Green Tea Augments Cognitive Function: An *In Silico* Model

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Adittya Sabhayasachi Khan[1,2], Rezowana Mannan[1,3], Laila Anjuman Banu[4], Sohidul Islam[2]* and Mahmud Hossain[1]*

[1] *Laboratory of Neuroscience and Neurogenetics, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000, Bangladesh.*
[2] *Department of Biochemistry & Microbiology, North South University, Bangladesh.*
[3] *Institute of Epidemiology, Disease Control & Research (IEDCR), Mohakhali, Dhaka-1212, Bangladesh.*
[4] *Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka-1000, Bangladesh.*

ABSTRACT: Recent scientific advancements have sparked an increasing trend of returning to nature. Scientists worldwide prefer natural medical derivatives over synthetic ones due to fewer side effects. Green tea is abundant in bioactive components and vitamins. Although most components of green tea were thought to be absorbed inadequately by oral administration, they are essential for better health. In the present study, an *in silico* approach was taken to evaluate the effect or correlation of bioactive components of tea on memory retention, cognitive performance, and prevention of neurodegenerative diseases that result in memory alterations, dementia, and cognitive dysfunction. Furthermore, binding of bioactive components with brain-specific proteins and possible alterations in those proteins due to tea components were illustrated. Four critical brain-specific proteins were evaluated in the present molecular analysis. Cyclooxygenase 1 (COX1), Acetylcholinesterase (AChE), Amyloid-β Precursor Protein (APP1), and Cytochrome P4502D6 (Cyp2D6) were the proteins involved. Their interaction with the bioactive components of green tea was evaluated using computational molecular docking analysis (CMDA). The bioactive molecules were Epigallocatechin gallate (EGCG), L-Theanine, Kaemferol, Coumarin, and Myricetin. The beneficial effect of green tea on memory was prioritized in this study. CMDA has shown possible inhibition of acetylcholinesterase, amyloid-β protein, cyclooxygenase 1, and Cytochrome P4502D6 (Cyp2D6). Bioactive components of green tea passed the blood-brain barrier and influenced short-term memory at low concentrations. Significant dosage or concentration in capsulated form might result in long-term effects since both bioavailability, and concentration of essential components of green tea are scarce.

KEYWORDS: *EGCG (Epigallocatechin Gallate), AChE (Acetylcholinesterase), Cyp2D6 (Cytochrome P4502D6), COX1 (Cyclooxygenase 1), APP1 (Amyloid-β Precursor Protein 1)*

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CORRESPONDING AUTHOR: Sohidul Islam and Mahmud Hossain, Department of Biochemistry & Microbiology, North South University and Laboratory of Neuroscience and Neurogenetics, Department of Biochemistry and Molecular Biology, University of Dhaka
Email: mahmudbio1480@du.ac.bd, sohidul.islam@northsouth.edu

Introduction

Tea is a traditional beverage served at every corner of Bangladesh. Due to its affordability, from mundane black tea to sophisticated green tea, tea is a traditional beverage for all ages and classes. However, local demand for affordable tea inhibits the production of high-quality tea for export. Tea is primarily prepared with warm water, milk, and sugar, along with introducing additives such as blood orange juice, tamarind, green chili pepper slices, citron slices, ginger, and spices (black cardamom, cinnamon, cloves, etc.) to make the drink flavorsome. The origin of tea dates to 207 BCE, when the plant was domesticated in the Han dynasty of China (Lu *et al.*, 2016). All types of tea originate from the same plant (*Camellia sinesis*), but the method of processing and degree of oxidation of tea leaves determines the variation among tea flavors. Green tea is prepared with the least amount of oxidation (10%) to preserve polyphenols, oolong tea is partially oxidized (10-85%), and black tea is fully oxidized (> 85%), containing the most caffeine and flavonoids (Cerbin-Koczorowska et al., 2021). According to some sources, green tea is referred to as non-fermented tea (Cabrera, Artacho and Giménez, 2006) known for its subtle and delicate flavor. On average, the caffeine percentage is less in green tea than in black tea. The flavor of green tea is vegetal, astringent, herbaceous, and earthy (Lee, Chambers and Chambers IV, 2014).

Green tea contains the non-protein amino acid Theanine or L-Theanine, free sugars, methyl xanthine or purine alkaloids such as caffeine, theobromine, theophylline, and phenolic acids such as gallic acid, catechins such as catechin (C), (+)-catechin (C), catechin gallate (CG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-gallocatechin gallate (GCG), (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) and flavonols such as myricetin, kaempferol, quercetin, chlorogenic acid, coumarylquinic acid or coumarin, and theogalli (Tounekti *et al.*, 2013; Peng *et al.*, 2008). Catechins such as epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epigallocatechin-3-gallate (EGCG) are prominent in green tea along with polyphenolic

