

**TJNPR\_MANUSCRIPT  
DIREVIEW**

# **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 on carbohydrate metabolism. Inhibition of these enzymes contribute to suppress increase in blood glucose level.. Soybean (*Glycine max*) seeds and ginger (*Zingiber officinale*) rhizome are herbs having activity as anti-diabetic. Their 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 suppress 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<sup>1</sup>. GLP-1 have a short half-life, approximately 2-5 minutes due to DPP-4 activity<sup>1,2</sup>. The inhibition of DPP-4 is effective to treat Type 2 Diabetes Mellitus (T2DM) due to GLP-1 bioavailability can be retained, moreover it was able to regulate blood glucose level<sup>3</sup>. Meanwhile,  $\alpha$ -glucosidase ( $\alpha$ -glucosidase) are enzyme that responsible for conversion carbohydrate into maltosa, dextrin and maltotriosa<sup>4</sup>. The location of enzyme 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  $\alpha$ -glucosidase become glucose and absorbed into blood circulation<sup>5</sup>. 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  $\alpha$ -glucosidase prevent the increase of blood glucose level especially for diabetes mellitus<sup>3,4</sup>.

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<sup>6</sup>. 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<sup>7,8</sup>.

*Glycine max* (*G. max*) or soybean and *Zingiber officinale* (*Z. officinale*) or ginger are functional food are known to cure some diseases empirically. Animal study showed *G. max* have a hypoglycemic activity<sup>9</sup>. Isoflavon compound like daidzein and genestein were predicted as a leads compound in *G. Max*<sup>10</sup>. Meanwhile, *Z. officinale* 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<sup>11</sup>. The anti-diabetes effect is thought due to active compounds such as phytosterols, flavonoids, saponins, phenols, and essential oils<sup>10,11,12</sup>. 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  $\alpha$ -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*

Ethanol 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<sub>2</sub>O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300  $\mu$ L/min. Hypersilgold as a stationary phase<sup>14</sup>. 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<sup>15</sup>. The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and  $\alpha$ -glucosidase with UniProt ID: O43451) is downloaded on the Uniprot website (<http://www.uniprot.org/>)<sup>16</sup>.

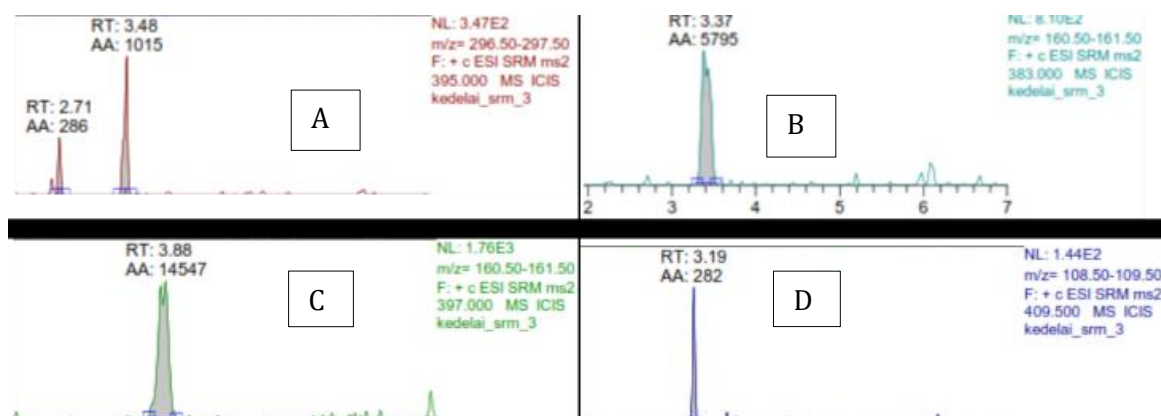
Show the crystal structure of the two enzymes here.

Moreover, protein homology on the Swiss Model (<http://www.swissmodel.expasy.org/interactive>), the modeling results can be downloaded in a PDB file<sup>17</sup>. 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  $\alpha$ -glucosidase.

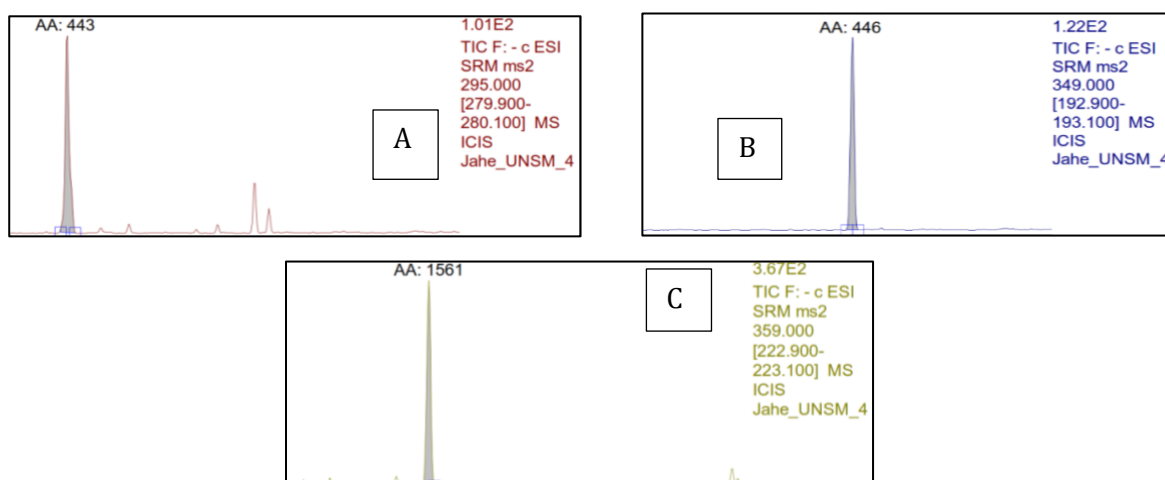
## Results and Discussion

### 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)  $\beta$ -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	Surface Area (AA)	Ethanol Extract of Soybean Seeds the significance of this column is not clear
<i>Glycine max</i>	Stigmasterol	395-297	395	1015	++
	Kampesterol	383-161	383	5795	++
	$\beta$ -Sitosterol	397-161	397	14547	+++
	Lanosterol	409-109	409	282	+
<i>Zingiber officinale</i>	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,  $\beta$ -Sitosterol, and Lanosterol. The highest level is  $\beta$ -Sitosterol, whereas the lowest level is Lanosterol<sup>18</sup>. 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<sup>19</sup>.

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<sup>20</sup>. The active substances of soybean seeds and ginger rhizome are secondary metabolites and have biological activity, moreover they can be used as candidates phytopharmaca<sup>21</sup>.

The main components of phytosterols in soybean seeds are  $\beta$ -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<sup>22</sup>. Meanwhile,  $\beta$ -Sitosterol acts as anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant<sup>23</sup>. 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<sup>24</sup>. Some studies both of *in vivo* and *in vitro* showed that lanosterol has activity as anti-cataract<sup>25</sup>. *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*<sup>26</sup>. *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  $\beta$ -cells pancreas*<sup>11,27</sup>. Other

research indicated that administration of ginger extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus<sup>28</sup>.

#### 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  $\alpha$ -glucosidase were evaluated by *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 ( $\mu$ M)	Surface Interaction ( $\text{\AA}$ )
<i>Glycine max</i>	Stigmasterol	-7.11	6.16 $\mu$ M	931.16
	$\beta$ -Sitosterol	-6.94	8.13 $\mu$ M	908.89
	Kampesterol	-6.84	9.74 $\mu$ M	877.50
	Lanosterol	-7.03	7.06 $\mu$ M	860.98
<i>Zingiber officinale</i>	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 $\mu$ M	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,  $\beta$ -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<sup>29</sup>. 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<sup>30</sup>. 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<sup>31</sup>. Based on those categories, stigmasterol and 12-shogaol have the 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 have the 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<sup>30</sup>. 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  $\alpha$ -glucosidase

<b>Herbs</b>	<b>Ligand</b>	<b>Binding Free Energy (Kcal/mol)</b>	<b>Inhibition Constant (<math>\mu</math>M)</b>	<b>Surface Interaction (<math>\text{Å}</math>)</b>
<i>Glycine max</i>	Stigmasterol	-10.57	17.8 nM	893.79
	$\beta$ -Sitosterol	-10.26	30.0 nM	902.57
	Kampesterol	-7.79	93.6 nM	872.98
	Lanosterol	-7.86	162.2 nM	894.78
<i>Zingiber officinale</i>	6-Gingerdiol	-5.56	83.39 $\mu$ M	755.71
	10-Gingerol	-4.74	336.65 $\mu$ M	820.43
	12 Shogaol	-5.07	192.37 $\mu$ M	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  $\alpha$ -glucosidase<sup>33</sup>. Based on inhibition constant value, stigmasterol and  $\beta$ -sitosterol more potential to inhibit activity of  $\alpha$ -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  $\beta$ -sitosterol and 12-shogaol against  $\alpha$ -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<sup>34</sup>. 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  $\alpha$ -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  $\alpha$ -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<sup>30,33</sup>. 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**

This research is supported financially by the Faculty of Medicine, Islamic University of Malang. Therefore, we are grateful for the funding and support of this research.

## **References**

1. Shubrook, J., Colucci, R., Guo, A., & Schwartz, F. Saxagliptin: A Selective DPP-4 Inhibitor for the Treatment of Type 2 Diabetes Mellitus. *Clinical Medicine Insights: Endocrinology And Diabetes*, 4, CMED.S5114. 2011. doi: 10.4137/cmed.s5114
2. Wang, Y., Li, L., Yang, M., Liu, H., Boden, H., and Yang, G. Glucagon-like peptide-1 receptor agonist versus insulin in inadequately controlled patient with type 2 diabetes mellitus: a meta-analysis of clinical trials. *Diabetes, Obesity and Metabolism*. 2011; 13: 972-981. PMID 21651690
3. Singh, A.K. Dipeptidyl peptidase-4 inhibitors: Novel mechanism of actions. *Indian J Endocrinol Metab*. 2014. 18(6): 753-759. PMID 25364668
4. You, Q., Chen, F., Wang, X., Jiang, Y., & Lin, S. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. *LWT - Food Science And Technology*, 2012; 46(1), 164-168. doi: 10.1016/j.lwt.2011.10.011

5. Zeng, L., Zhang, G., Lin, S. and Gong, D. 2016. Inhibitor Mechanisme of Apigenin in  $\alpha$ -Glucosidase & Synergi Analycis of Flafonoid. *Journal of Agricultural and Food Chemistry*, 2016; 64(37), pp.6939-6949
6. Soelistijo, SA. *Perkumpulan Endokrinologi Indonesia: Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia*, Edisi Pertama, PB PERKENI. Jakarta. 2015
7. Chang, C., Lin, Y., Bartolome, A., Chen, Y., Chiu, S., & Yang, W. Herbal Therapies for Type 2 Diabetes Mellitus: Chemistry, Biology, and Potential Application of Selected Plants and Compounds. *Evidence-Based Complementary And Alternative Medicine*, 2013, 1-33. doi: 10.1155/2013/378657
8. World Health Organization (WHO). *WHO Traditional Medicine Strategy 2014-2023*. World Health Organization. Geneva. 2013
9. Mustofa, M., Mukhtar, D., Susmiarsih, T., & Royhan, A. Pengaruh Kedelai (*Glycine max* (L) Merrill) terhadap Kadar Glukosa Darah dan Ekspresi Insulin Sel  $\beta$  Pankreas pada Tikus Diabetik. *Jurnal Kedokteran Yarsi*, 2010; 18(2), 094-103.
10. Kalaiselvan, V., Kalaivani, M., Vijayakumar, A., Sureshkumar, K. and Venkateskumar, K. Current knowledge and future direction of research on soy isoflavones as a therapeutic agents. *Pharmacognosy Reviews*, 2010; 4(8), p.111.
11. Dugasani, S., Pichika, M., Nadarajah, V., Balijepalli, M., Tandra, S., & Korlakunta, J. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *Journal Of Ethnopharmacology*, 2010; 127(2), 515-520. doi: 10.1016/j.jep.2009.10.004
12. Tiwari, V., Mishra, B., Dixit, A., Antony, J., Tiwari, R. and Sharma, N. Opportunity, challenge and scope of natural products in medicinal chemistry. Kerala, India. 2011; pp.367-383.

13. Yulianingtyas, A., & Kusmartono, B. Optimasi volume pelarut dan waktu maserasi pengambilan flavonoid daun belimbing wuluh (*averrhoa bilimbi* l.). *Teknik Kimia*, 2016; 10(2), 58-64.
14. McHale K, Sanders M. Quantitative LC-MS Screening for Illicit Drugs Using Ultrahigh Resolution Mass Analysis and Accurate Mass Confirmation. 2010
15. O'Boyle, N., Banck, M., James, C., Morley, C., Vandermeersch, T., & Hutchison, G. Open Babel: An open chemical toolbox. *Journal Of Cheminformatics*, 2011; 3(1). doi: 10.1186/1758-2946-3-33.
16. Putra, A. *Blog LIPI >> Sekali Lagi Tentang Docking*. U.lipi.go.id.2014. Retrieved 29 December 2020, from <http://u.lipi.go.id/1391883188>.
17. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F., de Beer, T., Rempfer, C., Bordoli, L., Lepore, R. and Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*. 2018. Retrieved 29 December 2020 From <https://academic.oup.com/nar/article/46/W1/W296/5000024>
18. KHALAF, I., CORCIOVĂ, A., VLASE, L., IVĂNESCU, B., & LAZĂR, D. LC/MS ANALYSIS OF STEROLIC COMPOUNDS FROM GLYCYRRHIZA GLABRA. *STUDIA UBB CHEMIA*, 2011; 56(3), 97 - 102.
19. Van Breemen, R., Tao, Y., & Li, W. Cyclooxygenase-2 inhibitors in ginger (*Zingiber officinale*). *Fitoterapia*, 2011; 82(1), 38-43. doi: 10.1016/j.fitote.2010.09.004
20. Bilbao, A. (2019). Proteomics Mass Spectrometry Data Analysis Tools. *Encyclopedia Of Bioinformatics And Computational Biology*, 84-95. doi: 10.1016/b978-0-12-809633-8.20274-4
21. Mo, S., Dong, L., Hurst, W., & van Breemen, R. Quantitative Analysis of Phytosterols in Edible Oils Using APCI Liquid Chromatography–Tandem Mass Spectrometry. *Lipids*, 2013; 48(9), 949-956. doi: 10.1007/s11745-013-3813-3

22. Batta, A., Xu, G., Honda, A., Miyazaki, T., & Salen, G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. *Metabolism*, 2006; 55(3), 292-299. doi: 10.1016/j.metabol.2005.08.024
23. Rudkowska, I., AbuMweis, S., Nicolle, C., & Jones, P. Cholesterol-Lowering Efficacy of Plant Sterols in Low-Fat Yogurt Consumed as a Snack or with a Meal. *Journal Of The American College Of Nutrition*, 2008; 27(5), 588-595. doi: 10.1080/07315724.2008.10719742
24. Nguyen, H., Neelakadan, A., Quach, T., Valliyodan, B., Kumar, R., Zhang, Z., & Nguyen, H. Molecular characterization of Glycine max squalene synthase genes in seed phytosterol biosynthesis. *Plant Physiology And Biochemistry*, 2013; 73, 23-32. doi: 10.1016/j.plaphy.2013.07.018
25. Daszynski, D., Santhoshkumar, P., Phadte, A., Sharma, K., Zhong, H., Lou, M., & Kador, P. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts. *Scientific Reports*, 2019; 9(1). doi: 10.1038/s41598-019-44676-4
26. Oboh, G., Akinyemi, A., & Ademiluyi, A. Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. *Rubra*) and white ginger (*Zingiber officinale* Roscoe) on Fe<sup>2+</sup> induced lipid peroxidation in rat brain in vitro. *Experimental And Toxicologic Pathology*, 2012; 64(1-2), 31-36. doi: 10.1016/j.etp.2010.06.002
27. Mahmudati, N. Seduhan Jahe Menurunkan Ekspresi TNF  $\alpha$  pada Tikus Putih yang Diberi Diet Tinggi Lemak (HFD). *Proceeding Biology Education Conference*, 2016; 13(1), 653-655.
28. Wei, C., Tsai, Y., Korinek, M., Hung, P., El-Shazly, M., & Cheng, Y. et al. 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *International Journal Of Molecular Sciences*, 2017; 18(1), 168. doi: 10.3390/ijms18010168

29. Utomo, Didik H, Nashi Widodo, and M Rifa'i. Identifications Small Molecules Inhibitor of p53-Mortalin Complex for Cancer Drug Using Virtual Screening. *Bioinformation*. 2012;8 426–9. PMID 22715313
30. Riyanti, S., Suganda, A.G., & Sukandar, E.Y. DIPEPTIDYL PEPTIDASE-IV INHIBITORY ACTIVITY OF SOME INDONESIAN MEDICINAL PLANTS. *Asian Journal of Pharmaceutical and Clinical Research*, 2016. 9, 375-377.
31. Bikadi, Z., Hazai, E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J. Cheminf.*2009;15 PMID 2820493
32. Damayanti, D., Utomo, D., & Kusuma, C. Revealing the potency of *Annona muricata* leaves extract as FOXO1 inhibitor for diabetes mellitus treatment through computational study. *In Silico Pharmacology*, 2017; 5(1). doi: 10.1007/s40203-017-0023-3
33. Rosiarto, B., Puspaningtyas, A., & Holidah, D. Studi Aktivitas Antioksidan Senyawa 1-(p-klorobenzoiloksimetil)-5-fluorourasil dengan Metode Molecular Docking dan Metode DPPH (Antioxidant Activity of 1-(p-chlorobenzoyloxymethyl)-5-Fluorouracyl Using Molecular Docking and DPPH Method). *Pustaka Kesehatan*, 2014; 2(1), 95-99.

