

The bioactive compounds in bilimbi leaves (*Averrhoa bilimbi* L.) as natural-based anticancer targeting on VEGFR-2: *In silico* studies

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Abstract

Cancer is characterized by uncontrolled cell proliferation. Over the past five years, cancer prevalence in Indonesia has risen from 1.4% to 1.49%. Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) plays a critical role in promoting tumor growth and metastasis by initiating angiogenesis, making it a promising target for anticancer agents. *Averrhoa bilimbi* L., a member of the Oxalidaceae family, contains secondary metabolites such as flavonoids, phenols, alkaloids, tannins, and coumarins, with its leaves reported to have antineoplastic activity, though the molecular mechanisms remain unclear. This study evaluates the activity of bioactive compounds in bilimbi leaves on VEGFR-2 through computational approaches. Drug-likeness and ADMET predictions were conducted using Mcule and PreADMET tools, followed by pharmacophore screening with LigandScout and molecular docking analysis with AutoDock. Among the 27 compounds analyzed using Lipinski's Rule of Five, ADMET predictions, and molecular docking, 2-(1-phenylethyl)-phenol emerged as the top candidate based on the identification of the hit from pharmacophore modeling with a Gibbs free energy of -7.78 kcal/mol and an inhibition constant of 1.99 μ M, as well as passing the criteria of Lipinski's rule with a favorable pharmacokinetic profile. Furthermore, this compound demonstrated interaction with the ASP A:1046 residue in VEGFR-2, similar to the reference drug Sorafenib, highlighting its potential as an anticancer agent. Our findings suggest that bilimbi leaves and their bioactive compounds merit further exploration as VEGFR-2 inhibitors for cancer therapy.

Keywords: bilimbi (*Averrhoa bilimbi* L.); computational studies; VEGFR-2

Introduction

Cancer is characterized by uncontrolled cell proliferation (Fatmawati and Wijaksono, 2020). In Indonesia, cancer prevalence increased from 1.4% to 1.49% over the past five years. Breast cancer is the most common cancer in Indonesian women, while lung cancer is predominant among men (Rahmawati *et al.*, 2023). Based on GLOBOCAN report, there are more than 408,661 new cases related to cancer and nearly 242,099 deaths caused by cancer in Indonesia in (Kemenkes, 2024). WHO showed that there were an estimated 20 million new cancer cases and 9.7 million deaths. Over 35 million new cancer cases are predicted in 2050, a 77% increase from the estimated 20 million cases in 2022 (WHO, 2024). The vascular endothelial growth factor receptor (VEGFR) plays a central role in tumor growth and metastasis, making it a promising target for anticancer therapies. VEGFR-2, in particular, is essential in stimulating endothelial cell proliferation, regulating blood vessel formation during embryonic development, and increasing vascular permeability in angiogenesis (Izzaturahmi *et al.*, 2023). VEGFR-2 expression is low in normal cells but highly upregulated in various cancers, including bladder carcinoma (50%), brain glioma (71.4%), breast cancer (64.5%), cervical cancer (73.3%), colon cancer (71.4%), esophageal cancer (100%), renal clear cell cancer (35%), NSCLC (54.2%), oral cancer, ovarian cancer (100%), pancreatic cancer (80%), prostate cancer (100%), and skin melanoma (Modi and Kulkarni, 2019).

Sorafenib, a tyrosine kinase inhibitor (TKI), is a well-known anticancer drug that targets VEGFR-2, suppressing tumor angiogenesis and cell proliferation (Izzaturahmi *et al.*, 2023). It works by inhibiting natural killer (NK) cell proliferation and their cytotoxic effects, reducing immune response to cancer (Zhu *et al.*, 2020). Preclinical studies have shown that Sorafenib effectively limits tumor growth across a wide range of cancers, including melanoma, renal, colon, pancreatic, hepatocellular, thyroid, ovarian, and non-small cell lung carcinoma, and in some cases, induces tumor regression (Gong *et al.*, 2017). However, the low solubility of Sorafenib, combined with its associated side effects (Tian *et al.*, 2022), underscores the importance of exploring alternative compounds with improved pharmacological profiles for effective cancer therapy.

Averrhoa bilimbi L., commonly known as bilimbi or belimbing wuluh in Indonesia, is a promising plant for further investigation. Belonging to the Oxalidaceae family, this plant is native to Indonesia and thrives in tropical climates. It is widely utilized by Indonesian communities for its medicinal properties. (Ismail *et al.*, 2019; Rozi *et al.*, 2022). Bilimbi leaves contain secondary metabolites such as flavonoids, phenols, alkaloids, tannins, and coumarins (Figure 1), compounds with demonstrated cytotoxic properties (Agastia *et al.*, 2021; Hasim *et al.*, 2019). Research has shown bilimbi leaf extracts to be effective against several cancer cell lines: MCF-7 (breast cancer) with an IC_{50} of 668.3 $\mu\text{g/ml}$, T47D (breast cancer) with an IC_{50} of 805.0 ± 4.3 $\mu\text{g/ml}$, and WiDr (colon cancer) cells with an IC_{50} of 1406.3 ± 3.9 $\mu\text{g/ml}$ (Utami *et al.*, 2023). Despite these findings, the molecular mechanism by which bilimbi leaf extracts inhibit cancer cell proliferation remains unknown. This study aims to evaluate bioactive compounds in bilimbi leaves for their potential as VEGFR-2 inhibitors by assessing drug-likeness properties and molecular interactions through *in silico* studies. This research is expected to provide scientific support for the potential use of bilimbi leaves as an anticancer agent targeting VEGFR-2.

