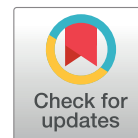


RESEARCH ARTICLE

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Exploring the anti-diabetic potential of stevia-derived compounds through PPAR- γ targeted molecular docking

Amalia Sonita Putri, Ertika Agtha Prawicha, Esterike Alfathien Putri, Indah Wulandari, Mutiara Anggun Saputri, Nadia Nur Syakilla, Putri Aulia Nurul Hidayati, Winni Nur Auli^{ID}, Anjar Hermadi Saputro*^{ID}

Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, Indonesia

*Corresponding author: Anjar Hermadi Saputro, Jl Terusan Ryacudu No 1, Way Huwi, Jati Agung, South Lampung 35365, Indonesia. Email: anjar.saputro@fa.itera.ac.id

Abstract: This study explores the potential of *Stevia rebaudiana* Bertoni-derived compounds as anti-diabetic agents by targeting the peroxisome proliferator-activated receptor gamma (PPAR- γ), a key regulator of glucose metabolism. Utilizing in silico molecular docking, we evaluated the binding affinities of four stevia-derived compounds (dulcoside A, steviol, isosteviol, steviolmonoside) and compared them to the native ligand (J35) and the well-known PPAR- γ agonist, rosiglitazone. Isosteviol exhibited the strongest binding affinity to PPAR- γ , with a binding energy of -8.89 kcal/mol, surpassing that of rosiglitazone (-8.26 kcal/mol) and closely following the native ligand (-9.01 kcal/mol). The interactions between isosteviol and PPAR- γ included multiple hydrogen bonds and hydrophobic interactions. These findings indicate that isosteviol, along with other stevia-derived compounds, has a potential as a natural anti-diabetic agent.

Keywords: diabetes, stevia, molecular docking, PPAR-gamma

Introduction

Diabetes mellitus (DM) is a chronic, lifelong condition characterized by hyperglycemia, or elevated blood sugar levels, due to metabolic dysfunction in the pancreas that leads to insufficient insulin production. If not managed effectively, diabetes can result in severe complications, including damage to large and small blood vessels, increasing the risk of cardiovascular diseases such as hypertension and heart attacks. In extreme cases, untreated diabetes can lead to lower limb amputations [1].

Indonesia ranks seventh globally, with approximately 10.3 million people diagnosed with DM. According to the Indonesian Ministry of Health, DM is currently the third leading cause of death in the country, following stroke and heart disease, with about 10 million Indonesians affected. Projections suggest that the number of diabetes cases could double or triple within the next decade. Globally, the World Health Organization (WHO) reports that over 422 million people are living with diabetes, a fourfold increase over the past 30 years. This disease now claims more than one million lives annually [2]. Conventional treatments for diabetes often involve synthetic drugs, which, while effective, can cause side effects and lead to long-term

dependence. Consequently, there is a growing interest in finding safer, more effective alternatives, including the use of natural compounds for anti-diabetic therapies.

One plant that has attracted attention in the search for natural anti-diabetic agents is *Stevia rebaudiana* Bertoni, commonly known as stevia. Stevia is a genus within the sunflower family, comprising about 200 species. Native to the Amambay region in northeastern Paraguay, stevia is now cultivated globally, including in parts of Asia, Europe, and Canada. The leaves of stevia are small, about 3-4 cm long, with a dense texture, elongated spatula-like shape, blunt ends, and serrated edges from the middle to the tips [3]. Stevia's remarkable sweetness is due to diterpene glycoside compounds, particularly stevioside and rebaudioside A, which are 110-270 times and 140-400 times sweeter than sucrose, respectively. Additionally, stevia leaf extract contains various bioactive compounds, including alkaloids, flavonoids, steviolmonoside, dulcoside A, rubusoside, steviolbioside, chlorophyll, xanthophylls, hydroxycinnamic acids (such as caffeic and chlorogenic acids), essential oils, soluble neutral oligosaccharides, amino acids, fats, and free sugars [3,4].

