A: 32; Leu A: 78
GW855857	-8.5	0.59079	His A: 83	**Alkyl; Pi-Alkyl:** Val A: 19; Lys A: 32; Phe A: 62; Leu A: 60; Leu A: 78; Ala A: 30; Ala A: 150 **Pi-Sigma:** Tyr A: 49; Leu A: 140 **Pi-Pi Stacked:** Tyr A: 82
Tannin	+4472.54	-	Gly A: 22	**Alkyl; Pi-Alkyl:** Cys A: 148 **Salt Bridge; Attractive Bridge:** Glu A: 45 **Covalent Bond:** Cys A: 148; Glu A: 45; Asp A: 151; Leu A: 60; Leu A: 140; Tyr A: 49; Val A: 19; Lys A: 32 **Unfavorable Bump; Unfavorable Donor-Donor; Unfavorable Positive-Positive:** Gly A: 12; Gly A: 14; Gly A: 17; Asp A: 81; Asp A: 151; Phe A: 16; Leu A: 60; Leu A: 140; Leu A: 152; Tyr A: 49; Tyr A: 82; His A: 83; Ile A: 11; Ile A: 149; Val A: 19; Val A: 31; Lys A: 32; Ala A; 30; Ala A: 150; Trp A: 20; Arg A: 21

Table 5. Molecular docking results (continuous).

Compound	Binding Energy (kcal/mol)	Ki (μM)	Amino Acid Interactions	
			Hydrogen Bond	Others
Kaempferol	-7.98	1.42	His A: 83; Asp A: 81; Asp A: 151; Ser A: 80; Ile A: 11; Tyr A: 49; Glu A: 45	**Carbon Hydrogen Bond:** Tyr A: 82 **Pi-Alkyl:** Ala A: 30; Ala A: 150; Leu A: 60; Leu A: 140; Val A: 19; Lys A: 32
Quercetin	-7.87	1.72	Asp A: 81; Ser A: 80; His A: 83; Glu A: 45; Ile A: 11	**Pi-Alkyl:** Leu A: 60; Leu A: 140; Lys A: 32; Ala A: 30; Ala A: 150; Val A: 19
Decanal	-5.05	198.98	His A: 83	**Alkyl; Pi-Alkyl:** Leu A: 60; Leu A: 78; Ala A: 30; Phe A: 62; Val A: 19; Lys A: 32 **Carbon Hydrogen Bond:** Asp A: 81 **Pi-Sigma:** Tyr A: 49
Saponin	+551.62	-	Gly A: 61; Gly A:86;	**Carbon Hydrogen Bond:** Lys A: 13; Gly A: 14; Glu A: 45 **Alkyl; Pi-Alkyl:** Tyr A: 49; Leu A: 60; Leu A: 78; Val A: 79 **Covalent Bond:** Leu A: 60; Tyr A: 49; Tyr A: 82; Gly A: 86; Ser A: 80; Val A: 79 **Unfavorable Bump:** Lys A: 32; Lys A: 137; Phe A: 62; Ser A: 80; Glu A: 84; Ile A: 11; Ile A: 63; His A: 83; Tyr A: 82; Asn A: 38
Dodecanal	-5.64	73.46	His A: 83	**Alkyl; Pi-Alkyl:** Leu A: 60; Leu A: 78; Ala A: 30; Phe A: 62; Val A: 19; Lys A: 32 **Pi-Sigma:** Tyr A: 49
Dodecyl ester	-6.52	16.73	-	**Alkyl; Pi-Alkyl:** Leu A: 60; Leu A: 78; Ala A: 30; Phe A: 62; Val A: 19; Lys A: 32 **Pi-Sigma:** Tyr A: 49
Lauric acid	-6.23	26.99	Tyr A: 49; Lys A: 32; Glu A: 45	**Alkyl; Pi-Alkyl:** Ala A: 30; Leu A: 60; Leu A: 78; Val A: 19; Phe A: 62
Lauryl alcohol	-5.76	59.87	His A: 83	**Alkyl; Pi-Alkyl:** Ala A: 30; Leu A: 60; Leu A: 78; Val A: 19; Phe A: 62; Lys A: 32 **Pi-Sigma:** Tyr A: 49
1-tetradecene	-5.91	46.82	-	**Alkyl; Pi-Alkyl:** Ala A: 30; Leu A: 60; Leu A: 140; Val A: 19; Phe A: 62; Lys A: 32; Ile A: 11; Tyr A: 82 **Pi-Sigma:** Tyr A: 49

