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Research Article

Binding Mode and Molecular Dynamics of Epigallocatechin and Epigallocatechin Gallate with Superoxide Dismutase-1

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Abstract

Superoxide dismutases (SODs) are metalloenzymes that defend the body against reactive oxygen species. Human cells have three distinct SODs, i.e., MnSOD, ECSOD, and Cu-ZnSOD (SOD1). *Camellia sinensis* L. leaves contain polyhydroxylated polyphenols in the form of catechins. This work aims to study the binding mode and molecular dynamics (MD) of epigallocatechin (EGC) and epigallocatechin gallate (EGCG) compared to ascorbic acid and SBL1 (native ligand) with SOD1. Molecular docking was performed using AutoDock 4.2. The complex stability and MM-GBSA were analyzed by trajectory analysis using Amber22. Both catechins demonstrated a binding mode with the enzyme, in terms of hydrogen bonds and hydrophobic interaction, similar to the SBL1. Meanwhile, ascorbic acid is only similar in hydrogen bonds. EGC and ascorbic acid possess a better binding affinity (-4.15 kcal/mol) compared to EGCG (-4.02 kcal/mol), thus the EGC/SOD1 complex was continued in 100 ns MD simulation. The MD simulation confirmed that EGC is more stable than the SBL1 with the RMSD average value of SBL1 and EGC being 1.1669 Å and 0.5607 Å, respectively. Taken together, this study confirms the antioxidant activity of catechins.
Keywords: Antioxidants, Catechins, Oxidative stress, Reactive oxygen species, Superoxide dismutase

Mode Pengikatan dan Dinamika Molekuler Epigalokatekin dan Epigalokatekin Galat dengan Superoksida Dismutase-1

Abstrak

Superoksida dismutase (SOD) adalah metaloenzim yang melindungi tubuh dari spesies oksigen reaktif. Sel manusia memiliki tiga jenis SOD meliputi MnSOD, ECSOD, dan Cu-ZnSOD (SOD1). Daun *Camellia sinensis* L. mengandung polifenol polihidroksilasi berupa katekin. Penelitian ini bertujuan untuk mempelajari mode pengikatan dan dinamika molekuler (DM) epigalokatekin (EGC) dan epigalokatekin galat (EGCG) dibandingkan dengan asam askorbat dan SBL1 (ligan *native*) pada SOD1. Penambatan molekul dilakukan menggunakan AutoDock 4.2. Stabilitas kompleks dan MM-GBSA dianalisis dengan analisis *trajectory* menggunakan Amber22. Kedua katekin menunjukkan mode pengikatan yang mirip dengan SBL1 pada ikatan hidrogen dan interaksi hidrofobik, sedangkan asam askorbat hanya pada ikatan hidrogen. EGC dan asam askorbat memiliki afinitas pengikatan yang lebih baik (-4,15 kkal/mol) dibandingkan EGCG (-4,02 kkal/mol) sehingga kompleks EGC-SOD1 dilanjutkan pada simulasi DM 100 ns. Simulasi DM menunjukkan bahwa EGC lebih stabil dibandingkan SBL1 dengan nilai rata-rata RMSD SBL1 dan EGC masing-masing sebesar 1,1669 Å dan 0,5607 Å. Hasil studi menyimpulkan adanya potensi aktivitas antioksidan pada katekin.
Kata Kunci: Antioksidan, Katekin, Stres oksidatif, Spesies oksigen reaktif, Superoksida dismutase

1. Introduction

Human cells have three distinct superoxide dismutase (SOD) enzymes, i.e., manganese SOD (MnSOD), extracellular SOD (ECSOD), and copper-zinc SOD (Cu-ZnSOD) or SOD1.[1] Human SOD1 preserves the cells in resistance to oxidative stress caused by reactive oxygen species (ROS).[2] SOD1 reveals a unique solvent-exposed tryptophan residue (Trp32) on the beta-sheet surface, which can be cleaved by oxidation thus potentiating cytotoxicity.[2,3,4,5] The monomer of SOD1 forms an eight-stranded beta-sheet topology, with two long loops sticking out from the beta-sheet contributing to the metal interaction and building the active pocket. The 'disulfide loop' creates a disulfide bond between Cys57 and Cys146 in the beta-sheet.[2]