compounds such as flavonoids and tannins (Cerbin-Koczorowska *et al.*, 2021). Green tea catechins are collectively known as polyphenols (Banerjee and Chatterjee, 2015). Green tea is also notable for vitamins such as vitamin A, E, and B complex (Fernández *et al.*, 2002), minerals, and trace elements such as K, Mn, Cr, Zn, and Ni (Komes *et al.*, 2010). Alkaloids such as L-Theanine, catechin epigallocatechin gallate, proanthocyanidins, and flavonols such as myricetin, kaempferol, and coumarin, among others, contribute to green tea's beneficial properties (Dietz and Dekker, 2017). The composition of bioactive molecules in tea depends on the age of the leaf, harvesting time, climate of production, and processing method. Green tea has the most polyphenol among all types of tea since it is least oxidized and thus retains polyphenols while impeding flavonoids (Stegemann, 2007). Proven benefits of the bioactive components of green tea include anti-obesity, anti-diabetic, anti-inflammatory, antioxidant, anti-cancer, antiviral, protection against metabolic and neurodegenerative diseases, and neuroprotective actions such as inhibition of neurodegenerative impairment, cognitive dysfunction, and memory loss (Cerbin-Koczorowska *et al.*, 2021; Pervin *et al.*, 2018). Proteins participating in memory alterations and cognitive dysfunction are considered. The four proteins are Amyloid-β Precursor Protein (APP), Cyclooxygenase 1 (Cox 1), Acetylcholinesterase (AChE), and CytochromeP450 2D6 (Cyp2D6). AChE hydrolyzes the neurotransmitter acetylcholine (Dvir *et al.*, 2010), COX converts arachidonic acid to prostaglandin (Miciaccia *et al.*, 2021), Cyp2D6 has a modulatory effect on dopamine concentration (Miksys and Tyndale, 2002), and APP is a membrane receptor maintaining synapses, memory formation and neuronal plasticity (Garcia-Osta and Alberini, 2009). The *in silico* model, demonstrated significant inhibition of AChE, Cyp2D6, and COX1, with partial inhibition of APP1 by the selected components of green tea.

This study focuses on the neuronal benefits of green tea by developing an *in silico* model to emphasize green tea's neuroprotective effects and emphasizing its five distinct components namely: coumarin, kaempferol, epigallocatechin (EGC), L-Theanine, and myricetin by Computational Molecular Docking Analysis (CMDA) on cognitive function, brain stimulation, and memory retention.

Materials and Methods

Preparation of protein structures

The crystal structures of Cyclooxygenase 1 (COX1), Cytochrome P450 (Cyp2D6), Amyloid-β Precursor Protein 1 (APP1), and Acetylcholinesterase (AChE) were collected from the Protein Data Bank (PDB) database (www.rcsb.org) (Figure 1). The PDB ids of the proteins and enzymes were 6y3c (COX1) (Miciaccia, M., Scilimati, 2021), 2F9Q (Cyp2D6) (Rowland, P., Bridges, 2006), 3PMR (APP1) (Lee, S., Ha, 2011), and 3LII (AChE) (Dvir, H., Sussman, 2010). By the use of Biovia Discovery Studio (Dassault Systèmes, 2020), all the water molecules and unnecessary polypeptide chains or atoms were removed, followed by the addition of polar hydrogens to evaluate the hydrogen bonds. The crystal structure was then saved in '.pdqt' format for further analysis.

Preparation of ligand structures

Green tea contains many bioactive molecules. Depending on the target of the experiment, five compounds were selected. The ligand structures of the five compounds were retrieved from the PubChem database (pubchem.ncbi.nlm.nih.gov). The bioactive compounds satisfy Lipinski's rule of five (Table 1). The files were downloaded in SDF format, and Open Babel of PyRx software (Dallakyan and Olson, 2015) was used to minimize energy and conversion of SDF files to PDB files and then into .pdbqt files. Finally, the ligands were ready for docking analysis.

Molecular docking

The proteins or enzymes were designated as macromolecules individually, followed by docking with five ligands on each turn. The grid box is a 3-dimensional box used to encapsulate the whole macromolecule. Care was taken to ensure that not a single portion or amino acid of the macromolecule crosses the gird box. Finally, a multi-ligand docking analysis was completed without any specific grid parameters. The docking analysis was imported from the PyRx software in a comma-separated value file (CSV). The CSV file was later converted to an excel file for feasibility. Each docking analysis gave nine respective binding energies for each ligand, the highest binding energy from each was taken into account for comparison.

Virtual screening

The docked ligands with the highest affinity were imported in PDB files for further analysis in the BIOVIA Discovery Studio. The highest affinity ligand was merged with the specific protein or enzyme for the hydrogen bonds and interacting amino acids. The more the hydrogen bonds, the higher was the interaction or the strength of interaction. Thus, enhanced interaction between ligand and protein and an elevated possibility of interaction in the biological system was estimated. Finally, PYMOL (Yuan, Chan and Hu, 2017) was used to evaluate the active site of molecular interactions, and 5Å (Angstrom unit) was taken to measure the possible amino acids interacting with the active site (Figure 4).

Results

The Computational Molecular Docking Analysis (CMDA) helped to understand the molecular basis of the mechanism of small therapeutic agents (Pervin et al., 2018). The present study used CMDA to evaluate the effects of catechin, flavonoids, and polyphenols on cognitive function, brain stimulation, and memory retention. CMDA was comprised of protein and ligand preparation followed by molecular docking and virtual screening. Molecular docking with the PyRx software (Dallakyan and Olson, 2015) provided the binding free energy and Rmsd/ub values (Tables 2 and 3). Virtual screening with BIOVIA Discovery Studio illustrated ligand binding with respective proteins, the preferable amino acid residues close to the ligand, and the hydrogen bond interactions between the respective ligands and proteins.

