Pharmacopore Modeling and Molecular Docking Studies on *Phyllanthus niruri* as a Target for Diabetes Mellitus

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**Abstract**

**Background:** *Phyllanthus niruri* is one of the herbal plants from the family of Euphorbiaceae and the extract of this plant was widely used in the preparation of various ayurvedic formulations for diabetic. **Objectives:** The main object of this study is to validate the ethnopharmological knowledge of *Phyllanthus niruri* against T2DM with the help of modern computational tools to explore the most potential active constituents through molecular docking (protein-ligand) and pharmacopore modeling.  

**Results:** The docking score result on different receptors for anti T2DM and bioactive compounds had shown that the compounds, namely phyllanthin, hinokinin and astragalin had best score among other docking scores on all evaluated receptors, while most significant effect were shown on PDB ID: 1US0 against internal ligand IDDD94.  

**Conclusion:** This study found phyllanthin, hinokinin, and astragalin had highest affinity for AR. This clearly indicate that phyllanthin from *Phyllanthus niruri* can be used against T2DM, especially through aldose reductase pathway.

**Introduction**

The dramatic increase in obesity and diabetes worldwide poses a huge socioeconomic burden to healthcare systems. In type 1 diabetes, autoimmune-mediated destruction of pancreatic beta-cell results in insulin deficiency. Obesity is one of the major causes of type 2 diabetes. In type 2 diabetes, a combination of peripheral insulin are amongst the paradox commonly encountered in the pathogenesis of the disease (Ndisang, J.F., 2015). Type 2 diabetes is the most commonly suffered by adult and teenagers. In this type of diabetes, the insulin cell targets lost the ability to response insulin normally or it was known as insulin resistance (Direktorat Bina Farmasi Komunitas, 2006).

Without a proper handling, diabetes mellitus can cause many chronic disease like cerebrovascular disease, coronary heart attack, limb vascular disease, eyes disease, kidney, dan and nerve. By controlling the glucose level, those chronic diseases can be prevented or stopped. Other factors such as genetic factor, environment, and life style also have a role in this pathogenetic disease (Soegondo, 2005).

WHO has estimated about 200 million people all over the globe are suffering diabetes and this figure is likely doubled by 2030. WHO said that the middle-income countries mostly contribute on the suffering number and the sufferer number in Indonesia about 8,4 million by 2000 and likely increased to be 21,3 million by 2030. The high death number put Indonesia on the 4th world ranking after US, India, and China (DepKes, R.I., 2004).

With advancement in modern medical sciences, a variety of major disease such as type 2 diabetes still prevail. Medicinal plants have the potential to prevent, control, or cure some of these diseases. Many of the plants have high medicinal values to be further exploration. However, determining specific components having specific pharmacological activity and their clinical effects still remains a challenge (Hua, G.S., 2015).

*Phyllanthus niruri* is one of the herbal plants from the family of Euphorbiaceae and the extract of this plant was widely used in the preparation of various ayurvedic formulations (Amin, Z.A., 2012). This medicinal plant reported to possess anti diabetic and kidney protective effect. Okoli, *et al.*, have investigated the phytochemical constituents and antidiabetic effect of this plant to the diabetic rat. Results suggest that extract of aerial parts of *P. niruri* has great potential as anti-diabetic remedy (Okoli, C.O., 2011).

Herbal medicine widely accepted in many countries. WHO said that many countries in Africa, Asia, and Latin America use the herbal medicine as
the complementary of their primer medicine. Who also recommends on using the traditional medicine such as medicinal plants for people’s health care, to prevent and cure the chronic and degenerative disease. Using of the traditional medicine is generally considered safer than the modern medicine. This is because traditional medicines have side effects that are relatively less than the modern medicine (WHO, 2005). The main object of this study is to validate the ethnopharmaceutical knowledge of Phyllanthus niruri against T2DM with the help of modern computational tools to explore the most potential active constituents through molecular docking (protein-ligand) and pharmacopoe modeling.

**Material And Methodology:**

**Receptor:**

There were four receptors involved in this study, i.e. insulin receptor (IR), aldose reductase (AR), protein tyrosine phosphatase 1-beta (PTP-1β), dipeptidyl peptidase-IV (DPP-IV), and. The three-dimensional crystal structure of those receptors was taken from Protein Data Bank (PDB) as is follows: IR (PDB ID: 1IR3), AR (PDB ID: 1USO), PTP-1β (PDB ID: 2F70), and DPP-IV (PDB ID: 3F8S). All of the PDB’s were loaded in the CLC Drug Discovery Workbench (CLCDDW).

