Trop J Nat Prod Res, October 2021; 5(10):1735-1742

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Molecular Docking of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome Components as Anti-Diabetic Through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase Enzymes

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ARTICLE INFO

Article history:
Received 27 March 2021
Revised 16 September 2021
Accepted 21 October 2021
Published online 02 November 2021

ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase (*α-glucosidase*) are enzymes involved in carbohydrate metabolism. Inhibition of these enzymes contribute to blood glucose level suppression. Soybean (*Glycine max*) seeds and ginger (*Zingiber officinale*) rhizome are herbs that have anti-diabetic activity. The mechanism of action, however, has not been thoroughly explored. This study aims to evaluate the anti-diabetic potentials of the chemical components in soybean seeds and ginger rhizome through inhibition activity of DPP-4 and *α*-glucosidase *in silico*. Soybean seed and ginger rhizome were extracted using the maceration method with ethanol solvent. Ethanol extract of soybean seeds and ginger rhizome were analysed using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The potency of active compounds from the plants on DPP-4 and *α-glucosidase* were evaluated by *in silico* study using web-based software (Docking server). Soybean seed were found to contain phytosterols, mainly beta sitosterol, campesterol, stigmasterol, and lanosterol. Meanwhile, ginger rhizome was found to contain 6-gingerdiol, 10-gingerol and 12-shogaol. Molecular docking study showed that stigmasterol and 12-shogaol strongly inhibits DPP-4 activity while stigmasterol and 6-Gingerdiol strongly inhibited *α*-glucosidase. This shows that both soybean seed and ginger rhizome potentially act as an anti-diabetic by inhibiting DPP-4 and *α*-glucosidase; however, soybean seed is more potent due to its ability to inhibit both of the tested enzymes.

Keywords: Anti-diabetic, *Glycine max*, *Zingiber officinale*, DPP-4, *α*-glucosidase.

Introduction

Glucagon-like Peptide-1 (GLP-1), which is a known incretin hormone synthesized from the lower gut, plays a role in modulating glycemic control and can be used in glucose-lowering medication for type-2 Diabetes Mellitus (T2DM).[1] However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively into inactive forms,[1] causing GLP-1 to have a short half-life (to approximately 2-5 minutes).[1,2] Effective use of GLP-1 in T2DM treatment requires increased GLP-1 bioavailability to regulate blood glucose level, in which DPP-4 inhibitors are used in conjunction. [3]
Meanwhile, alpha-glucosidases (*α*-glucosidases) are enzymes that are responsible for the conversion of complex carbohydrate into maltose, dextrin and maltotriose.[4] These enzymes are commonly found in the brush border of the small intestines, whereas the products of carbohydrate metabolism are delivered into the small intestinal mucosa, and absorbed into blood circulation.[5] Thus, *α*-glucosidases activity contributes in increasing blood glucose level post prandially, and needs to be controlled to avoid hyperglycemia. Inhibition of both DPP-4 and *α*-glucosidase could be used in preventing the increase of blood glucose level, especially for patients with T2DM.[3,4]

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Citation: Purnomo Y, Taufiq M, Wijaya AND, Hakim R. Molecular Docking of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome Components as Anti-Diabetic Through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase Enzymes. Trop J Nat Prod Res, 2021; 5(10):1735-1742. doi.org/10.26538/tjnpr/v5i10.7

