TJNPR_MANUSCRIPT DIREVIEW

Molecular Docking of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome components asAnti-diabetic through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase Enzymes

ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase (*α-glucosidase*) are enzymes involved on carbohydrate metabolism. Inhibition of these enzymes contribute to supress increase in blood glucose level.. Soybean (*Glycine max*) seeds and ginger (*Zingiber officinale*) rhizome are herbs having activity as anti-diabetic. There mechanism of action 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* by *in-silico* study. Soybean seed and ginger rhizome were extracted using the maceration method modified with ethanol solvent. Ethanolic extract of soybean seeds and ginger rhizome were analysed using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The potency of the components from plant parts on DPP-4 and *α-glucosidase* were evaluated by *in-silico* study using web-based software (Docking server).. Phytosterols were identified in soybean seeds, there are beta sitosterol, campesterol, stigmasterol, and lanosterol. Meanwhile in ginger rhizome were found 6-gingerdiol, 10-gingerol and 12-shogaol. Molecular docking study showed that stigmasterol and 12-shogaol supress DPP-4 activity strongly, however *α-glucosidase* are inhibited stronger by stigmasterol and 6-Gingerdiol. Soybean seed and ginger rhizome have potency as anti-diabetic through inhibitory activity of DPP-4 and *α-glucosidase*, however soybean seed more potent to inhibit both of the enzyme compare to ginger rhizome. The grammar is very poor. An English editor should correct the entire manuscript before review. The meaning of most sections of the write-up was lost due to poor expression.

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

Introduction

Incretin hormone especially GLP-1 has potency as anti-diabetes. However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively to become inactive forms[1]. GLP-1 have a short half-life, approximately 2-5 minutes due to DPP-4 activity[1,2]. The inhibition of DPP-4 is effective to treat Type 2Diabetes Mellitus (T2DM) due to GLP-1 bioavailability can be retained, moreover it was able to regulate blood glucose level[3]. Meanwhile, alpha-glucosidase (*α-glucosidase*) are enzyme that responsible for conversion carbohydrate into maltosa, dextrin and maltotriosa[4]. The location ofenzyme in the *brush border* of the small intestines and the product of carbohydrate metabolism is delivered to small intestinal mucosa.Therefore, they are hydrolyzed by *α-glucosidase* become glucose and absorbed into blood circulation[5]. It contributes an increase of blood glucose level post prandial and need to be controlled to avoid hyperglycemia. The inhibitory activity both of DPP-4 and *α-glucosidase* prevent the increase of blood glucose level especially for diabetes mellitus[3,4].

Oral Hypoglycemic Drug(OHD) is used to cure T2DM patient. They have activity by increasing of insulin secretion, repairing of insulin resistance and inhibiting of glucose absorption. However, there are many adverse reaction often be found on DM patient due to OHD using[6]. Moreover, it induces people to search alternative sources of medicine that derive from nature. Herbs is one of the natural product and becoming popular medications choices in the management of disease due to it has less adverse reaction and holistic care property[7,8].

Glycine max(G. max) or soybean and *Zingiber officinale(Z. officinalle)* or ginger are functional food are known to cure some diseases empirically.Animal study showed*G.max* have a hypoglycemic activity[9]. Isoflavon compound like daidzein and genestein were predicted as a leads compound in *G. Max*[10]. Meanwhile, *Z. officinalle* rhizome has anti-hyperlipidemic and antioxidant potential by gingerol and shogaol compounds. Other studies have reported that ginger has anti-diabetes and antioxidant effects so that it can help reduce blood glucose levels and also its complication[11]. The anti-diabetes effect is thought due to active compounds such as phytosterols, flavonoids, saponins, phenols, and essential oils[10,11,12]. Several studies have proven the potential of soybean seeds and ginger rhizome as anti-diabetes (cite atleast three of such studies), however the mechanism of action and active substances of soybean seed extract and ginger rhizome controlling blood glucose levels is still unknown clearly. The purpose of this study was to evaluate the mechanism of action of soybean seed extract and ginger rhizome as anti-diabetic through inhibitory activity both of DPP-4 and *α-glucosidase*using *in-silico* study. The grammar should be improved. English editor needed. This section should be rewritten to give a better flow.

Material and Methods

Preparation of Glycine max and Zingiber officinale Extract

Soybean seeds were obtained from Argo Mulyo variety with a letter of determination number 074/241/102.7/2017. Meanwhile, the ginger rhizome was obtained from Balai Materia Medika with a letter of determination 074/211/102.7/2017. Extraction of the two herbs using the maceration method with slightly modification. Both of soybean seed and ginger root were extracted by ethanol solvent at 50°C for 48 hours and shacked it. Give more details on the procedure from grinding/pulverization of the plant materials. This should go under the next heading.

Identification of active substances

Ethanolic extract of *G. max* seed and *Z. officinale* rhizome was performed a qualitative analysis using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific. The mobile phase of 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. Hypersilgold as a stationary phase[14].Complete the report and improve grammar.

Molecular docking study

The chemical structure of ligands (phytosterol compounds of soybean seeds and terpenoids from ginger rhizome) and reference drugs 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/)[16].

Show the crystal structure of the two enzymes here.

Moreover, protein homology on the Swiss Model (http://www.swissmodel.expasy.org/interactive), the modeling results can be downloaded in a PDB file[17]. The ligand-protein docking was performed using web-based software (dockingserver.com). 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 ethanolic extract of soybean seeds and ginger rhizomes using the LC-MS / MS method can be seen in figure 1, figure 2, and table 1.

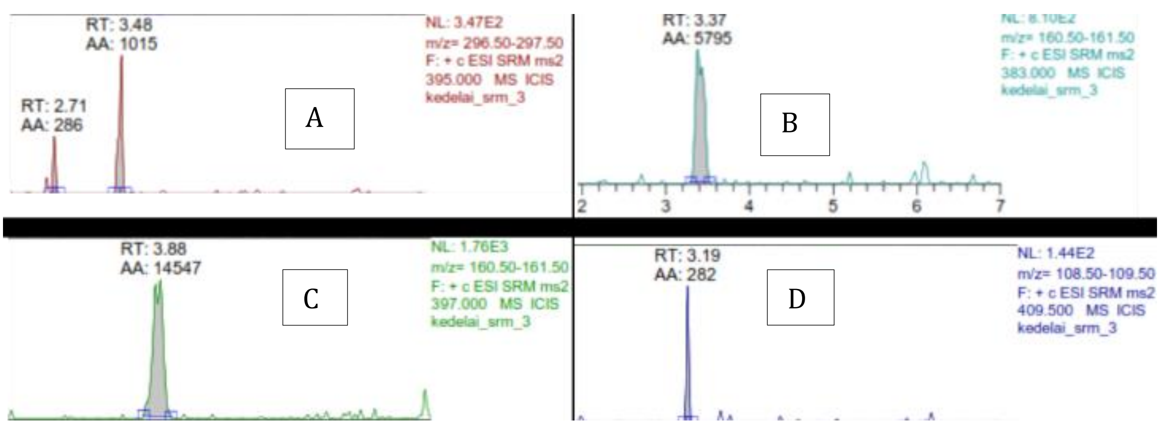

Figure 1. Chromatogram of active compound in ethanolic extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) β-Sitosterol, (D) Lanosterol Image quality is poor!

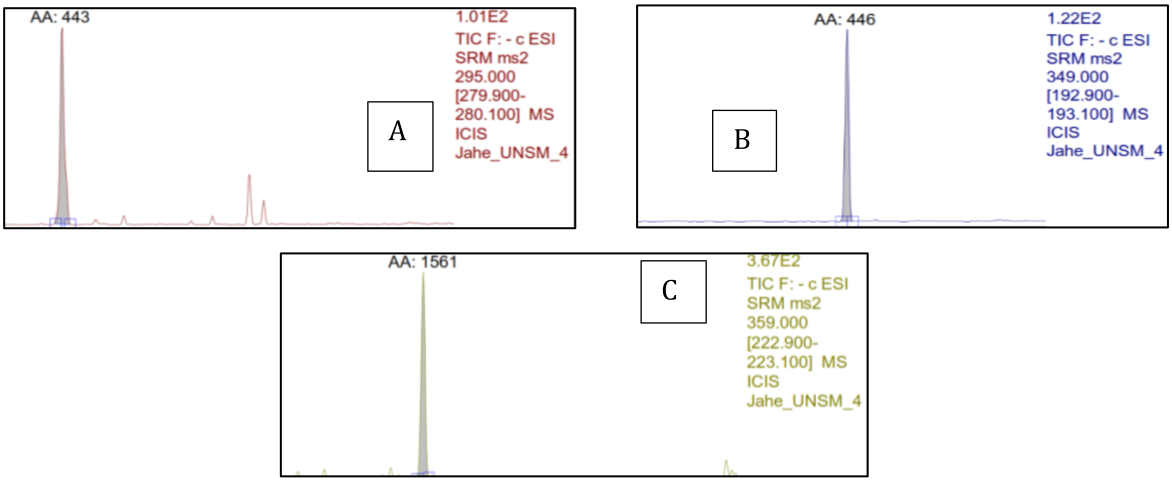

Figure 2.Chromatogram of active compound in ethanolic extract of Zingiber officinale rhizome. (A) 6-Gingerdiol, (B) 10-Gingerol, (C) 12-Shogaol

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)**	**Ethanol Extract of Soybean Seeds the significance of this column is not clear**
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	+++

Note: (-) None, (+) Weak, (++) Moderate, (+++)Strong

The qualitative analysis using LC-MS / MS showed that active substances contained in the ethanol extract of soybean seeds were Stigmasterol, Campesterol, β-Sitosterol, and Lanosterol. The highest level is β-Sitosterol, whereas the lowest level is Lanosterol[18]. Meanwhile, the active compounds contained in the ethanol extract of ginger root are 6-Gingerdiol, 10-Gingerol, and 12-Shogaol. The highest levels is 12-Shogaol, however the lowest level is has? 6-Gingerdiol [19].

