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RESEARCH ARTICLE

Determination of Potential Compounds of Stevia Leaves (*Stevia rebaudiana* Bertoni) Against DPP4 as Candidates for Antidiabetic Drugs

Effan Cahyati Junaedi[1, 2], Sandra Megantara[1], Resmi Mustarichie[1*]

1. *Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.*
2. *Department of Pharmaceutical Chemical Analysis, Faculty of Mathematics and Natural Science, Universitas Garut, Indonesia.*

*** Corresponding Author: Resmi Mustarichie**

Abstract

Diabetes mellitus is a metabolic disorder due to abnormalities in insulin secretion, insulin action, or both. Around 200 million people worldwide suffer from diabetes, most of which is type 2 diabetes. One of the targets of diabetes treatment is the DPP-4 enzyme which works to degrade incretin from the body. Sitagliptin, a DPP-4 inhibitor that has been approved for the treatment of type 2 diabetes. Besides using synthetic drugs, a biochemical compound can also be used for diabetes therapy, one of which is stevia leaf. This research was conducted to select compounds contained in stevia leaves based on their interaction with DPP4 in silico. The purpose of this study was to determine potential compounds from stevia leaves to be a candidate for antidiabetic drugs. Tests were carried out on DPP4 inhibitors, namely sitagliptin, and 10 compounds contained in stevia leaves. The results showed that there were three potential compounds namely isosteviol, steviol, and steviolmonoside. Testing the Lipinski's rule of five and pre-ADMET parameters implied that the three compounds had the potential to be used as candidates for antidiabetic drugs.

Keywords: *Stevia leaf, Stevia rebaudiana, Diabetes mellitus, DPP4, Molecular modeling.*

Introduction

Diabetes mellitus is a disease or group of metabolic disorders due to abnormalities in insulin secretion, insulin action, or both. This condition is characterized by high levels of blood sugar (hyperglycemia) with impaired carbohydrate, protein, and fat metabolism. The result is an increase in oxidative stress which triggers the development of other diseases [1]. WHO estimates that around 200 million people worldwide suffer from Diabetes and this number is likely to double by 2030 [2].

Around 90-95% of patients suffer from type 2 diabetes mellitus [3]. According to RISKESDAS data (National Basic Health Research) in 2018 the prevalence of diabetes mellitus at the age of 15 years in Indonesia increased from 6.9% in 2013 to 10.9% in 2018.

In addition to the examination of oral glucose tolerance, it is known that 30.8% of respondents experienced impaired fasting blood glucose and 26.3% experienced impaired glucose tolerance, which is stated as a prediabetic condition [4]. Efforts to control diabetes mellitus in patients can also be done in several ways, namely the regulation of diet, exercise, and the use of alternative herbs [5]. The use of herbs as an alternative has recently increased. Various natural products have been investigated for their effectiveness in treating health problems.

Therefore, it is necessary to study effective and potential bioactive compounds that can be used for treatment. One of them is an active compound for treating diabetes with specific molecular targets. One target of diabetes treatment is the DPP-4 enzyme.

Dipeptidyl peptidase-4 (DPP-4) is an adenosine deaminase binding protein that can inactivate various oligopeptides.

DPP-4 works to degrade incretin from the body, a hormone that regulates insulin secretion after meals to balance blood sugar levels. Thus, compounds that can suppress the work of DPP-4 can be an appropriate therapy for type 2 diabetes. DPP-4 inhibitors are the latest antidiabetic class [6].

Some compounds that are classified as DPP-4 inhibitors are sitagliptin, vildagliptin, and saxagliptin, alogliptin, linagliptin, gemigliptin, anagliptin, trelagliptin, omarigliptin, evogliptin, and gosogliptin [7]. Sitagliptin, a DPP-4 inhibitor that was approved for the treatment of type 2 diabetes in the United States in 2006. This drug can be used as monotherapy or in combination therapy with PPARg agonists. Therapy using sitagliptin results in an increase in ß cell function and a significant decrease in HbA1c [8].

Stevia rebaudiana Bertoni is a plant that is often used by industry as a natural sweetener instead of synthetic sweeteners. Besides having a sweet taste, stevia also has various benefits including antihyperglycemic, antihypertensive, anti-inflammatory, anti-tumor, anti-diarrhea, diuretic effect, and immunomodulatory [9].This study reports the determination of compound targets by molecular modeling as an initial screening for finding compounds from stevia leaves that have the potential as antidiabetic. So there will be antidiabetic drug candidates who work as DPP4 inhibitors for the next stage of testing.

