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Indonesian Black Tea (*Camellia sinensis*) as a Potential Acetylcholinesterase Inhibitor Against Alzheimer's Disease: Docking, DFT, and *In Vitro* Evidence

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder predominantly affecting the elderly and characterized by dementia. AD pathology involves impaired cholinergic neurotransmission, largely due to β-amyloid (Aβ) plaque accumulation, which inhibits choline acetyltransferase (ChAT) and reduces acetylcholine (ACh) levels. Acetylcholinesterase (AChE) contributes to AD progression by hydrolyzing ACh and promoting Aβ plaque formation, making it a key therapeutic target. This study investigated natural compounds from black tea (*Camellia sinensis*) as potential AChE inhibitors. Molecular docking analyses assessed interactions between bioactive compounds from aqueous black tea extracts and AChE, followed by evaluation of bioavailability, biological activity, toxicity, stability, and reactivity. Epigallocatechin gallate exhibited the strongest binding affinity (ΔG_{bind} = –12.2740 kcal/mol), forming extensive interactions with the catalytic active site located at the bottom of a deep and narrow gorge (~20 Å). Density Functional Theory (DFT) analysis confirmed its high stability and favorable reactivity in complex with AChE. *In vitro* validation using black tea extracts from Bogor, Indonesia, showed significant AChE inhibition with an IC_{50} value of 44.85 ± 1.48 µg/mL. These findings highlight the promising potential of Indonesian black tea as a natural alternative for Alzheimer's disease therapy.

Keywords: Acetylcholinesterase inhibitors, Alzheimer's disease, *Camellia sinensis,* Density Functional Theory*, in vitro*

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder primarily marked by the aggregation of β-amyloid (Aβ) proteins into insoluble plaques within the brain's extracellular matrix [1,2]. Under normal physiological conditions, soluble Aβ plays beneficial roles in regulating synaptic activity and plasticity, which are essential for learning and memory [3]. The peptide is encoded by exons 16–17 of the *APP* gene on chromosome 21, which produces three major isoforms, APP695, APP751, and APP770, through alternative splicing. APP695, the dominant

isoform in the brain, generates soluble Aβ via β-secretase (BACE1) cleavage through the amyloidogenic pathway [4-6].

At physiological levels, soluble Aβ contributes to maintaining normal neuronal function [4,5]; however, when present at elevated concentrations, it undergoes aggregation into toxic β-sheet fibrils that form senile plaques, subsequently inhibiting choline acetyltransferase (ChAT), damaging cholinergic neurons, and decreasing acetylcholine levels [6-7]. Additionally, elevated acetylcholinesterase (AChE) activity further aggravates cholinergic dysfunction, and AChE–Aβ complexes accelerate amyloid plaque formation [8].

Globally, AD affects over 50 million people, with prevalence projected to exceed 115 million by 2050 [9]. In Indonesia, dementia cases are expected to rise from 1.2 million in 2016 to 4 million by 2050 [10]. Besides genetic predisposition, factors such as poor lifestyle, social isolation, and depression contribute to disease progression [11]. The growing prevalence highlights the need for effective, safe, and affordable therapeutic options.

The first therapeutic approach developed for AD involved inhibiting Alzheimer's amyloidosis using peptide-based or natural compound-based agents derived from various natural sources [3,12,13]. Later, AChE inhibitors became the main therapeutic focus, enhancing cholinergic transmission by preventing acetylcholine hydrolysis [4,14,15]. The FDA-approved inhibitors—tacrine, donepezil, rivastigmine, and galantamine—alleviate symptoms but may cause adverse effects like hepatotoxicity [16-17], prompting exploration of safer natural alternative therapeutic candidates. This study is important as it provides a potential alternative therapy for Alzheimer's disease by evaluating the bioactivity of black tea, which is considered safer for long-term consumption.

Tea (*Camellia sinensis*), the world's second most consumed beverage, contains neuroprotective compounds [18] such as flavonoids [19], theaflavins [20-21], and L-theanine [22]. Black tea, rich in flavonoids and theaflavins formed during fermentation, shows potential as a natural AChE inhibitor [23,24]. However, studies on Indonesian black tea remain limited.

This study investigates the anti-Alzheimer's potential of bioactive compounds from Indonesian black tea through *in silico* molecular docking and *in vitro* AChE inhibition assays to identify safe and effective natural candidates for AD therapy.

2. RESEARCH METHODS

The study utilized various laboratory instruments, including glassware, heating and mixing equipment, measuring devices, and a UV-VIS Diode Array Elisa/Microplate Reader SpectroStar Nano (Serial no. 601-3354), along with a computer equipped with Minitab software for statistical analysis. For the *in silico* analysis, molecular docking was performed using YASARA Structure 19.9.17 (License No. 154638792) on a standard personal computer with sufficient computational resources to carry out the simulations. Discovery Studio 3.5 Client for 2D visualization of ligand–receptor interactions. These tools facilitated molecular modeling and computational interaction analyses essential to the research.

Materials

The materials used in this study included dried black tea crude material obtained from PT Perkebunan Teh Gunung Mas (Bogor), a research collaborator. This plantation is a certified producer with reliable quality, traceable sourcing, and export distribution extending to the Middle East. Using materials from two distinct highland regions also helps capture natural variation in phytochemical composition. Other materials used were distilled water, and a range of chemical reagents, such as the AChE enzyme assay kit (Abcam ab138871). For the *in silico* study, the 3D structure of donepezil—co-crystallized with the AChE receptor (PDB ID: 6O4W)—was used as the reference ligand. Additionally, 3D structures of natural compounds from black tea, based on secondary data reported by Hasan et al. (2022) [25], were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in *.sdf* format. The 3D structure of the AChE receptor enzyme was obtained from the Protein Data Bank in *.pdb format*, also under PDB ID: 6O4W.

