In Silico Analysis of Saponin Isolates from Mesocarp of Cucumber (Cucumis sativus L.) and Purple Eggplant (Solanum melongena L.) as Pancreatic Lipase Inhibitor

Mely Wijaya¹ and Subandi*¹

¹Department of Chemistry Universitas Negeri Malang, Jl. Semarang 5, Malang Indonesia

Corresponding author: subandi.fmipa@um.ac.id

Abstract. Currently orlistat has been widely used as anti-obesity drug, because of its activity as a pancreatic lipase inhibitor. Two saponin isolates, from cucumber mesocarp and purple eggplant, also proved to be active as pancreatic lipase inhibitors in vitro. Based on spectrophotometric analysis, the two saponin isolates are thought to be Silphioside F and Cesdiurins I-III. The purpose of this study is to confirm the ability of the two compounds as pancreatic lipase inhibitor through in silico analysis, relative to orlistat. This study uses Python Molecular Viewer (PyMol), Python Prescription (PyRx) 0.8, and Discovery Studio software. As a ligand, 3D structure of Silphioside F and Cesdiurins I-III have been used. The orlistat as a comparative ligand molecule have also been used. 3D structure of porcine pancreatic lipase has been used as receptor molecule. The result of the analysis shown that the binding site of pancreatic lipase is relatively same as orlistat of Cesdiurins I-III molecule, but different for the Silphioside F molecule. The data indicated that in inhibiting pancreatic lipase, the two isolate compounds used different mechanism. However, against pancreatic lipase, both molecules have greater binding affinities each, compared to orlistat, which were -9.7 kcal/mol for Silphioside F and -9.5 kcal/mol for Cesdiurins I-III, and orlistat only -7.6 kcal/mol. The latest data were in line with the in vitro analysis, that both isolates have greater inhibition power than orlistat.

1. Introduction

Obesity has become a global problem [1]. It is a risk factor for the emergence of degenerative diseases such as type 2 diabetes mellitus (T2DM), cardiovascular disease, cancer, and premature death [2,3,4]. As an anti-obesity drug, orlistat is a type of tetrahydrolipstatin, which is isolated from Actinomycetes (Streptomyces toxytricini), and works as a pancreatic lipase inhibitor [5]. The pancreatic lipase is an enzyme secreted by pancreas into the intestine and hydrolyzes 50-70% triglycerides into fatty acids [6]. Orlistat is a competitive inhibitor of pancreatic lipase. Its activity is determined by the presence of β-lactone rings in its carbon chain. The inhibitory activity of orlistat disappears when the lactone β ring is opened [7]. Orlistat consumption can cause several side effects such as hyperuricemia, diarrhea, nausea, myositis, stomach irritation, oily spots, flatulence, flatus, fecal incontinence, and dry skin [8]. Thus, many studies have used herbal sources to replace orlistat, as a pancreatic lipase inhibitor.

Previous studies have revealed the presence of saponins that active as pancreatic lipase inhibitor in methanol extracts of cucumber mesocarp [9] and purple eggplants [10]. The UV-Vis and FT-IR spectroscopic analysis suggested that the saponin isolated from cucumber mesocarp has a similar structure to saponin from Acanthopanax senticosus fruit, namely Silphioside F [11], while the saponin isolate from purple eggplant mesocarp has a similar structure to Cesdiurins I-III [12]. The purpose of
this study is to confirm the ability of the two saponin compounds as pancreatic lipase inhibitors relative to orlistat, through in silico analysis using molecular docking.

In molecular docking, the interaction between ligands and receptors will be studied by identifying the active site that matches the receptor, getting the best geometry of the ligand-receptor complex, and calculating the interaction energy in different ligands to get a more effective ligand [13]. By using molecular docking, the binding site and binding affinity of the two saponin isolate to the pancreatic lipase enzyme, relative to the standard ligand, orlistat, can be predicted.

2. Experiment Methods
This study used ASUSPRO P5430UA laptop with Intel® Core™ i7 6500U Processor, Windows 10 Pro-ASUS, Windows 10 Pro, memory DDR4 2133 MHz SDRAM, OnBoard Memory 4 GB, 1xDIMM socket for expansion up to 12 GB SDRAM, Python Molecular Viewer Program (PyMol), Python Prescription (PyRx) 0.8, and Discovery Studio.

The 3D structure of the receptor protein used was adjusted to the type of lipase used in in-vitro analysis [9,10], namely Pancreas Triacylglicerol Lipase P00591 (LIPP_PIG, with code 1ETH) downloaded from the UniProt Bank Protein Data database (https://www.uniprot.org/) in the PDB file format. The structure used is the A/C.chain

The 2D structure of orlistat and Silphioside F was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov), while the 2D structure of Cesdiurins I-III was obtained from Fouad et al. 2008 [12]. The 3D structure of orlistat, Silphioside F and Cesdiurins I-III were obtained from the PubChem database in Sybil Data File format (sdf).