In diabetes treatment, one promising approach targets the peroxisome proliferator-activated receptors

(PPARs), which are transcription factors regulating gene expression. PPARs are divided into three types: PPAR- γ , PPAR- α , and PPAR- β/δ , each playing a crucial role in various biological processes, including glucose metabolism [5]. Among these, PPAR- γ is a key target in developing anti-diabetic drugs due to its ability to enhance insulin sensitivity, reduce insulin resistance, and regulate glucose metabolism [6]. Consequently, research focusing on the potential of stevia-derived compounds to activate PPAR- γ could contribute significantly to developing innovative and effective anti-diabetic therapies.

This study employs *in silico* methods, utilizing computer simulations to predict the interactions between stevia-derived compounds and the PPAR- γ . Computational visualization of these interactions is instrumental in identifying the pharmacophore—a molecular framework that carries the essential features responsible for the compound's biological activity—thereby guiding the development of new therapeutic agents [7].

In this research, we explore the molecular docking of four compounds from stevia leaves—dolicuside A, steviol, isosteviol, and steviolmonoside—on the PPAR- γ receptor, examining their potential as anti-diabetic agents. The findings aim to enhance our understanding of how natural compounds can regulate glucose metabolism and contribute to the development of more effective diabetes treatments.

Methods

Software and databases

This research was conducted using a personal computer (PC) equipped with Windows 11 64-bit. Various software tools were employed to perform chemical computing studies, including AutodockTools from MGL Tools 1.5.7 for molecular docking, Avogadro for molecular visualization, RCSB PDB site for obtaining protein structures, MolView for ligand structure generation, SwissADME for format conversion, and BIOVIA Discovery Studio 2024 Client for interaction visualization. The three-dimensional macromolecular structure of PPAR- γ , along with the native ligand (2S)-2-[4-methoxy-3-({[4-(trifluoromethyl)benzoyl]amino}methyl)benzyl]pentanoic acid (J35), was obtained from the Protein Data Bank (PDB) under the code 3VJH.

Protein preparation

The initial step involved preparing the protein and native ligands for docking studies. The target protein, PPAR- γ , was retrieved from the RCSB Protein Data Bank with the PDB code 3VJH. Using AutodockTools, the protein structure was isolated by removing any co-crystallized ligands, water molecules, and other residues. The prepared protein structure and the native ligand were then saved in .pdbqt format for subsequent docking procedures.

Method validation

To ensure the accuracy of the docking procedure, method validation was performed by re-docking the native ligand (J35) into the PPAR- γ protein. The validation process was executed using AutodockTools (version 1.5.7), with the Root Mean Square Deviation (RMSD) as the primary validation parameter. An RMSD value of less than 2 Å was considered indicative of a valid docking method [8,9]. The validation process included preparing the protein and ligand, setting the grid box size, configuring docking parameters, and running the docking simulation to verify the method's validity.

Ligand preparation

The study involved the use of several ligands found in *Stevia rebaudiana*—dolicuside A, steviol, isosteviol, and steviolmonoside—as well as rosiglitazone, which served as a control. Ligand structures were initially obtained from MolView, and subsequently converted from .sdf to .pdb format using the SwissADME tool for compatibility with the docking software.

Molecular docking of target compounds

Molecular docking was carried out using AutodockTools (version 1.5.7). The previously prepared protein and ligand files, in .pdbqt format, were imported into the software. The grid box size, predetermined during the validation process, was set to 36x36x36 with coordinates X = -17.648, Y = 20.754, Z = -14.188. Docking was performed for each ligand, and the results were compared with those of other compounds to evaluate binding affinities and interactions.

Interaction visualization

The final step involved visualizing the interactions between the docked ligands and the PPAR- γ protein using BIOVIA Discovery Studio 2024. The docking results from AutodockTools (version 1.5.7) were used to generate ligand-protein complexes, which were then analyzed to identify key interactions, such as hydrogen bonds, hydrophobic interactions, and other relevant molecular interactions.

Results

Validation and docking results

Validation was performed by docking the native ligand, J35, to the PPAR- γ protein, ensuring the accuracy of the docking method. Subsequent docking experiments were conducted with the target compounds from stevia leaves—dolcусide A, steviol, isosteviol, and steviolmonoside—as well as an agonist the PPAR- γ , rosiglitazone. The binding energies obtained from these docking simulations are presented in Table 1.