Four active compounds were identified in *G. max* seed extract and three active substances in *Z. officinale* rhizome extract in ethanol. The active compound can be 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 global research or previous findings[20]. The active substances of soybean seeds and ginger rhizome are secondary metabolites and have biological activity, moreover they can be used as candidates phytopharmaca[21].

The main components of phytosterols in soybean seeds are β-sitosterol, campesterol, and stigmasterol. All of them are classified into secondary metabolite groups and have biological activity that can be used to cure diseases. Stigmasterol is one of the phytosterol groups in plants to maintain the balance of phospholipid membranes from plant cells which are chemically similar to cholesterol in animal cell membranes. Stigmasterol can inhibit cholesterol biosynthesis through the inhibition of sterol reductase in cells. On the other hand, stigmasterol has the potential to be anti-inflammatory, anti-tumor, anti-osteoarthritis, hypoglycemic and antioxidant effects[22]. Meanwhile, β-Sitosterol acts as anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant[23]. Campesterol plays a role in lowering blood cholesterol and has anti-carcinogenic effects. However, these compounds also have anti-angiogenic effects by inhibiting endothelial cell proliferation and capillary differentiation[24]. Some studies both of *in vivo* and *in vitro* showed that lanosterol has activity as anti-cataract[25]. *Ginger 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 ginger extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus*[28].

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

Activity of Soybean seed and ginger rhizome extracts both of on DPP-4 and *α-glucosidase* were evaluatedby *in-silico* approach and the results can be seen at Table 2 and Table 3.

Table 2. Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with DPP-4

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (µM)	Surface Interaction (Å)
Glycine max	Stigmasterol	-7.11	6.16 µM	931.16
	β-Sitosterol	-6.94	8.13 µM	908.89
	Kampesterol	-6.84	9.74 µM	877.50
	Lanosterol	-7.03	7.06 µM	860.98
Zingiber officinale	6-Gingerdiol	-2.92	7.26 mM	494.287
	10-Gingerol	-2.38	18.12 mM	679.164
	12 Shogaol	-3.13	5.11 mM	631.305
	Vildagliptin*	-7.76	2.03 µM	512.40

* *reference drug*

Show binding interactions

Docking studies showed that the lowest inhibitory constants and binding free energy in each herb were stigmasterol and 12-Shogaol, however the surface interaction was high actually. Meanwhile, β-Sitosterol, campesterol and lanosterol have a higher binding free energy score compare to stigmasterol and vildagliptin as a reference drug. 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.

In the pharmaceutical field, molecular docking is often used to screen and predict the potential candidates the drug target of ligands with a known structure, based on its free energy binding, inhibition constant, and surface interaction. The score of free energy binding represent of binding affinity of ligand to the target protein, the lower free energy binding, the higher binding affinity[29]. In addition, inhibition constant (Ki) can be predicted using bioinformatics approach to evaluate bioactivity of substances. The lowest Ki is the most potential active compound, however the toxicity must be tested[30]. Other parameter is surface interaction, it represents the molecular recognition between ligand and target protein. The higher value of surface interaction, the higher the interaction possibilities of compounds interacting with the target protein[31]. Based on those categories, stigmasterol and 12-shogaol havethe lowest value of inhibition constants activity on DPP-4,followed by lanosterol and 6-gingerdiol activity on DPP-4. It is related to binding free energy and surface interaction of these compounds. In this study, stigmasterol and 12-shogaol havethe highest value of surface interaction compare to the reference drug. A great result of surface interaction showed a stronger bond between ligand and protein target,moreover the biology activity is higher[30]. Based on the *in-silico* analysis, stigmasterol and 12-shogaol have the lowest value in binding free energy, meanwhile lanosterol and 6-gingerdiol were in the second position. The lowest value of binding free energy produces a strong binding of molecule, therefore it causes the potential biology activity. Free energy binding and surface interaction between ligand and protein target affects the inhibitory activity of*Glycine max* and *Zingiber officinale* extract on DPP-4.

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

Table 3. Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with *α-glucosidase*

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (μM)	Surface Interaction (Å)
Glycine max	Stigmasterol	-10.57	17.8 nM	893.79
	β-Sitoterol	-10.26	30.0 nM	902.57
	Kampesterol	-7.79	93.6 nM	872.98
	Lanosterol	-7.86	162.2 nM	894.78
Zingiber officinale	6-Gingerdiol	-5.56	83.39 μM	755.71
	10-Gingerol	-4.74	336.65 μM	820.43
	12 Shogaol	-5.07	192.37 μM	774.629
	Acarbose*	-7.99	140.00 nM	1033.81

* *reference drug*

Show binding interactions

In-silico studies indicated that stigmasterol and 6-gingerdiol have a low value of the inhibition constant, the binding free energy, and surface interaction compared to other active compound. Meanwhile, lanosterol and 10-gingerol have a higher value on binding free energy and inhibition constant than other substances in *G. max* and *Z. officinale* respectively. The differences in each parameter value cause the distinction of inhibitory activity on *α-glucosidase*[33]. Based on inhibition constant value, stigmasterol and β-sitosterol more potential to inhibit activity of *α-glucosidase*. Stigmasterol is stronger than active compound in ginger and acarbose also as a drug reference drug.

Based on active compound both of their extracts, stigmasterol in soybean and 6-gingerdiol in ginger have the lowest score of inhibition constant, followed by β-sitosterol and 12-shogaol against *α-glucosidase*. It is proved through the inhibitory activity of the ligands to the target protein was high. The lower Ki scores will indicate the high inhibitory activity against the target protein[34]. It is related to the free energy of binding and surface interaction of these substances. In this study, stigmasterol and 6-gingerdiol had a lower surface interaction score than some active compounds in both of herbs, however they have a high free energy binding value. Free energy of binding and surface interaction between molecule target and ligand influences the inhibitory activity of *α-glucosidase*. However, the lowest score of binding free energy results in a strong binding molecule, furthermore, it causes an increase in their biological activity of soybean seed and ginger rhizome 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 research also encouraged the *in vivo* and *in vitro* evaluation for the proposed designed compounds to validate the computational findings[30,33]. The overall grammar needs improvement to give meaning to the discussion.

Conclusion

Stigmasterol and 12-Shogaol strongly inhibit DPP-4 activity, meanwhile *α-glucosidase* activity was strongly inhibited by stigmasterol and 6-Gingerol. However, soybean seeds have more potential to inhibit both of the enzymes compare to ginger rhizomes. The conclusion is too scanty. State the aim of the study and the findings before the closing statement.

Conflict of interest

We declare that we have no conflict of interest

Authors' Declaration

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A. MANUSCRIPT

Journal	**Tropical Journal of Natural Product Research**
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B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Abstract	Needs to be rewritten. The grammar is poor.
Introduction	Better organization is required. Poor grammar.
Methodology	More information in the form of enzyme crystal structure should be included.
Results	The binding interactions between enzyme protein and ligands were not included. Please add.
Discussion	Binding interactions need to be shown and discussed.
Conclusion	Too short. More details on the findings should be added.
References	It did not follow the journal format.
Figures, Tables	Figure 1 is not as sharp as figure 2. Please improve the quality of Figure 1.

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.

Though the report has some interesting findings, the grammar could not clearly bring out these observations. The level of grammar in the entire text is not acceptable.

D. REVIEWER'S RECOMMENDATION
Please mark with "**X**" one of the options.

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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

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 modified with ethanol solvent. Ethanolic extract of soybean seeds and ginger rhizome were analysed using Liquid Chromatography-Mass Spectrometry (LC-MS/MS). The potency of the components from plant parts 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 glycaemic 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 maltosa, dextrin and maltotriosa.[4] These enzymes are commonly found in the *brush border* of the small intestines, whereas the products of carbohydrate metabolism are delivered in to 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]

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, as with other drugs, long-term consumption of these medications is often accompanied with various adverse 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 (G. max) or soybean and *Zingiber officinale (Z. officinalle)* or ginger are functional food known empirically to cure some diseases, which also has been backed by laboratory-based of *G. max* have shown it to be hypoglycemic,[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.

Material and Methods

Preparation of Glycine max and Zingiber officinale Extract

Soybean seeds were obtained from Argo Mulyo variety with a letter of determination number 074/241/102.7/2017. Ginger rhizome was obtained from Balai Materia Medika with a letter of determination 074/211/102.7/2017. Dried herbs or simplisia were pulverized by size reduction machine. Extraction of the two herbs using the maceration method with slight modifications. Both soybean seed and ginger root were extracted by ethanol solvent at 50℃ for 48 hours and shaken in rotary shaker.

Identification of active substances

Ethanolic extract of *G. max* seed and *Z. officinale* rhizome was 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 used were 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, i.e. firstly the tested compounds and the comparative compound 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 the tested compounds and the reference drug 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 reference drugs 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/).[16] The crystal structure both of the enzyme shown in figure 1 and figure 2 below.