3. Result and Discussion

3.1. Sterilization of 3D Structure of The Enzyme
The 3D structure of Pancreatic lipase enzyme must be sterilized to eliminate the water content. All the ligands contained in the enzyme. This process is carried out using PyMol software. The results can be seen in Figure 1.

![Sterilization results of the 3D structure of the porcine pancreatic lipase enzyme (Uniprot 2019) (surface form (a) and worm form (b))](image)

3.2. Ligand Preparation
The 2D structures of orlistat, Siphiosite F was made in PubChem and Cestiurins I-III [13] (Figure 2), then converted to 3D form using the PubChem program (Figure 3). The structure is saved in the form of a sdf file, and read by the PyRx program. Then, it is used for the docking process.
3.3. Binding Affinity Between Ligands and Pancreatic Lipase Enzyme Using Molecular Docking

Molecular docking process has been used to identify the activity of ligand compounds that has a potency as inhibitor against pancreatic lipase. Molecular docking analysis was performed using the Autodock Vina program in PyRx 0.8 software. This process produced the binding affinity (kcal/mol) value of a ligand to its receptor (enzyme). The higher absolute binding affinity value was predicted to have higher inhibitory activity against the enzyme. The result of this molecular docking analysis has shown that Silphioside F and Cesdiurins I-III, which have a higher binding affinity to pancreatic lipase than orlistat has (Table 1). This data suggested that inhibitory activity of both saponin against pancreatic lipase is higher than orlistat.

| Ligand              | Binding Affinity (kcal/mol) |
|---------------------|-----------------------------|
| Orlistat            | -7.6                        |
| Silphioside F       | -9.7                        |
| Cesdiurins I-III    | -9.5                        |

3.4. Binding Position of The Ligan on Pancreatic Lipase

The binding position of the ligand compound to the pancreatic lipase receptor was visualized using PyMol program, and the results were shown in Figure 4.

According to Figure 4, for the Pancreatic Lipase, the binding site of Silphioside F is different from the binding site of orlistat. The binding site of Cesdiurins I-III is relatively the same as orlistat. It has been known that the orlistat inhibitory mechanism is competitive [5]. This data suggests that the Silphioside F inhibition mechanism is non-competitive, while the inhibitory mechanism of Cesdiurins I-III is competitive.
Figure 4. Binding Position of (a) Orlistat, (b) Silphioside F and (c) Cesdiurins I-III against pancreatic lipase enzymes in pigs visualized with PyMol software.

3.5. The Interaction of Ligand Compounds to Amino Acid Residues on The Binding Site of The Enzyme

This visualization used the Discovery Studio program to find out the bonding distances, bond types, and amino acid residues involved in the interaction between the ligand and target protein. The results of the analysis were presented in Figure 5 and Table 2.

The number and type of amino acids involved in the binding to each ligand which is the binding position of Silphioside F, is different from orlistat. Whereas, Cesdiurins I-III binding position is relatively same with the residues of ALA261, HIS264, VAL261, and PRO261 (Table 2 and Figure 5).

The overall result of this analysis was in line to the previous data which shows that inhibitory activity of the both predicted saponin isolates were higher than orlistat, at the same mass [9,10]. In addition, the isolate from other sources that also contain Silphioside F has activity as pancreatic lipase inhibitor [11]. All the data suggested that the isolates which contain one or both of the predicted saponin have a potency as anti-obesity drug.
Figure 5. Types of bonding and amino acid residues involved in binding with pancreatic lipase (Orlistat (a), Silphioside F (b) and Cesdiurins I-III (c)).

Table 2. Types of numbers and bonding distances between Amino acid residues in pancreatic lipases involved and the intermolecular interactions with Orlistat, Silphioside F, and Cesdiurins I-III.

