Jurnal Ilmu Farmasi dan Farmasi Klinik
(Journal of Pharmaceutical Science and Clinical Pharmacy)
ISSN 1693-7899 (print) - 2716-3814 (online)
DOI: 10.31942/jiffk.v21i1.9529

In Silico Molecular Docking Study of Tulsi (*Ocimum sanctum L.*) Compounds to VEGFR2 (2XIR) as Potential Liver Cancer Angiogenesis Inhibitors

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ABSTRACT: Liver cancer development is highly dependent on angiogenesis, the formation of new blood vessels that supply nutrients and oxygen to cancer cells, facilitating tumor growth and metastasis. Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), a receptor tyrosine kinase, represents a promising anti-angiogenesis therapeutic target. Bioactive compounds from tulsi (*Ocimum sanctum* L.) possess potential anticancer properties, but their specific mechanisms against VEGFR2 in liver cancer require investigation. This study evaluated the interaction potential of tulsi bioactive compounds against VEGFR2 protein structure (PDB ID: 2XIR) through *in silico* molecular docking analysis. Drug-likeness evaluation was conducted based on Lipinski's rule of five and ADMET profiling. Molecular docking analysis revealed comparative binding performance between two tulsi compounds against VEGFR2. Cirsimaritin demonstrated significant inhibition potential with free binding energy (ΔG) of -9.06 kcal/mol, inhibition constant (Ki) of 226.95 μM, and stabilizing interactions with residues PHE1047, VAL848, LEU840, and CYS1045. The native ligand exhibited superior binding affinity with ΔG of -12.68 kcal/mol and Ki of 508.94 pM, indicating greater therapeutic potential for anti-angiogenic liver cancer treatment. Overall, this study is useful for the development of the potential of tulsi bioactive compounds as angiogenesis inhibitors and alternative natural ingredient-based therapies for liver cancer. Further *in vitro* and *in vivo* studies are needed to validate the anticancer activity and mechanism of angiogenesis inhibition by these compounds.

Keywords: *Ocimum sanctum* L; VEGFR2; Liver Cancer; Angiogenesis; Molecular docking

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Submitted: 26-05-2025; Received in Revised Form: 14-09-2025 Accepted: 10-11-2025

INTRODUCTION

Cancer is a disease characterized by abnormal uncontrolled cell division and is the second leading cause of death in the human population after cardiovascular disorders (Syamsi Dhuha et al., n.d., 2018). Liver cancer is a primary liver disease caused by malignant neoplasms. The development of liver cancer is highly dependent on the process of angiogenesis, which is the formation of new blood vessels that supply nutrients and oxygen to cancer cells, allowing tumor growth and metastasis. In 2020, liver cancer was the most commonly diagnosed cancer and the third leading cause of death from liver cancer (Sung et al., 2021). Liver cancer is also the second leading cause of premature death (Ferlay et al., 2021). Although the incidence and mortality from liver cancer have decreased in several East Asian countries including Japan, China, and the Republic of Korea, cases have increased in countries with previously low incidence such as the United States, Australia, and several other European countries (Arnold et al., 2020). Currently, many anticancer treatment methods have been developed, such as chemotherapy and radiotherapy. Among these methods, chemotherapy is an effective cancer treatment method, but the use of cytotoxic drugs used in chemotherapy to kill cancer cells can cause side effects and drug resistance (Lestari et al., 2024). Therefore, research on natural ingredients that have the potential as effective, selective, and minimal side effect anticancer agents continues to be carried out and is an important focus in the development of cancer therapy. It offers the potential for developing personalized medicine approaches that could improve treatment outcomes while minimizing adverse effects.

One of the targets of anti-angiogenesis therapy that has been widely studied is the Vascular Endothelial Growth Factor Receptor (VEGFR2). VEGFR2 is a tyrosine kinase receptor that plays an important role in the process of angiogenesis, which is the process of forming new blood vessels from existing ones. This receptor functions as the main mediator for signals originating from VEGF (Vascular Endothelial Growth Factor), especially VEGF-A, in vascular endothelial cells. So VEGFR2 is very important in regulating and facilitating the formation of new vascular tissue. VEGFR2 is not only involved in normal angiogenesis but also plays a role in pathological conditions such as cancer and cardiovascular disease. Overexpression of VEGFR-2 is observed in various types of cancer, namely breast cancer, cervical cancer, non-small cell lung cancer, hepatocellular carcinoma, renal carcinoma and the like (Modi & Kulkarni, 2019). In the context of cancer, increased VEGFR2 expression can trigger tumor angiogenesis, which supports the growth and spread of cancer cells. Because VEGFR2 is a major target in the development of anti-angiogenic therapy (Shibuya, 2011). VEGFR-2 is distributed mainly in blood vessel endothelial cells, lymphatic endothelial cells, and embryonic precursor cells, and has the ability to bind to VEGF-A, VEGF-C, and VEGF-D. The human VEGFR-2 gene, also known as KDR (kinase insert domain-containing receptor), is located at chromosome 4q11-12 locus and encodes 1356 amino acids for the full-length receptor (Wang et al., 2020).