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Therapy of T2DM usually requires an Oral Hypoglycemic Drug (OHD), which acts either by increasing of insulin secretion, repairing of insulin resistance and/or inhibiting of glucose absorption. However, long-term consumption of these medications is often accompanied with various adverse drug reactions, particularly in DM patients.[6] Improvement of such medications are sought from natural derivatives or natural products, or traditional herbs and remedies. Herbal remedies, in particular, is becoming a popular medication choice in the management of the disease as it is perceived to have less adverse reaction and a more holistic approach in treatment.[7,8]
Glycine max or soybean and *Zingiber officinale* or ginger are functional food known empirically to cure some diseases, and based on laboratory studies, have been shown to have hypoglycemic activity,[9] mainly due to the presence of isoflavons such as daidzein and genestein. [10] On the other hand, *Z. officinale* rhizome was shown to have anti-hyperlipidemic and antioxidant potential due to the presence of gingerol and shogaol compounds. Furthermore, other studies have reported that ginger also possesses anti-diabetic and antioxidant activity, and can help reduce blood glucose levels and as well as its complication caused by high glucose levels.[11] These anti-diabetic effects are thought to be due to active compounds such as phytosterols, flavonoids, saponins, phenols, and essential oils. Several studies have proven the potential of soybean seeds and ginger rhizome as anti-diabetes.[10-12] However, the mechanism of action and active substances of soybean seed extract and ginger rhizome responsible for controlling blood glucose levels are still unclear. The purpose of this study is to evaluate the mechanism of action of soybean seed extract and ginger rhizome as an anti-diabetic through inhibitory activity of both DPP-4 and *α*-glucosidase using *in silico* study.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Preparation of Glycine max and Zingiber officinale Extract
G.max seed and *Z.officinale* rhizome were obtained from Malang, East Java, Indonesia in June 2017. They were identified at Balai Materia Medika, Batu, Malang, East Java, Indonesia with voucher specimen numbers 074/241/102.7/2017 and 074/211/102.7/2017, respectivelly. Simplisia of herbs were pulverized by size reduction machine. *G.max* (20 g) and *Z.officinale* (20 g) were extracted using maceration method each with 100 mL ethanol as a solvent.

Identification of active substances
Ethanol extracts of *G. max* seed and *Z. officinale* rhizome were qualitative analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific, with Hypersil GOLD as the stationary phase. The mobile phase were used solution A (0.1% formic acid in H_2O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300 µL/min.[13]

Prediction of physicochemical property
Prediction of the physicochemical properties of the active compound was performed using the pkCSM online tool. Firstly, active compounds identified and references standard were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then stored as * .sdf file or * .pdb files. Secondly, all of active the compounds and the reference standard were translated into SMILES format using SMILES Translator Online Help. In the SMILES format, the compounds were processed using the pkCSM online tool to predict the physicochemical property.[14]

Molecular docking study
The chemical structure of ligands (phytosterol compounds of soybean seeds and terpenoids from ginger rhizome) and references standard were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov) with a 3D SDF file extension. Therefore, the file type was changed to a PDB extension file with the Open Babel software version 2.4.1.[15] The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and *α*-glucosidase with UniProt ID: O43451) is downloaded on the Uniprot website (http://www.uniprot.org/).The crystal structure both of the enzyme shown in Figure 1 and Figure 2 below.

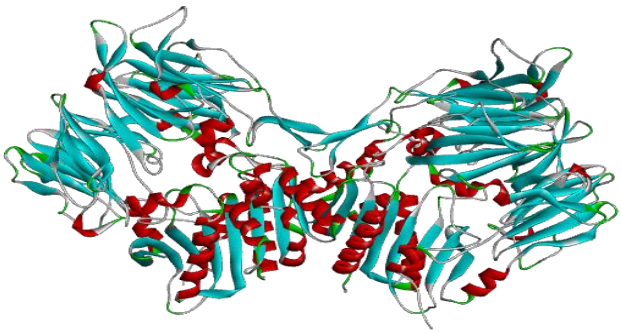

Figure 1: Crystal Structure of Dipeptidyl Peptidase-4

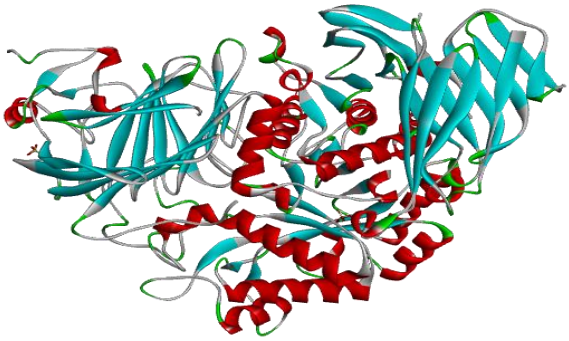

Figure 2: Crystal Structure of *α-glucosidase*

Moreover, protein homology on the Swiss Model (www.swissmodel.expasy.org/interactive), the modeling results can be downloaded in a PDB file. The molecular docking was performed using web-based software (dockingserver.com) by uploading both chemical compound and protein target on them. The prediction value of parameter include inhibition constant, free energy of binding, and surface interactions were observed by this method to examine their activity on DPP-4 and *α*-glucosidase.

