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IN SILICO SCREENING OF PHYTOCHEMICAL COMPOUND AS NOVEL NOX1 INHIBITOR VIA INHIBITING P47PHOX-P22PHOX COMPLEX

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ABSTRACT

The world's elderly population is increasing. Thus, in the future, degenerative diseases incidence will increase. Therefore, the development of anti-aging agent is required. NADPH oxidase (NOX), especially NOX1, has important roles in aging and degenerative diseases. NOX1 activation required interaction between p47phox and p22phox subunit. Based on that mechanism, compound that has ability to inhibit p47phox-p22phox complex development could become an anti-aging agent. The aim of this study is to find novel NOX1 inhibitors candidate from phytochemical database that work via inhibiting p47phox-p22phox complex development. Machine learning-based screening and molecular docking were used in this study. We screened phytochemicals from the PhytoHub database using machine-learning model with random forest method. The active compounds based on machine-learning-based screening were chosen as ligands for molecular docking.We found 7 compounds that could be NOX 1 inhibitor candidates from the machine-learning-based screening. Through blind molecular docking, we found that 6 of the 7 compounds were able to bind with the SH3 domain of p47phox. Those 6 compounds are 8,5'-Diferulic acid, Jaceosidin, Malvidin, Peonidin, Petunidin, and Tryptamine. Jaceosidin and petunidin are able to bind with both SH3B domain and polybasic region of p47phox. The conformation changes of both domains are required for p47phox activation. Inhibited p47phox could not bind p22phox. Thus, jaceosidin and petunidin might work as NOX1 inhibitor via inhibiting p47phox-p22phox complex development.

Keywords: Anti-aging., In-Silico., NADPH Oxidase 1.

INTRODUCTION

The world is facing increased of aging population. Elderly population is predicted to constitute 22% of total population in around 40 years. This amount is increased from 0.8 billion into 2 billion [1]. Therefore, the world will facing increased degenerative disease incidence. Based on this condition, development of anti-aging agent will be required.NADPH oxidase (NOX) is a membrane class enzyme that have role in converting NADPH oxidase into $NADP^+$ and superoxide anion. NOX has seven types including NOX 1-5 and DUOX 1&2 anion [2]. Several studies have also found that NOX, especially NOX1, plays role in several degenerative diseases progression including atherosclerosis, osteoarthritis, Alzheimer's disease, and osteoporosis [3] [4] [5] [6]. Research also found that mice with knockout NOX 1 gene have longer life-span [7].

NOX 1 activation requires translocation of cytosolic subunit into membrane and bind with membrane subunit. One of important cytosolic subunit is p47phox. During NOX1 activation, p47phox will bind with p22phox, one of the membrane subunits of NOX1. Inhibiting interaction between those subunits are able to prevent NOX 1 activity [8]. Research also shown that p22phox NOX polymorphism is correlated with speed of aging and risk of degenerative disease [9]. Based on those studies, development of NOX1 inhibitor has promising prospect in anti-aging field.

The main concern of drug development is the cost. Computational aided drug discovery can aid the process and cut the cost [10]. The Artificial intelligence (AI) technology usage can accelerate the process of finding new compounds that could be NOX1 inhibitor. One of AI technology that have promising role in drug development is machine-learning. Machine-learning-based screening could be used in detecting compound activity toward certain enzyme [10].

Phytochemical or plant-derived molecule could become base of development novel drug candidate. Therefore, this study aims to find novel NOX inhibitor candidate that work via inhibiting p47phox-p22phox

complex development. In this study, PhytoHub database (https://phytohub.eu/) was used for screening of novel NOX1 inhibitor candidate [11]. The screening was assisted by random forest machine-learning then the screening results were confirmed by molecular docking.

