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In Silico Study of Green Tea Leaf Compounds (Camellia sinensis) as Antibacterial Candidates against Streptococcus mutans

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Abstract. Streptococcus mutans is the primary bacterium responsible for dental caries. Caries can generally be treated with antibiotics; however, their use has resulted in increased resistance. Therefore, a natural product like green tea could serve as an alternative treatment for caries. This study aims to assess the potential of active compounds in green tea leaves (Camellia sinensis) against Streptococcus mutans using in silico methods. The in silico study involved molecular docking performed with PyRx, and ligand-protein interactions were visualized in 3D using Biovia to identify chemical bonds. The target protein utilized was 3AIC, and the compounds tested included epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC). Chlorhexidine and chloramphenicol were used as positive and negative controls. This study also includes assessments of physicochemical properties, toxicity, and predictions of biological activity. The results indicated that ECG exhibited the strongest binding affinity (-9.5 kcal/mol) and complied with Lipinski's Rule of Five. Toxicity predictions categorize catechin derivatives into toxicity classes 4 and 6, and based on biological activity, the catechin derivative compounds demonstrated antibacterial properties. In conclusion, epicatechin gallate showed promising results as a natural antibacterial candidate against Streptococcus mutans.

Keywords : Green tea, Streptococcus mutans, In silico, Molecular Docking

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Introduction

The current health condition of teeth and mouth is one of the critical indicators of overall body health. Tooth and mouth diseases are the most common problems worldwide, and dental caries causes them. Tooth and mouth diseases, which are the most common problems in the world, are dental caries [1]. Various types of bacteria have been known as microorganisms that cause caries [2]. Generally, the formation of caries results from the activity of the bacterium Streptococcus mutans [3]. Streptococcus mutans is an acidogenic bacterium with a strong organic acid production capacity and is aciduric, which allows it to survive for long periods at low pH [4].

The development of dental caries is associated with the formation of biofilm that affects large populations worldwide. Oral biofilm is a complex three-dimensional structure of various microorganisms that inhabit the oral cavity; if left untreated for a long time, the biofilm can mature and lead to the development of dental caries [5]. Enzymes are an essential target in efforts to inhibit bacterial biofilm formation. Extracellular enzymes produced by various bacteria, including Streptococcus mutans, are glucosyltransferase (GTF). This enzyme breaks down sucrose into glucose and fructose and forms a sticky biofilm chain [7].

The ability of Streptococcus mutans bacteria to form biofilms increases caries persistence, resulting in high antibacterial resistance. Antibacterials are substances that work to kill or interfere with the growth of bacteria. The substance works by interfering with the metabolism that occurs in bacteria when they are growing [7]. Streptococcus mutans has shown a resistance rate of about 50% to antibiotics such as Cefotaxime, Tetracycline, and Levofloxacin, while resistance to chloramphenicol antibiotics has reached 100% [8]. In addition, the mainstay antimicrobial agents for caries, such as chlorhexidine, also have limitations, such as causing side effects, mucosal pain, tooth discoloration, and disruption of oral microbial balance [9].

Drug resistance and side effects resulting from the use of synthetic drugs have led to an increasing demand for new and safe alternative drugs [10]. A promising alternative in overcoming resistance is using natural products as antibacterial agents. One example of a natural material that can be utilized as an antibacterial agent is green tea leaves. Unlike black tea and oolong tea, green tea undergoes minimal oxidation, thus preserving its natural antioxidants and polyphenols. Green tea has been considered medicinal since ancient times, with a significant increase in scientific research [11].

Green tea is considered to have oral health-promoting properties due to its rich polyphenol content [12]. Green tea (*Camellia sinensis*) shows antibacterial effects against various bacteria due to some of its ingredients [13]. The main content of green tea is polyphenols, with a 30-35% percentage. Polyphenols that are found in large amounts in tea plants are compounds that are included in flavonoids [7]. Green tea (*Camellia sinensis*) contains four main flavonoids, namely catechins, which are divided into epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [5]. Catechins such as EGC, ECG, and EGCG are the major antimicrobial compounds against various pathogenic organisms [14]. Based on previous research by Mufeena et al. (2024), green tea extract demonstrated antibacterial potential, exhibiting an inhibition zone against the S. mutans strain of 29±0.3 mm at a concentration of 2.5 mg/mL [15]. According to Efliani et al. (2023), the inhibition zone diameter criteria of ≥20 mm is categorized as very strong [40].

