
MOLECULAR DOCKING STUDIES ON ANTI-DIABETIC PROPERTIES OF PROPOLIS FROM STINGLESS BEE (*Tetragonula biroi* Friese)

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ABSTRACT

Diabetes mellitus is one of the leading causes of mortality among non-communicable diseases. The Philippines is reported to be one of the countries with high cases of diabetes mellitus. As such, there exists a need to improve current anti-diabetic drugs into safer, more accessible, and cheaper models. In this study, the potential anti-diabetic properties of compounds present in stingless bee propolis (apigenin, artemillin C, luteolin-5-methyl ether, pinobanksin-3-O-butyrate, pinobanksin-5,7-dimethyl ether, and pinocembrin-5-methyl ether) were screened through molecular docking. The relative binding affinities of the tested compounds against pre-defined binding sites in several diabetes-relevant enzymes was determined. For 11 β -hydroxysteroid dehydrogenase type 1, pinobanksin-3-O-butyrate had the best binding energy score of -9.2 ± 0.1 kcal/mol. For aldose reductase, both apigenin and luteolin-5-methyl ether had the best binding energy score of -9.7 ± 0.0 kcal/mol and -9.7 ± 0.1 kcal/mol, respectively. For glutamine: fructose-6-phosphate amidotransferase, both luteolin-5-methyl ether and pinobanksin-3-O-butyrate had the best binding energy score of -7.8 ± 0.1 kcal/mol and -7.8 ± 0.0 kcal/mol, respectively. For glucokinase, apigenin had the best binding energy score of -8.6 ± 0.1 kcal/mol. For N-terminal maltase glucoamylase, artemillin C had the best binding energy score of -7.6 ± 0.0 kcal/mol. And lastly for human pancreatic α -amylase, luteolin-5-methyl ether had the best binding energy score of -9.6 ± 0.1 kcal/mol. After docking, the ADMET properties (absorption, distribution, metabolism, excretion and toxicity) were determined using SwissADME. In terms of the ADMET properties, pinobanksin-3-O-butyrate showed the highest potential for drug development.

Key words: anti-diabetic drugs, diabetes mellitus, molecular docking, propolis, stingless bee

INTRODUCTION

Propolis, also known as bee glue, is a sticky resinous substance produced by bees. It serves to maintain structural integrity of the hive. It is utilized to protect the larvae, due to its antiseptic and antimicrobial action (Anjum et al., 2019). This bioactivity is due to propolis being sourced from a variety of plants, trees, and other flora, which contain a breadth of phytochemicals with different activities. Mostly, the substance consists of phenolics—flavonoids and phenolic acids—while other notable compounds include waxes, volatiles, minerals, and pollen residues (Farooqui & Farooqui, 2012). These compounds have been found

to exhibit antioxidant, anti-inflammatory, anti-tumor, anti-ulcer, anti-cancer, and antidiabetic activities (Sforcin & Bankova, 2011). Thus, propolis has found a variety of uses. It is present in dietary supplements, lotions, and antiseptics, for a few examples.

Diabetes mellitus (DM) are of 2 types (American Diabetes Association, 2014): DM that involves the breakdown of pancreatic β -cells in the system, which leads to an absolute loss of insulin (type 1) and DM that is due to insulin resistance due to unresponsiveness of target tissues (type 2). Recently, several pharmacological approaches have been studied for use in DM treatment such as 11β -hydroxysteroid dehydrogenase 1 inhibitors (Almeida et al., 2021), aldose reductase inhibitors (Jannapureddy et al., 2021), glutamine:fructose-6-phosphate amidotransferase inhibitors (Nakaishi et al., 2009), N-terminal maltase-glucoamylase inhibitors (Sim et al., 2010), pancreatic α -amylase inhibitors (Dandekar et al., 2021), glucokinase activators (Kamata et al., 2004) and many others. Diabetes mellitus is one of the leading causes of mortality among non-communicable diseases. Even the Philippines is not exempted to the problem. It is estimated that by 2030, Philippines will be among the top ten countries with the most cases of diabetes mellitus (Wild et al., 2004). As such, there exists a need to improve current anti-diabetic drugs into safer, more accessible, and cheaper models.

