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Prediction of toxicity and inhibition activities of a-glucosidase enzyme of the chemical compounds isolated from Indonesian medicinal plants using molegro virtual docking

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Abstract
Diabetes mellitus tipe 2 disebabkan oleh hormon insulin yang tidak mencukupi atau tidak efektif sehingga tidak dapat bekerja secara normal. Enzim a-glukosidase bekerja pada dinding usus halus, dimana enzim *a*-glukosidase akan memecah oligosakarida, atau polisakarida menjadi glukosa yang dapat diserap oleh usus halus sehingga terjadi peningkatan kadar glukosa dalam darah. Tumbuhan obat Indonesia diketahui banyak mengandung senyawa kimia aktif yang mampu menurunkan kadar glukosa dalam darah seperti daun teh (*Camellia sinensis*), daun yakon (*Smallanthus sonchifolius*), Daun stevia *(Stevia rebaudiana*) dan daun salam (*Syzigium polyanthum*). Penelitian ini bertujuan untuk memprediksi senyawa kimia dalam tumbuhan obat Indonesia yang berkhasiat sebagai antidiabetes secara *in silico* dengan menggunakan perangkat lunak Molegro Virtual Docking. Docking dilakukan terhadap 8 (delapan) senyawa kimia yang terdapat dalam tumbuhan obat Indonesia. Hasil pengujian menunjukkan bahwa senyawa theaflavin memberikan nilai reranke score (RS) paling rendah di reseptor □-glukosidase dengan nilai – 104,03 kkal/mol, sedangkan nilai RE akarbose – 74,51 kkal/mol dan miglitol – 73,32 kkal/mol.

Keywords: molecular docking, antidiabetes, enzim a-glukosidase, in silico, tumbuhan obat Indonesia

1. Introduction
Diabetes mellitus (DM) is defined as a disease or chronic metabolic disorder with multiple etiologies, characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid and protein metabolism as a result of insulin function insufficiency. Insufficiency of insulin function can be caused by disruption or deficiency of insulin production by beta Langerhans cells of the pancreas gland, or due to lack of responsiveness of the body's cells to insulin [1].

In the world, the number of diabetics is projected to increase from 171 million in 2000 to be 366 million cases in 2030. In Indonesia, the DM sufferers in 2006 were quite fantastic at 14 million people and the number has been estimated by World Health Organization (WHO) at 21.3 million people in 2030 [2].

Nowadays there are many antidiabetic chemicals found from natural materials. However, little is known about compounds that can inhibit the α-glucosidase enzyme selectively. Therefore, it is necessary to study the interaction modeling of several chemical structures specifically that are known to have antidiabetic activity through inhibition of the α-glucosidase enzyme. The method used to study the interaction of several natural substances which are known to inhibit the enzyme α-glucosidase by using computer applications in silico at the molecular level or known as molecular docking simulation method [3].

In silico test is done by docking molecules which will be predicted its activity with the selected target cell. Doking is an attempt to harmonize between ligands which are small molecules into target cells which are large protein molecules [4]. In silico test results in the form of bond energy values or Rerank Score (RS). Bond energy shows the amount of energy needed to form bonds between ligands and receptors. The smaller the bond energy, the more stable the bond is. The more stable the ligand's binding to the receptor, it can be predicted that its activity increases.

Some chemical compounds that will be tried in this molecular docking are two of the tea leaves (*Camellia sinensis*) namely theaflavin, and cathecin gallic [5, 6]; 3,5-dikafeoil acid quinic and chlorogenic acid from the leaves of yakon (*Smallanthus sonchifolius*) (*Smallanthus sonchifolius*) [7]; sterebin B, C; from stevia leaves (Stevia rebaudiana) (*Stevia rebaudiana*) [8]; and gallic acid and caffeic acid from bay leaves (*Syzigium polyanthum*) [9] The purpose of this study was to validate eight chemical compounds originating from Indonesian medicinal plants with the help of Molegro software Virtual Docker is expected to provide an overview of the interaction of active chemical compounds from plants with the enzyme α-glucosidase [10].

2. Experimental
2.1 Material
3 Dimension Structure of Macromolecules
The structure of protein receptor ligands consisting of the enzymes α-glucosidase and α-amylase taken from Protein Data Bank (PDB) with code 3L4W. The material used is a three-dimensional crystal structure of complex maltase-glucoamylase. The structure of the protein used is made in the condition "Preparation always" which is implemented in Molegro Virtual Docker.

