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A new candidate of calcium channel blocker in silico from *Tectona grandis* for treatment of gestational hypertension

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**Abstract.** Gestational Hypertension is one of the three main causes of maternal mortality in Indonesia. Nifedipine which blocks the Cav1.2 calcium channel has frequently been used to treat gestational hypertension. However the efficacy of nifedipine has not been established yet and the prevalence of gestational hypertension is still high (27.1 %). Indonesian herbal plants have potential to be developed as natural drugs. Molecular docking, a computational method, is very often used to depict interaction between molecules and target receptor. This study was therefore to identify Indonesian herbal plants that could inhibit the calcium channel in silico.

This was a bioinformatics study with molecular docking approach. Three-dimensional structure of human calcium channel Cav1.2 was determined by modelling with rabbit calcium channel (ID:5GJW) as template and using the SWISS MODEL software. Nifedipine was used as a standard ligand and obtained from ZINC database with the access code ZINC19594578. Active compounds of Indonesian herbal plants were registered in HerbalDB database and their molecular structure was obtained from PubChem. Binding affinity of human Cav1.2 model-ligand complexes were assessed using AutoDock Vina 1.1.2 software and visualization of molecular conformation used Chimera 1.10 and PyMol 1.3 softwares. The Lipinsky’s rules of five were used to determine active compounds which fulfilled drug criteria. The human Cav1-2 model had 72.35% sequence identity with rabbit Cav1.1. Nifedipine bound to the human Cav1.2 model with -2.1 kcal/mol binding affinity and had binding sites at Gln1060, Phe1129, Ser1132, and Ile1173 residues. A lower binding affinity was observed in 8 phytochemicals but only obtusifolin 2-glucoside (-2.2 kcal/mol) had similar binding sites as nifedipine did. In addition, obtusifolin 2-glucoside met the Lipinsky criteria and the molecule conformation was similar with nifedipine. From the HerbalDB database, obtusifolin 2-glucoside is found in *Tectona grandis*. Obtusifolin 2-glucoside computationally becomes a potential candidate of calcium channel blocker. In vitro assays should be performed to evaluate the antagonist effect of obtusifolin 2-glucoside on calcium channel Cav1.2.

**1. Introduction**

Gestational hypertension is one of the three main causes of maternal death in Indonesia. Based on Indonesian Health Profile (2014), the prevalence of gestational hypertension is 21.5% in 2010 and increases by 5.6 % in 2013 [1]. In short time, untreated gestational hypertension can cause pre-eclampsia, placental abruption, and cerebrovascular complications like intravascular coagulation [2-3].
Subsequently, premature birth, low birth weight, intrauterine fetal death and mental retardation are the most common complication that is found in babies who are delivered by pregnant women with gestational hypertension [3].

Pharmacological regimens which are currently available for treatment of gestational hypertension are methyldopa and nifedipine. Methyldopa is the first line drug for gestational hypertension but this drug has many side effects [5]. Although nifedipine is the second choice for gestational hypertension, it is commonly used in Indonesia. Nifedipine blocks calcium (Cav1.1 and Cav1.2) channels which results in systemic arterial dilation, increases oxygen transport to myocardial tissue and reduces total peripheral resistance, systemic blood pressure and afterload [6, 7]. Senadheera and co-workers (2013) have reported that the expression of Cav1.2 increases in uterine artery during pregnancy [8]. So, inhibition of Cav1.2 expression by nifedipine may have beneficial effects to treat gestational hypertension due to its tocolytic property and placental perfusion [2].

In some cases, extensive use of nifedipine, however, does not reduces the incidence of gestational hypertension, which leads to high morbidity and mortality rates. In addition, several side effects of nifedipine treatment has been reported including gingival hyperplasia, peripheral edema, vomiting, fatigue, constipation, bradycardia, atrioventricular block, and heart failure [9]. Therefore, it is required for development of new drugs as an alternative treatment for gestational hypertension.

Herbal plants have been used for human remedy either their natural products or derivatives for thousand years ago in the world including Indonesia [13]. Indonesia has more than 30.000 plants and around 9.600 of them are known to have pharmacological properties [14]. Digitalis purpurea and Allium sativum are identified to have anti-hypertension properties [15] but these plants have not been developed yet as an anti-hypertension drug.