Evaluation of protein structures

Four distinct proteins were evaluated in the CMDA. The proteins were extracted from RCSB (www.rcsb.org) and edited by BIOVIA Discovery Studio for molecular analysis.

Amyloid-β-Precursor Protein 1 (APP1) (Lee *et al.*, 2011), Acetylcholinesterase (AChE) (Dvir *et al.*, 2010), Cytochrome P450 (Cyp2D6) (Rowland *et al.*, 2006), and Cyclooxygenase (Cox1) were the proteins studied by *in silico* (Figure 1). Approximately 250 kinases and many receptors involved in regulating memory (for instance, extracellular signal-regulated kinase 1 and 2 (ERK1/2), cAMP-dependent protein kinase A (PKA), cGMP-dependent protein kinase G (PKG), HDAC2 receptor, and NMADR1 receptors) are excluded from this *in silico* model as most of their functions are still a riddle. Superoxide dismutase (SOD) was not considered as it affects amyloid-protein plaques and impairs other enzymatic functions (Massaad *et al.*, 2009), and has four distinct organelle-specific forms (Younus, 2018). The polypeptide chains of the proteins used in molecular docking analysis (Figure 1) were based on their ability to distort cognitive function when aggravated and participate in memory formation and retention. Herein, the four proteins play a prominent role in memory retention, cognitive function, and memory storage (Vauzour *et al.*, 2008).

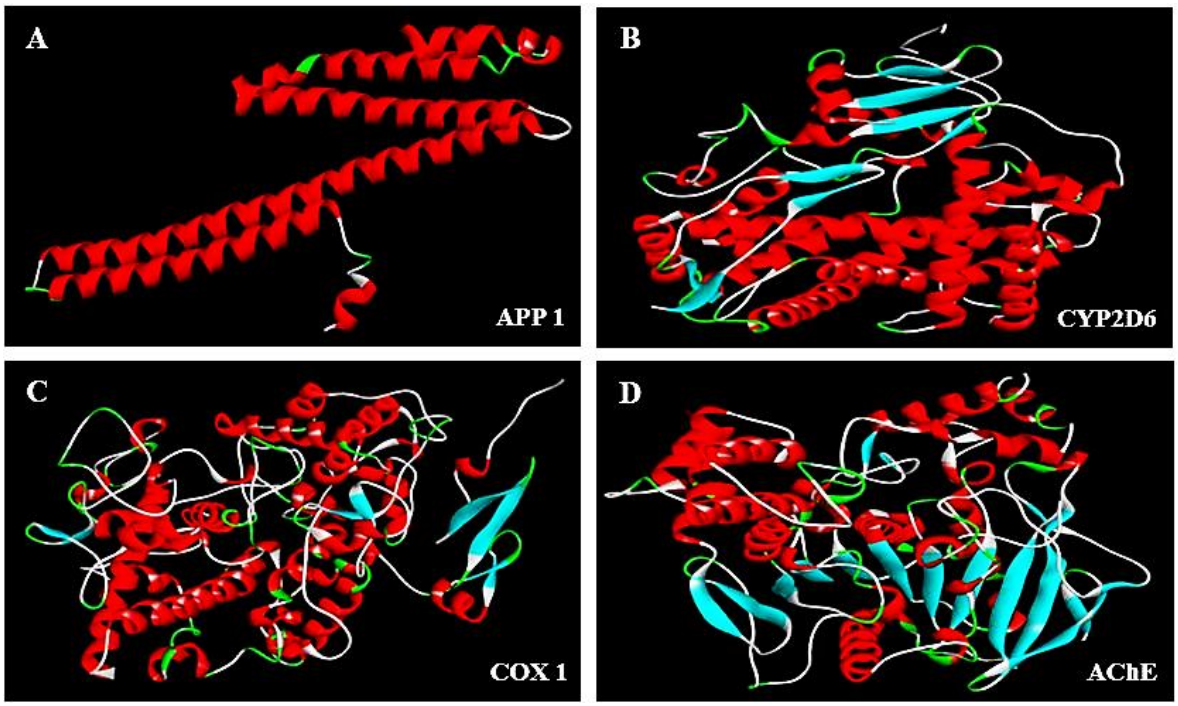

Figure 1. Polypeptide chains. **A**. APP1 facilitates trophic function in neurons and synapses, hippocampal deficiency of APP1 can lower neurite outgrowth (Müller and Zheng, 2012); **B.** COX1 catalyzes the conversion of free arachidonic acid into prostaglandin H_2, which in turn modulates memory in receptor-mediated mechanism (Miciaccia *et al.*, 2021); **C.** Cyp2D6 metabolizes xenobiotics and endogenous compounds such as dopamine to regulate memory (Rowland *et al.*, 2006); **D.** AChE hydrolyses the neurotransmitter acetylcholine and terminates the impulse transmission at cholinergic synapses (Dvir *et al.*, 2010).

Ligand preparation

Five ligands (Coumarin, Myricetin, Kaempferol, L-Theanine, and Epigallocatechin gallate (EGCG)) (Figure 2) structures for molecular docking analysis were collected from the PubChem database (pubchem.ncbi.nlm.nih.gov). The ligands were extracted in SDF format along with the essential pieces of information (Table 1). EGCG had the highest molecular weight and therefore expected to have the least bioavailability. On the contrary, EGCG had the most hydrogen bond acceptors, indicating that it can form the maximum number of hydrogen bonds. Coumarin had the lowest molecular weight but rarely interacts. Molecular docking analysis suggested that only L-Theanine has the negative XlogP3 value (Table 1). Followed by the physical properties, the two-dimensional structure of the ligands was exported in a PNG file where the structures showed the hydroxyl groups, charged groups, and chemical nature (Figure 2).