Identification of the Active Site of Acetylcholinesterase (AChE, EC 3.1.1.7)

Identifying the receptor's active site is essential for virtual screening, as it guides the evaluation of potential inhibitors. Acetylcholinesterase (AChE, EC 3.1.1.7) was chosen as the receptor. Its active site was identified through homology analysis using BLASTp against the Protein Data Bank (PDB). The AChE sequence (PDB ID: 6O4W) was retrieved and compared with 5–10 homologs. Multiple sequence alignment with Clustal Omega highlighted conserved residues in the active site, based on literature-reported [26].

Preparation of Ligand and Receptor Structures for Molecular Docking

This study utilized three ligand types: two reference ligands and test ligands. The reference ligands, donepezil and rivastigmine—both FDA-approved acetylcholinesterase (AChE) inhibitors—were used to validate the grid box and benchmark the effectiveness of test ligands [26]. In addition, donepezil

is the co-crystallized ligand in the crystallographic structure of AChE (PDB ID: 6O4W). The test ligands were natural compounds derived from black tea based on secondary data reported by Hasan et al. (2022) [25]. Their 3D structures were downloaded from PubChem (*.sdf*), energy-minimized using YASARA Structure, and saved in *.pdb* format for virtual screening. The AChE homodimer (PDB ID: 6O4W) was prepared by using only Chain A as the receptor, removing co-crystallized ligands, water molecules, Chain B, and other ligands, and adding hydrogens. The final receptor was saved in *.sce* format. These procedures followed Safithri et al. (2025) [26].

Validation of Docking Area (Grid Box) for Virtual Screening

The docking area (grid box) for test ligands was determined based on the optimal redocking position of the co-crystallized ligand, donepezil, on AChE (PDB ID: 6O4W), a critical step for virtual screening. Grid box validation was performed in YASARA Structure using the VINA method via the dock_run.mcr script. The grid was centered on donepezil, with sizes from 2.0 to 7.0 Å (0.5 Å intervals) and validated 100 times. Binding free energy (ΔG_{bind}) was calculated using the AMBER14 force field, and redocking accuracy was assessed by RMSD (< 2.0 Å) between redocked and crystal conformations. The final 3D visualization confirmed positional and conformational accuracy. This procedure followed Safithri et al. (2025) [26].

Virtual Screening

Virtual screening was conducted using the best grid box size determined from the validation step. Natural compounds found in black tea were screened and compared with donepezil, a known natural AChE inhibitor [27]. The geometrically optimized test ligands were docked to AChE using the *dock_runscreening.mcr* script based on the VINA method in YASARA Structure, employing the validated grid box size of 7 Å.

The binding free energy (ΔG_{bind}) between ligands and receptor was calculated using the AMBER14 force field. Each ligand was screened with 100 repetitions, and the best binding pose was selected. The output files included *.txt files* containing ΔG_{bind}, dissociation constant (K_d), and amino acid residue contact data, as well as *.yob files* containing docking poses.

Analysis of Virtual Screening Results

Ligand–receptor interactions were visualized in two dimensions using Discovery Studio and in three dimensions using YASARA Structure. Potential inhibitor candidates among the test ligands were analyzed based on ΔG_{bind} values, dissociation constants, amino acid residue interactions, and ligand docking positions relative to the co-crystallized ligand (donepezil) and the reference ligand (rivastigmine).

Analysis of Biological Activity (PASS), Drug-Likeness, DFT, and Toxicity

Drug-likeness was evaluated using Lipinski's Rule of Five (RO5) via SwissADME (http://www.swissadme.ch/) by inputting each compound's canonical SMILES. Biological activity and protein target predictions related to Alzheimer's disease therapy were obtained using PASS Online (https://www.way2drug.com/passonline/) and PASS Targets (https://www.way2drug.com/passtargets/). Human intestinal absorption (HIA) and blood–brain barrier (BBB) permeability were predicted using ADMETSAR 3.0, while carcinogenicity and toxicity were assessed with Protox 3.0. Quantum chemical calculations followed Neese et al. (2020) [28] using the DFT method (B3LYP-D3MBJ/6-31G) implemented in Orca 6.0.0 to determine HOMO and LUMO energies. These were further used to calculate the HOMO–LUMO gap (ΔE), chemical softness, hardness, global electrophilicity index, and electronegativity.

Extract Preparation

The sample extraction was conducted using the maceration method. A total of 10 g of simplicia was dissolved in 100 mL of 70% ethanol (1:10). The maceration process was repeated for 24 hours under dark conditions at room temperature. The mixture was then filtered through filter paper, and the residue was re-macerated with fresh solvent until the resulting filtrate no longer exhibited the characteristic color of the sample. All filtrates obtained were pooled and subsequently concentrated using a rotary evaporator at 50 °C until a paste-like extract was formed. The extraction was performed in duplicate, and the extract yield was expressed as a percentage calculated using the equation described by Safithri et al. (2022) [26] and Huda et al. (2022) [29], resulting in an average yield of 37.014 ± 0.298% (equation 1).

$$\text{Extract yeild (\%)} = \frac{\text{Weight of extract obtained (g)}}{\text{Weight of simplicia sample (g)}} \times 100 \quad \text{.....(1)}$$

Acetylcholinesterase (AChE) Enzyme Inhibition Assay (Abcam: ab138871)

The assay involved three types of solutions: sample, blank, and positive control. All solutions were incubated in the dark for 30 minutes before spectrophotometric analysis at 408 nm. The standard curve was prepared by combining 50 µL of ddH_2O, 50 µL of AChE solution, and 50 µL of ATCh mix. The ATCh mix contained acetylthiocholine (ATCh) as a synthetic substrate, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent) as a colorimetric indicator, and assay buffer.