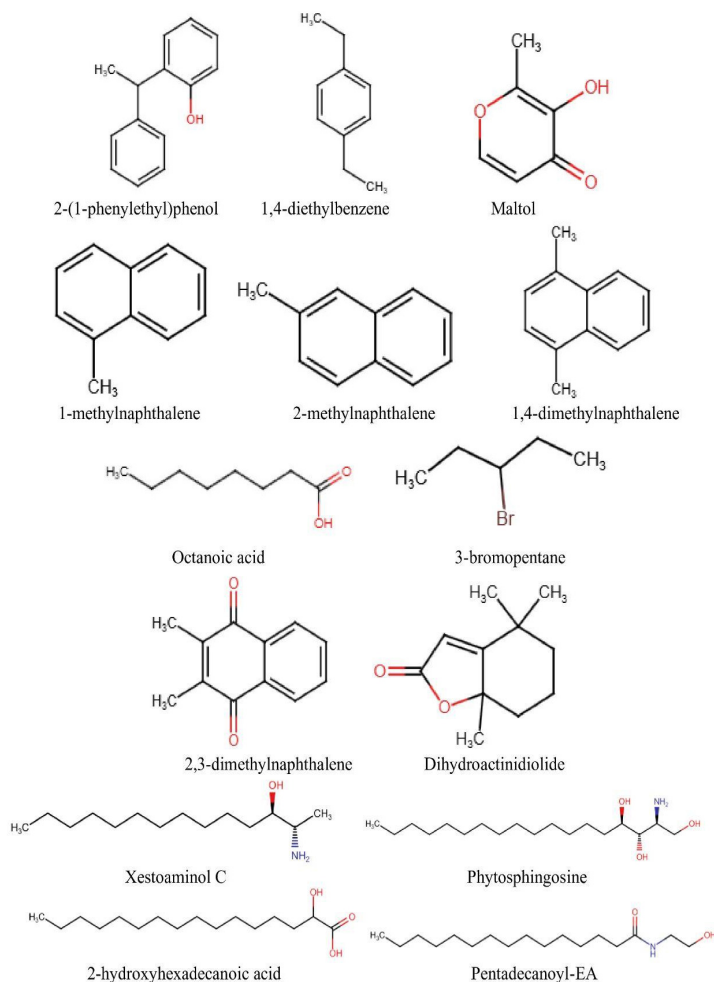
Materials and Methods

Materials and Tools

The study was conducted on a personal laptop with an 11th Gen Intel® Core™ i7-1195G7 processor, 16.0 GB RAM, and a Windows 11 64-bit operating system. The materials used include the VEGFR-2 protein with PDB ID 2P2I and its native ligands, retrieved from the Protein Data Bank (PDB), along with the reference drug Sorafenib and test compounds from *Averrhoa bilimbi* L., as shown in Figure 1. The compounds were

sourced from PubChem and optimized using Chem3D Pro 12.0 software with MM2 geometry optimization. Furthermore, the software and web tools used in this study were MarvinJS, LigandScout, BIOVIA Discovery Studio, PubChem, Protein Data Bank, the Mcule property calculator, Chem3D Pro 12.0, AutoDock 4.2.6, PreADMET, and dud.e.

The test ligands were selected based on previous research indicating their potential as anti-colorectal cancer agents (Pratama *et al.*, 2023). Colorectal cancer, or colon cancer, has been identified as one of the cancer types with significant VEGFR-2 overexpression, seen in approximately 71.4% of cases (Modi and Kulkarni, 2019). Additionally, the *A. bilimbi* L. compounds used in this study were identified through Liquid Chromatography-Quadrupole Time-of-Flight Tandem Mass Spectrometry (LC-QTOF-MS/MS) analysis of the n-butanol fraction of *A. bilimbi* L. leaves methanol extract (Ahmed *et al.*, 2018).