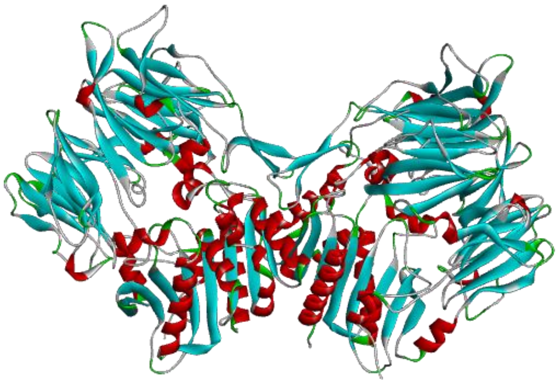


Figure 1. Crystal Structure of Dipeptidyl Peptidase-4

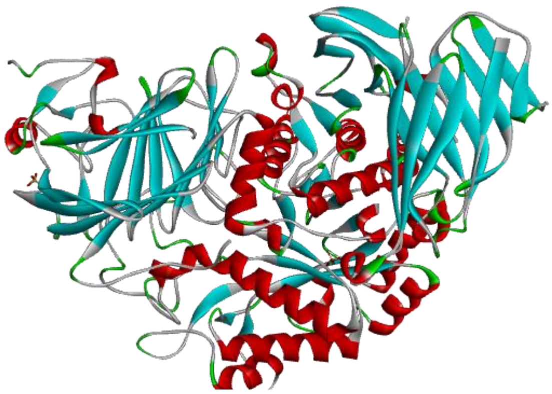


Figure 2. Crystal Structure of *α-glucosidase*

Moreover, protein homology on the Swiss Model (www.swissmodel.expasy.org/interactive), the modeling results can be downloaded in a PDB file.[17] The ligand-protein docking was performed using web-based software (dockingserver.com). 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 ethanolic extract of soybean seeds and ginger rhizomes using the LC-MS / MS method can be seen in figure 3, figure 4, and table 1.

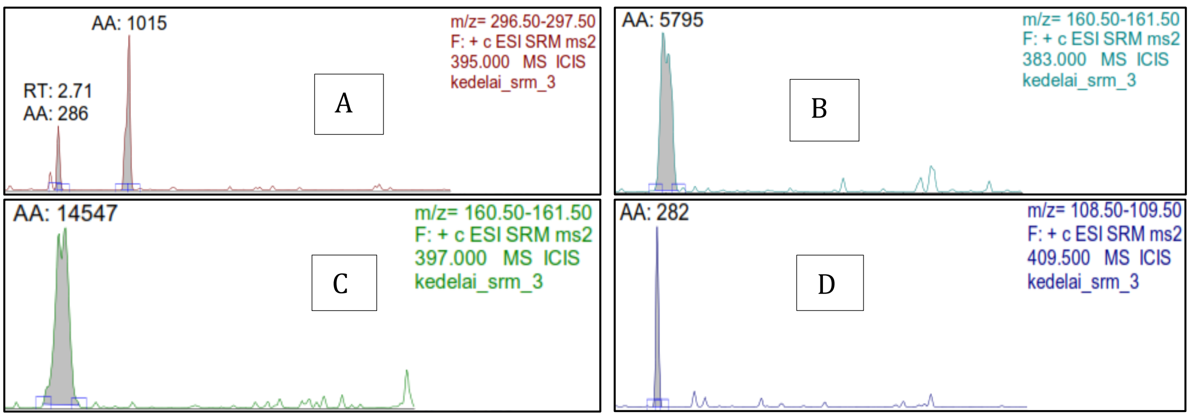


Figure 3. Chromatogram of active compound in ethanolic extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) β-Sitosterol, (D) Lanosterol

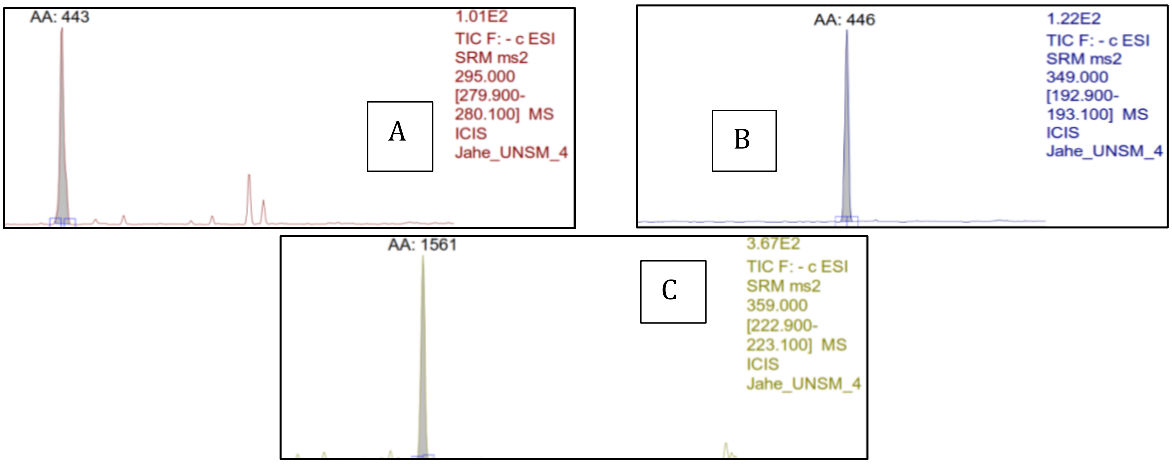


Figure 4.Chromatogram of active compound in ethanolic extract of Zingiber officinale rhizome. (A) 6-Gingerdiol, (B) 10-Gingerol, (C) 12-Shogaol

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

The qualitative analysis using LC-MS / MS showed that active substances contained in the ethanol extract of soybean seeds were Stigmasterol, Campesterol, β-Sitosterol, and Lanosterol. The highest level is β-Sitosterol, whereas the lowest level is Lanosterol.[18] Meanwhile, the active compounds contained in the ethanol extract of ginger root are 6-Gingerdiol, 10-Gingerol, and 12-Shogaol. The highest level is 12-Shogaol, whereas the lowest level is 6-Gingerdiol.[19]

Four active compounds were identified in *G. max* seed ethanolic extract and three active substances in *Z. officinale* rhizome ethanolic extract. These active compounds was 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 soybean seeds and ginger rhizome are secondary metabolites and have biological activity, moreover, they can be used as candidates phytopharmaca.[21]

As mentioned before, the main components of phytosterols in soybean 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 disease, whereas β-Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant[23] and 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 of *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 to be anti-inflammatory, anti-tumor, anti-osteoarthritis, hypoglycemic and antioxidant effects.[22] On the other hand,

Ginger 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 ginger extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.[2]

Prediction of physicochemical properties

The result of the *in silico* study of the physicochemical properties of *Glycine max* and *Zingiber officinale* active compound can be seen at table 2

Table 2. Prediction of physicochemical properties of active compound *G. max* and *Z. officinale* compound

Herbs	Active Compounds	MW	Log P	Fr. Csp3	Torsion	HBA	HBD	PSA (A^2)	Water Solubility
G. 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
Z. 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; LogP=logarithm of octanol/water partition coefficient; Torsion= bond between rotating atoms; HBA=H-bond acceptors; HBD=H-bond donors; PSA=polar surface activity

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 (> 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, others compound did not meet in 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 meet the Lipinski Rules of Five completely, meanwhile five others did not fulfill these rule.[29] Hence, it can be predicted that 6-gingerdiol and 10-gingerol will be easily absorbed and have high permeability.

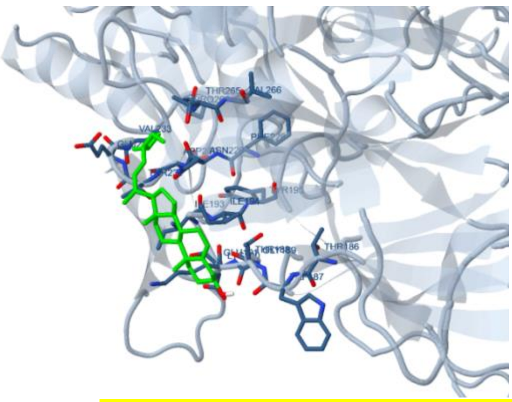
Molecular docking of Glycine max and Zingiber officinale on DPP-4

Activity of Soybean seed and ginger rhizome extracts both of on DPP-4 were evaluated by *in-silico* approach and the results can be seen at Table 3 and Figure 5.

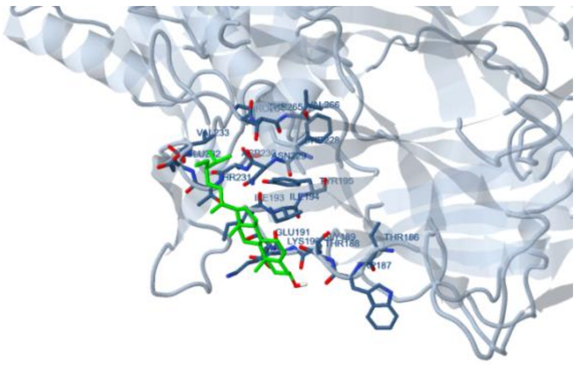
Table 3. Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with DPP-4

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (µM)	Surface Interaction (Å)
Glycine Max	Stigmasterol	-7.11	6.16 µM	931.16
	β-Sitosterol	-6.94	8.13 µM	908.89
	Kampesterol	-6.84	9.74 µM	877.50
	Lanosterol	-7.03	7.06 µM	860.98
Zingiber Officinale	6-Gingerdiol	-2.92	7.26 mM	494.287
	10-Gingerol	-2.38	18.12 mM	679.164
	12 Shogaol	-3.13	5.11 mM	631.305
	Vildagliptin*	-7.76	2.03 µM	512.40