Catechins are polyhydroxylated polyphenols contained in the leaves of *Camellia sinensis* L.[6] The structure of catechin is an important factor in its radical scavenging and metal chelating capacity. The antioxidant activity of catechins mostly depends on the number and location of hydroxyl moiety which allow catechins to work as metal ion chelators.[7]

Previous studies reported the molecular docking of catechins towards numerous proteins, such as serum albumin,[8] bovine β-lactoglobulin,[9] digestive enzyme trypsin,[10] SARS-CoV-2 spike glycoprotein,[11] however, no studies reported the molecular interaction of catechins with human SOD1. This work aims to study the binding mode and molecular dynamics of two major catechins, epigallocatechin (EGC) and epigallocatechin gallate (EGCG), with human SOD1 to explore their antioxidant mechanism further.

2. Method

2.1. Hardware

The hardware used was DESKTOP-HPAGIL, processor AMD Ryzen 5 3600, mainboard AsRock B550M Steel Legend, RAM 16GB DDR4 2600 MHz, SSD NVME ADATA XPG 1TB, VGA MSI Geforce RTX3060 Gaming X 12GB, system type 64-bit operating system, x64-based processor.

2.2. Software

The software used was Chimera 1.17.3 by Resource for Biocomputing, Visualization, dan Informatics (RBVI), AutoDockTools 1.5.7, and MGL tools 1.5.7, contained in AutoDock4 4.2.6 by The Scripps Research Institute, PyMOL-2.5.7 Edu, and BIOVIA Discovery Studio 2022, Amber 22.

2.3. Methods

2.3.1. Protein and Ligand Preparation

The protein is the 3D structure of human superoxide dismutase 1 in complex with a naphthalene-catechol, namely SBL1, (PDB ID: 5YTO), resolution 1.80 Å, PDB DOI: *https://doi.org/10.2210/pdb5YTO/pdb*, retrieved at the Protein Data Bank https://www.rcsb.org/. The proteins' natural ligand (SBL1) was separated before use.

EGCG and EGC in the 3D structure were obtained from the PubChem compound database (*https://pubchem.ncbi.nlm.nih.gov/*). The geometry energy of the ligand was optimized by parameterization method number 6 (PM6) in Gaussian09w. Hydrogen atoms and charges were added using Chimera. The charges were computed using the Gasteiger charge method.

2.3.2. Molecular Docking Simulation

Initially, the molecular docking method was validated by redocking the native ligand into its origin site, and the resulting pose was superimposed to the separated ligand, and both ligand molecules were calculated for their root mean square deviation (RMSD).[12,13,14,15]

Molecular docking simulation was carried out by employing AutoDock 4.2 with a grid box covering the native ligand position. Lamarckian Genetic Algorithm (LGA) parameters used were 100 runs, elitism of 1, a mutation rate of 0.02, a population size of 150, a crossover rate of 0.08, and 2.500.000 energy evaluations. The ligand conformation with the lowest free energy of binding (ΔG) obtained from the most favored cluster was selected for further analysis. The docking was visualized and ligand interaction features for each pose within the binding pocket were

determined automatically using BIOVA Discovery Studio.[16,17]

2.3.3. Molecular Dynamics (MD)

All MD simulations were performed using the AMBER22 molecular dynamics program. MD simulation of each ligand-protein complex was performed in five consecutive steps: (1) energy minimization, (2) NVT heating, (3) NPT equilibration, (4) NPT pre-production simulation, and (5) production simulation.[18]

The topology and coordinate file for the amino acid residues were generated using the force field ff19SB. The topology and parametrization of the ligands were computed using the General Amber Force Field (GAFF). The missing force field parameters of the ligands were generated by the Parmchk2 tool. Electrostatic force over a distance was determined using Particle Mesh Ewald Molecular Dynamics.[19] The system neutralization was carried out by adding sodium and chloride ions. Solvation was done using the TIP3P water cube model. The preparation steps included minimization, heating to 310ºK, and equilibration of temperature and pressure. MD was performed up to 50 ns and 2fs.[16,17,18,19,20]

2.3.4. Trajectory analysis and visualization

The resulting MD trajectories of the ligand-protein complexes were processed through the CPPTRAJ tool. The binding energy and the stability of the protein-ligand complex were determined using the MM/GBSA and were observed using Visual Molecular Dynamics (VMD).[18]

3. Results

The RMSD value between the co-crystal ligand and the redocked poses co-crystal ligand is 1.537 Å. RMSD value is less than 2 Å demonstrating the validity of the molecular docking simulation (Figure 1).