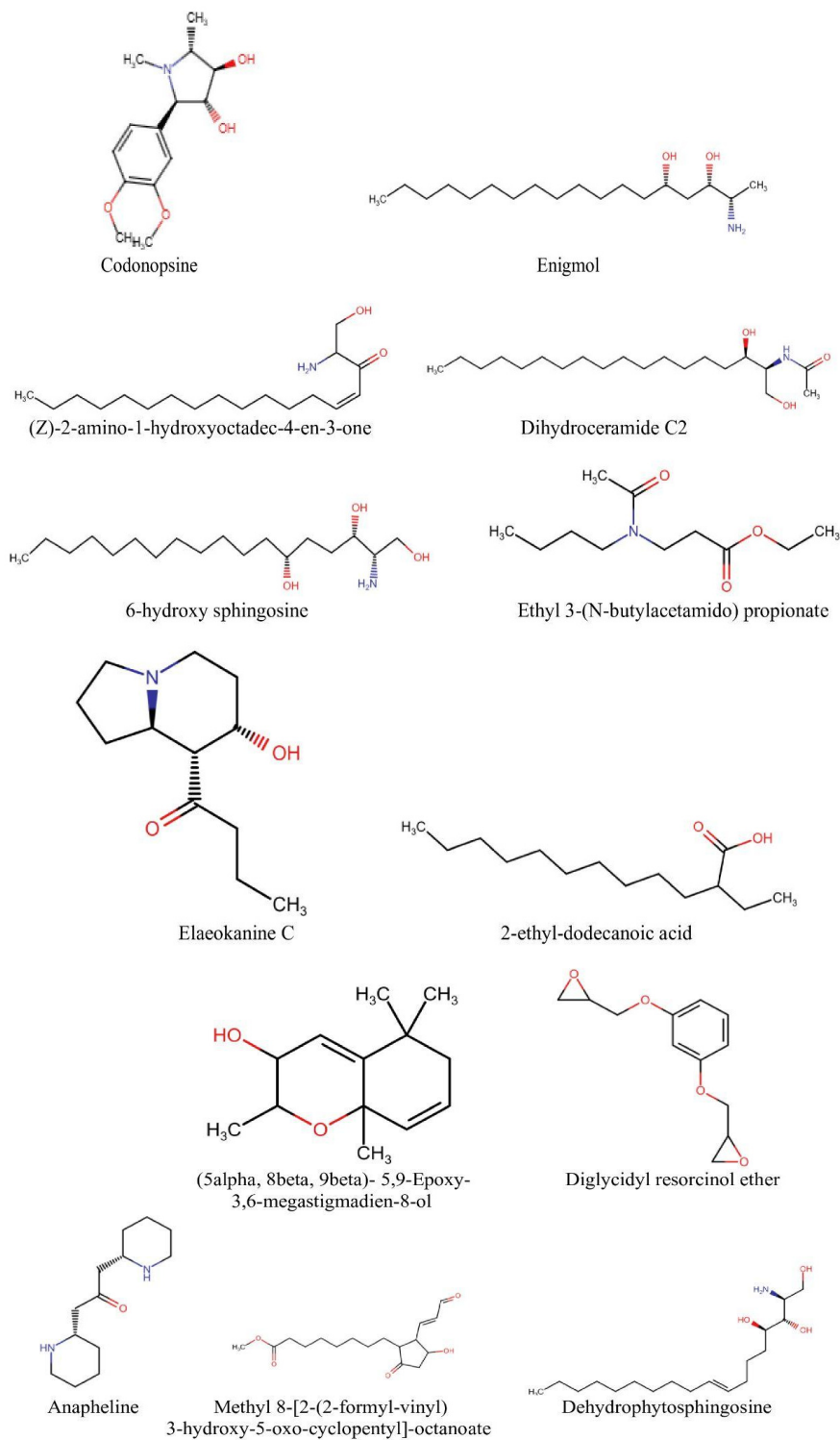


Figure 1. Chemical structure of bioactive compounds in the leaves of *A. bilimbi* L. used as test ligands

Lipinski's rule of five predictions

Lipinski's Rule of Five (RO5) predictions were determined by obtaining the SMILES format of each test compound from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) and inputting their 2-dimensional structural forms. The test compounds were then analyzed using the mcule property calculator (<https://mcule.com/apps/property-calculator>). The parameters assessed according to Lipinski's RO5 included molecular weight, log P, hydrogen bond donors, and hydrogen bond acceptors.

ADMET prediction

ADMET predictions were performed by retrieving test compounds from PubChem and analyzing them through the PreADMET site (<https://preadmet.webservice.bmdrc.org/>). The pharmacokinetic parameters assessed included human intestinal absorption (HIA), permeability to Caco-2 cells, blood-brain barrier (BBB) distribution, and plasma protein binding (PPB). Toxicity parameters, including the Ames test and rodent carcinogenicity, were also evaluated.

Pharmacophore screening

Pharmacophore screening began with the creation of an active/decoy database from the to the VEGFR-2 (PDB ID: 2P2I) that obtained from the dude site (<https://dude.docking.org/targets>). Ten pharmacophore models were generated using LigandScout. These models were then screened using the active and decoy database by copying features into the Screening Perspective. Receiver Operating Characteristic (ROC) curve plots were analyzed to select the best model. Hit compounds were identified using the test compound database, screened based on the best model without additional databases. The test compound database was loaded into the screening perspective, and the ligand-based perspective feature was used to transfer the best model for pharmacophore screening, yielding a final hit compound.

Molecular docking simulation

Prior conducting the molecular docking analysis, geometry optimization towards the ligands were carried out using molecular mechanics (MM2) calculations on Chem3D Pro 12.0. This step began by drawing each ligand's structure. Then, geometry optimization was performed by minimizing energy to minimum RMS gradient of 0.010 using MM2 calculations.

Later, the molecular docking validation began with setting the grid box's position and size by re-docking the native ligand to the VEGFR-2 receptor (PDB ID: 2P2I) using AutoDock 4.2.6. The grid box size and position were adjusted to ensure a reference RMSD value of ≤ 2 Å and a negative binding energy, with a Genetic Algorithm Lamarckian value of 10. The grid box parameters were as follows: Grid Box (x = 40; y = 48; z = 40), Grid Coordinates (x = 38.192; y = 35.014; z = 12.178), with a spacing of 0.375 Å.

Molecular docking simulations were performed on the prepared VEGFR-2 (PDB ID: 2P2I) and test ligands from *A. bilimbi* L. compounds. Receptor preparation was done using BIOVIA Discovery Studio by removing water molecules and native docking ligands. Kollman charges and hydrogen bonds were added to the receptor's polar regions. Ligand preparation involved the AutoDock, where Gasteiger charges were added, hydrogen bonds were included, and nonpolar hydrogens were merged. Molecular docking followed the similar steps as in the validation phase, utilizing a Genetic Algorithm Lamarckian value of 100. The results were analyzed using AutoDock to determine binding energies and inhibition constants. Visualization of ligand-receptor interactions in 2D and 3D was completed using the BIOVIA Discovery Studio.

Results

Lipinski's rule of five predictions

Lipinski's Rule of Five (RO5) predictions were performed to assess the physicochemical profile of the test compounds, helping to evaluate their drug-likeness for potential oral administration. Four key physicochemical parameters were considered in the RO5 predictions: molecular weight, log P, and hydrogen bond acceptors and donors, and it passed the criteria with the violations no more than 2. The results of the RO5 predictions, as shown in Table 1, The RO5 predictions demonstrate that all tested compounds from *A. bilimbi* L. fulfill the essential drug-likeness criteria for oral administration. The compounds meet the parameters for molecular weight, log P, hydrogen bond acceptors, and donors, indicating favorable physicochemical properties and suggesting their potential as orally administrable drugs (Ahmad *et al.*, 2023).

Table 1. Lipinski's rule of five prediction results of *A. bilimbi* L. leaves' bioactive compounds

Compound	Molecular weight (Da)	Log P	Hydrogen bond acceptor	Hydrogen bond donor	Lipinski violations
2-(1-phenylethyl) phenol PUBCHEM ID: 95322	198.26	3.54	1	1	0
1,4-diethylbenzene PUBCHEM ID: 7734	134.22	2.81	0	0	0
Maltol PUBCHEM ID: 8369	126.11	0.65	3	1	0
1-methylnaphthalene PUBCHEM ID: 7002	142.20	3.15	0	0	0
2-methylnaphthalene PUBCHEM ID: 7055	142.20	3.15	0	0	0
1,4-dimethylnaphthalene PUBCHEM ID: 11304	156.22	3.46	0	0	0
Octanoic acid PUBCHEM ID: 379	144.21	2.43	2	1	0
3-bromopentane PUBCHEM ID: 15738	151.04	2.57	0	0	0
2,3-dimethylnaphthalene PUBCHEM ID: 11386	156.22	3.46	0	0	0
Dihydroactinidiolide PUBCHEM ID: 6432173	180.24	2.44	2	0	0
Xestoaminol C PUBCHEM ID: 14756407	229.40	4.32	2	2	0
Phytosphingosine PUBCHEM ID: 122121	317.51	3.82	4	4	0