MATERIALS AND METHODS

Machine-learning-Based Screening

A machine-learning model was made using the python programming language. The SMILES structure of phytochemical compounds from the PhytoHub database were converted into morgan fingerprint and RDKIT molecular descriptors [12] [13]. The following molecular descriptors were used: molecular weight, number of hydrogen acceptors, number of hydrogen donors, total polar surface area, and partition coefficient. The random forest method was used as machine-learning method. The database of NOX1 inhibitors was acquired from CheMBL website (https://www.ebi.ac.uk/ chembl/). The CheMBL database contains 250 compounds that have been already tested on NOX1. That database consists of 89 active compounds, 125 non-active compounds, 2 compounds with non-determined activity, and 34 compounds with no information. The database was split into an 80% training set and a 20% test set. We trained the machine-learning model using the scikit-learn package [14]. Ten-fold cross-validation was performed to evaluate model quality. The active compounds based on machine-learning screening were chosen as ligands for molecular docking.

Ligand Preparation

Ligands structure were downloaded from the PubChem website (National Library of Medicine, Bethesda, Maryland, USA) in SDF format. Ligand energy was minimized with PyRx 0.8 software with a 200-step mff94 force field and steepest descent algorithm [15].

Protein Preparation

Auto-inhibited p47-phox structure (PDB id: 1NG2) was downloaded from the Protein Data Bank website (RCSB, San Diego, California, USA) [16] [17]. Protein was cleaned with Biovia Discovery Studio software (Dassault Systèmes, Paris, France). The polar hydrogen and Kolmann charge were added to the protein with Autodock Tools. Protein energy minimization was performed using the Swiss PDB Viewer [18].

Docking Procedure

Vina Wizard program on PyRx 0.8 was used in the docking process [19] [20]. The docking grid box is made to cover the whole protein's structure. Docking results were downloaded in .pdb and visualized using Biovia Discovery Studio Visualizer. Inhibitor candidates were assessed based on docking profiles and binding affinity. We also docked a known ligand (diapocynin) and five non-active compounds from the ChEMBL database [21].

Pharmacokinetic Profile Screening

The pharmacokinetic profile of the compounds were screened using the Swiss Admet website (http://www.swissadme.ch/) [22]. The compounds were considered good oral drug candidates if they fulfilled Lipinski rules of five [23].

RESULTS

Machine-learning-based Screening

Machine-learning-based screening has 80% total accuracy. Active class has 82% accuracy, and non-active class has 72% accuracy. The ROC curve shows that active and non-active class have 0.92 and 0.91 of area under the curve, respectively (Figure 1). After performing 10-fold cross-validation, we found no significant score difference between all 10 tests (Table 1). So, the model is not significantly affected by overfitting. From a total of 2727 compounds, machine-learning-based screening found 7 compounds that were detected as active NOX1 inhibitor candidates. Those compounds are 8,5'-Diferulic acid, Jaceosidin, Malvidin, Peonidin, Petunidin, Sinapic acid-O-sulfate, and Tryptamine (Figure 2).

Molecular Docking and Pharmacokinetic Prediction Results

Through blind docking, we found six of seven compounds are able to bind with the SH3 domains of p47phox (Figures 3 and 4). These compounds are: 8,5'-Diferulic acid, Jaceosidin, Malvidin, Peonidin, Petunidin, Sinapic, and Tryptamine. SH3 consists of SH3A and SH3B. Only Jaceosidin and Petunidin are able to bind with SH3B and the polybasic region (PBR). The change in PBR and SH3B domain conformation is required for p47phox-p22phox complex development [17]. Since jaceosidin and petunidin interact with both domains, they could inhibit the conformational change process of p47phox. Petunidin has the lowest binding affinity, while trypatamine has the highest binding affinity among all results (Table 2). The docking result of control is presented in Figure 3 and Table 2. All compounds fulfill the Lipinski rule of five, which is indicates that they could become oral drug candidates.