The molecular docking simulation of EGCG and EGC with SOD1 is summarized in Table 1. The binding affinity of SBL1, EGC, EGCG, and ascorbic acid were -7.8, 4.15, 4.02, and 4.15 kcal/mol. Both EGC and EGCG provided high similarity of the binding modes with SBL1.

The complex interaction between EGC and EGCG with SOD1 is depicted in Figure 2 and Figure 3. Although not of significant difference, EGC possesses a better binding

Table 1. The affinity in terms of the binding energy and residues involved in the interaction of EGCG and EGC, with SOD1 compared to the native ligand

Compound	Binding Affinity in terms of Docking Score (kcal/mol)	Residues involved in interaction
SBL1 (Native ligand)	-7.8	Hydrogen bond: Lys23, Pro28, Glu100 Hydrophobic interaction: Lys30, Trp32 Electrostatic: Glu21
EGC	-4.15	Hydrogen bond: Gln22, Lys23, Pro28, Glu100 Hydrophobic interaction: Lys30, Trp32 Electrostatic: Glu21
EGCG	-4.02	Hydrogen bond: Glu21, Lys23, Lys30, Glu100. Hydrophobic interaction: Lys30, Trp32. Electrostatic: Glu21, Lys30.
Ascorbic acid (Standard Drug)	-4.15	Hydrogen bond: Lys23, Glu24, Pro28, Lys30, Glu100. Hydrophobic interaction: None Electrostatic: None

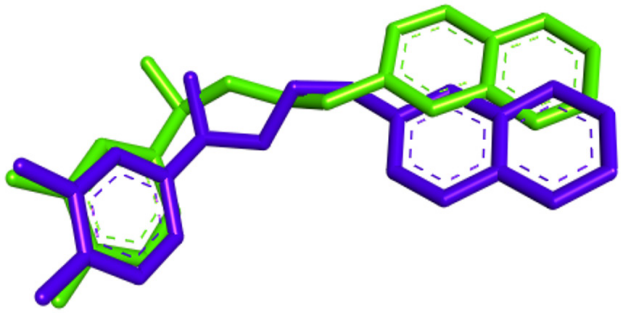

Figure 1. Superimposition of redocking the co-crystal ligands (green and ligand pose (blue) in the binding pocket of human SOD1 structure (PDB ID: 5YTO).

affinity (docking score of -4.15 kcal/mol) for human SOD1 compared to EGCG (docking score of -4.02 kcal/mol).

The RMSD of SBL1/SOD1 and EGC/SOD1 complex simulation within 100 ns is depicted in Figure 4B. Based on the graph, EGC is more stable than the native ligand, SBL1, with the RMSD average value of SBL1 and EGC being 1.1669 Å and 0.5607 Å, respectively.

The RMSF of the ligand/SOD1 complex of SBL1 and EGC is depicted in Figure 4B. The results revealed that the complex of EGC/SOD1 has similar fluctuation with SBL1/SOD1 during 50 ns in the MD simulation. Based on the graph, EGC/SOD1 provides the highest fluctuation in residue Glu 134, Thr137, and Lys13.

MM-GBSA analysis of SBL1 and EGC with human SOD 1 is summarized in Table 2. The MM-GBSA results of SBL and EGC demonstrated that the van der Waals, electrostatic, nonpolar solvation, and gas binding energies showed negative values. Polar solvation energy and solvation energy showed positive values. The binding energy of EGC is slightly higher than SBL1, -15.48 ± 0.26, and -16.89 ± 0.47 kJ/mol, respectively.