Results and Discussion

Identification of active substances in Glycine max and Zingiber officinale Extract
The active compounds from ethanol extract of soybean seeds and ginger rhizomes using the LC-MS/MS method can be seen in Figure 3, Figure 4, and Table 1.
The qualitative analysis using LC-MS/MS showed that active substances contained in the ethanol extract of *G. max* seeds were Stigmasterol, Campesterol, *β*-Sitosterol, and Lanosterol. The highest component is *β*-Sitosterol, whereas the lowest component is Lanosterol.[18] Meanwhile, the active compounds contained in the ethanol extract of *Z. officinale* root are 6-Gingerdiol, 10-Gingerol, and 12-Shogaol. The highest component is 12-Shogaol, whereas the lowest component is 6-Gingerdiol.[19]
Four active compounds were identified in *G. max* seed ethanol extract and three active substances in *Z. officinale* rhizome ethanol extract. These active compounds were determined by evaluating the value of the Selected Reaction Monitoring (SRM) on the chromatogram. SRM is a measurement parameter on LC-MS/MS to measure protein and active substances accurately and consistently. SRM is also used as a validation method to confirm the list of target proteins and active compounds obtained in research done globally or from previous findings.[20] The active substances of *G. max* seeds and *Z. officinale* rhizome are secondary metabolites and have biological activity, moreover, they can be used as candidates phytopharmaceuticals.[21]
As mentioned before, the main components of phytosterols in G. max seeds are β-sitosterol, campesterol, stigmasterol, and lanosterol. All of them are classified into secondary metabolite groups and have biological activity that can be used to cure various symptoms and diseases, whereas β-Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant[23] while Campesterol plays a role in lowering blood cholesterol and has anti-carcinogenic effects. These two compounds also have anti-angiogenic effects by inhibiting endothelial cell proliferation and capillary differentiation.[24] Some studies both in vivo and in vitro showed that lanosterol has activity as anti-cataract.[25] More importantly, stigmasterol is one of the phytosterol groups in plants used to maintain the balance of phospholipid membranes in plant cells and are chemically similar to cholesterol in animal cell membranes. Stigmasterol can inhibit cholesterol biosynthesis through the inhibition of sterol reductase in the cells. Furthermore, stigmasterol has the potential anti-inflammatory, anti-tumor, anti-osteoarthritis, hypoglycemic and antioxidant effects.[22] On the other hand, Z. officinale contains many active phenolic components such as gingerol and shogaol that have antioxidant and anti-cancer effects. Phenolic compounds have activity as antioxidants due to their ability to stabilize free radicals by providing hydrogen atoms to free radicals. Meanwhile, radicals derived from antioxidants of phenolic compounds are more stable than free radicals.[26] The results of pre-clinical study showed that gingerol and shogaol compounds in the ginger extract can increase insulin secretion through protection activity from free radical on β-cells pancreas.[11,27] Other research indicated that administration of Z. officinale extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.[28]

Prediction of physicochemical properties
The result of the *in silico* study of the physicochemical properties of *Glycine max* and *Zingiber officinale* active compound is presented in Table 2. It can be seen that the molecular weight values of the active compound ranged from 296 to 426 (less than 500), the value of log of the octanol/water partition coefficient (log P) ranged from 3.03 to 8.48

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ISSN 2616-0692 (Electronic)

(more than 5), the amount of HBD ranged from 1 to 3 (less than 5), and the amount of HBA ranged from 1 to 4 (less than 10). 6-gingerdiol and 10-gingerol meet Lipinski Rules of Five completely, meanwhile, other compounds did not meet the requirement in terms of their log P value only.

Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were analysed and it was concluded that a compound is more likely to have poor absorption or permeability if the molecular weight exceeds 500; the calculated octanol/water partition coefficient (log P) exceeds +5; there are more than 5 H-bond donors (HBD) expressed as the sum of O–H and N–H groups; and there are more than 10 H-bond acceptors (HBA) expressed as the sum of N and O atoms. The above analysis is called the Lipinski Rules of Five because all values are multiples of five.[29]

Based on Table 2, this means that 6-gingerdiol and 10-gingerol met the Lipinski Rules of Five completely, meanwhile five others did not fulfill the rule.[29] Hence, it can be predicted that 6-gingerdiol and 10-gingerol will be easily absorbed and have high permeability.