Materials and Method

Materials

3D complex DPP4 protein-Novel Heterocyclic DPP4 inhibitor with code 4A5S with RMSD 1.62Å downloaded from Protein Data Bank (www.rscb.org) in PDB format. 3D structure of test ligands (glucoside A, isosteviol, rebaudioside A, B, C, E, steviol, steviolmonoside, steviolbioside, stevioside) and comparative ligands (sitagliptin) were obtained from PubChem database with SDF format. This research was conducted using the Autodock Tools 4.0.1 program, Discovery Studio Visualizer 3.5, ChemDraw Ultra 12.

Method

Ligand and receptor complexes were separated using the Discovery Studio program and then stored in PDB format. Energy minimization of the comparative ligands and test ligands was carried out using the Chemdraw Ultra 12 program. The ligands and receptors were then prepared with the Autodock Tools 4.0.1 program by adding hydrogen atoms and the charge then stored in the pdbqt format.

Validation was then performed with native ligand redocking of DPP4 receptors with the Autodock Tools 4.0.1 program and the resulting grid parameters were used for the docking of comparative ligands and test ligands. Docking was performed on 1 comparison ligand and 10 test ligands with GA running 100 times. The resulting docking output was in the form of free bond energy (ΔG) and the value of the coefficient of inhibition (Ki). Pharmacokinetics, toxicity and Lipinski's rule of five tests were conducted online using the sites preadmet.bmdrc.kr and scfbio-iitd.res.in/software/drugdesign/lipinski.jsp.

Result and Discussion

The research aimed to obtain potential compounds that could be used as drug candidates from *S. rebaudiana* leaves by predicting their affinity and activity for DPP4 receptors. The DPP4 receptor downloaded, had 2 chains namely A and B. In this study, the selected DPP4 receptor part was chain B (Fig. 1).

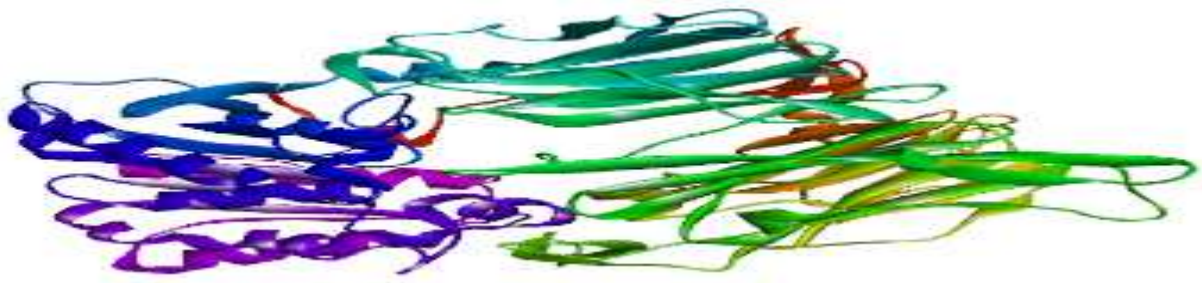


Fig. 1: 3D structure of DPP4 protein chain B with code 4A5S [10]

Validation was performed on the active site of the native ligand on crystallographic results [11]. The validation process was performed on the grid box coordinate settings x = 25.541; y = 65.708; z = 81.931; 40; with volumes x, y, z = 40 Å. The results of the validation by the redocking method showed an RMSD value of 0.463 Å (<2 Å) which indicated that the position of the atoms in the ligand from the redocking result was almost the same as the position in the crystallographic ligand (see Fig.2 and Table 1), so the 4A5S receptor could be used for the docking process.