Table 1. Docking results between ligands and protein (PDB ID 3VJH)

Compounds	Binding free energy (kcal/mol)
J35 (Native ligan)	-9.01
Dolcусide A	-6.48
Isosteviol	-8.89
Steviol	-8.29
Steviolmonoside	-7.82
Rosiglitazone	-8.26

The RMSD value calculated between the docked native ligand and the crystallographic position was 1.35 Å, confirming that the docking method was valid. The native ligand J35 exhibited a binding energy of -9.01 kcal/mol, serving as a reference for comparison.

Among the target compounds, isosteviol demonstrated the strongest binding affinity to PPAR- γ , with a binding energy of -8.89 kcal/mol, followed closely by steviol (-8.29 kcal/mol) and rosiglitazone (-8.26 kcal/mol). Dolcусide A had the weakest binding affinity, with a binding energy of -6.48 kcal/mol. These results indicate that isosteviol has the highest affinity for the PPAR- γ among the compounds tested, suggesting it as the most promising candidate for anti-diabetic activity.

Interaction analysis

The interactions between the ligands and PPAR- γ were further analyzed using BIOVIA Discovery Studio 2024 Client software, which provided 2D visualizations of the ligand-receptor interactions.

Figure 1A shows the 2D visualization of dolcусide A interacting with PPAR- γ , revealing multiple interactions including conventional hydrogen bonds, unfavorable donor-donor bonds, Pi donor hydrogen bonds, Pi-Sigma, Pi-Pi stacked, Pi-alkyl, and alkyl bonds. In conventional hydrogen bonds, involving amino acids Ile A:281, Phe A:282, Arg A:288, His A:323, His A:449, in unfavorable donor-donor bonds involving amino acids Cys A:285, in Pi bonds -sigma involves the amino acid Ile A:341, in stacked Pi-Pi bonds, Pi-Alkyl and alkyl involve the amino acids Ala A:292, Leu A:330, Phe A:36, Ile A:326, and Met A:364.

Figure 1B illustrates the interactions of isosteviol with PPAR- γ , showing conventional hydrogen bonds, carbon-hydrogen bonds, Pi-alkyl, and alkyl interactions. In the conventional hydrogen bond, it involves the amino acid Tyr A:327, in the carbon hydrogen bond it involves the amino acid Lys A:367. Meanwhile, the Pi-alkyl and alkyl bonds involve the amino acids Cys A:285, Leu A:330, Ala A:292, Ile A:326, Arg A:288, and His A:449.

Figure 1C depicts the steviol-PPAR- γ interactions, where conventional hydrogen bonds and alkyl bonds are prominent. In conventional hydrogen bonds, involving the amino acids Tyr A:327, Lys A:367, His A:449, and Ile A:326. Meanwhile, the alkyl bond involves the amino acids Cys A:285, Leu A:330, Ala A:292, Arg A:288, and Leu A:333.

Figure 1D shows steviolmonoside’s interactions with PPAR- γ , including conventional hydrogen bonds, carbon-hydrogen bonds, Pi-alkyl, and alkyl bonds. Conventional hydrogen bonds involve the amino acids Arg A:288 and Leu A:340. The carbon hydrogen bond involves the amino acid Phe A:282. The Pi-alkyl and Alkyl bonds involve the amino acids Cys A:285, Ala A: 292, Leu A:330, Ile A:326, Phe A:363, Met A:364, Lys A:367, and His A:449.

Figure 1E visualizes rosiglitazone’s interactions with PPAR- γ , displaying a range of interactions including van der Waals forces (the amino acid residue Gln A: 286), conventional hydrogen bonds (Cys A:285, His A:323, His A:449), Pi-sulfur, Pi-cation, Pi-Pi T-shaped, Pi-Pi stacked, amide-Pi stacked (Tyr A:327,