Promising progress may be found in exploring stingless bee propolis compounds for their anti-diabetic properties through molecular docking. In the Philippines, *Tetragonula biroi* (Friese) is the most common species. Alvarez et al. (2013) have identified six flavonoids and phenolic compounds from *T. biroi* propolis (apigenin, artepillin C, luteolin-5-methyl ether, pinobanksin-3-O-butyrate, pinobanksin-5,7-dimethyl ether, and pinocembrin-5-methyl ether) using LCMS analysis. Flavonoids and phenolic compounds have been reported to have biological activities such as antioxidant, anti-inflammatory, and anti-diabetic activities. However, the bioactivity of *T. biroi* propolis still has not been extensively studied in comparison to propolis from other countries. The goal of this study, then, is to utilize molecular docking to screen the six compounds present in *T. biroi* propolis for their binding affinity towards various diabetes-related enzymes and to predict their probability for eventual drug development.

MATERIALS AND METHODS

Ligand Preparation

Alvarez et al. (2013) have identified six compounds from *T. biroi* propolis using LCMS analysis. All of these compounds were used as ligands in this study. The spatial data file (SDF) of the compounds (apigenin, artepillin C, luteolin-5-methyl ether) was obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov>). Compounds with no existing SDF files in PubChem (pinobanksin-3-O-butyrate, pinobanksin-5,7-dimethyl ether, pinocembrin-5-methyl ether) were manually

drawn in Avogadro version 1.2.0 and their 3D geometries were optimized using the same software (Hanwell et al., 2012). Gasteiger charges and non-polar hydrogens were added to the ligand and the number of torsions were determined using AutoDock Tools v1.5.6 (Morris et al., 2009).

Receptors Preparation

The crystal structure of various diabetes-related enzymes – 11 β -hydroxysteroid dehydrogenase 1 (PID: 4K1L), aldose reductase (PID: 3G5E), glutamine:fructose-6-phosphate amidotransferase (PID: 2ZJ4), glucokinase (PID: 1V4S), N-terminal maltase gluco-amylase (PID: 2QMJ), and human pancreatic α -amylase (PID: 3BAJ) – were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (<http://www.rcsb.org>). Downloaded files were opened using BIOVIA Discovery Studio Visualizer 2021 wherein water molecules and ligands attached to the receptors were removed. Polar hydrogens and Kollman charges were added using AutoDock Tools v1.5.6 (Morris et al., 2009).

Molecular Docking

The Protein Data Bank, Partial Charge, and Atom Type (PDBQT) files of the ligands and receptors were generated using AutoDock Tools v1.5.6 (Morris et al., 2009). The same software was used to ensure that the grids were centered on and fully cover predetermined binding sites in the different enzymes. These binding sites were obtained from the top docks of a previously performed blind docking. The grid box used for each receptor are shown in **Figure 1**. Molecular docking simulations were carried out using AutoDock Vina. The docking simulations follows the rigid receptor-flexible ligand format. This kind of docking trades the higher accuracy of a flexible receptor for the lower computing time provided by a rigid receptor (Meng et al., 2011). All docking simulations were done in triplicate.

Analysis and Visualization

For each docking simulation, the binding conformation with the most negative binding energy score was considered for further analysis. The interactions between the receptors and ligands were identified using BIOVIA Discovery Studio Visualizer 2021.

