Analysis of Antidiabetic Potential of Palmitic Acid Compounds Through *In Silico* Activation

Ardellya Elfidaa Salsabila*, Nurlaili Susanti, Zulvikar Syambani Ulhaq

Department of Medical Education, Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jalan Locari, Tlekung, Junrejo, Batu, 65151, Indonesia

Email: ardellyadella@gmail.com

Abstract

Diabetes Mellitus is a condition where the body's sugar level exceeds the blood's normal limit. Type 2 Diabetes Mellitus is the most common type of diabetes and is a chronic disease caused by insulin resistance and beta-cell dysfunction, resulting in decreased insulin sensitivity. The management of diabetes Mellitus is in the form of oral hypoglycemic drugs. Still, these drugs have side effects such as gastrointestinal disturbances, nausea, vomiting, hypoglycemia, macrovascular disorders, microvascular disorders, etc. So, it is necessary to seek alternative drugs to increase insulin sensitivity to lower blood sugar levels which are safer using herbal plants. One of the suspected plants that can be an alternative medicine to reduce excess blood sugar levels is the dandang gendis plant (*Clinacanthus nutans*). The dandang gendis plant (*Clinacanthus nutans*) contains palmitic acid, which can reduce excess blood sugar levels in the blood. This study aims to determine the potential of palmitic acid compounds from the Dandang gendis plant as an *in silico* AMPK protein activator. Qualitative descriptive method using molecular docking *in silico* method to determine the affinity and interaction with palmitic acid compounds in the dandang gendis plant in activating AMPK protein. Docking between palmitic acid and AMPK produces an energy value (ΔGbind) of -5.3 kcal/mol. docking of metformin resulted in the lowest energy value (ΔGbind) of 5.1 kcal/mol. The bond produced by palmitic acid docking has a fairly strong affinity for the AMPK protein of -5.3 kcal/mol. So palmitic acid, an ingredient in the dandang gendis plant (*Clinacanthus nutans*) has the potential as an antidiabetic.

***Keywords**: Palmitic acid, AMPK, Dandang gendis plant (Clinacanthus nutans), antidiabetic, in silico*

Background

Diabetes mellitus (DM) is one of the focuses health problems in the world whose prevalence is increasing from year to year. The morbidity and mortality of diabetics in the world are also quite high. According to WHO (2016), there were about 1.6 million cases of death directly caused by diabetes and another 2.2 million deaths caused by high glucose levels in 2012. In Indonesia, there are 10.7 million cases of diabetic, of which Indonesia ranks the 7th most in Southeast Asia. Diabetes mellitus type 2 (DMT2) is the most common type of diabetes compared to other types (World Health Organization, 2016). DMT2 is a chronic metabolic disease characterized by

Hyperglycemia. This type of diabetes is often associated with insulin resistance and pancreatic beta-cell dysfunction (Bellou *et al*., 2018; Müller-Wieland *et al*., 2019). In type 2 diabetes, there is one enzyme molecule whose presence has a major effect on insulin resistance, namely adenosine monophosphate protein kinase (AMPK). AMPK is an enzyme involved in the regulation of metabolic homeostasis. It is known that AMPK activation in the liver can reduce gluconeogenesis and lipogenesis through the downregulation of certain genes. In muscle, AMPK activation increases glucose uptake, mitochondrial genes, and lipid oxidation. Therefore, AMPK plays an important role in the pathogenesis of T2DM. Treatment of DMT2 until now still needs attention.
Currently, the treatment of diabetes uses oral hypoglycemic drugs (Joshi *et al*., 2019). It is known that metformin is one of the most commonly used DMT2 hypoglycemic drugs and works in increasing insulin sensitivity. This is related to the activation of AMPK (Janani & Ranjitha Kumari, 2015; Nandipati *et al*., 2017). Therapy for T2DM is generally effective, but many unexpected side effects arise such as gastrointestinal disturbances, nausea, vomiting, dizziness, tremors, and hypoglycemia and some patients may be intolerant of side effects (Putra *et al*., 2017).
In this study, palmitic acid (PA) compound from the plant *Clinacanthus nutans* was used which is thought to have the potential as an antidiabetic. *Clinacanthus nutans* (CN) or known as Dandang gendis is an herbal plant that is widely used to treat various diseases in the community (Alam *et al*., 2016). The antidiabetic potential of *Clinacanthus nutans* (CN) leaves is currently under-studied and has not yet explained the mechanism of this plant in activating AMPK. Supported by the result of Imam's research (2019) which was tested on mice showed that the CN plant has the potential as an antidiabetic. In this study, type 2 DM model rats given CN leaf extract showed better glycemic control results and their lipid profile significantly improved (Imam *et al*., 2019).
Based on this explanation, further research is needed on the potential of palmitic acid as a ligand and AMPK protein as a receptor. *In silico* is an information technology-based method for creating computational models or simulations that can be used to predict, and suggest hypotheses regarding the latest advances in treatment and therapeutic discoveries (Ekins *et al*., 2007).

