IJCB
INDONESIAN JOURNAL OF COMPUTATIONAL BIOLOGY

Research Article
Vol.1 No.1 (2022), 1-6

In Silico Study of Sesquiterpene and Monoterpene Compounds from Valerian Roots (*Valerian officinalis*) As Acetylcholinesterase Inhibitor

Auliya Afinasari, Safira Aulia, Muhamad Dimas Syarhul, Shannon Maidelaine Prijadi, Levina Aristawidya, Syahrul Hidayat, Tantie Noer Apriliya, Muchtaridi*)

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Jawa Barat, Indonesia

**Corresponding Author : muchtaridi@unpad.ac.id*

Received: September 28, 2021; Accepted: September 13, 2022; Availabe Online: September XX, 2022

Alzheimer's is a central nervous system disease that can be treated with an acetylcholinesterase enzyme inhibitor drug (AChE), namely rivastigmine. Rivastigmine has side effects such as nausea, vomiting, loss of appetite, headache, weakness, and malaise. Alternative to deal with these side effects is an extracted compound from valerian root with bioactivity as an anti-dementia. The test compounds that performed the activity were 4 variations of sesquiterpenoids (volvalerenal H, volvalerenal I, volvalerenal J, volvalerenic acid K) and 1 monoterpenoid (densispicnins C). Objective: This study aimed to determine the molecular interaction of sesquiterpene and monoterpene compounds and pharmacokinetic of the best compound from molecular docking. Methods: This study was conducted with the stages of molecular docking simulation, prediction of pharmacokinetics and toxicity of compounds, and Lipinski's Rule of Five parameters. Result: Volvalerenal J was the best compound from molecular docking simulation with a Gibbs free energy value of -7,48 kcal/mol, an inhibition constant of 1,30 µM, and interactions with amino acid residues His447 and Ser203. The values of HIA and CaCo2 were 100% and 46,76%, with the values of PPB and BBB were 96,57% and 0,88%. Respectively, volvalerenal J is not mutated but a carcinogen and fulfills the rules of Lipinski. Conclusion: Compound volvalerenal J has the highest potential to be the best acetylcholinesterase (AChE) inhibitor among other compounds in the extract valerian root.

Keywords: AChE | Valerian Root | Alzheimer's | In silico

Based on data from the Alzheimer's Association, the number of Alzheimer's cases reaches 13.8 million worldwide [1]. This gradual neurodegenerative disease of the central nervous system arises because cholinergic activity in the brain decreases so that the amount of acetylcholine (ACh) will decrease. ACh is a neurotransmitter that plays a role in mediating the function of learning, memory, and delivering signals from the nervous system to muscle cells and AChE. AChE functions as a catalytic enzyme in the ACh hydrolysis process which produces two compounds, namely acetate and choline, which aims to stop the synaptic transmission process. Therefore, people with Alzheimer's disease will experience a decrease in the ability to remember and learn [2].

Based on WHO in 2004, Alzheimer's patients can be given AChE inhibitor drug therapy, one of which is rivastigmine which has a dose of 1.5 mg which is taken 2 times a day. However, this drug has some side effects, namely nausea, vomiting, loss of appetite, headache, weakness, and malaise[3]. To deal with these side effects, a study is needed to find out a good and appropriate alternative. One alternative that has been approved by the US FDA from 1981 to 2011 is to develop new drugs using plant extracts [4]. One of the plants known to have good pharmacological activity is valerian root (Latin: *Valeriana officinalis* var. latifolia).

Valerian root is a plant known as European valeriana variety which has in vivo activity as anti-HIV, antiarrhythmic, antidepressant, sedative, anxiolytic, and antidementia because it can nourish nerve cells and reduce AChE activity. AChE activity in valerian root is known to be present in five test compounds that have been tested in vitro and in vivo. In vitro test results of Volvalerenal J compound, resulted in an IC50 value of 4.679±0.068 M. For the in vivo assay with the navigation test method in mice, which resulted in a decrease in AChE ($P < 0.01$) [5]. Developing new drugs de novo requires high costs and takes a long time, so the in silico method can be used as an initial screening stage to get potential candidates to be developed as drugs.