**Ligands:**

The mol files and smile formula of ligands were obtained from CHEMSPIDER database (http://www.chemspider.com.). Structure of ligands were drawn using marvin sketch and energy minimization was done using MMFF94 force field which is embed in CLCDDW algorithm. This process only changes the overall position, rotation, and any dihedral angles in the ligand. It will not change bond lengths and angles, or change ring conformations. Energy minimization is done to help the docking programme for identifying the bioactive conformer from the local minima (Kaushik, P., 2014) and can repair distorted geometries by moving atoms to release internal constraints. The important advantage of CLCDDW is to help in determining connectivity, bond order, assigning atom hybridization, and creating explicit hydrogens of the imported ligands. The 2D structures of 19 ligands are illustrated in Table 1.

**Molinspiration:**

Molinspiration is an online tool that can calculate various molecular properties needed in Quantitative structure–activity relationship (QSAR), molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches and bioactivity prediction. The selected compounds in evaluation process of drug properties should follow the Lipinski rule of five include molecular mass less than 500 daltons, high lipophilicity less than 5, less than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors (Sakamoori, K., 2014). The compounds that fulfill the Lipinski rule can be potential safe drug for human.

**Validation and Analysis of Docked Receptor-Ligand Complex Structures:**

To ensure that ligands docked using CLCDDW have valid scores and accurate binding site with receptor. We firstly validated the docking score of the crystal structures (PDB: 1IR3, 1USO, 2F70, 3F8S) and their internal ligand. Respectively, IR (PDB ID: 1IR3) was docked with molecule ANP (phosphoaminophosphonic acid-adenylate ester); AR (PDB ID: 1USO) was docked with IDD594 (2-(4-bromo-2-fluoro-benzylthiocarbamoio)-5-(fluorophenoxy)-acetic acid); PTP1β (PDB ID: 2F70) was docked with UN608 (3-{[3-(sulfaomino-phenyl)-propio-nylamino]-methyl-phenyl}sulfamic acid; DPP-IV (PDB ID: 3F8S) was docked with PF2 (2-(4-((3,5-S)-5-[[3,3, difluoropyrroldin-yl]carbonyl]pyrroloidin-3-yl)piperazin-1-yl)pyrimidine). This stage had done as control docking models as illustrated in Table 2.

**Molecular Docking of Ligands:**

The docking process we used CLCDDW software package. Basic set ups like protein receptor preparation, ligand preparation, detecting cavities/binding sites, and targeted ligand docking performed. The docking parameters include population size 19, maximum iterations 2000, binding set radius 12Å, and ligand conformation flexibility. CLCDDW uses a standard precision mode to determine the favourable binding poses, which detects various flexible ligand conformations while holding protein as rigid structure during docking[15]. The docking score used in the Drug Discovery Workbench is the PLANTSPLPscore. This score has a good balance between accuracy and evaluation time. The score mimics the potential energy change, when the protein and ligand come together. This means that a very negative score corresponds to a strong binding and a less negative or even positive score corresponds to a weak or non-existing binding. Score= Starget-ligand + Stigand

The Starget – ligand term is a sum over contributions from all heavy atom contacts between the ligand and the molecules included in the binding site setup. It scores the complementarity between binding site and ligand by rewarding and punishing different types of heavy atom contacts (inter atom distance below ~5.5Å). Five different types of contacts are hydrogen bond interaction, lone-pair-metal ion interactions, and Non-polar interactions as rewarded contacts; and non-polar – polar contacts and repulsive contacts as punished contacts. The 3D visualization to know the residue interaction with ligand complex was performed by PyMol, and to
know the binding site was performed by CLCDDW. This visualization aimed to strengthen the evidence of residues which interacted with ligand.

**Pharmacophore Modeling:**

Pharmacophore is an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response. The structure-based pharmacophore model was generated using the LigandScout software package. LigandScout (Amresh, P., 2013) uses algorithm that allows automatic generation of 3D-pharmacophore from structural data of protein-ligand complex that was used to generate the most potential ligand that was determined by docking in previous stage. This software uses an algorithm that studies and interprets ligand-receptor interactions such as charge transfer, hydrogen bonds, and hydrophobic regions of their macromolecular environment from PDB files, allowing the automatic building of the pharmacophore model.