Lead- and Drug-likeness Properties

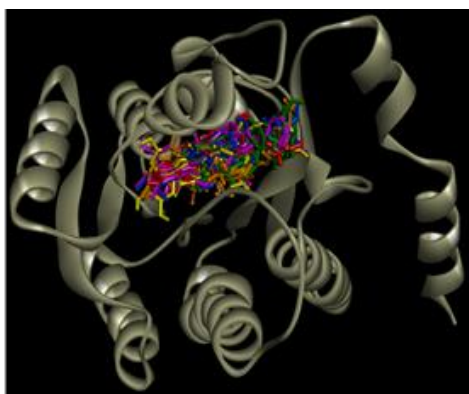
ADMET properties of the ligands were determined using SwissADME (Daina et al., 2017). The structure of each ligand was entered at the interface of the website (<https://swissadme.ch/>). These ADMET properties were used to predict the probability of the ligands for eventual drug development.

glucose and repairing pancreatic β -cells. On the other hand, Zhu et al. (2011) studied Chinese and Brazilian propolis and found that both inhibited body weight loss and blood glucose increase in diabetic rats. Furthermore, oxidative stress in the blood, liver, kidney was significantly reduced. As such, they believe that Chinese and Brazilian propolis can alleviate diabetes symptoms as well, due to the antioxidant properties of the bioactive compounds present. Based on these results, this study was directed to determine the anti-diabetic properties of the compounds present in *T. biroi* propolis for their binding activity towards various diabetes-related enzymes using a molecular docking approach.

In this study, six *T. biroi* propolis compounds were docked to six different diabetes-related enzymes. Each of the docking simulation returned 9 ligand-receptor docking results. The range of predicted binding energy scores for all the docking results are summarized in **Table 1**. Moreover, the location of all the docked ligands for each of the receptors are shown in **Figure 2**. These results show that all the ligands have a good binding affinity towards the binding site of each receptor since all showed similar values to that of the control. In molecular docking, the docked ligand with a more negative binding energy score is considered to have stronger binding towards the receptor (Sailah et al., 2021). The binding energy score of each of the docking simulations are summarized in **Table 2** as well as the binding energy score of the control used for each receptor.

The enzyme 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD1) is responsible for the conversion of cortisone to cortisol, which play important role in enhancing glucose production, regulating gluconeogenesis, and inhibiting glucose uptake (Almeida et al., 2021). The control used was carbenoxolone which is a steroidal derivative of glycyrrhettinic acid that has been investigated to inhibit 11β -HSD1. None of the propolis compounds exceeded the binding energy score of this control which is -9.3 ± 0.0 kcal/mol (**Table 2**). However, it is worth inspecting the ligands closest to the control's binding energy score such as pinobanksin-3-O-butyrate (-9.2 ± 0.1 kcal/mol). As shown in **Figure 3**, two oxygen atoms of this ligand form H-bond interaction with Ala 223 and Leu 171. Another oxygen atom exhibit carbon hydrogen interaction with Ser 170. Both of the aromatic rings form interactions with different amino acid residues. The pi electrons of one of the aromatic rings has interaction with the alkyl group of Ala 223. While in the other aromatic ring, its pi electrons have an interaction with the aromatic side chain of Tyr 177, alkyl group of Leu 126 and forms a pi-sigma interaction with Val 180.

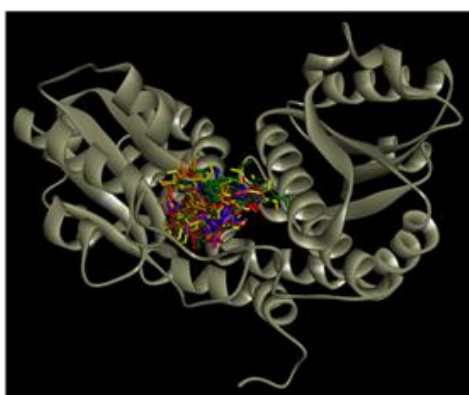
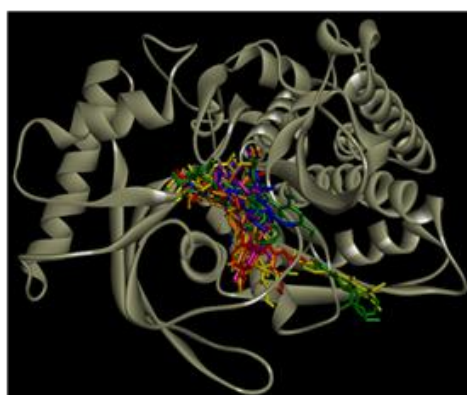
Aldose reductase catalyzes the conversion of glucose into sorbitol with NADPH as a cofactor. In a hyperglycemic system, the exhaustion of NADPH can affect other important biochemical pathways while the accumulation of sorbitol