Tulsi (*Ocimum sanctum* L.) is a plant species that is widely distributed in tropical areas including Indonesia and is generally known to the public as a herbal plant and food ingredient. Various scientific studies have shown that tulsi contains various secondary metabolites such as flavonoids, coumarins, neolignans, essential oils, triterpenoids, sesquiterpenoids, steroids, glycosides, and cerebrosides, which have a wide range of pharmacological activities such as anticancer, antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antistress, leishmanicidal, mosquito repellent, and radiation protection (Singh & Chaudhuri, 2018). Previous studies have also shown that tulsi extract has anti-inflammatory and antioxidant activities that can contribute to the inhibition of cancer cell

growth. However, the specific mechanism of action of tulsi bioactive compounds against VEGFR2 in liver cancer still needs further study.

Therefore, this study aims to evaluate the potential interaction of bioactive compounds from tulsi on the structure of the VEGFR2 protein (PDB ID: 2XIR) through *in silico* analysis using the molecular docking method. Some of the main bioactive compounds identified from tulsi include eugenol, ursolic acid, apigenin, caryophyllene, carvacrol, cirsimaritin, estragole, linalool, oleanolic acid, and rosmarinic acid (Hasan et al., 2023). This approach is expected to provide a deeper understanding of the potential of tulsi compounds as angiogenesis inhibitors and contribute to the development of more effective liver cancer therapies. The results of this study are expected to provide new insights into the use of natural ingredients as an alternative in cancer treatment.

METHODS

Tools and materials

A laptop with AMD Ryzen 3 5425U processor equipped with a Windows 10 64-bit operating system was used as hardware. While the software for computational chemistry studies includes AutoDockTools from MGLTools 1.5.6, BIOVIA Discovery Studio 2019 Client, RCSB PDB site, PubChem, and SwissADME. The materials used are data on metabolite compounds from tulsi leaves, namely eugenol, ursolic acid, apigenin, caryophyllene, carvacrol, cirsimaritin, estragole, linalool, oleanolic acid, rosmarinic acid. The target receptor VEGFR2 comes from the Protein Data Bank with the code 2XIR for the molecular docking studio (Izzaturahmi et al., 2023).

Research Stages

Lipinski's Rule of Five Predictions

Lipinski's Rule of Five prediction was performed to determine the physicochemical profile and drug-likeness of the tested compounds. Parameters include molecular weight, logP (partition coefficient), hydrogen bond donor, and hydrogen bond acceptor. The test was performed using the SwissADME site (http://www.swissadme.ch/) by uploading the SMILES code of each compound (Izzaturahmi et al., 2023).

Preparation of ligands and target proteins

The structure of VEGFR2 in complex with 00J (N,2-Dimethyl-6-{[7-(2-Morpholin-4-Ylethoxy)quinolin-4-Yl]oxy}-1-Benzofuran-3-Carboxamide) was downloaded from the Protein Data Bank (PDB) www.rcsb.org with PDB code 2XIR. The native ligands and target protein were anhydrated or removed from water molecules and separated using AutodockTools-1.5.6. Native ligands and target proteins were dehydrated and separated using ADT through the edit menu, delete submenu, then delete atomset after selecting water molecules with select from string feature and entering "HOH" or "WAT". The test ligands were obtained from the Pubchem website (https://pubchem.ncbi.nlm.nih.gov/), the structure was downloaded in MOL format with MolView (molview.org). The structure of the test ligand was optimized using Avogadro software. Preparation of the test ligand, native ligand, and target protein was carried out using AutodockTools-1.5.6. The test ligand and native ligand were given Gasteiger charges, hydrogen atoms were added, the compound was combined to become non-polar, and torsion was added. In the receptor preparation, Kollman charges were given and polar hydrogen atoms were added (Izzaturahmi et al., 2023).