Materials & Method

Tools

A PC with specification: Intel ® Core ™ i5-6200U CPU @2,30 GHz (4 CPUs), ~2,4 GHz and RAM 8 GB.

Software

The ChemPro 12.0 and ChemDraw Ultra 12.0 (http://www.perkinelmer.co.uk/category/chemdraw) programs for making 2D and 3D ligand structures. AutoDock Tools (https://autodocksuite.scripps.edu/) 1.5.6 (The Scripps Research Institute, USA) program for running molecular docking. The BIOVIA Discovery Studio 2020 R2 Client for visualizing the 3D structure and ligand-receptor interaction.

Procedure

Acquisition of Structure

Download AChE structure as PDB from RCSB Protein Data Bank (PDB) (http://www.rscb.org/) with code 6NTO which is completed with inhibitor A-230 molecule. Use BIOVIA Discovery Studio 2020 R2 Client for structure separation. Draw and optimize the 3D structure of ligand using ChemDraw Ultra 12.0 (PerkinElmer Inc) and Chem3D Pro 12.0. Rivastigmine was used for drug comparison and downloaded the structure

Molecular Docking Simulation

The docking simulations carried out with preparing proteins and ligands on AutodockTools 1.5.6 are saved in PDBQT format. Then set the grid box with dimensions 40 x 40 x 40 with coordinates x = -39,256; y = -17.598; and z = -26,206 at a distance of 0.375. Then, the system used setting 100 Lamarckian genetic algorithms with a population size of 150. Autodock 4.2.6 is used to perform molecular docking. Use Discovery Studio Visualizer to find affinity values and ligand-receptor interactions.

Pharmacokinetic and Toxicity Prediction

Prediction of pharmacokinetics (absorption, distribution, metabolism, and excretion) and toxicity, including Human Intestinal Absorption (HIA), CaCO2, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB), potential mutagens, and carcinogens with Adme webserver

(http://lmmd.ecust.edu.cn/admetsar2/).

Lipinski's Rules

The test is continued by adjusting the five Lipinski rules. Compounds that can be used as candidates for active drug compounds must have a molecular mass of fewer than 500 Daltons, high lipophilicity (expressed as LogP less than 5), less than five hydrogen bond donors, less than ten hydrogen bond acceptors, molar refractivity should be between 40- 130. Testing was carried out using a webserver (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp).

Results

Structure Accuision and Molecular Docking Simulation

This study used five active compounds (*volvalerenal H*, *volvalerenal I*, *volvalerenal J, volvalerenic acid K*) and 1 monoterpenoid (*densispicnins C*) from valerian root were tested to prove their potential as a therapy for Alzheimer's disease. The activities of the five active compounds will be compared and selected from the molecular docking simulation process. The results of the structure and other outcomes will be compared with the comparison drug, namely rivastigmine. Molecular docking simulations of 5 valerian root test compounds found that all test compounds except *Densispicnin* had better affinity energy values compared to rivastigmine.

Pharmacokinetic and Toxicity Prediction

The pharmacokinetic profile of the analyzed ligands consisted of absorption seen from HIA and CaCO2 values and distribution seen from PPB and BBB values. Then, the toxicity test consists of mutagenic and carcinogenic tests. The values of HIA, CaCO2, PPB, and BBB of volvalerenal compound J were 100%, 46.76 nm/sec, 96.57%, and 0.88%, respectively. Volvalerenal J compound is also not mutagenic, but potentially carcinogenic (Table 2).

Lipinski's rule

Lipinski's rule refers to the solubility and permeability of the test compound while in the gastrointestinal tract. This value can determine the bioavailability of a compound. Based on Lipinski's test results on the five compounds from valerian root, all the test compounds met the requirements (Table 3).

