Utilization of Nypa fruit in Alzheimer’s Disease: An In Silico Approach

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Abstract. This research aims to analyze the phytochemical potential of Nypa fruticans fruit for Alzheimer’s disease through molecular docking method. In 2007, it is estimated that 1 of 85 world population suffer from alzheimer and the number will be doubled in 20 years. The treatment of Alzheimer’s disease still relies on synthetic medicine. Indonesia as a mega-biodiversity country need to explore the natural resources for medicine, especially for Alzheimer’s disease treatment. Nypa fruticans fruit has several compounds like Kaempferol, Rutin, Quercetin, Chlorogenic Acid, Cinnamic Acid, Protocatechuic Acid, and Gallic Acid. These compounds could inhibit Acetylcholinesterase (AchE) enzyme activity. AchE is an enzyme that hydrolyzes acetylcholine to reduce the number of neurotransmitters. Biological activity of Nypa fruticans compound could be predicted with molecular docking and use score of binding affinity as a parameter for the ability on Acetylcholinesterase enzyme inhibition. The step consist of preparation of ligand and target protein, molecular docking, and drug-likeness test. The result shows that the compound of Nypa fruticans fruit potential for Alzheimer’s disease treatment, the compound who has the highest binding affinity is kaemferol of -9.6.

1. Introduction

1 of 85 world population in 2007 suffer from Alzheimer’s disease. It is estimated that the number will be doubled every 20 years, become 65.7 million in 2013 and 115.4 million people in 2050. In 2010, 57.7% of people with Alzheimer’s disease live in Indonesia and the percentage expected to increase to 63.4% in 2030 and 70.5% in 2050 [1]. Increasing of Alzheimer’s Disease (AD) patient not only effect on financial but also physiological and emotional burden from patient and caregiver [2].

Alzheimer’s disease drug development is performed by molecular therapy through Cholinesterase (Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE), Amyloidogenic secretase (β or γ-secretase), Aβ aggregation, phosphorylation and fibrillation of protein τ, and metal ion redox/reactive oxygen species (ROS). Inhibition of AchE being a key role to increase cholinergic transmission, reduce Aβ aggregation and formation of neurotoxic fibril on AD patients [3]. Inhibition of Acetylcholinesterase increases cholinergic synapse transmission by inhibiting acetylcholinesterase activity on the synapse gap lead to reduction of acetylcholine hydrolysis released by presynapse neuron [4]. AD synthetic drugs like donepezil, rivastigmine, or memantine has dangerous side effects include hepatotoxicity, low bioavailability, and harmful cholinergic side effect on peripheral nerve. Thus, the drug is dangerous for long-term treatment [5].

Indonesia as one of mega-biodiversity country has high plant abundance which can be utilized as a source of medicine. Mangrove becomes one of Indonesia forest ecosystems with high plant diversity. One of them is Nypa fruticans. Nypa fruticans is a mangrove plant with special adaptation to living in a near-deserted area such as river banks. Nypa fruticans classified in family Araceae and live in India, Malaysia, Indonesia, Philippines, and some region of Queensland, Australia. Nypa forest area in Indonesia reaches about 4.237.000 hectare, spread in Sumatera, Kalimantan, Sulawesi, Maluku, and Papua coastal. The sap is used as a beverage and the young fruit is eaten [6]. The usage of Nypa...
fruticans mostly as a beverage ingredient, even though the fruit has a great potential for Alzheimer’s therapy because of the compound in it. Besides, Nypa fruit doesn’t have any side effect on human health.

The phenolic compound in Nypa fruticans fruit is chlorogenic acid, protocatechuic acid, kaempferol, rutin, quercetin, hydroxybenzoic acid, cinnamic acid, and gallic acid. Phenol compound on unripe fruit is higher than ripe fruit [6].