Compose

Back Archive Move Delete Spam

Settings

- Inbox 923
- Unread
- Starred
- Drafts 5
- Sent
- Archive
- Spam
- Trash
- Less
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Mess...
- Unwanted

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com>  
 To: y\_purnomo92@yahoo.com  
 Wed, Mar 10, 2021 at 1:19 PM

Dear Dr Purnomo,

Thank you for your submission to the Tropical Journal of Natural Product Research ([www.tjnpr](http://www.tjnpr)) <https://www.scopus.com/sourceid/21100933230> SCOPUS Q3, published by the University of Benin and Natural Product Research Group.

Kindly send the names, affiliation and VALID email addresses and the URL of three potential reviewers, two from your country and the other from outside your country. The email addresses of the co-authors are also needed, stating also their roles in the study.

The peer-review process will commence immediately, as the manuscript will be passed to an editor for initial assessment as soon as possible. If there are any problems with your submission, we will contact you. Also, note that manuscripts submitted and undergoing peer review will not be accepted for withdrawal or retraction.

**Title:** Study on Molecular Docking of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome as Anti-diabetic through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase

Best regards  
 Abiodun

**Editor-in-Chief Tjnpr**

editor.tjnpr@gmail.com  
 +234 807 318 4488  
 + Add to contacts

They're made to last.  
 Learn more



- Compose
- Inbox 923
- Unread
- Starred
- Drafts 5
- Sent
- Archive
- Spam
- Trash
- Less
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Mess...
- Unwanted

← Back ↶ ↷ → Archive ↶ Move ↶ Delete Spam ...

Editorial decision on manuscript submitted 2 Yahoo/Inbox

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com>  
To: y\_purnomo92@yahoo.com

Sat, Mar 27, 2021 at 12:07 AM

Dear Dr Purnomo,

The manuscript submitted to the Tropical Journal of Natural Product Research [www.tjnpr.org](http://www.tjnpr.org) Q3 <https://www.scopus.com/sourceid/21100933230> has been carefully reviewed by competent experts.

Find attached the details of the decision.


Please send your response urgently to the editor-in-Chief, to enable us to process your manuscript for the next issue **Vol 5 issue 4, 2021**. Kindly acknowledge the receipt of the mail.

Title: Study on Molecular Docking of Soybean (*Glycine max*) Seed and Ginger (*Zingiber officinale*) Rhizome as Anti-diabetic through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase

Authors: Yudi Purnomo\*, Muhammad Taufiq, Afnan Nurman Dwi Wijaya, Reza Hakim

Decision: Accepted with moderate corrections

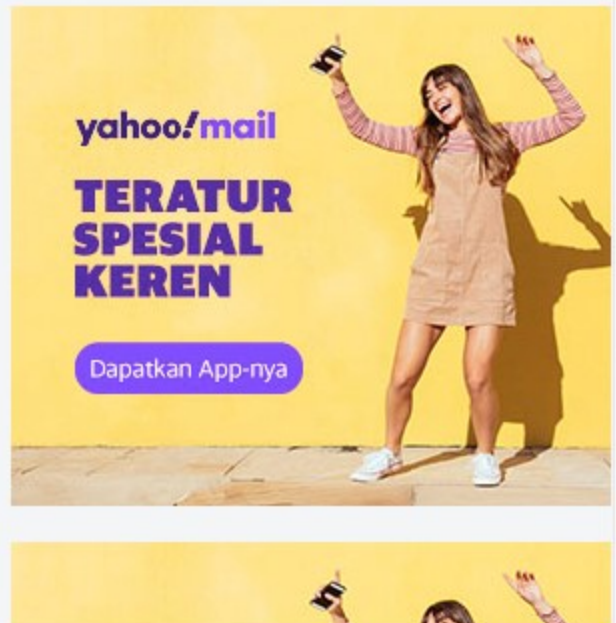
Congratulations

**Editor-in-Chief Tjnpr**  editor.tjnpr@gmail.com  
+234 807 318 4488  
[+ Add to contacts](#)

yahoo!mail

**TERATUR SPESIAL KEREN**

Dapatkan App-nya



**TJNPR\_HASIL  
KOMENTAR REVIEWER**

Compose

- Inbox 922
- Unread
- Starred
- Drafts 5
- Sent
- Archive
- Spam
- Trash
- Less
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Mess...
- Unwanted

Back Archive Move Delete Spam

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> To: yudi Purnomo Wed, Sep 1, 2021 at 4:13 PM

Review comments (Study on Molecular Docking of Soybean (Glycine max) Seed and Ginger (Zingiber officinale) Rhizome as Anti-diabetic through Inhibition of Dipeptidyl Peptidase 4 (DPP-4) and Alpha-Glucosidase)

Editorial comments to authors

Include date (Month and Year) of plant samples collection

For in-text references, the superscript numerals should come after the comma or full-stop.

There are grammatical errors that need to be corrected. Please submit for English Language editing.

Include a section for acknowledgements if any.

All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made.

All corrections/changes made in the manuscript should be highlighted in yellow colour when submitting the manuscript in the revised form on or before 15th September 2021.

The authors should carefully revise and correct the manuscript taking into consideration the comments of all the reviewers. 50% of the references cited should be between 2016-2020. The revised and corrected manuscript should be subjected to plagiarism checker (17% allowed in TJNPR) and English language editing. Evidence of the checks should be attached when submitting the revised/corrected manuscript.

**Editor-in-Chief Tjnpr**  
 editor.tjnpr@gmail.com  
 +234 807 318 4488  
 + Add to contacts



Learn more





1



2

The Editorial Team of the Tropical Journal of Natural Product Research kindly request you to review the enclosed article. Please complete the form and return to the Editor-in-Chief, [editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com); [editor.tjnpr@uniben.edu](mailto:editor.tjnpr@uniben.edu)

**A. MANUSCRIPT**

Journal	Tropical Journal of Natural Product Research
Manuscript Number	
Type of paper	
Title of paper	
Name of Authors	

**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.

## REVIEW FORM

The Editorial Team of the Tropical Journal of Natural Product Research kindly request you to review the enclosed article. Please complete the form and return to the Editor-in-Chief, [editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com); [editor.tjnpr@uniben.edu](mailto:editor.tjnpr@uniben.edu)

### A. MANUSCRIPT

Journal	<b>Tropical Journal of Natural Product Research</b>
Manuscript Number	
Type of paper	
Title of paper	
Name of Authors	

### 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.

You state the article should:

Publish as it is	
Accept with minor revisions (editor will check),specific comments to the editor below	
Accept with moderate revisions as recommended by reviewer	
Accept with major corrections (the article should be thoroughly changed)	<b>X</b>
<b>Full article</b>	
<b>Short communication</b>	
Reject for reasons noted by the reviewer (please be specific)	

**E. REVIEWER'S INFORMATION**

Name	<b>Dr. Chidi Edbert Duru</b>
Official title	<b>Senior Lecturer</b>
Affiliation	<b>Imo State University, Department of Chemisty.</b>
Specialization	<b>Physical Chemistry</b>
Country	<b>Nigeria</b>
E-mail	<b>chidiedbertduru@gmail.com</b>
Phone	<b>08037131739</b>
Signature	<b>CEDuru</b>

**TJNPR\_MANUSCRIPT  
DIREVISI**

# 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 on carbohydrate metabolism. Inhibition of these enzymes contribute to suppress 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

hp March 28, 2021 Deleted: Study on  
hp Deleted:  
hp Deleted: activity both of the enzyme  
hp Deleted: ing of  
hp Deleted: therefore it is potential as anti-diabetic  
hp Deleted: an  
hp Deleted:  
hp Deleted: however the mechanism of active compounds have  
hp Deleted: well  
hp Deleted: of  
hp Deleted: active  
hp Deleted: substances  
hp March 28, 2021 Deleted: herbs  
hp Deleted: Meanwhile, vildagliptin and acarbose are used as a



Yudi Purnomo\*

Faculty of Medicine, University of Islam Malang, Malang Indonesia

Corresponding email : [y\\_purnomo92@yahoo.com](mailto:y_purnomo92@yahoo.com)

Telephone Number: +62 812-3354-124

Muhammad Taufiq

Faculty of Medicine, University of Islam Malang, Malang Indonesia

Corresponding email : [toppek1@gmail.com](mailto:toppek1@gmail.com)

Telephone Number: +62 898-3444-502

Afnan Nurman Dwi Wijaya

Faculty of Medicine, University of Islam Malang, Malang Indonesia

Corresponding email : [afnannurman23@gmail.com](mailto:afnannurman23@gmail.com)

Telephone Number: +62 851-5641-9407

Reza Hakim

Faculty of Medicine, University of Islam Malang, Malang Indonesia

Corresponding email : [rezahakim@unisma.ac.id](mailto:rezahakim@unisma.ac.id)

Telephone Number: +62 856-3646-575

# 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 ( $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -glucosidase. This shows that both soybean seed and ginger rhizome potentially act as an anti-diabetic by inhibiting DPP-4 and  $\alpha$ -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,  $\alpha$ -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).<sup>1</sup> However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively into inactive forms,<sup>1</sup> causing GLP-1 to have a short half-life (to approximately 2-5 minutes).<sup>1,2</sup> 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.<sup>3</sup>

Meanwhile, alpha-glucosidases (*α-glucosidases*) are enzymes that are responsible for the conversion of complex carbohydrate into maltosa, dextrin and maltotriosa.<sup>4</sup> 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.<sup>5</sup> 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.<sup>3,4</sup>

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.<sup>6</sup> 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.<sup>7,8</sup>

*Glycine max* (*G. max*) or soybean and *Zingiber officinale* (*Z. officinale*) 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,<sup>9</sup> mainly due to the presence of isoflavons such as daidzein and genestein<sup>10</sup> 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.<sup>11</sup> 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.<sup>10-12</sup> 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  $\alpha$ -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°C for 48 hours and shaken in rotary shaker.

### *Identification of active substances*

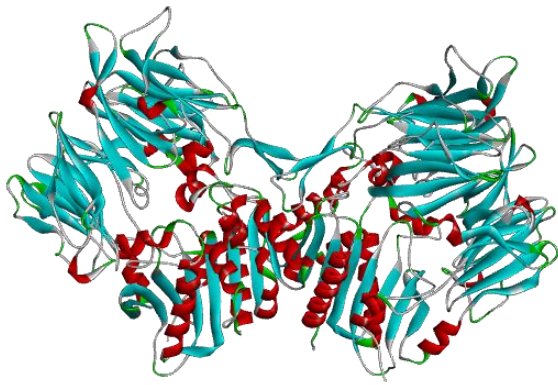
Ethanol extract of *G. max* seed and *Z. officinale* rhizome was qualitative analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific, with Hypersil GOLD as the stationary phase. The mobile phase used were solution A (0.1% formic acid in H<sub>2</sub>O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300  $\mu$ L/min.<sup>13</sup>

#### *Prediction of physicochemical property*

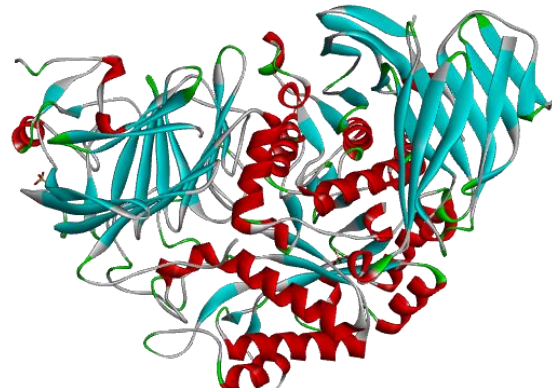
Prediction of the physicochemical properties of the active compound was performed using the pkCSM online tool, i.e. firstly the tested compounds and the comparative compound were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then 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.<sup>14</sup>

#### *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.<sup>15</sup> The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and  $\alpha$ -glucosidase with UniProt ID: O43451) is downloaded on the Uniprot website (<http://www.uniprot.org>).<sup>16</sup> The crystal structure both of the enzyme shown in figure 1 and figure 2 below.



**Figure 1.** Crystal Structure of Dipeptidyl Peptidase-4



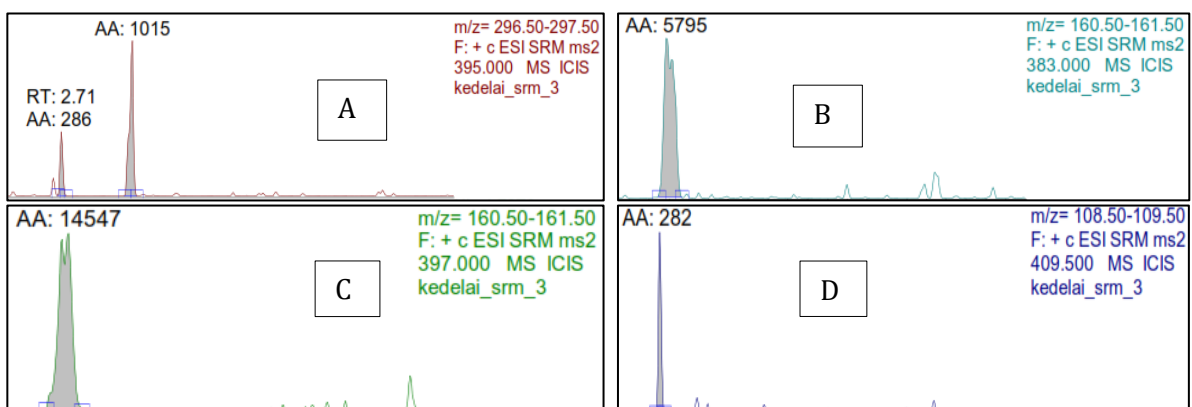
**Figure 2.** Crystal Structure of  $\alpha$ -glucosidase

Moreover, protein homology on the Swiss Model ([www.swissmodel.expasy.org/interactive](http://www.swissmodel.expasy.org/interactive)), the modeling results can be downloaded in a PDB file.<sup>17</sup> 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  $\alpha$ -glucosidase.

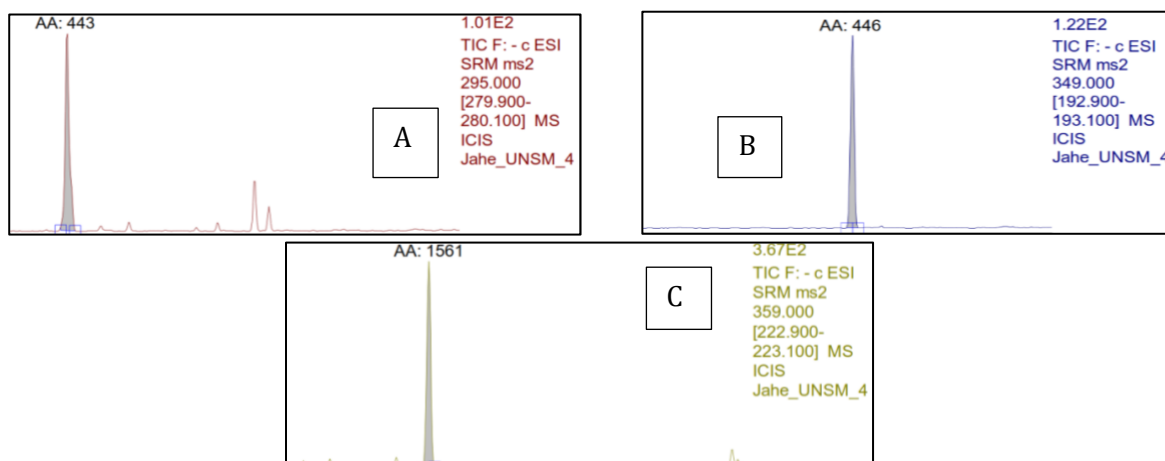
## Results and Discussion

### 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)  $\beta$ -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)
<i>Glycine max</i>	Stigmasterol	395-297	395	1015
	Kampesterol	383-161	383	5795
	$\beta$ -Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
<i>Zingiber officinale</i>	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,  $\beta$ -Sitosterol, and Lanosterol. The highest level is  $\beta$ -Sitosterol, whereas the lowest level is Lanosterol.<sup>18</sup> 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.<sup>19</sup>

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.<sup>20</sup> The active substances of soybean seeds and ginger rhizome are secondary metabolites and have biological activity, moreover, they can be used as candidates phytopharmaca.<sup>21</sup>

As mentioned before, the main components of phytosterols in soybean seeds are  $\beta$ -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  $\beta$ -Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant<sup>23</sup> 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.<sup>24</sup> Some studies both of *in vivo* and *in vitro* showed that lanosterol has activity as anti-cataract.<sup>25</sup> 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.<sup>22</sup> 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.<sup>26</sup> 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  $\beta$ -cells pancreas.<sup>11,27</sup> Other research indicated that administration of ginger extract



can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.<sup>2</sup>

### 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 <sup>2</sup> )	Water Solubility
<i>G. max</i>	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
<i>Z. officinale</i>	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.<sup>29</sup>

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.<sup>29</sup> Hence, it can be predicted that 6-gingerdiol and 10-gingerol will be easily absorbed and have high permeability.