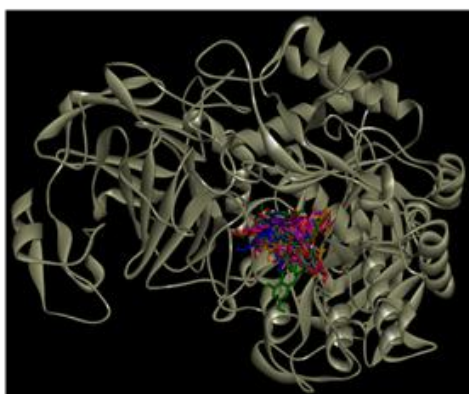
11β-hydroxysteroid dehydrogenase 1 (4K1L)



aldose reductase (3G5E)

glutamine:fructose-6-phosphate
amidotransferase (2ZJ4)

glucokinase (1V4S)



N-terminal maltase gluco-amylase (2QMJ)



human pancreatic α-amylase (3BAJ)

Figure 2. Location of all the docked ligands to the various diabetes-related enzymes (red: apigenin; orange: artepillin C; yellow: luteolin-5-methyl ether; green: pinobanksin-3-O-butyrate, blue: pinobanksin-5,7-dimethyl ether; violet: pinocembrin-5-methyl ether).

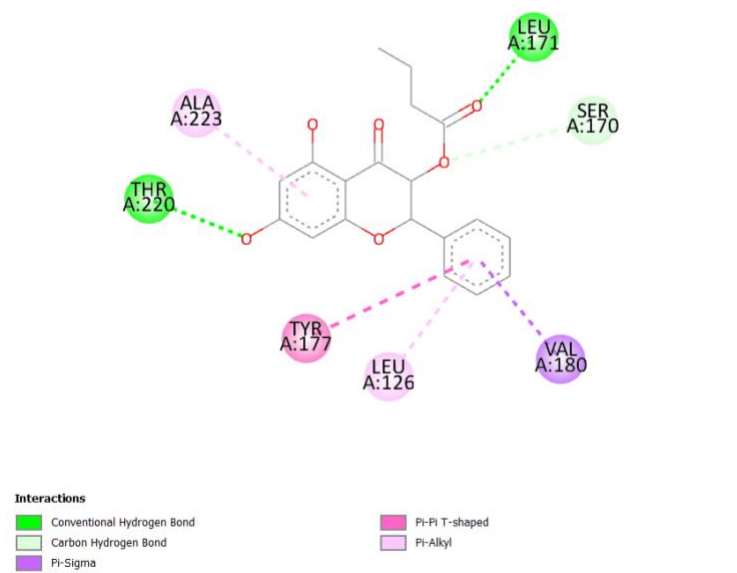


Figure 3. Ligand interaction diagram of pinobanksin-3-O-butyrate with 11 β -hydroxysteroid dehydrogenase type 1.

and fructose (the end-product of the polyol pathway) can degrade cellular performance in the body (Dunlop, 2000; Kawanishi et al., 2003). Epalrestat was used as the control for the docking with aldose reductase. It is a carboxylic acid-based drug commonly used to treat retinopathy and neuropathy (Grewal et al., 2016). None of the compounds in stingless bee propolis docked in aldose reductase were predicted to have a more negative binding energy score than that of epalrestat (-9.8 ± 0.1 kcal/mol). The binding energy score of apigenin and luteolin-5-methyl ether is the closest to that of the control which is -9.7 ± 0.0 kcal/mol and -9.7 ± 0.1 kcal/mol, respectively (**Table 2**). One oxygen atom of apigenin form H-bond interaction with Trp 111. The pi electrons of apigenin interact with the thiol group of Cys 80, alkyl group of Leu 300, and indole side chain of Trp 111. Moreover, its pi electrons also form a pi-hydrogen bond interaction with the thiol group of Cys 303 and a pi-pi T-shaped interaction with the phenyl side chain of Phe 122 (**Figure 4A**). On the other hand, luteolin-5-methyl ether forms an H-bond and a pi-sulfur interaction with Cys 80. Its pi electrons also interact with the aromatic rings of Trp 20, Trp 111 and Phe 122 as well as with the alkyl group of Trp 111 and Leu 300, and with the thiol group of Cys 303 (**Figure 4B**).