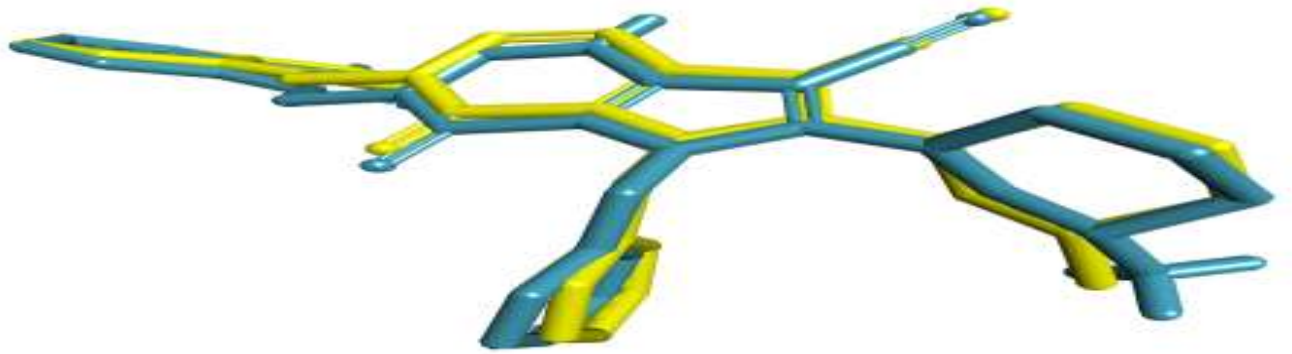
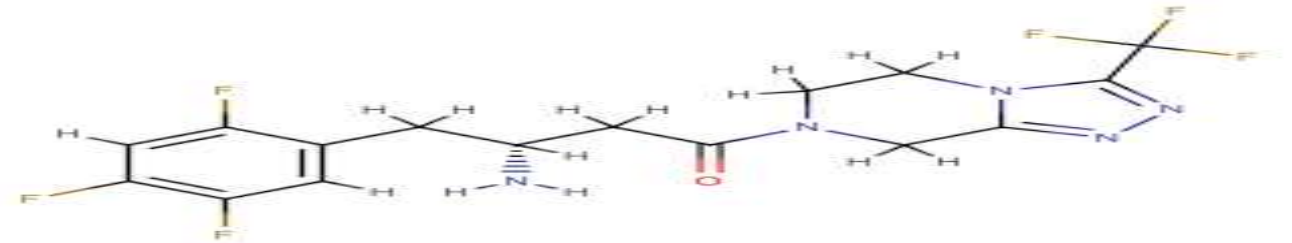


Fig. 2: Position of the ligand from crystallographic results with redocking results (yellow = crystallographic results and blue = redocking results)

Table 1: Results of the validation of native ligands with the DPP4 enzyme

Compounds	Amino Acid Residue Interactions		RMSD (Å)	ΔG (Kcal/mol)	KI (pM)
	Hydrogen Bonding	Van Der Waals Bonds (Hydrophobic)			
Native Ligan	Tyr 662, Tyr 631	Arg 669, Arg 125, Trp 659, Val 711, Val 656, Val 546, His 740, Gly 628, Gly 632, Asp 545, Asn 710	0,463	-14.69	17.16

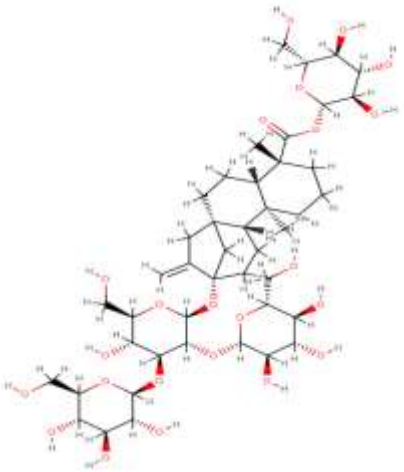
From the results it was seen that all amino acid residues were reconnected either by hydrogen bonding mechanisms or hydrophobic interactions between the ligands and receptors. RMSD value of less than 2 Å indicates the redocking was valid. The bond strength between native ligands and receptors is very strong was indicated by the small ΔG and Ki values. Geometry optimizations and comparison ligands were tested using Chemdraw ultra 12. The preparation was carried out using Autodock Tools 4.0.1 before in silico testing. Fig. 3 shows the structure of comparative ligands and test ligands.