Table 1. List of bioactive molecules of Green Tea and their biochemical properties

Compound name	PubChem CID	Molecular weight (g/mol)	XLogP3	Hydrogen bond donor	Hydrogen bond acceptor
Coumarin	323	146.14	1.4	0	2

Myricetin	5281672	318.23	1.2	6	8
Kaempferol	5280863	286.24	1.9	4	6
L-Theanine	439378	174.20	-3.6	3	4
Epigallocatechin gallate (EGCG)	65064	**458.4**	1.2	8	**11**

Molecular docking analysis and ligand-protein interactions

Molecular docking analysis was done using the PyRx software. Five ligands, epigallocatechin gallate, kaempferol, myricetin, coumarin, and L-Theanine, were extracted from the PubChem database and docked to four proteins: COX1, APP1, AChE, and Cyp2D6 individually and the binding energy of the ligands at various conformations was evaluated by PyRx software. The highest affinity ligand was individually merged with the protein structures using the BIOVIA Discovery Studio platform. The Discovery Studio evaluated the ligand-protein structure in two-dimensional (2D) and three-dimensional (3D) models, showing only the ligand-protein interaction while the rest of the protein was hidden. The 2D models generated using BIOVIA Discovery Studio were used to count the number of hydrogen bonds and detect the amino acids forming polar interactions and other bonds with the ligands. The bonds formed and their orientations of bonding are unique for each ligand. Each ligand binds differently with proteins, with a variation in interactions. Kaempferol had the highest binding affinity with COX1, followed by myricetin.

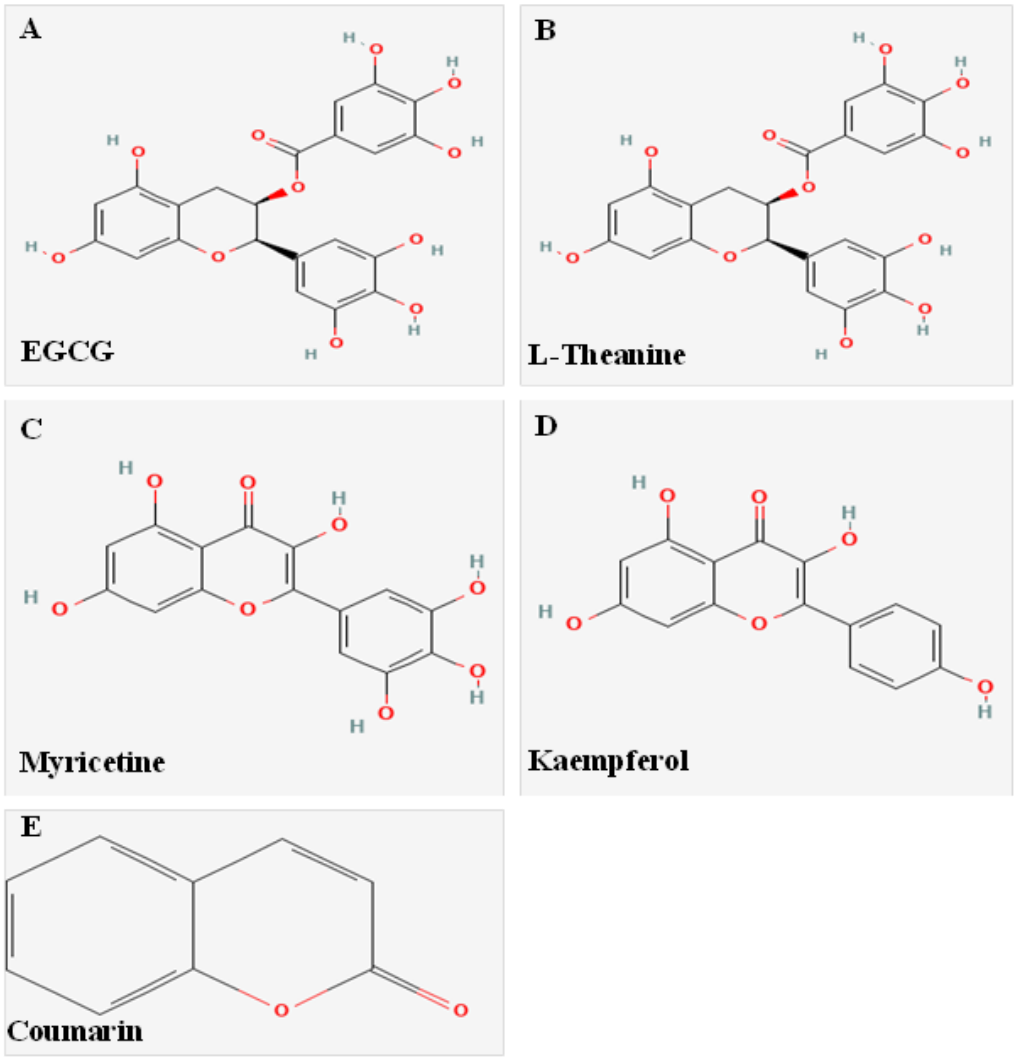

Figure 2. 2D structures of the bioactive molecules of green tea.