4. Discussion

The superimposition of redocking co-crystal ligands is very close to the ligand pose conformation (as shown in Figure 1); the RMSD value is only 1.537 Å. The RMSD value is lower than 1.5 or 2Å, indicating the docking procedure successfully predicted the

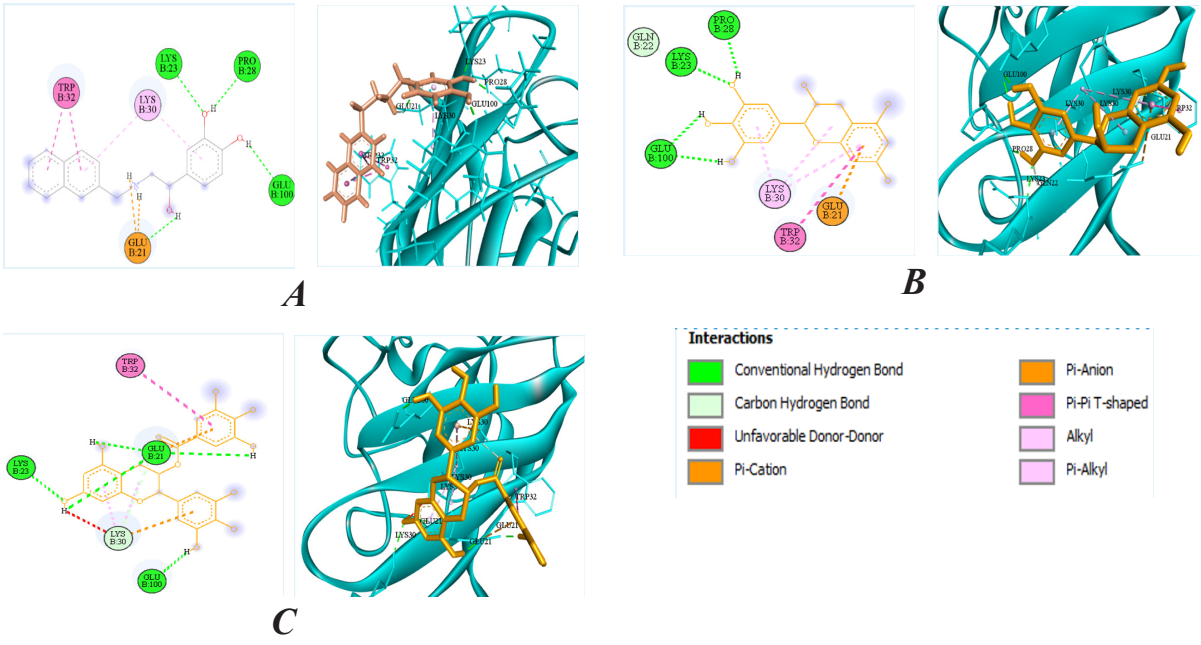

Figure 2. Molecular interaction of (A) SBL-1, (B) EGC, and (C) EGCG in the binding site of human SOD1 in 2D (left) and 3D (right)

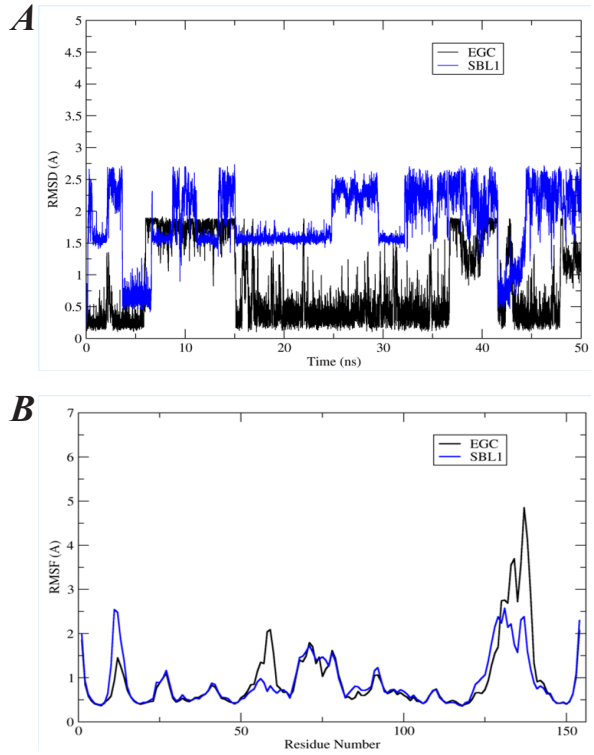

Figure 3. RMSD (A) and RMSF (B) graph of SBL-1 (blue) and EGC (black) in complex with SOD1

