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Article

In Silico Study of the Potential of Belimbing Wuluh (*Averrhoa bilimbi*) for the Treatment of Type 2 Diabetes Mellitus

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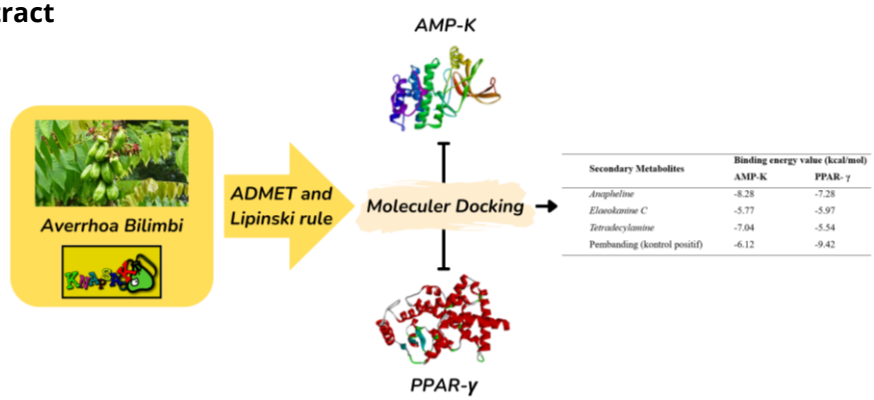
Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by prolonged elevated blood sugar levels due to impaired insulin secretion or function. Metformin is the first-line therapy for type 2 DM, but it has side effects such as nausea, vomiting, bloating, gastrointestinal disturbances, and hypoglycemia, which some patients may not tolerate. Averrhoa bilimbi has potential as an alternative therapy for type 2 DM. This study aims to identify secondary metabolites from bilimbi that have potential as antidiabetic agents by activating AMPK and PPAR-γ protein receptors through in silico studies. The study employed molecular docking methods between AMPK and PPAR-γ protein receptors with bilimbi test compounds and comparators. Test compounds were selected based on compliance with Lipinski's rule, pharmacokinetic predictions, and toxicity. Metformin and rosiglitazone were used as comparators. Screening results identified three bilimbi secondary metabolites: Anapheline, Elaeokanine C, and Tetradecylamine. Docking results showed binding energy values between AMPK protein receptor and Anapheline, Elaeokanine C, Tetradecylamine, and comparators were -8.28, -5.77, -7.04, and -6.12 kcal/mol, respectively. For the PPAR-γ receptor, the binding energy values were -7.28, -5.97, -5.54, and -9.42 kcal/mol, respectively. Anapheline and Tetradecylamine demonstrated potential as antidiabetic agents with 75% and 25% amino acid residue similarity to the comparator compounds.

Keywords: *: Antidiabetic, Averrhoa bilimbi, in silico, binding energy, docking*

Graphical Abstract



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Introduction

Diabetes Mellitus (DM) has become one of the top ten leading causes of death worldwide [1]. According to data compiled by the International Diabetes Federation (IDF) compiled data in 2021, estimating that approximately 537 million adults (ages 20–79) are living with diabetes. This number is projected to increase to 643 million by 2030 and 783 million by 2045[2]. Indonesia ranks sixth among countries with the highest number of diabetes patients in the world, with 20.4 million cases [3].

Diabetes mellitus is a disorder in the body's metabolic process characterized by elevated blood sugar levels that persist for an extended period. This condition can be caused by impairments in insulin secretion, insulin action, or both. Diabetes mellitus is divided into two types: type 1 and type 2 diabetes mellitus. Type 1 diabetes mellitus arises from an autoimmune reaction that attacks the pancreatic β cells, completely inhibiting insulin production [4]. Type 2 diabetes occurs because the pancreas loses its ability to respond effectively to stimulate insulin production, known as insulin resistance [5].

Metformin is the first-line therapy for type 2 diabetes mellitus that has been proven effective in controlling blood sugar levels [6]. However, it can cause side effects that should not be overlooked, such as nausea, vomiting, bloating, gastrointestinal disturbances, and hypoglycemia, which may be intolerable for some patients [7]. Therefore, it is necessary to develop alternative treatments from herbal plants with minimal side effects [8].