3D structure of ligand molecule
The comparative ligands used are two-dimensional and three-dimensional structures consisting of Acarbose (CID

41774) and Miglitol (CID 441314) which are downloaded from Pubchem NCBI in the form of Data Structure Format (SDF). The docking compound is an active compound contained in *Camelia sinensis*, *Smallanthus sonchifolius*, *Stevia rebaudiana* and *Syzigium polyanthum* which are thought to have antidiabetic activities [5, 9, 8, 7, 6].

2.2 Instrumentation
Asus Notebook (KCU7PBFB) E203M with Intel (R) Celeron (R) N4000 @ 1.10 GHz specifications, 4GB (3.83 gigabyte usable) RAM (Random 3.83 gigabyte usable) Notebook Operating System connected with AC / DC adapter and connected to the internet.

Software
The program used is Molegro Virtual Docker 5.0, Chem Office Professional 17 and PDB

Preparation of Macromolecular Structures
The macromolecular structure of a receptor ligand from a protein consisting of the enzymes α-glucosidase and α-amylase obtained from the Protein Data Bank (PDB) with the site http://www.rcsb.org/pdb/. The macromolecular identity used is 3L4W which is downloaded in * .pdb format. Protein macromolecules are separated from solvents and ligands, which are non-polar residues. Separation of macromolecules from molecules that are not needed is done using the Biovia Discovery studio 2020 program. The results of the separation are used for molecular docking. Separation results are saved in * pdb format. The material used is a three-dimensional maltase-glucoamylase complex crystal structure. The structure of the protein used is made in the condition "Preparation always" which is implemented in virtual Molegro.

Ligand Structure Preparation
Three-dimensional structure preparation was carried out on eight [8] chemical structures isolated from Indonesian medicinal plants (*Camellia sinensis*, *Smallanthus sonchifolius*, *Stevia rebaudiana* and *Syzigium polyanthum*). The active compound was made with the Chem 3D 17.0 program and the structure obtained is still in an unstable state, so energy minimization is done by clicking MM2 to be the most stable form is obtained. Then the file is saved in MDLMolFile.

Validation of Molecular Docking Method
Molecular docking validation is done to determine whether the protein used for molecular docking can be used or not. Validation is done by determining the value of RMSD (Root Mean Square Deviation). The RMSD evaluation is done by determining the RMSD value based on the range: RMSD ≤ 1Å for good or similar conformation, 1 Å <RMSD ≤ 2 Å for close to true conformation, 2 Å <RMSD ≤ 3 Å for conformation with errors and RMSD> 3 Å for poor conformation.

Molecular Docking
Molecular docking is carried out on 3L4W as a protein target by using the Molegro Virtual Docker (MVD) program to study the interaction of compounds with α-glucosidase. MVD is used because it has a higher docking accuracy compared to other docking products (MVD: 87%, Glide: 82%, Surflex: 75%, and FlexX 58%). The first step taken in the docking process is downloading ligands with receptors/proteins on RCSB PDB. The second step is opened by Molegro application, importing selected receptor molecules (uncheck water and cofactor) then selecting one of the comparative ligands both internal and external, and detecting the position of the receptor (where the drug will interact with the cavities of the ligand-receptor with how to click "toolbar preparation" and "detect cavities" and select one of the cavities where ligands and receptors are bound. Cavity volume is set with a minimum range of 10 Å and a maximum of 1000 Å. Detect cavities used are simple grid-based which depend on the surface of the molecule and or Van der Waals radius. The third step of the test compound molecules is imported using "preparation protein" and a docking process is carried out with the cavities that have been selected and seen interactions between bonds and selected interactions of the ligand receptor bonds with the lowest value. The fourth step is the detection of the chromophore group and determining the 3 points (compound atoms) where the drug will bind to the receptor, then the alignment process is carried out between the comparative ligand and the test ligand, so that the test ligand whose interaction is known cannot move dynamically, because when the ligand will enter the cavities will move dynamically and will affect the docking process. The docking process is repeated and checks the interaction between bonds. The parameter measured in the docking process is the energy value seen in the form of a Rerankee Score (RS). The smaller the Rerankee Score, the binding energy between the ligand and the receptor is more stable and that means the activity gets better. Then the Reranke Score is compared between the test ligand and the comparative ligand. If the Reranke Score is equal or lower, then the test ligand has a corresponding activity or is higher than the comparative ligand.