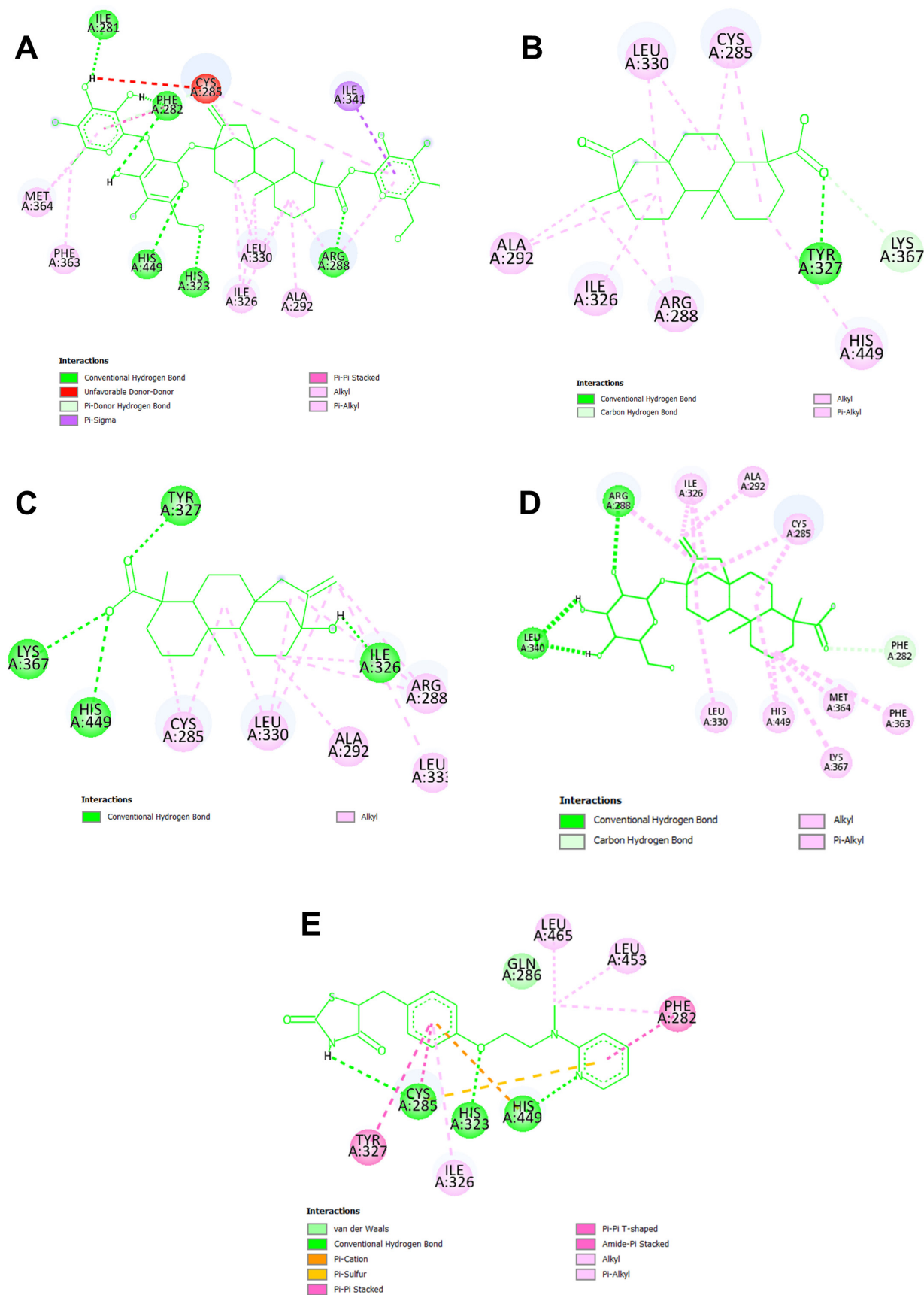


Figure 1. Two-Dimension visualization results of the interaction of compounds to protein. (A) dolcuside A, (B) isosteviol, (C) steviol, (D) steviolmonoside, (E) rosiglitazone on the PPAR-γ

Phe A:282), Pi-alkyl, and alkyl bonds (Leu A:465, Leu A:453, Ile A:326).

Among the five compounds tested, isosteviol demonstrated the highest number of residue interactions, involving eight residues with at least one hydrogen bond. This extensive interaction profile suggests a high binding affinity between isosteviol and the PPAR- γ .

Discussion

The study aimed to evaluate the potential of various compounds found in stevia leaves as PPAR- γ agonists, using molecular docking as the primary investigative method. The binding affinities of these compounds were compared to the native ligand J35 and the known PPAR- γ agonist, rosiglitazone.