Indonesia ranks second after Brazil in terms of biodiversity richness [9]. Indonesia has identified approximately 31,750 plant species, but only about 7,000 species are actually utilized as raw materials in the production of medicine [10]. One of the plants believed to have antidiabetic properties is the bilimbi plant (*Averrhoa bilimbi*) [11].

In silico studies are a strategy used in the discovery of compounds with potential as drug candidates. The application of in silico methods offers several advantages, such as reducing the need for equipment and materials and increasing cost efficiency in research [12]. This approach is also useful for predicting interactions between two types of molecules, such as between proteins and ligands [13]. Proteins that can be used as therapeutic targets for type 2 diabetes mellitus to stimulate insulin and prevent resistance include Adenosine Monophosphate Activated Protein Kinase (AMPK) [14] and Peroxisome Proliferator-Activated Receptor-Gamma (PPAR-γ) [6].

Based on the above description, bilimbi has the potential to be developed as an alternative therapy for individuals with diabetes mellitus (DM). Research on the potential active compounds in bilimbi through in silico methods has not yet been conducted. Therefore, it is important to explore the potential of these secondary metabolites as a solution for addressing type 2 diabetes mellitus.

Material and Methods

Materials and Instrumentation

The materials used include the three-dimensional structures of the proteins AMPK (code: 3aqv) and PPAR-γ (code: 2prg) obtained from the Protein Data Bank, secondary metabolite compounds of bilimbi acquired from the KNApSAcK database with the keyword *Averrhoa bilimbi*, and the control comparators metformin and rosiglitazone.

Methods

Prediction of Lipinski's Rule of Five and Pharmacokinetics, Toxicity of Secondary Metabolites of Averrhoa bilimbi. The prediction was performed using the pkCSM website(http://structure.bioc.cam.ac.uk/pkcsm) by inputting the SMILES code obtained from the KNApSAcK database. The results of the prediction indicated that the secondary metabolite compounds meet the pharmacokinetic requirements (absorption, distribution, metabolism, and excretion), toxicity, and Lipinski's rule.

Protein Receptor Preparation. The protein receptor was downloaded from the Protein Data Bank

(http://www.rcsb.org). Preparation was performed using BIOVIA Discovery Studio Visualizer 2021 by removing water molecules and ligands.

Natural Ligand Preparation. The ligand was separated from water molecules and protein using BIOVIA Discovery Studio Visualizer 2021, followed by the addition of hydrogen atoms.

Redocking (Validation). Autodock is an application used for the redocking process. Redocking is performed by re-docking the original ligand with the receptor protein to obtain the grid box coordinates, which serve as a reference for docking comparison ligands and secondary metabolite ligands. The rigid receptor molecule was subjected to the Lamarckian genetic algorithm (LGA) during the redocking and docking stages in order to find the best conformers; a maximum of 100 conformations were specified for each ligand. The maximum number of energy evaluations was raised to 2,500,000, the genetic generation limit was set at 100,000, and the population size was set at 150. Other parameters were kept at the default values of AutoDock 4.2. The docking methodology was evaluated through redocking to enhance result accuracy. The conformation with the lowest binding energy from the densest cluster was selected as the best docking result and further analyzed for hydrogen bond interactions [15]. The final results of the redocking are based on the conformation data with the lowest binding energy and an RMSD value of less than 2 Å.

Preparation of Reference and Test Ligands. The reference and test ligands were downloaded from the PubChem website by entering the SMILES code obtained from Knapsack. The resulting three-dimensional structures were then energy-optimized using Avogadro.

Docking with Secondary Metabolites of Averrhoa bilimbi and Comparators. The use of AutoDock in linking secondary metabolite compounds with AMP-Kinase and PPAR-γ proteins involves setting a grid box that is identical to the redocking/validation process. This approach ensures consistency in results during both docking and redocking, avoiding significant variations. The method for docking with secondary metabolites of belimbing wuluh (Averrhoa bilimbi) is the same as in the redocking method.

Study of Docking Interactions and Visualization. The application used to study interactions and visualizations is BIOVIA Discovery Studio Visualizer 2021. Observations of ligand-protein interactions can be examined through both 2D and 3D visualizations. This tool facilitates the analysis of interactions by clearly presenting various types of interactions, such as van der Waals forces, hydrophobic interactions, hydrogen bonds, and others, directly within its interface, making the process more efficient and insightful.