The binding energy differed slightly between kaempferol and myricetin with -9 and -8.0, respectively. EGCG formed the maximum number of four hydrogen bonds. Notably, coumarin did not form any hydrogen bonds (Suppl. Figure 1). L-Theanine, kaempferol, and myricetin formed 3 hydrogen

bonds (Table 2). Interestingly, both kaempferol and myricetin formed hydrogen bonds with gln461 and glu465.

Both EGCG and myricetin interacted with Cyp2D6 strongly with -7.8 and -7.5 binding energy, respectively. However, the number of hydrogen bonds formed by the two ligands differs by two, where EGCG forms seven, and myricetin forms five hydrogen bonds (Suppl. Figure 2). EGCG had a tendency to form hydrogen bonds using aromatic rings where the pi-electrons favor strong interactions. Coumarin and L-Theanine formed two hydrogen bonds despite their differences in binding energy with the presence of 'arg' in their vicinity. Notably, kaempferol formed four hydrogen bonds with the binding energy just below myricetin (Table 2).

The highest affinity binding energy of the ligands with Amyloid-β -Precursor Protein 1 (APP1) from PyRx was -6.6 with EGCG (Table 3). In APP1, the amino acids in close proximity to the ligand EGCG were arg429, his426, ala377, his430, and val373. Hydrogen bond interactions of the same ligand were arg429 (Suppl. Figure 3). Both L-Theanine and myricetin did not form any hydrogen bonds with APP1. EGCG had the highest binding affinity but forms only one hydrogen bond compared to kaempferol which has a binding affinity of -5.8 with two hydrogen bonds (Table 3). Interaction of myricetin and coumarin with APP1 was fairly non-existent but had the same amino acids in their vicinity.

EGCG and kaempferol interacted strongly with acetylcholinesterase (AchE), showing multiple interactions with nearby amino acids. L-Theanine formed a weak interaction along with a hydrogen bond. The highest affinity binding energy ligand with AChE was EGCG, with a score of -9.3. They were followed by myricetin with a score of -8.4. AChE has leu76, ser293, and arg296 in the close vicinity of ligands myricetin and EGCG. Both ser293 and arg296 made hydrogen bond interactions with the ligands (Table 3). Both EGCG and myricetin formed four hydrogen bonds (Suppl. Figure 4). Notably, L-Theanine and Kaempferol had the same number of hydrogen bonds with drastically different binding affinity and have tyr124 in their vicinity. Prevalence of the pi-alkyl bond was observed in the interactions Coumarin, Kaempferol, and L-Theanine demonstrated one, two, and two more hydrogen bonds with AChE, respectively (Table 3).

Table 2. Interactions of Ligands with COX1 and Cyp2D6

Tested compound	Protein/ Enzyme	Binding energy (kcal/mol)	Rmsd/ub	Residues close to the ligand	Hydrogen bond interaction
EGCG	**Cox1**	**-8.4**	33.092	Gln289, His207, Thr212, Glu494, Tyr 385	**Gln289, Thr212, Glu454, Tyr385**
Coumarin		-6.8	3.571	His388, Ala202, Met391	**None**
L-Theanine		-5.7	28.128	Tyr385, His388, Phe210, His386, Gln203	Tyr385, His388, Gln203
Kaempferol		**-9**	2.956	Ile46, Cys47, Gln461, Pro153, Cys36, Leu152, Glu465, Arg469, Gly45	Cys47, Gln461, Glu465
Myricetin		**-8.9**	2.949	Cys36, Ile46, Gln44, Glu465, Leu152, Pro153, Cys47, Cys36	**Gln461, Glu465, Ile46**
EGCG	**Cyp2D6**	**-7.8**	15.989	Tyr56, Lys404, His478, Phe366, Asp368, Gly367, Val485	**His478, Tyr56, Lys404, Phe366, Asp368, Gly367, Val485**
Coumarin		-6.6	21.749	Val119, Phe120, Arg441, Arg101, Leu444, Ala305	Arg441, Arg101
L-Theanine		-5.4	21.615	Met374, Arg101, Arg441, Val119, Leu444	Met374, Arg441
Kaempferol		-7.2	**44.128**	Met279, Asn285, Gly284, Pro 286, Arg115, Pro114	Met279, Asn285, Gly284, Pro286
Myricetin		**-7.5**	7.046	Ser288, Asn285, Ala282, Met 279, Glu280, Lys283, Asp292	**Ser288, Ala282, Asp292, Lys283, Glu280**

Table 3. Interactions of Ligands with APP1 and AChE

Tested compound	Protein/ Enzyme	Binding energy (kcal/mol)	Rmsd/ub	Residues close to the ligand	Hydrogen bond interaction
EGCG	**APP1**	**-6.6**	17.316	Arg429,His426, Ala377, His 430, Val373	**Arg429**
Coumarin		-5.5	3.865	Leu417, Val460	**None**
L-Theanine		-4	16.544	Leu417, Val460	Leu417
Kaempferol		**-5.8**	16.257	Met300, Arg379, Leu372, Ala 313	**Met300, Leu372**
Myricetin		-5.9	23.835	Not applicable	**None**
EGCG	**AChE**	**-9.3**	20.543	Trp286, Leu289, Gln291, Ser293, Leu 76, Tyr341, Arg298	**Gln291, Ser293, Arg 296,** Tyr341
Coumarin		-7.2	2.359	Tyr124, Trp86	Tyr124
L-Theanine		-5.4	6.406	Glu202, Phe297, Tyr124, His447	Glu202, His447
Kaempferol		-8.1	5.999	Ser293, Phe295, Val294, Trp286, Tyr 124, Phe 338, Tyr341	Ser293, Phe295
Myricetin		**-8.4**	6.243	Leu76, Tyr341, Trp286, Arg296, Phe 295, Ser293	**Arg296, Phe295, Ser 293, Trp286**