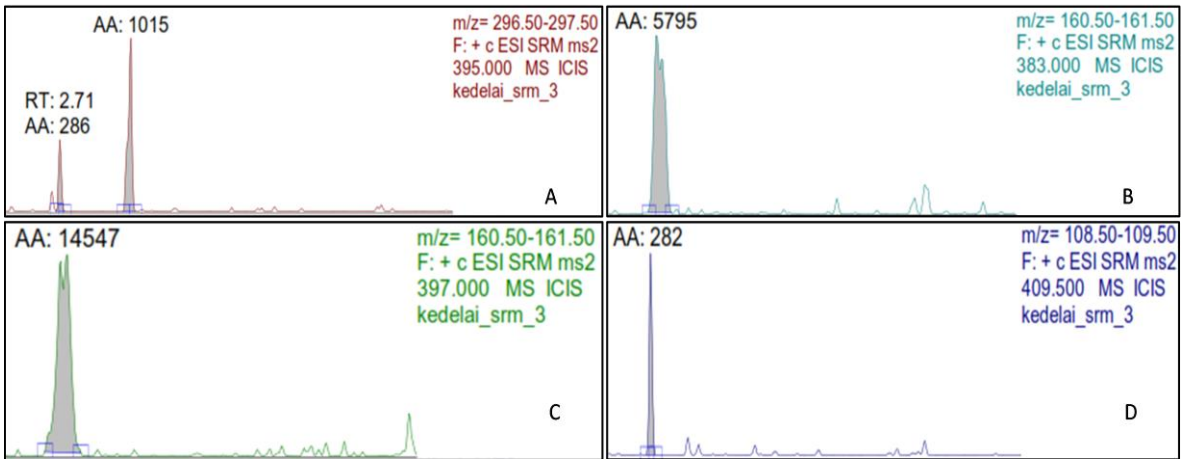


Figure 3: Chromatogram of active compound in ethanol extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) *ß*-Sitosterol, (D) Lanosterol

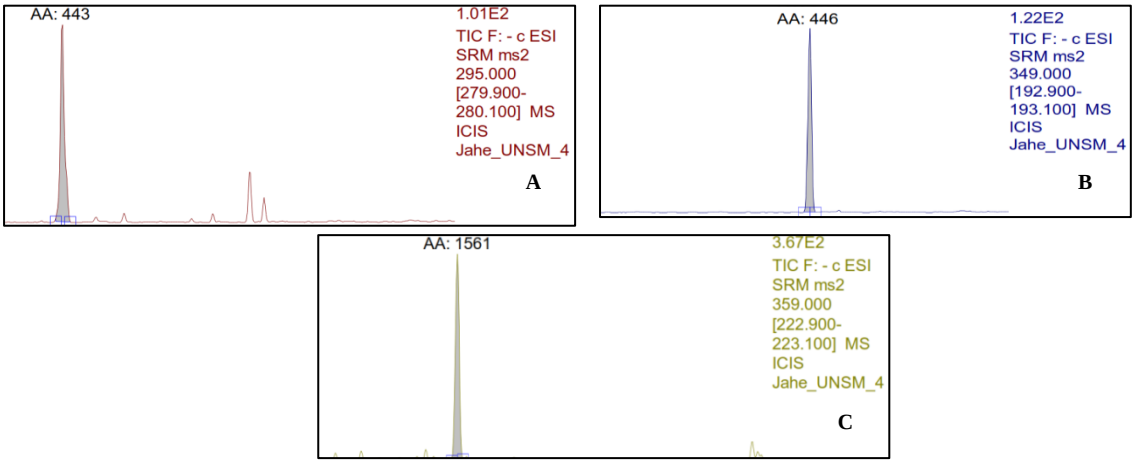


Figure 4: Chromatogram of active compound in ethanol extract of *Zingiber officinale* rhizome. (A) 6-Gingerdiol, (B) 10-Gingerol, (C) 12-Shogaol

Table 1: Active compound in *Glycine max* seed and *Zingiber officinale* rhizome extracts

Herbs	Active Compound	SRM Transition (*m/z*)	Identified SRM Score	Suraface Area (AA)
Glycine max	Stigmasterol	395-297	395	1015
	Kampesterol	383-161	383	5795
	β-Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
Zingiber officinale	6-Gingerdiol	295-280	295	443
	10-Gingerol	399-193	399	446
	12-Shogaol	359-223	359	1561

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Molecular docking of Glycine max and Zingiber officinale on DPP-4