2-hydroxyhexadecanoic acid PUBCHEM ID: 69417	272.42	4.52	3	2	0
Pentadecanoyl-EA PUBCHEM ID: 13849	285.47	4.58	3	2	0
Codonopsine PUBCHEM ID: 442631	267.32	0.74	5	2	0
Enigmol PUBCHEM ID: 11415391	301.51	4.85	3	3	0
(Z)-2-amino-1-hydroxyoctadec-4-en-3-one PUBCHEM ID: 5280901	297.48	4.83	3	2	0
Dihydroceramide C2 PUBCHEM ID: 6610273	343.54	4.72	4	3	0
6-hydroxy sphingosine PUBCHEM ID: 12991068	315.49	3.60	4	4	0
Ethyl 3-(N-butylacetamido)propionate PUBCHEM ID: 104150	215.29	1.59	4	0	0
Elaeokanine C PUBCHEM ID: 442855	211.30	1.14	3	1	0
2-ethyl-dodecanoic acid PUBCHEM ID: 102894	228.37	4.63	2	1	0
(5alpha, 8beta, 9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol PUBCHEM ID: 101415508	208.30	2.44	2	1	0
Diglycidyl resorcinol ether PUBCHEM ID: 7586	222.24	1.24	4	0	0
Anapheline PUBCHEM ID: 440934	224.34	2.28	3	2	0
Methyl 8-[2-(2-formyl-vinyl)3-hydroxy-5-oxo-cyclopentyl]-octanoate PUBCHEM ID: 5282975	310.38	2.21	5	1	0
Dehydrophytosphingosine PUBCHEM ID: 14757418	315.49	3.60	4	4	0

ADMET prediction

ADMET predictions were intended to determine the pharmacokinetic and toxicity profiles of drug compounds to ensure safety and efficacy of drug candidate compounds as therapy (Pantaleão *et al.*, 2022). Pharmacokinetic parameters of drug candidate compounds consist of ADME (absorption, distribution, metabolism, and excretion). Meanwhile, the safety parameters of the drug candidate compounds are presented

using the toxicity profiles. All bioactive compounds show favorable human intestinal absorption (HIA) values, ranging from 77.30% to 100%, indicating good absorption in the intestine. For the Caco-2 permeability parameter, all compounds fall within the medium permeability range. Nineteen compounds displayed strong plasma protein binding (PPB), with values over 90%, suggesting a high binding affinity, while the remaining eight compounds have weaker plasma protein binding with PPB values below 90%. Generally, PPB could affect compounds' distribution and efficacy, though weaker PPB levels in some compounds were potentially reduced the risk of protein-related side effects (Hasan and Herowati, 2024).

Additionally, 11 compounds have moderate blood-brain barrier (BBB) penetration potential, with BBB values between 0.1 and 2.0, while 15 compounds demonstrate high BBB penetration potential with values above 2.0 (Lestari *et al.*, 2023). For a VEGFR-2 inhibitor candidate from *A. bilimbi* L., a low BBB penetration is preferable. Further, two compounds show BBB values below 0.1, indicating low penetration ability across the blood-brain barrier. The identification of two compounds with low BBB penetration is encouraging, as this feature may reduce potential central nervous system side effects. In toxicity predictions, 11 compounds were identified as non-mutagenic, and 9 compounds were non-carcinogenic in rodent models (Table 2). However, several test ligands in this research may pose a risk of genetic mutations, highlighting the need for further safety and efficacy studies to fully assess their therapeutic potential before therapeutic use.

Table 2. ADMET results of *A. bilimbi* L. leaves' bioactive compounds

Compound	HIA	Caco-2	PPB	BBB	Mutagen	Carcinogen	
						Mouse	Rat
2-(1-phenylethyl)phenol	100.00	45.11	100.00	6.16	Mutagen	-	-
1,4-diethylbenzene	100.00	23.43	100.00	5.80	Mutagen	+	-
Maltol	90.48	2.29	91.34	0.34	Mutagen	-	+
1-methylnaphthalene	100.00	23.44	100.00	2.53	Mutagen	+	-
2-methylnaphthalene	100.00	23.47	100.00	2.53	Mutagen	-	-
1,4-dimethylnaphthalene	100.00	23.44	100.00	3.49	Mutagen	+	-
Octanoic acid	93.72	1.22	100.00	0.91	Mutagen	-	+
3-bromopentane	100.00	43.85	96.27	5.10	Mutagen	+	+
2,3-dimethylnaphthalene	100.00	23.47	100.00	3.49	Mutagen	-	-
Dihydroactinidiolide	100.00	48.56	60.12	0.82	Mutagen	+	-
Xestoaminol C	91.05	23.10	100.00	5.42	Non-mutagen	-	+
Phytosphingosine	77.30	12.96	100.00	2.36	Non-mutagen	+	-
2-hydroxyhexadecanoic acid	93.51	21.59	100.00	5.60	Mutagen	-	-

Pentadecanoyl-EA	90.76	29.37	100.00	8.01	Non-mutagen	-	-
Codonopsine	91.25	30.32	23.81	0.48	Mutagen	+	-
Enigmol	87.18	15.25	100.00	0.48	Non-mutagen	+	-
(Z)-2-amino-1-hydroxyoctadec-4-en-3-one	92.96	22.58	100.00	4.84	Non-mutagen	-	-
Dihydroceramide C2	86.93	25.71	100.00	6.05	Non-mutagen	-	-
6-hydroxy sphingosine	79.33	12.94	100.00	1,55	Non-mutagen	+	-
Ethyl 3-(N-butylacetamido) propionate	95.69	28.22	42.20	1.20	Non-mutagen	-	+
Elacokanine C	94.04	27.98	18.66	0,50	Non-mutagen	-	-
2-ethyl-dodecanoic acid	97.85	23.33	100.00	5.06	Mutagen	-	+
(5alpha, 8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	96.94	47.88	79.26	2.26	Mutagen	+	-
Diglycidyl resorcinol ether	97.81	35.80	58.30	0.01	Mutagen	+	-
Anapheline	90.02	39.97	10.00	1.03	Mutagen	-	-
Methyl 8-[2-(2-formyl-vinyl)3-hydroxy-5-oxo-cyclopentyl]-octanoate	92.24	19.97	89.04	0.02	Non-mutagen	+	+
Dehydrophytosphingosine	79.34	12.94	97.58	1.73	Non-mutagen	+	-

