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Article

The Potency of Bioactive Constituents in *Piper betle* L. for Alzheimer Targeting on Caspase-3: *In Silico* Studies

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Abstract

Alzheimer's disease (AD) is a neurodegenerative condition that can disrupt memory, cognition, and neurological functions, Recent studies highlight caspase-3 as a potential target, with several lines of evidence pointing to the enzyme's possible role in the onset of AD. Several findings revealed that betel leaf was also examined to treat AD by targeting acetylcholinesterase *in vitro* and *in silico*, yet no evaluation had not been done in caspase-3 activity. Using molecular docking, Lipinski's and PreADMET prediction, an in-silico analysis of compounds found in betel leaf (*Piper betle* L.) was conducted in order to determine whether these compounds could be applied as therapeutic candidates in the treatment of Alzheimer's. To ascertain the drug similarity and ADMET profile of the evaluated ligands, the Mcule and PreADMET sites were used in the studies, which were followed by the molecular docking simulation software AutoDock. The findings demonstrated that all the tested compounds passed the physicochemical features based on Lipinski rule. Further analysis then showed that arecoline bound to the critical amino acid that involved in caspase-3 inhibition. Further evaluation needs to be done to confirm the molecular mechanism of *P. betle* leaves to AD.

Keywords: Alzheimer's disease; Arecoline; Betel Leaves; Caspase-3; Molecular docking

1. INTRODUCTION

Alzheimer, a neurodegenerative illness, is primarily caused by the death of brain cells, which can disrupt memory, cognition, reasoning, language, and other neurological functions [1]. The symptoms of Alzheimer's disease (AD) include impaired memory and recognition, confusion, speech impairments, agitation, and hallucinations [2]. The etiology involves neuronal degeneration and shrinkage in the temporofrontal cortex, leading to inflammation,

accumulation of plaques of aggregated amyloid-β (Δβ) peptide, and the development of clusters of protein fragments and aberrant tangled fiber bundles known as intracellular neurofibrillary tangles [3]. Novel strategies utilizing treatments specifically targeting amyloids have demonstrated limited therapeutic advantages [4]. The preceding findings underscore the need of identifying novel molecular targets to enable the development of efficacious alternative treatments for AD. Recent research emphasizes caspase-3 as a prospective target, supported by multiple lines of evidence suggesting a potential involvement of this enzyme in the development of AD [5]. Studies have shown that caspase-3 is specifically activated in the cells of the hippocampus in transgenic animal models of AD. Moreover, this atypical activation of caspase-3 initiates changes in the connections between neurons that are associated with the beginning of impairments in memory in mice [6]. Active caspase-3 is increased before the formation of amyloid plaques and neurofibrillary tangles, and without causing cell death [7]. Increased levels of caspase-3 were also observed in the brains of patients with AD [8]. Hence, inhibiting caspase-3 at the initial stages of AD may be a viable approach to impede the progression of subsequent cellular mechanisms that ultimately result in neuronal degeneration during later phases of the disease. While inhibiting caspases is challenging because of their involvement in regular cellular activities, selectively and reversibly inhibiting caspase-3 to limit its increased activity in pathological situations could be a beneficial therapeutic strategy [9, 10].

Betel leaf (*Piper betle* L.), belongs to Piperaceae family, is one type of plant that has been widely used as a medicinal plant in society. Betel leaves contain a variety of secondary metabolites, including essential oils, terpinene, sesquiterpenes, phenylpropanes, and terpenes. There are also catechins and tannins which belong to polyphenolic compounds [11,12]. A review by Biswas et al. [13] reported several secondary metabolite constituents found in Betel leaf, including arecoline, eugenol, carvacrol, phytol, hydroxychavicol, piperine, and piperitol. Several pharmacological activities from Betel leaf that have been studied include antibacterial [14], antioxidant, anticancer [15], and other activities. Several reports revealed that betel leaf was also studied to treat AD by targeting acetylcholinesterase inhibition based on *in vitro* and *in silico* studies [16, 17]. In rats with aluminum chloride-induced AD, piper betle leaf extract enhanced learning and memory skills [18]. Based on the prior results which demonstrated the potency of *P. betle* in AD treatment, this work aims to explore the metabolites from *P. betle* that may interact with caspase-3 based on molecular docking analysis, supported by pharmacophore studies to find the potential lead compound that likely inhibit caspase-3 activity in regards to AD.

