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Molecular docking-based virtual screening of antidiabetic agents from Songga (*Strychnos lucida* R.Br.): an Indonesian native plant

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ABSTRACT

This study was carried out to predict the compounds derived from Songga that have potential as antidiabetic and predict their mechanism of action on various pathways of glucose regulation in diabetes mellitus by molecular docking. Molecular docking-based virtual screening was done by using AutoDock Vina software assisted by AutoDockTools. The test compounds used for virtual screening were obtained from literature studies and were combined with Lipinski's rule to select the compounds for the prediction of lead candidates that can be used in oral administration. The receptors used in this study were human aldose reductase, human maltase-glucoamylase, PPAR-gamma, pancreatic beta-cell SUR1, and human DPP-IV. The validation of the molecular docking method of five target receptors showed that RMSD values of human aldose reductase, human maltase-glucoamylase, PPAR-gamma, pancreatic beta-cell SUR1, and human DPP-IV were 0.6446 Å, 1.8668 Å, 0.2527 Å, 1.7452 Å, and 1.7439 Å, respectively. From the molecular docking-based virtual screening, we discovered that for each target protein, there were one to three optimal compounds that have the best interaction in our investigation. Those compounds were chlorogenic acid on human aldose reductase, phyllamycin A, chlorogenic acid, and brucine N-oxide on human maltase-glucoamylase, phyllamycin A on PPAR-gamma, strychnine N-oxide on pancreatic beta-cell SUR1 and strychnine on human DPP-IV with binding affinity value of -9.9 kcal/mol, -7.6 kcal/mol, -9.9 kcal/mol, -8.8 kcal/mol, and -6.2 kcal/mol, respectively. Several compounds are predicted to have potential to be developed as antidiabetic agents. However, further laboratory investigations like *in vitro* and *in vivo* assays need to be conducted.

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1. Introduction

Diabetes mellitus is one of the non-communicable diseases with the highest prevalence worldwide. Diabetes mellitus is a metabolic disease caused by inadequate control of blood glucose levels due to lack of insulin secretion, insulin resistance, or both (Kharroubi, 2015; Sapra and Bhandari, 2021). According to World Health Organization (2021), 422 million people across the globe suffer from diabetes mellitus with 1.6 million numbers of deaths each year. It is estimated that this number will continue to increase until 2025. In 2019, Indonesia was in the top ten countries with the highest number of diabetics in the world based on the International Diabetes Federation (IDF). The prevalence of diabetes mellitus in Indonesia increases every year (Kemenkes RI, 2020).

Currently, the treatment of diabetes mellitus is still using synthetic drugs like insulin and oral antidiabetic agents such as sulfonylureas, biguanides and glinides. However, the use of these drugs has serious side effects (Patel et al., 2012). Therefore, research on finding new antidiabetic agents that have minimal side effects and are more effective needs to be conducted. In this time, many natural products which have antidiabetic activity have been reported. Several plants have even been used empirically to treat diabetes mellitus (Malviya et al., 2010). One of the plants which is potential to be developed as an antidiabetic agent is songga (*Strychnos lucida* R. Br.).

Songga (*Strychnos lucida* R. Br.) is a plant from Loganiaceae family that grows in Bali and West Nusa Tenggara, Indonesia. Songga is traditionally used by the local people to prevent and manage several diseases including malaria, diabetes, inflammation, cancer, and cardiovascular problems (Setiawan and Rostiwati, 2014). Phytochemical studies reported that songga contains many active constituents such as beta-colubrine, brucine, brucine N-oxide, chlorogenic acid, diaboline, loganin, phyllamycin A, secoxyloganin, strychnine, strychnine N-oxide (Itoh et al., 2006; Manurung et al., 2019; Setiawan and Rostiwati, 2014). These compounds play an important role in giving pharmacological activities of songga, one of which is antidiabetic.

Several studies have been reported regarding the antidiabetic activity of songga. The infused water of songga could significantly reduce blood glucose level of alloxan-induced rat (Kurniadi, 2012). Recent study showed that dry extract of songga at 8.5 mg/kgBW was effective in decreasing blood glucose in streptozotocin-induced diabetes rat model (Hendarto et al., 2019). Another research also showed that ethyl acetate and *n*-hexane fractions of songga extract

at 900 ppm could inhibit the alpha-glucosidase enzyme by 33.39% and 34.23%, respectively (Rale et al., 2019).

Having said that, until now, research related to the exploration of compounds that is chemically and pharmacologically active as antidiabetic agents from songga plant has not been done. Therefore, this study was carried out to predict the compounds derived from songga that have potential as antidiabetic agents and predict their mechanisms of action on various pathways of glucose regulation in diabetes mellitus by molecular docking.

2. Materials and methods

2.1. Materials

The hardware used in this study was a unit of computer with Windows 10 Pro 64-bit operating system, Intel CoreTM i3, 4 GB of RAM, and 2 GB NVIDIA GEFORCE VGA.