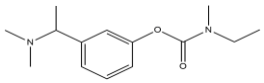
Discussion

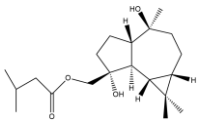
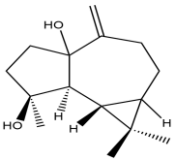
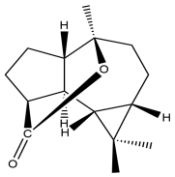
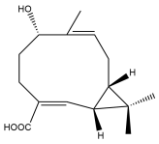
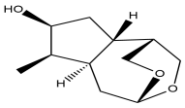
In this molecular docking simulation, rivastigmine is used as a comparison drug. Rivastigmine is a drug to treat mild to moderate Alzheimer's disease with an initial dose of 1.5 mg taken twice a day and the usual dose of 3-6 mg twice a day[6]. The mechanism of action of rivastigmine is to increase ACh production by inhibiting the AChE enzyme which functions as an ACh hydrolyzing enzyme[7]. This drug has several side effects, including nausea, vomiting, diarrhea, dyspepsia, and anorexia. US FDA controlled clinical trial data stated that of 1189 patients treated with rivastigmine (Exelon) at a dose of 6-12 mg daily, 47% of these patients experienced nausea and vomiting and 17% of patients experienced anorexia. In addition, 26% of patients also experienced weight loss on high doses of rivastigmine (more than 9 mg per day)[8]. Of these side effects, rivastigmine resulted in mortality risk of 12.62%. The risk of death was found to be greater than treatment using donepezil, which was 1.38%.[9].

Therefore, to deal with the risk of death from the use of the drug rivastigmine, the development of drugs derived from plants is carried out. In silico study of valerian root with autodock program to determine its potential against Alzheimer's disease. Based on the results of the molecular docking simulation which has been validated with an average RMSD (Root Mean Square Deviation) value from the number of runs of 100 data, it is 1.36. The RMSD value has met the acceptance requirements of 3.00 and indicates that the protein complex remains stable during the molecular docking simulation process.[10]. In addition to RMSD, another observed parameter of simulation results is the hydrogen bonding of key amino acids (catalytic). The amino acid residues possessed by AChE are His447, Ser203, Gly121, and Glu202. Meanwhile, the amino acid residues from the crystal structure and the validation results of volvaleneral J binding were His447 and Ser203 which were predicted to have stable interactions with the AChE enzyme.

The test compound is said to be potential, if a compound is able to hydrogen bond with the target amino acid residue. The more hydrogen bonds and hydrophobicity, the stronger the inhibitory activity on the target protein[11]. Hydrogen bonding is an interaction that contributes to the affinity of the molecule to the receptor in the formation of electrostatic interactions (acceptor and donor) provided that it has a distance of < 3.9 Å[12]. The distances for His447 and Ser203 bonds are 2.12 and 1.79, so that the data fulfill the requirements for residual distance.

Table 1 Result of each active compound from molecular docking

No	*Compound*	*Cluster*	*Binding Energy (ΔG) (kkal/mol)*	*KI (uM)*	*Hydrogens Binding*
1.	 Rivastigmin	77	-7,14	5,83	-

2.	 Volvalerenal H	38	-7,22	5,10	Ser125 Glu202
3. 4.	 Volvalerenal I	97	-7,48	3,32	Ser203
5.	 Volvalerenal J	100	-8,03	1,30	His447 Ser203
6.	 Volvalerenal Acid K	84	-7,27	4,27	Gly122 Glu202
7.	 Densispicnins	87	-5,89	8,48	His447