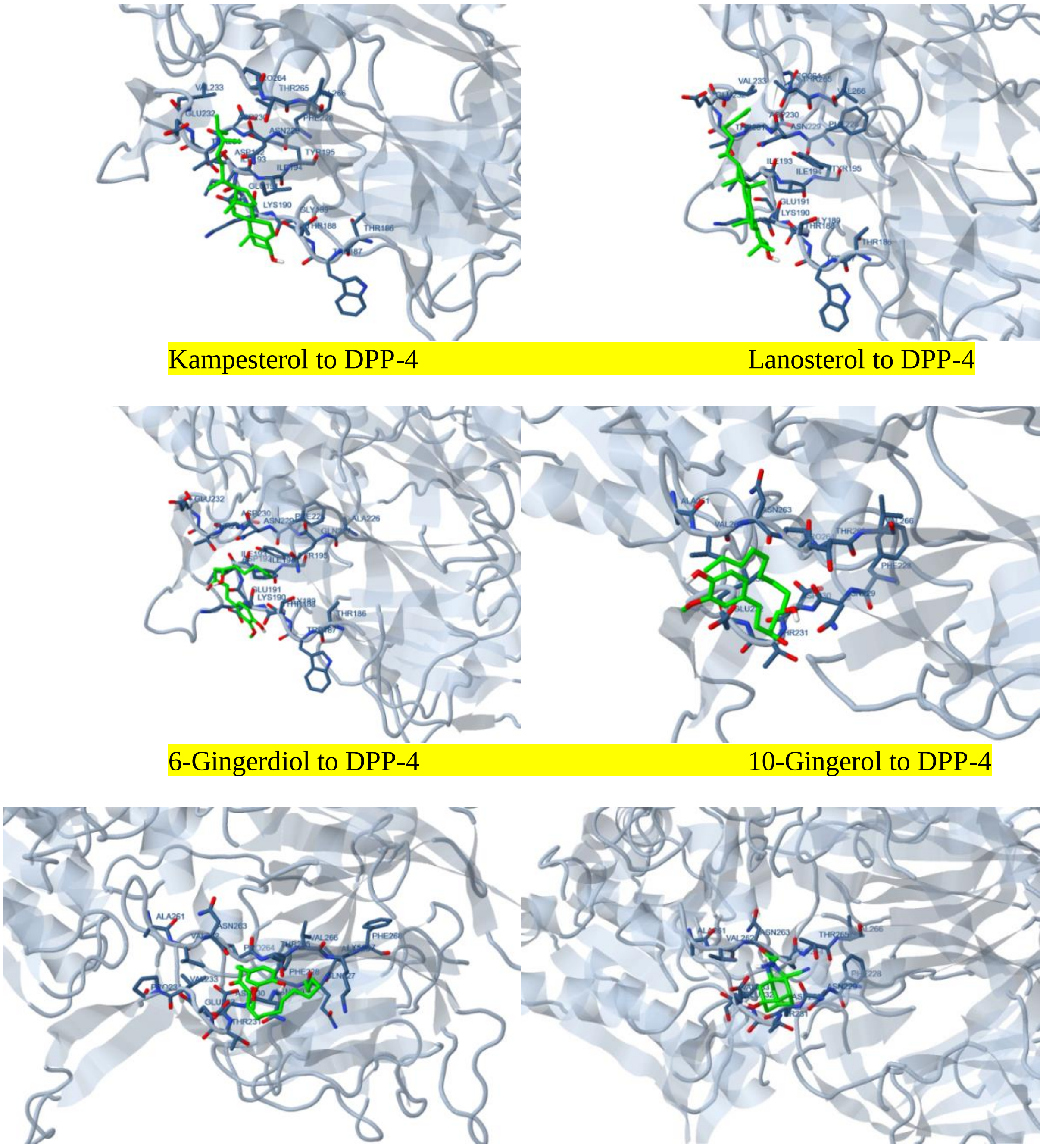
* *reference drug*



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

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 drug). 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.

In the pharmaceutical field, molecular docking is often used to screen and predict the potential candidates the 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 to this, bioinformatics can also evaluate the bioactivity of a substance by predicting its inhibition constant (Ki). The lowest Ki score would indicate to the most potentially active compound, however, the toxicity of said 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 occurs.[32]

Based on those categories, on 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 drug used. 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 *Glycine max* and *Zingiber officinale* extract on DPP-4.

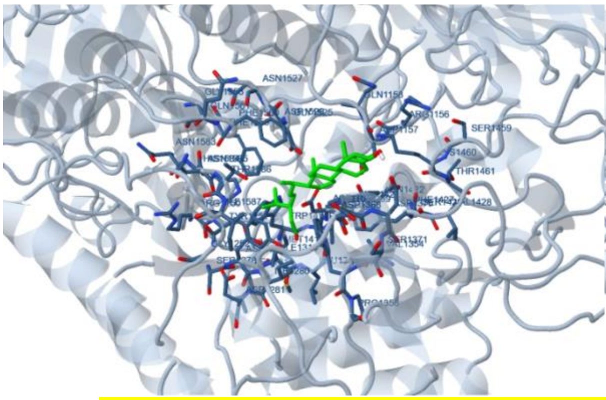
Molecular docking of Glycine max and Zingiber officinale on alpha-glucosidase

Activity of Soybean seed and ginger 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

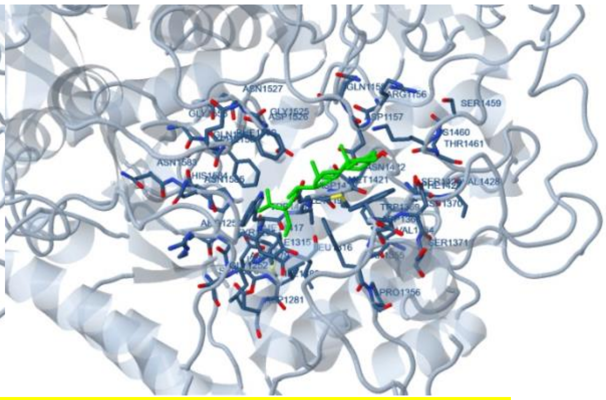
Table 4. Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with *α-glucosidase*

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (μM)	Surface Interaction (Å)
Glycine max	Stigmasterol	-10.57	17.8 nM	893.79
	β-Sitoterol	-10.26	30.0 nM	902.57
	Kampesterol	-7.79	93.6 nM	872.98
	Lanosterol	-7.86	162.2 nM	894.78
Zingiber officinale	6-Gingerdiol	-5.56	83.39 μM	755.71
	10-Gingerol	-4.74	336.65 μM	820.43
	12 Shogaol	-5.07	192.37 μM	774.629
	Acarbose*	-7.99	140.00 nM	1033.81