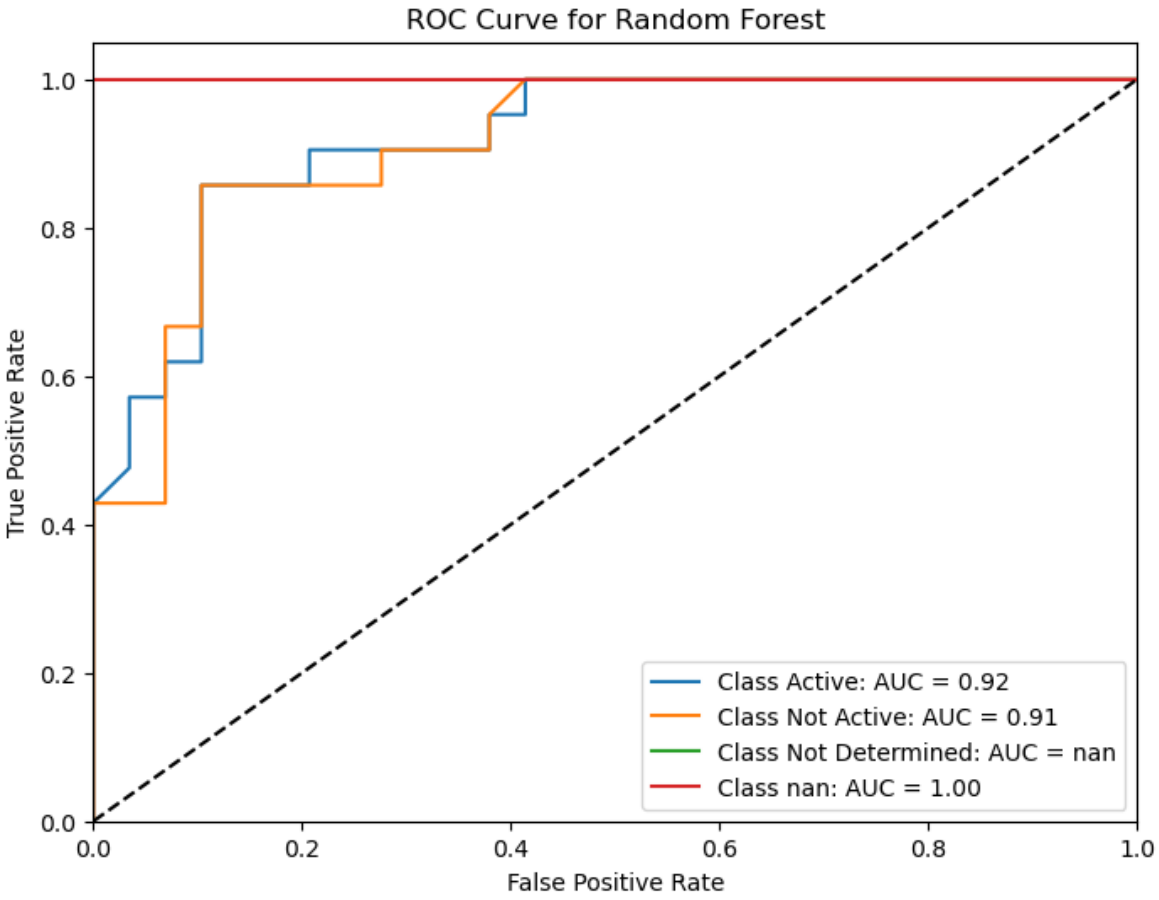

Figure 1. ROC curve of machine learning model.