However, further studies on the effectiveness of green tea's active compounds with antibiotics and conventional drugs through an in silico approach are needed to determine the potential of green tea as an antibacterial candidate. "In silico drug discovery" describes identifying and designing possible drug candidates using computer techniques. This strategy utilizes additional molecular modeling tools and computer-aided drug design methods. In silico drug discovery has evolved into an essential component of contemporary drug research because it can reduce the time and resources needed to discover and improve therapeutic candidates [16].

One of the in silico methods used is molecular docking. Molecular docking is a computationally based approach [17]. Molecular docking is widely used in modern drug design to investigate ligand conformations in target binding sites, as well as to estimate binding energy between ligands and receptors [18]. The reason for using molecular docking is that this method allows predictions of interactions between small molecules (ligands) and biomolecular

targets such as proteins or DNA, aiding in the identification of new drug candidates and understanding drug-receptor interactions [17]. Thus, it accelerates drug discovery at a lower cost than other methods. This study aims to analyze the potential of active compounds in green tea (*Camellia sinensis*) as antibacterial candidates against Streptococcus mutans through an in silico approach and to compare their effectiveness with reference ligands consisting of a positive control, chlorhexidine, and a negative control, chloramphenicol.

Experimental

The Tools and materials. The device used is an Asus laptop with the following specifications: Processor: AMD Ryzen 5 5600H (6 cores, 12 threads, clock up to 4.2 GHz). GPU: AMD Radeon Vega 7 (integrated). RAM: 16GB DDR4. Storage: 512 GB NVMe PCIe SSD. Supporting software in Pyrx, Biovia Discovery Studio, PubChem, SwissADME, Protox, and Way2Drug websites.

Sample Preparation Ligand and target protein. The stage produces output in the form of a compound ID and a 3D structure with a structural data format (sdf) for the target ligand. The steps to follow include accessing the PubChem database (https://pubchem.ncbi.nlm.nih./), downloading the ligand compound along with its 3D structure, and then accessing the RCSB Protein Data Bank database (https://www.rcsb.org/). The preparation of this target protein aims to obtain a 3D structure in Protein Data Bank (PDB) format for visualization purposes.

The test ligands of this study were green tea (*Camellia sinensis*) catechin derivative compounds in the form of epicatechin gallate (ECG), epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC). The comparator ligands used included the positive control drug chlorhexidine and the negative control chloramphenicol. Additionally, the negative ligand is the original ligand that binds to the target protein, alpha-acarbose. Native ligands are the original ligands that naturally bind to the target protein. These ligands are obtained after the protein is cleaned of receptors, water molecules, and other unnecessary ligands, using Discovery Studio Visualizer software. Subsequently, the ligand obtained from the PubChem database in sdf format was converted into PDB through a conversion process using the Open Babel plugin in PyRx software. Following this, the mmff94 minimization process was carried out. Afterward, the preparation was completed with AutoDock Tools, and the ready file was saved in .pdbqt format. Meanwhile, the preparation of the target protein, namely glucanosucrase with PDB code 3AIC on chain A, was conducted using the Biovia Discovery Studio Visualizer program. This preparation involves removing parts of the system that are not needed, including native ligands and water. Once completed, the protein was stored for later tethering.

Receptor Validation. Before performing the molecular docking process between the ligand and protein, validation is done first. Validation was done through AutoDock Vina software. This validation is done by re-docking the native ligand on the original protein that has been prepared [19]. This method validation aims to ensure that the method used in docking the native ligand can be trusted as a reference. The parameters used are Root Mean Square Deviation (RMSD), where the technique is said to be valid if RMSD < 2 Å [19]. An RMSD value of less than 2.0 Å is used as a threshold to state that the docking results are valid and accurate. Poses that show an RMSD of less than 2 Å are considered correct and acceptable docking poses because the positions of the simulated ligand are similar to those of the original ligand [35], while an RMSD > 2 Å means that the positions of the simulated ligand deviate significantly from the original positions in the crystal structure (native pose).

Molecular Docking Process. The molecular docking process is done by opening the PyRx 0.8.8 version software, inputting the target protein, and running molecular docking with coordinates adjusted in the receptor validation. 3AIC receptor has a binding site center with grid box parameters x = 191.7795, y = 45.0372, and z = 199.1373. Dimensions (Angstrom) x= 22.7224, y= 13.9927, and z= 25.2360. The docking results are expressed regarding binding affinity and amino acid residue interactions. The binding affinity is the most negative value because, according to Effendi et al. (2023), if the binding energy required is lower (more negative), the receptor-ligand bond becomes more stable. The drug candidate's activity will increase if the bond is stable [20]. Meanwhile, the docking results are visualized using Biovia Discovery Studio software.