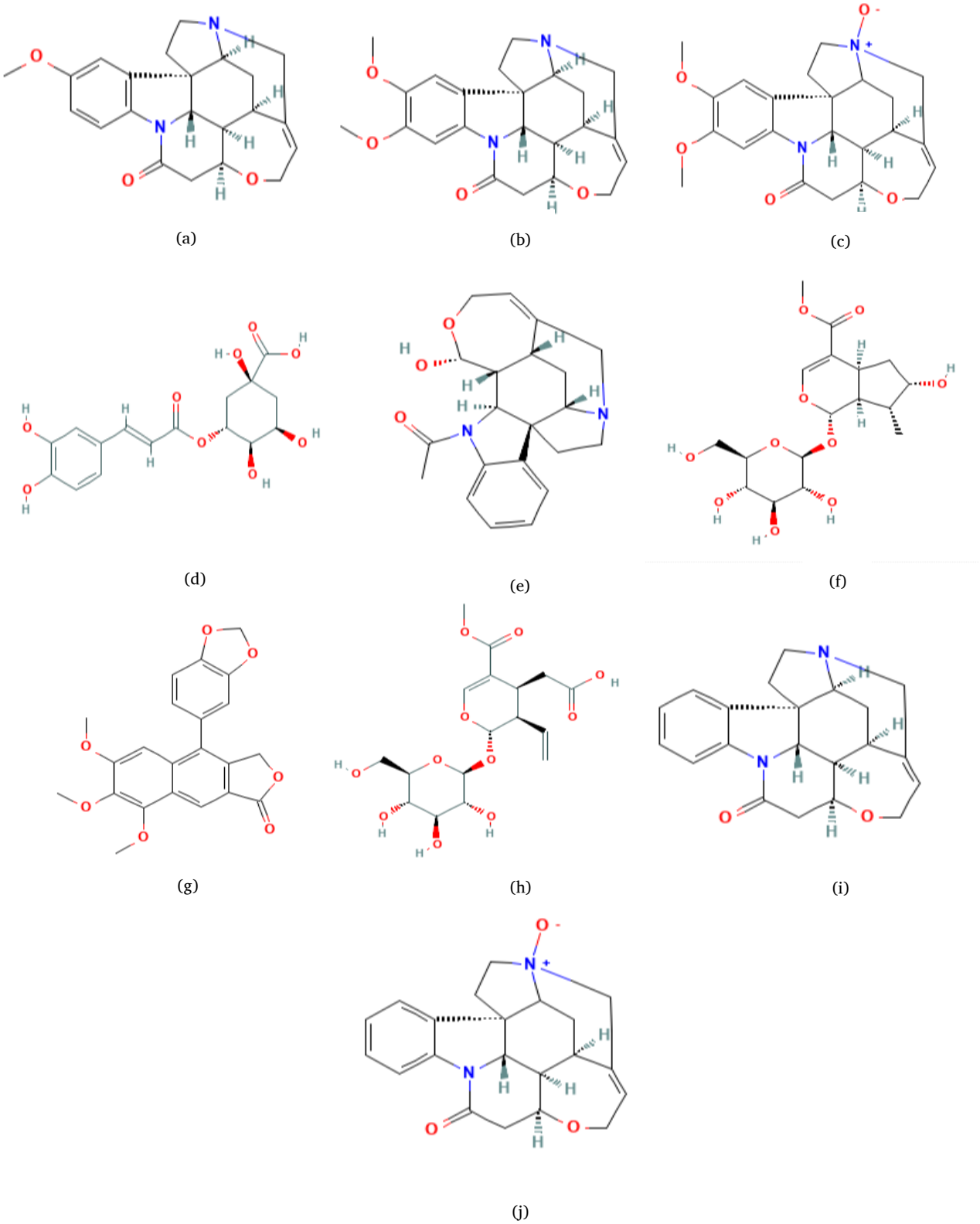


Fig. 1. Chemical structure of compounds derived from songga, (a) beta-colubrine, (b) brucine, (c) brucine N-oxide, (d) chlorogenic acid, (e) diaboline, (f) loganin, (g) phyllamycin A, (h) secoxyloganin, (i) strychnine, (j) strychnine N-oxide