Toxicity Test
The toxicity tests were performed using "Protox II" software with parameters including predictive levels of similarity, accuracy, LD_{50} values and prediction of toxicity classes. This prediction is done by inputting the ligand in * .sdf format. then the "tox prediction" menu is selected to see the criteria for the level of hepatotoxicity, carcinogenicity, mutagenicity, immunotoxicity and cytotoxicity of the target drug compound.

3. Results and Discussion
3.1 Docking Validation Results
RMSD validation results showed that the acarbose protein (CID 41774) has an RMSD value ranging from 6-8 Å, protein miglitol (CID 441314) has an RMSD value ranging from 0.9-1 Å. RMSD values can be obtained from the results of validation, and this shows that RMSD values of more than 3 mm have conformations with poor categories. If the docking process uses acarbose, it will get invalid results (positive Reranke Score). Therefore, miglitol ligands are used as native ligands which have same mechanism with acarbose as an α-glucosidase inhibitor.

Table 1: RMSD values from the validation results

RMSD (Angstrom)	MolDock Optimizer	MolDock SE	Iterated Simplex	GPU Screening (CUDA)
MolDock Score	1.14	1.01	1.15	3.22
MolDock (Grid) Score	0.51	1.13	0.72	0.70
PLANTS Score	0.59	1.10	0.53	0.65
PLANTS (Grid) Score	0.68	0.73	0.66	1.86

Docking validation is done by reassembling the original ligand 3L4W enzyme to its active site. The accepted criteria are set with a Root Mean Square Deviation (RMSD) value below 2.0 Å. The validation method can be seen from the RMSD value obtained from the reference ligand on the receptor. RMSD value of 0.51 indicates that (RMSD <2). MolDock (Grid) (scoring function) and MolDock Optimizer (algorithm) scores indicate that this method has a high validity value.

3.2 Preparation Results of Macromolecular and Ligand Structure

Macromolecular structure obtained from Protein Data Bank (PDB) is a crystal structure of a complex protein consisting of α-glucosidase and α-amylase enzymes from human maltase obtained from X-ray crystallography method with a resolution of 2 Å. The macromolecular identity chosen is miglitol with 3L4W code which can be seen in Figure 1

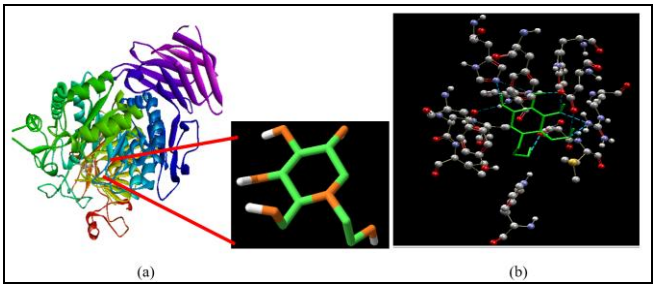


Fig 1: Visualization of 3D crystal structure of glucoamylase maltase with miglitol (a) conformation of the miglitol ligand view (b)

Macromolecules that are downloaded are bound to ligands and water molecules, so ligands and water molecules must be removed so that they do not interfere in the molecular docking process. The interaction of amino acids with miglytol can be seen in Figure 2.

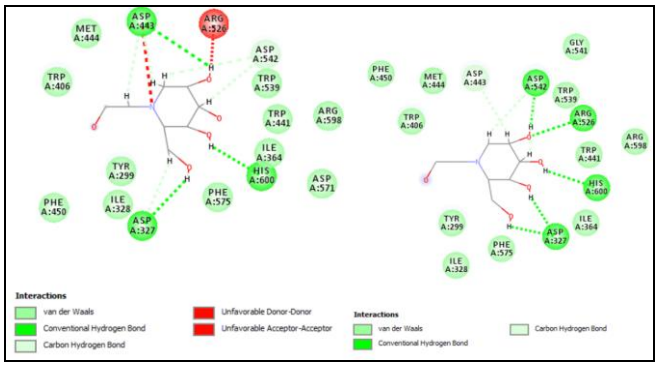


Fig 2: Interactions of amino acids with miglytol (native ligands) with 3L4W (a) and interactions of amino acids with miglytols resulting from docking validation (b)

The structure of the active compounds from Camelia sinensis, Smallanthus sonchifolius, Stevia rebaudiana and Syzigium polyanthum used is the result of library research. The structure used in this study is a 3D structure downloaded from the Pubchem website in * .sdf format. ligands that have been downloaded; the format is changed to * pdb.