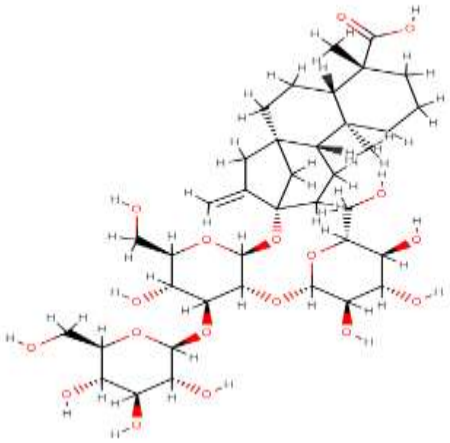
Sitagliptin

Dulcoside A

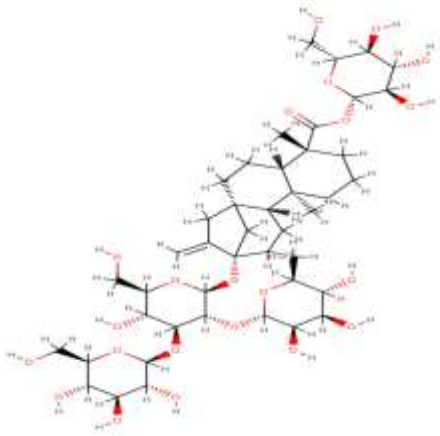
Isosteviol



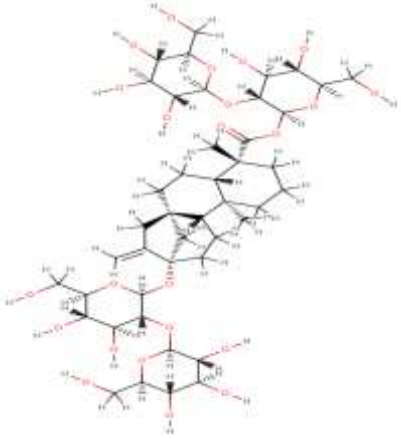
Rebaudioside A



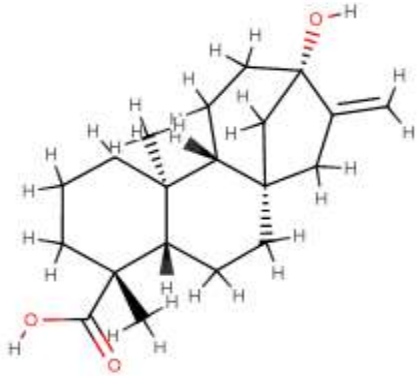
Rebaudioside B



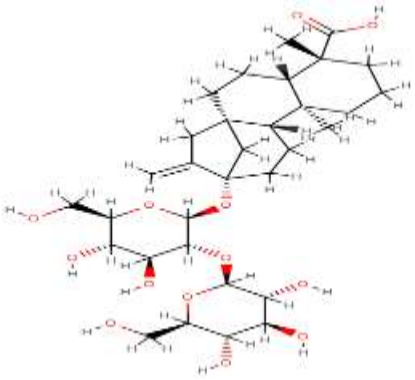
Rebaudioside C



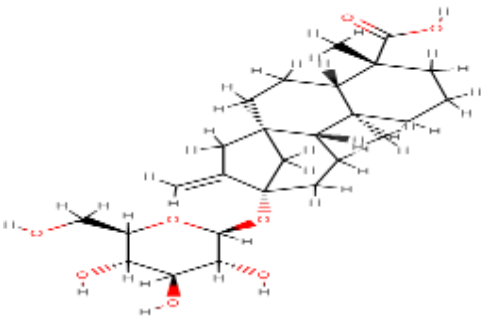
Rebaudioside E



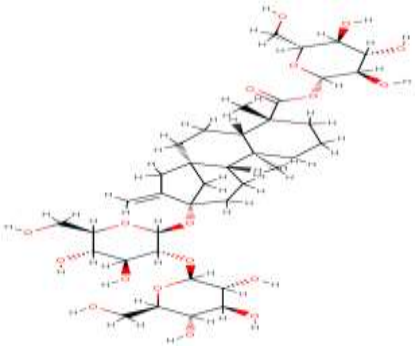
Steviol



Steviolbioside



Steviolmonoside



Stevioside

Fig. 3: Structure of comparative ligands and test ligands [12]

Then the docking of the comparative ligand and the test compound was carried out, belaying was done 100 times in one run. The result was chosen as a conformation that had the most cluster members to see several parameters including free energy, inhibition constants, and amino acids that interact and form bonds. The lowest free bond energy (ΔG) showed the strongest interaction with the receptor. The inhibition constant (KI) was a measure of the strength of the ligand in binding to the enzyme. Ligands with smaller KI values indicated greater binding affinity to inhibit the activity of an enzyme [13]. The bonds formed between DPP4 and each ligand are shown in Table 2.