Several animal experiments shows a protective effect from chlorogenic acid against oxidation on brain tissue. Chlorogenic acid can reduce oxidative stress induce by Fe^{2+} and Sodium Nitriprusside. Chlorogenic acid trigger neuron protection in mice brain by reduce acetylcholinesterase and butyrylcholinesterase enzyme activity. Inhibition of this enzyme leads to improve acetylcholine level in synapse, thus the interaction between neuron increased [7]. Moreover, kaempferol administration on rat given STZ (Streptozotocin) increase memory ability, slowly repair hippocampus CA1 cell and reduce cell death rate [8].

Meanwhile, Protocatechuic acid can reduce oxidative stress in mouse brain cells treatment. Protocatechuic acid can protect the brain from oxidative stress and apoptosis due to H_{2}O_{2}. Therefore, Protocatechuic acid can be used in the treatment of brain disorders. Giving Kaemferol to STZ-given mice (Streptozotocin) can improve memory storage, gradually repair cells in the hippocampus and reduce the number of cell deaths [8,9]. In addition, kaempferol is proven to repair memory damage due to β-Amyloid induction, also protects neuron cell damage due to oxidative stress through the mechanism of repairing neuron and mitochondrial cell membranes [10].

Quercetin compounds work by reducing extracellular β-amyloidosis, tauopathy, astrogliosis, microgliosis in the hippocampus and amygdala. By testing using a labyrinth test, it was found that giving quercetin contributed to improving cognitive performance, memory, and behavior in AD patients. The loss of AD histological signs is known as an effect of quercetin activity [11]. Another compound is Cinnamic acid, Cinnamic acid in diabetic rats can improve memory ability by giving a certain dose. The test results of brain homogenates of diabetic mice due to STZ showed cholinergic dysfunction, increased levels of lipid peroxidation and reactive oxygen species (ROS), decreased glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). Giving Cinnamic acid repairs the index in diabetic rats [12].

Rutin significantly increases GSH (Gluthathione) levels, GSH is an extracellular and intracellular antioxidant that is vital for oxidative stress. In addition, rutin additions significantly increased the activity of SOD (superoxide dismutase) in the brain of AD rats. SOD is a radical cleaning enzyme that converts superoxide to hydrogen peroxide [13]. The last compound is Gallic acid. Aβ (Beta Amyloid) that interferes with hippocampal synaptic plasticity due to the formation of senile plaques and neuronal apoptosis, through the addition of gallic acid in the area of plaque, causing AD (Alzheimer's disease) plaque loss. AD treatment in mice using Gallic acid is known to improve brain histology and electrophysiological damage depending on the dose given [14]. With the variety of potential compounds contained in the Nipah fruit, thus this study was conducted to prove this potential, so Nipah can be used as an alternative in the treatment of Alzheimer's disease.

With the variety of potential compounds contained in the Nipah fruit, thus this study was conducted to prove the phytochemical compound potential from Nipah fruit for Alzheimer’s disease through molecular docking method, so Nipah can be used as an alternative in the treatment of Alzheimer's disease.

2. Materials and Method

2.1. Ligand Preparation

Chemical structure of Nypa fruticans fruit collected from literature study, 3D chemical structure, ligand SMILES and ID number is taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Table 1). The ligand then processed with Avogadro and saved with PDB format.
Table 1. ID Number and Canonical Smiles of *Nypa fruticans* fruit compound

| Compound (control) | ID Number | Canonical SMILES |
|--------------------|-----------|------------------|
| Donepezil          | 3152      | COC1=C(C=C2(C=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC |
| Kaempherol         | 5280863   | C1=CC(CC=CC1C2=C(C=O)C3=C(C=C(C=C3O2)O)O)O |
| Rutin              | 5280805   | CC1C(C(C(C1)O)OC2C(C=C2)OC3=C(C=O)C=C(C=C3)O)O |
| Quercetin          | 5280343   | C1=CC(CC=CC=C1C2=C(C=O)C3=C(C=C(C=C3O2)O)O)O |
| Chrologenic acid   | 1794427   | C1(C(C(CC1(C=O)O)O)OC=CC2=CC=CC=CC2(C=C2)C(O)O)O |
| Cinnamic acid      | 444539    | C1=CC=C(C=C1)C=CC(C=O)O |
| Protocatechuic acid| 72        | C1=CC=C(C=C1C=O)O |
| Gallic acid        | 370       | C1=C(C=C(C=C1)C(O)O)C=O |