3.3 The Result of Toxicity Test

Ligand toxicity prediction aims to determine the character of the toxicology of each ligand, to predict the adverse effects caused if used in medicine. Toxicity prediction based on Benigni / Bossa rules is based on the presence of hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity which can be known through "protox II" by knowing based on similarity, accuracy, LD_{50} and its toxicity level. Table 1 shows the results of the prediction of the toxicity of 8 compounds derived from *Camelia sinensis, Smallanthus sonchifolius, Stevia rebaudiana* and *Syzigium polyanthum*.

Table 2: Toxicity prediction results for the chemical compounds of Indonesian medicinal plants

No	Compounds	Sources	Similarity (%)	Accuracy (%)	LD_{50} (mg/kg)	Prediction Toxicity class
1	Theaflavin	*Camellia sinensis*	79,38	69,26	2500	5
2	Cathecin gallate	*Camellia sinensis*	100	100	1000	4
3	Gallic Acid	*Syzigium polyanthum*	84,82	70,97	2000	4
4	Cafeic acid	*Syzigium polyanthum*	88,59	68,09	2980	5
5	Sterebin B	*Stevia rebaudiana*	66,66	68,07	37000	6
6	Sterebin C	*Stevia rebaudiana*	68,99	68,07	4800	5
7	3,5-Dicaffeoylquinic acid	*Smalanthus sonchifolius*	71,63	69,26	5000	5
8	Chlorogenic acid	*Smalanthus sonchifolius*	71,21	69,26	5000	5

The results of the toxicity tests on 8 chemical structures from Indonesian medicinal plants (Table 2) show that chemical compounds isolated from plants have efficacy as antidiabetic at this dose have mild acute toxicity levels (500-5000) and sterebin B has no harmful toxicity [11]

3.4 Molecular Docking Result

Molecular docking using the Molegro Virtual Docker can improve the accuracy and prediction of bond models. From the docking results we can get the value of free energy gibbs (ΔG) Gibbs free energy is the energy needed for ligands to interact with the receptor, the smaller the price of Gibbs free

energy (ΔG), the more stable the ligand bonds with the receptor. The docking result shows that the Gibbs free energy value of all test ligands is negative, this shows that the reaction that occurs is a spontaneous reaction. From the results of docking, the active compound which has the smallest Gibbs free energy value of each plant can be seen in Table 2. The value of Gibbs energy (ΔG) is used to determine the value of the inhibition constants, the smaller the value of inhibition constants, the the concentration of inhibitors is smaller to inhibit substrate [12].

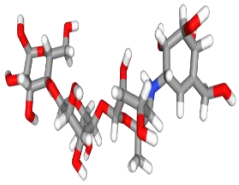
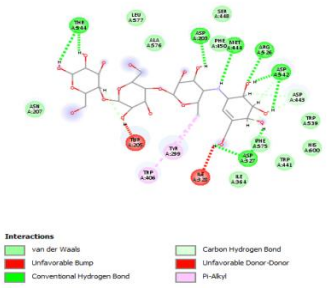
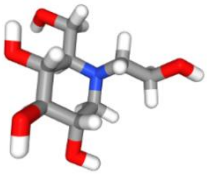
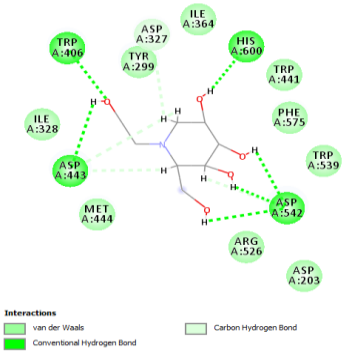
Table 3: Molecular docking result on 3L4W