Table 2: Results of tethering comparative ligands and test compounds with the DPP4 enzyme

Compound	Amino Acid Residue Interactions		ΔG (Kcal/mol)	KI (μM)
	Hydrogen Bonding	Van Der Waals Bonds (Hydrophobic)		
Sitagliptin	Tyr 547, lys 554, trp 629, val 546, Arg 125, Asn 710	Trp 627, Trp 659, Gly 628, Gly 632, Asp 545, Ser 630, Val 711, Val 656	-7.49	3.25
Dulcoside-A	Trp 629, Val 546, Gly 632, Arg 125, Tyr 547	Gly 633, Gly 628, Gly 741, Asp 545, Lys 554, Tyr 631, Tyr 662, Tyr 666, Asn 710, Glu 205, His 740	-5.29	133.26
Isosteviol	Lys 554, Arg 125	Gly 628, Gly 632, Ser 630, Tyr 631, Val 546	-8.44	0.652
Rebaudioside A	Lys 554, Trp 629, Tyr 662, Glu 205, Arg 125	Gly 628, Gly 633, Gly 632, Gly 741, Val 656, Val 711, Glu 206, His 740, Arg 669, Ser 630, Trp 659	-4.15	901.85
Rebaudioside B	Trp 629, Val 546, Arg 125, Ser 630, Asn 710	Gly 632, Gly 628, Lys 554, Asp 545, Tyr 752, Tyr 631, Glu 206, Glu 205, Val 711	-4.52	483.19
Rebaudioside C	Val 546, Lys 554, Ser 630, Trp 629, Arg 125, Tyr 547, Tyr 662, Glu 205	Gly 628, Gly 632, Gly 633, Ser 552, Arg 669, Glu 206, Tyr 666	-5.10	182.61
Rebaudioside E	Trp 629, Tyr 547, Tyr 662, Glu 205, Arg 125	Val 546, Gly 633, Gly 628, Trp 627, Trp 659, Ser 552, Phe 957, Asn 710, Tyr 666, Tyr 631	-4.02	1130
Steviol	Arg 125	Ser 630, Tyr 631, Gly 632, Gly 628, Lys 554, Val 546	-8.10	1.16
Steviolbioside	Tyr 662, Tyr 547, Glu 205, Lys 554	Glu 205, Arg 669, Glu 125, His 740, Ser 630, Glu 628, Glu 632, Val 546, Tyr 631, Tyr 666, Phe 357	-7.33	4.27
Steviolmonoside	Arg 125, Tyr 547, Arg 669, Glu 206	Phe 357, Asn 710, Gly 632, Glu 205, Ser 630, Tyr 631	-7.60	2.71
Stevioside	Lys 554, Trp 554, Tyr 662, Asn 710	Val 546, Val 656, Val 711, Gly 628, Gly 632, Gly 633, Arg 125, Glu 205, Trp 659	-7.02	7.10

Crystal 4-hidroksitamoksifen

The docking results showed that there were 6 hydrogen bonds and 8 hydrophobic interactions between the DPP4 enzyme and the comparative ligand/sitagliptin (Fig. 4). Isosteviol, steviol, and steviolmonoside compounds produce free bond energy and inhibition constants that were smaller than sitagliptin. As well as binding to several amino acid residues that were the same as sitagliptin (Fig.4,5,6,7). This implied that isosteviol, steviol, and steviolmonoside were potential candidates for antidiabetic drugs.

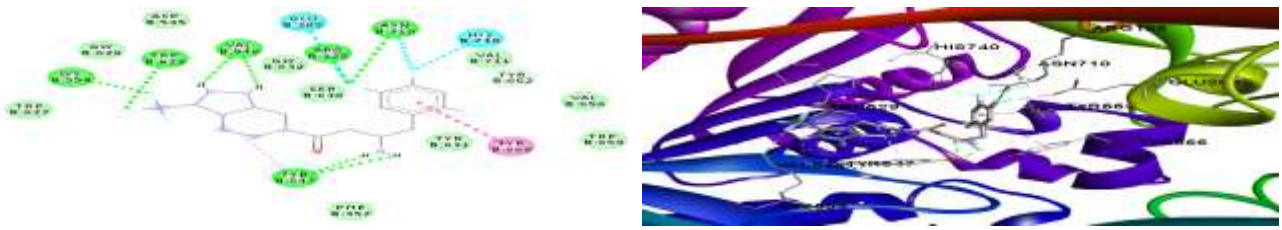


Fig. 4: Visualization of sitagliptin 2D (a) and 3D (b) docking results on the DPP4 enzyme