Materials and Methods

The ligands used in this study were palmitic acid as a test ligand with code 985 (Ismail *et al*., 2020), metformin as a comparison ligand with code 4091, native ligand ADP 1327, ADP 1328, and AMP contained in the protein structure of AMPK with code 2Y8L and ATP from protein crystal 2V92 with code 5957.

Ligand preparation
Palmitic acid and metformin ligands are downloaded via the PubChem website in *.sdf format and can be accessed at http://pubchem.ncbi.nih.gov.The ligand file is opened using the PyMOL application or UCSF Chimera (software discovery studio visualizer) and saved in PDB format (*.pdb).

Receptor protein preparation
AMPK code 2Y8L is downloaded in the form of a PDB file (*pdb) via the protein data bank (PDB) website in 3D. The downloaded file is then opened via PyMOL and Chimera software. Cleaning unnecessary chains and separating water molecules from AMPK protein so that it can be used in the molecular docking process. Addition of hydrogen atoms into AMPK to provide partial charges. The final result in cleaning and partial loading is stored in the format (*pdb).

Human intestinal absorption (HIA) test
The test is used to predict the absorption of palmitic acid compounds. It can be accessed through the online pre-ADMET site http://preadme.bmdrc.org/. The uploaded ligand structure is in the form of a file format (*.mol).

Lipinski rule of five test
This test predicts whether palmitic acid compounds used as oral drugs can work actively and can enter cells. Accessible via the SCFBio Lipinski website.

Molecular docking
Change the receptor and ligand data format which previously had *.pdb format to *pdbqt. Doing molecular anchoring using AutoDock Vina online software at Mcule by setting the active receptor site grid which is then running. The docking results are saved in PDB format and the binding affinity value data is stored in Microsoft Excel.

Docking result visualization
The docking results are visualized in 2D and 3D using discovery studio visualizer software and Ligplot+1.4.5.

Result and Discussion

Prediction of compound absorption with human intestinal absorption (HIA) parameters
The compound is said to have good absorption when the Human Intestinal Absorption (HIA) test value is more than 70%, moderate is 20-70%, and 0-20% low (Nerkar *et al*., 2012). In the HIA test, the percentage of the palmitic acid compound obtained is 98.29%. Based on these results, it can be shown that palmitic acid is a compound that can be well absorbed in the body. Meanwhile, metformin has a value percentage of 45.66%. This shows that metformin has the possibility of being a compound that is quite well absorbed in the body. Both palmitic acid and metformin are well absorbed and well enough in the intestines that they can be given orally.

Table 1. Human intestinal absorption (HIA) test results

Ligand	HIA (%)
Palmitic Acid	98.297110
Metformin	45.666887

Prediction of potential compounds passing cell membranes with Lipinski rule of five parameters
If a drug has been absorbed in the intestine and then enters the blood circulation, the drug will be distributed throughout the body tissues by penetrating the cell membrane so that the drug can reach the target receptor to be targeted (Tian *et al*., 2015). Lipinski's Rule of five can be used to determine solubility and permeability. of a ligand. It is said that a ligand can be well absorbed if it fulfills this rule (Ferdian *et al*., 2016). Lipinski Rule of five consists of 4 parameters, namely molecular weight <500, hydrogen bond donor (HBD) <5, hydrogen bond acceptor (HBA) <10, and logP coefficient <5. This analysis is called the Rule of five because the parameter criteria above have limitations with the number 5 or multiples thereof.

Table 2. Lipinski Rule of five test results

Ligand	Molecular weight (Da)	Hydrogen Bond Donor (HBD)	Hydrogen Bond Acceptor (HBA)	logP	*Molar Refractivity*
Palmitic Acid	556.00	1	2	5.55	77.94
Metformin	129.00	5	5	-1,24	37.22

Based on the table above, the data on palmitic acid compounds needed in the Lipinski Rule of five tests are obtained, namely the value of molecular weight 256, Hydrogen Bond Donor (HBD) 1, Hydrogen Bond Acceptors (HBA) 2, logP value 5.55 and molar refractivity 77.94, while The metformin compound data obtained were the molecular weight value 129, HBD 5, HBA 4, logP value -1.24 and molar refractivity 37.22.