Virtual screening is one of the common method for drug development which is started by searching small molecules [10]. Molecular docking is a computational method that is widely used to describe interaction between molecules and target receptor [11]. This method can certainly be used to investigate phytochemicals which are able to interact with target receptors [12]. Thus, the aim of this study was to explore Indonesian phytochemicals which can act as calcium channel blocker for development of gestational hypertension drug.

2. Methods

Three dimensional (3D) structure of Cav1.2 calcium channel, nifedipine, and Indonesian phytochemicals were used in this bioinformatics study with a molecular docking approach. Sequence target of human Cav1.2 protein was downloaded from Uniprot with ID: Q13936. Nifedipine was a standard ligand which was obtained from ZINC database with the access code ZINC19594578. Phytochemicals derived from Indonesian herbal plants should be registered in HerbalDB database and have 3D structures which were obtained from PubChem NCBI. Selected phytochemicals had to meet the Lipinski’s rule of five. Because 3D structure of human (h) Cav1.2 protein has not been established yet, this protein structure was made by modeling with the rabbit (r) Cav1.1 channel (ID: 5GJW) using SWISS-MODEL (http://swissmodel.expasy.org/). PyRx 0.8 and AutoDock Tools 1.5.6 softwares were used to molecularly dock phytochemicals with the hCav1.2 model while Chimera 1.10 and PyMol 1.3 softwares were used to visualize phytochemical- Cav1.2 binding complexes.

To optimize interaction between hCav1.2 model and nifedipine as the standard ligand, water molecules were removed from the hCav1.2 model and it was then added with hydrogen molecules. Molecular docking was run in the grid box position at X center = ±148.378, Y center = ±184.750 and Z center = ±174.310 to restrict area interaction between hCav1.2 model and nifedipine. Nifedipine was molecularly docked with the hCav1.2 model at least three times to get the root mean square deviation (RMSD) <2 Å as a valid conformation. Nifedipine- hCav1.2 binding complexes were then visualized using Chimera 1.10 and PyMol 1.3 and their binding sites were compared with the existing binding sites of rCav1.1 (Gln^{949}, Phe^{1008}, Ser^{1011}, and Ile^{1052}). Phytochemicals were finally docked with the hCav1.2 model and considered as calcium channel blocker when the docking score was lower or equal
to the docking score of nifedipine and they similarly bound to Cav1.2 at four residues after visualization with Chimera and PyMol softwares [16].

3. Results and Discussion

3.1 Human Cav1.2 Model

In this study, we have firstly demonstrated 3D structure of hCav1.2 which was generated from rCav1.1 as calcium channel template. The superposition showed that the hCav1.2 model had 72.35% similarity with rCav1.1 (Figure 1).

![Figure 1. Superposition of 3D structure between rCav1.1 and h Cav1.2. Homology modeling was performed using SWISS-MODEL and superposition was visualized using Chimera 1.10 software. Blue ribbon indicated 3D rCav1.1 structure. Red ribbon was hCav1.2 model, yellow sticks were nifedipine structure and yellow circle was calcium channel binding site.](image)

Figure 2 indicated sequence alignment between hCav1.2 and rCav1.1 which were comparable from residue 74 to 2.221. The binding sites of hCav1.2 were as same as binding sites of rCav1.1 and located at residues Gln1060, Phe1129, Ser1132 and Ile1173. The hCav1.2 model was then analyzed using homology modeling curve [17]. Our hCav1.2 model contained 2.221 amino acids with 72.35% sequence identity so that the created hCav1.2 model was located in a safe and represented the 3D hCav1.2 (Figure 3).
Figure 2. Sequence alignment between hCav1.2 (Q13936) and rCav1.1 (5GJW) primary structures. The binding sites of hCav1.2 were localized at Gln\textsuperscript{1060}, Phe\textsuperscript{1129}, Ser\textsuperscript{1132}, and Ile\textsuperscript{1173} (black boxes) and red boxes designated binding sites of rCav1.1 at Gln\textsuperscript{939}, Phe\textsuperscript{1008}, Ser\textsuperscript{1011}, and Ile\textsuperscript{1052}. Residue deletion or insertion were indicated by dash. Vertical bar designated identical amino acids and colon was amino acid with similar property whilst dot was different amino acids.

![Sequence alignment between hCav1.2 and rCav1.1](image1)

Figure 3. The homology modeling threshold (curved line) of hCav1.2 model. The red-cross mark showed the plot of residue number of hCav1.2 model and its sequence identity.