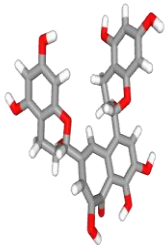
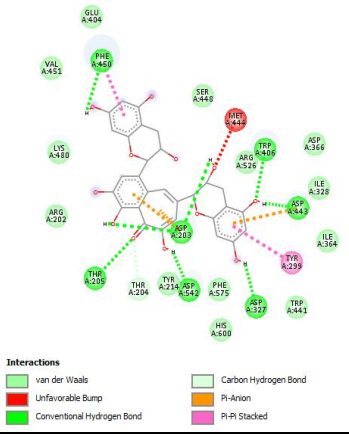
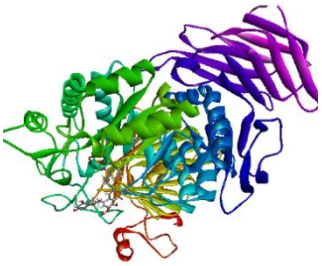
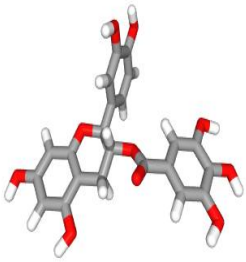
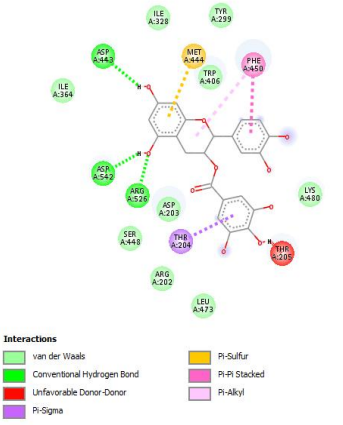
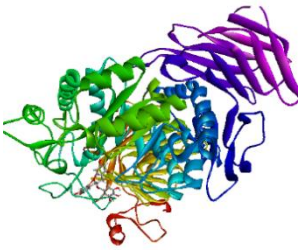
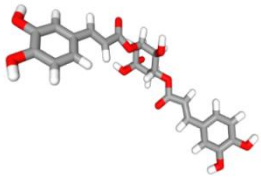
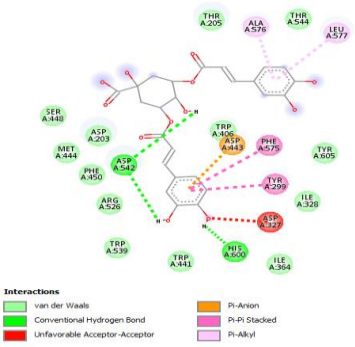
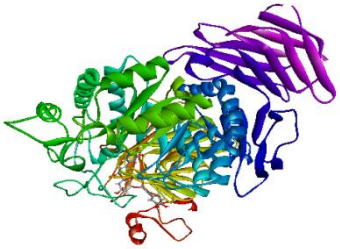
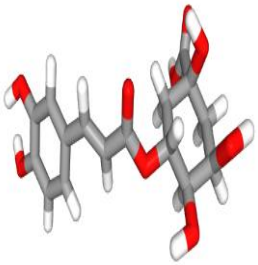
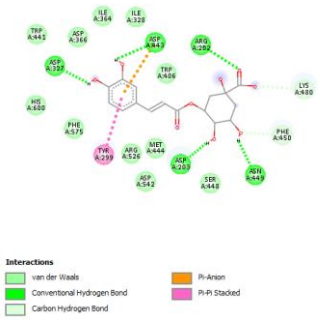
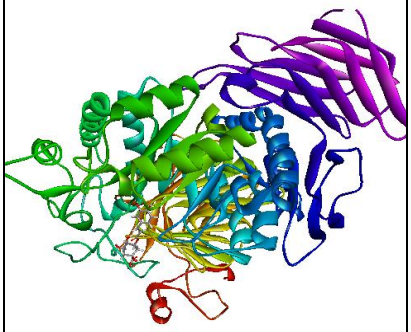
No.	Compounds	Sources	Reranke Score Kcal/mol
1	Acarbose	Chemical synthesis	-74.5088
2	Miglitol_	Chemical synthesis	-73.3232
3	Theaflavin	*Camellia sinensis*	-104.033
4	Catechin galat	*Camellia sinensis*	-93.3237
5	Gallic acid	*Syzigium polyanthum*	-71.3957
6	Caffeic acid	*Syzigium polyanthum*	-66.4984
7	Sterebin B	*Stevia rebaudiana*	-66.0308
8	Sterebin C	*Stevia rebaudiana*	-65.5351
9	3,5-Dicaffeoylquinic acid	*Smalanthus sonchifolius*	-91.3517
10	Chlorogenic acid	*Smalanthus sonchifolius*	-88.1028

