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In Silico Molecular Docking of Flavonoids from *Vitex trifolia* L. Leaves as Potential Inhibitors of EGFR Double Mutations

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ABSTRACT: The Legundi plant (*Vitex trifolia* L.) is known to contain flavonoid compounds that exhibit potential anticancer activity through the inhibition of cancer cell growth. This study aimed to evaluate the binding affinity of flavonoid compounds from *Vitex trifolia* L. leaves against the epidermal growth factor receptor (EGFR) with double mutations (L858R/T790M) using a molecular docking approach. An in silico study was conducted by docking flavonoid compounds with the EGFR protein (PDB ID: 3IKA) using the AutoDock Vina program. A total of 14 flavonoid compounds were analyzed to assess their binding affinity and interaction profiles. The docking results revealed that the compound 2-(2,4-dimethoxyphenyl)-7-hydroxy-5-methoxy-2,3-dihydro-4H-chromen-4-one ($C_{18}H_{18}O_6$) exhibited the lowest binding free energy, indicating the strongest affinity toward the target receptor. Furthermore, the interaction analysis showed that this compound formed multiple types of interactions with the EGFR active site, including hydrogen bonds, hydrophobic interactions, van der Waals forces, and ionic interactions. These findings suggest that flavonoid compounds from *Vitex trifolia* L. have potential as inhibitors of EGFR double mutations and may serve as promising candidates for further development as anticancer agents.

KEYWORDS: Binding affinity; EGFR; flavonoid; molecular docking; phytochemical.

1. INTRODUCTION

Medicinal plants have long served as valuable sources of therapeutic agents, particularly in traditional and complementary medicine systems. One such plant is *Vitex trifolia* L., commonly known as Legundi, which belongs to the genus *Vitex* comprising approximately 250 species of shrubs and small trees (Mottaghipisheh et al., 2024). This genus is widely distributed across tropical, subtropical, and warm temperate regions. *Vitex trifolia* L. has been extensively utilized in traditional medicine due to its diverse pharmacological properties. It has been reported to possess therapeutic effects in the treatment of dysentery, diarrhea, and stomach disorders, as well as exhibiting analgesic, anti-inflammatory, antimalarial, anticancer, and antibacterial activities. Additionally, it has been traditionally used for the management of scorpion stings and other inflammatory conditions (Garbi et al., 2015).

The pharmacological potential of *V. trifolia* is largely attributed to its rich content of secondary metabolites, particularly flavonoids, terpenoids, and other phenolic compounds. Among these, flavonoids have attracted considerable attention due to their well-documented biological activities, including antioxidant and anticancer effects (Fawwaz et al., 2024). Recent studies have demonstrated that extracts of *V. trifolia* exhibit cytotoxic activity against cancer cells, particularly those associated with mutations in the epidermal growth factor receptor (EGFR). EGFR is a critical transmembrane receptor involved in cell proliferation, differentiation, and survival, and its mutations—especially the L858R and T790M double mutations—are commonly associated with resistance to conventional tyrosine kinase inhibitors in cancer therapy (Fawwaz et al., 2025).

Previous in silico studies using molecular docking approaches have supported the potential of *V. trifolia* compounds, such as artemetin, casticin, and vitexylactone, as inhibitors of EGFR double mutations (Fawwaz et al., 2024; Fawwaz et al., 2025). These findings highlight the importance of exploring other bioactive constituents, particularly flavonoids, as potential therapeutic agents. In this context, flavonoid compounds identified from *V. trifolia* leaves through liquid chromatography–high resolution mass spectrometry (LC-HRMS) analysis provide a promising pool of candidates for further computational investigation targeting EGFR L858R/T790M mutations.

The application of in silico methods, particularly molecular docking, has become an essential tool in modern drug discovery. These computational approaches enable the prediction of ligand–receptor interactions, binding affinity, and molecular mechanisms with high efficiency and relatively low cost compared to experimental methods. Moreover, in silico studies facilitate the rapid screening and identification of potential bioactive compounds from natural sources, thereby accelerating the early stages of drug development (Al-Mahrami et al., 2024).