2.2. **Target Selection**
Prediction of protein target performed with Pharmmapper (http://lilab.ecust.edu.cn), SuperPred (http://prediction.charite.de), and Swiss target prediction (www.swistargetprediction.ch). The protein prediction then validated with UniProt (https://www.uniprot.org). The protein structure collected in Protein Data Bank (https://www.rcsb.org/) with PDB code 5HF5. The protein structure processed using PyMOL v1.7.4.5 to remove non-protein molecule. The target protein for this research is the Acetylcholinesterase enzyme.

2.3. **Molecular Docking**
Molecular docking performed with Vina Wizard feature integrated in PyRx 0.8. The ligand is Kaempferol, Rutin, Quercetin, Chlorogenic acid, Cinnamic acid, Protocatechuic acid, and Gallic acid. The protein target is the Acetylcholinesterase. Donepezil is used as ligand control for the docking process.

2.4. **Molecular Visualization and Small Molecule Interaction**
Interaction between ligand, protein target, and control are visualized and analyzed with PyMOL v1.7.4.5.

2.5. **Drug-Likeness Test**
Drug-likeness test using physiochemical properties of ligand and matched with physiochemical of registered drugs. Drug likeness test using Lipinski rule.

3. **Results and Discussion**

3.1. **Ligand Preparation**
The ligand is a active compound for being tested in target. Based on literature review, there are seven ligand to use in this research, that is Kaempferol, Rutin, Quercetin, Chlorogenic acid, Cinnamic acid, Protocatechuic acid, and Gallic acid. Donepezil is used a a control for the research. After being download from PubChem website, the ligand format should be converted from SDF to PDB format to make the molecular docking process easier. The 3d structure of each ligand are shown in figure 1.
Figure 1. Phytochemical compound of *Nypa fruticans* fruit. A. Kaempferol, B. Rutin, C. Quercetin, D. Chlorogenic Acid, E. Cinnamic Acid, F. Protocatechuic Acid, G. Gallic Acid, H. Donepezil (Control)

3.2. Target selection

The target used in this research is Acetylcholinesterase enzyme with PDB code 5HF5. Acetylcholinesterase enzyme inhibition has proven to be achievable as therapeutic target because cholinergic deficit is a consistent and early finding in Alzheimer’s Disease. There are two types of cholinesterase, that is acetylcholinesterase and butyrylcholinesterase. Acetylcholinesterase can hydrolyzes acetylcholine more quickly than butyrylcholinesterase [15]. After taken the target from Protein Data Bank, the molecule being clean up from water molecule and other residues by using PyMol v1.7.4.5. The result is shown in Figure 2.

Figure 2. Acetylcholinesterase enzyme after being processed with PyMol v1.7.4.5
3.3. Molecular Docking Result

In silico is a method which uses database and software to do research. One of in silico techniques is molecular docking. Molecular docking using computation method to predict potential activity from a compound before it is being tested. The advantage of this method is to anticipate the failure of in vivo results by predict compound potential activity.

Binding affinity result from molecular docking of *Nypa fruticans* fruit compound are shown in Table 2. Binding affinity is a score to measure the compound ability to bind with the receptor. If the value is lower, then the affinity between receptor and ligand is higher, and vice versa [16], from all of the compound, Kaempherol has the lowest binding affinity value and also the number is closest to Donepezil as control. It means that Kaempherol in *Nypa fruticans* fruit has the highest potency to become drug compound for Alzheimer’s disease treatment. The interaction between Kaempherol and Acetylcholinesterase enzyme are shown in Figure 1. From the picture, we could see that Kaempherol and Donepezil has similar binding site location.