AChE solutions were prepared in a concentration series: 500, 300, 150, 125, 100, 75, 50, 30, 10, 3, 1, and 0 mU/mL. These standards were also incubated in the dark for 30 minutes before spectrophotometric measurement at 408 nm. Acetylthiocholine is hydrolyzed by acetylcholinesterase to produce thiocholine containing a free thiol (-SH) group, which reacts with DTNB to form a yellow product, TNB^{2-} (5-thio-2-nitrobenzoic acid), that absorbs light at 408–412 nm. The intensity of the yellow color (measured at 408–412 nm) corresponds to AChE activity. The standard curve was constructed by plotting absorbance values against enzyme concentrations and used to determine enzyme activity in unknown samples or black tea water extracts in this study.

The percentage of acetylcholinesterase (AChE) inhibition was calculated using the equation (2).

$$\%\text{Inhibition} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100 \ldots\ldots (2)$$

where A_{control} represents the absorbance of the reaction mixture without the inhibitor, and A_{sample} is the absorbance obtained in the presence of the extract or the reference inhibitor. All measurements were performed in duplicate. The black tea extract was tested at concentrations of 0.1, 1, 5, 10, 20, 30, 40, and 50 µg/mL. Donepezil HCl, used as the standard AChE inhibitor, was evaluated at concentrations of 0.00625, 0.0125, 0.025, 0.05, 0.1, and 0.2 µg/mL.

The inhibition values were plotted against the logarithm of the concentration, and the half-maximal inhibitory concentration (IC_{50}) was determined using a four-parameter logistic (4PL) non-linear regression model [23]. IC_{50} values for both the extract and Donepezil HCl are reported as mean ± standard deviation (SD) based on duplicate measurements.

3. RESULTS AND DISCUSSION

Structural Characteristics of Acetylcholinesterase (AChE; 6O4W) and Grid Box Validation for Molecular Docking

Acetylcholinesterase (AChE; PDB ID: 6O4W) is a homodimeric enzyme linked by six hydrogen bonds, forming a quaternary structure [27,30]. Its active site is located ~20 Å beneath the surface and consists of the catalytic triad Ser203, His447, and Glu334 (**Figure 1**). The active-site gorge contains functionally important regions, including the peripheral anionic site (PAS), acyl binding pocket, oxyanion hole, and anionic subsite. PAS residues (Tyr72, Asp74, Tyr124, Ser125, Trp286, Tyr337, Tyr341) guide acetylcholine into the gorge and interact with amyloid-β, linking AChE activity to Alzheimer's disease (AD) pathology. The acyl binding pocket (Trp236, Phe295, Phe297, Phe338) near Ser203 accommodates the acetyl group, facilitating tetrahedral intermediate formation and product release, while the oxyanion hole (Gly121, Gly122, Ala204) and anionic subsite (Trp86, Tyr133, Glu202, Gly448, Ile451) stabilize transition states and maintain substrate orientation and catalytic efficiency [31-33].

The optimal grid box for molecular docking was determined based on ΔG_{bind}, RMSD, and ligand–receptor interaction similarity between re-docked and co-crystallized donepezil. All grid box sizes produced poses highly similar to the native ligand (RMSD 0.0011–0.0019 Å), with the 7 Å box selected for its larger volume and consistent interaction patterns [27] (ΔG_{bind} = –12.3500 kcal/mol; RMSD = 0.0011 Å; **Figure 2**).

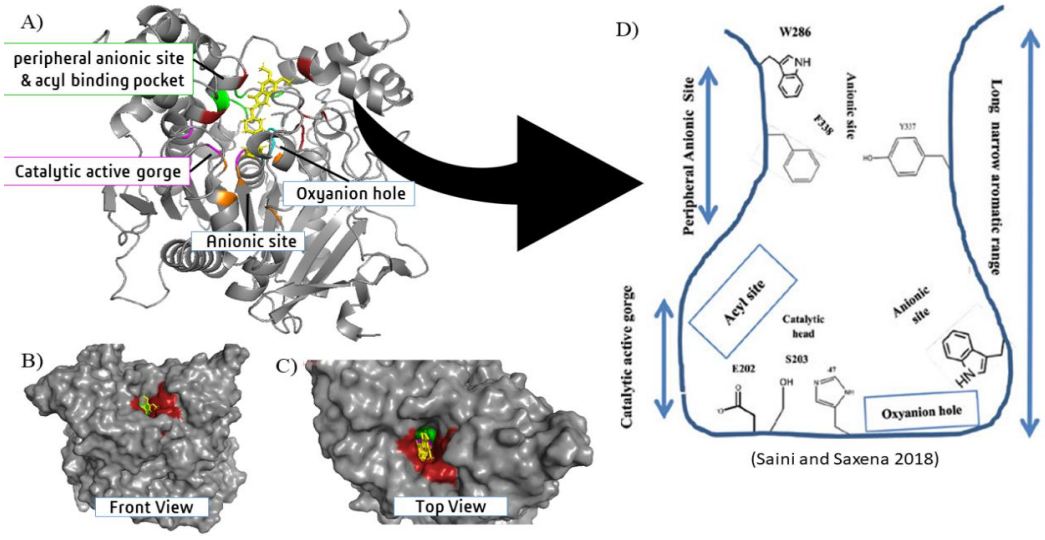

Figure 1. Active "gorge" site and other key residues of AChE (6O4W) [34-35]. (A) Active and functional regions shown in cartoon form; (B) Front view; (C) Top view; (D) Schematic representation of the AChE active gorge [34]. Green: acyl binding pocket; brown: peripheral anionic site (PAS); orange: anion subsite; cyan: oxyanion hole; magenta: active or catalytic site.