Based on these considerations, this study aims to explore the chemical constituents of *Vitex trifolia* L. leaf extract and to evaluate the binding affinity of its flavonoid compounds against EGFR double mutations (L858R/T790M) using a

molecular docking approach. This research is expected to provide scientific insights into the potential of Legundi-derived flavonoids as candidate anticancer agents targeting EGFR-mediated pathways.

2. EXPERIMENTAL SECTION

2.1. Study Location and Sample Collection

This research was conducted from November 2024 to January 2025 at the Chemistry Laboratory, Department of Pharmacy, Muhammadiyah University of Palopo, and at the National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia. Legundi leaf samples (*Vitex trifolia* L.) were collected from Leang-leang Village, Maros Regency.

2.2. Extraction

A total of 300 g of dried Legundi leaf samples were extracted using the maceration method with 96% ethanol as the solvent. The maceration process was carried out for 3 × 24 hours and repeated twice with occasional stirring to ensure optimal extraction. The resulting filtrates were combined and concentrated using an evaporator to obtain a crude extract.

2.3. Molecular Docking

2.3.1. Hardware and Software Preparation

The molecular docking study was performed using a notebook equipped with an Intel® Atom™ CPU N2600 processor (1.60 GHz), 2 GB RAM, and a Windows 7 (32-bit) operating system. The software utilized included PyRx (integrating AutoDock and AutoDock Vina), MarvinSketch (MarvinBean Suite), PyMOL, Protein–Ligand Interaction Profiler (PLIP), and UCSF Chimera.

2.3.2. Preparation of Receptors and Ligands

The receptor structure was obtained from the Protein Data Bank (PDB: 3IKA, **Figure 1**) and prepared using UCSF Chimera by removing native ligands and water molecules to yield a clean protein structure. The native ligand was separated and saved for validation purposes. Ligand structures of flavonoid compounds were generated and optimized using the MarvinBean Suite to obtain their three-dimensional conformations. Both receptor and ligand files were then converted into the appropriate formats for docking using AutoDock tools and Open Babel integrated within PyRx (Najib et al., 2025).

2.3.3. Molecular Docking and Data Analysis

Molecular docking simulations were performed to evaluate the binding affinity between ligands and the target protein, expressed as binding free energy (ΔG_bind) in kcal/mol (Rahmawati et al., 2025). The docking results were analyzed based on increasingly negative binding energy values, where lower ΔG_bind indicates stronger and more stable ligand–receptor interactions.

In addition, docking validation and analysis included the assessment of Root Mean Square Deviation (RMSD) values and the identification of molecular interactions between ligands and amino acid residues at the binding site. The docked conformations were ranked according to their ΔG_bind values from lowest to highest. A lower ΔG_bind value reflects a more stable complex, whereas higher values indicate weaker binding affinity (Najib et al., 2023).