| No | Compound            | Binding Affinity |
|----|---------------------|------------------|
| 1  | Donepezil (control) | -10.0            |
| 2  | Kaempherol          | -9.6             |
| 3  | Rutin               | -8.9             |
| 4  | Quercetin           | -8.6             |
| 5  | Chlorogenic acid    | -7.7             |
| 6  | Cinnamic acid       | -6.8             |
| 7  | Protocatechuic acid | -6.6             |
| 8  | Gallic acid         | -6.2             |

3.4. Ligand-Macromolecule Interaction Visualization

Ligand-macromolecule interaction visualization result is shown by Figure 3. Based on binding affinity value, kaempherol has the lowest binding affinity value compare to the other compound of *Nypa fruticans* fruit. From the visualization, the binding site of kaempherol and donepezil is similar to each other.

![Figure 3. The Binding site of Acetylcholinesterase (blue), kaempherol (yellow), and donepezil (pink) visualized with PyMol v1.7.4.5.](image-url)
3.5. Drug-likeness Test

After the molecular docking process, the next step is the drug-likeness test. Drug-likeness is a term to explain how physiochemical properties of a compound affect molecular properties in vivo. The majority rule for drug-likeness test using physiochemical properties of molecular structure and match with the registered drug. On of those rule is Lipinski rule, which is the molecular weight is \( \leq 500 \text{ kDa} \), LogP is \( \leq 5 \), Hydrogen bond donor is \( \leq 5 \) and Hydrogen bond acceptor is \( \leq 10 \). These criteria are similar with good drug oral bioavailability. The log value P states the solubility coefficient in fat/water which has a range of -0.4 - 5. The molecular weight of more than 500 Da cannot diffuse through the cell membrane. The high log P value indicates the more hydrophobic of the molecule. Molecules that are too hydrophobic tend to have high levels of toxicity because they will be retained longer in lipid bilayers and are more widely distributed in the body so that the selectivity of bonds to the target enzyme is reduced. The negative log P value is also not good because the molecule cannot pass through the lipid bilayer membrane. The number of donors and hydrogen bond acceptors describes the higher the hydrogen bond capacity, the higher the energy needed for the absorption process to occur. In general Lipinski's rules describe the solubility of certain compounds to penetrate cell membranes by passive diffusion [17].

The result of drug-likeness test for each compound are shown in Table 3. Based on the Lipinski rule, all of the compound qualify criteria, except Rutin, with molecular weight is more than 500 kDa so it is difficult to absorbed by the human body. Furthermore, hydrogen bond donor and acceptor criteria is not eligible. Commonly, Lipinski rule describes a solubility of a compound to absorbed by cell membrane through passive diffuse. Thus, it is suspected that Rutin could be absorbed by human body but not through passive diffuse.

| Compound               | Molecular weight | Hydrogen bond donor | Hydrogen bond acceptor | (LogP) | Information |
|------------------------|------------------|--------------------|------------------------|--------|-------------|
| Donepenzil (control)   | 415.95 g/mol     | 0                  | 4                      | 3.37   | ✓           |
| Kaempherol             | 286.24 g/mol     | 4                  | 6                      | -0.03  | ✓           |
| Rutin                  | 610.52 g/mol     | 10                 | 16                     | -3.89  | X           |
| Quercetin              | 302.24 g/mol     | 5                  | 7                      | -0.56  | ✓           |
| Chlorogenic acid       | 354.31 g/mol     | 6                  | 9                      | -1.05  | ✓           |
| Cinnamic acid          | 148.16 g/mol     | 1                  | 2                      | 1.90   | ✓           |
| Protocathecuic acid    | 154.12 g/mol     | 4                  | 3                      | 0.40   | ✓           |
| Gallic acid            | 170.12 g/mol     | 4                  | 5                      | -0.16  | ✓           |
4. Conclusion

Based on molecular docking result, Kaempherol on Nypa fruticans fruit has the lowest binding affinity value compare to the other molecule, thus it has the highest potential to use for Alzheimer’s disease. From drug-likeness test result, all of the compound qualify the criteria except Rutin. It is suspected that Rutin could be absorbed by human body but not through passive diffusion.

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