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Potential Asiatic Acid in *Centella asiatica* (L.) Urban Towards NMDA Receptors of Alzheimer Dementia Patients In Silico

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ABSTRACT

Alzheimer's dementia can occur due to an increase in the neurotransmitter glutamate which can affect synaptic plasticity. Centella asiatica (L.) Urban is considered as one of the herbal plants used in the treatment of various diseases, such as medicine for leprosy, thrush, fever, hypertension, diabetes, and anemia and has a high antioxidant effect, one of the active ingredients is asiatic acid. This research to determine asiatic acid potential for NMDA receptors in Alzheimer's dementia. This research is an experimental research using a computer. The applications used were PyMOL for ligand preparation, PyRx for docking, and LigPlot for amino acid residue analysis. The interaction of receptor ligand binding is seen from the binding affinity score, the smaller the binding affinity score, the greater the potential between the ligand-receptors to interact. The pharmacokinetics of a compound are based on Lipinski's rule. In this study, the binding affinity score of - 8.3 was obtained and the Lipinski test showed the result of 'yes'.

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disease, characterized by a progressive decline in cognitive function with a decrease in activities of daily living and behavioral changes[1]. The earliest symptom of Alzheimer's disease is dementia, which has a prevalence of 5% in people aged 65 years and over and increases to 30% in people aged 85 years and over. Alzheimer's disease is expected to double between 2008 and 2051 and become a major problem for the elderly (>65 years old)[2].

In Indonesia, there is an increase of life expectancy which is directly proportional to the

number of elderly people in Indonesia[3,4]. As a result, higher cases of dementia will be found due to the increase in the elderly population in Indonesia[8]. The estimated number of people with Alzheimer's dementia in Indonesia in 2013 reached one million people. The trend of people with Alzheimer's dementia in Indonesia is increasing every year[4].

Alzheimer's disease is a multifactorial disease, with no known single cause, and multiple risk factors are associated with disease progression[1]. Aging is the biggest risk factor for Alzheimer's disease. Most people over the age of 65 have a higher risk of Alzheimer's disease[5].

Aging can be defined as a progressive deterioration in biochemical and physiological efficiency. Aging is also a multifactorial process, which is based on mechanisms of altered metabolic homeostasis, inflammation, and/or redox processes in cells and tissues[6]. At present, the pathology of Alzheimer's disease is believed to be due to the accumulation of beta-amyloid peptide (Aβ*)* that causes plaques at synapses in the brain. The buildup of beta-amyloid plaques allows the availability of more glutamate so that it affects the decrease in the affinity of the NMDA (N-methyl-d-aspartate) receptors with Mg^{2+} which can cause the entry of Ca^{2+} into the postsynaptic, then affect synaptic plasticity and also survival neurons[7].

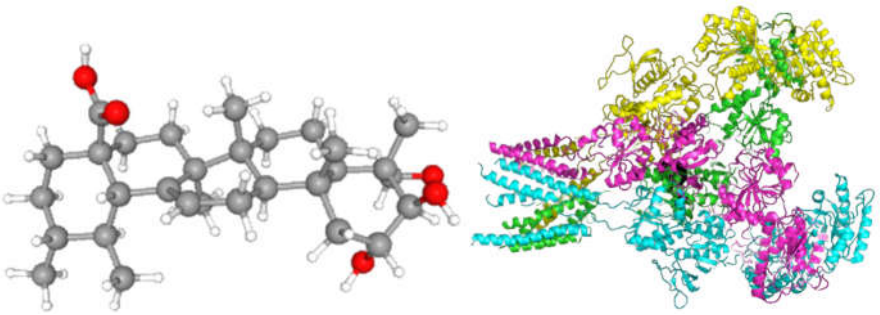
In Alzheimer's disease, the presence of *beta*-amyloid plaques in the synaptic can cause death after several years due to impaired motoric skills. The pathological process of Alzheimer's dementia occurs several years before the onset of a clear clinical picture. This causes people with Alzheimer's dementia to require comprehensive care and treatment, which leads to high treatment costs. In addition, the treatment of Alzheimer's dementia is only symptomatic and cannot be cured[8].