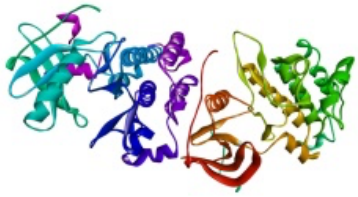


Figure 1. Receptor 3IKA as EGFR dual mutations

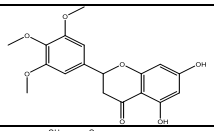
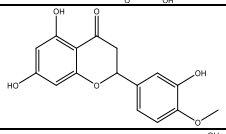
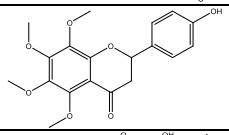
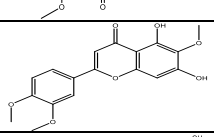
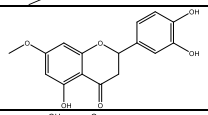
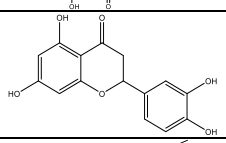
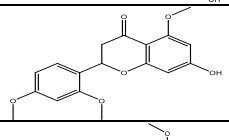
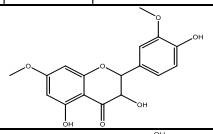
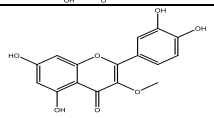
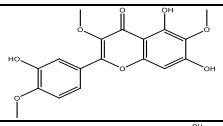
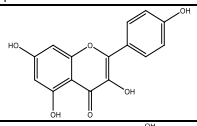
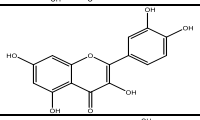
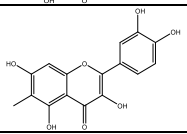
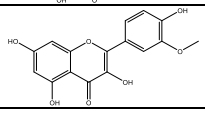
3. RESULTS AND DISCUSSION

Our previous study analyzed the chemical composition of *Vitex trifolia* L. using liquid chromatography–high resolution mass spectrometry (LC-HRMS). The results revealed that the identified compounds exhibited distinct retention times and peak areas in the chromatogram, corresponding to their respective chemical characteristics. A total of 130 chemical constituents were detected in the extract. Among these, approximately 24 compounds were classified as flavonoids, while around 70 compounds belonged to the terpenoid group. The remaining constituents were categorized into other classes, including alkaloids, polyphenols, and amino acids.

Subsequently, all identified compounds were processed using the ACD/ChemSketch software to generate their two-dimensional (2D) chemical structures, which were then saved in MDL Molfile format for further computational analysis. In this study, 24 flavonoid compounds from *V. trifolia* were selected and utilized for molecular docking analysis.

The results of the in silico molecular docking analysis between the chemical compounds of the ethanol extract of legundi leaves and the 3IKA enzyme receptor are presented in **Table 2**.

Table 2. The molecular docking analysis of flavonoid compounds of *Vitex trifolia*

No	Chemical Formula	Chemical Structure	ΔG (kcal/mol)
1	$C_{18}H_{18}O_7$		-8,0
2	$C_{16}H_{14}O_6$		-8,4
3	$C_{19}H_{20}O_7$		-7,6
4	$C_{18}H_{16}O_7$		-8,2
5	$C_{16}H_{14}O_6$		-8,4
6	$C_{15}H_{12}O_6$		-8,2
7	$C_{18}H_{18}O_6$		-8,5
8	$C_{17}H_{16}O_7$		-8,3
9	$C_{16}H_{12}O_7$		-8,0
10	$C_{18}H_{16}O_8$		-7,6
11	$C_{15}H_{10}O_6$		-8,4
12	$C_{15}H_{10}O_7$		-8,2
13	$C_{16}H_{20}O_7$		-8,4
14	$C_{16}H_{12}O_7$		-8,3

The molecular docking results demonstrated that one of the flavonoid compounds identified from the ethanol extract of *V. trifolia*, namely 2-(2,4-dimethoxyphenyl)-7-hydroxy-5-methoxy-2,3-dihydro-4H-chromen-4-one ($C_{18}H_{18}O_6$), exhibited the most favorable binding affinity toward the 3IKA receptor, with a binding free energy (ΔG) value of −8.5 kcal/mol. A more negative ΔG value indicates stronger binding affinity and greater stability of the ligand–receptor complex, suggesting that this compound has a high potential to interact effectively with the target protein. The docking pose and interaction profile of this compound with the 3IKA receptor are illustrated in **Figure 2**.