2.2. Methods

2.2.1. Ligand preparation

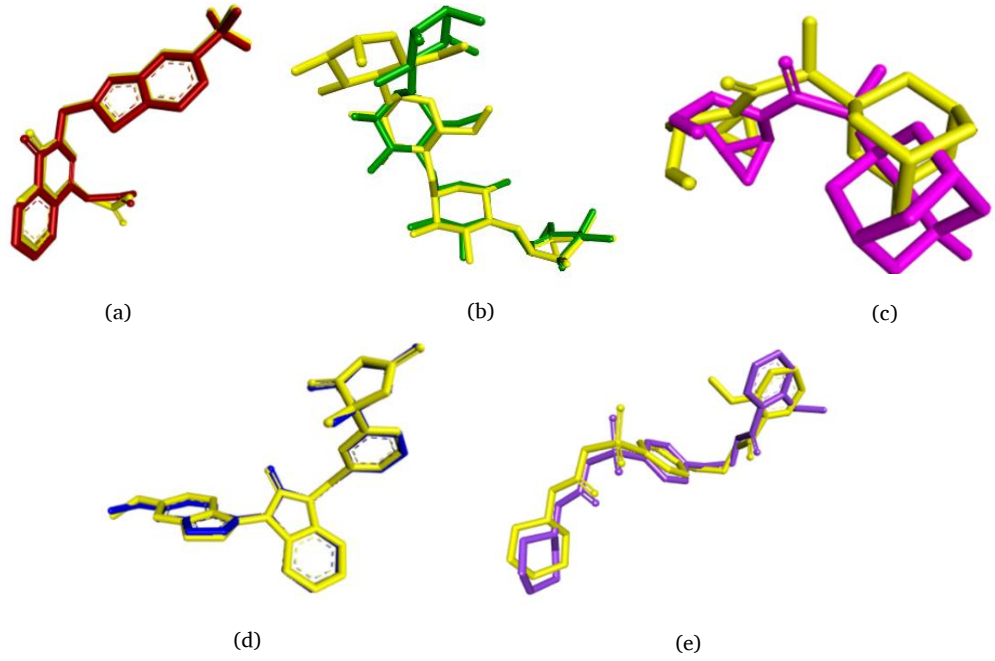
The test compounds used for virtual screening were obtained from literature studies. The Lipinski's rule had been applied to select the compounds for the prediction of lead candidates which can be used in oral administration. The number of chosen natural products derived from songga was ten compounds. The 3D conformations of all ten target compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov).

2.2.2. Protein structure preparation

The receptors used in this study were Human Aldose Reductase (PDB ID: 2HV5) (Steuber et al., 2006), Human Maltase-Glucoamylase (PDB ID: 2QMJ) (Sim et al., 2008), PPAR-gamma (PDB ID: 3TY0) (Liu et al., 2011), the pancreatic beta-cell SUR1 (PDB ID: 6PZA) (Martin et al., 2019), and human DPP-IV (PDB ID: 3BJM) (Metzler et al., 2009). The structure of these target proteins was obtained from Protein Data Bank (PDB) (ww.rscb.org). The proteins were selected based on their role in diabetes mellitus. The proteins downloaded from PDB were then prepared using BIOVA Discovery Studio software. In the preparation of proteins, water molecules, ligands and other heteroatoms were removed from the protein structures. On the other hand, polar hydrogen atoms were added to the protein structures.

2.2.3. Molecular docking study

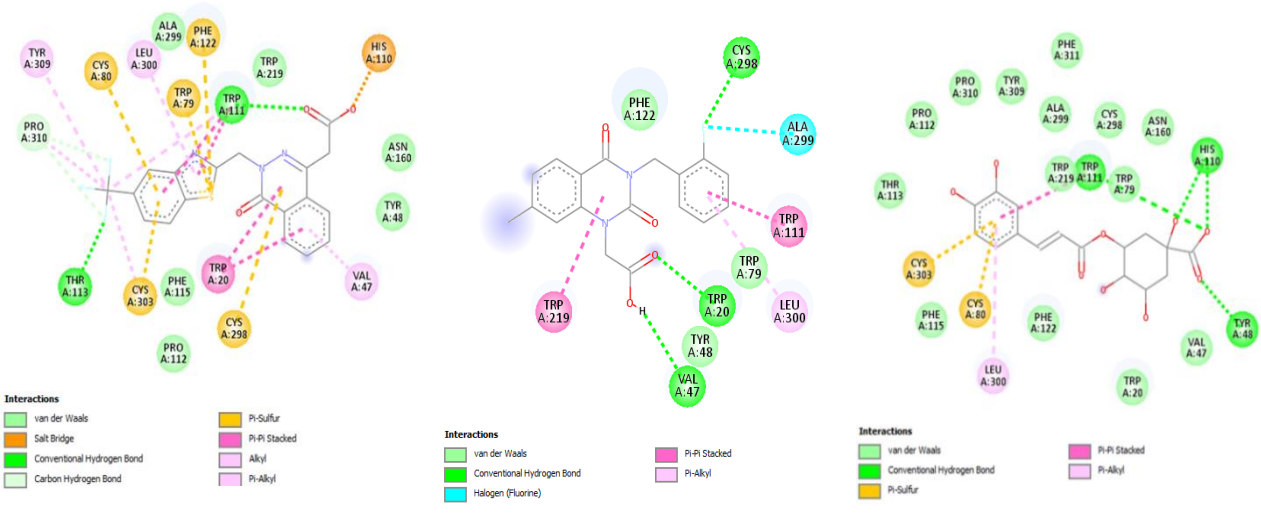
The molecular docking study was carried out by using AutoDock Vina software assisted by AutoDockTools. The protein active site was determined following to the native ligand binding site of each protein. Molecular docking parameters were used according to the default value. The coordinate of the gridbox and adjustment of x, y and z-axes can be seen in Table 1 with spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was used in docking process with 100 runs of genetic algorithm parameter; 150 population size; 2.500.000 the number of evaluations; default mutation rate and crossover rate. The molecular docking method are valid if the RMSD value obtained from the re-docking of native ligand is less than 2 Å (Morris et al., 2009).