Results and Discussions

Searching for secondary metabolite compounds.

The results of the search for secondary metabolite compounds from *Averrhoa bilimbi* in the Knapsack database, using the keyword *Averrhoa bilimbi*, revealed the presence of 22 secondary metabolites (Table 1).

Table 1. Secondary Metabolites of *Averrhoa bilimbi* [16]

No	ID	Secondary Metabolites
1	19519-53-0	*Anapheline*
2	26989-20-8	Codonopsine
3	33023-03-9	Elaeokanine C
4	142741-31-9	Afzelechin 3-O-alpha-L-rhamnopyranoside
5	613253-63-7	Cucumerin A
6	2765-11-9	Pentadecanal
7	629-54-9	Palmitic acid amide
8	1160155-55-4	14-Methyl-8-hexadecen-1-ol
9	2874-75-1	2-Ethyldodecanoic acid
10	764-67-0	2-Hydroxyhexadecanoic acid
11	24546-19-8	7-Hexadecen-1-ol
12	101-90-6	Diglycidyl resorcinol ether
13	2304-80-5	Dihydroceramide C2
14	3687-54-5	4-Hydroxy-8-sphingenine
15	52304-36-6	Ethyl 3-(N-butylacetamido) propionate
16	862472-69-3	Enigmol

No	ID	Secondary Metabolites
17	34227-09-3	Isoavocadienofuran
18	3999-01-7	Linoleamide
19	17352-32-8	Nonadecanal
20	554-62-1	Phytosphingosine
21	2016-42-4	*Tetradecylamine*
22	129825-28-1	Xestoaminol C

Screening using Lipinski's Rule. All obtained secondary metabolite compounds were then screened for drug likeness based on Lipinski's Rule of Five. This rule states that a compound is considered drug-like if it has a molecular weight (MW) of less than 500 Daltons, a log P value of less than 5, fewer than 5 hydrogen bond donors (HBD), and fewer than 10 hydrogen bond acceptors (HBA) [17] (Table 2). A molecular weight exceeding 500 Da indicates that the compound may not penetrate cell membranes. A higher log P value indicates increased hydrophobicity of the molecule; compounds with excessive hydrophobicity tend to exhibit higher toxicity. The number of hydrogen bond donors and acceptors describes the capacity for hydrogen bonding: a higher capacity requires more energy for absorption to occur [18].

Table 2. Lipinski's Rule Screening of Secondary Metabolite Compounds

No.	Secondary Metabolites	Lipinski Screening				Description
		MW	Log p	HBA	HBD	
		<500	<5	<10	<5	
1	*Anapheline*	224.348	1.6199	3	2	Qualified
2	Codonopsine	267.325	0.8006	5	2	Qualified
3	Elaeokanine C	211.305	1.2008	3	1	Qualified
4	Afzelechin 3-O-alpha-L-rhamnopyranoside	420.414	0.6923	9	6	Not qualified
5	Cucumerin A	552.532	1.9491	11	8	Not qualified
6	Pentadecanal	226.404	5.2765	1	0	Not qualified
7	Palmitic acid amide	255.446	4.953	1	1	Qualified
8	14-Methyl-8-hexadecen-1-ol	254.458	5.482	1	1	Not qualified
9	2-Ethyldodecanoic acid	228.376	4.628	1	1	Qualified
10	2-Hydroxyhexadecanoic acid	272.429	4.5231	2	2	Qualified
11	7-Hexadecen-1-ol	240.431	5.236	1	1	Not qualified
12	Diglycidyl resorcinol ether	222.24	1.2418	4	0	Qualified
13	Dihydroceramide C2	567.984	10.5672	3	3	Not qualified
14	4-Hydroxy-8-sphingenine	315.498	2.895	4	4	Qualified
15	Ethyl 3-(N-butylacetamido) propionate	215.293	1.5882	3	0	Qualified
16	Enigmol	301.515	4.1466	3	3	Qualified
17	Isoavocadienofuran	246.394	5.6851	1	0	Not qualified
18	Linoleamide	279.468	5.2852	1	1	Not qualified
19	Nonadecanal	282.512	6.8369	1	0	Not qualified
20	Phytosphingosine	317.514	3.119	4	4	Qualified
21	*Tetradecylamine*	213.409	4.6462	1	1	Qualified
22	Xestoaminol C	229.408	3.6154	2	2	Qualified