Method validation

The validation process is carried out as an initial step in the molecular docking simulation to determine the position and size of the grid box to be used. Validation is carried out by re-docking the native ligand to the receptor using the AutoDockTools-1.5.6 application. The grid position and size are adjusted to obtain a reference RMSD value of ≤2 Å. RMSD values below 2 Å indicate a good accuracy between the conformation of the docked ligand and its original crystallographic structure. In this study, the grid box used has dimensions of 52×30×30 Å with the grid center positioned at coordinates X: 17.996, Y: 25.972, and Z: 39.032 (Shamsian et al., 2023).

Molecular docking simulation and visualization

Molecular docking simulation of the test ligand was performed on the prepared receptor. This stage was carried out with the same stages as the validation stage using a Lamarckian genetic algorithm value of 100. The results of the analysis used AutoDockTools-1.5.6 to see the binding energy and inhibition constant. Then the docking results were visualized using the BIOVIA Discovery Study 2019 application to see the interaction of the ligand with the receptor in the form of 2D and 3D visualizations (Izzaturahmi et al., 2023).

Prediction of pharmacokinetic and toxicity properties

Prediction of pharmacokinetic properties (Absorption, Distribution, Metabolism, and Elimination) and toxicity was carried out using pkCSM tools (https://biosig.lab.uq.edu.au/pkcsm) with parameters: Human Intestinal Absorption (HIA), Caco-2, Plasma Protein Binding (PPB), Brain Blood Barrier (BBB), inhibition of CYP2C19, CYP2C9, CYP2D6, CYP3A4 proteins, total clearance, Renal OCT2 Substrate. For toxicity, see AMES and LD50 Rat (Naufa et al., 2021).

RESULT AND DISCUSSION

Lipinski's rule of five predictions

Based on Ro5 (Lipinski's rule of five) shown in table 1 and figure 1, drug compounds must have a molecular weight (MW) of less than 500 g/mol, logP of less than 5, and acceptors (HBA) and hydrogen bond donors (HBD) of less than 10 and 5, respectively (Lipinski et al., 2012). The test results based on predictions from Lipinski's rule of five are listed in table 1, which can be interpreted that of the 10 tulsi plant compounds that have met the Rule of Five (RO5) criteria. A total of 10 compounds mostly show compliance with these requirements, indicating that these compounds are likely to be developed as oral drugs. However, the ursolic acid compound shows a slight deviation, where ursolic acid has a log p value of 5.93 which exceeds the limit which should be at a value of less than 5, although other characteristics are still within the permitted range. However, the compound still meets other criteria so that it does not immediately reduce its pharmacological potential and is likely to be well absorbed if developed as an oral drug.

Protein and ligand preparation

The protein used is the VEGFR2 receptor which forms a complex with 00J (N,2- Dimethyl6-{[7-(2-Morpholin-4-Ylethoxy)quinolin-4-Yl]oxy}-1-Benzofuran-3 Carboxamide) as its native ligand. 00J is an oral, potent ATP competitive inhibitor of the VEGFR family. It inhibits VEGFR2 phosphorylation and has greater selectivity towards VEGFR2 than other kinases (Bruce et al., 2016). The target protein is separated from its native ligand (figure 2) to obtain target protein structure without a ligand complex. Optimization of 3d structures of 10 bioactive compounds (test ligands) from tulsi (*Ocimum sanctum* L.) is performed to ensure a stable conformation (free from steric tension), accurate electron charge

distribution, and protonation of functional groups, so that predictions of ligand-receptor binding affinity are more accurate and free from bias due to geometric or energy errors.

Table 1 Lipinski's Rule of Five (RO5) Prediction Results

No	Compound	Molecular weight (Da)	Log P	Hydrogen Bonds		Drug-likeness
				Donor	Acceptor	
1.	Native Ligand	461.51	3.45	1	7	Yes
2.	Eugenol	164.2	2.25	1	2	Yes
3.	Ursolic acid	456.70	5.93	2	3	Yes
4.	Apigenin	270.24	2.11	3	5	Yes
5.	Caryophyllene	204.35	4.24	0	0	Yes
6.	Carvacrol	150.22	2.82	1	1	Yes
7.	Cirsimaritin	314.29	2.46	2	6	Yes
8.	Estragole	148.20	2.47	0	1	Yes
9.	Linalool	154.25	2.70	1	1	Yes
10.	Oleanolic acid	456.70	3.94	2	3	Yes
11.	Rosmarinic acid	360.31	1.48	5	8	Yes