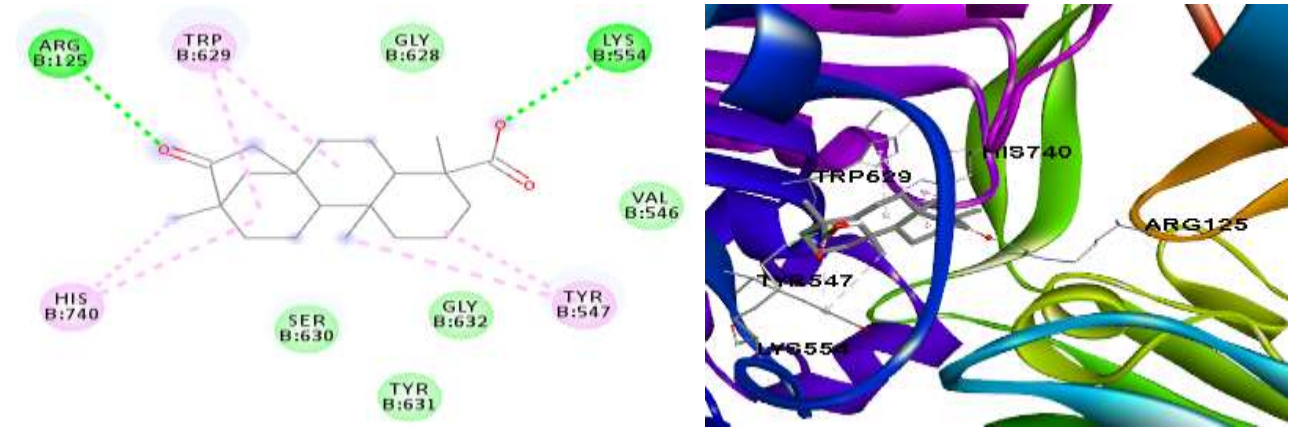


Fig. 5: Visualization of isosteviol 2D (a) and 3D (b) docking results on the DPP4 enzyme

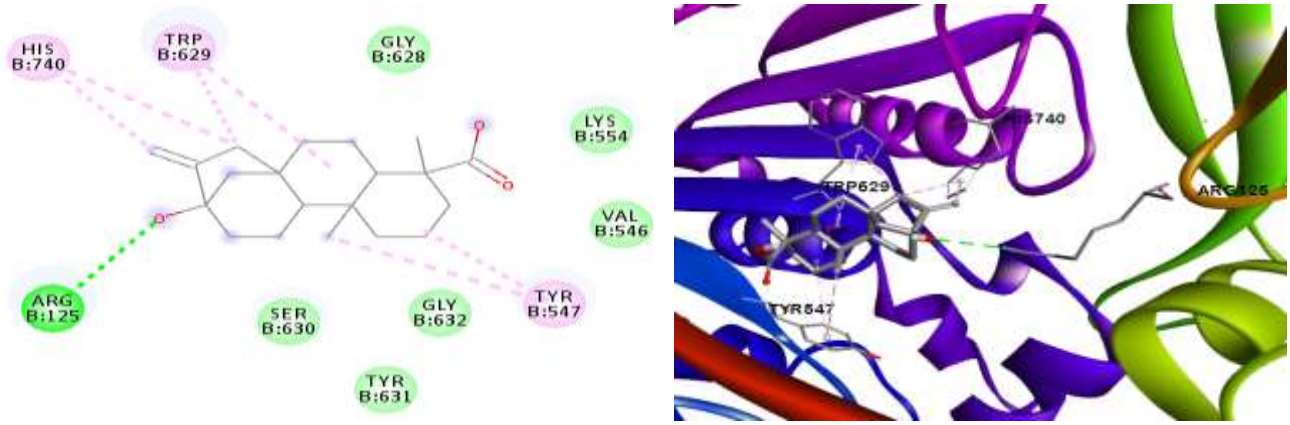


Fig. 6: Visualization of steviol 2D (a) and 3D (b) docking results on the DPP4 enzyme