When comparing pharmacokinetic and docking results, a compromise pattern was observed between absorption potential and receptor binding affinity. Kaempferol and quercetin showed moderate absorption (HIA 79.44% and 63.49%) but achieved the strongest binding energy (-7.98 and –7.87 kcal/mol) and the lowest inhibition constant (1.42 µM and 1.72 µM). On the other hand, compounds with excellent absorption such as decanal and lauryl alcohol showed weaker binding affinities (-5.05 and -5.76 kcal/mol). These results indicate that optimal pharmacokinetic

Figure 2. Molecular docking visualization of kaempferol, A) 2D diagram of kaempferol on TGF-βR1 active sites; B) 3D diagram of kaempferol on TGF-βR1 active sites.

parameters do not always correlate with stronger receptor interactions, emphasizing the importance of balancing both aspects in the early stages of drug discovery. Overall, kaempferol and quercetin showed the most balanced profile, combining acceptable pharmacokinetic characteristics with strong receptor binding affinity. These findings justify further structural visualization to analyze specific binding interactions.

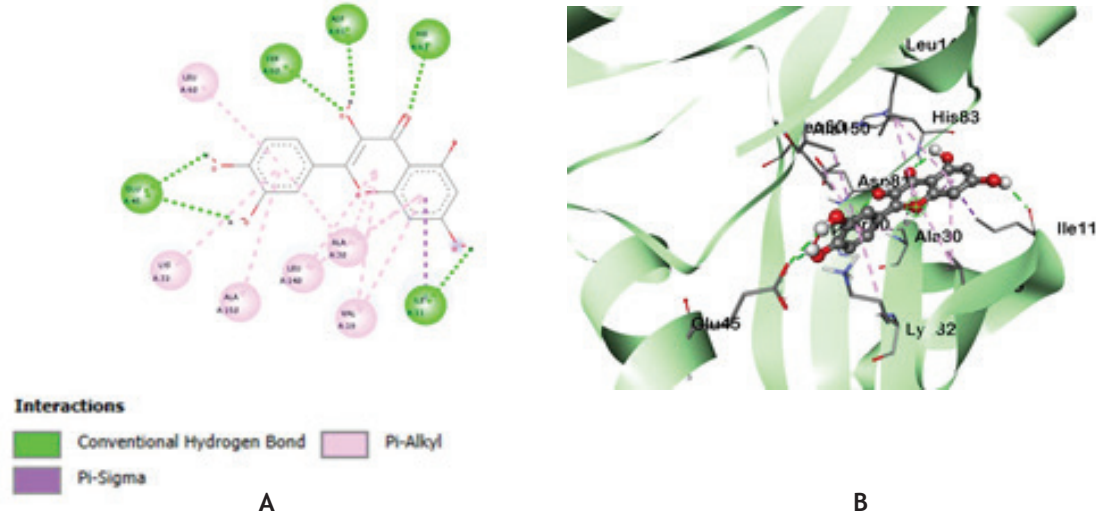
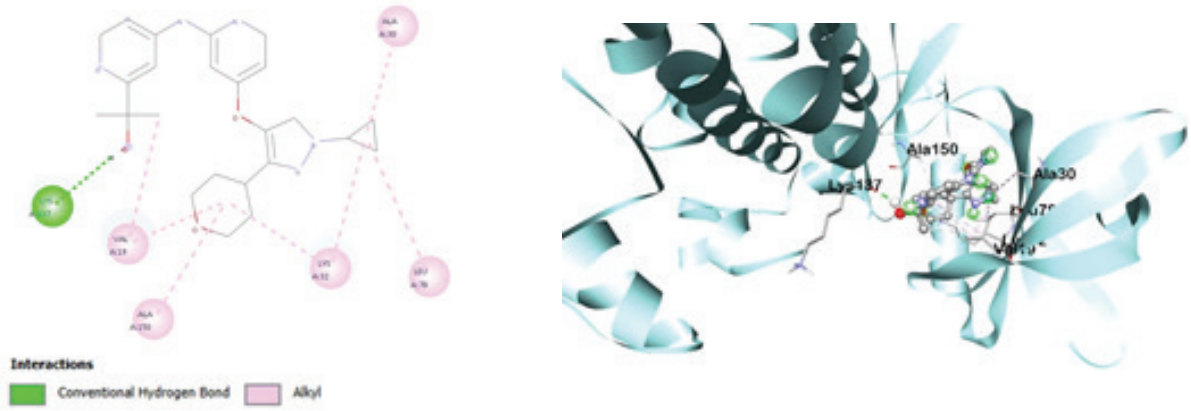


Figure 3. Molecular docking visualization of quercetin: A) 2D diagram of quercetin on TGF-βR1 active sites; B) 3D diagram of quercetin on TGF-βR1 active sites.



A B

Figure 4. Molecular docking visualization of LY3200882: A) 2D diagram of LY3200882 on TGF-βR1 active sites; B) 3D diagram of LY3200882 on TGF-βR1 active sites.