Molecular weight describes the size of a molecule, the larger the size of the molecule, the more it affects the compound in penetrating the cell membrane. It is said that absorption in the intestines and blood vessels of the brain takes place well when the molecular weight of the ligand is less than 500. The palmitic acid compound obtained a molecular weight of 256 which indicates that this compound complies with the Lipinski molecular weight criteria, while the molecular weight of the drug metformin also meets the molecular weight criteria. that is equal to 129. It is known that hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) correlates with intermolecular bonds. The value of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) can affect the compound in reaching its target cell, if the value of HBD and HBA is greater than more hydrogen bonds are formed and slow the compound to reaching its target so that the Lipinski Rule of five rules, the values of HBD and HBA are limited (Ekins *et al*., 2005; Abad-Zapatero, 2007; Lipinski *et al*., 2012). The values of HBD and HBA on palmitic acid compounds obtained values of 1 and 2 which indicate that the palmitic acid compound meets the criteria for HBD less than 5 and HBA less than 10. In metformin, the HBD value is 5 which means it does not meet the criteria because the value is not below five or equal to five, while the HBA value is 5 which means it is by the HBA criteria less than 10.

Lipophilicity or LogP describes the lipophilicity of a compound. A positive LogP value indicates the non-polar nature of the compound while a negative value indicates the polarity of the compound. The higher the lipophilicity of a compound, the better the ability of the ligand to penetrate the lipid bilayer of the cell membrane (Ekins *et al*., 2005; Abad-Zapatero, 2007; Lipinski *et al*., 2012). Based on the data obtained, the LogP value of the palmitic acid compound is 5.55, which means that this compound does not meet the criteria because the LogP value is more than 5 while the LogP value for metformin is -1.24, which means it is in accordance with the criteria because the value is below 5. A drug is declared capable of penetrating cell membranes if it meets at least 2 of the Lipinski Rule of five (Tian *et al*., 2015). Research conducted by Ismail et al, by using the same ligand, namely palmitic acid, three of the four criteria obtained have the same values as those obtained in this study, using different software, namely SwissADME, including molecular weights 256 Da, HBD 1, HBA 2 and LogP 4,19 (Ismail *et al*., 2020).

Based on the data obtained above, the compound palmitic acid violates 1 of the 4 Lipinski Rule of five criteria, namely the value of LogP equal to 5.55 which should be less than 5. The same is the case with the comparison compound, namely metformin which also violates 1 of 4 criteria, namely the HBD value of 5 which should have an HBD value of <5. However, this can still be tolerated and it can be said that the compound can penetrate the cell membrane so that the ligand can bind to the target receptor because it has fulfilled at least 2 of the Lipinski Rule of five rules. Both palmitic acid and metformin are safe for consumption and used as oral drugs.

Optimization and preparation of three-dimensional structures of palmitic acid compounds and AMPK receptors

The three-dimensional structure of the palmitic acid test compound obtained from the https://pubchem.ncbi.nlm.nih.gov/ page with code 985 was downloaded and the storage format changed from *.sdf to *.pdb format by using the Discovery Studio Visualizer software. Furthermore, assisted with AutoDock software to do geometry optimization to get the lowest energy of the test compound. The macromolecular structure used in the docking process was downloaded from the Protein Data Bank (PDB) page https://www.rcsb.org/ with code 2Y8L (**Figure 1**). The macromolecular structure is bound in the form of a water molecule and its native ligand. Water molecules and native ligands were removed from the macromolecules because they could interfere with the docking process (**Figure 2**). After that, optimization is carried out in the form of adding a partial load so that it can be used in the docking process (**Figure 2**).