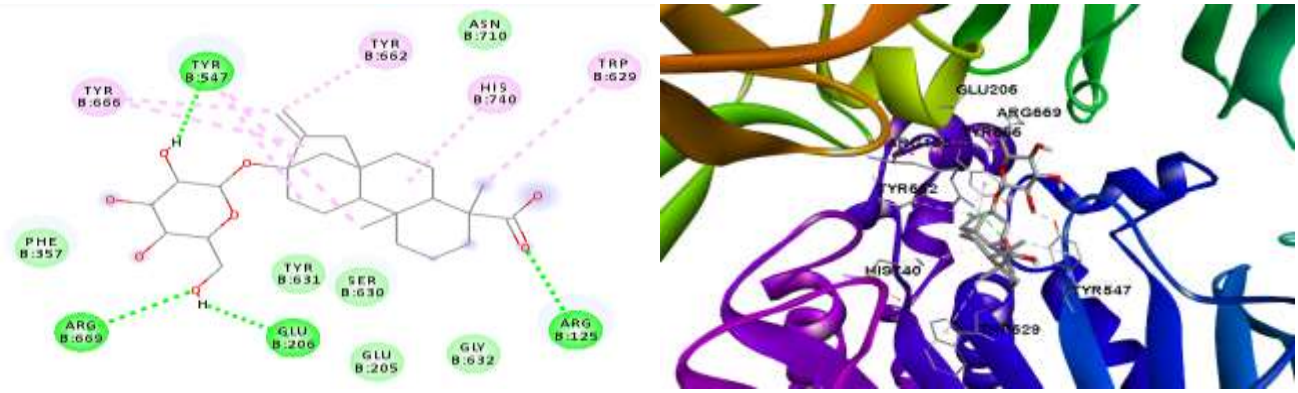


Fig. 7: Visualization of steviolmonoside 2D (a) and 3D (b) docking results on the DPP4 enzyme

Testing of pharmacokinetic parameters and toxicity was carried out through the pre-ADMET site [14-18]. The results of the sitagliptin, isosteviol, steviol, and steviolmonoside compounds are presented in Table 3.

Table 3: Pre-ADMET Test Results

Compound	Absorption		Distribution	Toxicity	
	Caco2 (nm.Sec-1)	HIA (%)	PPB (%)	Carcinogenicity	Mutagenicity
Sitagliptin	21,6827	97,05249	54,32294	No	Yes
Isosteviol	19,7064	97,67445	100	No	No
Steviol	16,4437	95,24048	100	No	Yes
Steviolmonoside	14,6581	65,79098	80,44304	No	Yes

The results showed that the three selected compounds had medium permeability to Caco-2 cells. Isosteviol and steviol compounds can be absorbed either through the digestive tract, comparable to sitagliptin. While steviolmonoside was included in moderate absorption. Isosteviol and steviol had a strong bond with plasma protein (PPB)> 90%

strong bond while steviolmonoside was classified as weak [18, 19].

Table 4: Compound Testing Results Based on Lipinski's Rule of Five rules

Compound	BM	Log P	Hydrogen Bond	
			Acceptor	Donor
Sitagliptin	407	2,017	5	2
Isosteviol	480	1,979	8	5
Steviol	318	4,155	3	2
Steviolmonoside	318	4,443	3	1

Lipinski's rule of five was a rule for evaluating the use of compounds as oral preparations. Where the parameters that must be met include a molecular weight of fewer than 500 Daltons, hydrogen bond donors not more than 5, hydrogen bond acceptors not more than 10, and LogP values, not more than 5 [20]. Of the 3 compounds that had been selected, it was known that the three compounds meet the Lipinski's rule of five. So combine with the above data it can be stated that the three compounds can be used as drug candidates by oral use.

Conclusion

Based on the results of the study it could be concluded that the compounds contained in stevia leaves (*Stevia rebaudiana* Bertoni) in silico were able to interact with the DPP4 enzyme where compounds that had the potential to become candidates for antidiabetic drugs were isosteviol, steviol, and steviolmonoside. The compound bound to amino acid residues that also bound sitagliptin, namely Arginine (Arg 125). Isosteviol had noncarcinogenic and mutagenic characters while steviol and steviolmonoside had mutagenic characteristics such as comparative drugs.