Note: HIA: Human Intestinal Absorption, PPB: Plasma Protein Binding, BBB: Blood Brain Barrier

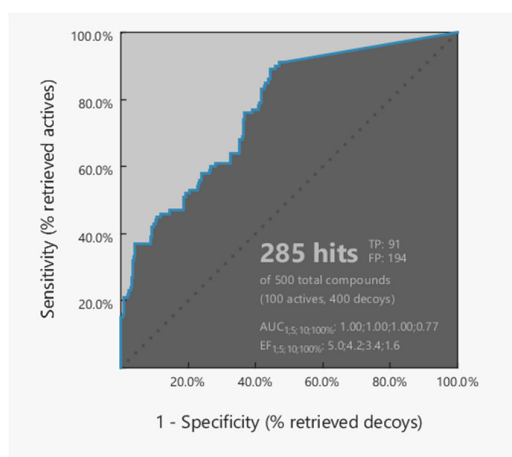
Pharmacophore screening

To identify the pharmacophore features of the native ligand and the test ligands in relation to the target receptor, pharmacophore screening was conducted in the LigandScout application using the VEGFR-2 protein (PDB ID: 2P21). This particular receptor was chosen due to the availability of its target database on the dude site (<https://www.dude.docking.org>), which facilitated the determination of pharmacophores for 27 compounds derived from *A. bilimbi* L. leaves in order to identify hit compounds. A "hit compound" refers to a compound with pharmacophore groups similar to those of the native ligand, displaying the desired biological activity against the target receptor (Katsuno *et al.*, 2015).

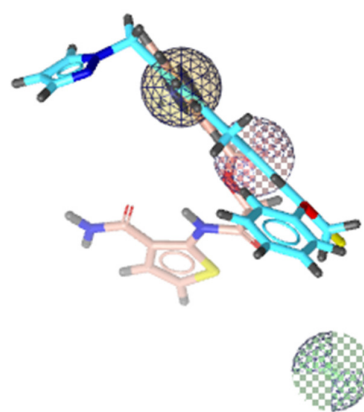
From the 10 pharmacophore models generated, model 2 (Figure 2A), with an AUC value of 0.77, was selected to determine the pharmacophore features of the test ligands, as shown in Table 3. Model 2 was chosen over the other models due to its receiver operating characteristic (ROC) curve, which displayed the highest AUC value. A pharmacophore screening model is considered accurate when the AUC value exceeds 0.70; therefore, model 2 was deemed reliable for screening the test ligands.

Table 3. Pharmacophore screening validation results from *A. bilimbi* L. leaves' bioactive compounds

Pharmacophore Model	Hits	True Positive	False Positive	AUC
1	278	87	171	0.74
2	285	91	194	0.77
3	283	91	192	0.75
4	279	89	190	0.73
5	281	89	192	0.75
6	280	89	191	0.75
7	275	88	187	0.71
8	275	89	186	0.73
9	280	91	189	0.76
10	283	90	193	0.74



(A)



(B)

Figure 2. Pharmacophore screening results

The figure contains the result of pharmacophore screening ROC curve and its pharmacophore visualization; (A) Pharmacophore screening validation curve (ROC curve model 2); (B) Visualization of pharmacophores in model 2 (Hit molecule CHEMBL224889 and ZINC07679665)

Using pharmacophore model 2, specific hit compounds CHEMBL224889 and ZINC07679665 were identified, featuring pharmacophore groups that include 1 hydrophobic group, 1 hydrogen bond acceptor, and 1 hydrogen bond donor (Figure 2B). These hit compounds were identified based on the presence of essential pharmacophore features required by model 2. The screening revealed three hit compounds that demonstrated similarity to the native ligand in pharmacophore features and interactions for VEGFR-2 activity. These compounds—codonopsine, diglycidyl resorcinol ether, and 2-(1-phenylethyl)phenol—achieved pharmacophore fit scores of 34.61, 33.93, and 34.13, respectively.

Molecular docking

Before performing molecular docking simulations on the test compounds from *A. bilimbi* L., the docking protocol was validated to locate the protein's active site and set up a grid box to facilitate the docking process between the protein and the test ligands (Allo *et al.*, 2023). The validation results showed a clear overlay (Figure 3) with a root mean square deviation (RMSD) score of 0.63 Å. RMSD values are crucial in assessing docking accuracy, with successful bonding mode predictions typically indicated by an RMSD value of ≤ 2 Å. Since the RMSD value obtained was lower than 2 Å, the docking protocol validation was deemed acceptable for this study. The active compounds from *A. bilimbi* L. were then docked with the VEGFR-2 receptor to predict their conformation, binding energy, and inhibition constants in interaction with the receptor. Docking analysis of 27 compounds from *A. bilimbi* with the VEGFR-2 receptor identified the most frequently interacting amino acid residues as CYS A:1045, VAL A:848, LYS A:868, ALA A:866, LEU A:1035, and VAL A:899 (Table 4). Notably, both the native ligand and the VEGFR-2 inhibitor drug Sorafenib exhibited conventional hydrogen bonding with ASP A:1046, a key amino acid residue critical for VEGFR-2 inhibition (Abdallah *et al.*, 2022).