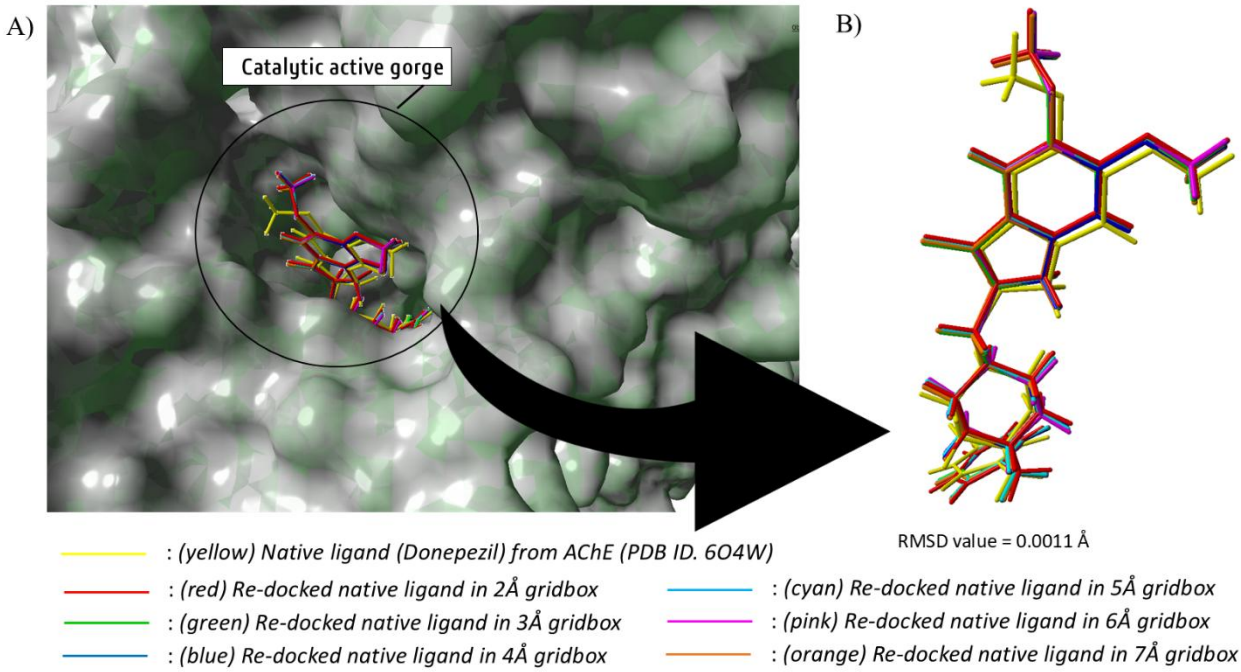

Figure 2. Re-docking of the native inhibitor (donepezil) against AChE during grid box validation. (A) Result using AutoDock Vina; (B) Result using YASARA Structure. Red ball-and-stick: native donepezil; yellow ball-and-stick: re-docked donepezil.

Evaluation of Selected Compounds Following Docking

Virtual screening of 20 bioactive compounds obtained from secondary data reported by Hasan et al. (2022) [25], identified several potential acetylcholinesterase (AChE; PDB ID: 6O4W) inhibitors using YASARA Structure. Donepezil exhibited a ΔG_{bind} of –12.350 kcal/mol, while test ligands ranged from –5.981 kcal/mol (L-theanine) to –12.274 kcal/mol (Epigallocatechin gallate, EGCG). Lower ΔG_{bind} values indicate stronger receptor binding [35-37]. EGCG showed the closest ΔG_{bind} to donepezil, followed by Chlorogenic Acid (–10.858 kcal/mol), Theaflavin (–10.544 kcal/mol), Theaflavin-3-O-gallate (–10.892 kcal/mol), and Theaflavin-3-gallate (–10.857 kcal/mol). These results align with prior findings that catechins and theaflavins constitute the predominant polyphenols in black tea [38]. The strong *in silico* affinity observed in this study is consistent with previously reported AChE inhibitory activities, where EGCG, theaflavin, and theaflavin-3-gallate exhibited IC_{50} values of 30.99 ± 1.5 µg/mL, 16.64 ± 0.4 µg/mL, and 2.38 ± 0.08 µg/mL, respectively [23].

EGCG exhibited the most potent inhibitory potential, forming eight hydrogen bonds with critical residues including Tyr72, Asp74, Ser125 (Peripheral Anionic Site), Trp86 (anion subsite), Trp286 (PAS), and Gly122 (oxyanion hole). It also engaged in multiple hydrophobic interactions, π–π stacking, π–π T-shaped contacts, and Van der Waals interactions with His447 and the acyl-binding pocket (**Figure 3**; **Table 1**), stabilizing the complex and potentially hindering acetylcholine access. Donepezil and rivastigmine (**Figure 4**), in contrast, formed only a single hydrogen bond with a critical residue, including Tyr124 and Phe295.