Activity of *G.max* seed and *Z.officinale* rhizome extracts on DPP-4 were evaluated by *in silico* approach and the results can be seen at Table 3 and Figure 5. Docking studies showed that the lowest inhibitory constants and binding free energy in each herb were stigmasterol and 12-Shogaol, although the surface interaction was high. Meanwhile, *β*-Sitosterol, campesterol and lanosterol have a higher binding free energy score compare to stigmasterol and vildagliptin (a reference standard). On the other hand, vildagliptin indicated binding free energy lower than 12-shogaol. The difference in the value of each parameter causes differences in inhibitory activity on DPP-4.[30]

In the pharmaceutical field, molecular docking is often used to screen and predict the potential candidate drug target of ligands with a known structure, based on its free energy binding, inhibition constant, and surface interaction. The free energy binding score represents the binding affinity of a ligand to a target protein, whereas the lower the free energy binding score, the higher binding affinity.[30] In addition, bioinformatics can also evaluate the bioactivity of a substance by predicting its inhibition constant (Ki). The lowest Ki score would indicate the most potentially active compound, however, the toxicity of compound must also be tested.[31] Evaluation of surface interaction represents the molecular recognition between a ligand and a target protein; the higher the surface interaction value, the higher the possibility of an interaction of compounds with a target protein.[30,32]

Based on these criteria, DPP-4, stigmasterol and 12-shogaol have the lowest Ki value, followed by lanosterol and 6-gingerdiol. This is also supported by free binding energy and surface interaction scores of these compounds. Both stigmasterol and 12-shogaol had a higher score of surface interaction compared to the reference standard. This may indicate a stronger bond between ligand and protein target which would correspond to higher biological activity.[31]

Stigmasterol and 12-shogaol was also found to have the lowest value in binding free energy, followed by lanosterol and 6-gingerdiol. A low free binding energy score indicates a strong binding affinity of a ligand to a protein target which potentially indicates some biological activity. The free energy binding and surface interaction scores may indicate some inhibitory activity of the ligands from *G. max* and *Z. officinale* extract on DPP-4.

Table 2: Prediction of physicochemical properties of active compound *Glycine max* and *Zingiber officinale* compound

Herbs	Active Compounds	MW	Log P	Fr. Csp3	Torsion	HBA	HBD	PSA (A^2)	Water Solubility
Glycine max	Stigmasterol	412.69	7.80	0.86	5	1	1	20.23	-5.47
	Kampesterol	400.68	7.63	0.93	5	1	1	20.23	-5.79
	β-Sitosterol	414.71	8.02	0.93	6	1	1	20.23	-6.19
	Lanosterol	426.72	8.48	0.87	4	1	1	20.23	-7.20
Zingiber officinale	6-Gingerdiol	296.40	3.03	0.65	10	4	3	69.92	-4.11
	10-Gingerol	350.49	4.79	0.67	14	4	2	66.76	-6.17
	12-Shogaol	360.53	6.38	0.61	15	3	1	46.53	-7.19

MW = Molecular weight; Log P = logarithm of octanol/water partition coefficient; Torsion = bond between rotating atoms; HBA = H-bond acceptors; HBD = H-bond donors; PSA = polar surface activity

Table 3: Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with DPP-4

Herbs	Ligand	inding Free Energy (Kcal/mol)	Inhibition Constant (µM)	Surface Interaction (Å)
Glycine max	Stigmasterol	-7.11	6.16	931.16
	β-Sitosterol	-6.94	8.13	908.89
	Kampesterol	-6.84	9.74	877.50
	Lanosterol	-7.03	7.06	860.98
Zingiber officinale	6-Gingerdiol	-2.92	7260	494.287
	10-Gingerol	-2.38	1812	679.164
	12 Shogaol	-3.13	5110	631.305
	Vildagliptin*	-7.76	2.03	512.40

* *Reference standard*

Molecular docking of Glycine max and Zingiber officinale on alpha-glucosidase

Activity of *G.max* seed and *Z.officinale* rhizome extracts both of on *α-glucosidase* were evaluated by *in-silico* approach and the results can be seen at Table 4 and Figure 6.