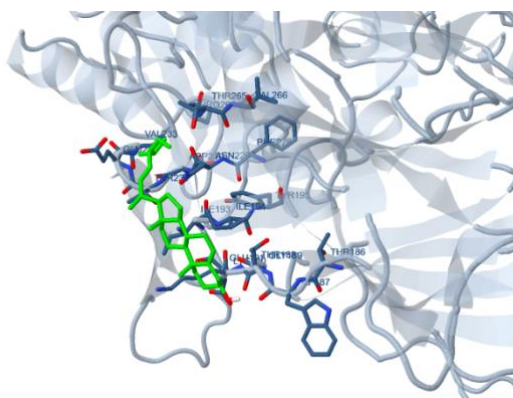
#### Molecular docking of *Glycine max* and *Zingiber officinale* on DPP-4

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.

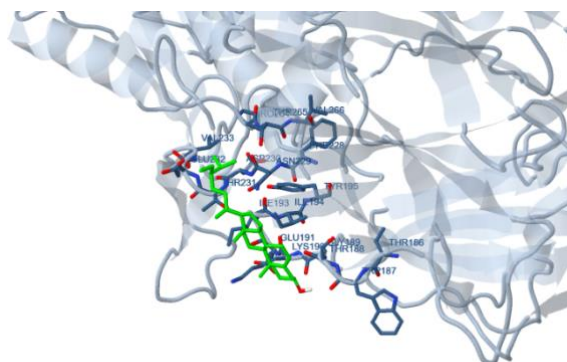
**Table 3.** Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with DPP-4

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant ( $\mu\text{M}$ )	Surface Interaction ( $\text{\AA}$ )
<i>Glycine Max</i>	Stigmasterol	-7.11	6.16 $\mu\text{M}$	931.16
	$\beta$ -Sitosterol	-6.94	8.13 $\mu\text{M}$	908.89
	Kampesterol	-6.84	9.74 $\mu\text{M}$	877.50
	Lanosterol	-7.03	7.06 $\mu\text{M}$	860.98
<i>Zingiber Officinale</i>	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 $\mu\text{M}$	512.40

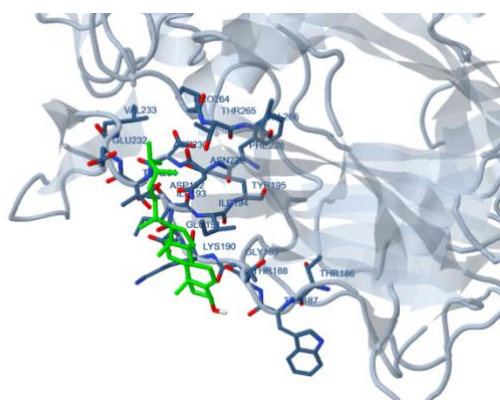
\* reference drug



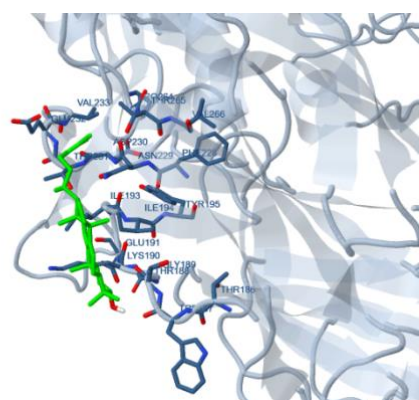
Stigmasterol to DPP-4



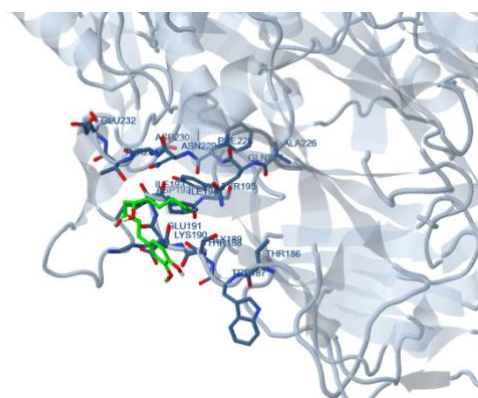
$\beta$ -Sitosterol to DPP-4



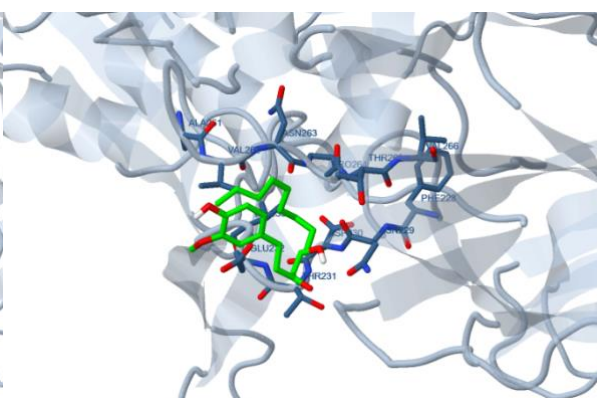
Kampesterol to DPP-4



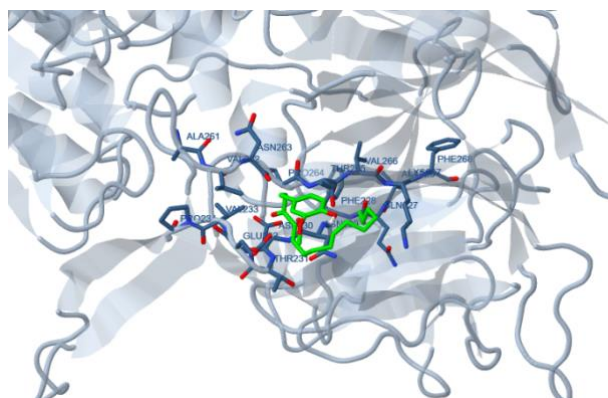
Lanosterol to DPP-4



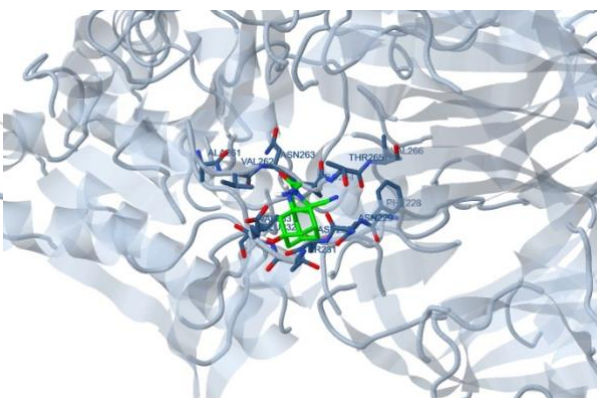
6-Gingerdiol to DPP-4



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,  $\beta$ -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.<sup>30</sup> In addition to this, bioinformatics can also evaluate the bioactivity of a substance by predicting its inhibition constant (K<sub>i</sub>). The lowest K<sub>i</sub> score would indicate to the most potentially active compound, however, the toxicity of said compound must also be tested.<sup>31</sup> 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.<sup>32</sup>

Based on those categories, on DPP-4, stigmaterol and 12-shogaol have the lowest K<sub>i</sub> value, followed by lanosterol and 6-gingerdiol. This is also supported by free binding energy and surface interaction scores of these compounds. Both stigmaterol 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.<sup>31</sup>

Stigmaterol 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.

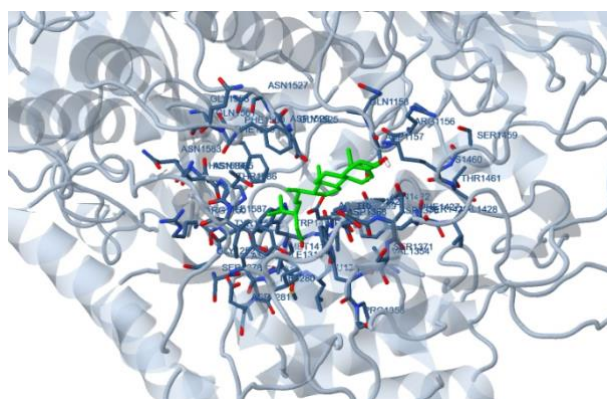
*Molecular docking of Glycine max and Zingiber officinale on alpha-glucosidase*

Activity of Soybean seed and ginger rhizome extracts both of on  $\alpha$ -glucosidase were evaluated by *in-silico* approach and the results can be seen at Table 4 and Figure 6

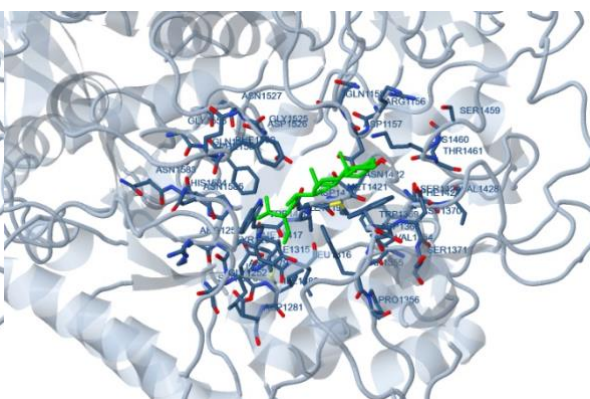
**Table 4.** Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with  $\alpha$ -glucosidase

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant ( $\mu$ M)	Surface Interaction ( $\text{\AA}$ )
<i>Glycine max</i>	Stigmasterol	-10.57	17.8 nM	893.79
	$\beta$ -Sitosterol	-10.26	30.0 nM	902.57
	Kampesterol	-7.79	93.6 nM	872.98
	Lanosterol	-7.86	162.2 nM	894.78
<i>Zingiber officinale</i>	6-Gingerdiol	-5.56	83.39 $\mu$ M	755.71
	10-Gingerol	-4.74	336.65 $\mu$ M	820.43
	12 Shogaol	-5.07	192.37 $\mu$ M	774.629
	Acarbose*	-7.99	140.00 nM	1033.81

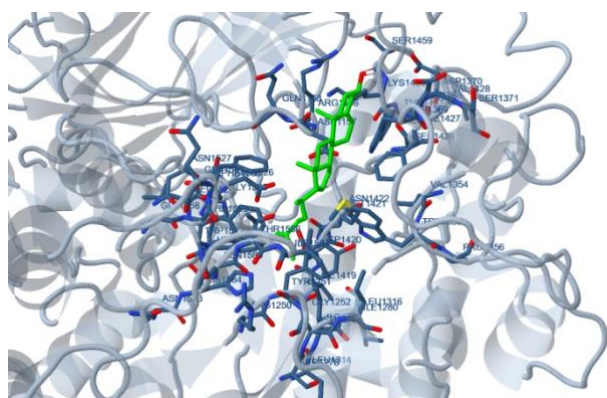
\* reference drug



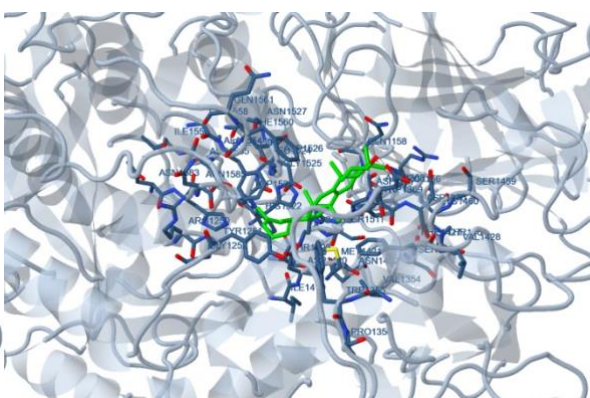
Stigmasterol to  $\alpha$ -glucosidase



$\beta$ -Sitosterol to  $\alpha$ -glucosidase



Kampesterol to  $\alpha$ -glucosidase



Lanosterol to  $\alpha$ -glucosidase



protein was high. The lower  $K_i$  scores indicate a high inhibitory activity against the target protein.<sup>35</sup> 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  $\alpha$ -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  $\alpha$ -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.<sup>31,34</sup>

## Conclusion

This study aims to evaluate the activity of the compounds extracted from soybean seeds and ginger rhizome against DPP-4 and  $\alpha$ -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  $\alpha$ -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

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

### References

1. Deacon C. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology*. 2020; 16(11): 642-653.
2. Singh S, Wright E, Kwan A, Thompson J, Syed I, Korol E et al. Glucagon-like peptide-1 receptor agonists compared with basal insulins for the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes, Obesity and Metabolism*. 2016; 19(2): 228-238.
3. Gallwitz B. Clinical Use of DPP-4 Inhibitors. *Frontiers in Endocrinology*. 2019; 10.
4. Pereira A, Banegas-Luna A, Peña-García J, Pérez-Sánchez H, Apostolides Z. Evaluation of the Anti-Diabetic Activity of Some Common Herbs and Spices: Providing New Insights with Inverse Virtual Screening. *Molecules*. 2019; 24(22): 4030.
5. Zeng L, Zhang G, Lin S, Gong D. Inhibitory Mechanism of Apigenin on  $\alpha$ -Glucosidase and Synergy Analysis of Flavonoids. *Journal of Agricultural and Food Chemistry*. 2016; 64(37): 6939-6949.



6. Soelistijo, SA. Perkumpulan Endokrinologi Indonesia: **Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia, Edisi Pertama, PB PERKENI. Jakarta. 2015**
7. Pang G, Li F, Yan Y, Zhang Y, Kong L, Zhu P **et al.** Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chinese Medical Journal*. 2019; 132(1): 78-85.
8. **World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023. World Health Organization. Geneva. 2013**
9. Mustofa, M., Mukhtar, D., Susmiarsih, T., & Royhan, A. **Pengaruh Kedelai (*Glycine max* (L) Merrill) terhadap Kadar Glukosa Darah dan Ekspresi Insulin Sel  $\beta$  Pankreas pada Tikus Diabetik. *Jurnal Kedokteran Yarsi*. 2010; 18(2): 094-103.**
10. Pabich M, Materska M. Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. *Nutrients*. 2019; 11(7): 1660.
11. Bischoff-Kont I, Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. *Pharmaceuticals*. 2021; 14(6): 571.
12. Tiwari V, Mishra B, Dixit A, Antony J, Tiwari R and Sharma N. Opportunity, challenge and scope of natural products in medicinal chemistry. Kerala, India. 2011; p 367-383.
13. Yulianingtyas A & Kusmartono B. **Optimasi volume pelarut dan waktu maserasi pengambilan flavonoid daun belimbing wuluh (*Averrhoa bilimbi* L.). *Teknik Kimia*, 2016; 10(2): 58-64.**
14. Nicklaus M. Online SMILES Translator [Internet]. Cactus.nci.nih.gov. 2020 [cited 4 September 2021]. Available from: <https://cactus.nci.nih.gov/translate>
15. McHale K, Sanders M. Quantitative LC-MS Screening for Illicit Drugs Using Ultrahigh Resolution Mass Analysis and Accurate Mass Confirmation. **2010**
16. O'Boyle N, Banck M, James C, Morley C, Vandermeersch T, Hutchison G. Open Babel: An open chemical toolbox. *Journal of Cheminformatics*. 2011; 3(1).

17. Putra, A. Blog LIPI >> Sekali Lagi Tentang Docking. U.lipi.go.id.2014. Retrieved 29 December 2020, from <http://u.lipi.go.id/1391883188>.
18. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F., de Beer, T., Rempfer, C., Bordoli, L., Lepore, R. and Schwede, T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Research*. 2018. Retrieved 29 December 2020 from <https://academic.oup.com/nar/article/46/W1/W296/5000024>
19. Khalaf I, Corciovă A, Vlase I, Ivănescu B & Lazăr D. LC/MS Analysis of Sterolic Compounds from *Glycyrrhiza Glabra*. *Studia ubb chemia*. 2011; 56(3): 97 - 102.
20. Fikri F, Saptarini N, Levita J. The Inhibitory Activity on the Rate of Prostaglandin Production by *Zingiber officinale* var. Rubrum. *Pharmacology and Clinical Pharmacy Research*. 2016; 1(1).
21. Bilbao A. Proteomics Mass Spectrometry Data Analysis Tools. *Encyclopedia of Bioinformatics and Computational Biology*. 2019: 84-95.
22. Skubic C, Vovk I, Rozman D, Križman M. Simplified LC-MS Method for Analysis of Sterols in Biological Samples. *Molecules*. 2020; 25(18): 4116.
23. Zhu H, Chen J, He Z, Hao W, Liu J, Kwek E et al. Plasma Cholesterol-Lowering Activity of Soybean Germ Phytosterols. *Nutrients*. 2019; 11(11): 2784.
24. Trautwein E, Vermeer M, Hiemstra H, Ras R. LDL-Cholesterol Lowering of Plant Sterols and Stanols-Which Factors Influence Their Efficacy? *Nutrients*. 2018; 10(9): 1262.
25. Nguyen H, Neelakadan A, Quach T, Valliyodan B, Kumar R, Zhang Z et al. Molecular characterization of *Glycine max* squalene synthase genes in seed phytosterol biosynthesis. *Plant Physiology and Biochemistry*. 2013; 73: 23-32.
26. Daszynski D, Santhoshkumar P, Phadte A, Sharma K, Zhong H, Lou M et al. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts. *Scientific Reports*. 2019; 9(1).