Alzheimer's disease based on its pathophysiology is an oxidative stress disorder, which occurs when the production of ROS (reactive oxygen species) or free radicals in the body is not balanced with the antioxidant defenses in the body, so an exogenous antioxidant diet is needed. *Centella asiatica* is one of the natural antioxidants that has been widely used by the community as an herbal plant to cure various diseases[9,10]. One of the bioactive components contained in *Centella asiatica* is asiatic acid, which is an antioxidant that is useful in the treatment of Alzheimer's disease[10]. In this study, in silico precilinical test were carried out using computing to predict the potential of asiatic acid that can be found in the *Centella asiatica* in Alzheimer's dementia patient by blinding to the NMDA receptors.

METHODS

The scope of this research is bioinformatics. This type of research is experimental research with *in silico* computation. The equipment used is divided into two groups, namely hardware and software. The hardware used is a Lenovo Ideapad 3 laptop with AMD Ryzen 3 5300U

specifications, 8GB RAM, 512GB SSD, AMD radeon graphics, Windows 10, Display 14 "FHD Led. The software used are *PyMOL*, *PyRx*, and *LigPlot*. The materials used in this study are the molecular structure of 7EOT (receptors) which can be downloaded from the www.rscb.org/pdb and have been prepared using the *PyMOL* compounds asiatic acid which can be downloaded from the https://pubchem.ncbi site. nlm.nih.gov, both in 3D.



(a) (b)

Figure 1. (a)The structure of asiatic acid; (b)7EOT structure of the NMDA

In the docking process, the ligand and receptor compound format was changed, from pdb to pdbqt format. Then, the grid box maximization is performed to determine the ligand rotation space with respect to the receptor position so that the ligand can rotate freely to find the most stable place for the receptor. This study is included in the blind docking because there is no grid box on the NMDA. The docking is done using the *PyRx* software.

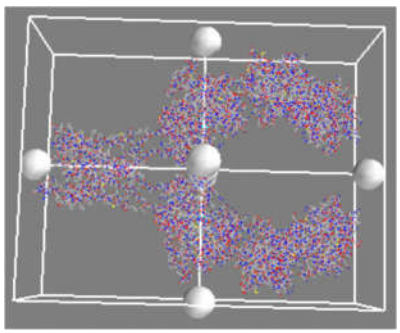


Figure 2. Inquity grid box on NMDA receptors

Amino acid residue analysis was performed using the *LigPlot*. The Lipinski test was carried out through the website http://www.swissadme.ch/index.php, which aimed to determine the toxicity and absorption of a ligand compound according to Lipinski's rules.

RESULTS AND DISCUSSION

Centella asiatica (L.) Urban is a wild plant that has quite good prospects as a medicinal plant[10]. The medicinal and biological activity of *Centella asiatica* is known for its function, both on the plant as a whole and on its extracts, namely ethanol extract and water extract[11]. *Centella asiatica* aqueous extract can attenuate cognitive impairment in mice with *β-amyloid* accumulation, but the mechanism is unknown[12,13]. *Centella asiatica* ethanol extract can improve the antioxidative defense system by supressing neurotoxicity induced by *β-amyloid*[9].

In this study, asiatic acid as the main ingridient in *Centella asiatica* is said to have neuroprotective benefits in Alzheimer's dementia patient, which was carried out using computing. Molecular docking using *PyRx* software was carried out between ligand compound asiatic acid and the NMDA derived from the prepared 7EOT structure, the binding affinity was -8.3. The implementation of the docking is that the smaller the binding affinity, the higher the affinity between the ligand and the receptor. On the other hand, the higher the binding affinity, the lower the affinity between the ligand and the receptor[10].