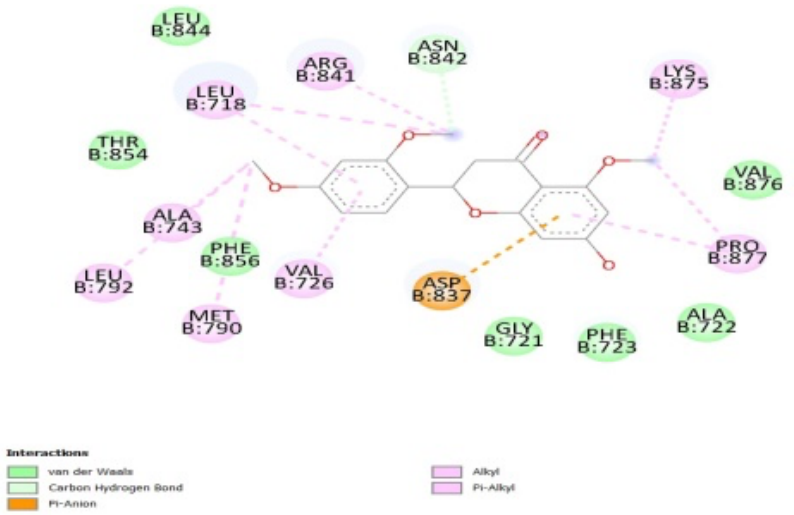


Figure 2. Docking molecular of the sample *(2-(2,4-Dimethoyphenyl)-7-hydroxy-5-methoxy-2,3-dihydro-4H- chromen -4-one)* with the 3IKA receptor.

The interaction analysis revealed that the ligand forms multiple types of non-covalent interactions with the receptor, including hydrogen bonds, hydrophobic interactions, electrostatic (ionic) interactions, and van der Waals forces. Hydrogen bonds play a crucial role in stabilizing ligand–receptor complexes due to their relatively strong and directional nature, typically formed between a hydrogen donor (X–H, where X is an electronegative atom) and an electron-rich acceptor atom. In this study, a hydrogen bond was observed between the ligand and ASN B-842 with a bond distance of 3.52 Å, indicating a stable interaction within the active site.

Hydrophobic interactions also contributed significantly to the binding stability (Wati, et al., 2026). These interactions arise from nonpolar regions of the ligand and receptor, promoting close contact in aqueous environments. The ligand formed hydrophobic interactions with several amino acid residues, including VAL B-876, ALA B-743, MET B-790, LEU B-792, LEU B-718, ARG B-841, LYS B-875, PRO B-877, and VAL B-726. The presence of multiple hydrophobic contacts suggests that the ligand is well accommodated within the hydrophobic pocket of the receptor, enhancing binding affinity.

In addition, an electrostatic (ionic) interaction was observed between the ligand and ASP B-837. Electrostatic interactions arise from the attraction between oppositely charged groups and contribute to the specificity and strength of ligand binding. Furthermore, van der Waals interactions were identified with residues LEU B-844, THR B-854, PHE B-856, GLY B-721, PHE B-723, and ALA B-722. Although individually weak, these interactions collectively play a significant role in stabilizing the ligand–receptor complex, particularly when numerous contacts are involved. When compared to the reference, olmutinib, which formed only two hydrogen bonds with LEU B-718 (2.66 Å) and GLY B-719 (3.63 Å), the identified flavonoid compound demonstrated a more extensive interaction network. The greater number and diversity of interactions observed in the test compound indicate a stronger and more stable binding mode within the active site of the 3IKA receptor.

Overall, the results suggest that the flavonoid compound from *V. trifolia* possesses significant potential as a bioactive ligand targeting the 3IKA receptor. The combination of favorable binding energy and multiple stabilizing interactions highlights its promise as a candidate for further investigation. These findings support previous reports that flavonoids can effectively interact with protein targets involved in disease pathways, reinforcing the potential of natural products as sources of novel therapeutic agents. However, further studies, including molecular dynamics simulations and in vitro or in vivo validation, are necessary to confirm the stability and biological activity of this compound.

4. CONCLUSION

Among the compounds identified in the ethanol extract of *Vitex trifolia* L. leaves, 2-(2,4-dimethoxyphenyl)-7-hydroxy-5-methoxy-2,3-dihydro-4H-chromen-4-one ($C_{18}H_{18}O_6$) exhibited the most favorable binding affinity in silico, with a binding free energy (ΔG) value of −8.5 kcal/mol.

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Ethical Approval: Not applicable

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