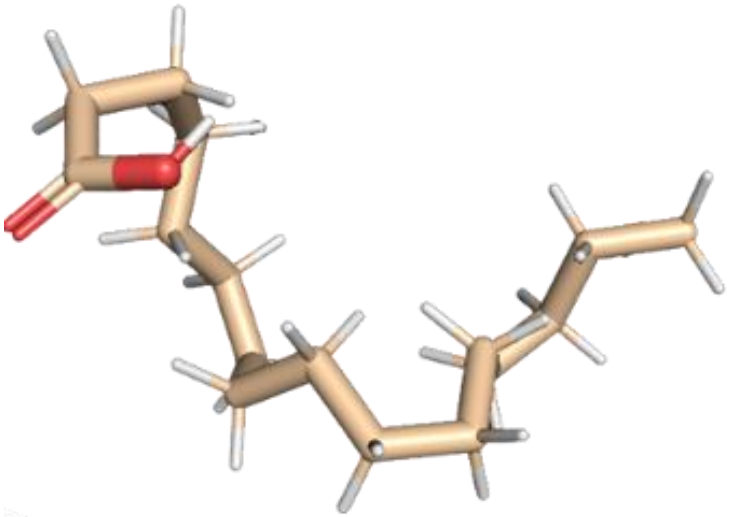
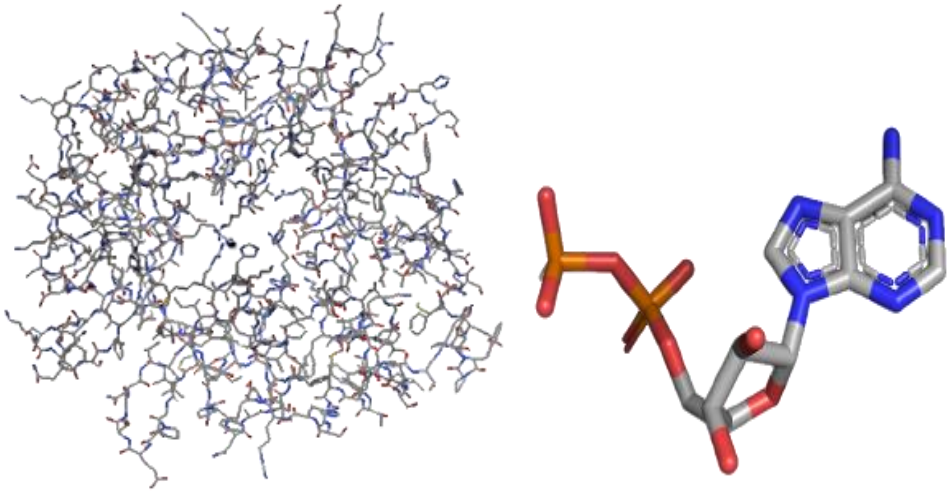


Figure 1. Palmitic acid optimization results – Palmitic acid compounds that have obtained the lowest energy of the test compound with the help of AutoDock



A **B**

Figure 2. (A) Results of optimization of AMPK structure (B) Native ligand

Ligand-receptor molecular docking test results

The Molecular Docking test was carried out using several software including PyMOL, Chimera, AutoDock, and Discovery Studio Visualizer. The lowest root means score deviation (RMSD) obtained through the docking process between AMPK and native ligands ADP 1327 and ADP 1328, was 1.078 and 1.136, respectively. for native ligands, ADP 1327 and ADP 1328 have the lowest binding affinity (ΔGbind) values of -7.9 kcal/mol and -8.3 kcal/mol. The molecular docking test is said to be valid if the RMSD value is below 2.5 Å (Baber *et al*., 2009). This indicates that the molecular docking method is valid and meets the validation parameter requirements because the RMSD value is less than 2.5 Å.

Table 3. Results of native ligand docking validation

No.	Native ligand	Binding affinity (Kcal/mol)	RMSD (Å)
1	ADP 1327	-7.6	1.078 Å
2	ADP 1328	-8.0	1.136 Å

Docking simulations were carried out between the test compound and the comparison compound against AMPK, using the region and the anchoring center (grid box) adjusted to the complex

ligand-binding region (native ligand) at the 2Y8L receptor and have been validated. All ADP, AMP, and ATP ligands produce negative ΔGbind values, in the sense that binding of these ligands occurs spontaneously and results in non-covalent binding interactions. The smallest value of ΔG ATP is -8.6 kcal/mol, lower than the value of ΔG AMP of -8.3 kcal/mol and ΔG ADP of -7.6 kcal/mol and -8.3 kcal/mol. The results of docking between palmitic acid and AMPK resulted in a ΔGbind value of -5.3 kcal/mol (**Table 4**). Meanwhile, metformin has a ΔGbind value of -5.1 kcal/mol. Based on these data, the two compounds above have binding affinity values that are greater and closer to the value of ΔG AMP or at least have a value of around ΔG ADP which is predicted to trigger AMPK activation and prevent AMPK dephosphorylation. On the other hand, if the value of the two compounds is smaller or closer to the value of ΔG ATP, it is predicted that it will inhibit AMPK activation and trigger AMPK dephosphorylation. This prediction leads to the natural state of AMPK regulation of the subunit where attachment of AMP to this subunit will trigger the phosphorylation of Thr172 residues on the subunit so that AMPK is active. ADP attachment is known to only play a role in preventing AMPK dephosphorylation. Unlike the case with ATP, if ATP attaches to the y subunit, it will inhibit the phosphorylation of the Thr172 residue so that AMPK is inactive. AMP has a role as an allosteric activator of AMPK, ADP as an active structural defense so that AMPK remains active while ATP acts as an allosteric inhibitor of AMPK (Carling, 2005; Hardie, 2008; 2015; Lim *et al*., 2010; Xiao *et al*., 2013; Calabrese *et al*., 2014). Based on data on ΔGbind values, palmitic acid and metformin compounds are predicted to potentially trigger AMPK activation and prevent AMPK dephosphorylation because their ΔGbind values are closer to AMP values or at least around ΔG ADP and greater than ΔG ATP.