Interaction and Visualization. Ligand-protein interaction occurs when the molecular dock-

ing process has been completed. This method is done through Biovia software, which displays a 3D visualization of molecular complexes from docking simulation results. The chemical bond interaction is identified to determine the interaction position and the type of chemical bond formed in the ligand-protein complex from the molecular docking results.

Prediction of Physicochemical Properties. Prediction of ligand compounds' properties aims to identify a compound's ability to become a promising drug candidate by fulfilling the parameters of Lipinski's Law 5. Lipinski's Law of Five stipulates that an orally active drug should not have more than one violation of 5 criteria, namely molecular weight greater than 500 Da, log P value greater than 5, H-bond donors or Hydrogen Bond Donors (HBD) greater than five and H-bond acceptors or Hydrogen Bond Acceptors (HBA) greater than 10 [21]. The steps taken to predict physicochemical properties are to access the SwissADME web server through (http://www.swissadme.ch/) and run it on the web server to get data on a query compound's ability to become a promising drug candidate.

Predicted toxicity. Toxicity prediction using the Protox website is done to determine the toxicity level of a compound by looking at the toxicity level class. The lower the class, the more toxic the compound is. The step that must be done is to access the Protox web server via (https://tox.charite.de/protox3/index), then run the web server to get the toxicity data of a compound. LD50 (Lethal Dose 50%) toxicity parameters are classified based on the Globally Harmonized System (GHS), which is divided into several classes, namely toxicity classes 1 to VI. All toxicity classes use LD50 thresholds of 5, 50, 300, 2000, and 5000 mg/kg body weight, with details of class I (fatal if ingested), with a range of LD50 values ≤ 5 mg/kg. Class II (fatal if ingested) with a value range of 5 < LD50 ≤ 50 mg/kg, class III (toxic if ingested) with a value range of 50 < LD50 ≤ 300 mg/kg, class IV (dangerous if ingested) with a value range of 300 < LD50 ≤ 2000 mg/kg, class V (can be hazardous if ingested) with a value range of 2000 < LD50 ≤ 5000 mg/kg, and class VI (non-toxic) with a value range of LD50 > 5000 mg/kg [19].

Prediction of Biological Activity (Antibacterial and Antibiotic). Prediction of biological activity using the Way2Drug website is done to determine the biological activity of a compound by looking at the active probability (Pa) and inactive probability (Pi). If Pa>Pi, it is likely that the compound has the desired activity. The steps that must be taken are accessing the Way2Drug web server via (https://www.way2drug.com/passonlin/), then running on the web server to get biological activity data. According to Syaqila et al. (2024), if the Pa value ≥ 0.3, it can be said that the opportunity has been proven in silico; if the Pa value ≥ 0.7, it can be said that the chance has been demonstrated in laboratory tests [22].

Data Analysis. The data analysis used in this study is the interpretation of molecular docking results and molecular dynamics simulations based on affinity scores, binding energies, and types of molecular interactions. Biovia Discovery Studio (BDS) uses software to create graphical representations of molecular structures and simulation results. The visualization tools in BDS are designed to help researchers understand and communicate the results of molecular simulations, docking studies, and other molecular analyses. The advantage of Discovery studio software is that it can display interaction results in 2D and 3D [23].

Result and Discussion

The results of the validation can be seen in Table 1. The validation results using the redocking method obtained an average value of 0.2954 Å, which indicates that the validation results have a pretty good accuracy, or can be said to be valid because the RMSD value is below 2.0 Å. It is essential to obtain the minimum RMSD value because it is an indicator of the accuracy and validity of the ligand position [24]. The purpose of this method validation is to ensure that the method used in docking native ligands can be trusted as a reference, so that the molecular docking process can be carried out on each protein with the same settings in the validation.