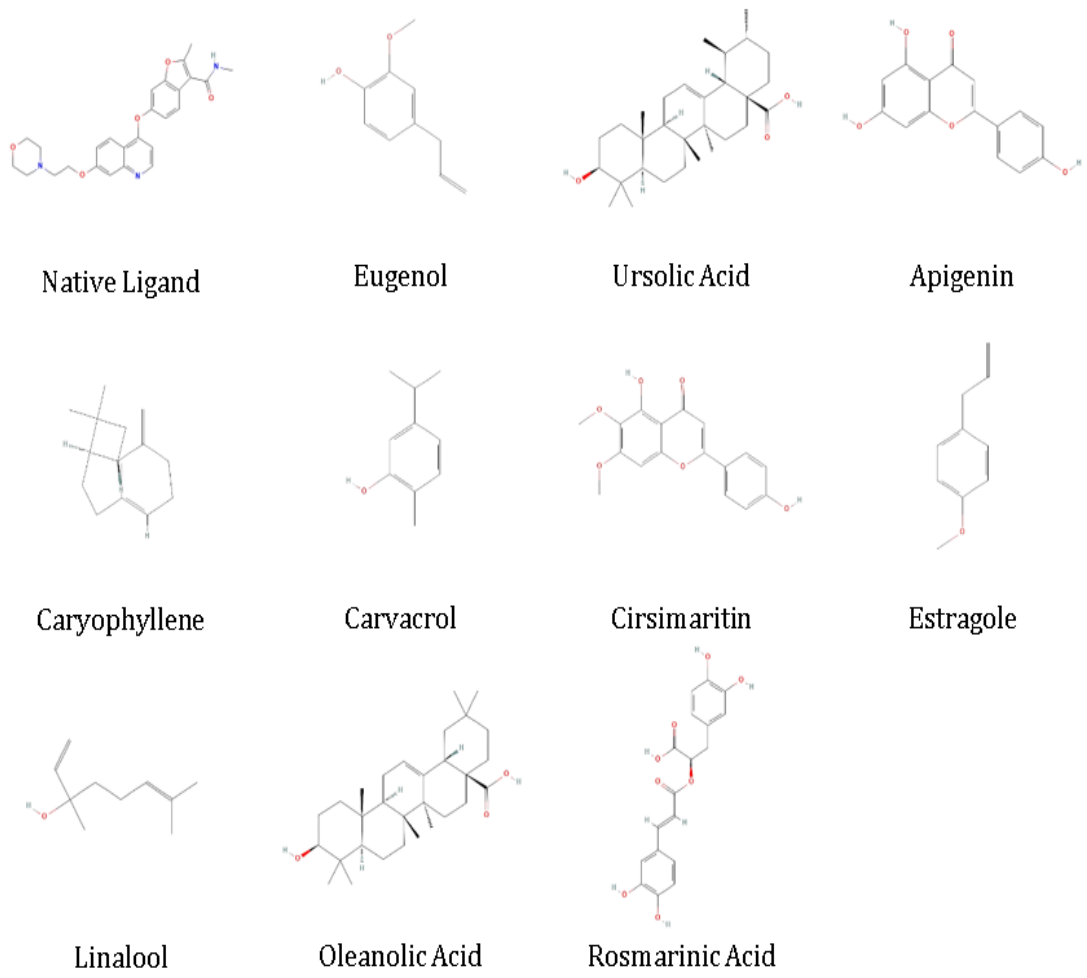

Figure 1. Structure of native ligand and compounds

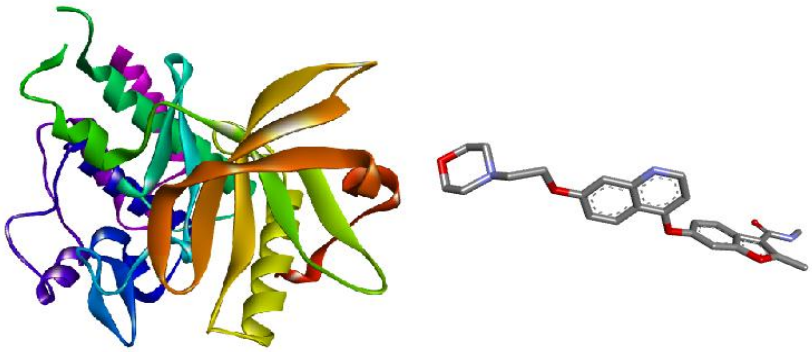

Figure 2. The separated structures of target proteins and its native ligand

Method validation

Based on the method validation results of the molecular docking shown in figure 3, the target protein used was VEGFR2 with PDB ID 2XIR, and the native ligand was 00J [N,2-Dimethyl-6-{[7-(2-Morpholin-4-ylethoxy)quinolin-4-yl]oxy}-1-benzofuran-3-carboxamide]. The docking process was conducted using a grid box size of 52 × 30 × 30 Å, with the grid center positioned at coordinates X: 17.996, Y: 25.972, and Z: 39.032. Redocking of native ligand to the target protein produced an RMSD value of 1.56 Å (<2 Å) and binding energy of the native ligand is −12.68 kcal/mol, which indicates that the docking method and parameters used have high accuracy in reproducing the position and orientation of native ligands in the active site of the protein. This validation strengthens the reliability of the docking protocol to predict ligand-receptor interactions computationally before being applied to new test ligands.

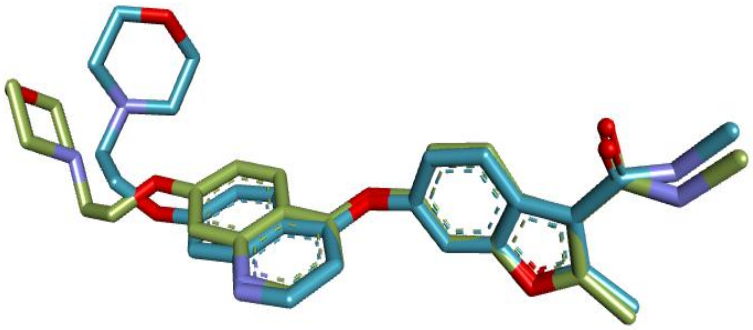

Figure 3. Overlay Re-Docking of ligand (Blue) with Native Ligand (Green)

Table 2. Molecular Docking Simulation Output

Compound	Free Binding Energy (Kcal/mol)	KI	Interaction	
			Hydrogen Bonds	Non-Covalent Bonds
Native ligand	-12.68	508.94 pM	ASN923, CYS919, ASP1046, GLU885, PHE921, GLU917	VAL914, LYS868, VAL916, VAL848, PHE1047, VAL899, CYS1045, LEU840, PHE918, LEU1035, ALA866
Eugenol	-5.21	152.36 uM	CYS919*, GLY922	LEU1035*, ALA866*, VAL848*, PHE1047*, CYS1045*, CYS919
Ursolic acid	-8.45	637.29 nM	ASN923*, PHE921*, ARG842, ALA1050	PHE918, LEU840*, PHE1047*