(a) (b) (c)

(d) (e)

Fig. 2. Visualization of the results of docking method validation, (a) re-docking of 2HV5 native ligand, (b) re-docking of 2QMJ native ligand, (c) re-docking of 3BJM native ligand, (d) re-docking of 3TY0 native ligand, (e) re-docking of 6PZA native ligand. Note: yellow for native ligand and other colors for re-docking ligand



(a) (b) (c)

Fig. 3. Interactions of native ligand (a), zenarestat (positive control) (b), and chlorogenic acid (c) with 2HV5.

3. Results and discussion

3.1. Ligand Selection

Table 1. Adjustment of coordinate and size of the gridbox

PDB ID	Coordinate			Size		
	Center X	Center Y	Center Z	Size X	Size Y	Size Z
2HV5	17.032	-6.715	13.582	48	48	48
2QMJ	-21.727	-6.323	-5.281	48	48	48
3BJM	67.237	71.209	69.791	16	16	26,67
3TY0	13.95	7.22	11.672	48	48	48
6PZA	203.088	281.744	219.146	48	48	48

Table 2. The Lipinski's rule screening result of songga derived compounds

Test Compound	BM	H donor	H acceptor	Log P
Loganin	390.4	5	10	-1.4
Phyllamycin A	394.4	0	7	4
Strychnine N-oxide	350.4	0	3	1.4
Strychnine	334.4	0	3	1.9
Secoxyloganin	404.4	5	11	-1.6
Diaboline	352.4	1	4	0.7
Chlorogenic acid	354.31	6	9	-0.4
Brucine	394.5	0	5	1
Brucine N-oxide	410.5	0	5	0.4
Beta colubrine	364.4	0	4	1.9

The literature studies revealed that many chemical compounds have been isolated from various parts of songga such as beta-colubrine, brucine, brucine N-oxide, chlorogenic acid, diaboline, loganin, phyllamycin A, secoxyloganin, strychnine, and strychnine N-oxide (Itoh et al., 2006; Manurung et al., 2019; Setiawan and Rostiwati, 2014). The chemical structure of some compounds derived from songga can be seen in Fig. 1.

All compounds were then analyzed according to the Lipinski's rule. As shown in Table 2, all ten compounds met the criteria of Lipinski's rule where all compounds have BM <500, H donor <5, H acceptor <10, and log P <5. This results showed that all compounds are predicted to have drug-likeness properties. Drug-likeness refers to the similarity of a compound to an oral drug. This is indicated that the adsorption, distribution and permeability of all test compound similar to that of an oral drug.

3.2. Molecular docking-based virtual screening

The first step in molecular docking is a validation of the docking method. This method validation was applied by re-docking each native ligand to the target receptors.

Table 3. The docking scores of songga derived compounds

Test Compounds	Receptors/Binding energy (kcal/mol)				
	2HV5	2QMJ	3TY0	6PZA	3BJM
Loganin	-6.8	-7.0	-7.8	-6.8	-5.4
Phyllamycin A	-8.0	-7.6	-9.9	-8.2	19.9
Strychnine N-oxide	-9.5	-7.2	-7.5	-8.8	-4.0
Strychnine	-9.2	-7.5	-7.5	-8.7	-6.2
Secoxyloganin	-6.3	-6.2	-7.8	-6.5	-4.6
Diaboline	-7.7	-7.4	-8.0	-8.1	-4.9
Chlorogenic acid	-9.9	-7.6	-8.1	-7.9	-5.2
Brucine	-8.1	-7.3	-7.5	-8.3	1.7
Brucine N-oxide	-8.2	-7.6	-8.4	-8.0	-0,9
Beta colubrine	-9.0	-7.2	-8.0	-8.1	-5.5
Positive control					
Vildagliptin					-5.4
Miglitol		-5.4			
Gliclazide				-8.4	
Zenarestat	-8.5				
Pioglitazone			-8.7		
Native ligand	-12,6	-7.6	-11.7	-8.7	-4.2