Other top ligands—Chlorogenic Acid, Theaflavin, Theaflavin-3-O-gallate, and Theaflavin-3-gallate—also showed favorable binding. Theaflavin-3-O-gallate formed four hydrogen bonds, including Phe295 (acyl binding pocket), supported by π–π interactions (**Table 1**). Chlorogenic Acid established nine hydrogen bonds, including with Gly121, Gly122 (oxyanion hole), and Ser203 (active site). Theaflavin-3-gallate formed six hydrogen bonds with PAS residues, while Theaflavin formed only two. According to Luo et al. (2024) [39], thearubigins, theaflavins, and its derivatives are the major polyphenols in black tea [40,41], formed during enzymatic fermentation catalyzed by polyphenol oxidase and peroxidase—processes absent in green tea. This oxidation converts catechins into benzotropolone-based dimers, producing theaflavins and thearubigins [39,42]. Among these, theaflavins contribute most to black tea's color, flavor, and bioactivity, including antioxidant, anti-inflammatory, and neuroprotective effects [41,43-46].

Overall, EGCG displayed the broadest interaction network spanning both catalytic and peripheral sites, with binding affinity comparable to Donepezil and exceeding Rivastigmine. Tea polyphenols are generally divided into (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-

epicatechin (EC) [45]. EGCG, a polyphenol from the flavan-3-ols subclass, is abundant in green tea but present in smaller amounts in black tea [42] and exhibits antioxidant [47], anti-inflammatory [48], and neuroprotective properties [49-50].

The extensive phenolic hydroxyl groups of EGCG enable multiple hydrogen bonds, hydrophobic, and Van der Waals interactions, stabilizing the AChE complex and supporting its neuroprotective potential. These findings align with prior studies demonstrating EGCG's ability to prevent amyloid aggregation and remodel misfolded proteins [49,50], highlighting its promise as a natural anti-Alzheimer's agent.

Table 1. ΔG_{bind}, Dissociation constant, and interactions of selected ligands against AChE (PDB: 6O4W)

Compound	ΔG_{bind} (kcal/mol)	Dissociation constant (µM)	Total of H-Bond	H-bond (length in Å)	Hydrophobic & other Interactions
Donepezil (Native ligand/Co-cristall ligand)	-12,350	0,885	2	Phe295 (1,91), *Ser293 (2,99)*	• **Pi-Pi Stacked :** His447, Trp286 • **Pi-Sigma :** Phe338, Tyr341 • **Pi-Cation :** Trp86, Tyr337 • **VdW :** Tyr72, Tyr124, Glu202, Ser203, Leu289
Rivastigmine (reference ligand)	-8,055	1246	1	Tyr124 (2,13)	• **Pi-Pi Stacked & Pi-Pi T-Shaped :** Tyr337, Phe338 • **Pi-Alkyl :** Trp86, His447, Trp286, Phe297, Tyr341 • **VdW :** Asp74, Gly121, Glu202, Ser203, Ala204, Phe295,Arg296
Epigallocatechin gallate	-12,274	1	8	Tyr72 (3,00), Asp74 (2,60), Trp86 (1,77), Gly122 (2,26), Gly126 (3,02), *Ser125 (2,23), Trp286 (2,91, 3,29)*	• **Pi-Pi Stacked** : Tyr341, Tyr124, Trp86, Trp286 • **Pi-Pi T-Shaped :** Tyr341, Tyr337, Phe338 • **VdW :** Gln71, Gly121, Leu130, Phe295, Phe297, His447 • **Unfavorable Acceptor-Acceptor :** Asn87
Theaflavin-3-O-gallate	-10,892	10	4	*Val294 (2,49),* Phe295 (2,44), Arg296 (2,34), Gly342 (2,92)	• **Pi-Pi Stacked :** Tyr72, Trp286 • **Pi-Pi T-Shaped :** Trp286 • **Alkyl & Pi-Alkyl:** Leu76 • **Unfavorable Donor-Donor (& Acceptor-Acceptor) :** Glu292
Chlorogenic Acid	-10,858	11	7	Gly121 (2,58), Gly122 (2,86), Tyr124 (2,66; *2,88)* Ser203 (2,71, 2,59), Ser293 (2,15, 2,83), Arg296 (2,18)	• **Pi-Pi Stacked :** Trp286 • **Pi-Alkyl :** Phe338 • **VdW:** Gly120, Val294, Leu289, Phe297, Phe295, Tyr341
Theaflavin-3-gallate	-10,857	11	6	Tyr72 (2,39), Tyr124 (2,04), Gln291 (2,34), Ser293 (2,48), *Val294 (2,36), Tyr341 (2,49)*	• **Pi-Pi Stacked :** Trp286 • **Pi-Alkyl :** Leu76, Leu289 • **VdW :** Asp74, Phe295, Phe297

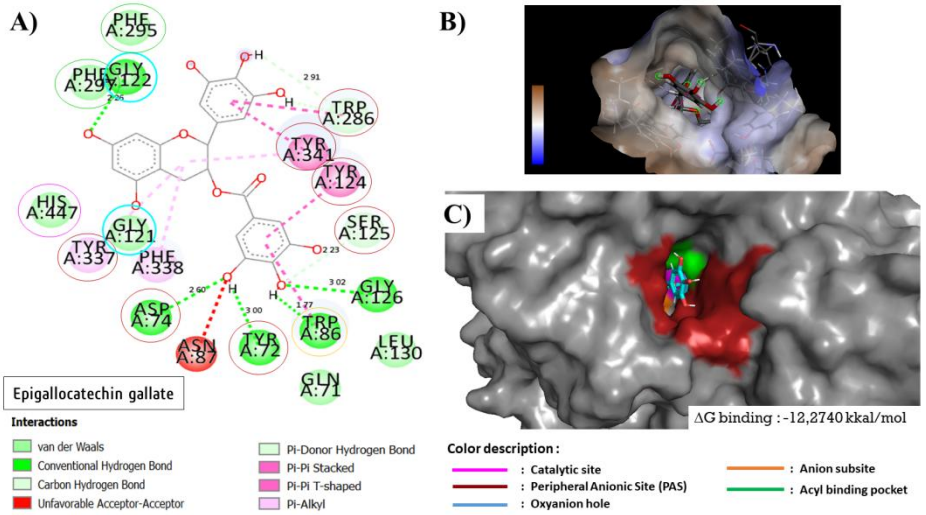

Figure 3. 2D and 3D visualization of Epigallocatechin gallate docking with AChE (6O4W).