Apigenin	-5,73	63.24 uM	ASP1046*, VAL899, ILE1025	HIS1026, LEU1019, VAL898, ILE892 LEU889, VAL889
Caryophyllene	-6.90	8.72 uM	-	PHE1047*, CYS1045,* VAL899*, VAL848*, LYS868, ALA866*, VAL916*, LEU889, VAL914*
Carvacrol	-5.87	49.85 uM	GLU917*, CYS919*	VAL916*, VAL848*, LEU840*, LEU1035*, PHE918*, ALA866*, CYS1045*, PHE 1047*
Cirsimaritin	-9,06	226.95 nM	CYS919*, ASP1046*	CYS1045*, VAL916*, VAL899*, PHE1047*, VAL848*, ALA866*, LEU1035*, LEU840*
Estragole	-5.43	105.32 uM	CYS919*	LEU1035*, ALA866*, VAL848*, PHE1047*, CYS1045*
Linalool	-5.33	124.66 uM	GLU917*	VAL848*, CYS1045*, PHE1047*, LEU1035*, CYS919
Oleanolic acid	-7.81	1.87 uM	ALA1050, ASN923, PHE921*	PHE1047*, LEU840*, PHE918*
Rosmarinic acid	-8,25	896.28 nM	GLU885*, ASN923*, LEU840, CYS919*	VAL916*, LYS868*, VAL848*, LEU840*

*Similarity of binding to amino acid residues with native ligand

Molecular Docking Simulation

The table 2 presents the molecular docking results of several compounds compared with the native ligand. Among them, Ursolic acid (-8.45 kcal/mol), Oleanolic acid (-7.81 kcal/mol), and Cirsimaritin (-9.06 kcal/mol)* showed the lowest binding energies, indicating stronger binding affinity to the target protein than the native ligand (-12.68 kcal/mol, 508.94 pM). These compounds also share several similar interacting residues (e.g., ASN923, PHE921, CYS919, and ASP1046), suggesting comparable binding modes. Other compounds such as Caryophyllene (-6.90 kcal/mol) and Rosmarinic acid (-8.25 kcal/mol) also exhibited good affinities. Overall, the results indicate that these phytochemicals, particularly Cirsimaritin, Ursolic acid, and Oleanolic acid, demonstrate promising binding potential comparable to or stronger than the native ligand.