Table 4. Validation results of the test compound docking with comparison

No.	Ligand	Binding affinity (Kcal/mol)	RMSD (Å)
1	ATP	-8.5	1.158 Å
2	AMP	-8.0	0.464 Å
3	Palmitic acid	-5.0	1.708 Å
4	Metformin	-4.6	1.504 Å

The binding formed at the AMPK receptor and all ligands used were analyzed and visualized in 2D using LigPlot+ 1.4.5 software. The bond interactions analyzed are in the form of hydrogen bonds and hydrophobic bonds. It can be seen in the results of the binding of ATP (**Figure 3**) that hydrogen bonds and hydrophobic bonds are produced more than ADP, which is more than AMP. One of these conditions can occur because ATP has more phosphate groups than ADP more than AMP. The phosphate group has an O atom that can form hydrogen bonds, as well as hydrophobic bonds. The more bonds formed, the more negative ΔG values are produced (de Beer *et al*., 2010). This can be seen in **Table 4** which shows that ATP does have a more negative ΔG value than ADP than AMP.

The similarity of the interaction between the receptor and the ligand is carried out because it can be a mimetic indicator between the test ligand and the known role of the ligand. The high similarity presentation shows the similarity of properties so that it can be predicted to have the same role as the comparison ligand (Ferdian *et al*., 2016). The interaction similarity data in **Table 5** shows that metformin has residues that tend to have a greater similarity to AMP than to ATP. It was characterized by the presence of the same residue which was only found in AMP ligands, namely, Ser225 and Ser315. Meanwhile, the palmitic acid residues formed tend to have a greater similarity to ADP and ATP. However, most of these residues tend to be closer to ADP than to ATP because the hydrogen bonds present in this compound are also found in ADP hydrogen bonds, including His297, Ser241, and Arg298. Both metformin and palmitic acid have residues with greater similarity to AMP and ADP than ATP, which means that both compounds are predicted to be able to act as allosteric activators of AMPK by direct binding. Based on the greater similarity of metformin to AMP compared to the similarity of palmitic acid, which is greater than ADP is greater, ATP is greater than AMP, then palmitic acid compounds can be said to be able to activate

AMPK *in silico* but not as good and effective as metformin which has the potential to be an anti-diabetic compound.