Three test compounds showed the closest similarity to the native ligand and Sorafenib in terms of interactions with amino acid residues: codonopsine, diglycidyl resorcinol ether, and 2-(1-phenylethyl)phenol (Table 5). Codonopsine exhibited three common interactions with the native ligand, including a conventional hydrogen bond at ASP A:1046 and hydrophobic pi-alkyl and alkyl interactions at LEU A:889 and LEU A:1035. Codonopsine also demonstrated two similar interactions with Sorafenib at ASP A:1046 (conventional hydrogen bond) and VAL A:916. Conversely, diglycidyl resorcinol ether shared two similar interactions with the native ligand (conventional hydrogen bond at ASP A:1046 and a carbon hydrogen bond at ALA A:866), suggesting that codonopsine may have slightly stronger inhibition potential than diglycidyl resorcinol ether for VEGFR-2.

Among the hit compounds, 2-(1-phenylethyl)phenol showed the greatest potential as a VEGFR-2 inhibitor due to its lowest inhibition constant (1.99 μ M) and binding energy (-7.78 kcal/mol). Additionally, this compound exhibited the highest similarity in interactions with both the native ligand and Sorafenib. Specifically, 2-(1-phenylethyl)phenol displayed three matching interactions with the native ligand (conventional hydrogen bond at ASP A:1046 and pi-alkyl and alkyl hydrophobic interactions at VAL A:899 and LEU A:1035). It also shared three interactions with Sorafenib: a carbon hydrogen bond at ASP A:1046 and hydrophobic interactions at ALA A:866 and VAL A:848.

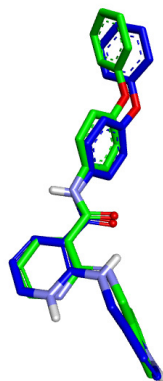


Figure 3. Visualization of native ligand N-(4-phenoxyphenyl)-2-[(pyridin-2-ylmethyl) amion]-nicotinamide re-docking overlay result

Table 4. Molecular docking results from *A. bilimbi* L. leaves' bioactive compounds

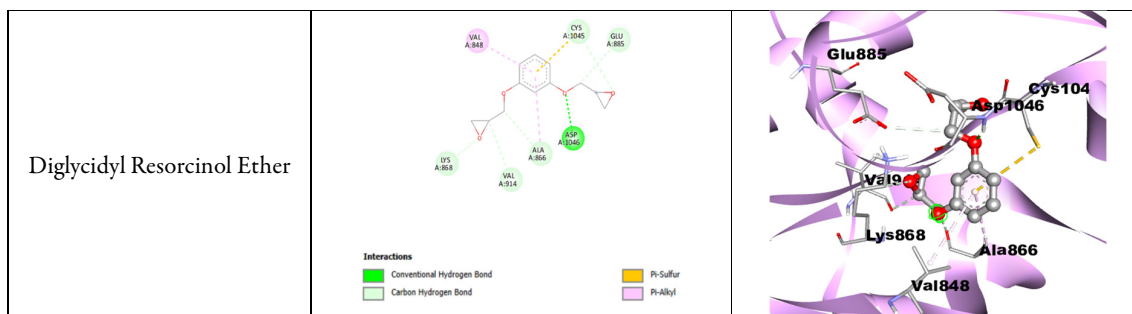
Compound	Binding Energy (kcal/mol)	Inhibition constant (Ki)	Interactions with amino acid	
			Hydrogen bond	Other bond
N-(4-phenoxyphenyl)-2-[(pyridin-2-ylmethyl) amion]nicotinamide (native ligand)	-11.78	2.3 nM	Conventional Hydrogen Bond ASP A: 1046 GLU A: 885 Carbon Hydrogen Bond ALA A: 866 GLU A: 917	Pi-Sigma LEU A: 1019 Pi-Alkyl LEU A: 1035 LEU A: 840 LEU A: 889 VAL A: 899 CYS A: 1024 Unfavorable Donor-Donor CYS A: 919
Sorafenib (reference drug)	-10.51	19.75 nM	Conventional Hydrogen Bond ASP A: 1046 CYS A: 919	Halogen (Fluorine) CYS A: 1045 ILE A: 1044 Pi-Pi T-Shaped PHE A: 1047 Pi-Alkyl, Alkyl VAL A: 916 ALA A: 866 VAL A: 848 VAL A: 898 LEU A: 1019 Pi-Sigma LEU A: 889
2-(1-phenylethyl)phenol	-7.78	1.99 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-cation LYS A: 868 Pi-Sigma PHE A: 1047 VAL A: 916 Pi-Alkyl, Alkyl LEU A: 1035 CYS A: 919 VAL A: 899

				CYS A: 1045 ALA A: 866 VAL A: 848
1,4-diethylbenzene	-5.20	153.94 μ M	-	Pi-Alkyl, Alkyl LEU A: 840 CYS A: 919 PHE A: 918 ALA A: 866 LEU A: 1035 CYS A: 1045 VAL A: 916
Maltol	-4.50	500.46 μ M	Conventional Hydrogen Bond GLU A: 917 CYS A: 919	Alkyl VAL A: 916 VAL A: 899 CYS A: 1045 LEU A: 1035
1-methylnaphthalene	-6.31	23.64 μ M	-	Pi-Alkyl VAL A: 848 ALA A: 866 Pi-Sigma VAL A: 916 Pi-Cation LYS A: 868 Pi-Sulfur CYS A: 1045
2-methylnaphthalene	-6.36	21.70 μ M	-	Pi-Alkyl, Alkyl VAL A: 848 ALA A: 866 LEU A: 1035 Pi-Sigma VAL A: 916 PHE A: 1047 Pi-Cation LYS A: 868 Pi-Sulfur CYS A: 1045
1,4-dimethylnaphthalene	-6.55	15.91 μ M	-	Pi-Alkyl, Alkyl VAL A: 848 ALA A: 866 PHE A: 1047 LEU A: 889 VAL A: 889 Pi-Sigma VAL A: 916 Pi-Cation LYS A: 868 Pi-Sulfur CYS A: 1045
Octanoic acid	-4.60	425.79 μ M	Conventional Hydrogen Bond CYS A: 919 GLU A: 917	Pi-Alkyl, Alkyl LYS A: 868 VAL A: 848 VAL A: 916 LEU A: 1035