Pharmacokinetics and toxicity prediction. The secondary metabolite compounds that pass the Lipinski's rule screening are those that meet all the requirements of Lipinski's rules. Out of 22 secondary metabolite compounds screened, 13 met the criteria. After screening for drug-likeness, pharmacokinetic prediction was conducted to determine the pharmacokinetic profile of each compound, including Absorption, Distribution, Metabolism, Excretion, and Toxicity. PkCSM is a website that is often used to predict the pharmacokinetic properties and toxicity of a substance that has the potential to become a new drug. Access to this web server is free (http://structure.bioc.cam.ac.uk/pkcsm). From the pharmacokinetic and toxicity screening of the 13 compounds, only 3 secondary metabolite compounds met the requirements: *Anapheline*, Elaeokanine C, and *Tetradecylamine* (Table 3). The criterion for each parameter is that a compound is considered to have good absorption if the absorption value is >80%, and poor absorption if it is <30%. The intestine is the primary site for the absorption of orally administered drugs [19].

The next pharmacokinetic prediction parameter is distribution. Distribution refers to the process by which a drug enters the bloodstream. The greater the extent of drug distribution throughout the body, the more rapidly the drug reaches its site of action and the quicker its effects are felt. The parameter used is VDss (volume of distribution at steady state). A compound is considered to have a low volume of distribution if the Log VDss value is < -0.15, and high if it is > 0.45. The volume of distribution (VDss) is the theoretical volume required for the total dose of a drug to be distributed evenly so that it achieves a concentration equal to that in plasma. A higher VD indicates that a larger proportion of the drug is distributed in tissues rather than in plasma [20].

The distributed drug is then metabolized. Metabolism generally occurs in the liver with the assistance of cytochrome P450 enzymes. Cytochrome P450 (CYP) is a crucial detoxification enzyme in the body that oxidizes xenobiotics for excretion. Cytochrome P450 enzymes are responsible for the metabolism of many drugs, including CYP1A2, CYP2C9, CYP2D6, and CYP3A4/5, which account for approximately 72% of all drug metabolism. Therefore, it is important to evaluate the potential of compounds to inhibit cytochrome P450, which in this study is represented by the CYP2D6 and CYP3A4 isoforms.

The next pharmacokinetic parameter is excretion. The process of excreting a compound can be assessed by measuring the Total Clearance (CLTOT) constant. CLTOT represents a combination of hepatic clearance (metabolism in the liver and bile) and renal clearance (excretion via the kidneys). This is related to bioavailability and is crucial for determining the dosage required to achieve a steady-state concentration. The value of CLTOT can be used to predict the rate of excretion of the compound. An important toxicity parameter is LD_{50}, which is defined as the dose of a test substance, determined through statistical calculation, that causes the death of 50% of test animals when administered orally.

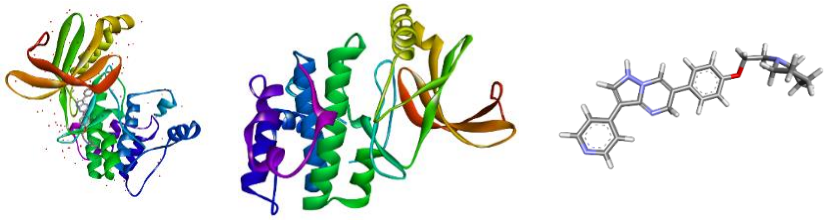
Preparation of protein receptors and natural ligands.

After pharmacokinetic prediction screening is conducted on a compound, the next step is to prepare it for molecular docking. The docking process begins with validation, which involves separating the protein from its natural ligand and then re-docking the separated protein and ligand. The validation phase starts with the preparation of the protein. The BIOVIA Discovery Studio app is used for protein and ligand preparation because it provides a variety of advanced tools for molecular manipulation, such as protein structure cleaning, hydrogen addition, geometry optimization, and active pouch detection [21]. Protein preparation involves the removal of water molecules, natural ligands, and other complex compounds present [22], [23]. The prepared protein is then used for validation by performing redocking with its natural ligand. The natural ligand is first prepared by removing proteins and other complex molecules except for the ligand (Figure 1 and Figure 2).