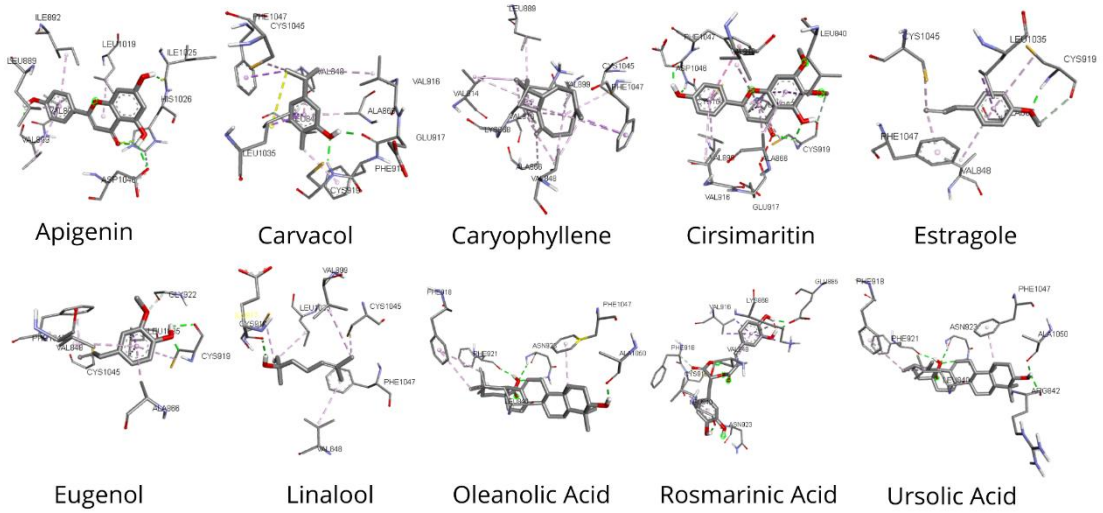

Figure 4. 3D visualization with amino acid residues

Figure 4 illustrates the 3D visualization of ligand protein interactions, showing how each compound binds to the amino acid residues at the active site. The images highlight hydrogen bonds and noncovalent interactions that contribute to the binding stability and orientation of the ligands within the protein pocket.

In molecular docking, several key parameters are evaluated to measure the strength and mechanism of the interaction between the ligand and the target protein. The free energy of binding (ΔG) being the main parameter describing the stability of the ligand-protein complex, is calculated based on the thermodynamic affinity between the two molecules. A more negative ΔG value indicates a more stable and spontaneous bond. The inhibition constant (Ki) is derived from ΔG to estimate the ligand's inhibitory potential on protein activity, where a low Ki value indicates a stronger inhibitory ability. In addition, the number and configuration of hydrogen bonds are analyzed to understand the specificity of the ligand towards the active site, as hydrogen bonds play an important role in determining the orientation and strength of the interaction. Additional parameters such as the type of non-covalent interaction, such as hydrophobic, electrostatic, or van der Waals bonds, and the 3D orientation of the ligand at the active site (Hakiki et al., 2024).

Although cirsimaritin shows a binding energy (ΔG) of −9.06 kcal/mol, which is less negative than the native ligand of VEGFR2 (−12.68 kcal/mol), its corresponding inhibition constant (Ki ≈ 227 nM) already falls in the nanomolar range (100 nM–5 μM), indicating meaningful binding affinity and making it a credible early hit candidate (Hughes 2011).

This confidence stems from its ability to form key hydrogen bonds with CYS919 in the hinge region and ASP1046 in the DFG motif, which is two critical residues that most validated VEGFR2 inhibitors use to achieve strong and specific binding (Li, et al. 2014).