				ALA A: 866 PHE A: 1047 CYS A: 1045
3-bromopentane	-3.65	2.13 mM	Conventional Hydrogen Bond CYS A: 919 GLU A: 917	Pi-Alkyl, Alkyl LYS A: 868 VAL A: 848 VAL A: 916 ALA A: 866 VAL A: 899
2,3-dimethylnaphthalene	-6.57	15.37 μ M	-	Pi-Alkyl, Alkyl LEU A: 840 VAL A: 914 VAL A: 848 ALA A: 866 Pi-Sigma LYS A: 868 VAL A: 916 PHE A: 1047 Pi-Sulfur CYS A: 1045
Dihydroactinidiolide	-6.36	21.80 μ M	Conventional Hydrogen Bond LYS A: 868	Pi-Alkyl, Alkyl CYS A: 1045 VAL A: 916 VAL A: 899 ALA A: 866 VAL A: 848 Pi-Sigma PHE A: 1047
Xestoaminol C	-6.31	23.74 μ M	Conventional Hydrogen Bond CYS A: 919 GLU A: 917	Pi-Alkyl, Alkyl LEU A: 840 PHE A: 918 CYS A: 1045 PHE A: 1047 VAL A: 848 VAL A: 916 ALA A: 866 VAL A: 899 VAL A: 914 LYS A: 868
Phytosphingosine	-5.89	48.12 μ M	Conventional Hydrogen Bond CYS A: 1045 ASP A: 1046 Pi Donor Hydrogen Bond PHE A: 1047	Pi-Alkyl, Alkyl LEU A: 889 VAL A: 899 VAL A: 916 VAL A: 914 ILE A: 1044 HIS A: 1026 LEU A: 1019
2-hydroxyhexadecanoic acid	-6.63	13.81 μ M	Conventional Hydrogen Bond HIS A: 1026	Pi-Alkyl, Alkyl CYS A: 919 LEU A: 840 ALA A: 866 VAL A: 848 VAL A: 916

				VAL A: 899 LEU A: 1035 PHE A: 918 PHE A: 1047 CYS A: 1045 LEU A: 889
Pentadecanoyl-EA	-6.53	16.23 μ M	Conventional Hydrogen Bond GLU A: 885 Carbon Hydrogen Bond ALA A: 866 VAL A: 914 LYS A: 868	Pi-Alkyl, Alkyl HIS A: 1026 ALA A: 1050 VAL A: 898 VAL A: 899 LEU A: 889 CYS A: 1045
Codonopsine	-7.16	5.64 μ M	Conventional Hydrogen Bond ASP A: 1046 ILE A: 1044 VAL A: 899	Pi-Alkyl, Alkyl LEU A: 889 LEU A: 1035 CYS A: 1045 VAL A: 916 Pi-Cation, Pi-Anion GLU A: 885 LYS A: 868
Enigmol	-6.91	8.61 μ M	Conventional Hydrogen Bond CYS A: 1045 ILE A: 1044	Pi-Alkyl, Alkyl VAL A: 848 ALA A: 866 LEU A: 1035 LYS A: 868 VAL A: 899 VAL A: 916 LEU A: 889 VAL A: 914 Unfavorable Donor-Donor HIS A: 1026
(Z)-2-amino-1-hydroxyoctadec-4-en-3-one	-7.30	4.43 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl VAL A: 899 PHE A: 1047 LEU A: 889 CYS A: 1045 VAL A: 916 VAL A: 848 ALA A: 866
Dihydroceramide C2	-7.15	5.74 μ M	Conventional Hydrogen Bond ASP A: 1046 GLU A: 917 Carbon Hydrogen Bond LYS A: 868	Pi-Alkyl, Alkyl ILE A: 892 ALA A: 1050 LEU A: 1019 VAL A: 899 LEU A: 889 CYS A: 1024 VAL A: 916 HIS A: 1026

				CYS A: 1045
6-hydroxy sphingosine	-6.86	9.31 μ M	Conventional Hydrogen Bond VAL A: 899 GLU A: 885 VAL A: 914 ALA A: 866	Pi-Alkyl, Alkyl LEU A: 899 LEU A: 1019 VAL A: 898 ILE A: 888 ILE A: 892 HIS A: 1026
Ethyl 3-(N-butylacetamido) propionate	-6.09	34.28 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl LEU A: 889 VAL A: 916 VAL A: 899 ALA A: 866 LYS A: 868 Pi-Sigma PHE A: 1047
Elaeokanine C	-6.58	14.96 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl VAL A: 914 VAL A: 848 VAL A: 916 LYS A: 868 PHE A: 1047
2-ethyl-dodecanoic acid	-6.46	18.38 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl VAL A: 899 VAL A: 916 VAL A: 898 LEU A: 889 ALA A: 1050 ILE A: 1044 HIS A: 1026
5alpha, 8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8-ol	-7.80	1.91 μ M	Conventional Hydrogen Bond ASP A: 1046 GLU A: 885	Pi-Alkyl, Alkyl CYS A: 1045 LYS A: 868 VAL A: 916 VAL A: 848 VAL A: 899 ALA A: 866 Pi-Sigma PHE A: 1047
Diglycidyl resorcinol ether	-6.24	26.58 μ M	Carbon Hydrogen Bond ALA A: 866 VAL A: 914 LYS A: 868 CYS A: 1045 GLU A: 885 Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl VAL A: 848 Pi-Sulfur CYS A: 1045
Anapheline	-6.89	8.93 μ M	Conventional Hydrogen Bond ASP A: 1046	Pi-Alkyl, Alkyl CYS A: 1045 VAL A: 899



Discussion

The current study conducts the analysis of *A. bilimbi* L. compounds as potential VEGFR-2 inhibitors and combines several *in silico* approaches, including Lipinski's Rule of Five (RO5) screening, ADMET predictions, pharmacophore modeling, and molecular docking validation, to assess their drug-likeness and interaction profiles. Based on Lipinski's Rule of Five (RO5) screening and ADMET predictions, Codonopsine has the most promising physicochemical and pharmacokinetic properties among 26 other test compounds in *A. bilimbi* L since it fulfill all Lipinski's RO5 parameters in order to be administered orally. Furthermore, Codonopsine has high intestinal absorption value, medium Caco-2 permeability, low plasma protein binding, and moderate brain blood barrier value, indicating its safety according to ADMET predictions. However, Codonopsine and several other *A. bilimbi* L. compounds needs to be assessed through safety and efficacy studies due to its mutagenicity.