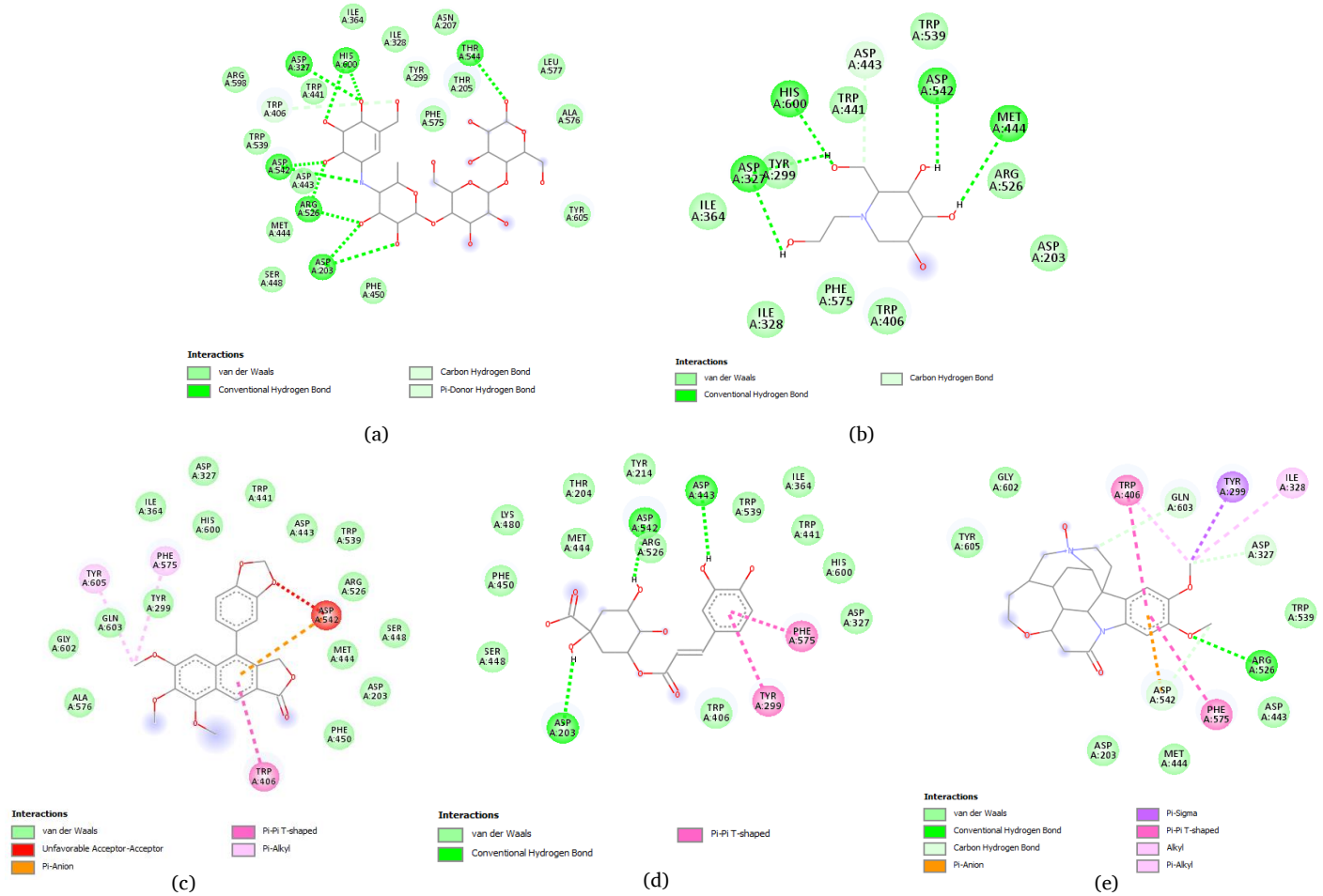
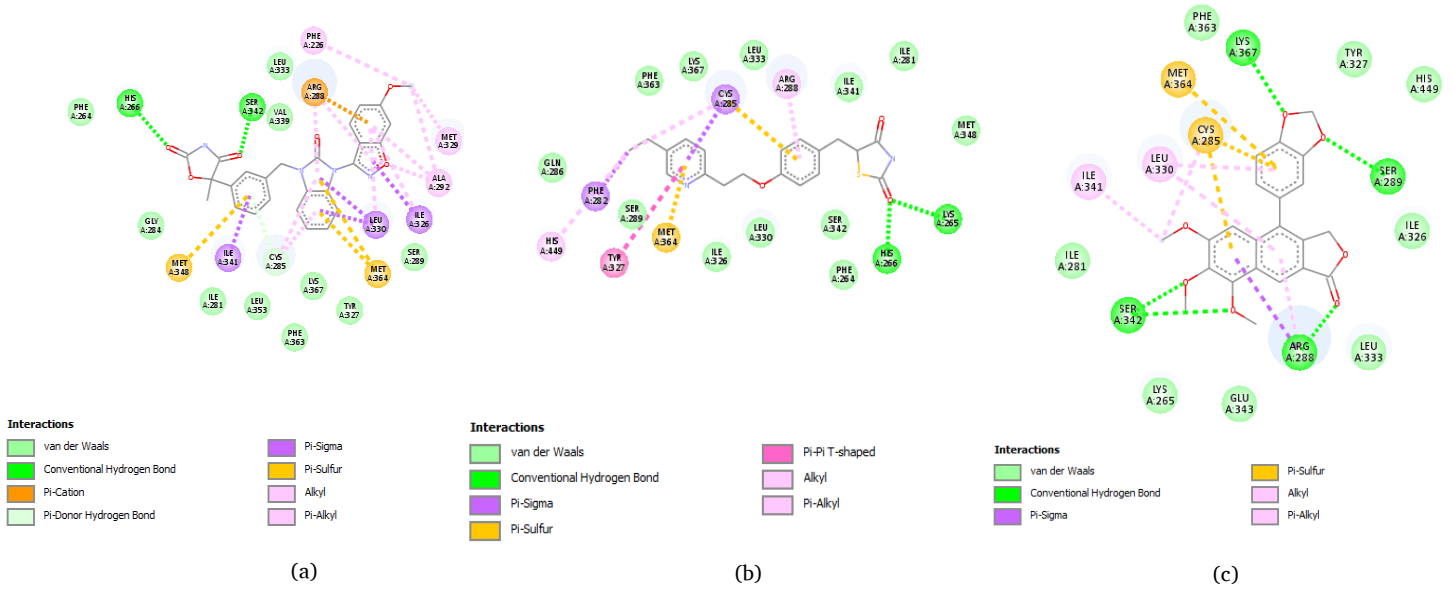