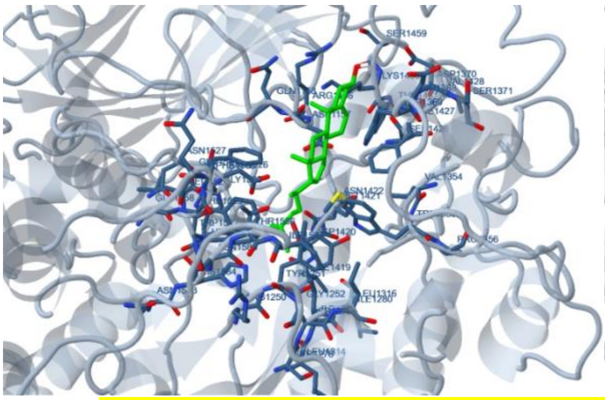
* *reference drug*



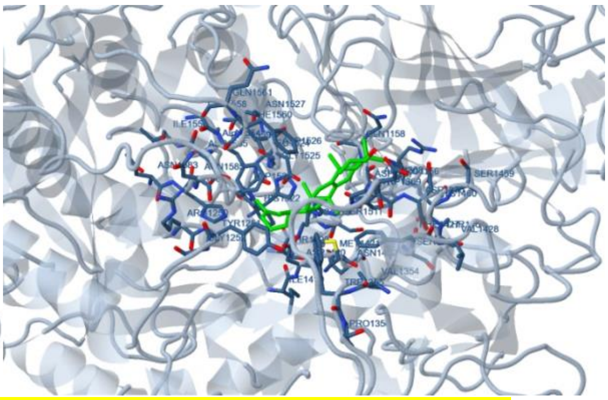
Stigmasterol to *α-glucosidase*



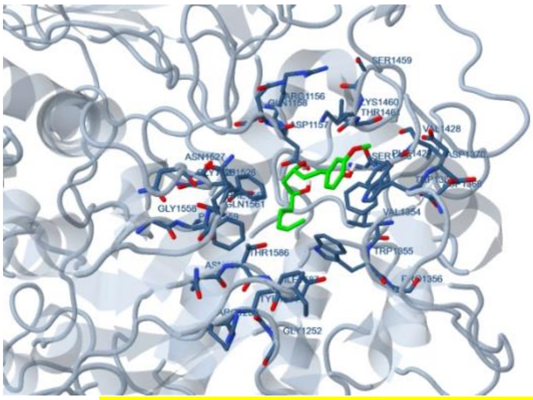
β-Sitosterol to *α-glucosidase*



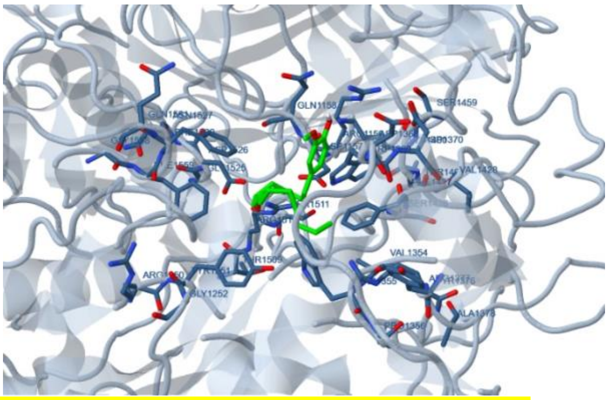
Kampesterol to *α-glucosidase*



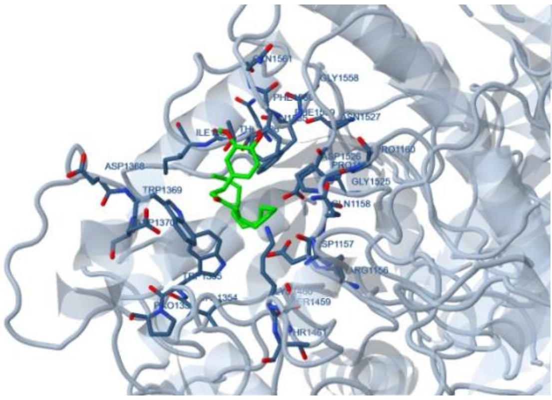
Lanosterol to *α-glucosidase*



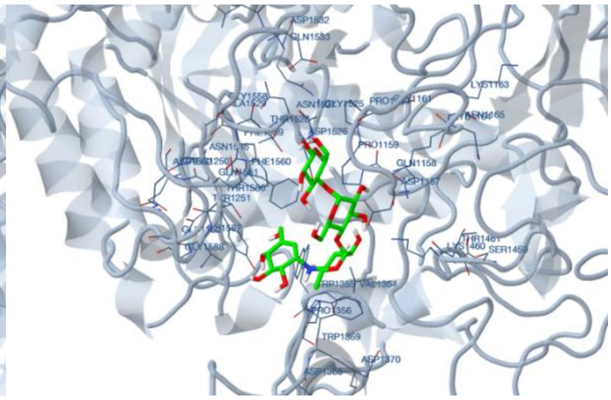
6-Gingerdiol to *α-glucosidase*



10-Gingerol to *α-glucosidase*



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*

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 and Ki compared to than 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 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 ginger as well as acarbose (which was used as a drug reference drug).

Based on the active compounds found in both of the extracts, stigmasterol in soybean and 6-gingerdiol in ginger 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 some active compounds in both herbs, however they had a high free energy binding score. Free energy binding and surface interaction scores between a target molecule and ligand influences the inhibitory activity against *α-glucosidase*. However, a lower free energy binding score would result the ligand to strongly bind to a target molecule and an increase in their biological activity. In this case, soybean seed and ginger 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 research also encouraged the *in vivo* and *in vitro* evaluation for the proposed designed compounds to validate the computational findings.[31,34]

Conclusion

This study aims to evaluate the activity of the compounds extracted from soybean seeds and ginger rhizome against DPP-4 and *α-glucosidase* using an in-silico evaluation. Soybean seeds were found to contain phytosterols, mainly beta sitosterol, campesterol, stigmasterol, and lanosterols, while ginger 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, soybean seeds was found to have more potential to be used as a drug candidate for T2DM as it was able to inhibit both enzymes compared to ginger rhizomes.

Conflict of interest

We declare that we have 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.

Aknowledgements

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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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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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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.

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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.

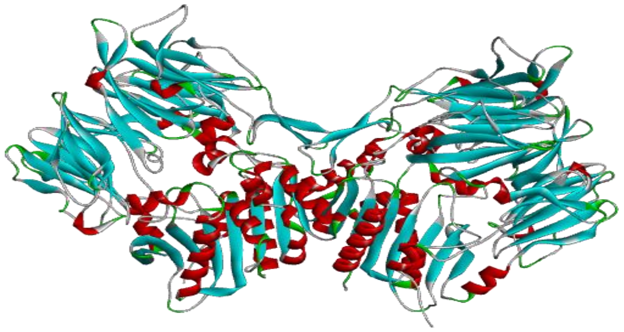

Figure 1: Crystal Structure of Dipeptidyl Peptidase-4

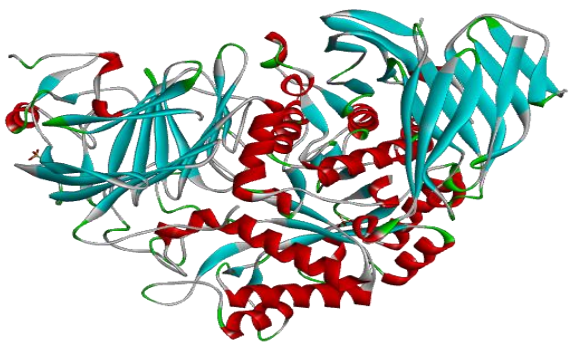

Figure 2: Crystal Structure of *α-glucosidase*

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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(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.

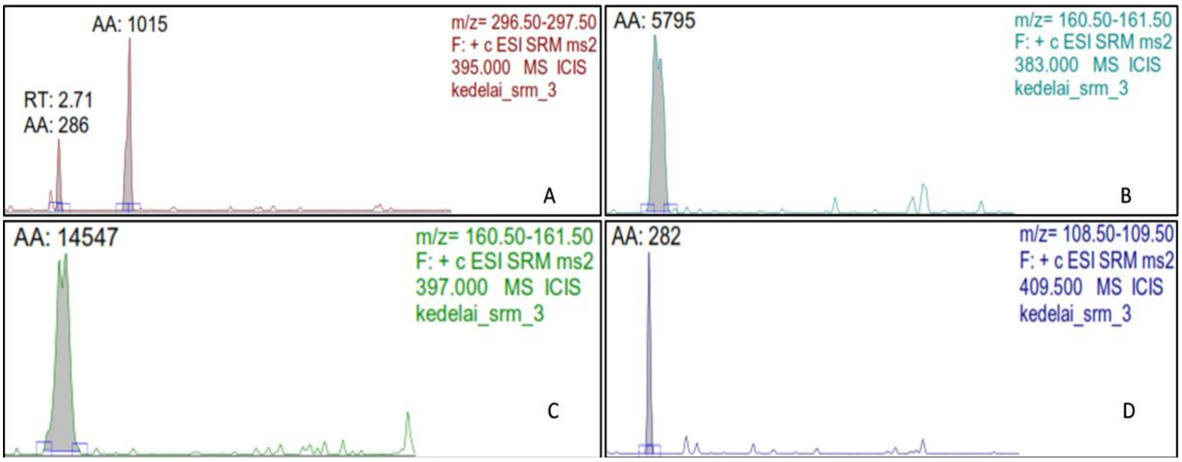

Figure 3: Chromatogram of active compound in ethanol extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) β-Sitosterol, (D) Lanosterol

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

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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

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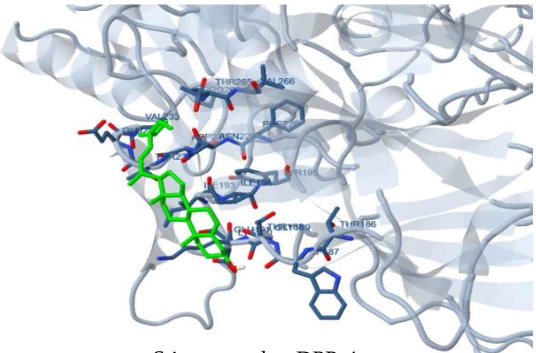
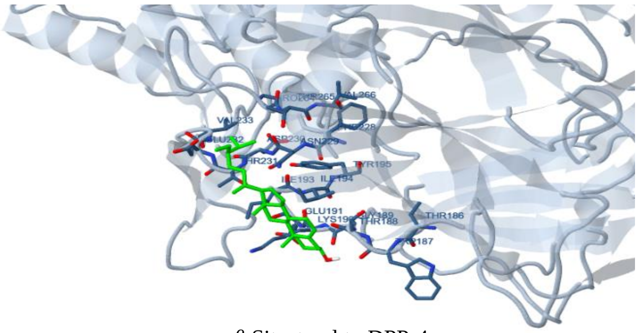
scores between a target protein and ligand influences the inhibitory activity against *α*-glucosidase. However, a lower free energy binding score would result in the ligand strong binding to a target molecule and an increase in their biological activity. In this case, *G. max* seed and *Z. officinale*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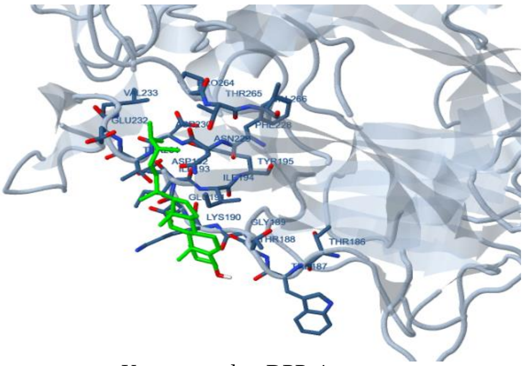
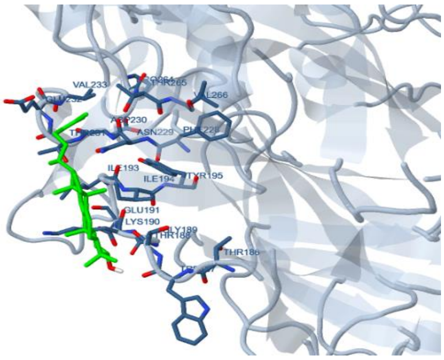
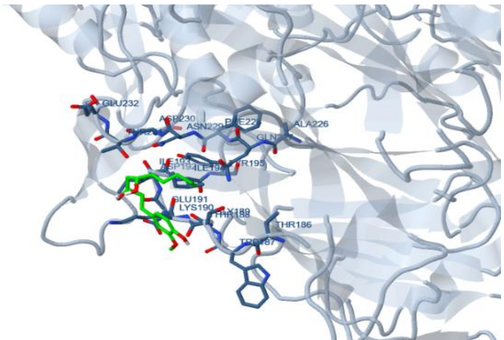
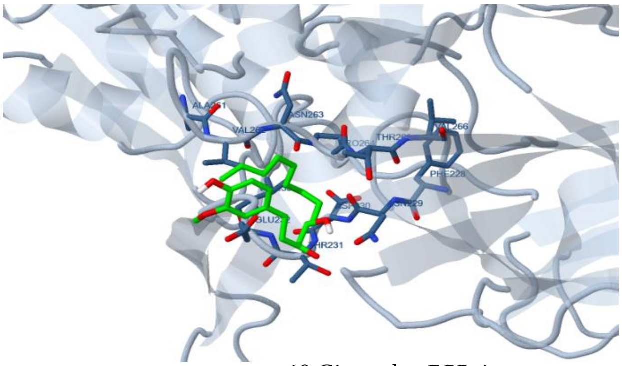
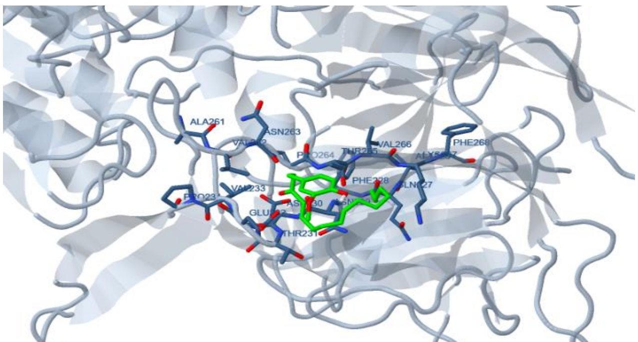