Interest has also raised recently on the inhibition of glutamine:fructose-6-phosphate amidotransferase (GFAT) as a form of supplementary diabetes treatment. This protein is the first and the rate-limiting enzyme in the hexosamine biosynthesis pathway, whose high flux may be linked to a rise in

The strong binding of this ligand is due to various interactions such as two H-bonding with Tyr 215. Its pi electrons also interact with the alkyl group of Pro 66, Val 62, Ile 211, Val 452, Val 455 and Ala 456. Moreover, it also exhibits a pi-pi T-shaped interaction with the aromatic side chain of Tyr 214. The pi electrons of Tyr 214 also interact with the hydroxyl group of apigenin (**Figure 6**).

Maltase glucoamylase (MGAM) is an alpha-glucosidase in the brush border of the small intestine, together with sucrase isomaltase. Both enzymes catalyze the final steps of carbohydrate digestion in the intestine. Thus, their inhibition aids in decreasing blood glucose levels in the body (Sim et al., 2010). The control used for MGAM docking was acarbose, a pseudotetrasaccharide that competes against oligosaccharides thus preventing its cleavage into monosaccharides. Among the propolis ligands, artepillin C had the most negative binding energy score which is -7.6 ± 0.0 kcal/mol compared to that of the control (-7.2 ± 0.0 kcal/mol; **Table 2**). This ligand forms four H-bond interaction with Asp 203, Asp 542 and Gln 603. The pi electrons of the aromatic ring also interact with the aromatic ring of Trp 406 and anion side chain of Asp 542. Moreover, some of the ligands alkyl groups also interact with Tyr 299, Ile 328, Ile 364, Trp 406 and Phe 450 (**Figure 7**).

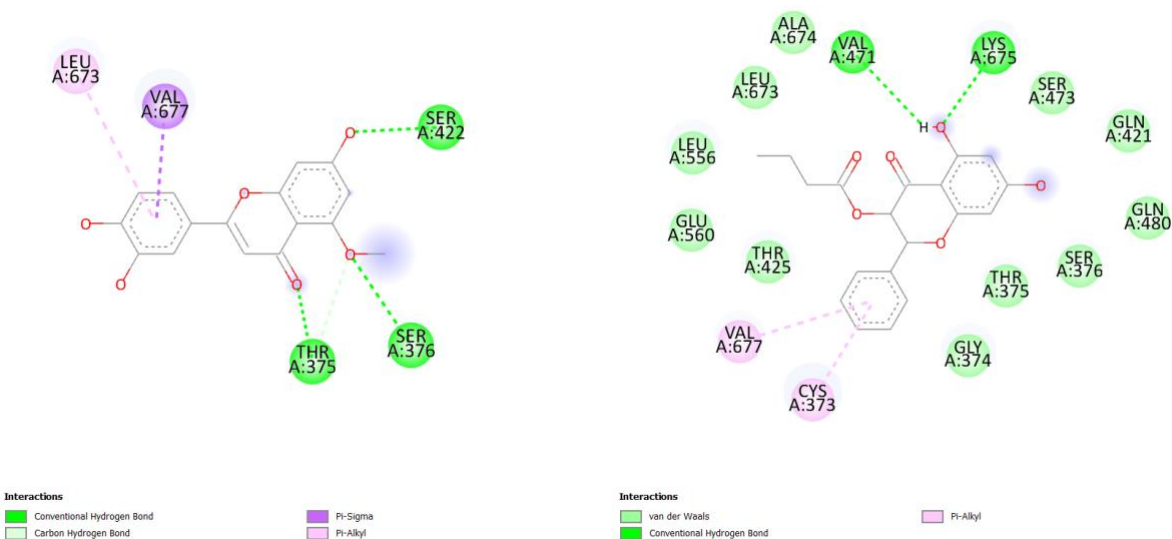


Figure 5. Ligand interaction diagram of (A) luteolin-5-methyl ether and (B) pinobanksin-3-O-butyrate with glutamine:fructose-6-phosphate amidotransferase.