Molecular docking results include binding affinity values (Table 2) and ligand interactions with amino acid residues in the target protein. Based on the research, it is known that the ECG compound has a binding affinity of -9.5 kcal/mol and EGCG -9.3 kcal/mol, the value is higher than the native ligand, which is -8.8 kcal/mol, and the positive control comparison ligand, chlorhexidine of -7.6 kcal/mol, and the negative control, chloramphenicol of -6.6. This indicates that the ECG and EGCG compounds have

Table 1. Receptor validation results using Discovery Studio software

Native ligand	*alpha-acarbose*			Average
	Replication I	Replication II	Replication III	
RMSD Value Å	0.2271	0.3256	0.3336	0.2954

Table 2: Binding Affinity Values

Ligand	*Binding Affinity* (kcal/mol)	Hydrogen Bond	Hydrophobic Bond	Electrostatic
Native Ligand (*alpha-acarbose*)	-8.8	ASP A:424, THR A:426, GLY A:429, TYR A:430, ASP A:480, ASN A:481, GLU A:515,	-	-
epicatechin gallate (ECG)	-9.5	ASN A:481, TRP A:517, SER A:589, GLN A:592, ASP A:909, ASN A:914	LEU A:433, ALA A:478, TRP A:517, TYR A:916	ASP A:588, ASP A:909
epigallocatechin gallate (EGCG)	-9.3	ASN A:481, GLN A:592, ASP A:593, ASN A:862, ASP A:909	LEU A:433, TRP A:517, TYR A:916	ASP A:588, ASP A:909
epicatechin (EC)	-8.2	TYR A:430, ALA A:478, GLN A:588, GLN A:592, ASP A:909, ASN A:914	LEU A:433, ALA A:478, TRP A:517,	ASP A:588, ASP A:909
epigallocatechin (EGC)	-8.1	ASN A:481, TYR A:517, GLN A:592, ASN A:862, ASN A:914	LEU A:433, TYR A:916	ASP A:588, ASP A:909
Positive Control (Chlorhexidine)	-7.6	GLU A:515, ASP A:909	LEU A:382, LEU A:434,	ASP A:593
Negative Control (Chloramphenicol)	-6.6	GLU A:515, ASP A:477, ASN A:481, GLN A:592, ASN A:862	-	ASP A:588, ASP A:909

Description: : Residue similar to the native ligand
: Residues similar to the positive control comparator ligand (Chlorhexidine)

more substantial interaction potential with the target protein compared to the native ligand and the comparator ligand. According to previous research by Han et al. (2023), EGCG was able to bind to the glucose transport domain with a free binding energy of -9.9 kcal/mol, while ECG showed the same affinity with a binding energy of -9.8 kcal/mol [12].

Interactions include hydrogen bonds, hydrophobic bonds, unfavorable interactions, and electrostatic interactions. The interaction formed between the ECG compound and the EGCG compound with the target protein has similarities, namely the formation of 1 hydrogen bond with an amino acid residue similar to the native ligand, namely (ASN A: 481). It has one hydrogen bond with an amino acid residue identical to the chlorhexidine positive control comparator ligand (ASP A: 909). While the EC compound has one hydrogen bond with an amino acid residue similar to the native ligand, namely (TYR A: 430), it has one hydrogen bond with an amino acid residue identical to the chlorhexidine positive control comparator ligand, namely (ASP A: 909). Then the EGC compound has one hydrogen bond with an amino acid residue similar to the native ligand (ASN A: 481). Of the four compounds, it is known that all test compounds except the EGC compound have one hydrophobic bond similar to the chlorhexidine positive control comparator ligand, namely (TRP A: 517), and have 2 ASP electrostatic interactions (A: 588, ASP A: 909).

The interaction between ligand and receptor involves several essential residues. As a result of