Kampesterol to DPP-4


Lanosterol to DPP-4


6-Gingerdiol to DPP-4


10-Gingerol to DPP-4


12-Shogaol to DPP-4

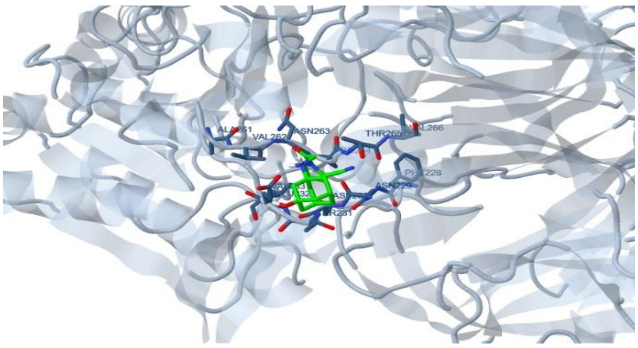

Vildagliptin to DPP-4

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

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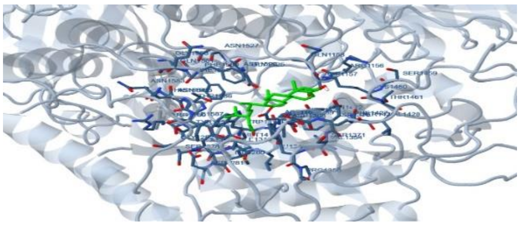
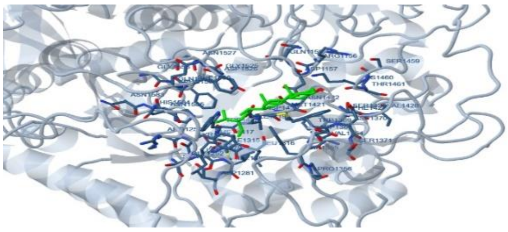
Table 4: Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with *α-glucosidase*

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

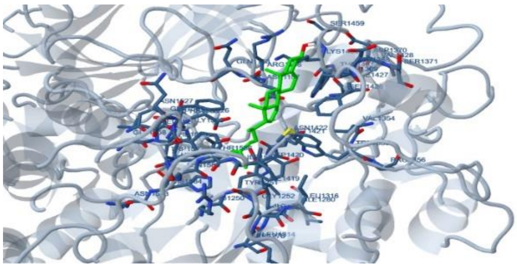
**Reference standard*



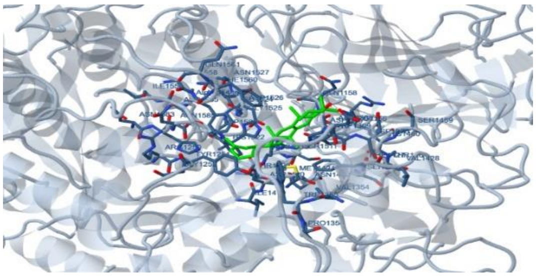
Stigmasterol to *α-glucosidase*



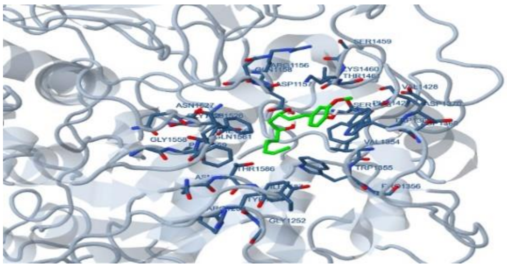
β-Sitosterol to *α-glucosidase*



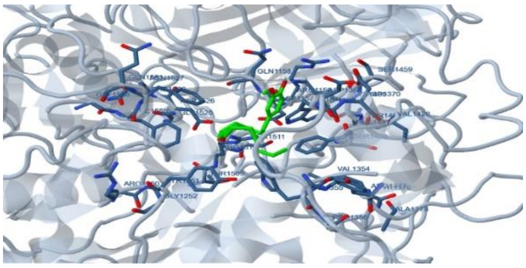
Kampesterol to *α-glucosidase*



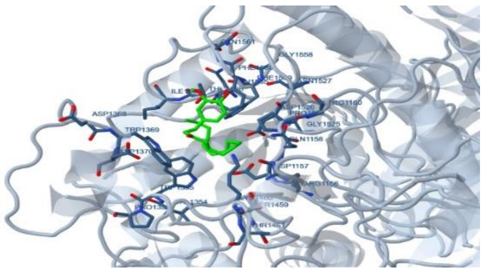
Lanosterol to *α-glucosidase*



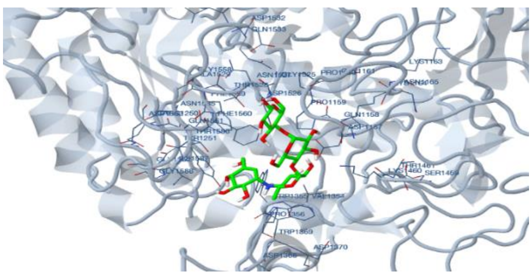
6-Gingerdiol to *α-glucosidase*



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*

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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.

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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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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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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.

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Material 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.

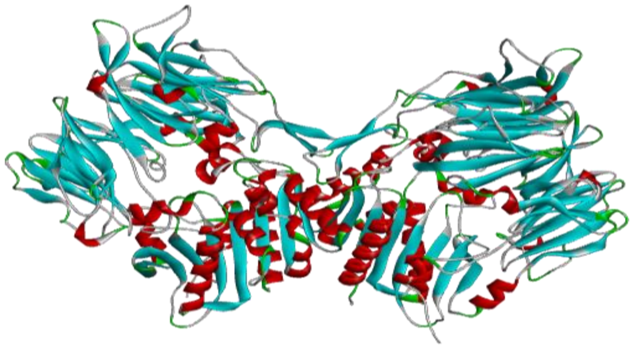

Figure 1: Crystal Structure of Dipeptidyl Peptidase-4

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

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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 (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.

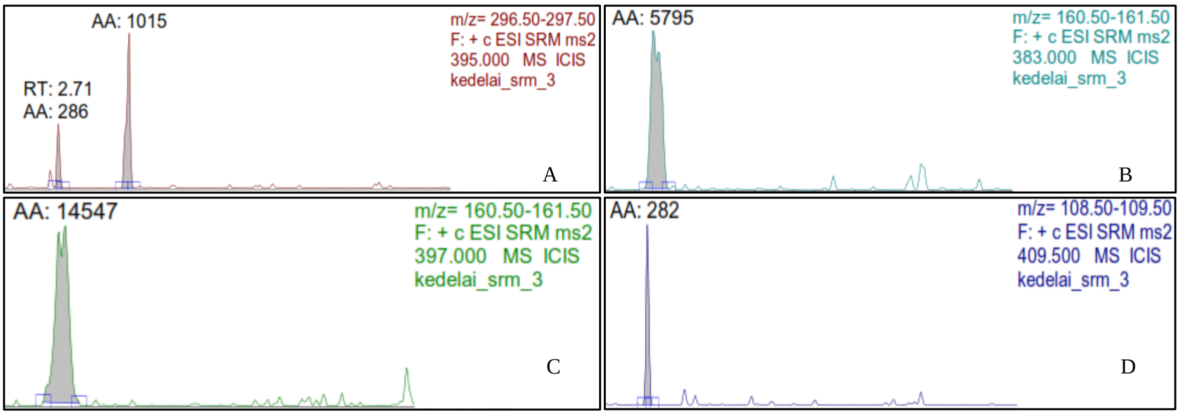

Figure 3: Chromatogram of active compound in ethanol extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) *β*-Sitosterol, (D) Lanosterol

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)
	Stigmasterol	395-297	395	1015
Glycine	Kampesterol	383-161	383	5795
max	*β*-Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
Zingiber	6-Gingerdiol	295-280	295	443

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officinale	10-Gingerol	399-193	399	446
	12-Shogaol	359-223	359	1561

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; LogP=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

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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 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]