**RESULTS AND DISCUSSION**

Out of 19 constituents which had done molecular docking process, only 15 constituents passed the Lipinski rule of five. Docking result between ligand and receptor were analyzed apart from docking energy, binding modes, and interactions of each ligand with the residue of IR (PDB ID: 1IR3), AR (PDB ID: 1US0), PTP-1β (PDB ID: 2F70), and DPP-IV (PDB ID: 3F8S) were analyzed by CLC Drug Discovery Workbench (CLCDDW).

**Insulin Receptor (IR):**

PLANTSPLP score of internal ligand ANP and IR (PDB ID: 1IR3) was -47.31, while three bioactive compounds of *Phyllanthus niruri*, namely lupeol acetate, lupeol, and astragalin had score -75.44, -74.78 and -47.51, respectively. Amino acid residue which involved interaction with internal ligands and most of the bioactive compounds in 1IR3 were Met 1079, Asp 1083, Met 1076, Met 1139, Glu 1077, Lys 1030, Ser 1006.

**Aldose Reductase (AR):**

PLANTSPLP score of internal ligand IDD594 and AR (PDB ID: 1US0) was -80.35, while three bioactive compounds, namely phyllanthin, hinokinin, and astragalin had score -94.47, -90.11 and -89.19, respectively. Amino acid residue which involved interaction with internal ligands and most of the bioactive compounds in 1US0 were Phe 122, Trp 20, Trp 79, Val 47, Tyr 48, Ala 299, Leu 300, Phe 115, Thr 113, Tyr 309, and Trp 111, respectively.

**Dipeptidyl Peptidase-IV (DPP-IV):**

PLANTSPLP score of internal ligand UN608 and DPP-VI (PDB ID: 2F70) was -36.86, while three bioactive compounds, namely astragalin, lupeol, and hinokinin had score -69.98, -64.10 and -62.04, respectively. Amino acid residue which involved interaction with internal ligands and the bioactive compounds in 1US0 were Arg 221, Ser 216, Asp 181, Arg 24, Arg 254, Ile 219, Glu 262, Tyr 46, Phe 182, Ala 217, Gly 220, Glu 207, Ser 80, Leu 204, and Arg 79, respectively. In this case, the amino acid residues involves interaction between DPP-VI and UN608 are completely different.

**Protein Tyrosine Phosphatase 1-beta (PTP-1β):**

PLANTSPLP score of internal ligand PF2 and PTP-1β (PDB ID: 3F8S) was -55.41, while three bioactive compounds that had highest score among other compounds, namely ellagic acid, astragalin, and kaempferol had score -45.69, -41.12 and -37.74, respectively. Amino acid residue which involved interaction with internal ligands and most of the bioactive compounds in 3F8S were Asp 663, Glu 205, Glu 206, Ser 209, Phe 357, Tyr 662, Ser 630, Val 711, Val 656, Tyr 666, and Trp 659, respectively. The PLANTSPLP score showed that internal ligand had higher value than top three bioactive compounds which had docked previously. In this case, the bioactive compounds in *Phyllanthus niruri* can not be considered as inhibitor of PTP-1β.

The docking score result on different receptors for anti T2DM and bioactive compounds had shown that the compounds, namely phyllanthin, hinokinin and astragalin had best score among other docking score on all evaluated receptors, while most significant effect were shown on PDB ID: 1US0 against internal ligand IDD594. Comparison of interactions between the internal ligand and phyllanthin docked with amino acid residue 1US0 was given in Figures 1(a) – 1(b).

A molecular docking is aimed to know the specific region of the target protein, expected to be the binding site[11]. This process was generated by CLC Drug Discovery Workbench where uses a specific algorithm to identify the binding site of the receptor. The area which identified as binding site is called binding pocket. The study found that the binding site of phyllanthin and IDD594 almost exactly in same site (see Figures 2(a) – 2(b). *Phyllanthus niruri* has a long history in herbal medicine systems in every tropical country where it grows. Its mainly uses are for many types of biliary and urinary conditions including kidney and gallbladder stones; but, it is also widely used for diabetes (Taylor, L., 2003). This study focused on the active constituents in it against T2DM. We used modern computational chemistry tools to explore the most potential active constituents through molecular docking (protein – ligand) and pharmacophore modeling.
Table 1: 2D structures of 19 chemical compounds from *Phyllanthus niruri*