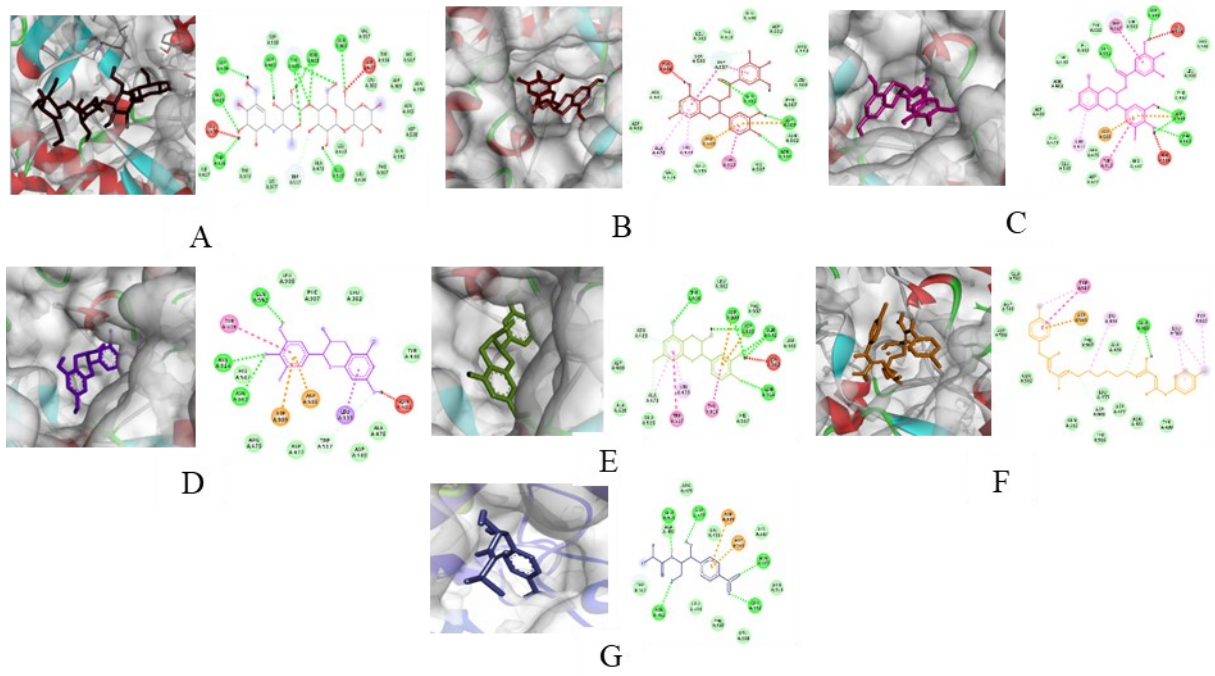

Figure 1. Interaction of 3AIC Target Protein with (A) Native ligand, (B) ECG, (C) EGCG, (D) EC, (E) EGC, (F) Positive Control (Chlorhexidine), and (G) Negative Control (Chloramphenicol)

hydrogen bonding and hydrophobic interactions, there are always residues in every interaction of ligands and receptors. These residues have a crucial role in the binding site area. Hydrogen bonding and hydrophobic interactions play a vital role in determining the stability of the ligand to the receptor, as well as the strength and characteristics of the interaction between the two [25]. While electrostatic interactions are interactions between opposite charges that can help maintain molecular conformation in binding between proteins and ligands [26].

The same interaction in the test compound, the native ligand, and the positive control comparator ligand indicates that the interaction is on the same active side of the 3AIC target protein with similar interaction strength. Based on these results, it can be predicted that the ECG and EGCG compounds have inhibitory activity against the target protein, resembling the native ligand and the positive control comparator ligand (chlorhexidine). This indicates that the content of active compounds in green tea leaves has potential as an antibacterial because it has affinity and chemical interaction with the target protein 3AIC. Visualization of molecular docking results can be seen in Figure 1.

Based on the results of the Physicochemical Properties Screening and Drug Enzymology Analysis (Table 3) conducted through the SwissADME website, it is known that the compounds Epicatechin gallate, Epicatechin, Epigallocatechin, Epigallocatechin gallate, and the negative control comparator ligand (chloramphenicol) fulfill aspects of Lipinski's Law of Five. This suggests the possibility that the ligands are clinically active when administered orally due to their good absorption and permeability.

Native ligands, epigallocatechin gallate compounds, and positive control comparator ligands

Table 3. Results of Physicochemical Properties Screening and Drug Similarity Analysis

Ligand	BM (g/mol)	Log P	HBA	HBD	Lipinski	Bioavailability Score	GI Absorption
Native Ligand	645.60	-6,41	19	14	No.	0,17	Low
ECG	442, 37	1,30	10	7	Yes	0,55	Low
ECGC	458, 37	1,00	11	8	No.	0,17	Low
EC	290, 27	0,85	6	5	Yes	0,55	High
EGC	306, 27	0,42	7	6	Yes	0,55	High
Chlorhexidine	505.45	3,14	4	6	No.	-	-
Chloramphenicol	323.13	0,53	5	3	Yes	0,55	High