Visualization of Molecular Docking Results

Based on the results of previous testing, kaempferol (Figure 2) and quercetin (Figure 3) demonstrated the most promising performance. The 2D and 3D visualizations of their interactions with the target receptor are presented below, along with the comparative ligand LY3200882 (Figure 4).

DISCUSSION

The screening of ten compounds from *Etlingera elatior* was carried out to predict the physicochemical properties of test compounds in the form of pharmacokinetic properties, toxicity, and compatibility with Lipinski's Rule of Five and ADME-Tox predictions. These tests screen compounds to prevent the failure of compound development into drugs due to low permeation or absorption of target compounds as oral drugs (Harvey and Champe, 2013). This research was conducted by screening and determining compounds that can be used as tumor suppressants through an *in-silico* approach using pharmacophore modelling, molecular docking, ADME-Tox prediction, and plasma protein binding evaluation. Using Lipinski's Rule of Five revealed that tannins and saponins did not meet multiple criteria, suggesting poor oral bioavailability. Quercetin, dodecyl ester, and 1-tetradecene violated only one rule and are still considered acceptable. ADME-Tox predictions further indicated that tannin and saponin had 0% HIA, confirming poor intestinal absorption. Kaempferol and quercetin demonstrated moderate absorption and Caco-2 permeability, with quercetin having the lowest intestinal permeability. In terms of distribution, most compounds exhibited strong plasma protein binding, except kaempferol and especially saponin. Four compounds, including kaempferol and quercetin, showed limited blood-brain barrier penetration, which may be beneficial depending on the therapeutic goal. Toxicity analysis revealed that quercetin and dodecanoic acid are predicted mutagens, while kaempferol, dodecyl ester, and 1-dodecanol tested positive for carcinogenicity in mice, warranting further evaluation before development. Mutagenic and carcinogenic parameters were predicted using Pre ADMET with a threshold of 0.5 to distinguish between positive and negative values. LY3200882, used as a positive control, fulfilled all Lipinski criteria and showed favourable ADME-Tox prediction results with its low blood-brain barrier value, suggesting poor ability to penetrate the blood-brain barrier. Although ADME-Tox analysis predicted possible mutagenicity/carcinogenicity in certain compounds, these results remain

computational estimates and should be verified through experimental ADME-Tox assays, as in silico tools primarily function as early screening tools rather than definitive toxicity confirmations.

The pharmacophore model used had an AUC of 0.94, indicating good discrimination ability. A pharmacophore model is declared good and valid if it has an AUC value of more than 0.5, and the better if it is close to 1 (Lestari, *et al*., 2024). When a pharmacophore fit score value is over 50%, the compound has high pharmacological activity. However, if the fit score value obtained ranges between 35-50%, the compound has less pharmacological activity. However, some studies report that a fit score of around 44-45% is good enough for certain active compounds (Fariha, *et al*., 2024). Kaempferol and quercetin had fit score values of ~47%, suggesting moderate pharmacological potential. Although only these two matched the pharmacophore model, all compounds underwent molecular docking as the inclusion of other compounds helps broaden the scope of discovery, especially for potential new or unconventional inhibitors that may work through non-classical interaction pathways or bind near the heme group or at distant allosteric binding sites in the protein (Guttman and Kerem, 2022).

Molecular docking is used to understand and predict molecular recognition between a ligand and its target protein, both in terms of structure (possible binding modes) and energetics (predicted binding affinity). The main focus of molecular docking is to evaluate how well a ligand fits and interacts with the binding site of the target protein (Stanzione, *et al*., 2021). The success of the docking method can be seen from how similar the ligand positions are or overlap with each other. One of the parameters seen in this validation is the RMSD value, where the RMSD value below 2 Å indicates that the docking method used is quite accurate (Siswanto, *et al*., 2019). Docking results show 0.96 Å RMSD, confirming the reliability of the docking method. Two compounds present positive binding energy values, namely tannin (+3140 kcal/mol) and saponin (+551.62 kcal/mol), these two compounds are certainly not suitable to become drug candidates with TGF-βRI receptor targets, as those numbers indicate the amount of energy required for binding into the protein which is not ideal in drug discovery (Fadlan and Nusantoro, 2021). Among the tested compounds, kaempferol and quercetin exhibited the lowest binding energies (−7.98 and −7.87 kcal/mol, respectively) and low inhibition constants (1.42 μM and 1.72 μM), suggesting favourable interaction with TGF-βR1. These values were the closest to LY3200882 (-8.39 kcal/mol; 0.71167 μM), although still categorized as moderate in comparison.