Table 1. Ten-fold cross-validation results

Run	Score
1	0.76
2	0.88
3	0.92
4	0.88
5	0.96
6	0.92
7	0.80
8	0.84
7	0.80
10	0.80

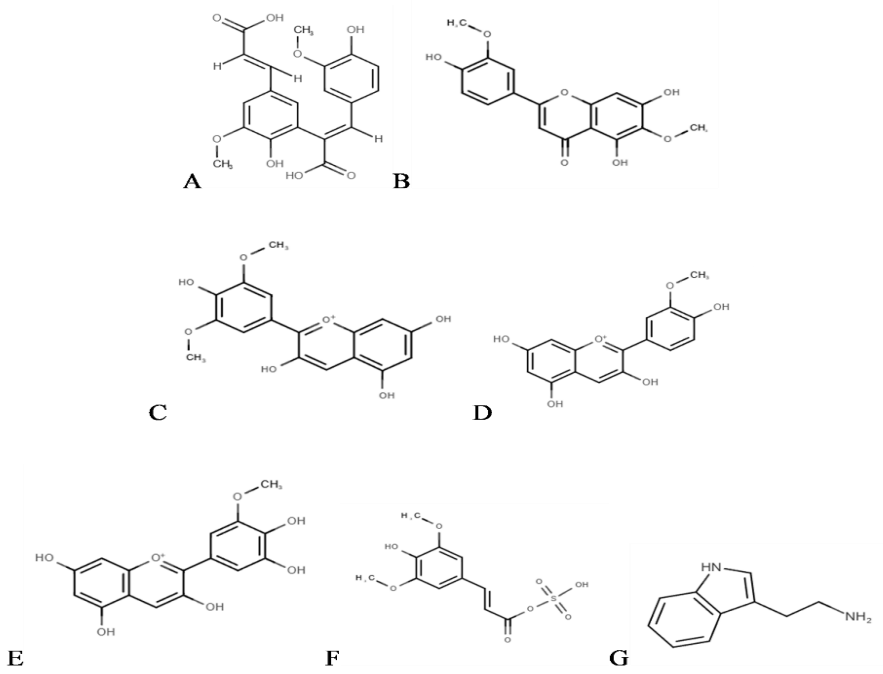

Figure 2. Seven NOX1 inhibitor candidates that predicted with machine-learning-based screening. (A) 8,5'-Diferulic acid, (B) Jaceosidin, (C) Malvidin, (D) Peonidin, (E) Petunidin, (F) Sinapic acid-O-sulfate, (G) Tryptamine.