3.2 Validation of Nifedipine-hCav1.2 Binding Complexes

After five times docking, nifedipine bound to hCav1.2 model with -2.1 kcal/mol binding energy and RMSD value = 0 (Figure 4). The localization of binding sites was the same as the binding sites in the sequence alignment. Hydrogen bond was observed at residue Gln\textsuperscript{1060} and Van der Waals bond was at Phe\textsuperscript{1129}, Ser\textsuperscript{1132}, and Ile\textsuperscript{1173}.

![Nifedipine-hCav1.2 Binding Complexes](image2)
Figure 4. a) Visualization of nifedipine-hCav1.2 binding complexes with Chimera 1.10. White boxes showed interaction between nifedipine (grey bar) and hCav1.2 model (blue ribbon) at Gln\(^{1060}\), Phe\(^{1129}\), Ser\(^{1132}\), and Ile\(^{1173}\) residues. Yellow lines were atom interaction between nifedipine and hCav1.2 model. b) Nifedipine bound to hCav1.2 model at Gln\(^{1060}\) which was visualized using PyMol 1.3. Green: carbon (C), red: oxygen (O), white: hydrogen, blue: nitrogen (N), yellow dashes: detailed interaction between nifedipine and hCav1.2 model at Gln\(^{1060}\) residue.

4. Docking of Indonesian Phytochemicals and hCav 1.2

A total of 6,776 phytochemicals registered in the HerbalDB database, 517 met the Lipinski’s rule of five and had their 3D structures. After molecularly docked with hCav1.2 model, it revealed 66 compounds with lower binding energy, compared to nifedipine. There were only 8 phytochemicals that bound to hCav1.2 at Gln\(^{1060}\), Phe\(^{1129}\), Ser\(^{1132}\), and Ile\(^{1173}\) residues (Table 1). Among these phytochemicals, morindone, obtusifolin 2-glucoside, actinodaphnine, oxonantenine, and gibberellin A44 had optimal lipophilicity. Obtusifolin 2-glucoside had five H-bond donors and ten H-bond acceptors. The conformation of obtusifolin 2-glucoside fitted with nifedipine (Figure 5a). This compound had Van der Waals bond at Gln\(^{1060}\), Phe\(^{1129}\), and Tyr\(^{1169}\) residues while the hydrogen bonds were at Gln\(^{1060}\), Met\(^{1126}\), Tyr\(^{1130}\), and Ser\(^{1132}\) (Figure 5b). Additional binding sites of obtusifolin 2-glucoside to hCav1.2 was observed at Met\(^{1126}\), Thr\(^{1130}\), and Tyr\(^{1169}\) residues but the specific function is unknown [18].

In contrast to obtusifolin 2-glucoside, actinodaphnine had two H-bond donors and five H-bond acceptors. It had Van der Waals bond at Gln\(^{1060}\), Phe\(^{1129}\), and Leu\(^{1128}\) residues and hydrogen bond at Ser\(^{1132}\) residue. Oxonantenine did not have H-bond donor but had six H-bond acceptors. This compound bound to Cav1.2 at Gln\(^{1060}\), Phe\(^{1129}\), and Ser\(^{1132}\) residues with hydrogen bond and at Phe\(^{1129}\) residue with Van der Waals bond. Morindone had three H-bond donor and five acceptors. It interacted with hCav1.2 at Phe\(^{1129}\) residue with Van der Waals bond and at Gln\(^{1060}\) and Ser\(^{1132}\) residues with hydrogen bond. However, their conformation did not fit with nifedipine.

Obtusifolin-2-glucoside which is a secondary metabolite belongs to glycoside group and is found in Tectona grandis (teak). Some studies has showed that this compound has antiviral effect against Chikungunya virus, antiplasmodial property, antibacterial effect, analgesics, and anti-inflammation [19-22].
Table 1. Binding energy, sites and Lipinski’s criteria of phytochemicals and hCav1.2 model