The docking grid, centred on native ligand GW855857, was applied uniformly across all ligands, including LY3200882, ensuring consistent treatment for valid comparative analysis, even though the grid may not align with common ATP binding sites. The grid centered on GW855857 was selected because the ligands occupy the canonical ATP binding pocket in the TGF-βR1 kinase domain between the N and C lobes. The docking results for kaempferol and quercetin overlap with GW855857 in this pocket. This indicates similar hydrogen bonding and hydrophobic interactions, confirming that docking was performed in the active site capable of kinase inhibition. Through previous research, it was shown that even if the binding site does not align with the classically known ATP binding pocket, it can still be modified into an effective drug (Ding and Xue, 2024). Using the TGF-βR1 receptor file with PDB ID 3HMM and the comparative ligand LY3200882, the active site of the receptor for the pharmacological effect of tumor suppression were mediated by hydrogen interactions at Lys A: 137 and alkyl interactions at Val A: 19; Ala A: 30; Ala A: 150; Lys A: 32; and Leu A: 78. Of the ten compounds tested, those with the most similar active site were dodecyl ester, lauric acid, kaempferol, and quercetin. Upon evaluating compound suitability based on Lipinski's Rule of

Five, ADME/Tox prediction, binding affinity values and inhibition constants for the selection of oral drug candidate compounds, it can be concluded that quercetin and kaempferol have the best potential due to binding affinities below -7.5 kcal/mol and low Ki (inhibition constant) values indicating potential effectiveness with sufficient number of overlapping active sites. On the other hand, dodecyl ester and lauric acid have less potential with ADME-Tox risks, less drug-like behavior, and show weaker binding affinity, although they involve residues that are more aligned with the ATP binding site. These compounds may serve as structural guides for the design of fragment-based or hybrid molecules targeting the active site of TGF-βR1.

A previous in silico study with the same compounds, quercetin and kaempferol, reported similar binding affinities (−8.9 and −7.6 kcal/mol, respectively) and inhibitor constants (1.26 μM and 3.57 μM) against TGF-β1 (PDB: 3TZM) (Suryono, *et al*., 2025). TGF-βs (TGF-β1, β2, and β3) are ligand isoforms that bind to the extracellular domain of TGF-βRII, which then recruits and activates TGF-βR1. This receptor complex triggers downstream signalling via canonical (Smad-dependent) and non-canonical pathways, ultimately regulating cell growth, apoptosis, and processes involved in cancer progression (Leonardo-Sousa, *et al*., 2025). Such results prove that both compounds have strong interactions within the TGF-β signalling pathway, either through ligand-receptor interference with their binding affinity to TGF-β1 (PDB: 3TZM) or receptor inhibition mechanisms in the TGF-βR1 kinase domain (PDB: 3HMM).

Although kaempferol and quercetin demonstrated the most favorable pharmacophore fit and docking affinities among the tested compounds, these results remain predictive because molecular docking and pharmacophore models simplify protein–ligand interactions and do not fully account for protein flexibility, solvent effects, entropy, or metabolic processes (Sacan, *et al*., 2012). Likewise, ADME-Tox predictions, including mutagenicity or carcinogenicity alerts, serve only as early screening tools and must be validated experimentally. Therefore, to confirm whether these compounds truly act as TGF-βR1 inhibitors, further studies are required. These should begin with *in vitro* kinase inhibition assays to measure direct binding and inhibition of TGF-βR1 enzyme activity. This can be followed by cell-based assays, such as evaluating Smad2/3 phosphorylation or using a TGF-β reporter gene system, to confirm whether the compounds block TGF-β signaling inside living cells. In parallel, cytotoxicity and selectivity tests on both cancer and normal cell lines should be performed to assess safety and specificity. Finally, experimental ADME-Tox studies, including Caco-2 permeability, plasma protein binding, metabolic stability, and genotoxicity tests, are essential before progressing to animal models.

CONCLUSION

The results from computational approaches, including Lipinski's Rule of Five, ADMET analysis, pharmacophore screening, and molecular docking, kaempferol has the best binding affinity to TGF-βR1, as indicated by the binding value of –7.98 kcal/mol, moderate interactions with the active site of TGF-βR1, along with an inhibition constant of 1.42 μM. These findings indicate that among the ten compounds assessed, kaempferol and quercetin are the most promising to advance beyond this initial *in silico* screening stage as potential TGF-βR1 targeting tumour suppressor candidates. However, as this research remains at the computational stage, experimental validation is essential. Subsequent studies should include TGF-βR1 kinase inhibition assays, cellular pathway analyses, cytotoxicity, and selectivity testing in both cancerous and non-cancerous cells, as well as experimental ADME-Tox assessments and possible structural optimisation. Thus, the present study serves as an early and time-efficient step that supports and guides further drug discovery rather than providing definitive therapeutic confirmation.

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