27. Deoranto P, Dewi I, Citraresmi A, Sari I, Dewi C. Antioxidant analysis of instant herbal beverages ingredients. IOP Conference Series: Earth and Environmental Science. 2021; 733(1): 012133.
28. Mahmudati, N. **Seduhan Jahe Menurunkan Ekspresi TNF  $\alpha$  pada Tikus Putih yang Diberi Diet Tinggi Lemak (HFD).** Proceeding Biology Education Conference, 2016; 13(1): 653-655.
29. Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*. 2012; 64: 4-17.
30. Wei C, Tsai Y, Korinek M, Hung P, El-Shazly M, Cheng Y **et al.** 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *International Journal of Molecular Sciences*. 2017; 18(1): 168.
31. Utomo D, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. *Bioinformation*. 2012; 8(9): 426-429.
32. Riyanti S, Suganda AG & Sukandar EY. Dipeptidyl Peptidase-IV Inhibitory Activity of Some Indonesian Medicinal Plants. *Asian Journal of Pharmaceutical and Clinical Research*. 2016; 9: 375-377.
33. Bikadi Z, Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *Journal of Cheminformatics*. 2009; 1(1).
34. Damayanti D, Utomo D, Kusuma C. Revealing the potency of *Annona muricata* leaves extract as FOXO1 inhibitor for diabetes mellitus treatment through computational study. *In Silico Pharmacology*. 2017; 5(1).
35. Rosiarto B, Puspaningtyas A & Holidah D. **Studi Aktivitas Antioksidan Senyawa 1-(p-klorobenzoiloksimetil)-5-fluorourasil dengan Metode Molecular Docking dan Metode**

**DPPH** (Antioxidant Activity of 1-(p-chlorobenzoyloxymethyl)-5-Fluorouracyl Using Molecular Docking and DPPH Method). Pustaka Kesehatan, 2014; 2(1): 95-99.

**yahoo!mail** Editor-in-Chief Tjnpr Add keywords Advanced

yudi Home

Compose

- Inbox 922
- Unread
- Starred
- Drafts 5
- Sent
- Archive
- Spam
- Trash
- Less
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Mess...
- Unwanted

Back Archive Move Delete Spam ...

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Tue, Sep 14, 2021 at 2:24 AM

To: yudi Purnomo

Send the other file containing the responses to the reviewers comments

Best regards

Abiodun

---

**Professor Abiodun Falodun, PhD**

Editor-in-Chief:  
 Tropical Journal of Natural Product Research (TJNPR)  
 Head, Natural Product Research Group, University of Benin  
 Email: [editor.tjnpr@uniben.edu](mailto:editor.tjnpr@uniben.edu); [editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com)  
[www.tjnpr.org](http://www.tjnpr.org) **SCOPUS, SCImago SJR Q4 0.13**  
<https://www.scopus.com/sources.uri>

Professor of Pharmaceutical Chemistry  
 Fellow, Fulbright (USA)  
 Deputy Vice-Chancellor (Academic) 2014-2016  
 Faculty of Pharmacy  
 University of Benin  
 Phone: +234-807-318-4488;  
 email: [faloabi@uniben.edu](mailto:faloabi@uniben.edu); [abiodun.falodun@fulbrightmail.org](mailto:abiodun.falodun@fulbrightmail.org)  
[Google Scholar Citations](https://scholar.google.com/citations?user=...)  
 SCOPUS [https://www.scopus.com/authid/detail.uri?](https://www.scopus.com/authid/detail.uri?https://orcid.org/0000-0002-2829-2205&authid=12794226500#top)

Settings

**Editor-in-Chief Tjnpr**

[editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com)  
 +234 807 318 4488  
 + Add to contacts

[Learn more](#)

Home Insert Design Layout References Mailings Review View

Times New Ro... 12 A A Aa A

B I U abc X<sub>2</sub> X<sup>2</sup> A A

AaBbCcDdEe Normal No Spacing AaBbCcDc Heading 1 AaBbCcDdEe Heading 2 AaBbCcDc Title AaBbCcDdEe Subtitle AaBbCcDdEe Subtle Emph...

Styles Pane

**Point-to-point responses**

Reviewer

No	Issues	Responses
<u>Abstrak</u>	Needs to be rewritten. The grammar is poor.	<u>I have repaired it</u>
<u>introduction</u>	Better organization is required. Poor grammar.	<u>I already revised it</u>
<u>Methodology</u>	More information in the form of enzyme crystal structure should be included.	<u>I have added it</u>
<u>Result</u>	The binding interactions between enzyme protein and ligands were not included. Please add.	<u>I have added it and also the physicochemical properties of ligand to support the molecular docking</u>
<u>Discussion</u>	Binding interactions need to be shown and discussed.	<u>I have added it</u>
<u>Conclusion</u>	Too short. More details on the findings should be added	<u>I already fixed it</u>
<u>Reference</u>	It did not follow the journal format	<u>I have repaired it</u>
<u>Figures, Tables</u>	Figure 1 is not as sharp as figure 2. Please improve the quality of Figure 1.	<u>I have repaired it</u>

Compose

- Inbox 999+
- Unread
- Starred
- Drafts 6
- Sent
- More
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Messages
- Unwanted

Back Archive Move Delete Spam

**Editor-in-Chief Tjnpr** <editor.tjnpr@gmail.com> Tue, Sep 28, 2021 at 4:46 AM ☆  
 To: yudi Purnomo

Please seek for the assistance of a science English editor. Prof Shittu ([olalekan.shittu@uniben.edu](mailto:olalekan.shittu@uniben.edu)) or Dr Vincent ([vincent.imieje@gmail.com](mailto:vincent.imieje@gmail.com)) may help you. Attach a certificate of English editing when submitting the revised manuscript

Best regards  
 Abiodun

---


**Professor Abiodun Falodun, PhD**  
 Editor-in-Chief:  
 Tropical Journal of Natural Product Research (TJNPR)  
 Head, Natural Product Research Group, University of Benin  
 Email: [editor.tjnpr@uniben.edu](mailto:editor.tjnpr@uniben.edu); [editor.tjnpr@gmail.com](mailto:editor.tjnpr@gmail.com)  
[www.tjnpr.org](http://www.tjnpr.org) **SCOPUS, SCImago SJR Q4 0.13**  
<https://www.scopus.com/sources.uri>

Professor of Pharmaceutical Chemistry  
 Fellow, Fulbright (USA)  
 Deputy Vice-Chancellor (Academic) 2014-2016  
 Faculty of Pharmacy



**Managing Editor**  
**TJNPR**  
[p.editor.tjnpr@gmail.com](mailto:p.editor.tjnpr@gmail.com)  
 + Add to contacts

**Make sense of your SCOM monitoring**



**Enhance your SCOM monitoring, with a fast, interactive dashboard.**

SquaredUp [Sign Up >](#)

**Make sense of your SCOM monitoring**



**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* by *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.



**TJNPR\_GALLEY PROOF**

Compose

- Inbox 999+
- Unread
- Starred
- Drafts 6
- Sent
- More
- Views Show
- Folders Hide
- + New Folder
- Notes
- Sent Messages
- Unwanted

Back Archive Move Delete Spam

Galley PROOF OF YOUR ARTICLE 2


**Managing Editor TJNPR** <p.editor.tjnpr@gmail.com>  
 To: yudi Purnomo  
 Cc: Editor-in-Chief Tjnpr  
 Wed, Oct 27, 2021 at 5:02 AM

Dear Author,  
 Find attached the galley proof of your article titled "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"

We request you go through carefully to ensure no error has been made.


Also respond to the comments indicated in the galley proof.

**Please, return the corrected galley proof as quickly as possible (on Wednesday 27th October, 2021).**



**Managing Editor TJNPR**  
 p.editor.tjnpr@gmail.com  
 + Add to contacts

**Make sense of your SCOM monitoring**



**Enhance your SCOM monitoring, with a fast, interactive dashboard.**  
 SquaredUp

**Make sense of your SCOM monitoring**



# Tropical Journal of Natural Product Research



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

*Original Research Article*

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

Yudi Purnomo\*, Muhammad Taufiq, Afnan N. D. Wijaya, Reza Hakim

*Faculty of Medicine, University of Islam Malang, Malang Indonesia*

### ARTICLE INFO

*Article history:*

Received 27 March 2021

Revised 16 September 2021

Accepted 21 October 2021

Published online xxxxxxxxxxxx

### 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



## 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

Yudi Purnomo\*, Muhammad Taufiq, Afnan N. D. Wijaya, Reza Hakim

Faculty of Medicine, University of Islam Malang, Malang Indonesia

### ARTICLE INFO

#### Article history:

Received 27 March 2021

Revised 16 September 2021

Accepted 21 October 2021

Published online 02 November 2021

**Copyright:** © 2021 Purnomo *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase ( $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -glucosidase. This shows that both soybean seed and ginger rhizome potentially act as an anti-diabetic by inhibiting DPP-4 and  $\alpha$ -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,  $\alpha$ -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).<sup>1</sup> However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively into inactive forms,<sup>1</sup> causing GLP-1 to have a short half-life (to approximately 2-5 minutes).<sup>1,2</sup> 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.<sup>3</sup> Meanwhile, alpha-glucosidases ( $\alpha$ -glucosidases) are enzymes that are responsible for the conversion of complex carbohydrate into maltose, dextrin and maltotriose.<sup>4</sup> 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.<sup>5</sup> Thus,  $\alpha$ -glucosidases activity contributes in increasing blood glucose level post prandially, and needs to be controlled to avoid hyperglycemia. Inhibition of both DPP-4 and  $\alpha$ -glucosidase could be used in preventing the increase of blood glucose level, especially for patients with T2DM.<sup>3,4</sup>

\*Corresponding author. E mail: [y\\_purnomo92@yahoo.com](mailto:y_purnomo92@yahoo.com)  
Tel: +62 812-3354-124

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

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

Therapy of T2DM usually requires an Oral Hypoglycemic Drug (OHD), which acts either by increasing of insulin secretion, repairing of insulin resistance and/or inhibiting of glucose absorption. However, long-term consumption of these medications is often accompanied with various adverse drug reactions, particularly in DM patients.<sup>6</sup> 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.<sup>7,8</sup> *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,<sup>9</sup> mainly due to the presence of isoflavons such as daidzein and genestein.<sup>10</sup> 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.<sup>11</sup> 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.<sup>10-12</sup> 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  $\alpha$ -glucosidase using *in silico* study.

## 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, respectively. 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

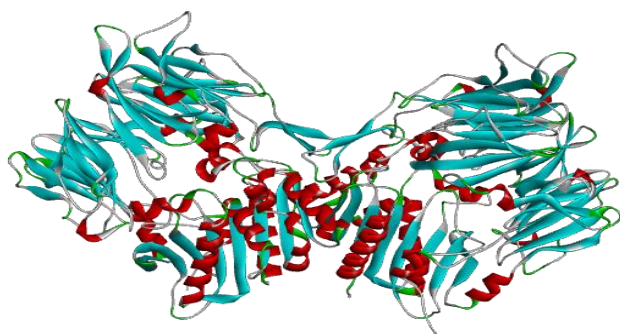
Ethanol extracts of *G. max* seed and *Z. officinale* rhizome were qualitative analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific, with Hypersil GOLD as the stationary phase. The mobile phase were used solution A (0.1% formic acid in H<sub>2</sub>O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300  $\mu$ L/min.<sup>13</sup>

### Prediction of physicochemical property

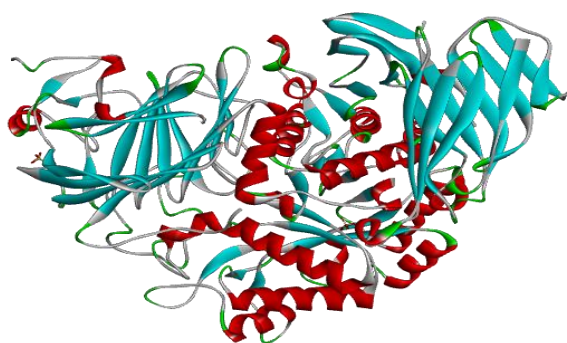
Prediction of the physicochemical properties of the active compound was performed using the pkCSM online tool. Firstly, active compounds identified and references standard were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then 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.<sup>14</sup>

### 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.<sup>15</sup> The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and  $\alpha$ -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  $\alpha$ -glucosidase

Moreover, protein homology on the Swiss Model ([www.swissmodel.expasy.org/interactive](http://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](http://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  $\alpha$ -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,  $\beta$ -Sitosterol, and Lanosterol. The highest component is  $\beta$ -Sitosterol, whereas the lowest component is Lanosterol.<sup>18</sup> 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.<sup>19</sup>

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.<sup>20</sup> 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.<sup>21</sup>

As mentioned before, the main components of phytosterols in *G. max* seeds are  $\beta$ -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  $\beta$ -Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant<sup>23</sup> 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.<sup>24</sup> Some studies both in vivo and in vitro showed that lanosterol has activity as anti-cataract.<sup>25</sup> 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.<sup>22</sup> 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.<sup>26</sup> 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  $\beta$ -cells pancreas.<sup>11,27</sup> Other research indicated that administration of *Z. officinale* extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.<sup>28</sup>

### Prediction of physicochemical properties

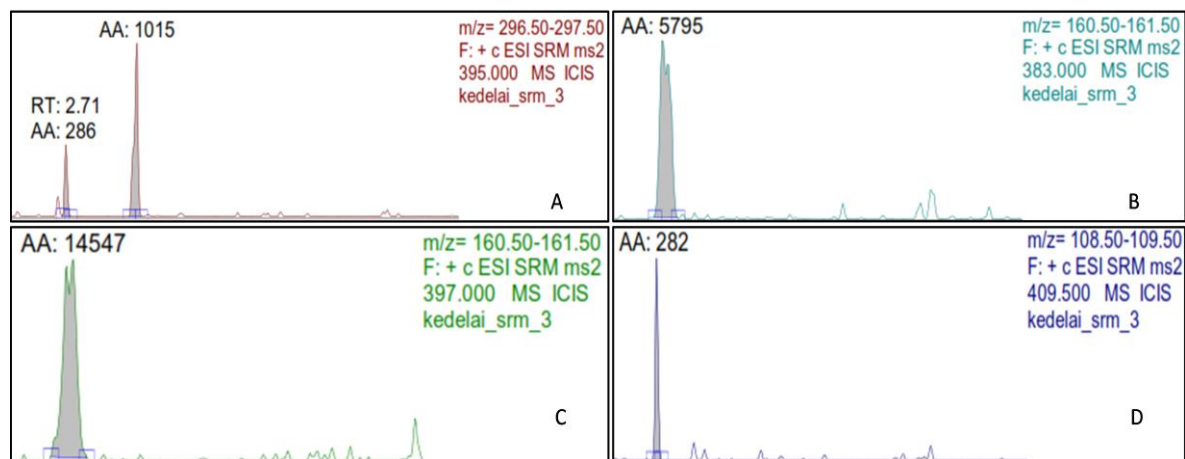
The result of the *in silico* study of the physicochemical properties of *Glycine max* and *Zingiber officinale* active compound is presented in Table 2. It can be seen that the molecular weight values of the active compound ranged from 296 to 426 (less than 500), the value of log of the octanol/water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

(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.

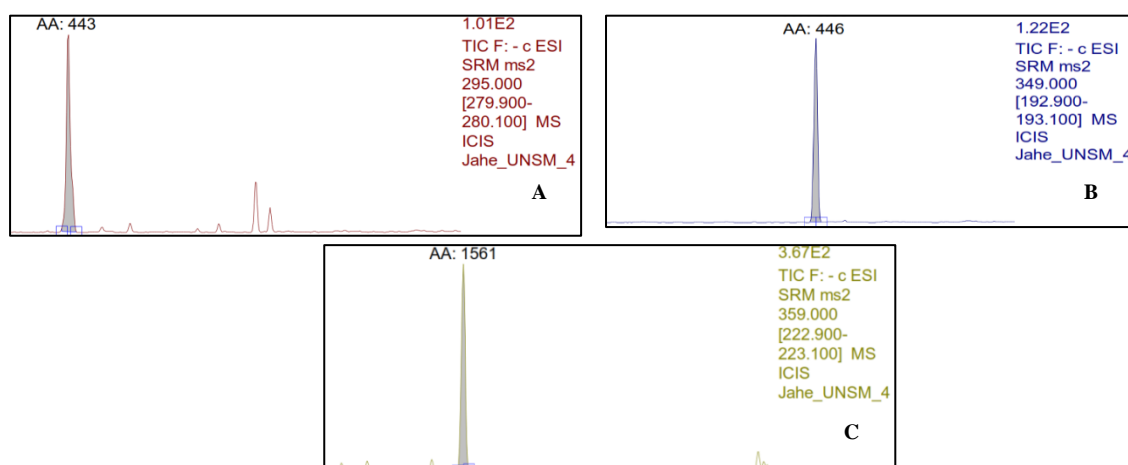
Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were 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.<sup>29</sup>

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.<sup>29</sup> 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) Kampesterol (C)  $\beta$ -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
<i>Glycine max</i>	Kampesterol	383-161	383	5795
	$\beta$ -Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
<i>Zingiber officinale</i>	6-Gingerdiol	295-280	295	443
	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,  $\beta$ -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.<sup>30</sup>

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.<sup>30</sup> 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.<sup>31</sup> 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.<sup>30,32</sup> 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.<sup>31</sup> 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 (Å <sup>2</sup> )	Water Solubility
<i>Glycine max</i>	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
	$\beta$ -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
<i>Zingiber officinale</i>	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 ( $\mu$ M)	Surface Interaction (Å)
<i>Glycine max</i>	Stigmasterol	-7.11	6.16	931.16
	$\beta$ -Sitosterol	-6.94	8.13	908.89
	Kampesterol	-6.84	9.74	877.50
	Lanosterol	-7.03	7.06	860.98
<i>Zingiber officinale</i>	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  $\alpha$ -glucosidase were evaluated by *in-silico* approach and the results can be seen at Table 4 and Figure 6.