| No | Pubchem Code | Compound Name       | Average Docking Score | Binding site (with Pymol) | Bond Type                  |
|----|--------------|---------------------|-----------------------|---------------------------|----------------------------|
| 1  | 4485         | Nifedipine          | -2.1 \(\pm\) 0.000   | Gln-1060, Phe-1129, Ser-1132, Ile-1173 | Hydrogen Van der Waals     |
| 2  | 442756       | Morindone           | -2.2 \(\pm\) 0.000   | Gln-1060, Ser-1132 Met-1125, Phe-1129 | Hydrogen Van der Waals     |
| 3  | 442761       | Obtusifolin 2-glucoside | -2.2 \(\pm\) 0.000  | Gln-1060, Met-1126, Phe-1129, Tyr-1130, Ser-1132, Tyr-1169 | Hydrogen Van der Waals     |
| 4  | 72276        | (-)-Epicatechin     | -2.2 \(\pm\) 0.173   | Gln-1060, Phe-1129, Ser-1132 | Hydrogen Van der Waals     |
| 5  | 13964005     | BR-xanthone A       | -2.3 \(\pm\) 0.000   | Gln-1060, Leu-1128, Ser-1132 Met-1125, Phe-1129 | Hydrogen Van der Waals     |
| 6  | 160502       | Actinodaphnine      | -2.3 \(\pm\) 0.000   | Ser-1132 Gln-1060, Phe-1129 | Hydrogen Van der Waals     |
| 7  | 3084224      | Oxonantenine        | -2.3 \(\pm\) 0.000   | Gln-1060, Ser-1132 Phe-1129 | Hydrogen Van der Waals     |
| 8  | 392169       | Thwaitesixanthone   | -2.3 \(\pm\) 0.000   | Gln-1060, Ala-1124, Met-1126, Leu-1128 | Hydrogen Van der Waals     |
| 9  | 46173798     | Gibberellin A44     | -2.3 \(\pm\) 0.000   | Gln-1060, Ser-1132 Phe-1129 | Hydrogen Van der Waals     |

| No | Pubchem Code | Compound Name      | Molecule Formula   | Molecular weight (<500g/mol) | H-Bond Donor (<5) | H-Bond Acceptor (<10) | Lipinski’s Rule of Five |
|----|--------------|-------------------|--------------------|------------------------------|-------------------|------------------------|------------------------|
| 1  | 4485         | Nifedipine        | C17H18N2O6         | 346.339                      | 1                 | 7                      | 2.2                    |
| 2  | 442756       | Morindone         | C15H10O5           | 270.24                       | 3                 | 5                      | 3.3                    |
| 3  | 442761       | Obtusifolin 2-     | C22H22O10          | 446.408                      | 5                 | 10                     | 1.2                    |
| 4  | 72276        | (-)-Epicatechin   | C15H14O6           | 290.271                      | 5                 | 6                      | 0.4                    |
| 5  | 13964005     | BR-xanthone A     | C23H24O6           | 396.439                      | 2                 | 6                      | 4.8                    |
| 6  | 160502       | Actinodaphnine    | C18H17NO4          | 311.337                      | 2                 | 5                      | 2.4                    |
| 7  | 3084224      | Oxonantenine      | C19H13NO5          | 335.315                      | 0                 | 6                      | 3.3                    |
| 8  | 392169       | Thwaitesixanthone | C23H20O5           | 376.408                      | 1                 | 5                      | 5                      |
| 9  | 46173798     | Gibberellin A44   | C20H26O5           | 346.423                      | 2                 | 5                      | 1.6                    |
Figure 5. Visualisation of obtusifolin 2-glucoside- hCav1.2 binding complexes. (a) Superposition of obtusifolin 2-glucoside (cream-coloured) and nifedipine (purple) in hCav1.2 model with Chimera 1.10; (b) Binding sites at Gln^{1060}, Met^{1126}, Phe^{1129}, Thr^{1130}, Ser^{1132}, Tyr^{1169} residues (yellow dashes). Green: carbon (C), red: Oxygen (O), white: Hydrogen (H), blue: Nitrogen (N), yellow dashes: interaction between atom

5. Limitation of The Study
Because biochemical properties of human Cav1.2 channel are limited, it is likely that the endogenous structure and function of calcium channels in both species differ. Secondly, AutoDockVina software is a rigid receptor-flexible ligand program which is unable to describe receptor-macromolecule interaction inside the human body since endogenous receptors have flexibility to interact with macromolecules.
6. Conclusion
Obtusifolin 2-glucoside becomes a potential candidate of calcium channel blocker in silico. In vitro assays should be performed to evaluate the antagonist effect of obtusifolin 2-glucoside on hCav1.2 channel.

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