Moreover, cirsimaritin engages multiple hydrophobic and van der Waals interactions with residues such as CYS1045, VAL916, VAL899, PHE1047, VAL848, ALA866, LEU1035, and LEU840, which overlap extensively with those contacted by the native ligand, suggesting it can adopt a similar binding mode and gain substantial stabilization from hydrophobic packing (Rampogu, et al. 2019). While the free energy difference of about 3.6 kcal/mol corresponds to an estimated ∼450-fold lower affinity than the native ligand, such differences are within the known error margins of docking scoring functions, which often mispredict by 1–3 kcal/mol and do not fully capture solvation, entropy, or protein flexibility (Pinzi and Rastelli 2019). Therefore, the combination of a nanomolar Ki, reproduction of crucial hinge and activation loop contacts, and a native-like hydrophobic footprint provides a mechanistic rationale for viewing cirsimaritin as a promising scaffold for VEGFR2 inhibition, warranting further validation through molecular dynamics, MM-GBSA rescoring, and biochemical or cellular inhibition assays. Other compounds such as Oleanolic acid (ΔG -7.81; Ki 1.87 μM), Rosmarinic acid (ΔG -8.25; Ki 896.28 nM) and Ursolic acid (ΔG -8.45; Ki 637.29 nM) also have low ΔG values and low Ki, supported by hydrogen interactions with key residues such as ALA1050, ASN923, and GLU885.

Prediction of pharmacokinetic and toxicity properties

The ADMET prediction results in Table 3 show that most compounds demonstrate good absorption, as indicated by high HIA and Caco-2 permeability values. Compounds such as ursolic acid, oleanolic acid, and caryophyllene exhibit excellent absorption profiles, while rosmarinic acid shows lower absorption. In terms of distribution, all compounds show moderate to low BBB permeability, suggesting limited central nervous system penetration. Regarding metabolism, only apigenin, cirsimaritin, and the native ligand interact with certain CYP enzymes, indicating possible metabolic activity, while others do not. Most compounds also show low total clearance and are not substrates for renal OCT2, suggesting favorable excretion properties. For toxicity, almost all compounds are classified as nonmutagenic, except for eugenol, estragole, and the native ligand, which are mutagenic. Based on LD50 values, all compounds fall within a relatively safe range, with rosmarinic acid having the highest LD50 (2.811), indicating the lowest toxicity among the tested compounds. Absorption prediction consists of HIA (Human Intestinal Absorption) and Caco-2 parameters. The Human Intestinal Absorption parameter identifies potential drug candidates and predicts their activity effects (Viana Nunes et al., 2020). HIA is the result of the sum of bioavailability and absorption evaluated from the ratio of excretion through urine, bile, and feces (Nursamsiar et al., 2016). HIA values in the range of 70-100% indicate good absorption, values in the range of 20-70% indicate moderate absorption, and values below 20% indicate poor absorption (Azzahra et al., 2021). Of the 10 compounds, 9 of them meet the good HIA value with the HIA requirement of 70-100%.

Table 3. Prediction of ADMET result

Compounds	Absorption		Distribution		Metabolism				Excretion		Toxicity	
	HIA	Caco-2	VDss	BBB	CYP2C19	CYP 2C9	CYP 2D6	CYP 3A4	Total clearance	Renal OCT2 substrate	Mutagen	LD50
Native Ligan	92.995	0.675	0.913	-0.999	No	Yes	No	Yes	0.852	No	Mutagen	2.653
Eugenol	92.041	1.559	0.24	0.374	No	No	No	No	0.282	No	Mutagen	2.118
Ursolic acid	100	1.171	-1.088	-0.141	No	No	No	No	0.083	No	Non-Mutagen	2.346
Apigenin	93.25	1.007	0.822	-0.734	Yes	No	No	No	0.566	No	Non-Mutagen	2.45
Caryophyllene	94.845	1.423	0.652	0.733	No	No	No	No	1.088	No	Non-Mutagen	1.617

Compounds	Absorption		Distribution		Metabolism					Excretion	Toxicity	
	HIA	Caco-2	VDss	BBB	CYP2C19	CYP 2C9	CYP 2D6	CYP 3A4	Total clearance	Renal OCT2 substrate	Mutagen	LD50
Carvacrol	90.843	1.606	0.512	0.407	No	No	No	No	0.207	No	Non-Mutagen	2.074
Cirsimaritin	93.987	1.022	0.001	-0.59	Yes	Iya	No	No	0.587	No	Non-Mutagen	2.254
Estragole	94.536	1.41	0.401	0.601	No	No	No	No	0.332	No	Mutagen	1.899
Linalool	93.163	1.493	0.152	0.598	No	No	No	No	0.446	No	Non-mutagen	1.704
Oleanolic acid	99.558	1.168	-1.009	-0.143	No	No	No	No	-0.081	No	Non-mutagen	2.196
Rosmaniric acid	32.516	-0.937	0.393	-1.378	No	No	No	No	0.25	No	Non-mutagen	2.811