2. MATERIALS AND METHODS

2.1. Material

The materials used for this study were target Caspase-3 that obtained from PDB (PDB ID code 3KJF), as well as selected bioactive compounds from *P. betle*, including arecoline, eugenol, carvacrol, hydroxychavicol, phytol, piperine, and piperitol.

2.2. Instrument

The hardware used is a personal laptop with AMD Ryzen 5 5500U CPU @ 2.10 GHz RAM 8.00 GB processor specifications with Windows 10 64-bit operating system.

2.3. Method

2.3.1. Lipinski rule of five analysis

Lipinski's Rule of Five prediction was performed to determine the drug-likeness profile of the bioactive substances from *P. betle*. This analysis was conducted through the Mcule webtool (https://mcule.com/apps/property-calculator/) by submitting test compounds that have been made 2D structures first using ChemDraw. Lipinski's Rule of Five parameters that were assessed including: hydrogen bond donor, hydrogen bond acceptor, molecular weight, and log P value.

2.3.2 ADMET prediction

ADMET (Adsorption, Distribution, Metabolism, Excretion, Toxicology) prediction profile was conducted to determine the physicochemical profile and toxicity of the test compound. This analysis was carried out through the PreADMET website (http://www.preadmet.webservice.bmdrc.org/). The parameters analyzed include the %HIA (Human Intestinal Absorption), Caco-2 (Cancer coli-2), BBB (Blood-Brain Barrier) penetration, PPB (Plasma Protein Binding) values, as well as the results of toxicity tests with the Ames test and with rodent carcinogenicity.

2.3.3 Molecular docking study

The caspase-3 (PDB ID: 3KJF) structure was downloaded first through the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank site (https://www.rcsb.org/) and then prepared by removing the water molecule and separating the natural ligand structure from the receptor using the BIOVIA Discovery Studio 2020. Test ligands and comparison ligands were also prepared using the same application by minimizing the energy of the ligand first using Chem3D Pro 12.0. Furthermore, ligand preparation was continued with the AutoDock application to add hydrogen, combine compounds into non-polar and give Gasteiger charges to the ligand and then input torque into it. Meanwhile, the receptor preparation was done by adding polar hydrogen atoms and giving Kollman charges to the receptor that has been separated from its natural ligand using the Autodock 4.2.7.

Validation docking was done first to determine the position and size of the grid box that will be used in the molecular tethering simulation. This validation was done by re-bonding the natural ligand to the receptor using the AutoDock, and then adjusting the size and position of the grid box so that when running with a genetic algorithm value of 10, the validation results that can be accepted are if the Reference RMSD value was less than 2 Å and the bond energy was negative or smaller. In this study, the grid box used was as follows, grid box (x = 34; y = 26; z = 30) and grid Coordinate (x = 22.27; y = -4.534; z = 10.75) with a distance of 0.375 Å. Next, the molecular docking step of the test ligand to the receptor or target protein was carried out using the same steps as in the validation stage but using the Lamarckian Genetic Algorithm which was worth 100. The molecular docking results were then analyzed using AutoDock to determine the best binding energy value and KI (inhibition constant) of each ligand tested. The results of the analysis were then visualized using the BIOVIA Discovery Studio 2020 to obtain 2D and 3D visualizations of the test ligands that had been tethered to the receptor.

3. RESULT AND DISCUSSION

3.1. The physiochemical characteristics of compounds in P. betle based on Lipinski rule

The parameters used in this prediction include molecular weight, log P value, hydrogen bond donor and hydrogen bond acceptor. From this online test, the results are shown in Table 1. It is known that all test compounds from Betel Leaf (*Piper betle* L.) which are used as candidate drug compounds meet the requirements of Lipinski's rule. Based on these findings, those constituents found in *P. betle* leaf can be potentially developed for oral administration.

3.2. The pharmacokinetic prediction from the bioactive compounds in P. betle

In the determination of ADMET, the parameters seen include %HIA and Caco-2 values to see absorption, %PPB and BBB values to see the distribution of test compounds in the body and determination of the toxicity of compounds that can be known from mutagen properties and carcinogens from compounds using the Ames test and rodent carcinogenicity. The results of online analysis using the PreADMET (https://preadmet.webservice.bmdrc.org/) show the results as in Table 2. In the distribution parameter, it is known that high %PPB and BBB are owned by eugenol, carvacrol, phytol, and hydroxychavicol. The determination of toxicity showed vary results either in mutagenic characteristics or carcinogenic effects in animals.