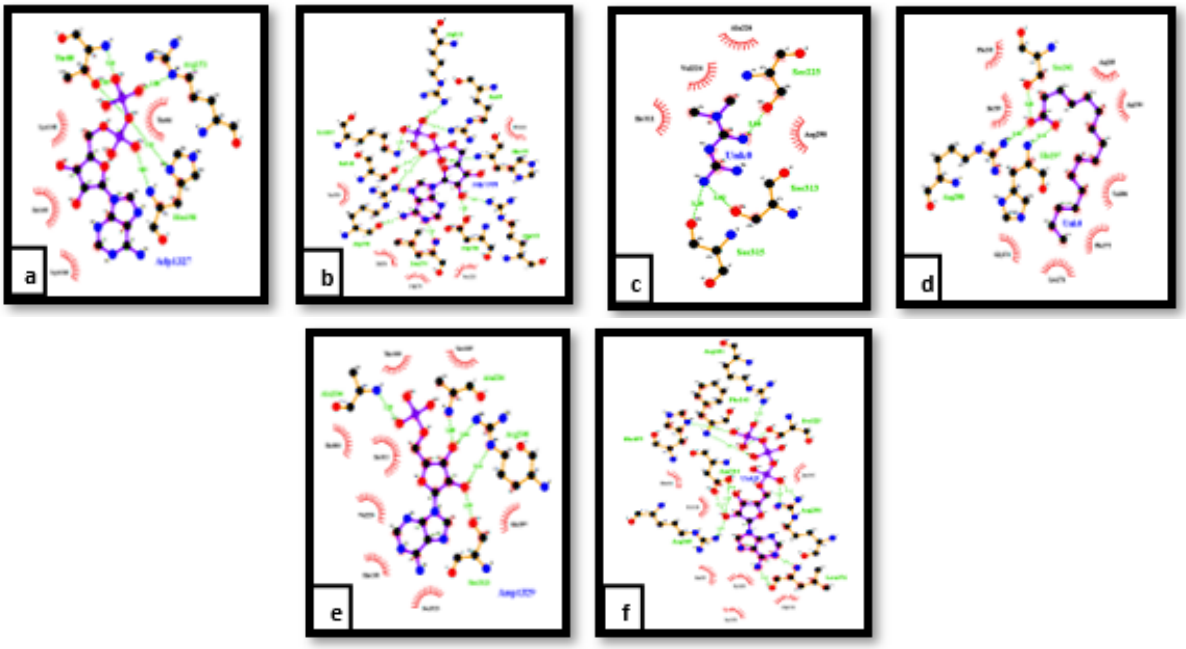


Figure 3. Hydrophobic and hydrogen bonds between AMPK receptors and ligands a) ADP 1237; b) ADP 1238; c) metformin; d) palmitic acid (PA); e) AMP; and f) ATP

Table 5. Amino acid residues that form hydrogen bonds and hydrophobic bonds

Ligand Model	Interacting Residual	
	Hydrogen Bonds	**Hydrophobic Bonds**
ADP 1237	His150; Thr88; Arg151	Lys148; Ile149; Lys126; Thr86
ADP 1238	Arg151; Arg69; Arg298; His297; Lys169; Ser241; Leu276; Asp244	Val296; Ile239; Gly274; Val275; Phe243
ATP	Arg151; Arg268; Arg298; Phe243; Ser225; Leu276; Asp244; His297	His150; Lys242; Ser241; Ile239; Val296; Val275; Gly224
AMP	Ala204; Ala226; Arg298; Ser313	Thr199; Ser225; Ile203; Ile311; Val224; His150; Ser315; His297
Metformin	Ser225; Ser313; Ser315	Arg298; Val224; Ile311; Ala226
Palmitic Acid	His297; Ser241; Arg298	Phe243; Ile239; Arg268; Asp244; Val296; Phe272; Leu276; Gly274

Apart from the binding energy produced by palmitic acid to AMPK protein, palmitic acid is one of the ingredients found in the dandang gendis plant (*Clinacanthus nutans*). This can be proven in a previous study by Murugesu *et al*. in 2019 which proved that palmitic acid compounds were contained in the dandang gendis plant (Murugesu *et al*., 2019). In the research of Ismail *et al*. in 2020, it was proven that the palmitic acid content in the dandang gendis plant (*Clinacanthus nutans*) had a fairly high level. compared to other types of plants which is 23.84% (Ismail *et al*., 2020).

Previous research by Dewinta *et al*. in 2020 that the administration of ethanol extract of dandang gendis leaves (*Clinacanthus nutans*) has potential as an antidiabetic by lowering blood sugar levels in diabetic white Wistar rats. This is because the leaves of dandang gendis (*Clinacanthus nutans*) contain flavonoids and fatty acids as antioxidants that can lower blood sugar levels (Dewinta *et al*., 2020). Another research conducted by Panggabean *et al*. in 2014 examined the "Activity Test of Increasing Insulin Sensitivity of Black Cumin Seed Extract (*Nigella sativa*) Through Measurement of Tyrosine Concentration Phosphorylated Insulin Receptor Substrate-1 (IRS-1)" that the administration of black cumin extract which has various contents, one of which is palmitic

acid can increase insulin sensitivity and lower blood glucose levels. In this study, it was found that giving black cumin extract to diabetic rats for 30 days was able to increase phosphorylated tyrosine levels on insulin receptor substrate-1 (IRS-1), insulin sensitivity and significantly reduce blood glucose levels at a dose of 96 mg/kg BW (Panggabean *et al*., 2014). The possible mechanism of action of palmitic acid in *Clinacanthus nutans* is as an AMPK agonist but needs to be proven in further studies *in vivo*. Further research is needed on the mechanism of action of other compounds in *Clinacanthus nutans* against AMPK.

Conclusion

Based on the results of the study, it can be concluded that the compound palmitic acid from the plant extract of *Clinacanthus nutans* has antidiabetic potential through *in silico* binding of the receptor-ligand to the AMPK active site. Palmitic acid compounds from plant extracts of *Clinacanthus nutans* can also be well absorbed in the body and can be used as oral drugs through the Human Intestinal Absorption (HIA) test and the Lipinski Rule of five tests.