Virtual screening of ligand-protein interactions in 3D modeling by PYMOL

The ligands with the highest affinity binding energy, docked to the proteins were extracted in .pdb format. The ligands were then merged with the respective proteins to evaluate the active site of the ligand-protein interactions (Figure 4).

EGCG bound with AChE, and the amino acids close to the ligand varied within the biological system according to orientation and physical interactions (Figure 3). The ligands could bind to multiple sites on the proteins depending on the cellular orientation of the protein. The Computational analysis summarized the possible interactions, where only one of the active sites of ligand-protein interaction can be visualized at a time. Due to PyRx docking and nine possible interaction sites (Figure 3), only one site was stable enough to become an active site (Figure 4).

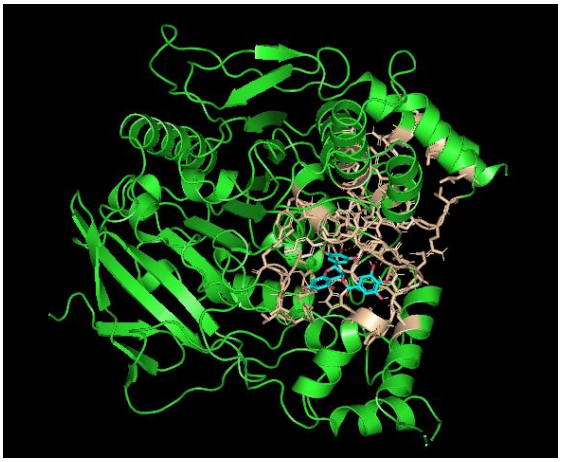

Figure 3. The active site of EGCG with acetylcholinesterase. Green represents polypeptide chains, wheat represents the site of possible interaction, and cyan represents the ligand. The stability of molecular interaction is determined by the number of polar interactions and hydrogen bonds formed.