Table 3. Pharmacokinetic and Toxicity Screening of Secondary Metabolite Compounds [25]

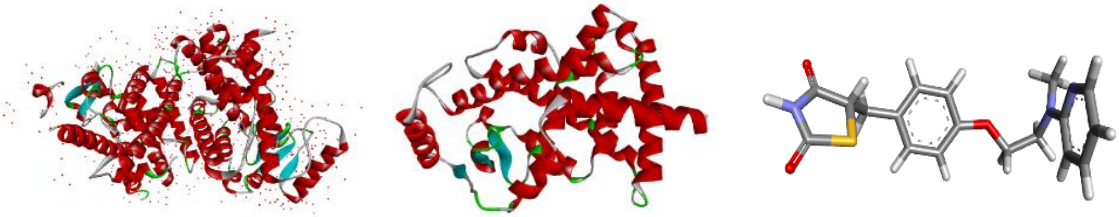
No.	Secondary Metabolites	ADME (Absorption, Distribution, Metabolism, Excretion) Screening								Description
		IAH (% Absorbed)	VDss (log L/kg)	CYP2D6 substrate	CYP3A4 substrate	CYP2D6 inhibitor	CYP3A4 inhibitor	TC (log ml/min/kg)	LD50 (mol/kg)	
1	*Anapheline*	93.738	0.908	No	No	No	No	1.272	2.483	Qualified
2	Codonopsine	75.276	0.24	No	No	No	No	0.824	2.396	Not Qualified
3	Elaeokanine C	85.76	0.545	No	No	No	No	1.003	2.03	Qualified
4	Palmitic acid amide	90.399	0.319	No	Yes	No	No	1.837	1.802	Not Qualified
5	2-Ethyldodecanoic acid	94.023	-0.638	No	No	No	No	1.701	1.611	Not Qualified
6	2-Hydroxyhexadecanoic acid	90.023	-0.708	No	No	No	No	1.832	1.371	Not Qualified
7	Diglycidyl resorcinol ether	92.38	0.072	No	Yes	No	No	0.267	2.13	Not Qualified
8	4-Hydroxy-8-sphingenine	90.898	-0.466	No	Yes	No	No	1.314	3.769	Not Qualified
9	Ethyl 3-(N-butylacetamido) propionate	94.929	-0.155	No	No	No	No	0.832	2.2	Not Qualified
10	Phytosphingosine	94.24	-0.307	No	No	Yes	No	1.43	1.782	Not Qualified
11	*Tetradecylamine*	89.411	0.933	No	Yes	No	No	1.338	2.375	Qualified
12	Xestoaminol C	91.165	0.278	No	No	No	No	1.31	2.512	Not Qualified
13	Enigmol	92.061	-0.074	No	No	No	No	1.363	3.723	Not Qualified

Description: IAH = Intestinal Absorbsi Human; TC = Total Clerance



a B c

Figure 1. Protein and Ligand Preparation (3AQV) (a) Protein before preparation; (b) Protein after preparation; (c) Natural ligand after preparation

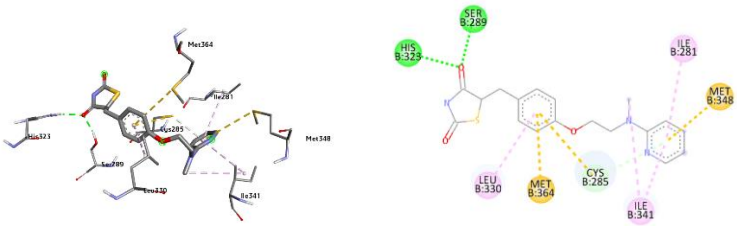


a b c

Figure 2. Protein and Ligand Preparation (2PRG) (a) Protein before preparation; (b) Protein after preparation; (c) Natural ligand after preparation

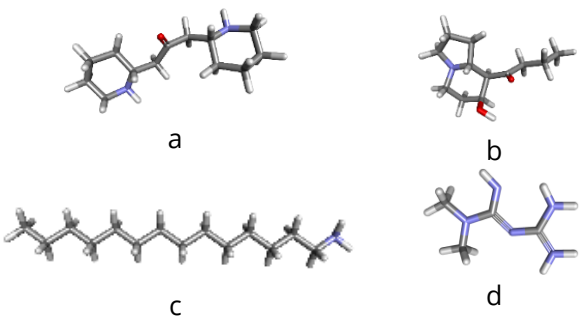
a b

Figure 3. Interaction of Protein and Natural Ligand (AMPK); (a) Three-Dimensional Form (b) Two-Dimensional Form



a b

Figure 4. Interaction of Protein and Natural Ligand (PPAR-γ); (a) Three-Dimensional Form (b) Two-Dimensional Form.



a b c d

Figure 5. Results of Preparation of Test and Reference Compounds: (a) *Anapheline*; (b) Elaeokanine C (c) *Tetradecylamine* (d) Metformin

Redocking (Validation).