Acknowledgment

The author expresses his gratitude along with the prayers and hopes of *Jazakumullah Ahsanal Jaza* 'to all those who have helped the completion of this thesis. The author expresses his gratitude to dr. Nurlaili Susanti, M. Biomed and dr. Zulvikar Syambani Ulhaq, M. Biomed, Ph.D as the thesis supervisor who has provided direction and guidance so that the author can arrange this research properly. The author's love goes to both parents who always provide prayers, material and non-material support, and their endless blessings to the author.

References

Abad-Zapatero, C. (2007). A Sorcerer's apprentice and The Rule of Five: from rule-of-thumb to commandment and beyond. *Drug Discovery Today*, **12**(23–24), 995–997. https://doi.org/10.1016/j.drudis.2007.10.022

Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A. S., Khatib, A., *et al*. (2016). Clinacanthus nutans: A review of the medicinal uses, pharmacology and phytochemistry. *Asian Pacific Journal of Tropical Medicine*, **9**(4), 402–409. https://doi.org/10.1016/j.apjtm.2016.03.011

Baber, J. C., Thompson, D. C., Cross, J. B., & Humblet, C. (2009). GARD: A generally applicable replacement for RMSD. *Journal of Chemical Information and Modeling*, **49**(8), 1889–1900. https://doi.org/10.1021/ci9001074

de Beer, S., Vermeulen, N., & Oostenbrink, C. (2010). The Role of Water Molecules in Computational Drug Design. *Current Topics in Medicinal Chemistry*, **10**(1), 55–66. https://doi.org/10.2174/156802610790232288

Bellou, V., Belbasis, L., Tzoulaki, I., & Evangelou, E. (2018). Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS ONE*, **13**(3), 1–27. https://doi.org/10.1371/journal.pone.0194127

Cahyaningsih, D. T. & Muwahhid, S. L. (2021). KKN UNS COVID-19: Upaya Pencegahan COVID-19 di Dukuh Wirocanan RT 3/4, Kertonatan, Kartasura, Sukoharjo. *Proceedings National Conference PKM Center*, **1**(1), 23-29.

Calabrese, M. F., Rajamohan, F., Harris, M. S., Caspers, N. L., Magyar, R., Withka, J. M., *et al*. (2014). Structural basis for AMPK activation: Natural and synthetic ligands regulate kinase activity from opposite poles by different molecular mechanisms. *Structure*, **22**(8), 1161–1172. https://doi.org/10.1016/j.str.2014.06.009

Carling, D. (2005). AMP-activated protein kinase: Balancing the scales. *Biochimie*, **87**(1), 87–91. https://doi.org/10.1016/j.biochi.2004.10.017

Dewinta, N. R., Mukono, I. S., & Mustika, A. (2020). Pengaruh Pemberian Ekstrak Dandang Gendis (Clinacanthus nutans) Terhadap Kadar Glukosa Darah pada Tikus Wistar Model Diabetes Melitus. *Jurnal Medik Veteriner*, **3**(1), 76-81. https://doi.org/10.20473/jmv.vol3.iss1.2020.76-81

Ekins, S., Mestres, J., & Testa, B. (2007). In silico Pharmacology for Drug Discovery: Applications to Targets and Beyond. *British Journal of Pharmacology*, **152**(1), 21-37. https://doi.org/10.1038/sj.bjp.0707306

Ekins, S., Nikolsky, Y., & Nikolskaya, T. (2005). Techniques: Application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends in Pharmacological Sciences*, **26**(4), 202–209. https://doi.org/10.1016/j.tips.2005.02.006

Ferdian, P. R. (2016). Potensi senyawa asam lemak rantai pendek sebagai aktivator langusung AMPK secara in silico untuk terapi sindrom metabolik. *Thesis.* Bogor: IPB University.

Hardie, D. G. (2008). AMPK: A key regulator of energy balance in the single cell and the whole organism. *International Journal of Obesity*, **32**(Suppl 4), S7–S12. https://doi.org/10.1038/ijo.2008.116

Hardie, D. G. (2015). AMPK: Positive and negative regulation, and its role in whole-body energy homeostasis. *Current Opinion in Cell Biology*, **33**, 1–7. https://doi.org/10.1016/j.ceb.2014.09.004

Imam, M. U., Ismail, M., George, A., Chinnappan, S. M., & Yusof, A. (2019). Aqueous leaf extract of Clinacanthus nutans improved metabolic indices and sorbitol-related complications in type II diabetic rats (T2D). *Food Science and Nutrition*, **7**(4), 1482–1493. https://doi.org/10.1002/fsn3.988