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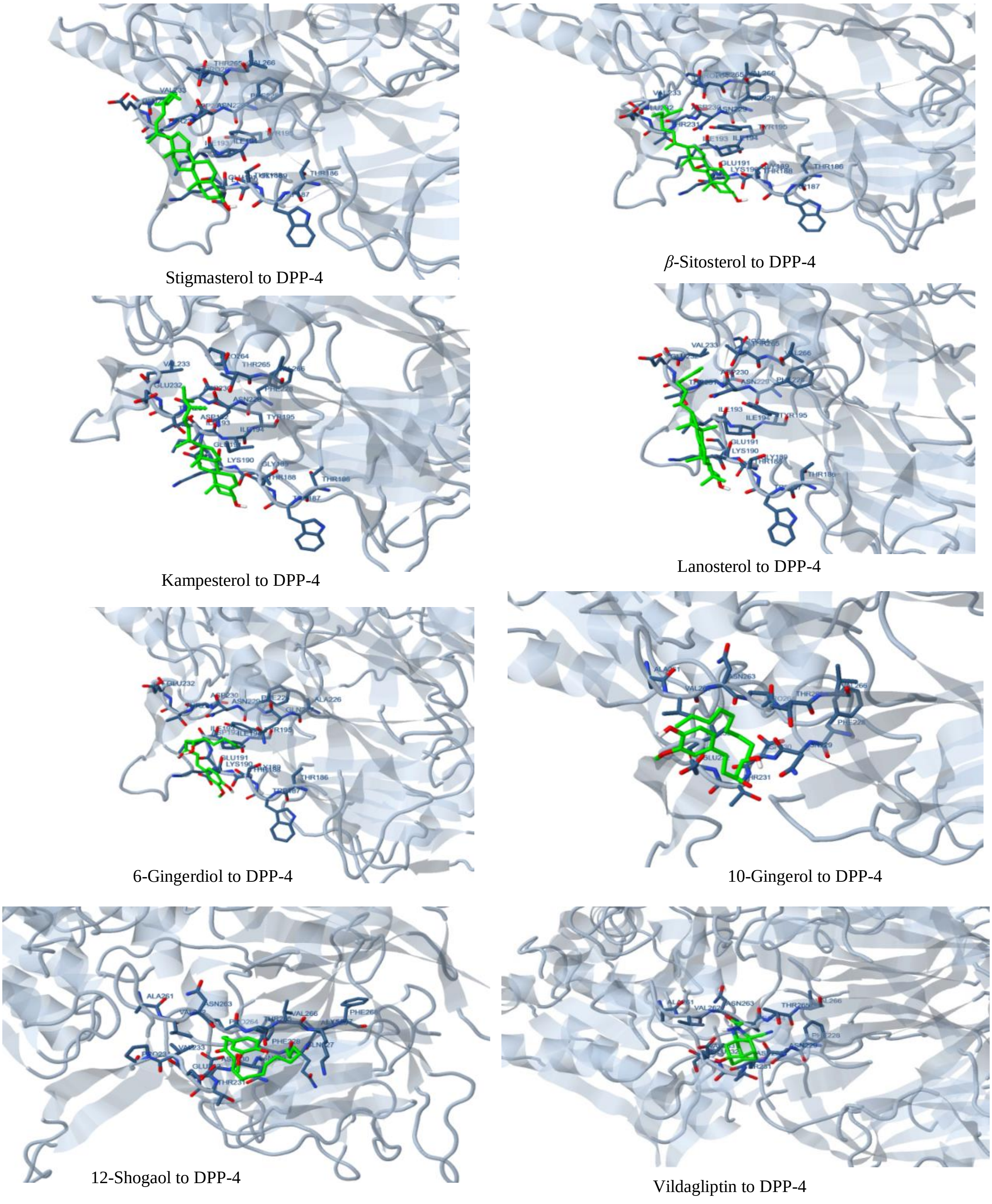

Figure 5: Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4

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

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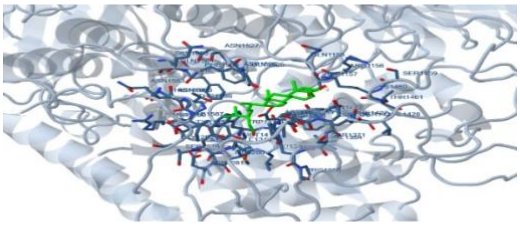
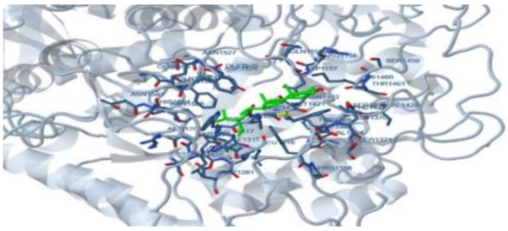
	Lanosterol	-7.86	162.2	894.78
Zingiber officinale	6-Gingerdiol	-5.56	83390	755.71
	10-Gingerol	-4.74	336650	820.43
	12 Shogaol	-5.07	192370	774.629
	Acarbose*	-7.99	140.0	1033.81

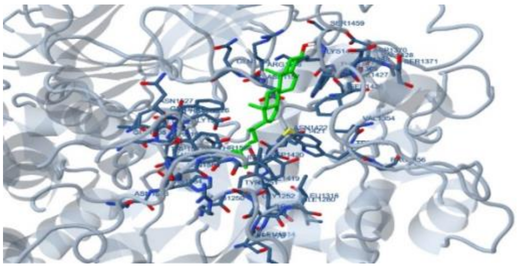
**Reference standard*



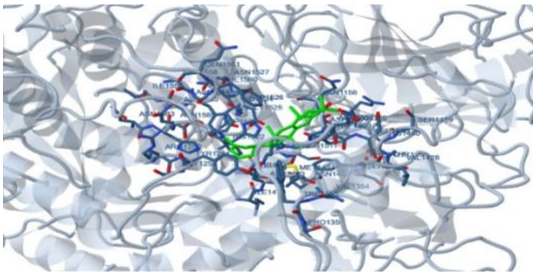
Stigmasterol to *α-glucosidase*



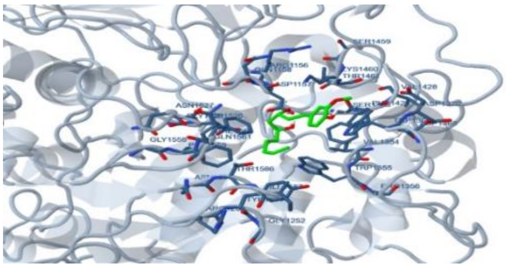
β-Sitosterol to *α-glucosidase*



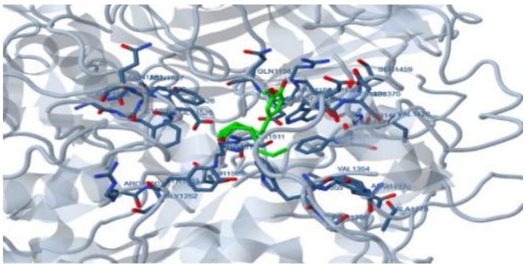
Kampesterol to *α-glucosidase*



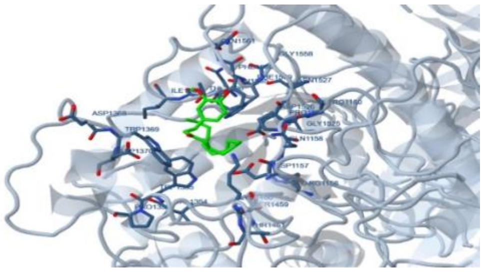
Lanosterol to *α-glucosidase*



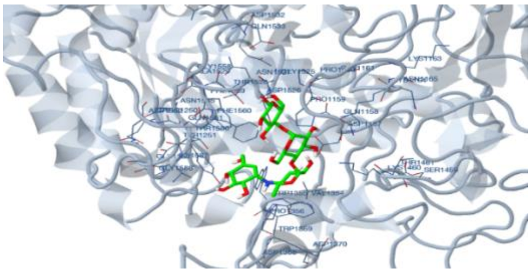
6-Gingerdiol to *α-glucosidase*



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

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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.

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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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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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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.

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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.

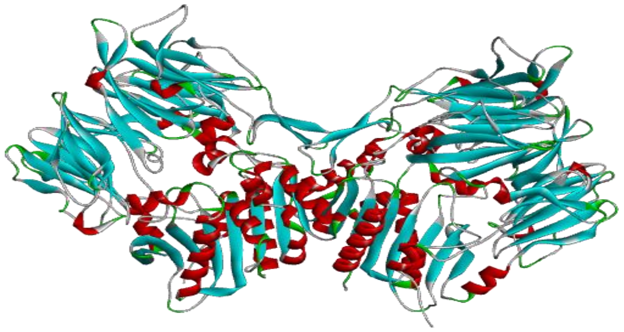

Figure 1: Crystal Structure of Dipeptidyl Peptidase-4

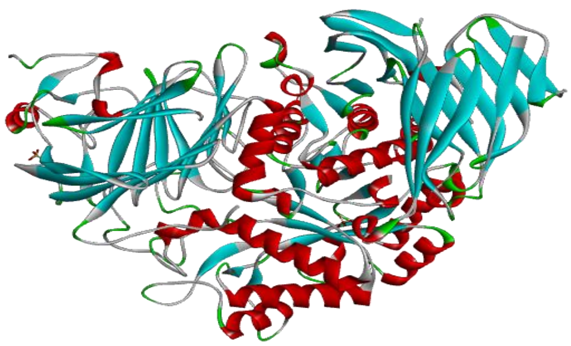

Figure 2: Crystal Structure of *α-glucosidase*

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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(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.