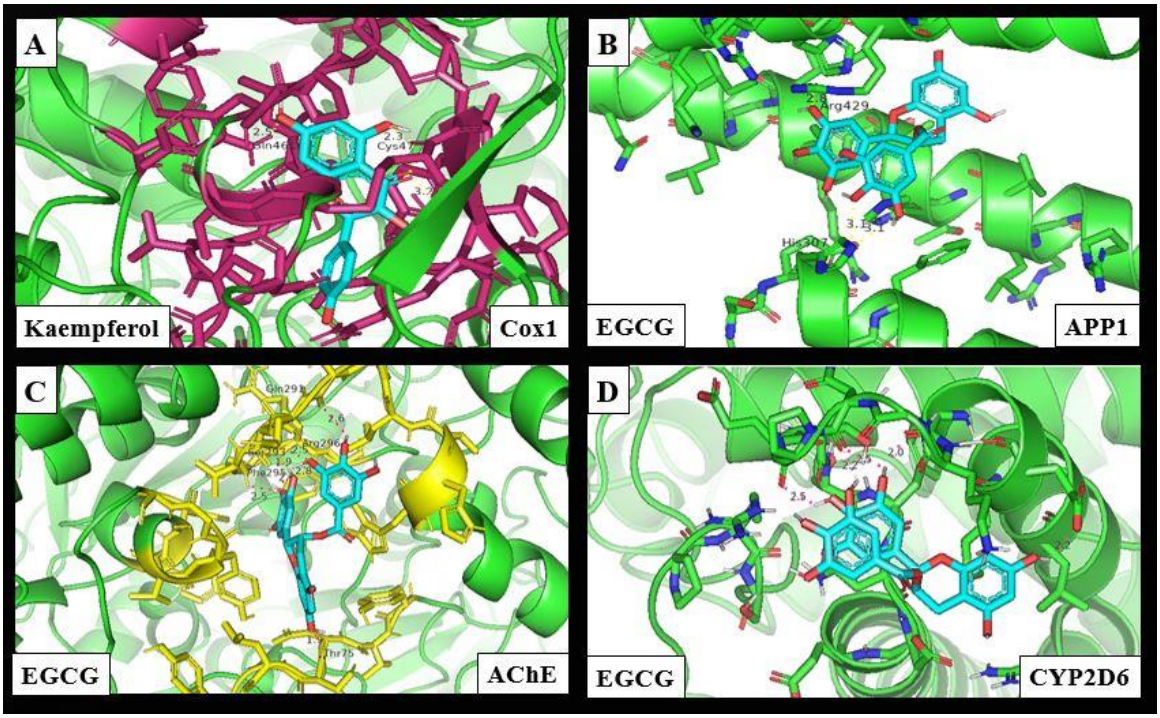

Figure 4: Stable active sites of the ligands. Active site representation was done by measuring the polar interactions. The angstrom length of the hydrogen bond was evaluated between 2.6 and 3.3. PYMOL was used to find the polar interactions of the ligands with the proteins, followed by the labeling of interacting amino acids. **A.** The interactions of Kaempferol with COX1 were illustrated. Kaempferol interacted prominently with COX1. There were two hydrogen bond interactions. Cysteine initiates most bonds; **B.** Dynamic interactions of APP1 and EGCG were evaluated. Polypeptide chains of APP1 were unique in their molecular composition. There were various interactions with multiple hydrogen bonds; **C.** The amino acids associated with EGCG, and AchE interactions were shown. The active site of interaction of EGCG ligand with AChE showed six polar interactions, of which three were hydrogen bonds; **D.** Cyp2D6 and EGCG illustrated multiple polar interactions, but only two of the interactions fall in the range of the hydrogen bond. All the interactions and hydrogen bonds were labeled and measured using the PYMOL.

Discussion

The amygdala, hippocampus, and cerebellum are the brain regions responsible for memory processing. A number of studies have proven that the amygdala influences stress, aggression responses, and fear memories. In contrast, the hippocampus influences declarative, episodic, spatial, and recognition memory, along with the processing of memories and converting new learning to long-term memory, cerebellum influences procedural memories, motor learning, and classical conditions (Fenker *et al*., 2005).

Factors such as alcohol, stress, drug abuse, etc., can relate to cognitive dysfunction and memory impairment. In their experiment with green tea polyphenols and alcohol-induced

impairment, Yong Zhang et al. suggested that green tea polyphenols can revert the alcohol-induced reduction of neurons, behavioral changes, morphological changes, learning, and memory deficit in the hippocampus region (Zhang *et al*., 2018). Furthermore, green tea polyphenols can enhance NDDAR1 (N-methyl-D-aspartic acid receptor) protein expression and cAMP-responsive element-binding protein (CREB) phosphorylation, thus ameliorating learning and memory (Zhang *et al*., 2018). NMDRA1 maintains long-term potentiation, plasticity, learning, and memory, whereas CREB regulates synaptic connections and long-term memory. EGCG is a probable medicament of memory enhancement and alteration of alcohol damage (Rahmani *et al.*, 2015).

Green tea's polyphenols can help with protein kinase activation or inhibition, protein acetylation and glycation, enzymatic activity changes, redox status, signaling cascades, and gene transcription regulation (Lorenz, 2013). Mario Lorenz demonstrated in a study that EGCG inhibits amyloid-β and α-synuclein fibrillogenesis by binding to them (Lorenz, 2013). The binding of EGCG to brain-specific proteins culminates in non-toxic aggregates and neuronal protection (Lorenz, 2013).

EGCG is an inhibitor of the brain's COX (cyclooxygenase) enzyme (Rahmani *et al.*, 2015). Cyclooxygenase is an enzyme that converts first-stage arachidonic acid (AA) to prostaglandin E2 (PGE2) (Miciaccia *et al.*, 2021), which in turn acts as a retrograde messenger and activates presynaptic PGE2 subtype 2 (EP2) receptors and therefore regulates the synaptic transmission, plasticity, and memory acquisition (Heysieattalab *et al.*, 2021). It has been found that bioactive molecules of green tea, such as catechins, play a significant role in managing telomerase activity, thus providing genetic material protection and antioxidant properties thus regulating damage to macromolecules (Chu *et al*., 2017).

Acetylcholinesterase of the hippocampus cholinergic system hydrolyses the neurotransmitter acetylcholine to choline and acetate. Catechins of green tea can inhibit lipid peroxidation and induce acetylcholinesterase activity, consequently providing neuroprotection (Schimidt *et al.*, 2021). EGCG and its metabolites, and L-Theanine are green tea's most promising bioactive components. Despite the fact that bioactive components of green tea are organic, they have hydroxyl groups facilitating hydrogen bond formation, and polar interaction, and are thus absorbed inadequately orally due to their hydrophilic nature. EGCG is an ester of EGC {(-)-epigallocatechin} and GA (gallic acid). Cytochromes P450 (CYP) are responsible for metabolizing endogenous and exogenous compounds, and only a minor amount of CYP is found in the brain (Miksys and Tyndale, 2002). Sharon L. Miksys et al. suggested the immunological staining of CYP2D6 in Purkinje cells and hippocampus neurons (Miksys and Tyndale, 2002). CYP2D6 has a modulatory effect on dopamine metabolism (Miksys and Tyndale, 2002). Dopamine has an adaptive response to motivation, neuronal connection, adaptive memory, long-term memory, and cognitive functions (Shohamy and Adcock, 2010). A plethora of neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, Huntington's disease, and Amyotrophic lateral sclerosis are characterized by the accumulation of fibrous proteins such as amyloid-β, α-synuclein, and superoxide dismutase, causing inflammation and elevated expression of pro-apoptotic proteins, leading to oxidative stress (Pervin *et al.*, 2018). The catechins in GT can inhibit both amyloid-β and tau proteins.