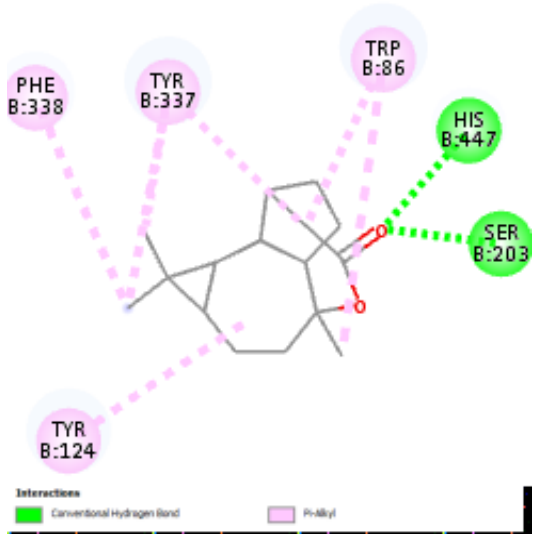


Figure 1 Interaction of *volvalerenal J* with AChE enzyme

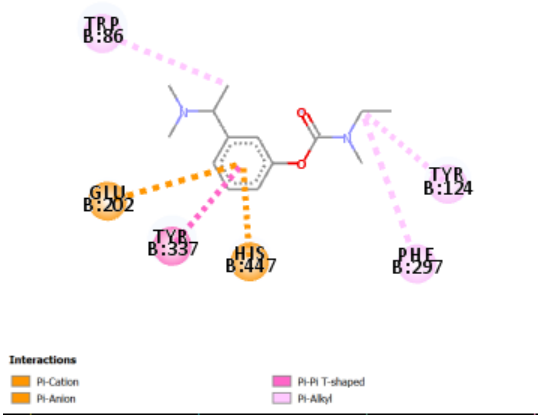


Figure 2 Interaction of rivastigmin with AChE enzyme

Based on the results of molecular docking in Table 1, the compound of valerian root extract that has the most potential as an AChE inhibitor is volvalerenal J. This is because the compound has the most hydrogen bonds with AChE catalytic amino acid residues compared to rivastigmine (comparison drug) and the other four test compounds. . ThE volvalerenal compound J can hydrogen bond with two amino acid residues, His447 and Ser203 (Figure 1), whereas rivastigmine does not form hydrogen bonds with the catalytic amino acid residue AChE (Figure 2).

Besides being seen from its potential for hydrogen bonding with amino acid residues, it is also necessary to look at the value of the free energy (ΔG) and the inhibition constant (KI) of the test compound. The free energy is a parameter of conformational stability between the ligand and the enzyme and is directly related to the inhibition constant. The lower the value of free energy (ΔG), the ligand-enzyme interaction tends to be stable[13]. The molecular docking simulation results in Table 1 show that volvalerenal J has lower G and KI values than rivastigmine and other test compounds, namely -8.03 kcal/mol and 1.30 M.

Thus, volvalerenal J has a more stable interaction and has the potential as an AChE inhibitor to treat Alzheimer's disease.Furthermore, predictions of the absorption, distribution, metabolism, and excretion, and toxicity (ADMET) of a compound in drug discovery and development need to be carried out to avoid drug pharmacokinetic problems and prevent drug development failure. The ADMET parameters used were Human Intestinal Absorption (HIA), CaCO2 cell model, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB), cytochrome P450 (CYP) inhibitors, potential mutagens, and carcinogens.

Human Intestinal Absorption (HIA) and CaCO2 cell model are absorption parameters used to predict and identify drug candidates by oral and transdermal administration. Parameters of HIA (Human Intestinal Absorption) are divided into three categories of absorption in the intestine, namely compounds that are well absorbed (70-100%), moderate (20-70%), and low (0-20%). Likewise, the CaCO2 cell model divides compounds into three categories of permeability capabilities, namely high (>70 nm/sec), moderate (4-70 nm/sec), and low (< 4 nm/sec) [14]

Table 2 Pharmacokinetic and Toxicity Prediction of Each Active Compound and Drug Comparison

No	Compound	Absorption		Distribution		Metabolism					Toxicity	
		HIA (%)	CaCO2 (nm/sec)	PPB (%)	BBB (%)	CYP2C9	CYP2C19	CYP2D6	CYP3A4	CYP3A4	Mutagent	Carcinogen
1.	Rivastigmine	99,41	47,61	26,4	1,16	No	No	No	No	Yes	Mutagent	Positive
2.	Volvalerenal H	91,90	24,33	91,5	1,64	Yes	No	No	Yes	Yes	Mutagent	Positive
3.	Volalerenal I	90,70	26,54	75,9	3,21	Yes	No	No	Yes	Yes	Mutagent	Positive
4.	Volvalrenal J	100	46,76	96,6	0,87	Yes	No	No	Yes	Yes	Non-Mutagent	Positive
5.	Volvalerenal Acid K	95	4,45	100	1,18	Yes	No	No	No	Yes	Mutagent	Negative
6.	Densispicnis	93,78	24,55	26,94	0,41	Yes	Yes	No	Yes	Yes	Mutagent	Negative