AutoDock 4.2 is often used in in silico research due to its reliable and flexible ability to accurately and efficiently perform molecular docking using genetic algorithms and Monte Carlo simulations, support the analysis of ligand-receptor interactions, and predict binding affinity and molecular conformation with wide validation in various scientific studies [24]. The prepared protein and natural ligand were then validated by re-docking the ligand to the protein, returning it to its original position. The validation results showed that the ligand successfully returned to its initial position, as evidenced by Root Mean Square Deviation (RMSD) values of 0.48 Å and 0.86 Å. Validation is considered successful if the RMSD value is less than 2 Å. An RMSD value close to 0 indicates that the ligand can be accurately returned to its original position (Table 4). Another parameter is the Grid Parameter File (GPF) from the validation, which contains information about the grid box size and grid box position. The grid box size describes the dimensions of the grid box, while the grid box position represents the ligand's coordinates within the three-dimensional space, expressed as X, Y, Z coordinates. The interactions between the natural ligand and the protein obtained from the validation process can also be visualized using the Biovia Discovery Studio software.

Table 4. Results of the Redocking Process

Parameters	PPAR-γ	AMPK
Grid Box Size (Å)	40 x 40 x 40	40 x 40 x 40
Grid Box Position	X : 59.415	X : -6.735
	Y : -5.607	Y : 44.128
	Z : 42.406	Z : 7.029
Spacing	0.375	0.375
RMSD	0.86 Å	0.48 Å
Binding energy	-9,42 kkal/mol	-8,89 kkal/mol

Based on the visualization results, it was observed that the natural ligand AMPK interacted with 11 amino acid residues: SER 97, VAL 96, GLU 94, VAL 30, MET 93, LYS 107, ALA 43, LEU 146, LYS 45, ALA 156, and MET 164 (Figure 3). In contrast, the natural ligand PPAR-γ interacted with 8 amino acid residues: SER 289, HIS 323, CYS 285, MET 348, MET 364, ILE 341, LEU 330, and ILE 281 (Figure 4). The types of bonds observed in the natural ligand AMPK included 3 hydrogen bonds and 9 hydrophobic bonds, totaling 12 bonds. Meanwhile, the natural ligand PPAR-γ exhibited 3 hydrogen bonds and 4 hydrophobic bonds, totaling 7 bonds.

Preparation of test and comparison compounds.

The test and reference compounds were prepared for docking by optimizing their molecular structures through energy minimization using Avogadro, an advanced cross-platform molecular editor and visualizer designed for computational chemistry, molecular modeling, bioinformatics, and related fields[26]. Energy minimization aims to obtain molecules with a stable structure in three-dimensional form. The prepared test and reference compounds were then used for docking with the target protein (Figure 5).

Docking with test and comparator compounds.

The binding energy values are the result of the docking process between the test compounds and the reference compounds. Docking between the AMPK protein receptor with the test and reference compounds yielded binding energy values of -8.28 kcal/mol for *Anapheline*, -5.77 kcal/mol for Elaeokanine C, -7.04 kcal/mol for *Tetradecylamine*, and -6.12 kcal/mol for the reference compound (metformin). Subsequently, docking between the PPAR-γ protein receptor with the test and reference compounds yielded binding energy values of -7.28 kcal/mol for *Anapheline*, -5.97 kcal/mol for Elaeokanine C, -5.54 kcal/mol for *Tetradecylamine*, and -9.42 kcal/mol for the reference compound (rosiglitazone). Binding energy provides insight into the stability of the interaction between a ligand and its receptor. The lower the binding energy value, the more stable the interaction and the higher the likelihood of the ligand interacting with the receptor [12].