| Ligand       | Types and Residues of Amino Acids Involved | Bonding Distance (Å) | Interaction Type                      |
|--------------|-------------------------------------------|----------------------|---------------------------------------|
| Orlistat     |                                           |                      |                                       |
| VAL A:260    |                                           | 4.05                 | Alkyl, Pi-Alkyl, Carbon Hydrogen Bond |
|              |                                           | 4.37                 | Alkyl, Pi-Alkyl, Carbon Hydrogen Bond |
| HIS A:152    |                                           | 2.58                 | Conventional hydrogen bond            |
| PHE A:78     |                                           | 2.01                 | Conventional hydrogen bond, Carbon Hydrogen Bond |
| PRO A:181    |                                           | 4.41                 | Alkyl, Pi-Alkyl                       |
| HIS A:264    |                                           | 5.03                 | Attractive Charge                     |
|              |                                           | 3.89                 | Attractive Charge                     |
|              |                                           | 3.01                 | Carbon Hydrogen Bond                  |
| LEU A:154    |                                           | 2.86                 | Conventional hydrogen bond            |
| Silphioside F|                                           |                      |                                       |
| VAL A:21     |                                           | 2.01                 | Conventional hydrogen bond            |
| PRO A:187    |                                           | 4.87                 | Alkyl                                 |
|              |                                           | 5.21                 | Alkyl                                 |
|              |                                           | 5.05                 | Alkyl                                 |
| PRO A:16     |                                           | 4.72                 | Alkyl                                 |
| LEU A:189    |                                           | 3.83                 | Alkyl                                 |
|              |                                           | 5.20                 | Alkyl                                 |
|              |                                           | 4.65                 | Alkyl                                 |
| Cesdiurins I-III |                                         |                      |                                       |
| PRO A:181    |                                           | 5.31                 | Alkyl, Pi-Alkyl                       |
| HIS A:264    |                                           | 4.74                 | Alkyl, Pi-Alkyl                       |
| VAL A:260    |                                           | 4.71                 | Alkyl, Pi-Alkyl                       |
| ALA A:261    |                                           | 3.92                 | Alkyl, Pi                            |
|              |                                           | 5.19                 | Alkyl, Pi                            |
| ILE A:79     |                                           | 4.03                 | Alkyl, Pi                            |
| PHE A:78     |                                           | 4.05                 | Alkyl, Pi                            |
|              |                                           | 5.39                 | Alkyl, Pi                            |
|              |                                           | 5.37                 | Alkyl, Pi                            |
|              |                                           | 4.49                 | Alkyl, Pi                            |
| PHE A:216    |                                           | 4.64                 | Alkyl, Pi                            |
|              |                                           | 4.32                 | Alkyl, Pi                            |
|              |                                           | 3.75                 | Alkyl, Pi                            |
|              |                                           | 5.01                 | Alkyl, Pi                            |
4. Conclusion

The result of this in silico analysis has shown that the binding site for Cesdiurins I-III molecule to pancreatic lipase is relatively same as orlistat. However, for the Silphioside F molecule, the binding site was different. The data indicated that in inhibiting pancreatic lipase, the two isolate compounds used different mechanism. However, against pancreatic lipase, both molecules have greater binding affinities, compared to orlistat, which are -9.7 kcal/mol for Silphioside F and -9.5 kcal/mol for Cesdiurins I-III), and -7.6 kcal/mol for Orsilat. The latest data are in line with the in vitro analysis, in which both isolates have greater inhibition power than orlistat.

References

[1] Barry M Pompkin and Collen M Doak 1998 Nutrition Reviews 56 106–114.
[2] Vasanti S. Malik, Barry M. Popkin, George A. Bray, Jean-Pierre Després, Frank B. Hu 2010 Contemporary Reviews in Cardiovascular Medicine 121 1356-1364.
[3] Hu FB. 2008. Obesity Epidemiology. New York, NY: Oxford University Press.
[4] Darusman L.K., Pradono D.I., and Susanti A.I 2011 Jurnal Natur Indonesia 13 142-154.
[5] Filippatos T. D, Derdemezis C. S., Gazi I. F, Na-kou E. S, Mikhalidis D. P, and Elisaf M. S. 2008 Drug Safety 31 53-65.
[6] Delphin D.V, Haripriya R, Subi S, Jothi D and P. Thirumalai Vasan 2014 World Journal Of Pharmacy And Pharmaceutical Sciences 3 1041-1048.
[7] Birari RB and Bhutani KK 2007 Drug Discov Today 12 879 [PMID: 17933690].
[8] Yamamoto M, Shimura S, Itoh Y, Ohsaka T, Ega-wa M, and Inoue S 2000 International Journal of Obesity and Related Metabolic Disorders 24 758-764.
[9] Subandi, Wijaya M, Sudarmo Tatas P B and Suarsini E 2018 AIP Conference Proceedings 2021, 070016.
[10] Subandi, Zakiyaturrodliyah L, and Sudarmo Tatas P B 2019 IOP Conference Series: Materials Science and Engineering 509 012139
[11] F. Li, W. Lì, H. Fu, Q. Zhang and K. Koike. 2007. Chem. Pharm. Bull. 55 1087-1089.
[12] Fouad M.A., Mohamed K.M., Kamel M.S., Matsunami K. 2008. Cesdiurins I-III, steroidal saponins from Cestrum diurnum L. Journal of Natural Medicines PubMed
[13] Mukesh B. Rakesh K 2011 International Journal of Research in Ayurveda & Pharmacy. 2 1746-1751.