Human pancreatic α -amylase (HPA) is one of two amylases in the body, the other being salivary α -amylase. These two enzymes catalyze the hydrolysis of starch into oligosaccharides that are further degraded into glucose (Dandekar et al., 2021). Its inhibition aids in decreasing blood glucose levels in the body. Acarbose was also used as control in docking with HPA. All of the propolis compounds except for artemillin C resulted to a more negative binding energy score compared to that of the control (-7.9 ± 0.0 kcal/mol). Luteolin-5-methyl ether had the most negative binding energy score which is -9.6 ± 0.1 kcal/mol (**Table 2**). The strong binding of this ligand is due to two H-bond interaction with Gln 63 and Asp 197. Moreover, its pi electrons also interact with the aromatic side chain of Trp 59 and Tyr 62. There is also an unfavorable donor-donor interaction between one of the hydroxyl group of luteolin-5-methyl ether and His 299 (**Figure 8**).

The Lipinski's rule-of-five (Ro5) is used to assess the drug-likeness of chemical compounds and potential medicines. The ligands that comply with Lipinski's Ro5 or having no more than one violation is considered to have the potential to become a drug (Sailah et al., 2021; Yang et al., 2020). **Table 3** shows the predicted ADMET properties of the propolis compounds based on Swiss ADME. All of the compounds are found to be soluble in water and exhibit good gastrointestinal absorption. However, only two of the propolis compounds do not penetrate the blood brain barrier (BBB). Since the target of this study are diabetes-related enzymes, it is preferential that these compounds are not BBB permeant since it can have secondary effects once it is in the brain. Based on the results, all of the propolis compounds inhibited one or more of the cytochrome P450 enzymes particularly CYP1A2, CYP2C19, CYP2C9, CYP2D6, and/or CYP3A4. Cytochrome P450 is an important detoxifying enzyme that works by oxidizing foreign organic compounds and facilitate its excretion (Foroozesh et al., 2019). Thus, it would be best if compounds do not inhibit CYP450 enzymes because these enzymes are responsible for drug metabolism. Even though these compounds inhibit CYP450 enzymes, all of the propolis compounds show similar properties to drugs (drug-likeness) as they do not violate Lipinski's Ro5 and all have a bioavailability of 0.55 or higher. Moreover, with the exception of artemillin C, all of the other compounds comply with lead-likeness. Lead-likeness is the structural and physicochemical similarity of a potential drug to a reference (lead) compound for further drug development (Polinsky, 2008). These results suggest that these compounds may exhibit similarities to an orally active drug and can be used for drug development. In terms of the ADMET properties, pinobanksin-3-O-butyrate showed the highest potential for drug development.

SUMMARY AND CONCLUSION

The potential anti-diabetic properties of compounds present in stingless bee propolis (apigenin, artepillin C, luteolin-5-methyl ether, pinobanksin-3-O-butyrate, pinobanksin-5,7-dimethyl ether, and pinocembrin-5-methyl ether) were screened through molecular docking with diabetes-relevant enzymes. Results showed that all ligands have good binding affinities to pre-defined binding sites. Thus, these compounds can be a potential anti-diabetic drug. Among the ligands used, pinobanksin-3-O-butyrate showed the highest potential for drug development due to its predicted high GI absorption, good water solubility and bioavailability score, non-BBB permeant, and does not violate Lipinski's Ro5.

It is recommended to use other software for molecular docking as this could determine whether or not the software used in this study affected the results. Moreover, molecular dynamics may be performed to further validate the docking results.