The results of the visualization of amino acid residues were carried out using the *LigPlot* with a 2D display. In the visualization results of the interaction between amino acid residues and asiatic acid got 1 hydrogen bond at Pro516 residue and 8 hydrophobic bonds at Ser687, Trp731, Phe758, Leu517, Phe484, Arg523, Thr518, and Ser688 residue.

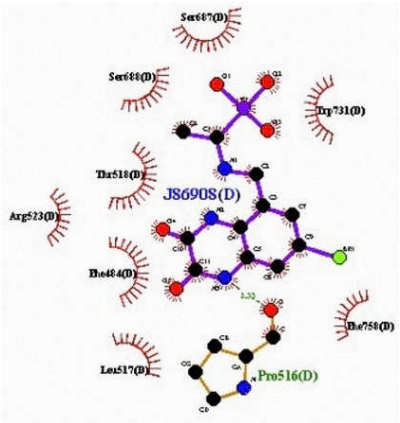


Figure 3. Visualization of amino acid redisues

Next, a drug-likeness to determine the physiochemical properties of the compounds affecting molecular properties in vivo, using Lipinski's rule: number of hydrogen proton donor groups ≤5, molecular weight ≤500 kDa, logP ≤5, logP ≤10[14].

Table 1. Lipinski test results

Compound Molecular	***Molecular Weight***	***Hydrogen Bonded Proton Donor***	***Hydrogen Bonded Acceptor Donor***	***Logarithm of Partition Coefficient in water and 1-octanol (LogP)***	***Lipinski***
Asiatic acid	488,70 g/mol	4	5	4,14	Yes

Ligands with a molecular weight of 500 kDa penetrate cell membranes more easily than ligands with a molecular weight of >500 kDa. The logP value is related to the polarity of the ligand in fats, oils, and non-polar solvents. Ligands with logP values >5 will easily penetrate the lipid bilayer and are widely distributed in the body. This causes a decrease in the sensitivity of the ligand binding to the target molecule and an increase in the toxicity of the ligand. The smaller the logP value, the more ligands tend to be water soluble and hydrophobic. The logP value should not be negative because it cannot cross the lipid bilayer membrane. The number of hydrogen bonds in the donor and acceptor correlates with the biological activity of a ligand or drug, where the higher the hydrogen bonding capacity, the higher the energy required for the absorption process to occur[14].

Based on the results of the Lipinski test data, the asiatic acid in *Centella asiatica* (L.) Urban meets the criteria of Lipinski's rule so that it has the potential to be easily absorbed and has high permeability.

CONCLUSION AND RECOMMENDATIONS

Based on the results of this research can conclude that:

1. In the result of test docking between compounds asiatic acid with NMDA receptors obtained a binding affinity -8.3, which indicates that there is a strong bond between the NMDA receptors with asiatic acid ligand. So, in this study it can be concluded that the asiatic acid in Centella asiatica (L.) Urban can block the binding of NMDA with glutamate.
2. The asiatic acid has a molecular weight of 488.70 g/mol, hydrogen bond proton donor 4, hydrogen bond proton acceptor 5, and LogP 4.14. Based on this value, the asiatic acid ligands comply with Lipinski's rules so that it has the potential that asiatic acid can be easily absorbed and have high permeability.
3. From the docking and Lipinski test results, there is potential for asiatic acid to block NMDA receptors from binding to the neurotransmitter glutamate excessively. This affects the distribution of Ca^{2+} into the postsynaptic so as not to trigger neuronal cell death so that it can play a role in the treatment of patients with Alzheimer's dementia.

Based on the research that has been done, suggestions for further research are as follows:

1. Research can be continued by using more complex methods to determine the comparative bond between molecules.
2. Further research is needed by conducting a safety test process on *asiatic acid* in *Centella asiatica* (L.) Urban on a laboratory scale (*in vitro* and *in vivo*) to obtain more accurate results.
3. Developed with other substrates and other ligands to develop drug discovery in the future

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