Table 1. The Lipinski rule analysis from the bioactive compounds in *P. betle*

Substance	Molecular weight (< 500 Da)	Log P (<5)	Hydrogen bond		Conclusion
			Donor (<5)	Acceptor (< 10)	
Arecoline	155.19	0.36	0	3	Passed
Eugenol	164.20	2.13	1	2	Passed
Carvacrol	150.22	2.82	1	1	Passed
Hydroxychavicol	150.17	1.53	2	2	Passed
Phytol	296.53	6.36	1	1	Passed
Piperine	285,.34	2.94	0	4	Passed
Piperitol	356.40	3.20	1	6	Passed

Table 2. The ADMET prediction from the bioactive compounds in P. betle

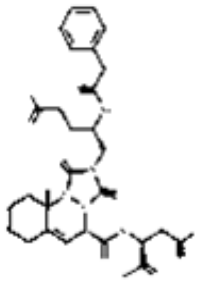
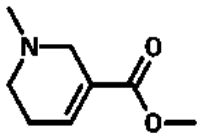
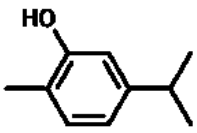
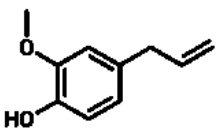
Substance	Absorption		Distribution		Toxicity		
	HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen	
						Mouse	Rat
Arecoline	100	26.32	8.13	1.05	mutagen	-	-
Eugenol	96.77	46.88	100	2.25	mutagen	+	+
Carvacrol	100	38.01	100	6.38	mutagen	-	-
Hydroxychavicol	89.41	18.40	100	3.39	mutagen	+	-
Phytol	100	37.61	100	19.08	non-mutagen	+	-
Piperine	98.18	52.38	90.45	0.05	mutagen	+	-
Piperitol	96.06	50.74	81.92	0.02	mutagen	-	-

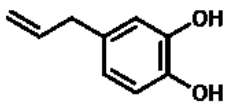
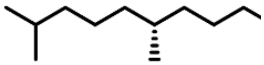
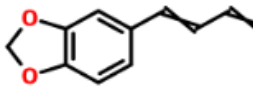
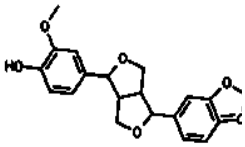
3.3. Molecular docking studies

The structure of caspase-3 in the complex with B93 ligand as its inhibitor (PDB code: 3KJF) was opted for in silico study due to the parameters were suitable for analysis, with a resolution of 2.0 Å, free R-values of 0.206, and working R-values of 0.181. The best ligand-docking conformation was selected, with the RMSD value of 1.78 Å (< 2.0 Å), indicating the validity of the protocol. All the tested compounds were docked to caspase-3, as seen in Table 3, piperitol was found to exhibited lowest binding score (-5.17 kcal/mol). In addition, carvacrol had the smallest KI value of 2.9

nM, which indicated the low KI value may lead to greater affinity of the ligand to the macromolecule. The binding affinity value indicates the ability of the ligand to bind with the receptor.

Table 3. The molecular docking results

No	Compound and chemical structure	Binding energy (kcal/mol)	Ki	Interaction with amino acid	
				Hydrogen bond	Others
1.	B92 (native ligand) 	-11.29	3.28 nM	ARG A:64 ARG B:207 CYS A:163 HIS A:121 SER B:205 SER B:209	Carbon-hydrogen bond Cation–π Sigma–π TYR B: 204 Alkyl–π PHE B:256 TRP B:206
2.	Arecoline 	-3.21	4.47 nM	ARG A:64 ARG B:207 CYS A:163 HIS A:121 SER B:205 SER B:209	Carbon hydrogen bond Cation–π Sigma–π TYR B:204 Alkyl–π PHE B:256 TRP B:206
3.	Carvacrol 	-3.46	2.9 nM	ILE B:265 PRO B:263	Alkyl, Alkyl–π CYS B:264
4.	Eugenol 	-3.3	3.8 µM	ILE B:267 SER B:267	Alkyl VAL B:266 Sigma–π MET B:233