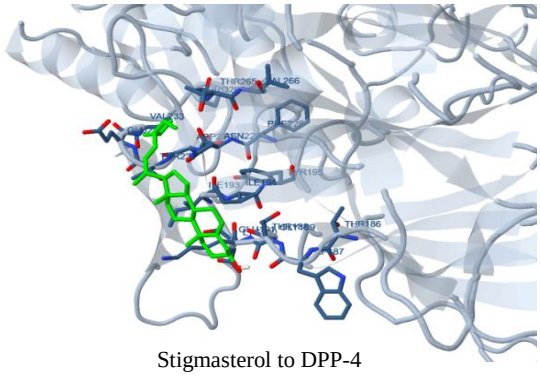
In-silico studies indicates that stigmasterol and 6-gingerdiol have a lower Ki, free energy binding, and surface interaction scores compared to other active compounds. Meanwhile, lanosterol and 10-gingerol was found to have a higher free energy binding score compared to other compounds in *G. max* and *Z. officinale*. The differences in each parameter value causes the differences of the inhibitory activity against *α*-glucosidase.[34] Based on inhibition constant (Ki) value, stigmasterol and *β*-sitosterol had higher inhibitory activity against *α-glucosidase*, and stigmasterol was found to be even stronger than other active compounds found in *Z. officinale* as well as acarbose (which was used as a reference standard).

Based on the active compounds in both of the extracts, stigmasterol in soybean and 6-gingerdiol in *Z. officinale* had the lowest Ki score, followed by *β*-sitosterol and 12-shogaol, against *α*-glucosidase. This shows that the inhibitory activity of the ligands to the target protein was high. The lower Ki scores indicate a high inhibitory activity against the target protein.[35] This is also shown by free energy binding and surface interaction scores of these substances. In this study, stigmasterol and 6-gingerdiol had a lower surface interaction score than other compounds in both herbs, however they had a high free energy binding score. Free energy binding and surface interaction

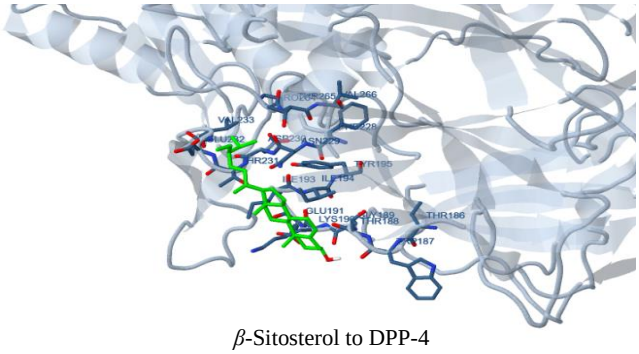
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

scores between a target protein and ligand influences the inhibitory activity against *α*-glucosidase. However, a lower free energy binding score would result in the ligand strong binding to a target molecule and an increase in their biological activity. In this case, *G. max* seed and *Z. officinale* rhizome extracts were able to inhibit the *α*-glucosidase enzyme.

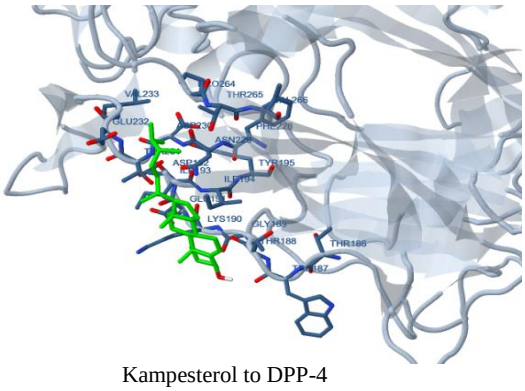
Docking molecular research is widely used to predict potential drug candidates in the pharmaceutical field. The orientation of the binding of this active substance to the molecular target indicates activity and affinity as a possible drug candidate. Moreover, the present research encouraged the *in vivo* and *in vitro* evaluation for the proposed designed compounds to validate the computational findings.[31,34]