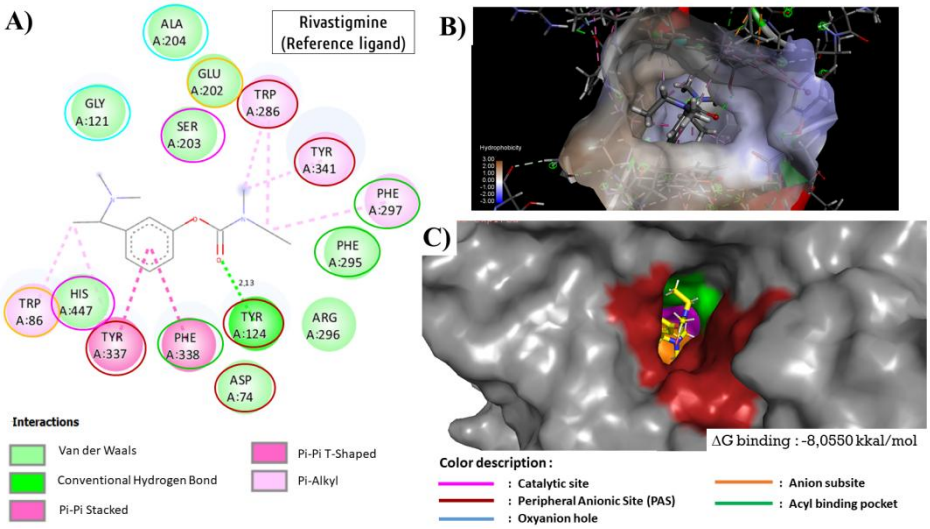

Figure 4. 2D and 3D visualization of Rivastigmine (reference ligand) docking with AChE (6O4W).

PASS, Druglikeness, Carcinogenicity, Toxicity, and Quantum Mechanical Properties

The inhibitory potential of black tea compounds against acetylcholinesterase (AChE; PDB ID: 6O4W) was evaluated using molecular docking, PASS Online, and PASS Target analyses. These structure–activity relationship (SAR)-based models showed high predictive reliability (>95%) [48]. Reference ligands donepezil and rivastigmine validated the system, with donepezil showing the highest probability of activity (Pa = 0.788) as a cholinergic AChE inhibitor, while rivastigmine exhibited a lower Pa value (0.227).

Epigallocatechin gallate (EGCG) emerged as a promising β-amyloid antagonist (Pa = 0.436) among the ligands selected through molecular docking, interacting with calcium channels and amyloid precursor-binding proteins. Theaflavin-3-O-gallate and theaflavin-3-gallate exhibited the highest Pa value (0.584) as amyloid aggregation inhibitors, whereas chlorogenic acid was predicted as a dementia-modulating compound (Pa = 0.258). PASS analysis confirmed that all four compounds had anti-Alzheimer's potential (Pa = 0.2–0.5; Pi < 0.5), consistent with docking results indicating strong receptor affinity.

ADMET predictions using SwissADME and ProTox 3.0 revealed that only EGCG and chlorogenic acid met Lipinski's Rule of Five, suggesting better drug-likeness and bioavailability (**Figure 5**). Theaflavin derivatives violated the rule due to high molecular weight and hydrogen bonding. Reference drugs showed high human intestinal absorption (HIA) and blood–brain barrier (BBB) permeability, while tea polyphenols displayed lower values. Nevertheless, all ligands were predicted to be non-carcinogenic and of low toxicity (classes IV–V).

Despite favorable docking and PASS predictions, the black tea compounds exhibited limited systemic bioavailability and poor blood–brain barrier (BBB) permeability, which may restrict their efficacy within the central nervous system (CNS). Nevertheless, other studies have reported that

theaflavin, one of the major constituents of black tea, not only accelerates functional recovery of the sciatic nerve but also effectively mitigates oxidative stress [51]. These limitations are typical of polyphenolic compounds and may be addressed through nanoformulation or structural modification to enhance brain delivery [52]. Nanoformulation approaches, in particular, offer promising strategies to overcome these pharmacokinetic constraints. Encapsulation of bioactive compounds into nanocarriers such as lipid-based nanoparticles, polymeric nanoparticles, nanoemulsions, or solid lipid nanoparticles has been shown to significantly improve solubility, stability, and resistance to gastrointestinal degradation. Moreover, nanocarriers can be engineered with surface modifications (e.g., PEGylation, ligand-functionalization targeting transferrin or lactoferrin receptors) to facilitate transport across the BBB via receptor-mediated transcytosis [53]. These systems not only enhance bioavailability but may also prolong circulation time and enable controlled release, thereby increasing the likelihood of achieving therapeutically relevant concentrations in the brain. Consequently, integrating nanoformulation strategies may substantially improve the translational potential of black tea polyphenols as neuroprotective agents for Alzheimer's disease [54].