Ursolic acid has a perfect HIA value of 100%, which means it can be used as a candidate for oral drugs because it can be absorbed well by the intestines. The Caco-2 parameter shows the permeability of a compound to the intestinal epithelium which is used as a model for the selection of oral administration route drug candidates (Viana Nunes et al., 2020). The compound that has the highest Caco-2 value is the carvacrol compound with a value of 1,606 cm/sec. This indicates good permeability. Distribution predictions showed six compounds had BBB values of 0.1-2.0, indicating good penetration into the central nervous system, while the other four compounds were <0.1, indicating no target in the CNS. Drug distribution is affected by binding with albumin in the blood (Azzahra et al., 2021).

Prediction of compound metabolism was conducted based on their potential interaction with cytochrome P450 (CYP) enzymes, particularly CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Most of the compounds were not identified as substrates of these enzymes, suggesting a low potential for metabolic interactions. However, certain compounds, such as apigenin and cirsimaritin, were identified as inhibitors of CYP2C19 and CYP2C9, indicating their potential to inhibit the metabolism of other drugs processed via the same enzymatic pathways. In addition, the compound (N,2-Dimethyl-6-{[7-(2-Morpholin-4-ylethoxy)quinolin-4-yl]oxy}-1-benzofuran-3-carboxamide), which acts as the native ligand, was also found to be an inhibitor of CYP2C9 and CYP3A4. Therefore, this compound may potentially interact with other drugs metabolized by these enzymes (Sativa et al., 2024).

The excretion parameters were evaluated based on total clearance values and the involvement of the renal transporter OCT2. Clearance reflects the rate at which a compound is eliminated from the body, with higher values indicating faster elimination. Caryophyllene exhibited a relatively high clearance value (1.088), whereas oleanolic acid showed a negative value (-0.081), suggesting slower elimination. No compounds were identified as substrates of OCT2, indicating that renal excretion via this pathway is likely not a dominant route (Sativa et al., 2024).

The toxicity tests used in this study were the Ames test and LD50. The Ames test was performed to determine whether the compounds under investigation are mutagenic or non-mutagenic, by assessing their ability to induce mutations in cellular DNA, both *in vitro* and *in vivo* which may affect DNA structure. LD50 refers to the amount of a compound required to cause death in 50% of a test animal population. The results of mutagenicity testing showed that almost all of the compounds exhibited no mutagenic potential. The LD50 values (mol/kg) were used to evaluate acute toxicity, the higher the value, the lower the compounds toxicity. The compound with the highest LD50 was rosmarinic acid (2.811), while caryophyllene had the lowest LD50 (1.617), although it still falls within a relatively safe range (Mujtahid et al., 2024).

CONCLUSION

Based on the research, it was found that the molecular docking results of cirsimaritin compounds from tulsi showed significant inhibitory potential against VEGFR2 receptors with a free binding energy (ΔG) of -9.06 kcal/mol and an inhibition constant (Ki) of 226.95 μM, and their interaction with residues on PHE1047, VAL848, LEU840, and CYS1045 strengthened the stability of ligand-protein complexes, thus having potential as anticancer therapy. ADMET predictions further support this potential, as cirsimaritin meets Lipinski's rules, demonstrates good absorption (HIA 93.99%, Caco-2 1.022 cm/s), is non-mutagenic with a high LD50 (2.254 mol/kg), and shows minimal CYP450 interaction, suggesting good oral availability, safety, and low drug interaction risk. However, further *in vitro* and *in vivo* studies are needed to validate the anticancer activity and mechanism of angiogenesis inhibition by these compounds.

ACKNOWLEDGMENT

The author would like express gratitude to Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, South Lampung, Indonesia.

AUTHOR CONTRIBUTION

MZAR: Conducted molecular docking tests, data interpretation, and writing the manuscript
KTL: Conducted molecular docking tests, data interpretation, and writing the manuscript
MAP:Conducted molecular docking tests, data interpretation, and writing the manuscript
NMA: Conducted molecular docking tests, data interpretation, and writing the manuscript
FA: Conducted molecular docking tests, data interpretation, and writing the manuscript
NN: Data interpretation, writing the manuscript, proofread the manuscript
WNA: Supervise work and proofread the manuscript

CONFLICT OF INTEREST

None to declare

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