No	Compound and chemical structure	Binding energy (kcal/mol)	Ki	Interaction with amino acid	
				Hydrogen bond	Others
5.	Hydroxychavicol 	-3.1	5.31 nM	ILE B:265 SER B:267	Donor Hydrogen Bond
6.	Phytol 	-2.68	10.94 µM	PRI B:263	Alkyl CYS B:264 MET B:233 MET B:268 VAL B:266
7.	Piperine 	-3.3	3.83 µM	ASN B:240 ILE B:265 THR B:237	Alkyl MET B:233
8.	Piperitol 	-5.17	161.88 µM	ASN B:240	Carbon Hydrogen Bond ILE B:265 SER B:267 Alkyl, Alkyl–π MET B:268 VAL B:266

A study by Ahmad et al. [19] demonstrated that the critical site for the inhibitory activity of caspase-3 is at His-121, Ser-205, and Arg-207. Our results revealed that among all the tested compounds, only arecoline along with the native ligand B92 had molecular interaction with caspase-3 at His-121 and Ser-205. Studies have shown that arecoline attenuates the memory impairment in cuprizone-induced mice [20], and randomized clinical trial showed low-dosed arecoline infusion improved psychomotor performance which attributed in Alzheimer patients [21]. In other study that also used *P. betle* for Alzheimer targeting in acetylcholinesterase, piperin was found to be potential in targeting the receptor [22]. Still, further studies are urged to be conducted to confirm the potency of *P. betle* in management of Alzheimer disease.

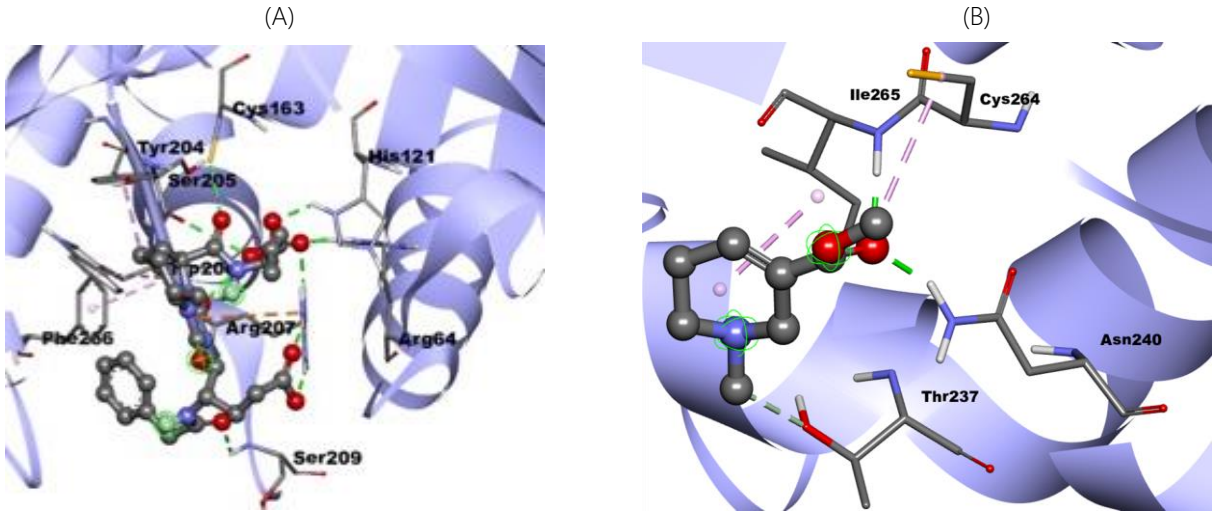


Figure 1. The visualization following molecular docking assay of the native ligand B92 (A) and arecoline (B) against caspase-3

4. CONCLUSION

Taken together, our study demonstrated that arecoline in *P. betle* may act as the caspase-3 inhibitor that could be further evaluated for its activity in Alzheimer disease.

AUTHOR CONTRIBUTION: Conceptualization, D.N. F.O.M, and F.M.M.; A.C.N.P, R.R.A, S.K.S, and N.D.R, formal analysis, investigation, data curation; A.C.N.P, R.R.A, S.K.S, and N.D.R, writing—preparation of original draft, F.O.M, F.M.M, D.N., writing—reviewing and editing, D.N.; supervision. All authors have read and approved the published version of the manuscript.

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