<table>
<thead>
<tr>
<th>(-) Gallicatcchin</th>
<th>(+) Gallicatcchin</th>
<th>Astragalin</th>
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<tbody>
<tr>
<td>Brevifolin</td>
<td>Butyrolactone</td>
<td>Catechin</td>
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<tr>
<td>Corilagin</td>
<td>Cymene</td>
<td>Ellagic Acid</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>Gallic acid</td>
<td>Geranin</td>
</tr>
<tr>
<td>Hinokinin</td>
<td>Kaemferol</td>
<td>Lupeol</td>
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<tr>
<td>Lupeol asetat</td>
<td>Phylanthin</td>
<td>Sesamin</td>
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<tr>
<td>Salisylic Acid Methyl Ester</td>
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Table 2: PLANTSPLP score of internal ligand and receptor-ligand complex structures docking.

<table>
<thead>
<tr>
<th>PDB ID</th>
<th>Internal ligand</th>
<th>PLANTSPLP score</th>
</tr>
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<tbody>
<tr>
<td>1IR3</td>
<td>ANP</td>
<td>-47.31</td>
</tr>
<tr>
<td>1US0</td>
<td>IDDS94</td>
<td>-80.35</td>
</tr>
<tr>
<td>2F70</td>
<td>UN608</td>
<td>-36.86</td>
</tr>
<tr>
<td>3F8S</td>
<td>PP2</td>
<td>-35.41</td>
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A protein-ligand docking program consists of two essential components, sampling and scoring. Sampling means to the generation of putative ligand binding orientations/conformations near a binding site of a protein (receptor). Scoring is the prediction of the binding tightness for individual ligand conformations with a physical or empirical energy function. The top conformation, namely the one with the lowest energy score is predicted as the binding mode. Scoring type in this study is the PLANTSPLP score which has a good balance between accuracy and evaluation time. The primary aim of selecting the four different receptors (IR, AR, PTP-1β, and DPP-IV) of T2DM was to get the best pathway through active constituents as ligands.

Through molecular docking, this study had found that aldose reductase was the most active to constituents of Phyllanthus niruri. Increasing of aldose reductase activity is one of the several mechanisms that have been implicated in the development of various secondary complications of diabetes. According to result of molecular docking, the active constituents that had high binding affinity against AR are namely phyllanthin, hinokinin, and astragilin, respectively. Then this result was validated by pharmacophore model which predicted Tyr 48 and Trp 111 are essential for H-bonding between internal ligand and AR, and TYR 309 are essential for H-bonding between phyllanthin and AR. Tyr 48 and Trp 111 made hydrophobic interactions in molecular docking between phyllanthin and AR, and TYR 309 also made hydrophobic interaction in molecular docking between internal ligand and AR (See Figures 3(a) – 3(b)).

This result had shown the hydrophobic interaction was essential on the docking of phylanthin and internal ligand. The interactions between ligands and the hydrophobic side chains of proteins contribute significantly to the binding free energy (Bronowska, A. K., 2011). The hydrophobic residues mutually will repel water and other polar groups and results in a net attraction of the non-polar groups of ligand. Aromatic rings of tryptophan and phenylalanine, which are available in phylanthin and internal ligand pharmacophore modelling, could make interactions with aromatic moieties of ligand, so it will contribute in binding free energy. Although there is not similarity H-bonding between phyllanthin and internal ligand of AR in pharmacopore modelling, H-bonding and similarity of hydrophobic interactions with internal ligand, have to consider to state phylanthin as potential inhibitor of AR.
Conclusion:
19 active constituents had involved in this study through molecular docking and pharmacopore modelling. Molecular docking used 4 different receptors that responsible to T2DM. This study found phyllanthin, hinokinin, and astragilin had highest affinity for AR. Pharmacopore modelling was done with help of Liganscout predicts that hydrophobic interactions of phyllanthin and AR may contribute as potential inhibitor. Moreover, antidiabetic effect has been shown in several study in wet laboratory (Okoli, C.O., 2010). This clearly indicate that phyllanthin from Phyllanthus niruri can be used against T2DM, especially through aldose reductase pathway. This study results may lay base of further exploration of the Phyllanthus niruri for its antidiabetic potential.

Fig. 3: Pharmacophore model of 1US0 (a) internal ligand (b) phyllanthin.

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REFERENCES


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