Alzheimer's disease drugs are targeted to enter the central nervous system, so candidate drug compounds are expected to have a large BBB value. Compounds with a PPB value of more than 90% were classified as compounds that were able to bind strongly to plasma protein, while compounds with a PPB value of less than 90% were classified as compounds that were weakly bound to plasma protein.

Compounds with a BBB value > 2.0 indicate that the compound can penetrate the blood-brain barrier well, a BBB value of 0.1-2.0 indicates that the compound has moderate penetration of the blood-brain barrier, and a BBB value < 0.1 which indicates that the compound can low penetration of the blood-brain barrier [14]. Based on the prediction results of ADMET in Table 2, volvaleneral J compound is predicted to be able to bind strongly to plasma proteins and has moderate penetration of the brain barrier.

Cytochrome P450 (CYP) inhibitor is a parameter to determine the ability of drug metabolism. CYP inhibition can have both positive and negative effects in medicinal and clinical use. The positive effects that can be produced include prolonging the pharmacological effect, while the negative effects that can be caused include increasing the toxic effect [15].Based on the prediction results of ADMET in Table 2, volvaleneral J compound is predicted to inhibit CYP2C9, CYP3A4, and CYP3A4 substrates.Prediction of mutagens

and carcinogens of drug compounds is carried out to determine the toxicity properties of potential drug candidates. Based on Table 2, volvaleneral J compound is not potentially mutagenic but has the potential to be carcinogenic.

Lipinski's Rule of Five test is used to determine hydrophilic or hydrophobic properties of drug candidates[16]. Lipinski's Rule of Five or RO5 is a parameter to predict the eligibility of a drug product to be administered by oral route. *Lipinski's Rule of Five* states that an orally active drug must fulfill at least three of the following criteria, molecular weight less than 500 g/mol, calculated lipophilicity (Log P) not greater than 5, number of hydrogen-bond acceptors not more than 10, and number of hydrogen-bond donors not more than 5[17]. Based on Table 3, Volvalerenal J compound has fulfilled the criteria. Therefore, Volvalerenal J compound has good bioavailability and it is suitable to be an oral drug. Volvalerenal J compound should be further investigated in vitro and in vivo as lead compounds inhibit Acetylcholinesterase. Chen *et al* had investigated the fifth extract compound from Valerian root at low dose (0,65 mg/kg.d), middle dose (1,30 mg/kg.d) and high dose (2,60 mg/kg.d). Based on the research, Volvalerenal J compound showed an IC_{50} value of 4,679±0,068 µM and decreased AChE ($P<0,01$) by navigation test[5].

Table 3 Result of Lipinski's Rule

No.	Compound	Molecule Weight (g/mol)	H-Acceptor Binding	H-Donor Binding	Log P	Description
1.	Volvalerenal H	338,48	4	2	3,72	Fulfil
2.	Volvalerenal I	236,35	2	2	2,74	Fulfil
3.	Volvalerenal J	234,33	2	0	2,76	Fulfil
4.	Volvalerenal Acid K	250,33	3	2	2,43	Fulfil
5.	Densispicnis	198,26	3	1	2,20	Fulfil

Conclusion

The best valerian root compounds from the molecular docking simulation is volvalerenal J compound with low bond-free energy and inhibition constant, i.e. -8.03 kcal/mol and 1.30 M. The catalytic amino acid residues from volvalerenal J are His447 and Ser203. Volvalerenal J has more potential as an AChE enzyme inhibitor in Alzheimer's disease because it has more hydrogen bonds J than the reference drug (rivastigmine). However, the results of pre-ADMET shows that volvalerenal J has carcinogenic properties, therefore it ought to be tested *in vitro* and *in vivo* to ensure the activity of these compounds as AChE inhibitor in the treatment of Alzheimer's disease.