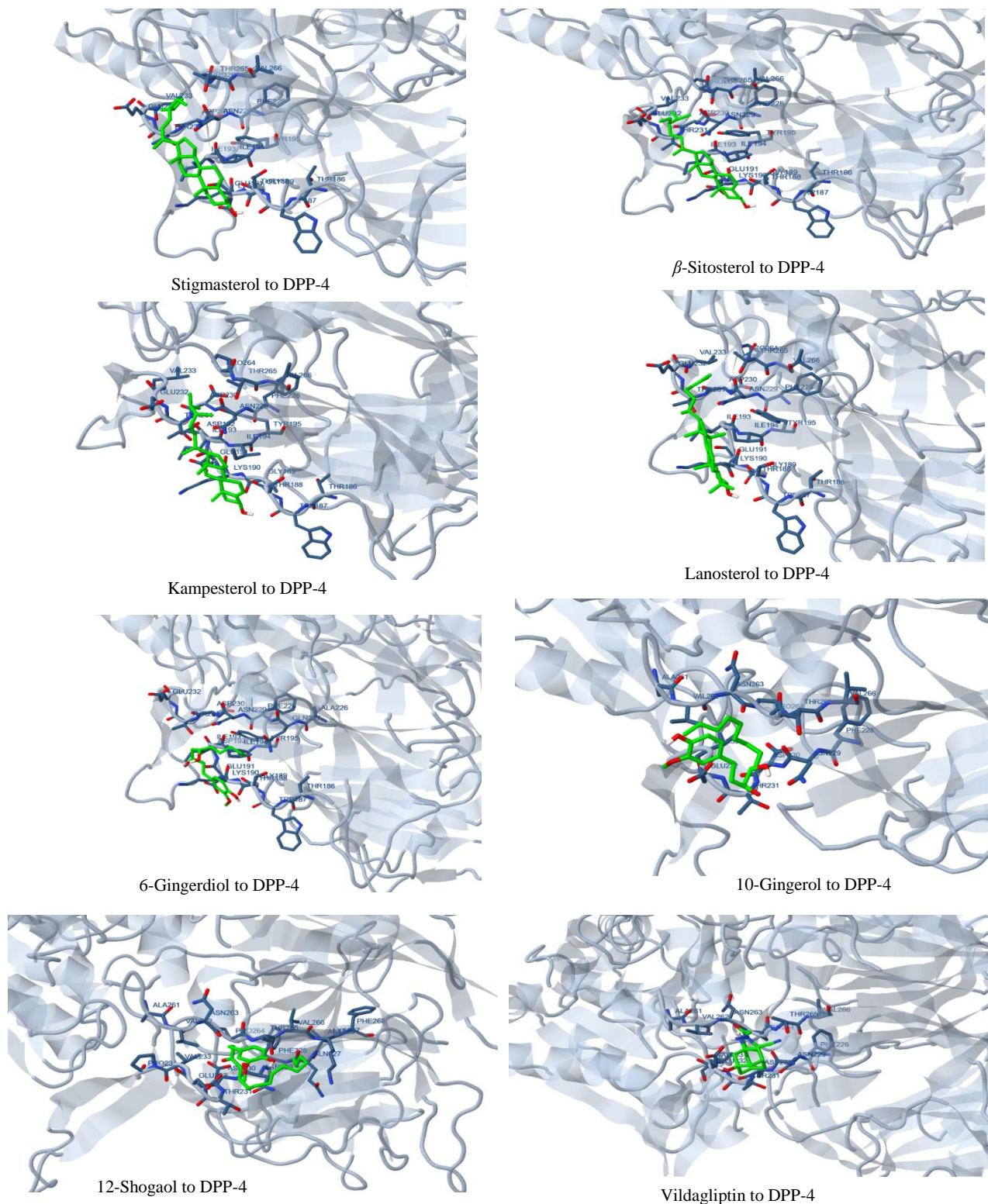
*In-silico* studies indicates that stigmasterol and 6-gingerdiol have a lower Ki, free energy binding, and surface interaction scores compared to other active compounds. Meanwhile, lanosterol and 10-gingerol was found to have a higher free energy binding score compared to other compounds in *G. max* and *Z. officinale*. The differences in each parameter value causes the differences of the inhibitory activity against  $\alpha$ -glucosidase.<sup>34</sup> Based on inhibition constant (Ki) value, stigmasterol and  $\beta$ -sitosterol had higher inhibitory

activity against  $\alpha$ -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  $\beta$ -sitosterol and 12-shogaol, against  $\alpha$ -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.<sup>35</sup> 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  $\alpha$ -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  $\alpha$ -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.<sup>31,34</sup>



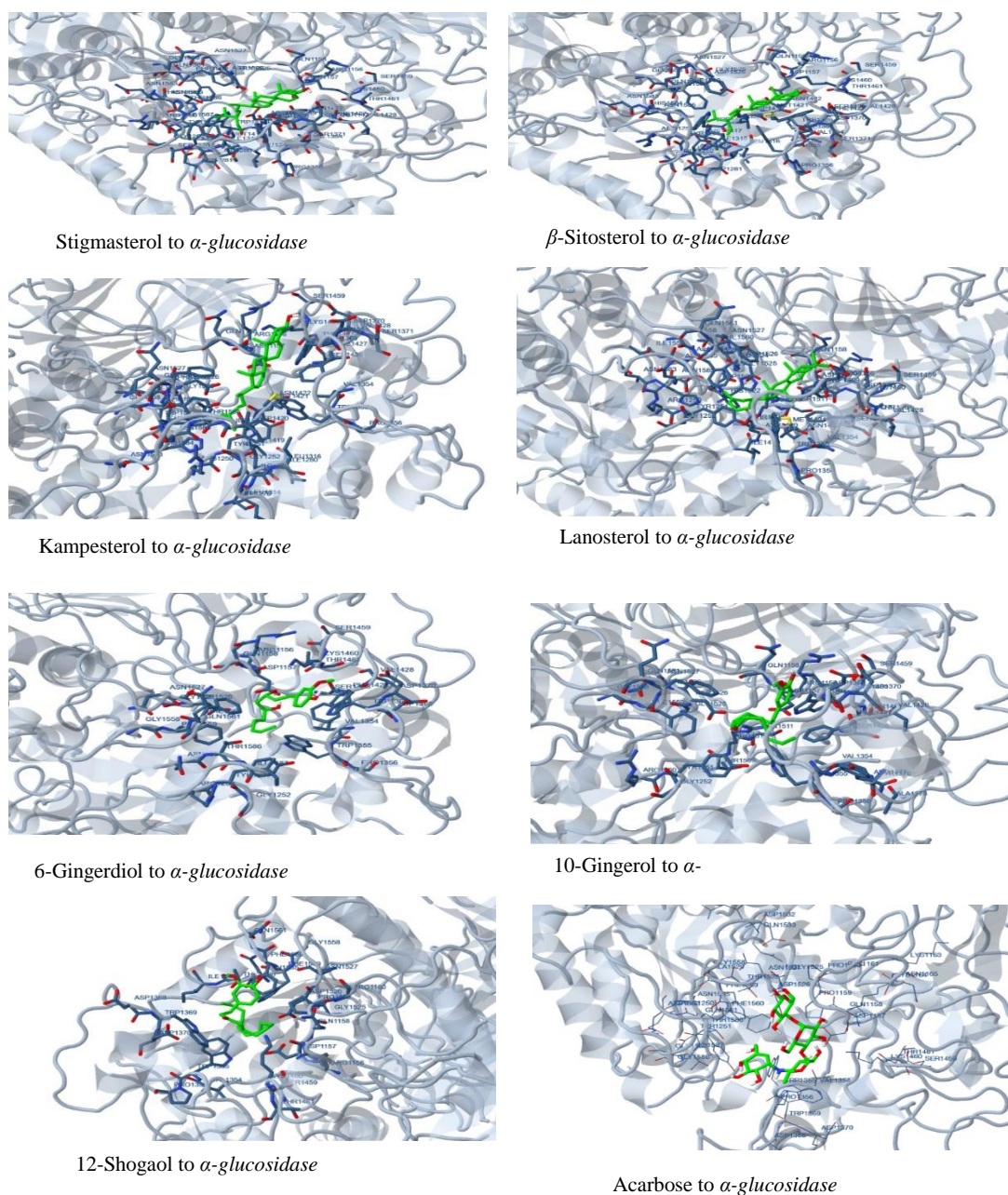
**Figure 5:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4



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

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (nM)	Surface Interaction (Å)
<i>Glycine max</i>	Stigmasterol	-10.57	17.8	893.79
	$\beta$ -Sitosterol	-10.26	30.0	902.57
	Kampesterol	-7.79	93.6	872.98
	Lanosterol	-7.86	162.2	894.78
<i>Zingiber officinale</i>	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

**Figure 6:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with  $\alpha$ -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  $\alpha$ -glucosidase activity was strongly inhibited by stigmasterol and 6-Gingerol. However, *G. max* seeds was found to have more potential to be used as a drug candidate for diabetes as it was able to inhibit both enzymes compared to *Z. officinale* rhizomes.

## Conflict of interest

The authors declare no conflict of interest

## Authors' Declaration

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

## Acknowledgements

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

## References

- Deacon C. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2020; 16(11): 642-653.
- Singh S, Wright E, Kwan A, Thompson J, Syed I, Korol EE, Waser NA, Yu MB, Juneja R. Glucagon-like peptide-1 receptor agonists compared with basal insulins for the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes, Obes Metab*. 2016; 19(2):228-238.
- Gallwitz B. Clinical Use of DPP-4 Inhibitors. *Front Endocrinol (Lausanne)*. 2019; 10(1):1-8.
- Pereira A, Banegas-Luna A, Peña-García J, Pérez-Sánchez H, Apostolides Z. Evaluation of the Anti-Diabetic Activity of Some Common Herbs and Spices: Providing New Insights with Inverse Virtual Screening. *Molecules*. 2019; 24(22): 4030.
- Zeng L, Zhang G, Lin S, Gong D. Inhibitory Mechanism of Apigenin on  $\alpha$ -Glucosidase and Synergy Analysis of Flavonoids. *J Agric Food Chem*. 2016; 64(37): 6939-6949.
- Soelistijo, SA. Consensus of the Management and Prevention of Type 2 Diabetes Mellitus in Indonesia (1<sup>st</sup> Ed.). Jakarta: PB Perkeni; 2015. p6-65
- Pang G, Li F, Yan Y, Zhang Y, Kong L, Zhu P, Wang KF, Zhang F, Liu B, Lu CL. Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chin Med J (Engl)*. 2019; 132(1):78-85.
- World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023 [Internet]. World Health Organization (WHO). Geneva; 2013. Available from: [http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1) (Accessed 04.09.2021)
- Mustofa M, Mukhtar D, Susmiarsih T, Royhan A. Effect of Soybean (*Glycine max* (L) Merrill) on Blood Glucose Levels and Expression of Pancreatic B Cell Insulin in Diabetic Rats. *J Kedokt Yars.β* 2010; 18(2):094-103.
- Pabich M and Materska M. Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. *Nutr*. 2019; 11(7): 1660.
- Bischoff-Kont I and Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. *Pharmaceuticals (Basel)*. 2021; 14(6):571p.
- Tiwari V, Mishra B, Dixit A, Antony J, Tiwari R, Sharma N. Opportunity, Challenge and scope of natural products in medicinal chemistry. *Kerala: Res Signpost*; 2011; 103-121 p.
- Yulianingtyas Y and Kusmartono B. Optimization of Volume of solvent and Maceration Time of Flavonoid Extraction of Belimbing Wuluh Leaves (*Averrhoa Bilimbi* L.). *J Tek Kim*. 2016; 10:58-64.
- Nicklaus M. Online SMILES Translator [Internet]. Cactus.nci.nih.gov. 2020 [cited 4 September 2021]. Available from: <https://cactus.nci.nih.gov/translate>
- Seo CS and Shin HK. Liquid chromatography tandem mass spectrometry for the simultaneous quantification of eleven phytochemical constituents in traditional Korean medicine, sogunjung decoction. *Processes*. 2021; 9(1):1-10.
- O'Boyle N, Banck M, James C, Morley C, Vandermeersch T, Hutchison G. Open Babel: An open chemical toolbox. *J Cheminfo*. 2011; 3(1):33.
- Putra AMJ. Once Again About Docking [Internet]. LIPI. 2014. Available from: <http://u.lipi.go.id/1391883188>. Accessed 04.09.2021.
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 2018; 46(W1):W296-303.
- Khalaf I, Corciovă A, Vlase I, Ivănescu B and Lazăr D. LC/MS Analysis of Sterolic Compounds from *Glycyrrhiza Glabra*. *Stud Univ Babeş-Bolyai Chem*. 2011; 3(1):97-102.
- Fikri F, Saptarini N, Levita J. The Inhibitory Activity on the Rate of Prostaglandin Production by *Zingiber officinale* var. Rubrum. *Pharmacol Clin Pharm Res*. 2016; 1(1):33-41.
- Bilbao A. Proteomics Mass Spectrometry Data Analysis Tools. *Encyclopedia of Bioinformatics and Computational Biology: ABC of Bioinformatics*. Elsevier Ltd; 2018. 84-95p.
- Skubic C, Vovk I, Rozman D, Križman M. Simplified LC-MS Method for Analysis of Sterols in Biological Samples. *Molecules*. 2020; 25(18):2-10
- Zhu H, Chen J, He Z, Hao W, Liu J, Kwek E Ma KY, Bi Y. Plasma Cholesterol-Lowering Activity of Soybean Germ Phytosterols. *Nutrients*. 2019; 11(11):2-18.
- Trautwein E, Vermeer M, Hiemstra H, Ras R. LDL-Cholesterol Lowering of Plant Sterols and Stanols-Which Factors Influence Their Efficacy? *Nutrients*. 2018; 10(9):1262.
- Nguyen H, Neelakadan A, Quach T, Valliyodan B, Kumar R, Zhang Z and Nguyen HT. Molecular characterization of *Glycine max* squalene synthase genes in seed phytosterol biosynthesis. *Plant Physiol Biochem*. 2013; 73(1):23-32.
- Daszynski D, Santhoshkumar P, Phadte A, Sharma K, Zhong H, Lou MF and Kador PF. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts. *Sci Rep*. 2019; 9(1):8459.
- Wei C, Tsai Y, Korinek M, Hung P, El-Shazly M, Cheng YB, Wu YC, Hsieh TJ, Chang FR. 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *Int J Mol Sci*. 2017; 18(1):168.
- Mahmudati N. Ginger (*Zingiber officinale*) extract decrease TNF expression of rat induced by High Fat Diet (HFD). *Proc Biol Educ Conf, FKIP UNS-2016*. 2016; 13(1):653-5.
- Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2012; 64(1):4-17.
- Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-diabetic potential of Urena lobata leaf extract through inhibition of Dipeptidyl Peptidase-IV activity. *Asian Pac J Trop Biomed*. 2015; 5(8): 645-649.
- Utomo D, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. *Bioinformation*. 2012; 8(9):426-429.
- Riyanti S, Suganda AG and Sukandar EY. Dipeptidyl Peptidase-IV Inhibitory Activity of Some Indonesian Medicinal Plants. *Asian J Pharm Clin Res*. 2016; 9(2): 375-377.

33. Bikadi Z and Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J Cheminfo*. 2009; 1(1): 15.
34. Purnomo Y, Makdasari J, Fatahillah FI. Inhibitory activity of Urena lobate leaf extract on alpha-amylase and alpha glucosidase: *in vitro* and *in silico* approach. *J Basic Clin Physiol Pharmacol* . 2021; 32(4):889-894.
35. Rosiarto B, Puspaningtyas A, Holidah D. Study of Antioxidant Activity of Compound 1-(p-chlorobenzoyloxymethyl)-5-fluorouracil with Molecular Docking Method and DPPH. *Med Rep*. 2014; 2(1):95-99.

**TJNPR\_GALLEY PROOF  
DIREVISI**



## 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

Yudi Purnomo\*, Muhammad Taufiq, Afnan N. D. Wijaya, Reza Hakim

Faculty of Medicine, University of Islam Malang, Malang Indonesia

## ARTICLE INFO

## Article history:

Received 27 March 2021

Revised 16 September 2021

Accepted 21 October 2021

Published online xxxxxxxxxxxx

## ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase ( $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -glucosidase. This shows that both soybean seed and ginger rhizome potentially act as an anti-diabetic by inhibiting DPP-4 and  $\alpha$ -glucosidase; however, soybean seed is more potent due to its ability to inhibit both of the tested enzymes.