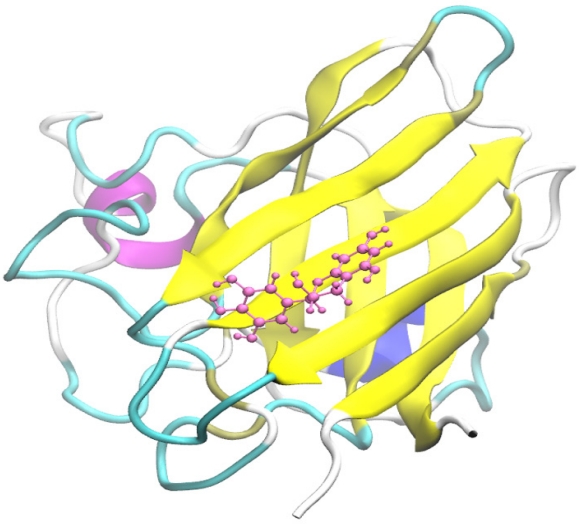

Figure 4. The EGC/SOD1 complex after MD simulation during 50 ns

experimental binding mode and is practicable to generate a physiologically relevant pose that is suitable for further studies.[21,22] Our molecular docking simulation revealed that EGC possesses a better binding affinity for human SOD1 compared to EGCG.

SBL1, as a native ligand, interacts with SOD1 by building hydrogen bonds with Lys23, Pro28, and Glu100. SBL1 also contacts with residue Lys30 and Trp32 by hydrophobic interaction and Glu21 by electrostatic interaction. Ascorbic acid as antioxidant drug standard only creates hydrogen bond with Lys23, Glu24, Pro28, Lys30, Glu100.

Based on our result, EGC (Figure 2) and EGCG (Figure 3) provided high similarity binding modes with SBL1 by interaction type and amino acid residue to which they bind. Hydroxyl group is responsible to build hydrogen bond with some amino acid. Ortho hydroxyl of EGC interacts with Lys23, Pro28 and Glu100 by conventional hydrogen bond and with Gln22 by carbon hydrogen bond. Ortho hydroxyl of EGCG binds Glu21 and Glu100 and meta hydroxyl of EGCG binds Glu21 and Lys23 by building conventional hydrogen bond. Glu21 interacts with EGC and EGCG through π-anion and π-cation electrostatic interaction. According to previous study, the carboxyl of Glu residue can interact with π-electron cloud of the benzene ring through electrostatic interactions.[23]

Table 2. MM-GBSA energy summary of SBL1 and EGC in complex with human SOD1

	Ligand	
	SBL1	EGC
VDWAALS (kJ/mol)	-21.15 ± 0.46	-14.02 ± 0.33
EEL (kJ/mol)	-33.66 ± 1.17	-57.35 ± 1.06
EGB (kJ/mol)	41.44 ± 1.00	58.86 ± 0.85
ESURF (kJ/mol)	-3.52 ± 0.06	-2.97 ± 0.03
Δ Ggas (kJ/mol)	-54.81 ± 1.20	-71.37 ± 0.95
Δ Gsolv (kJ/mol)	37.92 ± 0.97	55.89 ± 0.84
Δ Gbind (kJ/mol)	-16.89 ± 0.47	-15.48 ± 0.26

EGCG and EGC build hydrophobic interaction with Lys30 and Trp32 of SOD 1. The aromatic moiety of Trp32 establishes π-π interactions with the aromatic groups of EGC and EGCG and plays an important role in complex stabilization. Aromatic rings interact through various conformations, such as T-shaped conformation, face-to-face conformation, and offset stacking conformation. Previous quantum calculations have shown that T-shaped and offset stacking conformations are favored due to their lower electron repulsion.[24] Galloyl group and meta-hydroxyl group in EGCG reveal unfavorable donor-donor with Lys30 residue which indicates the repulsion between an atom and two molecules. Repulsion of unfavorable interactions will reduce the stability of the ligand-protein complex and will affect to the binding affinity score of EGCG.[25] Unfavorable donor-donor may cause lower binding affinity score of EGCG than EGC.