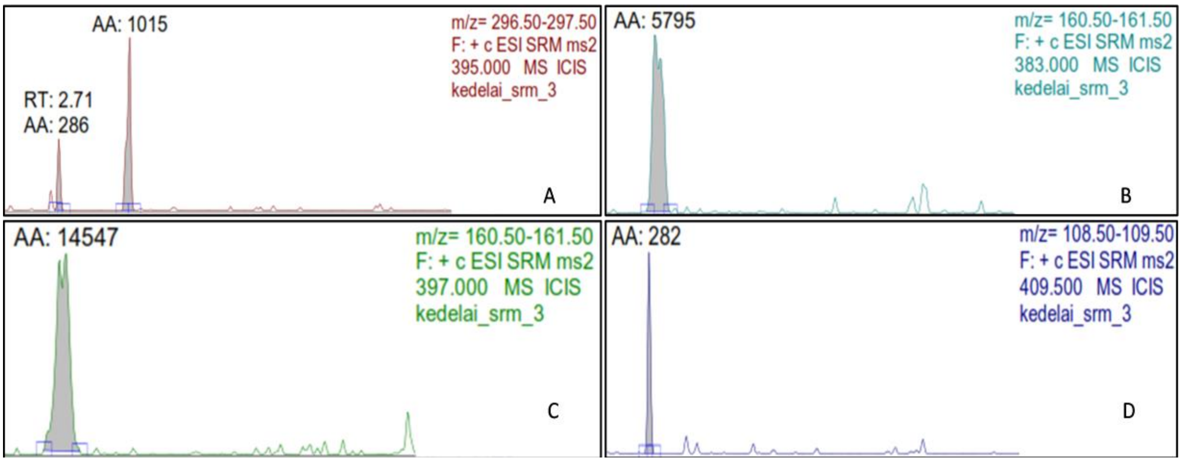

Figure 3: Chromatogram of active compound in ethanol extract of *Glycine max* seed. (A) Stigmasterol, (B) Campesterol, (C) β-Sitosterol, (D) Lanosterol

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

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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 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]

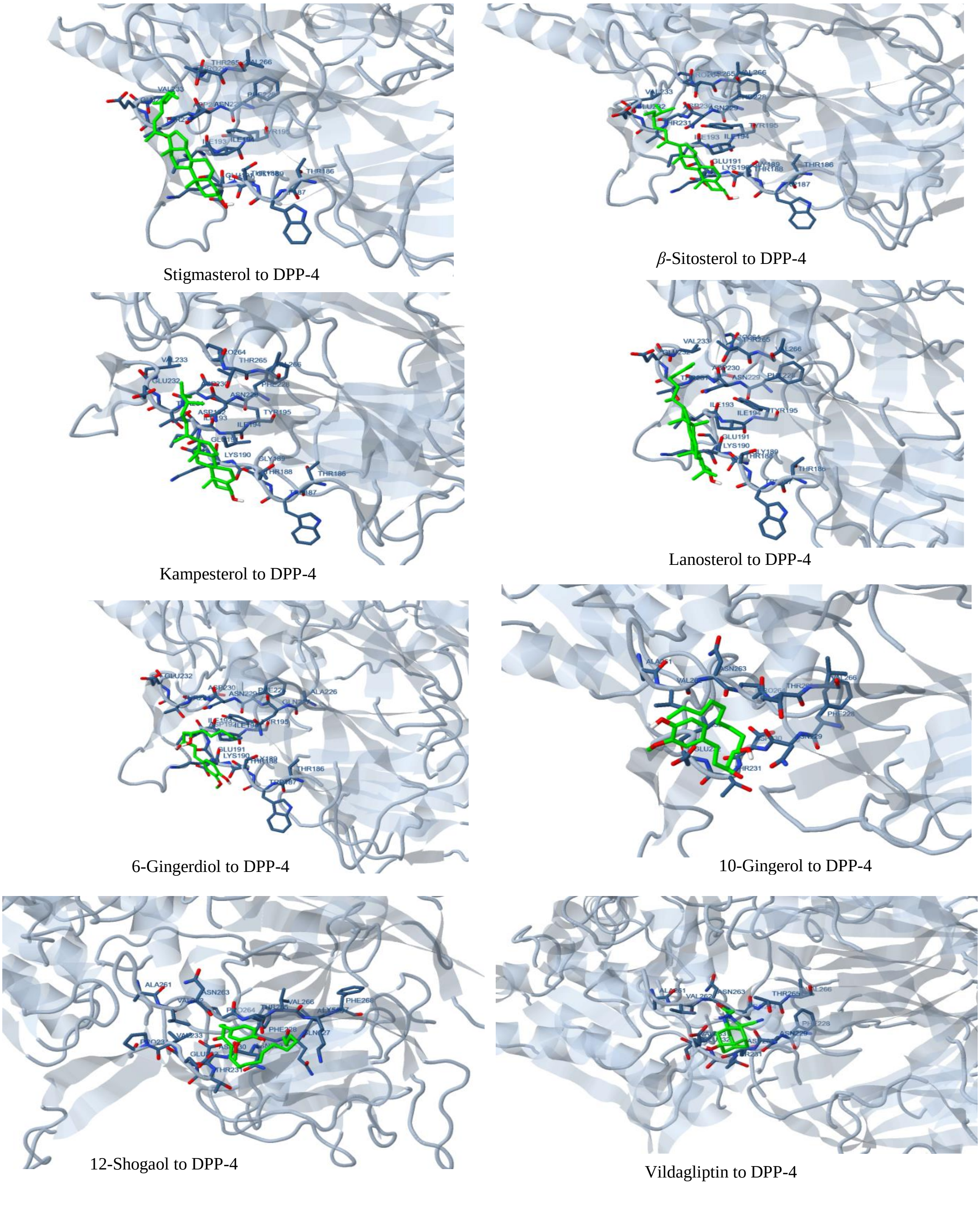

Figure 5: Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4

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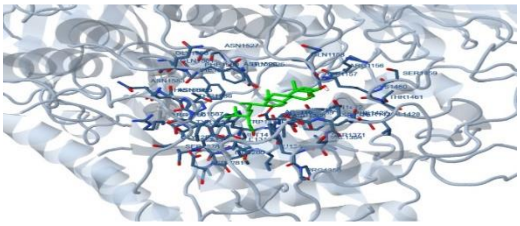
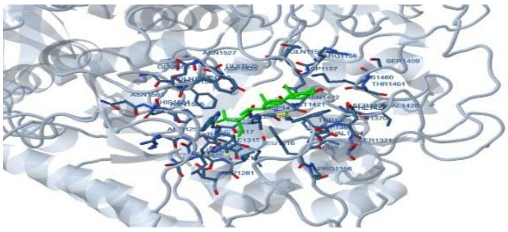
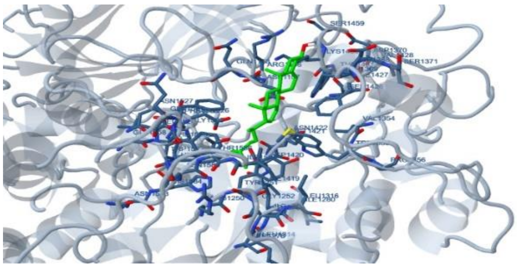
Table 4: Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with *α-glucosidase*

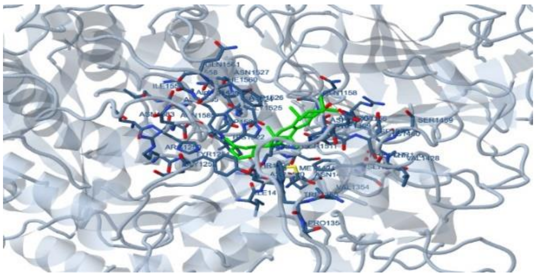
Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (nM)	Surface Interaction (Å)
Glycine	Stigmasterol	-10.57	17.8	893.79
max	*β*-Sitoterol	-10.26	30.0	902.57
	Kampesterol	-7.79	93.6	872.98
	Lanosterol	-7.86	162.2	894.78
Zingiber	6-Gingerdiol	-5.56	83390	755.71
officinale	10-Gingerol	-4.74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

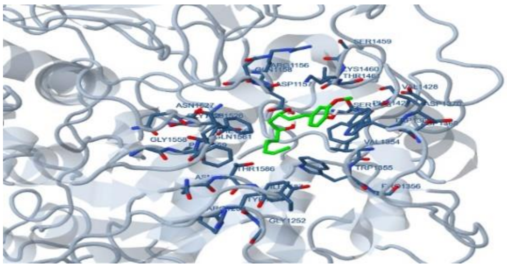
**Reference standard*

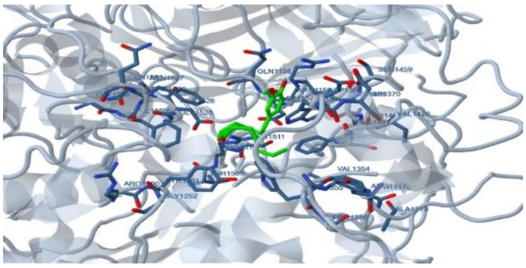

Stigmasterol to *α-glucosidase*

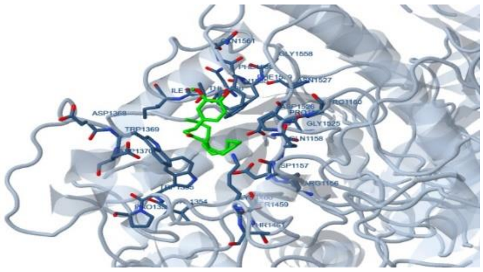

β-Sitosterol to *α-glucosidase*


Kampesterol to *α-glucosidase*


Lanosterol to *α-glucosidase*


6-Gingerdiol to *α-glucosidase*


10-Gingerol to *α-*


12-Shogaol to *α-glucosidase*

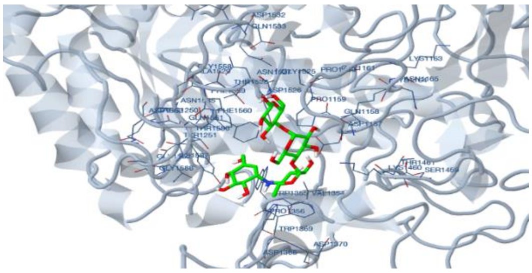

Acarbose to *α-glucosidase*

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

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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.

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