**Copyright:** © 2021 Purnomo *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Keywords:** Anti-diabetic, *Glycine max*, *Zingiber officinale*, DPP-4,  $\alpha$ -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).<sup>1</sup> However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively into inactive forms,<sup>1</sup> causing GLP-1 to have a short half-life (to approximately 2-5 minutes).<sup>1,2</sup> 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.<sup>3</sup> Meanwhile, alpha-glucosidases ( $\alpha$ -glucosidases) are enzymes that are responsible for the conversion of complex carbohydrate into maltose, dextrin and maltotriose.<sup>4</sup> 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.<sup>5</sup> Thus,  $\alpha$ -glucosidases activity contributes in increasing blood glucose level post prandially, and needs to be controlled to avoid hyperglycemia. Inhibition of both DPP-4 and  $\alpha$ -glucosidase could be used in preventing the increase of blood glucose level, especially for patients with T2DM.<sup>3,4</sup>

\*Corresponding author. E mail: [y\\_purnomo92@yahoo.com](mailto:y_purnomo92@yahoo.com)  
Tel: +62 812-3354-124

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

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

Therapy of T2DM usually requires an Oral Hypoglycemic Drug (OHD), which acts either by increasing of insulin secretion, repairing of insulin resistance and/or inhibiting of glucose absorption. However, long-term consumption of these medications is often accompanied with various adverse drug reactions, particularly in DM patients.<sup>6</sup> 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.<sup>7,8</sup>

*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,<sup>9</sup> mainly due to the presence of isoflavons such as daidzein and genestein.<sup>10</sup> 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.<sup>11</sup> 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.<sup>10-12</sup> 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  $\alpha$ -glucosidase using *in silico* study.

## 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, respectively. 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

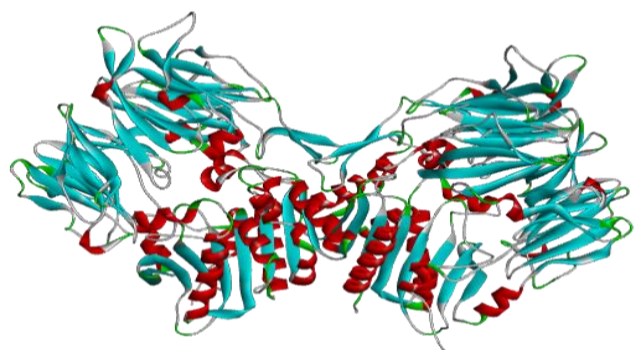
Ethanol extracts of *G. max* seed and *Z. officinale* rhizome were qualitative analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific, with Hypersil GOLD as the stationary phase. The mobile phase were used solution A (0.1% formic acid in H<sub>2</sub>O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300  $\mu$ L/min.<sup>13</sup>

### Prediction of physicochemical property

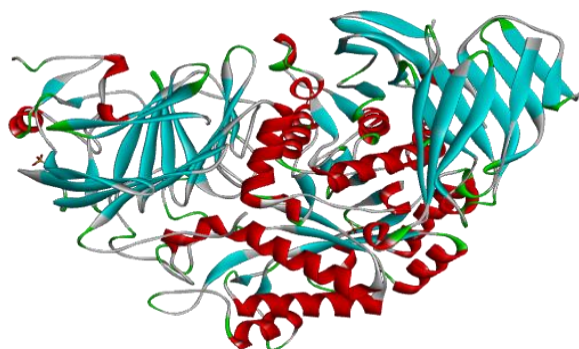
Prediction of the physicochemical properties of the active compound was performed using the pkCSM online tool. Firstly, active compounds identified and references standard were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then 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.<sup>14</sup>

### 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.<sup>15</sup> The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and  $\alpha$ -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 $\alpha$ -glucosidase

Moreover, protein homology on the Swiss Model ([www.swissmodel.expasy.org/interactive](http://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  $\alpha$ -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,  $\beta$ -Sitosterol, and Lanosterol. The highest component is  $\beta$ -Sitosterol, whereas the lowest component is Lanosterol.<sup>18</sup> 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.<sup>19</sup>

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.<sup>20</sup> 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.<sup>21</sup>

As mentioned before, the main components of phytosterols in *G. max* seeds are  $\beta$ -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  $\beta$ -Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant<sup>23</sup> 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.<sup>24</sup> Some studies both in vivo and in vitro showed that lanosterol has activity as anti-cataract.<sup>25</sup> 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.<sup>22</sup> 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.<sup>26</sup> 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  $\beta$ -cells pancreas.<sup>11,27</sup> Other research indicated that administration of *Z. officinale* extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.<sup>28</sup>

### Prediction of physicochemical properties

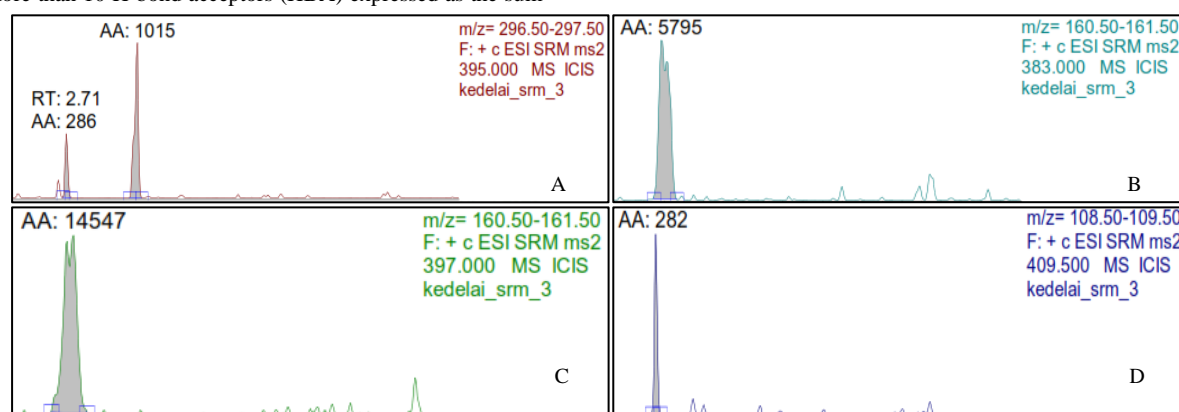
The result of the *in silico* study of the physicochemical properties of *Glycine max* and *Zingiber officinale* active compound is presented in Table 2. It can be seen that the molecular weight values of the active

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 (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.

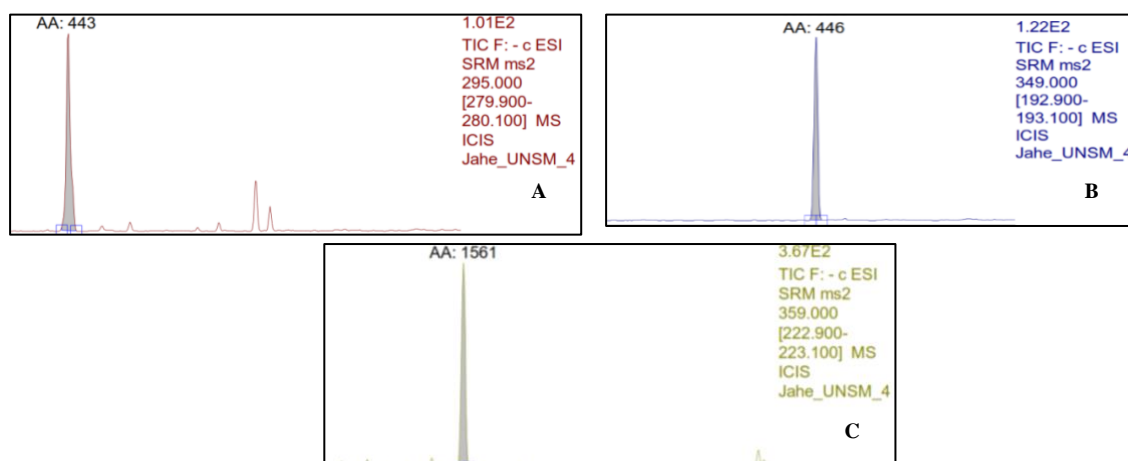
Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were 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.<sup>29</sup>

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.<sup>29</sup> 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)  $\beta$ -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
<i>Glycine</i>	Kampesterol	383-161	383	5795
<i>max</i>	$\beta$ -Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
<i>Zingiber</i>	6-Gingerdiol	295-280	295	443

<i>officinale</i>	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,  $\beta$ -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.<sup>30</sup>

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.<sup>30</sup> 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.<sup>31</sup> 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.<sup>30,32</sup> 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.<sup>31</sup> 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 (Å <sup>2</sup> )	Water Solubility
<i>Glycine max</i>	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
	$\beta$ -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
<i>Zingiber officinale</i>	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 ( $\mu$ M)	Surface Interaction (Å)
<i>Glycine max</i>	Stigmasterol	-7.11	6.16	931.16
	$\beta$ -Sitosterol	-6.94	8.13	908.89
	Kampesterol	-6.84	9.74	877.50
	Lanosterol	-7.03	7.06	860.98
<i>Zingiber officinale</i>	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  $\alpha$ -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  $\alpha$ -glucosidase.<sup>34</sup> Based on inhibition constant (Ki) value, stigmasterol and  $\beta$ -sitosterol had higher inhibitory activity against  $\alpha$ -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).

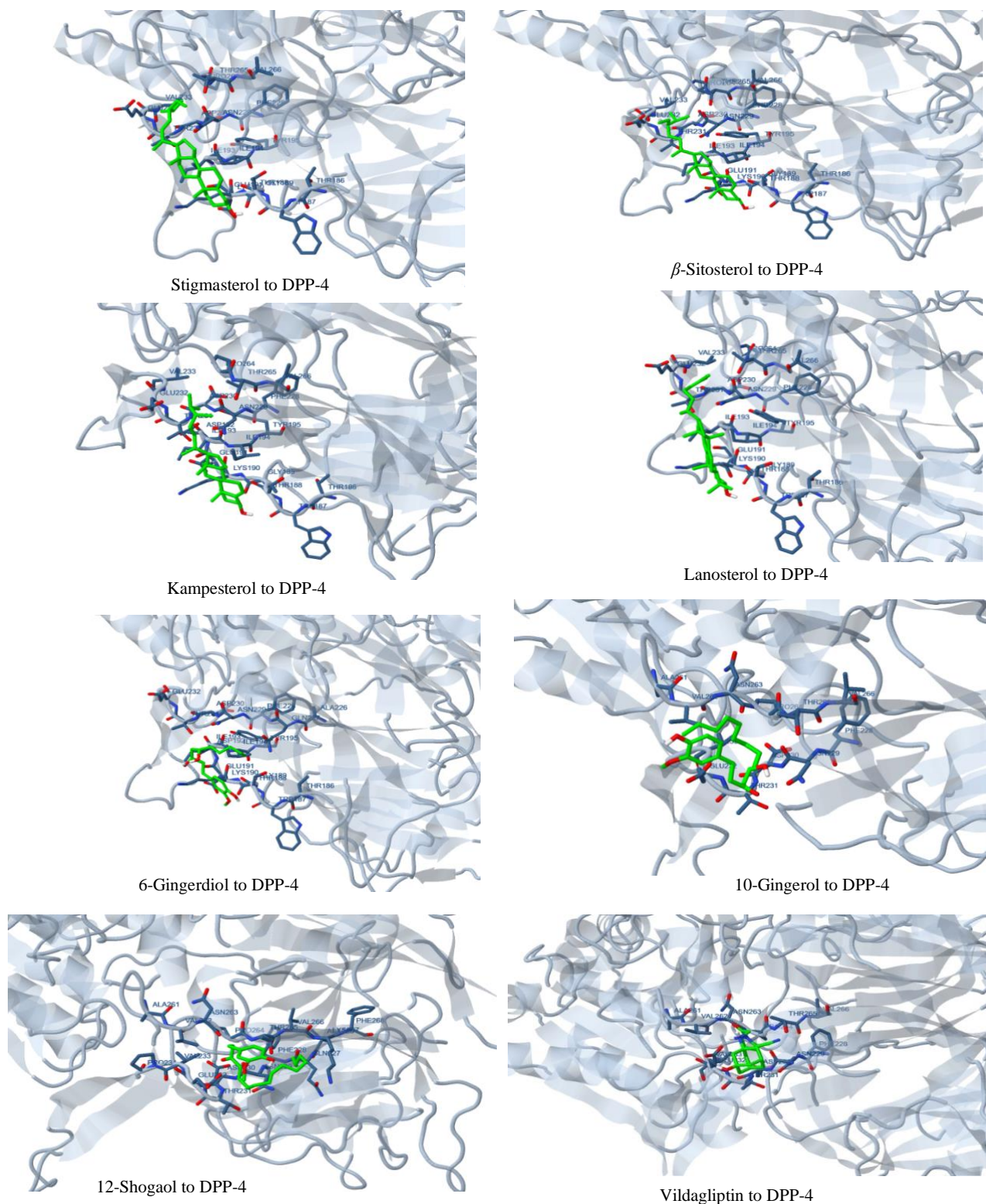
Based on the active compounds in both of the extracts, stigmasterol in soybean and 6-gingerdiol in *Z. officinale* had the lowest Ki score, followed by  $\beta$ -sitosterol and 12-shogaol, against  $\alpha$ -glucosidase. This shows that the inhibitory activity of the ligands to the target protein



was high. The lower  $K_i$  scores indicate a high inhibitory activity against the target protein.<sup>35</sup> 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  $\alpha$ -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  $\alpha$ -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.<sup>31,34</sup>

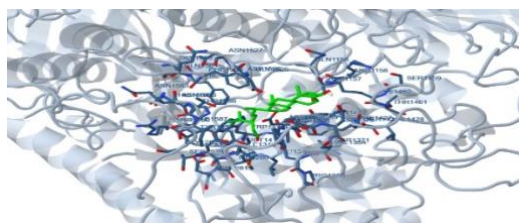
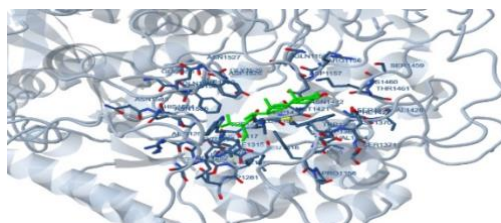
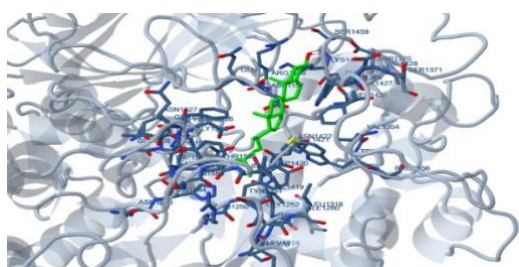
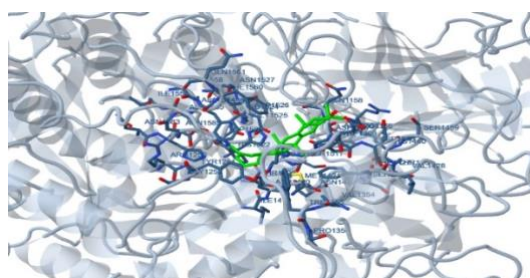
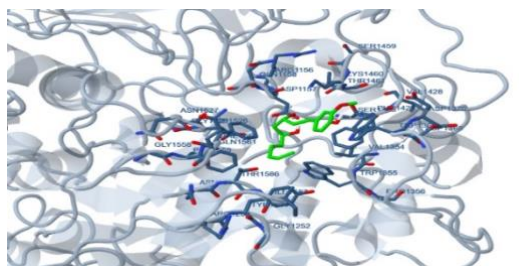
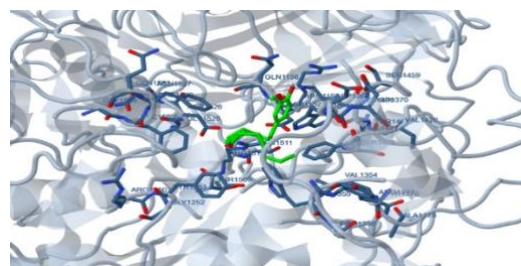
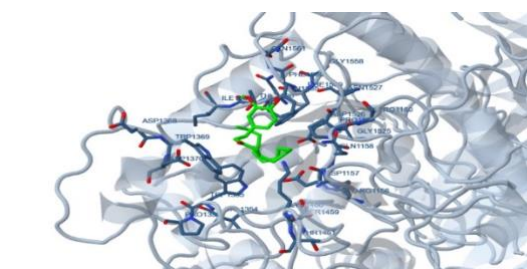
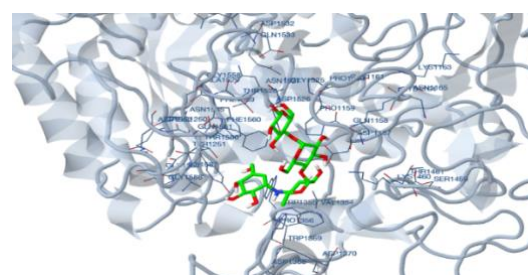


**Figure 5:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4  
**Table 4:** Molecular docking of active substances in *Glycine max* and *Zingiber officinale* extracts with  $\alpha$ -glucosidase

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (nM)	Surface Interaction (Å)
<i>Glycine</i>	Stigmasterol	-10.57	17.8	893.79
<i>max</i>	$\beta$ -Sitosterol	-10.26	30.0	902.57
	Kampesterol	-7.79	93.6	872.98

	Lanosterol	-7.86	162.2	894.78
<i>Zingiber</i>	6-Gingerdiol	-5.56	83390	755.71
<i>officinale</i>	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  $\alpha$ -glucosidase $\beta$ -Sitosterol to  $\alpha$ -glucosidaseKampesterol to  $\alpha$ -glucosidaseLanosterol to  $\alpha$ -glucosidase6-Gingerdiol to  $\alpha$ -glucosidase10-Gingerol to  $\alpha$ -12-Shogaol to  $\alpha$ -glucosidaseAcarbose to  $\alpha$ -glucosidase**Figure 6:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with  $\alpha$ -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  $\alpha$ -glucosidase activity was strongly inhibited by stigmasterol and 6-Gingerol. However, *G. max* seeds was found to have more potential to be used as a drug candidate for

diabetes as it was able to inhibit both enzymes compared to *Z. officinale* rhizomes.