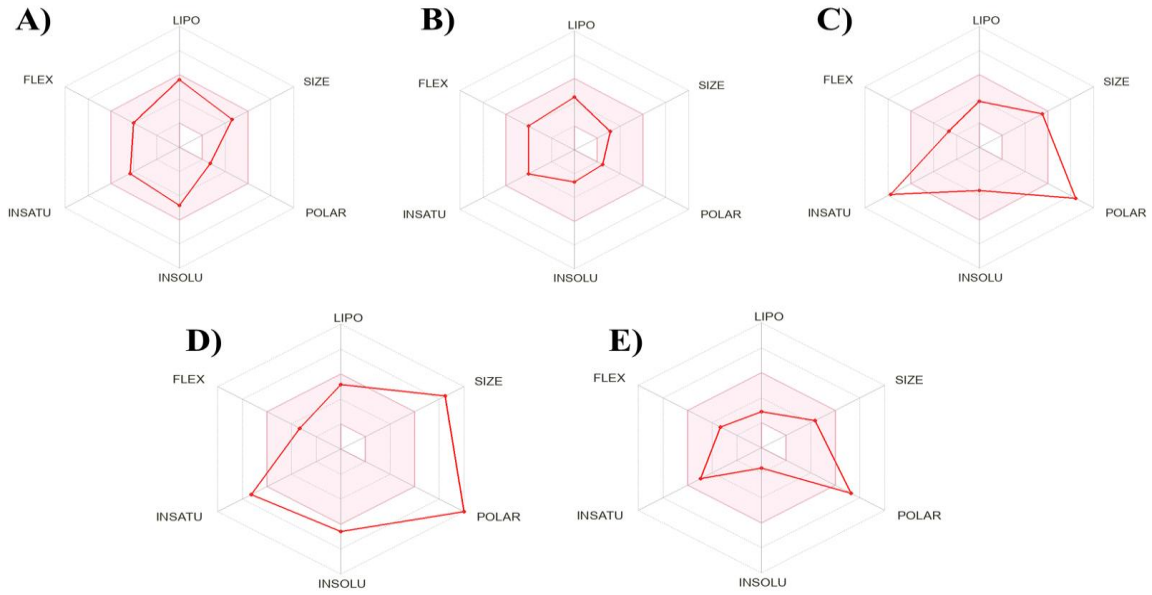

Figure 5. Visualization of bioavailability radar results generated using SwissADME for (A) Donepezil, (B) Rivastigmine, (C) Epigallocatechin gallate, (D) Theaflavin-3-O-gallate, and (E) Chlorogenic Acid.

Table 2. HOMO-LUMO energy values and other properties.

Ligands	Homo (eV)	Lumo (eV)	Energy gap (eV)	I (eV)	A (eV)	μ (eV)	χ (eV)	η (eV)	S (eV)	ω (eV)
Donepezil	-5.72	-1.09	4.63	5.72	1.09	-3.40	3.40	2.32	0.22	2.50
Rivastigmine	-5.75	0.13	5.88	5.75	-0.13	-2.81	2.81	2.94	0.17	1.35
Epigallocatechin gallate	-5.53	-1.11	4.42	5.53	1.11	-3.32	3.32	2.21	0.23	2.50
Chlorogenic Acid	-6.05	-2.15	3.90	6.05	2.15	-4.10	4.10	1.95	0.26	4.31

To further elucidate their electronic characteristics, frontier molecular orbital (FMO) analysis was performed using density functional theory (DFT). This approach provides insight into molecular reactivity and stability by evaluating the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) (37). The HOMO energy reflects the electron-donating capacity, while the LUMO energy represents the electron-accepting ability [55]. Among the tested ligands, EGCG exhibited the highest HOMO value (–5.53 eV), indicating superior electron-donating potential. Conversely, chlorogenic acid had the lowest HOMO (–6.05 eV) and LUMO (–2.15 eV)

values, suggesting high molecular stability and the strongest electron-accepting capacity (**Table 2**). These findings support the observed docking interactions and highlight EGCG and chlorogenic acid as the most promising candidates for further development as anti-Alzheimer's agents. Overall, EGCG and chlorogenic acid showed the most promising pharmacological profiles among the tested black tea compounds, indicating their potential as natural AChE inhibitors and anti-Alzheimer's agents for further *in vitro* and *in vivo* exploration.

Acetylcholinesterase (AChE) Enzyme Inhibition

The inhibitory activity of the black tea water extract originating from Bogor, Indonesia, was evaluated to validate the *in silico* predictions. The water extract exhibited concentration-dependent inhibition of AChE activity. The Bogor black tea extract showed strong inhibitory effects, with an IC_{50} value of 44.85 ± 1.48 µg/mL (**Table 3**; **Figure 6**). When compared with Donepezil (**Table 4**, **Figure 7**), a standard AChE inhibitor and commercially used drug for Alzheimer's disease, the IC_{50} value of the Bogor black tea extract was considerably higher, indicating that Donepezil retains a stronger inhibitory potency. However, relative to other studies, the Bogor black tea extract demonstrated a more favorable AChE inhibitory profile, with a lower IC_{50} than that reported by Okello et al. (2004) [57], which was 60 µg/mL, and slightly higher than the IC_{50} value reported by Piyasena et al. (2025) [58], which was 36.75 µg/mL.

Table 3. IC_{50} value of black tea from Bogor, Indonesia.

Black Tea	Concentration (µg/mL)								IC_{50} (µg/mL)	Mean of IC_{50}	SD
	0,1	1	5	10	20	30	40	50			
Replicate 1	1,58	6,00	11,69	16,75	30,96	33,18	50,55	52,09	43,81	44,85	1,48
Replicate 2	0,64	5,09	11,45	16,85	30,21	32,43	50,55	52,49	45,90		

Table 4. IC_{50} value of Donepezil as a reference AChE inhibitor.