Based on the binding energy values obtained in Table 5, the compounds that show potential as antidiabetic agents are *Anapheline* and *Tetradecylamine*. These compounds have lower

binding energy values compared to metformin, the reference compound, although they are not superior to rosiglitazone.

Table 5. Binding energy values from the docking process

Secondary Metabolites	Binding energy value (kcal/mol)	
	AMPK	PPARγ
Anapheline	-8.28	-7.28
Elaeokanine C	-5.77	-5.97
Tetradecylamine	-7.04	-5.54
Comparator (positive control)	-6.12	-9.42

Interaction and visualization studies.

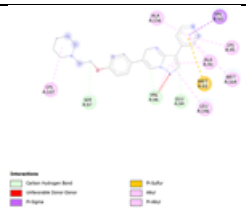
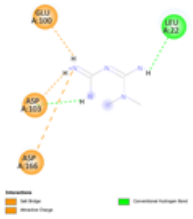
The interaction results between *Anapheline* and the AMPK protein receptor revealed four hydrogen bonds and one hydrophobic bond, with the amino acid residues involved in these interactions being GLU 100, ASP 103, SER 165, ASP 166, and MET 164. In contrast, the interaction between *Tetradecylamine* and the AMPK protein receptor resulted in two hydrogen bonds and fourteen hydrophobic bonds, with the amino acid residues involved being ASP 100, GLU 100, VAL 30, ALA 43, ALA 156, LEU 146, MET 164, LYS 45, MET 163, and LEU 146 (Table 6). *Anapheline* shares 75% of its amino acid residues with the reference compound metformin, while *Tetradecylamine* shares only 25%. The similarity in amino acid residues with the reference compound suggests that the test compounds may inhibit the activity of the target protein and potentially exhibit similar activity to the reference compound [27].

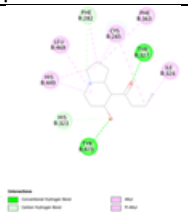
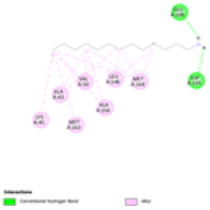
Based on the docking results presented in Table 5, it was found that the number of bonds between the ligand and the protein does not affect the binding energy (ΔG). However, the magnitude of the inhibition constant significantly influences the binding energy (ΔG). The lower the binding energy and inhibition constant values, the higher the ligand's affinity, as the non-covalent interactions between the compound and the receptor become more stable and stronger. A lower (negative) binding energy value indicates that less energy is required for the ligand-protein interaction, resulting in a more stable bond between the ligand and the protein [12].

The lower binding energies of *Anapheline* and *Tetradecylamine* relative to metformin in the context of AMPK receptor interaction. Lower binding energy often correlates with stronger ligand-receptor affinity, suggesting these compounds could be more effective at activating AMPK pathways, which is relevant in glycemic control for type 2 diabetes management. This finding underscores a promising therapeutic potential of compounds in *Averrhoa bilimbi*, but we recognize that binding energy alone may not fully predict clinical efficacy. Thus, it is crucial to set specific binding energy thresholds that could more accurately reflect therapeutic viability in clinical settings.

Our study has not yet been tested in laboratory or clinical settings on humans. However, we found research indicating that the results from in silico methods correlate strongly with those obtained from in vivo or in vitro methods. The flavan-3-ol compound tested using in silico analysis yielded a binding energy value of -10.24 kcal/mol, which is lower than the positive control, quercetin, at -8.95 kcal/mol. Furthermore, when tested in vitro using p-nitrophenyl-α-D-glucopyranoside as a substrate, the flavan-3-ol compound demonstrated the ability to inhibit the activity of the enzyme α-glucosidase in hydrolyzing p-nitrophenyl-α-D-glucopyranoside into p-nitrophenol, with inhibitory activity even superior to that of the standard quercetin [29]. Supporting this approach, Setyani [30] successfully isolated flavonoid compounds from yellow root with structures similar to rutin and quercetin. Although the binding affinity of these compounds was lower than that of the natural ligand, in vivo tests revealed significant antidiabetic activity.

Table 6. Analysis of docking results between protein receptors and test and reference compounds.