Trp32 and Glu21 are essential amino acids in the stabilization and activation processes of SOD1. Interaction between ligand and SOD1 in Trp32 can protect Trp32 from the oxidation process, which leads to the formation of mutations and triggers amyotrophic lateral sclerosis (ALS). Apart from that, ligand can also assist in the process of maturation and activation of SOD in conditions of deficiency of copper and zinc and intra-subunit disulfide bonds.[26] EGCG and EGC also have similar hydrogen bond with SBL 1 in Lys23 and Glu100. Lysine as a charged residue and glutamate as a polar residue play a crucial role in generating a positive electric field in the channel leading to the mature SOD1 active site and elevate enzyme function by providing electrostatic direction to anions $\bullet O^{2-}$ Solvent-accessible active sites catalyze Cu^{2+}.[27]

The EGC/SOD1 complex, with a better binding affinity (lower docking score) than EGCG/SOD1, was continued in MD simulation to investigate the conformational stability and time-dependent ligand binding ability in the binding pocket. An RMSD plot was observed to measure the deviation of the complex ligand-protein atom in the binding pocket during simulation. The RMSD value was used to predict the complex stability that formed during the simulation. In Figure 4A, both ligands SBL1 and EGC can create stable complexes with SOD1, as indicated by a fluctuation RMSD value ≤ 2Å (the acceptable range is 1-3Å).[28,29,30] The complex of EGC/SOD1 is more stable than the SBL1/SOD1 complex, as indicated by a lower RMSD value.

RMSF value was calculated to examine the fluctuation of amino acid residues of a complex during simulation. A low RMSF value revealed more rigid amino acid residues than a high RMSF value.[31] Based on Figure 4 the fluctuation pattern between EGC and SBL1 is similar. Complex EGC/SOD1 showed mayor fluctuation in Cys58, Thr59, Gly130, Gly131, Asn132, Glu133, Glu134, Ser135, Thr136, Lys137, Thr138, Gly139, dan Gln154 residues. Meanwhile the mayor fluctuation of SBL1/SOD1 is in Met1, Gly11, Asp12, Gly128, Lys129, Gly130, Gly131, Asn132, Glu133, Thr136, Lys137 and Gln154. Interestingly, the amino acid residue that binds the ligand showed minor fluctuation in the RMSF range value is 0.4–0.8. Minor fluctuation revealed the interaction between ligand and amino acid residue is stable.

MM-GBSA analysis was observed to determine the binding free energy of the MD trajectory of the ligand-protein complex.[31] The MM-GBSA calculation was performed within 0–100 ns. The van der Waals energy of SBL1 and EGC was -21.15 ± 0.46 and -14.02 ± 0.33, respectively. Electrostatic energy was -33.66 ± 1.17 for SBL1 and -57.35 ± 1.06 for EGC. The surface energy was -3.52 ± 0.06, while gas and solvation energies were -54.81 ± 1.20 and -71.37 ± 0.95 for SBL1, also 37.92 ± 0.97 and 55.89 ± 0.84 for EGC. The total binding energy for SBL1 and EGC was -16.89 ± 0.47 and -15.48 ± 0.26, indicating both SBL1 and EGC create a stable complex with human SOD1. The EGC/SOD1 complex after 50 ns MD simulation is depicted in Figure 4.

Previous studies have attempted to study the interaction of SOD1 and EGCG using ESI-IM-MS methods. EGCG-SOD1 complexes were detected under native ESI-

MS conditions and maintained in the gas phase, and conformational changes of SOD1 upon binding to catechin were examined using traveling wave ion mobility (IM) spectroscopy. The hydroxyl groups in EGCG can increase stability and prevent SOD1 aggregation through the hydrogen bonds they form.[32] Intra-peritoneal administration of catechins can increase antioxidant activity in wistar rats by upregulating SOD enzymes expression.[33] It has been reported that catechins (0.2%) in the polyphenol fraction isolated from green tea in drinking water can upregulate SOD activity in mice within 30 days.[34]

5. Conclusion

Catechins are the most abundant polyphenols in tea (*Camellia sinensis* L.) leaves. Our molecular docking study confirmed that EGC and EGCG, the two major catechins in tea, demonstrated a binding mode with human superoxide dismutase 1 (SOD1), in terms of hydrogen bonds in Lys23 and Glu100 and hydrophobic interaction in Lys30 and Trp32, similar to the native ligand (SBL1). EGCG shows unfavorable interaction which affected to the complex stability and pharmacology activity. Of the two catechins, EGC possesses a better binding affinity and binding modes for human SOD1 compared to EGCG. The molecular dynamics simulation revealed that EGC is more stable than SBL1, thus confirming the antioxidant activity of catechins in *C. sinensis* L.

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