The RMSD value obtained from the docking of J35, the native ligand, was 1.35 Å, which is well within the acceptable range (≤ 2 Å) for docking validation. This indicates that the docking protocol used in this study was reliable and could accurately simulate the interactions between PPAR- γ and the test compounds. The binding energy of J35 was found to be -9.01 kcal/mol, serving as a reference for comparing the affinities of other ligands. PPAR- γ is a group of nuclear receptor proteins in metabolism. PPAR- γ is a regulator of lipid and glucose metabolism that can be used to improve insulin and glucose parameters and increase whole body insulin sensitivity [10].

Among the stevia-derived compounds, isosteviol demonstrated the strongest binding affinity to PPAR- γ with a binding energy of -8.89 kcal/mol, closely approaching that of the native ligand J35. This value is also more negative than rosiglitazone (with binding energy -8.26 kcal/mol). This suggests that isosteviol has a significant potential to interact effectively with the PPAR- γ , making it a promising candidate for further investigation as an anti-diabetic agent. Isosteviol is a derivative of stevioside, which can inhibit the formation of reactive oxygen species (ROS) [11].

Steviol also showed strong binding affinities, with values of -8.29 kcal/mol. This result indicates that steviol has a comparable interaction strength with PPAR- γ to that of rosiglitazone, an agonist of PPAR- γ . Steviolmonoside and dolcicide A exhibited weaker binding energies of -7.82 kcal/mol and -6.48 kcal/mol, respectively, suggesting lower but still potentially relevant interactions.

The 2D visualizations revealed specific interactions between the ligands and the PPAR- γ , further elucidating the molecular basis of their binding affinities. Isosteviol showed the most extensive interactions, forming conventional hydrogen bonds, carbon hydrogen bonds, and Pi-alkyl bonds with key amino acids such as Tyr A:327 and Lys A:367. These interactions likely contribute to its strong binding affinity, as more interacting residues often lead to a more stable and robust ligand-receptor complex. In comparison, rosiglitazone formed a variety of interactions, including van der Waals forces, Pi-Sulfur, Pi-Cation, and multiple Pi-Pi interactions, which are critical for its binding efficacy.

Steviol and steviolmonoside formed fewer interactions but still demonstrated conventional hydrogen bonds and alkyl bonds that contribute to their moderate binding affinities. Dolcicide A, with the weakest binding energy, showed fewer and less significant interactions, which may account for its lower affinity.

The findings of this study suggest that isosteviol, with its strong binding affinity and extensive interactions with PPAR- γ , holds potential as a lead compound for developing new anti-diabetic therapies. The comparable binding affinity of isosteviol and steviol to rosiglitazone indicates that it may also be a viable candidate, particularly given its natural origin, which could offer a favorable safety profile.

While the docking study provides valuable insights, it is important to acknowledge that molecular docking alone cannot fully predict the pharmacological efficacy of a compound. In vitro and in vivo studies are necessary to validate the anti-diabetic potential of these compounds, particularly to assess their effects on glucose and lipid metabolism and their overall safety profile. Future research should also explore the bioavailability, metabolism, and potential side effects of these compounds in biological systems. Additionally, structure-activity relationship (SAR) studies could be conducted to optimize these compounds for better efficacy and selectivity towards PPAR- γ .

Conclusion

This study successfully identified isosteviol as a promising candidate for anti-diabetic drug development due to its strong binding affinity to PPAR- γ . Steviol

and steviolmonoside also show potential, though further research is necessary to fully understand their therapeutic potential. These findings contribute to the growing body of research exploring natural compounds as viable alternatives or complements to existing anti-diabetic therapies.

Acknowledgment

None.

Funding

The research we conducted did not receive funding from any party.

Declaration of interest

The authors declare no conflict of interest.

Author contributions

AHS, WNA conceptualized the study design, ASP, EAPa, EAPi, IW, MAS, NNS, PANH investigated the data, ASP, EAPa, EAPi wrote original draft, AHS, WNA, MAS, NNS, PANH reviewed and edited final version, AHS and WNA supervised all experiments. All authors have read the final manuscript.

Received: June 24, 2024

Revised: September 1, 2024

Accepted: September 2, 2024

Published online: September 3, 2024

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