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References

1. Abd El-Karim S S, Anwar M M, Syam Y M, Nael M A, Ali H F, Motaleb M A (2018) Rational design and synthesis of new tetralin-sulfonamide derivatives as potent anti-diabetic and DPP-4 inhibitors: 2D & 3D QSAR, in vivo radiolabeling and biodistribution studies, Bioorg Chem., 81: 481-93. doi: 10.1016/j.bioorg.2018.09.021.
2. Sharma P, Joshi T, Joshi T, Chandra S, Tamta S (2020) In silico screening of potential antidiabetic phytochemicals from Phyllanthus emblica against therapeutic targets of type 2 diabetes, J. Ethnopharmacol., 248: 112-268. doi: 10.1016/j.jep.2019.112268
3. Liu Q, Chen L, Hu L, Guo Y, Shen X (2010) Small molecules from natural sources, targeting signaling pathways in diabetes. Biochimica et Biophysica Acta - Gene Regulatory Mechanisms, 1799(10-12): 854-65. https://doi.org/10.1016/j.bbagrm.2010.06.004
4. Kemkes RI (2018) Hasil Utama Riskesdas. (cited 2020 May 15). Available from:http://www.kesmas.kemkes.go.id/assets/upload/dir_519d41d8cd98f00/files/Hasil-riskesdas-2018_1274.pdf
5. Sena CM, Bento CF, Pereira P, Seiça R (2010) Diabetes mellitus: new challenges and innovative therapies, EPMA J., 1(1): 138-63. doi: 10.1007/s13167-010-0010-9
6. Kerru N, Singh-Pillay A, Awolade P, Singh P (2018) Current anti-diabetic agents and their molecular targets: A review. Eur J. Med. Chem., 152: 436-488. doi: 10.1016/j.ejmech.2018.04.061.
7. Gallwitz B (2019) Clinical use of DPP-4 inhibitors. Front. Endocrinol., 10:Article 389. DOI: 10.3389/fendo.2019.00389
8. Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE (2006) Effect of the dipeptidyl peptidase-4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care, 29(12):2632-7. https://doi.org/10.2337/dc06-0703
9. Chatsudthipong V, Muanprasat C (2009) Steviosi+de and related compounds: therapeutic benefits beyond sweetness,

Pharmacol Ther., 121(1):41-54. doi: 10.1016/j.pharmthera.2008.09.007.

10. Ostermann N, Kroemer M, Zink F, Gerhartz B, Sutton JM, Clark DE, et.al (2012) 4A5S Crystal Structure of Human DPP4 in Complex with a Noval Heterocyclic DPP4 Inhibitor. DOI: 10.2210/pdb4A5S/pdb
11. Bissantz C, Folkers G, Rognan D (2000) Protein-Based Virtual Screening of Chemical Databases. 1. Evaluation of Different Docking / Scoring Combinations, J. Med. Chem., 43(25):4759-67. doi: 10.1021/jm001044l.
12. National Library for Medicine. Compound. In PubChem Database. (cited 2020 May 15). Available from: http://pubchem.ncbi.nlm.nih.gov/compound/Stevioside
13. Muchtaridi M, Yusuf M (2018) Teori dan Praktek Penambatan Molekul (Molecular Docking). Bandung: Unpad Press.
14. Madden JC (2010) In-silico approaches for predicting ADME properties in Recent Advances in QSAR Studies: Methods and Applications, edited by Tomasz Puzyn, Jerzy Leszczynski, Mark T. D. Cronin Springer, 283-301.
15. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, et.al (2010) Admet SAR: a comprehensive source and free tool for evaluating chemical ADMET properties. J. Chem. Inf. Model., 52(11):3099-105.
16. Cramer GM, Ford RA, Hall RL (1978) Estimation of Toxic Hazard - A Decision Tree Approach. Food and Cosmetics Toxicology, 16: 255-76.
17. Praceka MS, Megantara S, Mustarichie R (2020) Molecular Modeling of Anti-Alopecia Compounds Found in Sauropus Androgynus, Journal of Global Pharma Technology, 12(02): 1939-47.
18. Teotia P, Prakash Dw S, Dwivedi N (2018) In silico Molecular Docking and ADME/Tox Study on Benzoxazole Derivatives Against Inosine 5'-Monophosphate Dehydrogenase. Asian Journal of Biotechnology, 10(1):1-10. https://doi.org/10.3923/ajbkr.2018.1.10
19. Nursamsiar N, Surantaatmadja SI, Tjahjono DH (2015) Absorption, Distribution, and Toxicity Prediction of Curculigoside A and its Derivatives, 23rd International Conference on Computation for Science and Technology (ICCST-3) 32-35. https://doi.org/10.2991/iccst-15.2015.7
20. Tan HL, Chan KG, Pusparajah P, Lee LH, Goh BH (2016) Gynura procumbens: An overview of the biological activities. Frontiers in Pharmacology, 7(MAR). https://doi.org/10.3389/fphar.2016.00052