Donepezil HCl	Concentration (µg/mL)						IC_{50} (µg/mL)	Mean of IC_{50}	SD
	0,00625	0,0125	0,025	0,05	0,1	0,2			
Replicate 1	1,1834	24,4576	44,9704	78,5010	87,9684	96,4286	0,0291	0,0289	0,0003
Replicate 2	1,9646	23,9686	45,5796	78,9784	88,4086	97,2332	0,0287		

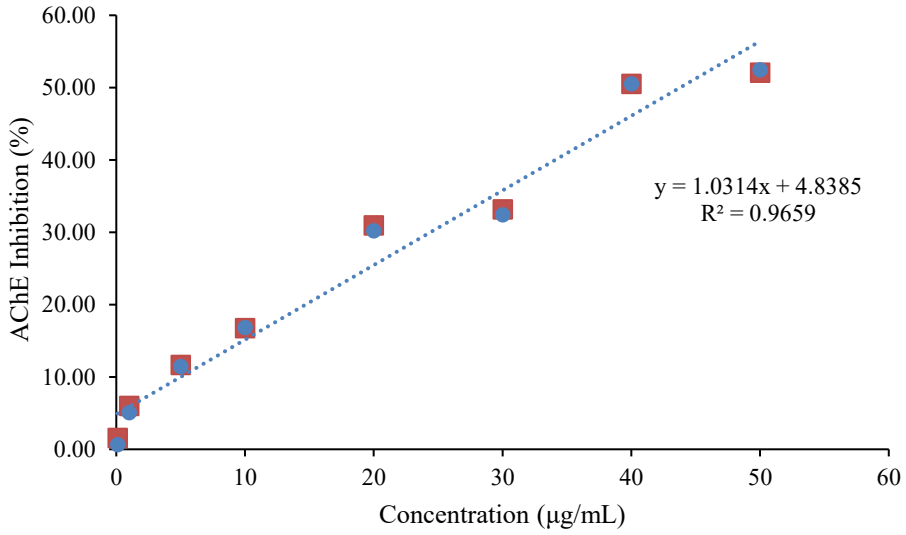

Figure 6. Inhibition curve of AChE by black tea water extract from Bogor. Red squares represent the first replicate, and blue circles represent the second replicate.

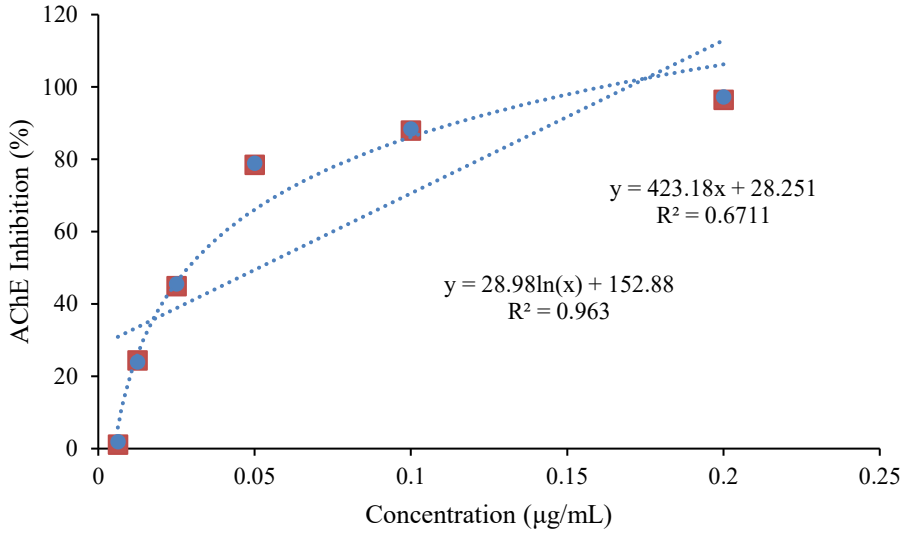

Figure 7. Inhibition curve of AChE by Donepezil as positive control (standard AChE inhibitor). Red squares represent the first replicate, and blue circles represent the second replicate.

The *in vitro* assay results supported the computational findings. These data confirm the anti-Alzheimer's potential of Indonesian black tea, particularly through the stable and reactive characteristics of EGCG and chlorogenic acid as indicated by DFT energy-gap (eV) analysis. The affinity and stability of EGCG, one of the major constituents of black tea, toward AChE are also consistent with the findings of Samanta et al. (2022), who reported an AChE inhibitory IC_{50} value of 30.99 ± 1.5 µg/mL. Using this integrated approach, the study effectively identified candidate compounds with potential anti-Alzheimer's activity, highlighted their interactions within the AChE active-site gorge, and reinforced their relevance as prospective therapeutic agents.

4. CONCLUSIONS

Our *in silico* analyses identified several polyphenolic compounds from Indonesian black tea with strong anti-Alzheimer's potential through acetylcholinesterase (AChE) inhibition. Epigallocatechin gallate exhibited the most favorable binding affinity (ΔG_{bind} = –12.2740 kcal/mol), followed by theaflavin derivatives and chlorogenic acid, all of which interacted effectively within the AChE active-site gorge. These compounds also demonstrated favorable PASS predictions and toxicity profiles, although their bioavailability remained limited. DFT analysis revealed lower energy gaps compared with reference inhibitors, indicating higher molecular reactivity and greater binding stability. *In vitro* assays supported the computational predictions, with black tea extract from Bogor showing strong AChE inhibitory activity consistent with previous studies, although its inhibition capacity remained lower than that of Donepezil. Overall, these findings highlight Indonesian black tea as a promising natural source of AChE inhibitors for Alzheimer's therapy. Future research should prioritize strategies to improve the bioavailability of these compounds.

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