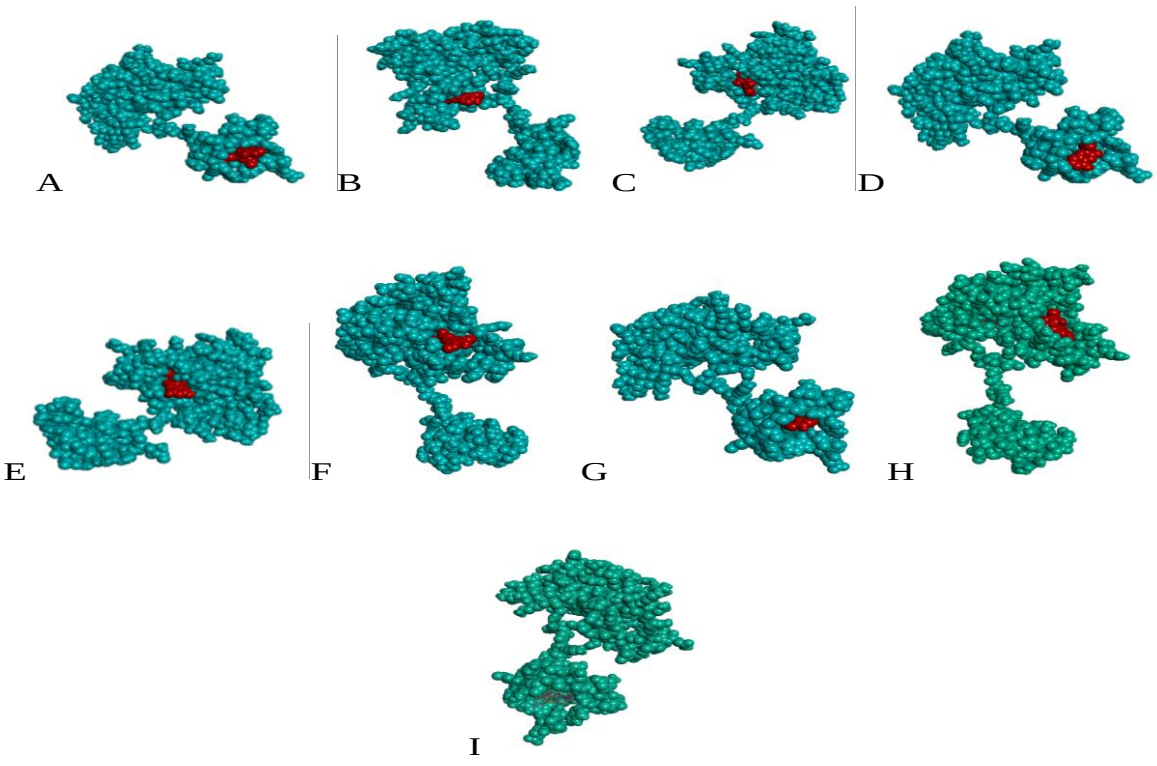

Figure 3. Molecular docking result. (A) 8,5'-Diferulic acid, (B) Jaceosidin, (C) Malvidin, (D) Peonidin, (E) Petunidin, (F) Sinapic acid-O-sulfate, (G) Tryptamine, (H) Positive control (Diapocynin), (I) Negative Control. Green: p47phox (PDB id: 1NG2), Red: ligand (selected compounds and positive control), Grey: negative control.

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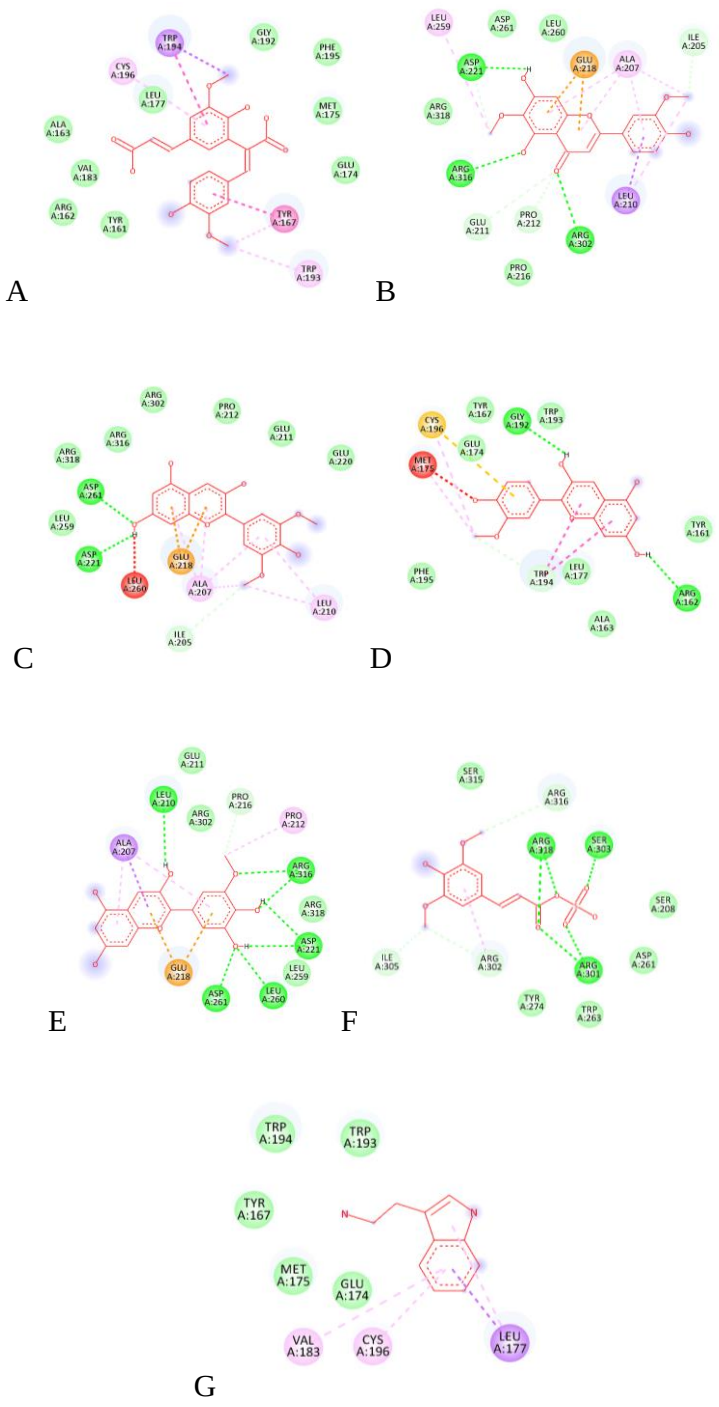