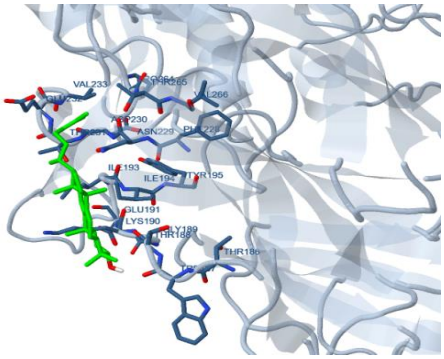
Stigmasterol to DPP-4



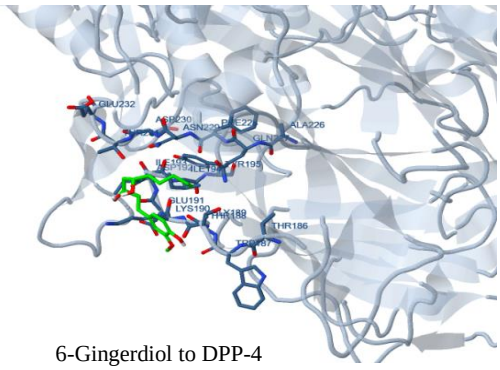
β-Sitosterol to DPP-4



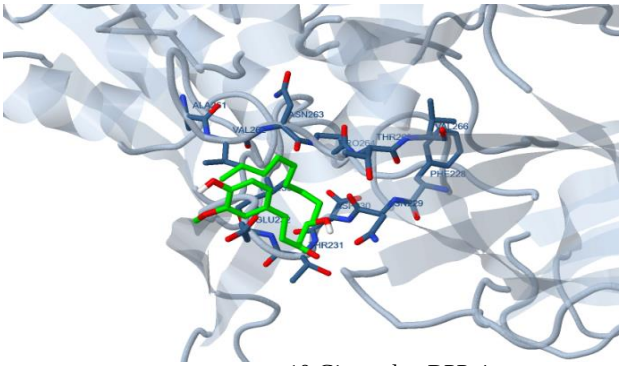
Kampesterol to DPP-4



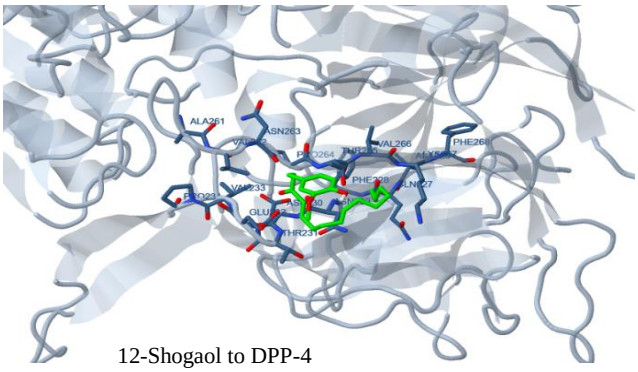
Lanosterol to DPP-4



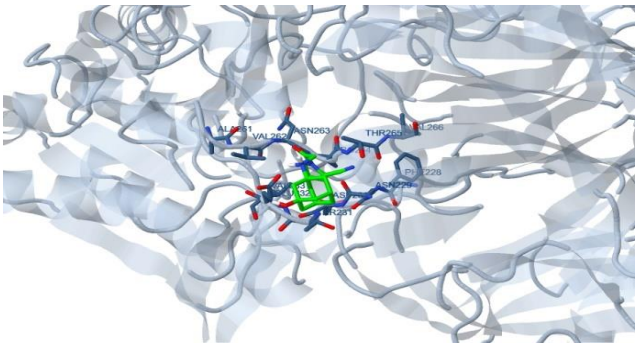
6-Gingerdiol to DPP-4



10-Gingerol to DPP-4



12-Shogaol to DPP-4



Vildagliptin to DPP-4

Figure 5: Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4

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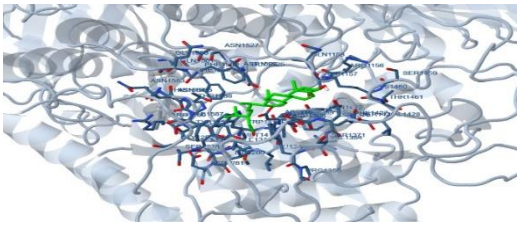
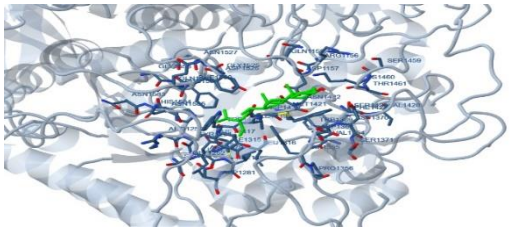
Table 4: Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with *α-glucosidase*