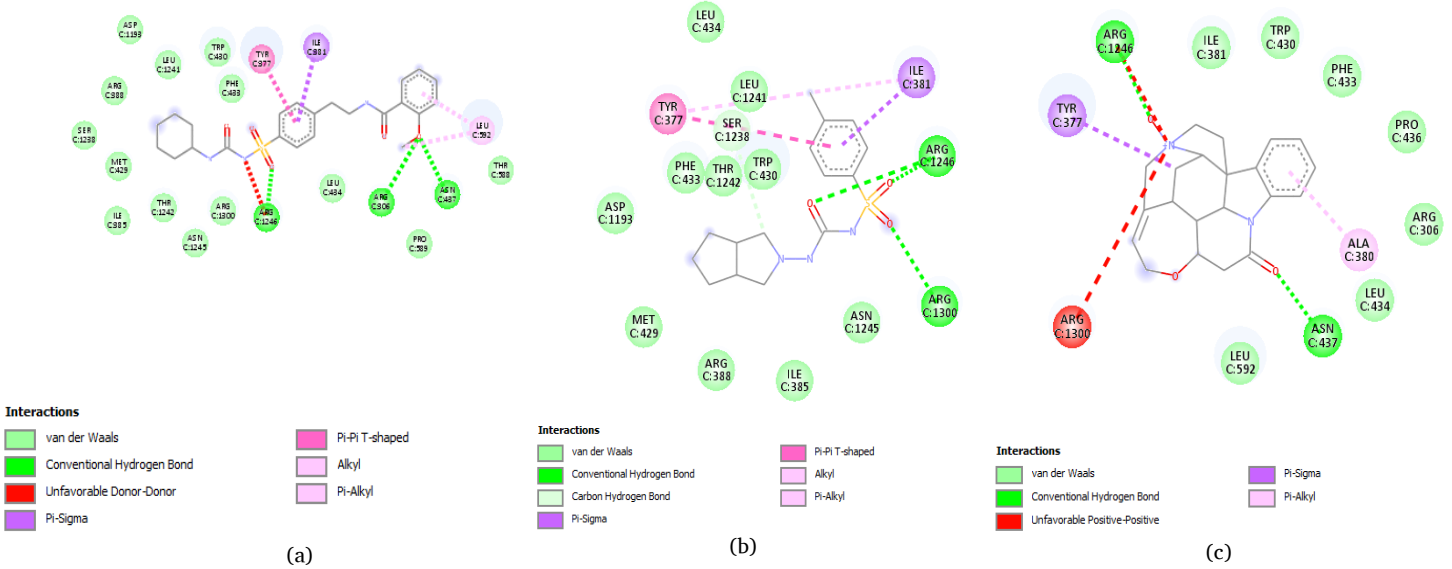