The blood-brain barrier (BBB) is a dynamic system that separates peripheral blood from neural tissues in the central nervous system. To make changes to cognition, one must cross the BBB. BBB forms a microenvironment for neuronal function and regulates the entry of ions, and molecules, from peripheral blood. It is a dynamic system composed of gap junctional proteins, astrocytes, pericytes, immune cells, and an extracellular matrix (Banks, 2008). In an experiment conducted by Monira Pervin et al. on oral administration of 100mg/kg ECGC in rats, only 0.01% reached various brain regions while ~4.95% reached systemic circulation and intestinal microbial metabolism of EGCG to its metabolites, elevates the bioavailability (Pervin *et al.*, 2019). It was shown in the same study that EGCG and its metabolites promote neurite outgrowth and prevent cognitive dysfunction or impairment. Most of the components of green tea can cross the blood-brain barrier. The bioavailability of the components depends on molecular weight, the number of hydroxyl groups, and conjugation (Dietz and Dekker, 2017).

Enzyme inhibitory activity is indirectly proportional to the binding free energy value (Yasmin *et al.*, 2017). Thus, the greater the binding free energy value, the lesser the inhibition. Although this does not prove the upregulation of the enzymes, other factors might play crucial roles. In a nutshell, compounds with higher binding energy have lower enzyme activity (Penta *et al.*, 2014). Likewise, the number of hydrogen bonds dictates the strength of binding, and the higher the number of hydrogen bonds, the stronger the force of attraction. In the above *in silico* model, ECGC has a binding energy of -8.4 whereas L-Theanine has -5.7 for COX1. In the case of Cyp2D6, the binding energy is -7.8 for EGCG, and -5.4 for L-Theanine. Similarly, for APP1 it is -6.6 for ECGC, and -4 for L-Theanine. Moreover, binding free energy with AchE is -9.3 for ECGC and -5.4 for L Theanine. The two bioactive components of green tea (EGCG and L-Theanine) act with a drastic difference with the same proteins or enzymes. The pattern of binding free energy shows that the regulatory effect might be interrelated to their upregulation or inhibition at specific times or periods.

Neurotransmitter acetylcholine plays a pivotal role in effectuating cognitive function. The cholinergic system mediates the neuronal concentration of acetylcholine in the hippocampus. Acetylcholinesterase is a key mediator of hydrolysis of acetylcholine (Amin *et al.*, 2021; Abu-Aisheh *et al.*, 2019). Acetylcholinesterase, acetylcholine, and cholinergic neurons regulate long-term memory, semantic memory, age-related dementia, and episodic memory (Hornick *et al.*, 2011). Lower concentrations of acetylcholine favor memory consolidation, whereas higher concentrations favor memory encoding (Haam and Yakel, 2017). Acetylcholinesterase inhibition by bioactive molecules substantiates memory formation. Both EGCG and myricetin form four hydrogen bonds with a binding affinity of -9.3 and -8.4, respectively. Notably, L-Theanine and Kaempferol form the same number of hydrogen bonds with drastically different binding affinity. Coumarin or 2H-1-benzopyran-2-one of green tea can inhibit acetylcholinesterase by binding to the active site. Molecular docking analysis typifies the binding of

coumarin to AchE and its inhibition. Coumarin also inhibits amyloid-β aggregation and dimerization. In the molecular docking and virtual screening assay, the binding free energy of coumarin ranges from -5.5 to -7.2. -7.2 binding free energy is seen for AChE with three hydrogen bonds.

Myricetin is another flavonoid with binding free energy ranging from -8.9 and -.5.9. A significant interaction of myricetin is observed between COX1, Cyp2D6, and AChE to give -8.9, -7.5, and -8.4 binding energy, respectively. The observation correlates with the potent antioxidant properties and reduction of reactive oxygen species in the brain (Ramezani *et al.*, 2016), dopaminergic activity by COX1 (Wang *et al.*, 2017), and finally, suppression of memory impairment by AchE inhibition (Dhanraj *et al.*, 2018).

The Binding free energy of kaempferol ranges from -9 to -5.8. The strongest docking energy is observed with COX1 followed by AChE with -8.1 binding energy. Kaempferol is known to interact similarly to the other flavonoid discussed in this study. Kaempferol reduces neuronal inflammation (Lei *et al.*, 2012), neurodegenerations, hippocampus degeneration, and memory impairment and improves memory retention (El-kott *et al.*, 2020).

The effect of L-Theanine depends on its interaction with the caffeine of green tea. Together they can alter mood and cognition. L-Theanine interacts poorly with the proteins evaluated in this study.

In brief, EGCG showed prominent results. The binding free energy of EGCG is the highest among all the ligands in all the given proteins except COX1. Kaempferol has the most binding free energy with COX1. Kaempferol and myricetin follow a pattern of falling after EGCG in the other cases. The bioavailability of EGCG is expected to be the least due to its molecular weight. However, coumarin and L-Theanine have a low molecular weight, but the interactions are insignificant. The impact of EGCG, myricetin, and kaempferol should be significant on memory proteins once they cross the blood-brain barrier.

Conclusion

Green tea's bioactive components show potential modulation of neurological disorders and can be used as a supplement. Since, these molecules can bypass the blood-brain barrier, it might suppress neurodegeneration and improve the cognitive functions, and memory by interacting with hippocampal proteins. Further *in vivo* analysis is necessary to elucidate the mechanism of these bioactive components.

Conflicts of Interest

The authors declare no conflict of interest regarding this work.

Supplementary Figure

Supplementary data associated with this article can be found in the online version at: doi.org/10.3329/brc.v8i2.60642

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