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (nM)	Surface Interaction (Å)
Glycine	Stigmasterol	-10.57	17.8	893.79
max	*β*-Sitoterol	-10.26	30.0	902.57
	Kampesterol	-7.79	93.6	872.98
	Lanosterol	-7.86	162.2	894.78
Zingiber	6-Gingerdiol	-5.56	83390	755.71
officinale	10-Gingerol	-4.74	336650	820.43
	12 Shogaol	-5.07	192370	774.629
	Acarbose*	-7.99	140.0	1033.81

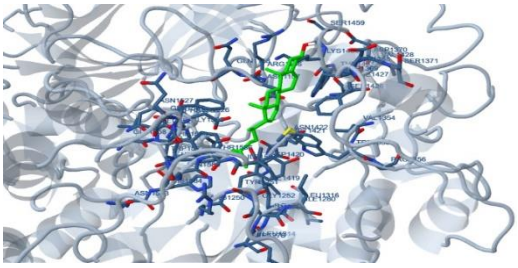
**Reference standard*



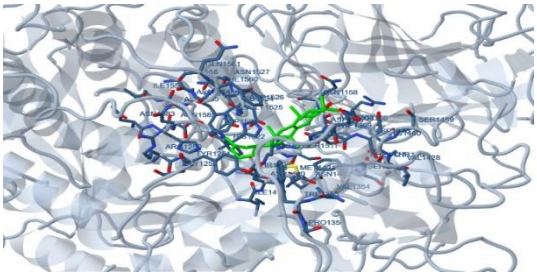
Stigmasterol to *α-glucosidase*



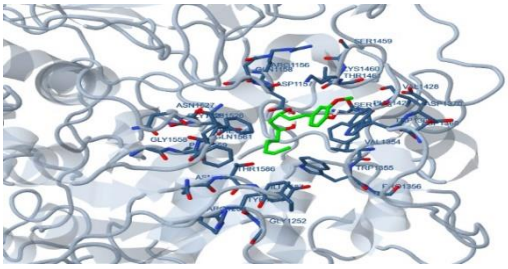
β-Sitosterol to *α-glucosidase*



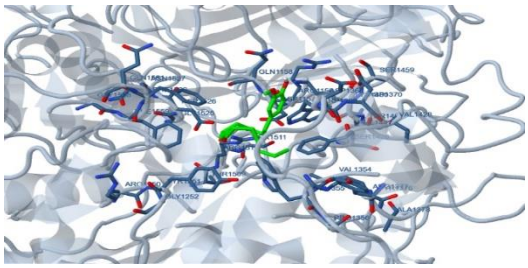
Kampesterol to *α-glucosidase*



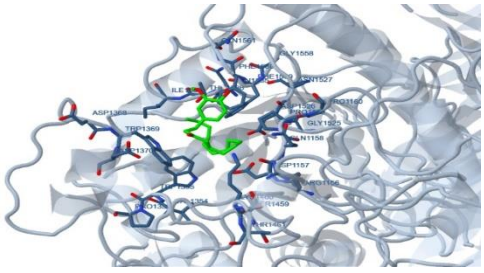
Lanosterol to *α-glucosidase*



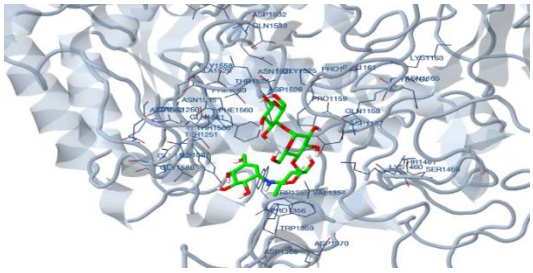
6-Gingerdiol to *α-glucosidase*



10-Gingerol to *α-*



12-Shogaol to *α-glucosidase*



Acarbose to *α-glucosidase*

Figure 6: Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with *α-glucosidase*

Conclusion

G. max seeds were found to contain phytosterols, mainly beta sitosterol, campesterol, stigmasterol, and lanosterols, while *Z.officinale* contained 6-gingerdiol, 10-gingerol and 12-shogaol. Based on *in silico* evaluation, DPP-4 was strongly inhibited by stigmasterol and 12-shogaol, while *α*-glucosidase activity was strongly inhibited by stigmasterol and 6-Gingerol. However, *G. max* seeds was found to have more potential to be used as a drug candidate for diabetes as it was able to inhibit both enzymes compared to *Z. officinale* rhizomes.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research is supported financially by the Faculty of Medicine, University of Islam Malang.

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ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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