Compound/ Ligand	Inhibition Constant (μM)	Binding Energy (kcal/ mol)	Amino Acid Residue	2D Interaction
AMPK	303.49	-8.89	SER 97, VAL 96, GLU 94, VAL 30, MET 93, LYS 107, ALA 43, LEU 146, LYS 45, ALA 156, MET 164	 3 Hydrogen Bonding 9 Hydrphobic Bond
PPAR- γ	151.91	-9.42	SER 289, HIS 323, CYS 285, MET 348, MET 364, ILE 341 LEU 330, ILE 281	 3 Hydrogen Bonding 4 Hydrphobic Bond
Metformin	32.43	-6.12	ASP 103, GLU 100, ASP 166, LEU 22	 2 Hydrogen Bonding and Elactrostatistical bonding 1 Elactrostatistical bonding 2 Hydrogen Bonding
Anapheline with AMPK protein	0.845	-8.28	GLU 100, ASP 103, SER 165, ASP 166, MET 164	 4 Hydrogen Bonding 1 Hydrphobic Bond
Anapheline with PPAR- γ protein	4.6	-7.28	TYR 327, TYR 473, CYS 285, ARG 288, LEU 453, ILE326, LEU 330, PHE 282, HIS 449	 3 Hydrogen Bonding 6 Hydrphobic Bond

Compound/ Ligand	Inhibition Constant (μM)	Binding Energy (kcal/ mol)	Amino Acid Residue	2D Interaction
Elaeokanine C with AMPK protein	59.43	-5.77	VAL 96, VAL 30, ALA 43, ALA 156, LEU 22, MET 164, ILE 77, LEU 146	 1 Hydrogen Bonding 8 Hydrphobic Bond
Elaeokanine C with PPAR- γ protein	42.33	-5.97	TYR 327, TYR 473, HIS 323, PHE 282, CYS 285, LEU 469, ILE 326, PHE 363, HIS 449	 4 Hydrogen Bonding 8 Hydrphobic Bond
Tetradecylamine with AMPK protein	6.88	-7.04	ASP 100, GLU 100, VAL 30, ALA 43, ALA 156, LEU 146, MET 164, LYS 45, MET 163, LEU 146	 2 Hydrogen Bonding 14 Hydrphobic Bond
Tetradecylamine with PPAR- γ protein	86.87	-5.54	CYS 285, SER 289, LEU 453, LEU 469, LEU 330, MET 364, PHE 282, HIS 323, PHE 363, HIS 449, TYR 473	 3 Hydrogen Bonding 15 Hydrphobic Bond

The analysis showed that the bound amino acids were similar to those in the natural ligand, suggesting that the similarity in bound amino acids may influence the biological activity. Similarly, Renganathan [28] conducted an in vivo study and found that the antihyperglycemic activity of certain compounds was comparable to that of acarbose. In silico analysis identified two active compounds, hexadecanoic acid and (Z)-octadec-9-enoic acid, with binding affinities of −1.313 and −1.266 kcal/mol, respectively. While these compounds were not directly compared with the natural ligand or acarbose, the similarity of the bound amino acids to those in the binding pocket supported the hypothesis that similar bound amino acids contribute to similar biological activities [31]. These findings highlight the complementary nature of in silico, in vitro, and in vivo analyses in evaluating the pharmacological potential of active compounds and suggest that the structure-activity

relationship plays a crucial role in the biological efficacy of these compounds.

Conclusions

Based on the molecular docking analysis, secondary metabolites from Averrhoa bilimbi, specifically Anapheline and Tetradecylamine, show potential as antidiabetic agents. This is indicated by their lower binding energy values of -8.28 kcal/mol and -7.04 kcal/mol, respectively, compared to metformin, which was used as the reference compound for the AMPK protein receptor. However, these compounds are not as effective as rosiglitazone, which served as the reference compound for the PPAR-γ protein receptor. Additionally, both compounds share 75% and 25% amino acid residue similarity with the reference compounds.

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Author Contibutions

Conceptualization, R.A.D.; Methodology, D.A.P.; Software, S.S.; Validation, S.S. and N.Z.W.K.; Investigation, R.A.D..; Resources, A.M.S.; Data Curation, D.A.P.; Writing – Original Draft Preparation, R.A.D.; Writing – Review & Editing, S.S. and N.Z.W.K.; Visualization, R.A.D.; Supervision, S.S and H.; Project Administration, A.M.S and H.

Conflict of Interest

There are no significant conflicts

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