REFERENCES

- ALMEIDA C, MONTEIRO C, SILVESTRE S. 2021. Inhibitors of 11β -hydroxysteroid dehydrogenase Type 1 as potential drugs for type 2 diabetes mellitus—A Systematic review of clinical and in Vivo preclinical studies. *Scientia Pharmaceutica* 89(1): 5.
- ALVAREZ P, CRUZ M, MICOR J, FAJARDO JR A, CERVANCIA C, HIZON-FRADEJAS A. 2013. Identification of flavonoids and phenolic compounds in propolis from stingless bee (*Tetragonula biroi* Friese) nests and extracts from five tree sources using tandem liquid chromatography–mass spectrometry. *The Philippine Entomologist* 27(1): 91–99.
- AMERICAN DIABETES ASSOCIATION. 2014. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 37(Suppl_1): S81–S90.
- ANJUM SI, ULLAH A, KHAN KA, ATTAULLAH M, KHAN H, ALI H, BASHIR MA, TAHIR M, ANSARI MJ, GHARAMH HA, ADGABA N, DASH CK. 2019. Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences* 26(7): 1695–1703.
- BIOVIA, Dassault Systèmes. 2021. Discovery Studio Visualizer v21.1.0.20298. San Diego: Dassault Systèmes.
- BUSE MG. 2006. Hexosamines, insulin resistance, and the complications of diabetes: Current status. *American Journal of Physiology. Endocrinology and Metabolism* 290(1): E1–E8.

- KAMATA K, MITSUYA M, NISHIMURA T, EIKI J, NAGATA Y. 2004. Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure* 12(3): 429–438.
- KAWANISHI K, UEDA H, MORIYASU M. 2003. Aldose reductase inhibitors from the nature. *Current Medicinal Chemistry* 10(15): 1353–1374.
- KITAMURA H. 2019. Effects of propolis extract and propolis-derived compounds on obesity and diabetes: Knowledge from cellular and animal Models. *Molecules* 24(23): 4394.
- LI W, ZHANG X, SUN Y, LIU Z. 2020. Recent clinical advances of glucokinase activators in the treatment of diabetes mellitus type 2. *Die Pharmazie* 75(6): 230–235.
- MENG XY, ZHANG HX, MEZEI M, CUI M. 2011. Molecular docking: A powerful approach for structure-based drug discovery. *Current Computer-Aided Drug Design* 7(2): 146–157.
- MORRIS GM, HUEY R, LINDSTROM W, SANNER MF, BELEW RK, GOODSSELL DS, OLSON AJ. 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry* 30(16): 2785–2791.
- NAKAISHI Y, BANDO M, SHIMIZU H, WATANABE K, GOTO F, TSUGE H, KONDO K, KOMATSU M. 2009. Structural analysis of human glutamine:fructose-6-phosphate amidotransferase, a key regulator in type 2 diabetes. *FEBS Letters* 583(1): 163–167.
- NNA VU, ABU BAKAR AB, RIZMAN M, MD LAZIN MRML, MOHAMED M. 2018. Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology* 120: 305–320.
- POLINSKY A. 2008. Chapter 12—Lead-Likeness and Drug-Likeness. In: Wermuth CG (ed), *The Practice of Medicinal Chemistry (Third Edition)*. Academic Press. pp. 244–254
- PUJIRAHAYU N, BHATTACHARJYA DK, SUZUKI T, KATAYAMA T. 2019. α -Glucosidase inhibitory activity of cycloartane-type triterpenes isolated from Indonesian stingless bee propolis and their structure–activity relationship. *Pharmaceuticals* 12(3): 102.
- SAILAH I, TUMILAAAR S, LOMBOGIA L, CELIK I, TALLEI T. 2021. Molecular docking and dynamics simulations study of selected phytoconstituents of “Pangi” (*Pangium edule* Reinw) leaf as anti-SARS-CoV-2. *Philippine Journal of Science* 150(5): 925–937.