Figure 4. Interaction profile of blind docking result. (A) *8,5'-Diferulic acid*, (B) *Jaceosidin*, (C) *Malvidin*, (D) *Peonidin*, (E) *Petunidin*, (F) *Sinapic acid-O-sulfate*, (G) *Tryptamine*.

Table 2. Docking Results. Blue: SH3B, Red: PBR.

Compounds	*Binding Affinity*	Amino Acid Residue
8,5'-Diferulic acid	-7.6	Tyr167, Trp194, Cys196, Trp193
Jaceosidin	-7.5	**Leu259**, Asp221, Glu218, Ala207, Leu 210, **Arg302**, **Arg316**
Malvidin	-6.8	**Asp261**, Asp221, **Leu260**, Glu218, Ala207, Leu210
Peonidin	-7.5	Met175, Cys196, Gly192, Arg162, Trp194
Petunidin	-8.0	Ala207, Leu210, Pro212, **Arg316**, Asp221, **Leu260**, **Asp261**, Glu218
Sinapic acid-O-sulfate	-6,3	**Arg318**, **Ser303**, **Arg301**
Tryptamine	-5.2	Val183, Cys196, Leu177
Diapocynin	-6.9	**Ser303**, **Arg302**, **Arg316**, **Arg318**, **Tyr274**, **Asp243**

DISCUSSION

This study's weakness is the lack of a proper docking validation process because there is no ligand-p47phox crystallography complex. The docking validation process requires the re-docking of known ligand and protein from ligand and protein crystallography complex to assess the value of the root mean square difference (RMSD) [24]. To overcome that weakness, we have performed molecular docking on a known active inhibitor (diapocynin) and five non-active compounds. The result shows that all non-active compounds have very different configurations or binding positions compared to diapocynin (Table 2, Figures 3, and Figure 4).

If the docking result is compared with the positive control, the docking results of jaceosidin and petunidin are similar to the docking results of diapocynin, which is able to bind with both the SH3B domain and PBR. Diapocynin is known as a NADPH oxidase inhibitor that inhibits the interaction between p47phox and p22phox Another in silico study by Perez *et al.* also showed that diapocynin interacts with the SH3B domain and PBR despite using the difference docking method [21]. Diapocynin is also known for its anti-aging ability [25]. Before entering the body, diapocynin can be found as apocynin. Myeloperoxidase turn apocynin into diapocynin [26]. The study by Sun *et al.* reported that apocynin prevents and reverses mesenchymal stem cell aging [25]. Another study found that apocynin treatment was able to prevent mitochondrial dysfunction in aging mice [27]. Since jaceosidin and petunidin interactions with p47phox have similar interactions with diapocynin, they could be anti-aging agent candidates that work via NOX1 inhibition.

CONCLUSION

From the 2727 compounds in the Phytohub database, we found two compounds that could be anti-aging candidates by inhibiting NOX1 activity. Those two compounds are jaceosidin and petunidin. Further experimental research is required to clarify these findings.

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