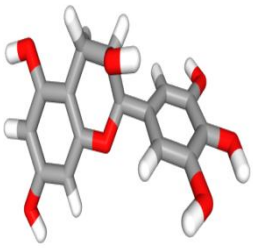
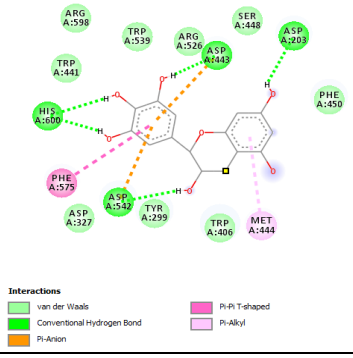
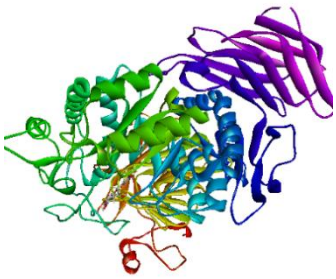
Table 3 shows that chemical compounds isolated from medicinal plants, have a score that is not much different, even better than comparative compounds used in the activity of inhibiting the enzyme α-glucosidase.

Comparative compounds used are commercially available inhibitors such as acarbose and miglitol which have several advantages and disadvantages. Acarbose can inhibit α-glucosidase and to a lesser extent, α-amylase but is reported with gastrointestinal disorders [13]. Miglitol inhibits α-glucosidase exclusively whereas the previous molecule is systematically absorbed [14] and more importantly in the side effect profile compared to acarbose and miglitol [3]. However, miglitol is not metabolized and excreted rapidly by the kidneys [14]. On the other hand, α-amylase inhibitors are expected to be a better PPHG suppressor because it will inhibit maltose accumulation thereby preventing side effects such as abdominal pain, flatulence and diarrhea [15]. Compounds derived from nature, are expected to be safer and have smaller side effects

Table 4: Interaction of receptors with ligands and hydrogen bonds resulting from docking

No.	Compound	Interaction of receptors with ligands in 2D	Hydrogen Bound	Number of interactions
1	**Acarbose** **-74.5088** 			Arg526, **Asp542,** Thr544, Asp203, Met444, A27
2	**Miglitol_nativeligand** **-73.3232** 			Asp443, Trp Sp3406, His600, **Asp542**

3	Theaflavin -104.033 			Phe450, Thr205, **Asp203, Asp542,** Asp327, **Asp443,** Trp406
4	Catechin gallate -93.3237 			**Asp443, Asp542, Arg526**
5	3,5-Dicaffeoylquinic acid -91.3517 			**Asp443, Asp542, Arg526**
6	Chlorogenic acid -88.1028 			**Asp327, Asp443, Asp203,** Asn449, Arg202

7	Epigallocatechin -81.5424 			**His600, Asp542, Asp203, Asp443**

Based on the results of molecular docking, it appears that there are several compounds have large molecular weights higher than 500 such as sterebin B and C, so that during the docking process, no poses appear, even the docking process stops. This is in line with the Lipinski (RO5) rule that is, if a drug compound is to be optimally absorbed by the body, the compound must, among other things, have a molecular weight of less than 500 [16]. If the predicted drug compound does not meet one of Lipinski's rules, then the drug is not effectively used orally and is recommended to be used by injection [17]. Dicaffeoylquinic acid, catechin gallate, chlorogenic acid, epigallocatechin and theaflavin have lower rerankee scores than miglitol and acarbose. This shows that chemical compounds derived from plants have the potential to be candidates for new drugs and have better pharmacological activity compared to positive control of acarbose or miglitol. This prediction can be used as a basis for further research in enzymatic and other testing in vitro.

4. Conclusion

From the docking results, it can be concluded that the chemical compounds from plants in Indonesia that have the best efficacy compared to the positive control of acarbose and miglitol are compounds derived from the leaves, namely Theaflavin with a rating score of -104,033. Theaflavin compound is a group of polyphenol compounds that have been known as antioxidant agents derived from tea leaves. The interaction between miglitol as an α-glucosidase native ligand inhibitor occurs in ASP 327 HIS 600 ASP 443. Theaflavin also shows potential as an α-glucosidase inhibitor, interaction with residues of ASP 327 ASP 542 THR 205 ASP 203 PHE 450 TRP 406 ASP 443.

Acknowledgement

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