Ismail, N. Z., Md Toha, Z., Muhamad, M., Kamal, N. N. M., Zain, N. N. M., & Arsad, H. (2020). Antioxidant Effects, Antiproliferative Effects, and Molecular Docking of Clinacanthus nutans Leaf Extracts. *Molecules*, **25**(9), 2067. https://doi.org/10.3390/molecules25092067

Janani, C. & Kumari, B. D. R. (2015). PPAR gamma gene - A review', *Diabetes and Metabolic Syndrome*, **9**(1), 46–50. https://doi.org/10.1016/j.dsx.2014.09.015

Joshi, T., Singh, A. K., Haratipour, P., Sah, A. N., Pandey, A. K., Naseri, R., *et al*. (2019). Targeting AMPK signaling pathway by natural products for treatment of diabetes mellitus and its complications. *Journal of Cellular Physiology*, **234**(10), 17212–17231. https://doi.org/10.1002/jcp.28528

Kurnia, K. A., Harimurti, S., Yung, H. K., Baraheng, A., Alimin, M. A. S., Dagang, N. S. M., *et al*. (2019). Understanding the effect of pH on the solubility of Gamavuton-0 in the aqueous solution: Experimental and COSMO-RS modelling. *Journal of Molecular Liquids*, **296**, 111845. https://doi.org/10.1016/j.molliq.2019.111845

Lim, C. T., Kola, B., & Korbonits, M. (2010). AMPK as a mediator of hormonal signalling. *Journal of Molecular Endocrinology*, **44**(2), 87–97. https://doi.org/10.1677/jme-09-0063

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2012). Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Advanced Drug Delivery Reviews*, **64**, 4-17. https://doi.org/10.1016/j.addr.2012.09.019

Müller-Wieland, D. & Kommission Labordiagnostik in der Diabetologie der Deutschen Diabetes Gesellschaft (DDG) und der Deutschen Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL). (2019). Definition, Classification and Diagnosis of Diabetes Mellitus. *Der Diabetologe*, **15**(2), 128–134. https://doi.org/10.1007/s11428-019-0460-1

Murugesu, S., Khatib, A., Ahmed, Q. U., Ibrahim, Z., Uzir, B. F., Benchoula, K., *et al*. (2019). Toxicity study on Clinacanthus nutans leaf hexane fraction using Danio rerio embryos. *Toxicology Reports*, **6**, 1148–1154. https://doi.org/10.1016/j.toxrep.2019.10.020

Nandipati, K. C., Subramanian, S., & Agrawal, D. K. (2017). Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. *Molecular and Cellular Biochemistry*, **426**, 27–45. https://doi.org/10.1007/s11010-016-2878-8

Nerkar, A. G., Kudale, S. A., Joshi, P. P., & Chikhale, H. U. (2012). In silico screening, synthesis and pharmacological evaluation of novel quinazolinones as NMDA receptor inhibitors for anticonvulsant activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4**(3), 449–453.

Panggabean, F. K., Triastuti, E., & Yunita, E. P. (2014). Uji Aktivitas Peningkatan Sensitivitas Insulin Ekstrak Biji Jintan Hitam (Nigella sativa) melalui Pengukuran Konsentrasi Tirosin Terfosforilasi Insulin Reseptor Substrat-1 (terhadap Tikus Wistar Model Diabetes Mellitus Tipe 2). *Majalah Kesehatan*, **1**(1), 53-59.

Putra, R. J. S., Achmad, A., & Rachma, H. P. (2017). Kejadian Efek Samping Potensial Terapi Obat Anti Diabetes Pada Pasien Diabetes Melitus Berdasarkan Algoritme Naranjo. *Pharmaceutical Journal of Indonesia*, **2**(2), 45–50.

Siswandono. (2015). *Kimia Medisinal Jilid Satu (2nd edition)*. Surabaya: Universitas Airlangga.

Tian, S., Wang, J., Li, Y., Li, D., Xu, L., & Hou, T. (2015). The application of in silico drug-likeness predictions in pharmaceutical research. *Advanced Drug Delivery Reviews*, **86**, 2-10. https://doi.org/10.1016/j.addr.2015.01.009

World Health Organization. (2016). *Diabetes*. https://www.who.int/diabetes/country-profiles/idn_en.pdf

Xiao, B., Sanders, M. J., Carmena, D., Bright, N. J., Haire, L. F., Underwood, E., *et al*. (2013). Structural basis of AMPK regulation by small molecule activators. *Nature Communications*, **4**, 3017. https://doi.org/10.1038/ncomms4017