**Conflict of interest**

The authors declare no conflict of interest

**Authors' Declaration**

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

### Acknowledgements

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

### References

- Deacon C. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2020; 16(11): 642-653.
- Singh S, Wright E, Kwan A, Thompson J, Syed I, Korol EE, Waser NA, Yu MB, Juneja R. Glucagon-like peptide-1 receptor agonists compared with basal insulins for the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes, Obes Metab*. 2016; 19(2):228-238.
- Gallwitz B. Clinical Use of DPP-4 Inhibitors. *Front Endocrinol (Lausanne)*. 2019; 10(1):1-8.
- Pereira A, Banegas-Luna A, Peña-García J, Pérez-Sánchez H, Apostolides Z. Evaluation of the Anti-Diabetic Activity of Some Common Herbs and Spices: Providing New Insights with Inverse Virtual Screening. *Molecules*. 2019; 24(22): 4030.
- Zeng L, Zhang G, Lin S, Gong D. Inhibitory Mechanism of Apigenin on  $\alpha$ -Glucosidase and Synergy Analysis of Flavonoids. *J Agric Food Chem*. 2016; 64(37): 6939-6949.
- Soelistijo, SA. Consensus of the Management and Prevention of Type 2 Diabetes Mellitus in Indonesia (1<sup>st</sup> Ed.). Jakarta: PB Perkeni; 2015. p6-65
- Pang G, Li F, Yan Y, Zhang Y, Kong L, Zhu P, Wang KF, Zhang F, Liu B, Lu CL. Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chin Med J (Engl)*. 2019; 132(1):78-85.
- World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023 [Internet]. World Health Organization (WHO). Geneva; 2013. Available from: [http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1) (Accessed 04.09.2021)
- Mustofa M, Mukhtar D, Susmiarsih T, Royhan A. Effect of Soybean (*Glycine max* (L) Merrill) on Blood Glucose Levels and Expression of Pancreatic B Cell Insulin in Diabetic Rats. *J Kedokt Yarsi* 2010; 18(2):094-103.
- Pabich M and Materska M. Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. *Nutr*. 2019; 11(7): 1660.
- Bischoff-Kont I and Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. *Pharmaceuticals (Basel)*. 2021; 14(6):571p.
- Tiwari V, Mishra B, Dixit A, Antony J, Tiwari R, Sharma N. Opportunity, Challenge and scope of natural products in medicinal chemistry. Kerala: Res Signpost; 2011; 103-121 p.
- Yulianingtyas Y and Kusmartono B. Optimization of Volume of solvent and Maceration Time of Flavonoid Extraction of Belimbing Wuluh Leaves (*Averrhoa Bilimbi* L.). *J Tek Kim*. 2016; 10:58-64.
- Nicklaus M. Online SMILES Translator [Internet]. Cactus.nci.nih.gov. 2020 [cited 4 September 2021]. Available from: <https://cactus.nci.nih.gov/translate>
- Seo CS and Shin HK. Liquid chromatography tandem mass spectrometry for the simultaneous quantification of eleven phytochemical constituents in traditional Korean medicine, sogunjung decoction. *Processes*. 2021; 9(1):1-10.
- O'Boyle N, Banck M, James C, Morley C, Vandermeersch T, Hutchison G. Open Babel: An open chemical toolbox. *J Cheminfo*. 2011; 3(1):33.
- Putra AMJ. Once Again About Docking [Internet]. LIPI. 2014. Available from: <http://u.lipi.go.id/1391883188>. (Accessed 04.09.2021).
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer F.T, de Beer T.A.P, Rempfer C, Bordoli L, Lepore R, Schwede T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 2018; 46(W1):W296-303
- Khalaf I, Corciovă A, Vlase I, Ivănescu B and Lazăr D. LC/MS Analysis of Sterolic Compounds from *Glycyrrhiza Glabra*. *Stud Univ Babes-Bolyai Chem*. 2011; 3(1):97-102.
- Fikri F, Saptarini N, Levita J. The Inhibitory Activity on the Rate of Prostaglandin Production by *Zingiber officinale* var. Rubrum. *Pharmacol Clin Pharm Res*. 2016; 1(1):33-41.
- Bilbao A. Proteomics Mass Spectrometry Data Analysis Tools. *Encyclopedia of Bioinformatics and Computational Biology: ABC of Bioinformatics*. Elsevier Ltd; 2018. 84-95p.
- Skubic C, Vovk I, Rozman D, Križman M. Simplified LC-MS Method for Analysis of Sterols in Biological Samples. *Molecules*. 2020; 25(18):2-10
- Zhu H, Chen J, He Z, Hao W, Liu J, Kwek E Ma KY, Bi Y. Plasma Cholesterol-Lowering Activity of Soybean Germ Phytosterols. *Nutrients*. 2019; 11(11):2-18.
- Trautwein E, Vermeer M, Hiemstra H, Ras R. LDL-Cholesterol Lowering of Plant Sterols and Stanols-Which Factors Influence Their Efficacy? *Nutrients*. 2018; 10(9):1262.
- Nguyen H, Neelakadan A, Quach T, Valliyodan B, Kumar R, Zhang Z and Nguyen HT. Molecular characterization of *Glycine max* squalene synthase genes in seed phytosterol biosynthesis. *Plant Physiol Biochem*. 2013; 73(1):23-32.
- Daszynski D, Santhoshkumar P, Phadte A, Sharma K, Zhong H, Lou MF and Kador PF. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts. *Sci Rep*. 2019; 9(1):8459.
- Wei C, Tsai Y, Korinek M, Hung P, El-Shazly M, Cheng YB, Wu YC, Hsieh TJ, Chang FR. 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *Int J Mol Sci*. 2017; 18(1):168.
- Mahmudati N. Ginger (*Zingiber officinale*) extract decrease TNF expression of rat induced by High Fat Diet (HFD). *Proc Biol Educ Conf, FKIP UNS-2016*. 2016; 13(1):653-5.
- Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2012; 64(1):4-17.
- Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-diabetic potential of Urena lobata leaf extract through inhibition of Dipeptidyl Peptidase-IV activity. *Asian Pac J Trop Biomed*. 2015; 5(8): 645-649.
- Utomo D, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. *Bioinformation*. 2012; 8(9):426-429.
- Riyanti S, Suganda AG and Sukandar EY. Dipeptidyl Peptidase-IV Inhibitory Activity of Some Indonesian Medicinal Plants. *Asian J Pharm Clin Res*. 2016; 9(2): 375-377.
- Bikadi Z and Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J Cheminfo*. 2009; 1(1): 15.
- Purnomo Y, Makdasari J, Fatahillah FI. Inhibitory activity of Urena lobate leaf extract on alpha-amylase and alpha glucosidase: *in vitro* and *in silico* approach. *J Basic Clin Physiol Pharmacol*. 2021; 32(4):889-894.
- Rosiarto B, Puspaningtyas A, Holiday D. Study of Antioxidant Activity of Compound 1-(p-chlorobenzoyloxymethyl)-5-fluorouracil with Molecular Docking Method and DPPH. *Med Rep*. 2014; 2(1):95-99.



**TJNPR\_MANUSCRIPT  
DIPUBLIKASIKAN**

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

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



# Tropical Journal of Natural Product Research



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

*Original Research Article*

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

Yudi Purnomo\*, Muhammad Taufiq, Afnan N. D. Wijaya, Reza Hakim

*Faculty of Medicine, University of Islam Malang, Malang Indonesia*

### ARTICLE INFO

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

### ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase ( $\alpha$ -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



## 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

Yudi Purnomo\*, Muhammad Taufiq, Afnan N. D. Wijaya, Reza Hakim

Faculty of Medicine, University of Islam Malang, Malang Indonesia

### ARTICLE INFO

#### Article history:

Received 27 March 2021

Revised 16 September 2021

Accepted 21 October 2021

Published online 02 November 2021

**Copyright:** © 2021 Purnomo *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### ABSTRACT

Dipeptidyl Peptidase-4 (DPP-4) and alpha-glucosidase ( $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -glucosidase. This shows that both soybean seed and ginger rhizome potentially act as an anti-diabetic by inhibiting DPP-4 and  $\alpha$ -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,  $\alpha$ -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).<sup>1</sup> However, GLP-1 is metabolized by Dipeptidyl peptidase-4 (DPP-4) excessively into inactive forms,<sup>1</sup> causing GLP-1 to have a short half-life (to approximately 2-5 minutes).<sup>1,2</sup> 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.<sup>3</sup> Meanwhile, alpha-glucosidases ( $\alpha$ -glucosidases) are enzymes that are responsible for the conversion of complex carbohydrate into maltose, dextrin and maltotriose.<sup>4</sup> 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.<sup>5</sup> Thus,  $\alpha$ -glucosidases activity contributes in increasing blood glucose level post prandially, and needs to be controlled to avoid hyperglycemia. Inhibition of both DPP-4 and  $\alpha$ -glucosidase could be used in preventing the increase of blood glucose level, especially for patients with T2DM.<sup>3,4</sup>

\*Corresponding author. E mail: [y\\_purnomo92@yahoo.com](mailto:y_purnomo92@yahoo.com)  
Tel: +62 812-3354-124

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

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

Therapy of T2DM usually requires an Oral Hypoglycemic Drug (OHD), which acts either by increasing of insulin secretion, repairing of insulin resistance and/or inhibiting of glucose absorption. However, long-term consumption of these medications is often accompanied with various adverse drug reactions, particularly in DM patients.<sup>6</sup> 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.<sup>7,8</sup>

*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,<sup>9</sup> mainly due to the presence of isoflavons such as daidzein and genestein.<sup>10</sup> 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.<sup>11</sup> 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.<sup>10-12</sup> 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  $\alpha$ -glucosidase using *in silico* study.



## 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, respectively. 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

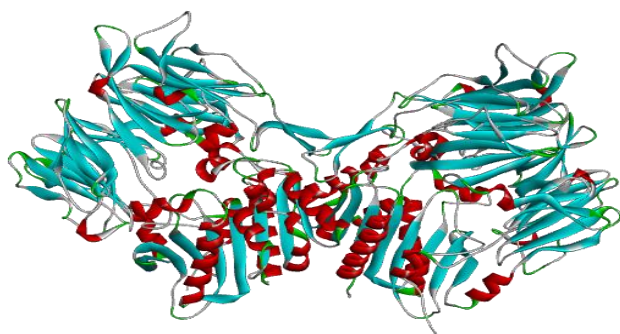
Ethanol extracts of *G. max* seed and *Z. officinale* rhizome were qualitative analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) TSQ Quantum Access Max Thermo Scientific, with Hypersil GOLD as the stationary phase. The mobile phase were used solution A (0.1% formic acid in H<sub>2</sub>O) and solution B (0.1% formic acid in acetonitrile) with a flow rate of 300 µL/min.<sup>13</sup>

### Prediction of physicochemical property

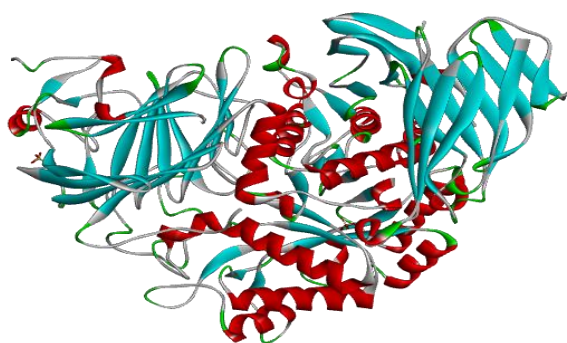
Prediction of the physicochemical properties of the active compound was performed using the pkCSM online tool. Firstly, active compounds identified and references standard were drawn as 2D molecular structures with ChemBio Draw Ultra and copied into ChemBio 3D Ultra to create a 3D structure, and then 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.<sup>14</sup>

### 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.<sup>15</sup> The FASTA format of the target protein (DPP-4 with UniProt ID: P27487 and  $\alpha$ -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  $\alpha$ -glucosidase

Moreover, protein homology on the Swiss Model ([www.swissmodel.expasy.org/interactive](http://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](http://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  $\alpha$ -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,  $\beta$ -Sitosterol, and Lanosterol. The highest component is  $\beta$ -Sitosterol, whereas the lowest component is Lanosterol.<sup>18</sup> 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.<sup>19</sup>

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.<sup>20</sup> 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.<sup>21</sup>

As mentioned before, the main components of phytosterols in *G. max* seeds are  $\beta$ -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  $\beta$ -Sitosterol is known to act as an anti-cholesterol, anti-inflammatory, immunomodulatory, and antioxidant<sup>23</sup> 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.<sup>24</sup> Some studies both in vivo and in vitro showed that lanosterol has activity as anti-cataract.<sup>25</sup> 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.<sup>22</sup> 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.<sup>26</sup> 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  $\beta$ -cells pancreas.<sup>11,27</sup> Other research indicated that administration of *Z. officinale* extract can reduce cholesterol, glucose, and triglyceride levels in experimental animals induced by Diabetes Mellitus.<sup>28</sup>

### Prediction of physicochemical properties

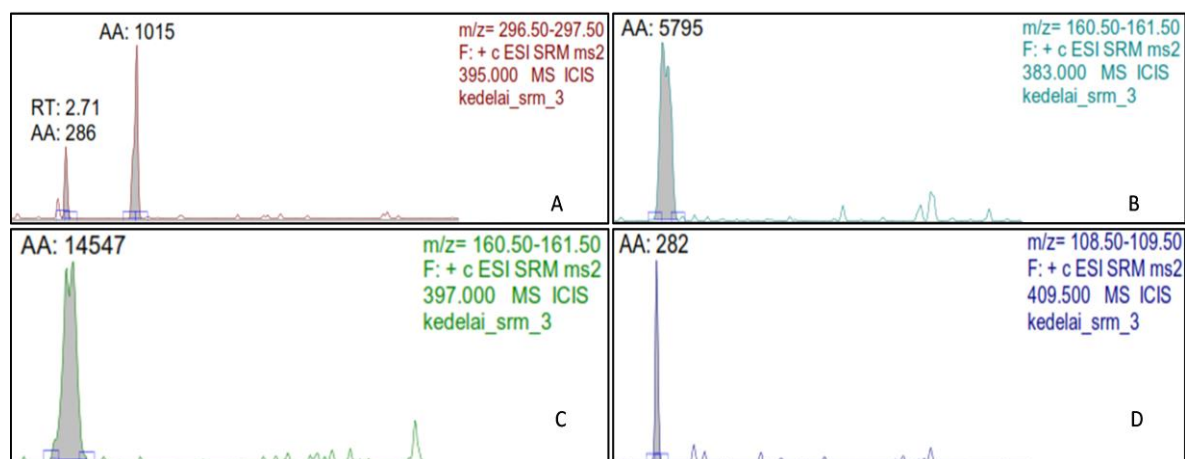
The result of the *in silico* study of the physicochemical properties of *Glycine max* and *Zingiber officinale* active compound is presented in Table 2. It can be seen that the molecular weight values of the active compound ranged from 296 to 426 (less than 500), the value of log of the octanol/water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

(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.

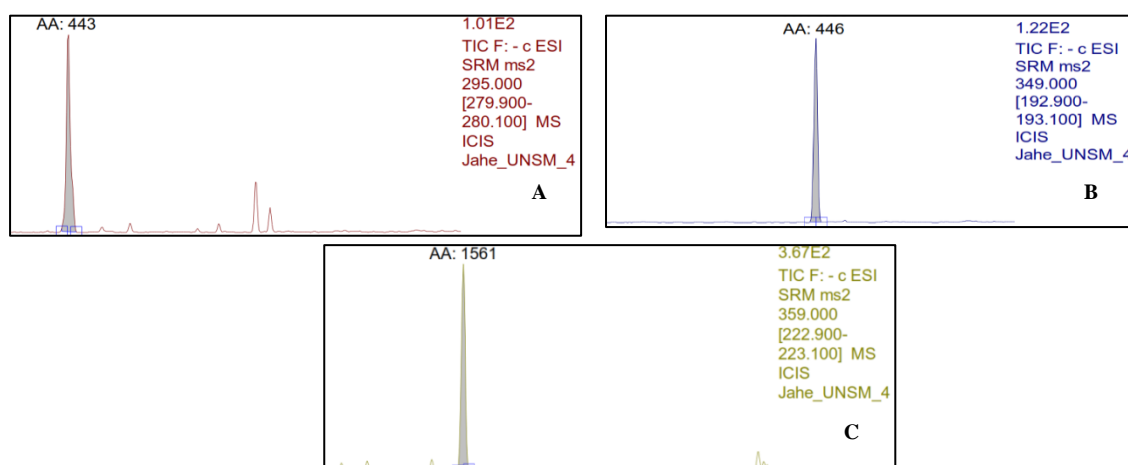
Chemical databases contain many of molecules that could be suitable ligands for an enzyme. However, no matter how good the fit with the protein target, the candidate molecule is of no use if the absorption is poor or if the drug is eliminated too slowly from the body. The World Drugs Index database were 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.<sup>29</sup>

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.<sup>29</sup> 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) Kampesterol (C)  $\beta$ -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
<i>Glycine max</i>	Kampesterol	383-161	383	5795
	$\beta$ -Sitosterol	397-161	397	14547
	Lanosterol	409-109	409	282
<i>Zingiber officinale</i>	6-Gingerdiol	295-280	295	443
	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,  $\beta$ -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.<sup>30</sup>

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.<sup>30</sup> 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.<sup>31</sup> 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.<sup>30,32</sup> 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.<sup>31</sup> 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 (Å <sup>2</sup> )	Water Solubility
<i>Glycine max</i>	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
	$\beta$ -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
<i>Zingiber officinale</i>	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 ( $\mu$ M)	Surface Interaction (Å)
<i>Glycine max</i>	Stigmasterol	-7.11	6.16	931.16
	$\beta$ -Sitosterol	-6.94	8.13	908.89
	Kampesterol	-6.84	9.74	877.50
	Lanosterol	-7.03	7.06	860.98
<i>Zingiber officinale</i>	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  $\alpha$ -glucosidase were evaluated by *in-silico* approach and the results can be seen at Table 4 and Figure 6.