Fig. 4. Interactions of native ligand (a), miglitol (positive control) (b), phyllamycin A (c), chlorogenic acid (d), brucine N-oxide (e) with 2QMJ receptor.



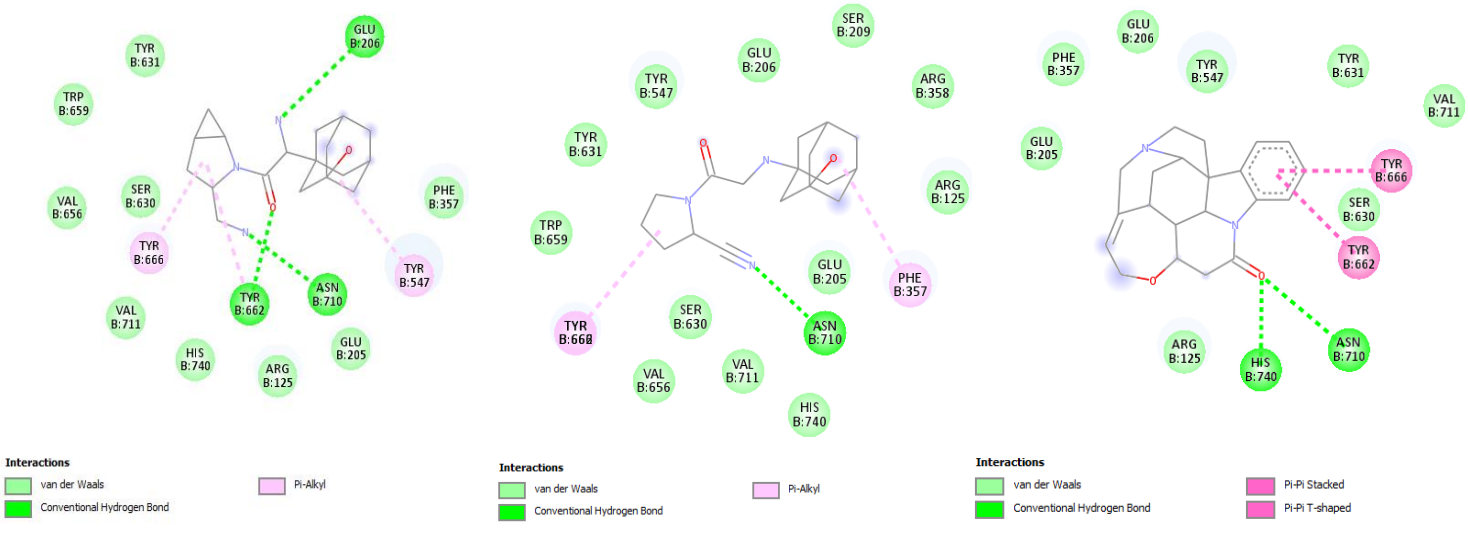
(a) (b) (c)

Fig. 5. Interactions of native ligand (a), pioglitazone (positive control) (b), and phyllamycin A (c) with PPAR-gamma receptor (3TY0).



(a) (b) (c)

Fig. 6. Interactions of native ligand (a), gliclazide (positive control) (b), and strychnine n-oxide (c) with the pancreatic beta-cell SUR1 (6PZA).



(a) (b) (c)

Fig. 7. Interactions of native ligand (a), vildagliptin (positive control) (b), and strychnine (c) with human DPP-IV (3BJM).

The RMSD value obtained from re-docking of Human Aldose Reductase (PDB ID: 2HV5), Human Maltase-Glucoamylase (PDB ID: 2QMJ), PPAR-gamma (PDB ID: 3TY0), the pancreatic beta-cell SUR1 (PDB ID: 6PZA), and human DPP-IV (PDB ID: 3BJM) were 0.6446 Å, 1.8668 Å, 0.2527 Å, 1.7452 Å, and 1.7439 Å, respectively. These results showed that the docking method is valid. After the validation of the docking method was done, evaluation of target compounds was conducted. In order to get the best compound, the screening results of target compounds obtained from the molecular docking were further analyzed by observing the values of the smallest binding energy. The test compounds which have lower binding energy values than native ligand indicated that the binding strength of those compounds to the receptor was better (Muttaqin et al., 2020). In addition, the analysis was also performed by observing the interactions that occur between ligands and amino acids residues in receptors. The binding energy values of the test compounds, native ligand and positive control can be seen in Table 3.

The result in Table 3 showed that the test compounds have different binding affinity on each receptor. Although there are several compounds that have potential for more than one receptor, several other compounds work specifically on one particular receptor.

The docking results of songga derived compounds against Human Aldose Reductase revealed that chlorogenic acid has the most negative free binding energy compared to any other test compounds and zenarestat as positive control. Hence, it is predicted that chlorogenic acid has potential as Human Aldose Reductase inhibitor. Human Aldose Reductase is an enzyme that catalyzes glucose conversion to sorbitol in the glucose metabolism polyol pathway. Inhibiting Human Aldose Reductase can reduce the flux of sorbitol as a cause of complication in diabetics (Singh et al., 2015).

Some compounds have the same affinity for acarbose as 2QMJ native ligand. Phyllamycin A, chlorogenic acid, and brucine N-oxide are three compounds that have more negative free binding energy than the positive control, miglitol. As demonstrated in Fig. 4, these substances have the capacity to block the 2QMJ because of their interactions with amino acids residues of the protein. 2QMJ is one of the two enzymes that catalyze the final glucose-releasing phase in starch digestion. By blocking this enzyme, the degradation of polysaccharide into monosaccharides can be diminished, resulting in the decrease in the blood glucose level (Sim et al., 2008). Phyllamycin A, chlorogenic acid, and brucine N-oxide are predicted to be potential 2QMJ inhibitors.