Pharmacophore screening, conducted using VEGFR-2 as the target receptor, aids in identifying compounds with similar pharmacophore groups to the native ligand, thus targeting similar biological activity. Among the 10 generated pharmacophore models, model 2, with an AUC value of 0.77, was selected for its high reliability in distinguishing active compounds from decoys, according to ROC analysis (Sangande and Unepetty, 2021; Wang *et al.*, 2020). This model identified ChEMBL224889 and ZINC07679665 as hit compounds, which exhibit key pharmacophore features: one hydrophobic group, one hydrogen bond acceptor, and one hydrogen bond donor, all critical for binding efficacy. Furthermore, three compounds—codonopsine, diglycidyl resorcinol ether, and 2-(1-phenylethyl)phenol—demonstrate significant similarity to the native ligand's pharmacophore, indicating a strong likelihood of VEGFR-2 inhibition.

Docking of the *A. bilimbi* compounds with VEGFR-2 revealed frequent interactions with key residues, including CYS A:1045, VAL A:848, and ASP A:1046, with ASP A:1046 being particularly important for inhibitory action, as shown by both the native ligand and Sorafenib as the reference drug that also targeted on VEGFR-2 (Nursamsiar *et al.*, 2020). Moreover, a study showed that Sorafenib as a VEGFR-2 inhibitor drug also interacts with VEGFR-2 (PDB ID: 2P2I) through ASP A: 1046, making this amino acid residue as the key interaction for the inhibition of VEGFR-2 (Izzaturahmi *et al.*, 2023). Among the tested compounds, 2-(1-phenylethyl)phenol exhibited the lowest inhibition constant (1.99 μ M) and binding energy (-7.78 kcal/mol), along with three matching interactions with both the native ligand and Sorafenib, suggesting that it may be the most potent VEGFR-2 inhibitor. Both pharmacophore screening and molecular docking studies results showed correlation in terms of binding interactions, where hit compounds retrieved from the pharmacophore screening exhibit lower binding energy and inhibition constant (K_i), while also possessing conventional hydrogen bond interaction at ASP A: 1046, indicating their potency to inhibit the VEGFR-2. This correlation might be connected to each method's principle where pharmacophore screening involves the discovery of a hit compound, which is a compound that has the desired activity at the target of interest (Chan *et al.*, 2024). Meanwhile, molecular docking involves predicting the predominant binding modes of a ligand with a protein

of known three-dimensional structure (Pawar *et al.*, 2023). Therefore, if a compound is considered as a hit compound, it will tend to show better molecular docking results in terms of binding energy and inhibition constant as well as bonding similarity towards the native ligand since it suggests the compound's ability to exert a certain activity.

Despite identifying hit compounds, two non-hit compounds demonstrated smaller inhibition constants and lower binding energy values compared to 2-(1-phenylethyl)phenol. These compounds, methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate and (5 α , 8 β , 9 β)-5,9-epoxy-3,6-megastigmadien-8-ol, exhibited binding energy values of -8.48 kcal/mol and -7.80 kcal/mol, with inhibition constant values of 604.75 nM and 1.91 μ M, respectively. However, based on interaction patterns, methyl 8-[2-(2-formyl-vinyl)-3-hydroxy-5-oxo-cyclopentyl]-octanoate did not bind to ASP A:1046, a residue critical for inhibiting VEGFR-2 activity. Similarly, (5 α , 8 β , 9 β)-5,9-epoxy-3,6-megastigmadien-8-ol showed limited similarity in interactions with the native ligand and Sorafenib. Consequently, neither compound shares the interaction pattern necessary to replicate the activity of the native ligand. A lower binding energy alone is insufficient to predict a compound's efficacy if it does not interact with key amino acid residues akin to native ligands. Thus, these compounds cannot be considered to exhibit the same activity as the native ligand or Sorafenib (Amrulloh *et al.*, 2023). However, it is important to note that *A. bilimbi* L. leaves contain a significant amount of oxalic acid (5.45 g/100 g), which could potentially lead to acute oxalate nephropathy (Gupta *et al.*, 2016; Sá *et al.*, 2019). Therefore, further research is needed to evaluate the safety of utilizing active compounds from *A. bilimbi* L. leaves for anticancer therapy targeting VEGFR-2. To mitigate the potential side effects of *A. bilimbi* leaves, an appropriate extraction method should be considered before consumption. Traditionally, *A. bilimbi* leaves have been used in herbal medicine, such as in the form of a tea decoction for antihypertensive purposes, following recommended dosages (Salam, 2023). Taken together, the *in silico* findings suggest that codonopsine, diglycidyl resorcinol ether, and particularly 2-(1-phenylethyl)phenol show promising potential as VEGFR-2 inhibitors. Further validation of the molecular docking study through dynamic simulations is essential to elucidate the stability of the compound interactions. This should be complemented by experimental assays based on the activity toward VEGFR2, followed by pharmacodynamic studies and toxicity assays to confirm the efficacy and safety of *A. bilimbi* for therapeutic applications.

Conclusions

Based on computational studies of 27 active compounds in *A. bilimbi* leaves, three compounds, named codonopsine, diglycidyl resorcinol ether, and 2-(1-phenylethyl)phenol were identified as having the potential to inhibit the VEGFR-2 receptor. Among these, 2-(1-phenylethyl)phenol demonstrated the best potential as a VEGFR-2 inhibitor, with an inhibition constant of 1.99 μ M and a binding energy of -7.78 kcal/mol. Further research is needed to determine the appropriate dosage and assess the toxicity of *A. bilimbi* compounds to evaluate their safety and effectiveness as candidates for cancer therapy targeting VEGFR-2 inhibition.

Authors' Contributions

Conceptualization: DN, FOM, FMF; Investigation and Data Curation: DPFI, AC, NFS, AA; Methodology: FOM and FMF; Supervision: DN; Writing - original draft: AC, NFS, DPFI, AA; Writing - review and editing: DN, FOM, FMF. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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