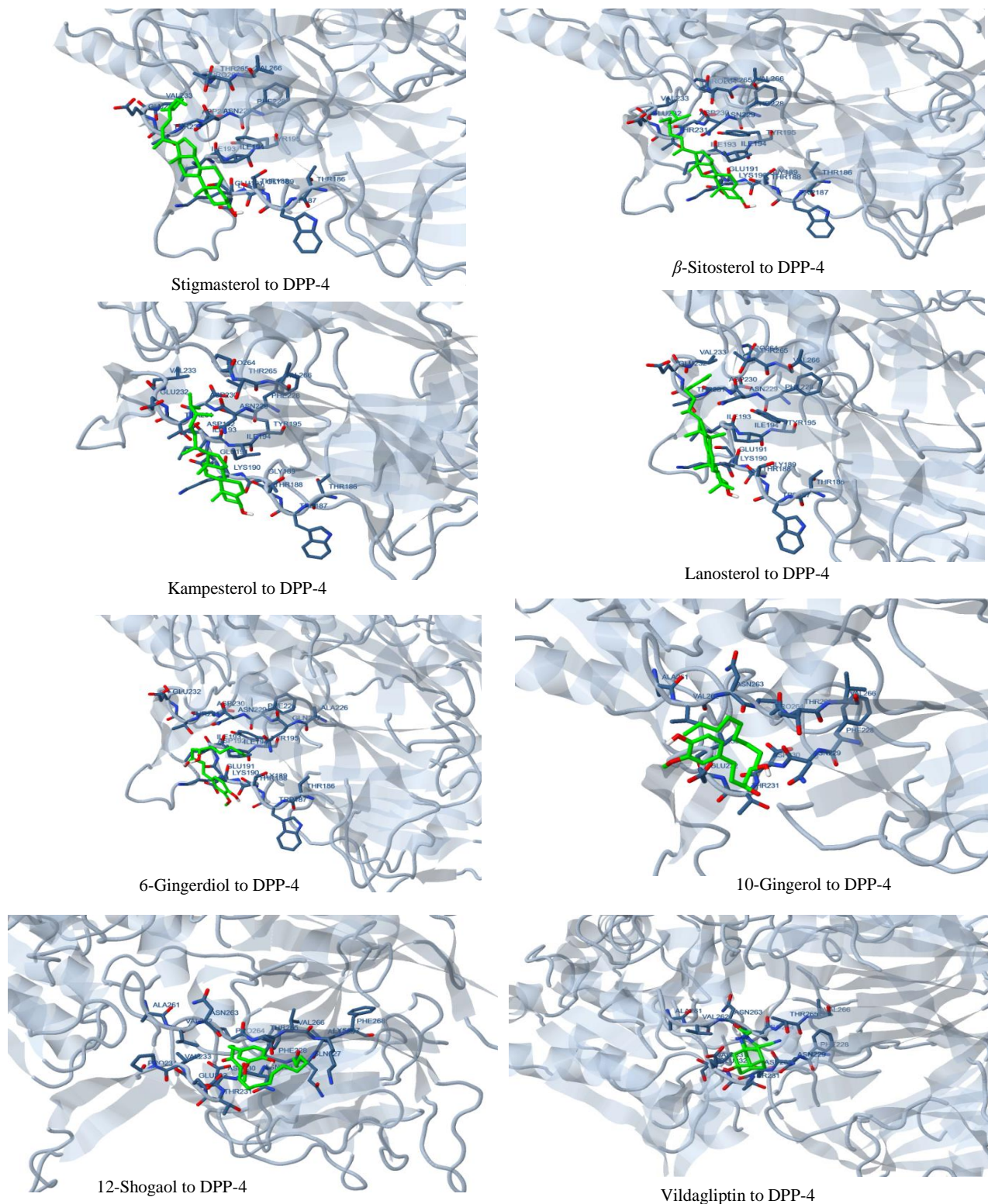
*In-silico* studies indicates that stigmasterol and 6-gingerdiol have a lower Ki, free energy binding, and surface interaction scores compared to other active compounds. Meanwhile, lanosterol and 10-gingerol was found to have a higher free energy binding score compared to other compounds in *G. max* and *Z. officinale*. The differences in each parameter value causes the differences of the inhibitory activity against  $\alpha$ -glucosidase.<sup>34</sup> Based on inhibition constant (Ki) value, stigmasterol and  $\beta$ -sitosterol had higher inhibitory

activity against  $\alpha$ -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  $\beta$ -sitosterol and 12-shogaol, against  $\alpha$ -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.<sup>35</sup> 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  $\alpha$ -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  $\alpha$ -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.<sup>31,34</sup>

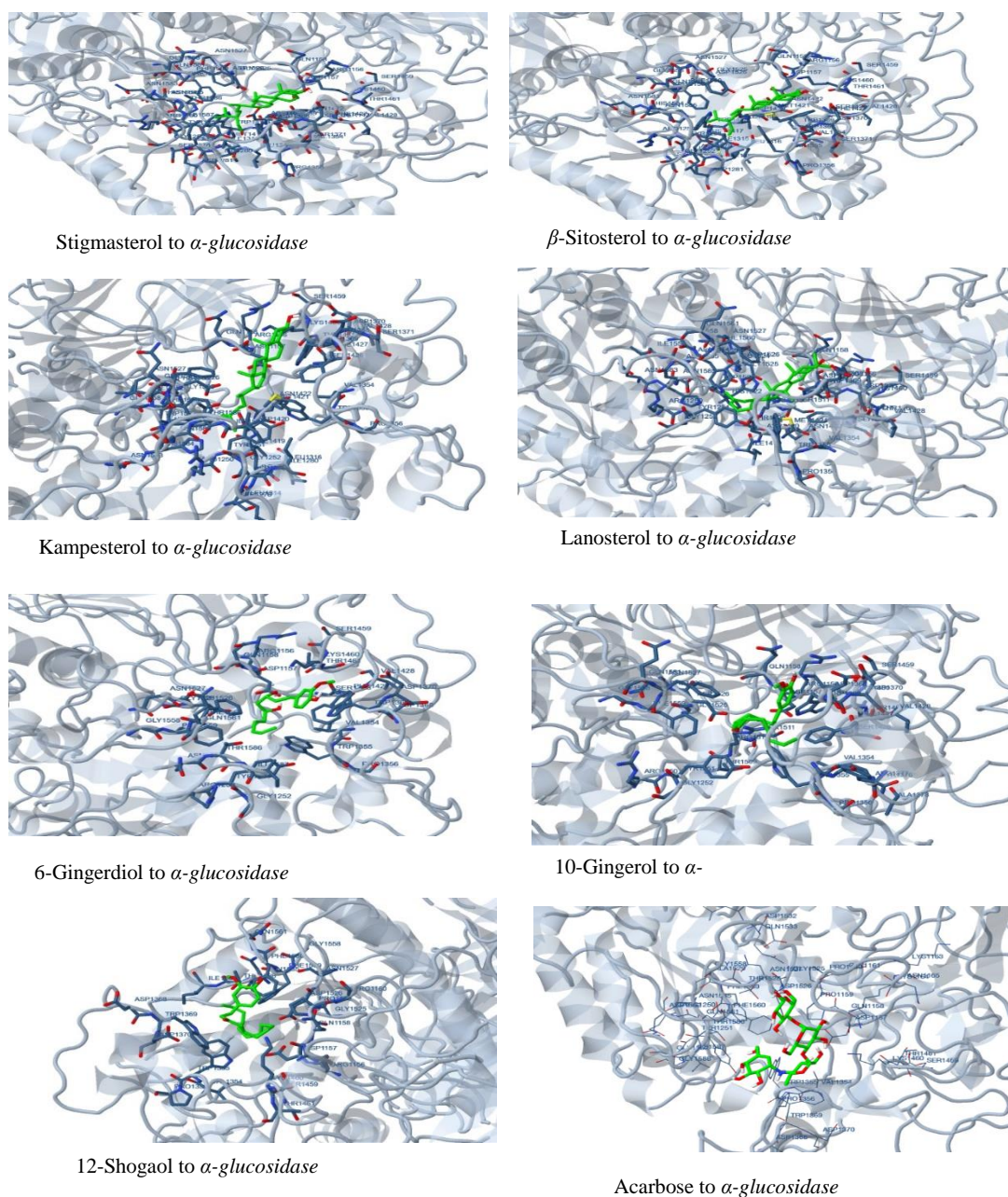


**Figure 5:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with DPP-4

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

Herbs	Ligand	Binding Free Energy (Kcal/mol)	Inhibition Constant (nM)	Surface Interaction (Å)
<i>Glycine max</i>	Stigmasterol	-10.57	17.8	893.79
	$\beta$ -Sitosterol	-10.26	30.0	902.57
	Kampesterol	-7.79	93.6	872.98
	Lanosterol	-7.86	162.2	894.78
<i>Zingiber officinale</i>	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

**Figure 6:** Binding interactions between active compound of *Glycine max* and *Zingiber officinale* with  $\alpha$ -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  $\alpha$ -glucosidase activity was strongly inhibited by stigmasterol and 6-Gingerol. However, *G. max* seeds was found to have more potential to be used as a drug candidate for diabetes as it was able to inhibit both enzymes compared to *Z. officinale* rhizomes.

## Conflict of interest

The authors declare no conflict of interest

## Authors' Declaration

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

## Acknowledgements

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

## References

- Deacon C. Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*. 2020; 16(11): 642-653.
- Singh S, Wright E, Kwan A, Thompson J, Syed I, Korol EE, Waser NA, Yu MB, Juneja R. Glucagon-like peptide-1 receptor agonists compared with basal insulins for the treatment of type 2 diabetes mellitus: a systematic review and meta-analysis. *Diabetes, Obes Metab*. 2016; 19(2):228-238.
- Gallwitz B. Clinical Use of DPP-4 Inhibitors. *Front Endocrinol (Lausanne)*. 2019; 10(1):1-8.
- Pereira A, Banegas-Luna A, Peña-García J, Pérez-Sánchez H, Apostolides Z. Evaluation of the Anti-Diabetic Activity of Some Common Herbs and Spices: Providing New Insights with Inverse Virtual Screening. *Molecules*. 2019; 24(22): 4030.
- Zeng L, Zhang G, Lin S, Gong D. Inhibitory Mechanism of Apigenin on  $\alpha$ -Glucosidase and Synergy Analysis of Flavonoids. *J Agric Food Chem*. 2016; 64(37): 6939-6949.
- Soelistijo, SA. Consensus of the Management and Prevention of Type 2 Diabetes Mellitus in Indonesia (1<sup>st</sup> Ed.). Jakarta: PB Perkeni; 2015. p6-65
- Pang G, Li F, Yan Y, Zhang Y, Kong L, Zhu P, Wang KF, Zhang F, Liu B, Lu CL. Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chin Med J (Engl)*. 2019; 132(1):78-85.
- World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023 [Internet]. World Health Organization (WHO). Geneva; 2013. Available from: [http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090\\_eng.pdf?ua=1](http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf?ua=1) (Accessed 04.09.2021)
- Mustofa M, Mukhtar D, Susmiarsih T, Royhan A. Effect of Soybean (*Glycine max* (L) Merrill) on Blood Glucose Levels and Expression of Pancreatic B Cell Insulin in Diabetic Rats. *J Kedokt Yars.* 2010; 18(2):094-103.
- Pabich M and Materska M. Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. *Nutr*. 2019; 11(7): 1660.
- Bischoff-Kont I and Fürst R. Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. *Pharmaceuticals (Basel)*. 2021; 14(6):571p.
- Tiwari V, Mishra B, Dixit A, Antony J, Tiwari R, Sharma N. Opportunity, Challenge and scope of natural products in medicinal chemistry. *Kerala: Res Signpost*; 2011; 103-121 p.
- Yulianingtyas Y and Kusmartono B. Optimization of Volume of solvent and Maceration Time of Flavonoid Extraction of Belimbing Wuluh Leaves (*Averrhoa Bilimbi* L.). *J Tek Kim*. 2016; 10:58-64.
- Nicklaus M. Online SMILES Translator [Internet]. Cactus.nci.nih.gov. 2020 [cited 4 September 2021]. Available from: <https://cactus.nci.nih.gov/translate>
- Seo CS and Shin HK. Liquid chromatography tandem mass spectrometry for the simultaneous quantification of eleven phytochemical constituents in traditional Korean medicine, sogunjung decoction. *Processes*. 2021; 9(1):1-10.
- O'Boyle N, Banck M, James C, Morley C, Vandermeersch T, Hutchison G. Open Babel: An open chemical toolbox. *J Cheminfo*. 2011; 3(1):33.
- Putra AMJ. Once Again About Docking [Internet]. LIPI. 2014. Available from: <http://u.lipi.go.id/1391883188>. Accessed 04.09.2021.
- Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, de Beer TAP, Rempfer C, Bordoli L, Lepore R, Schwede T. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 2018; 46(W1):W296-303.
- Khalaf I, Corciovă A, Vlase I, Ivănescu B and Lazăr D. LC/MS Analysis of Sterolic Compounds from *Glycyrrhiza Glabra*. *Stud Univ Babeş-Bolyai Chem*. 2011; 3(1):97-102.
- Fikri F, Saptarini N, Levita J. The Inhibitory Activity on the Rate of Prostaglandin Production by *Zingiber officinale* var. Rubrum. *Pharmacol Clin Pharm Res*. 2016; 1(1):33-41.
- Bilbao A. Proteomics Mass Spectrometry Data Analysis Tools. *Encyclopedia of Bioinformatics and Computational Biology: ABC of Bioinformatics*. Elsevier Ltd; 2018. 84-95p.
- Skubic C, Vovk I, Rozman D, Križman M. Simplified LC-MS Method for Analysis of Sterols in Biological Samples. *Molecules*. 2020; 25(18):2-10
- Zhu H, Chen J, He Z, Hao W, Liu J, Kwek E Ma KY, Bi Y. Plasma Cholesterol-Lowering Activity of Soybean Germ Phytosterols. *Nutrients*. 2019; 11(11):2-18.
- Trautwein E, Vermeer M, Hiemstra H, Ras R. LDL-Cholesterol Lowering of Plant Sterols and Stanols-Which Factors Influence Their Efficacy? *Nutrients*. 2018; 10(9):1262.
- Nguyen H, Neelakadan A, Quach T, Valliyodan B, Kumar R, Zhang Z and Nguyen HT. Molecular characterization of *Glycine max* squalene synthase genes in seed phytosterol biosynthesis. *Plant Physiol Biochem*. 2013; 73(1):23-32.
- Daszynski D, Santhoshkumar P, Phadte A, Sharma K, Zhong H, Lou MF and Kador PF. Failure of Oxysterols Such as Lanosterol to Restore Lens Clarity from Cataracts. *Sci Rep*. 2019; 9(1):8459.
- Wei C, Tsai Y, Korinek M, Hung P, El-Shazly M, Cheng YB, Wu YC, Hsieh TJ, Chang FR. 6-Paradol and 6-Shogaol, the Pungent Compounds of Ginger, Promote Glucose Utilization in Adipocytes and Myotubes, and 6-Paradol Reduces Blood Glucose in High-Fat Diet-Fed Mice. *Int J Mol Sci*. 2017; 18(1):168.
- Mahmudati N. Ginger (*Zingiber officinale*) extract decrease TNF expression of rat induced by High Fat Diet (HFD). *Proc Biol Educ Conf, FKIP UNS-2016*. 2016; 13(1):653-5.
- Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2012; 64(1):4-17.
- Purnomo Y, Soeatmadji DW, Sumitro SB, Widodo MA. Anti-diabetic potential of Urena lobata leaf extract through inhibition of Dipeptidyl Peptidase-IV activity. *Asian Pac J Trop Biomed*. 2015; 5(8): 645-649.
- Utomo D, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. *Bioinformation*. 2012; 8(9):426-429.
- Riyanti S, Suganda AG and Sukandar EY. Dipeptidyl Peptidase-IV Inhibitory Activity of Some Indonesian Medicinal Plants. *Asian J Pharm Clin Res*. 2016; 9(2): 375-377.

33. Bikadi Z and Hazai E. Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. J Cheminfo. 2009; 1(1): 15.
34. Purnomo Y, Makdasari J, Fatahillah FI. Inhibitory activity of Urena lobate leaf extract on alpha-amylase and alpha glucosidase: *in vitro* and *in silico* approach. J Basic Clin Physiol Pharmacol . 2021; 32(4):889-894.
35. Rosiarto B, Puspaningtyas A, Holidah D. Study of Antioxidant Activity of Compound 1-(p-chlorobenzoyloxymethyl)-5-fluorouracil with Molecular Docking Method and DPPH. Med Rep. 2014; 2(1):95-99.