On PPAR-gamma receptor, only phyllamycin A has smaller binding affinity value than positive control pioglitazone, despite the binding strength of this compound with PPAR-gamma receptor being weaker than that of the native ligand. Pioglitazone is a synthetic drug for PPAR-gamma that can improve insulin sensitivity and uptake by increasing the glucose transporter, decreasing free fatty acid, enhancing insulin signalling, reducing tumor necrosis factor alpha and remodelling of adipose tissue (Smith, 2001). By having better binding affinity than pioglitazone, it could be predicted that phyllamycin A has the potential to be developed as PPAR-gamma ligand as an antidiabetic agent.

The next target protein in this study is the pancreatic beta-cell SUR1 (6PZA). This receptor is responsible for insulin secretion. As shown in Table 3, strychnine N-oxide has the highest binding affinity compared to the other compounds as well as native ligand and positive control, gliclazide. This indicated that strychnine N-oxide binds better to 6PZA than do the native ligand and positive control. This result showed that strychnine N-oxide may have a function and mechanism of action which are similar to those of gliclazide and other sulfonylureas in controlling blood glucose levels. They increase the secretion of insulin by binding to SUR1 receptor and block the inflow of potassium through the ATP-dependent channel. Inhibition of the potassium inflow will reduce the intracellular potassium concentration which leads to cell membrane depolarization. This prevents the calcium diffusion into the cytosol and increases the inflow of calcium into beta-cells. As a result, actomyosin filaments contract, leading to insulin exocytosis (Sola et al., 2015).

The last protein used in this study is human DPP-IV. The result of molecular docking of songga-derived compounds against this protein showed that some compounds have better binding affinity than the native ligand and the positive control. However, only strychnine has the greatest binding strength compared to the native ligand and the positive control, vildagliptin. This can be seen from the free binding energy of strychnine which has more negative binding energy than positive control. The result indicated that strychnine has potential to bind with human DPP-IV and to be developed as an antidiabetic agent. In addition, strychnine is also predicted to have the same mechanism of action as vildagliptin in lowering blood glucose levels which are inhibiting the human DPP-IV enzyme.

In addition, the potential activity of the test compounds against each protein is also influenced by the interaction that occur between the ligands and amino acid residues. In this study, the results revealed that every ligand has different types of interaction that is also mediated by different amino acid residues. In Human Aldose Reductase, the amino acid residues that interact with ligands were Thr 113, Pro 310, Tyr 309, Cys 80, Leu 300, Ala 299, Trp 79, Phe 122, Trp 111, Trp 219, His 110, Asn 160 Tyr 48 Val 47, Cys 298, Pro 112, Phe 115 and Cys 303. while in Human Maltase-Glucoamylase were Tyr 605, Phe 450, Asp 203, Ser 448, Met 444, Arg 526, Asp 443, Asp 542, Trp 539, Trp 406, Arg 598, Trp 441, Asp 327, His 600, Ile 364, Tyr 299, Phe 575, Thr 205, Thr 544, Asn 207, Leu 577, Ala 576. In other protein, such as PPAR-gamma receptor, there were Phe 264, His 266, Ser 342, Val 339, Leu 333, Phe 226, Arg 288, Met 329, Ala 292, Ile 326, Leu 330, Ser 289, Met 364, Tyr 327, Lys 367, Phe 363, Cys 285, Leu 353, Ile 341, Ile 281, Met 348, Gly 284. In the pancreatic beta-cell SUR1, there were several amino acid residues which interact with ligand, such as Arg 1246, RG 1300, Asn 1245, Thr 1242, Ile 989, Met 429, Ser 1238, Arg 988, Asp 1193, Leu 1241, Trp 430, Phe 433, Tyr 977, Ile 981, Leu 382, Thr 388, Asn 437, Pro 389, Arg 906, Leu 434. The amino acid residues that act in human DPP-IV were Glu 206, Phe 357, Tyr 547, Glu 205, Asn 710, Tyr 662, Arg 125, His 740, Val 711, Tyr 666, Ser 630, Val 656, Trp 659, Tyr 631. All these amino acid residues interact with the ligand by direct and indirect interaction. The most occurred interactions were hydrogen bond, van der walls, alkyl, and pi. These interactions play an important role in stability of the bond between ligand and protein (Afriza et al., 2018).

4. Conclusions

Molecular docking-based virtual screening is capable to predict the compounds that have potential as antidiabetic agents. Those compounds have different binding affinities on every protein. Some compounds bind specifically on one particular protein, such as strychnine N-oxide on 6PZA, and strychnine on 3BJM. Several compounds have the same potential on one protein, like phyllamycin A, chlorogenic acid, and brucine N-oxide on 2QMJ. These compounds are predicted to be developed as potential antidiabetic agents. However, further laboratory investigations like *in vitro* and *in vivo* assay need to be conducted.

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Conflict of interest

Authors declare no conflict of interest.

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