References

1. Alzheimer's Association. Alzheimer's Diseases Fact and Figures [diunduh 22 Maret 2021]. Tersedia dari https://alz-journals.onlinelibrary.wiley.com/doi/full/10.1002/alz.12068.
2. Ghajarbeygi, Peyman, Ashraf Hajhoseini, Motahare-Sadat Hosseini, Anoosheh Sharifan. An In Vitro and In Vivo Cholinesterase Inhibitory Activity of Pistacia khinjuk and Allium sativum Essential Oils. *Journal of Pharmacopuncture*. 2019;22 (4):231-238.
3. DiPiro JT, Yee GC, Haines MPST, Nolin TD, Ellingrod V. 2020. *Pharmacotherapy A Pathophysiologic Approach Eleventh Edition*. Inggris : Mc-Graw Hill.
4. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. *Journal of Natural Products*. 2012;4: 425-440.
5. Chen HW, He XH, Yuan R, Wei BJ, Chen Z, Dong JX, Wang J. Sesquiterpenes and a monoterpenoid with acetylcholinesterase (AChE) inhibitory activity from *Valeriana officinalis var. latiofolia* in vitro and in vivo. *Fitoterapia*. 2016;110:142-149.
6. PIONAS BPOM. Rivastigmin [diunduh 27 Juni 2021]. Tersedia secara online di http://pionas.pom.go.id/monografi/rivastigmin.
7. Patel PH, Gupta V. Rivastigmine. [diunduh 27 Juni 2021]. Tersedia secara online di https://www.ncbi.nlm.nih.gov/books/NBK557438/.
8. US Food and Drug Administration. EXELON® (Rivastigmine tartrate) Capsules, for Oral Use and EXELON® (Rivastigmine tartrate) Oral Solution [diunduh 27 Juni 2021]. Tersedia dari https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/020823s036,021025s024lbl.pdf.
9. Kazmierski, J., Messini-Zachou, C., Gkioka, M., and Tsolaki, M. The Impact of a Long-Term Rivastigmine and Donepezil Treatment on All-Cause Mortality in Patients With Alzheimer's Disease. *American Journal of Alzheimer's Disease & Other Dementias®*. 2018;33(6): 385–393.
10. Ramírez D, Caballero J. Is it reliable to take the molecular docking top scoring position as the best solution without considering available structural data. *Molecules*. 2018;23(5):1038.
11. Syahputra G, Ambarsari L, Sumaryada T. Simulasi Docking Kurkumin Enol Bisdemetoksikurkumin dan Analoginya sebagai Inhibitor Enzim 12-Lipoksigenase. *Jurnal Biofisika*. 2014;10(1):55-67.
12. Bissantz, C., G.Folkers, D.Rognan. Protein-based Virtual Screening of Chemical Data Bases: Evaluation of Different Docking/Scoring Combinations. *Journal of Medicinal Chemistry*. 2011;43: 4759-4767.

13. Laksmiani NPL, Paramita NLPV, Wirasuta IMAG. In Vitro and Silico Antioxidant Activity of Purified Fractions from Purple Sweet Potato Ethanolic Extract. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2016;8:177-181.
14. PreADME. *ADME Prediction* [diunduh 26 Maret 2021]. Tersedia online di https://preadmet.bmdrc.kr/adme-prediction/.
15. Rahayu, M. dan Solihat, M. F. 2018. *Toksikologi Klinik*. Jakarta: Kementerian Kesehatan RI
16. Rukmono, Rendra, Inarah F, Hafrizal R, Mitra H. Virtual Screening Metabolit Aktif Senyawa Asam Dari Pacar Air Terhadap Reseptor Sulfurlinuera. *Jurnal Kesehatan*. 2021;1(1):16-29.
17. Lipinski, C. A. Rule of Five in 2015 and Beyond: Target and Ligand Structural Limitations, Ligand Chemistry Structure and Drug Discovery Project Decisions. *Advanced Drug Delivery Reviews*. 2016;101:34-41.