(chlorhexidine) violate aspects of Lipinski's Law of Five. This is because the epigallocatechin gallate compound has more than one parameter value that does not meet Lipinski's Law of Five, the parameters are the number of donor hydrogen bonds > 5 and the number of acceptor hydrogen bonds > 10. Based on the results, only the native ligand and the positive control, chlorhexidine, have a molecular weight of more than 500 Da. Natural ligands and chlorhexidine violate Lipinski's rules because natural ligands have a molecular weight > 500 g/mol, hydrogen bond donors (HBD) > 5, and hydrogen bond acceptors (HBA) > 10. Meanwhile, the positive control, chlorhexidine, violates these rules due to a molecular weight > 500 g/mol and hydrogen bond donors (HBD) > 5. Compounds will be challenging to absorb and have low permeability if they have a molecular weight greater than 500 g/mol [27]. Furthermore, the higher the capacity for hydrogen bonding, the greater the energy required for absorption. This is related to the number of hydrogen bond donors and acceptors [28].

The Log P value results from calculating the partition coefficient of fat solubility in water. LogP values greater than 5 will cause drug compounds to tend to have a high level of toxicity, because they will be retained longer in the lipid layer and distributed more widely in the body, so the selectivity of binding to receptors is reduced. However, a logP value that is too negative is also unfavorable, because the molecule is too hydrophilic, so it cannot pass through the lipid layer. Therefore, a drug compound will have good absorption or permeation if the log P value is less than 5 and greater than -0.4 [29]. Based on the results, it is known that all test ligands have a Log P value of less than 5 and greater than -0.4, except the native ligand.

The value of donor and acceptor hydrogen bonds is closely related to the biological activity of a molecule. The number of donor and acceptor hydrogen bonds will impact the compound's absorption process, where too large an amount can increase the energy required, thus slowing down the compound in reaching the target. The following result is the bioavailability score. Based on the results, it is known that native ligands and EGCG compounds have a bioavailability score of 0.17, and ECG, EC, EGC, and negative control comparator ligands have a value of 0.55. The bioavailability score in silico is a predictive value that shows how likely a compound can work well in the body after being given orally. A bioavailability score of 0.55 confirms that a compound has good absorption after oral administration, namely, 55% can enter and work in the body. However, for a bioavailability score of 0.17, it is estimated that only about 17% can enter the blood and work in the body [30].

The GI Absorption results show that the native ligand, ECG and EGCG compounds have low GI Absorption, while the EC, EGC, and negative control comparator ligands have high GI Absorption. GI Absorption is the ability of a compound to be absorbed by the gastrointestinal tract, especially the intestines, after being taken orally. Most compounds do not fully have high GI Absorption experimentally [31]. High GI Absorption indicates that the gastrointestinal tract (gut) easily absorbs the compound after oral administration. Conversely, low GI Absorption suggests that a compound is difficult to be absorbed by the gastrointestinal tract (intestine) after oral administration, so only a small amount of the compound can be absorbed.

Based on the results of toxicity (Table 4) prediction with the Protox website, which is a website for predicting toxicity and several toxicological endpoints for several test compounds [32]. Toxicity testing was conducted to compare the toxicity levels between the test compounds from green tea leaves with the positive control ligand, chlorhexidine, and the negative control, chloramphenicol. Additionally, toxicity testing through computational prediction assists in risk assessment without animal

Table 4. Toxicity Test Results

Ligand	LD_{50}	Toxicity Class	Hepatoxicity	Carcinogenic
Native ligand	24000	VI	Yes	No.
ECG	1000	IV	No.	No.
EGCG	1000	IV	No.	No.
EC	10000	VI	No.	No.
EGC	10000	VI	No.	No.
Control+	1100	IV	No.	No.
Control -	1500	IV	No.	No.

testing [33].

The results indicate that compound EC has an LD50 of 10000 mg/kg with a toxicity class of class VI. Compound EGC has an LD50 of 10000 mg/kg with a toxicity class of VI. Then, for the positive comparison ligand (chlorhexidine), it has an LD50 of 1100 mg/kg with a toxicity class of IV. For the negative comparison ligand (chloramphenicol), it has an LD50 of 1500 mg/kg with a toxicity class of IV. Based on the parameters, it is known that the native ligand is included in toxicity class VI, which means it is non-toxic. At the same time, the active compounds from green tea leaves, namely ECG and EGCG, are classified into toxicity class IV, which means the compounds are likely to be harmful if ingested.

Previous research by Boudou et al. (2025) showed that compounds in green tea leaves, such as EGCG, generally exhibit a favorable safety profile, with green indicators in most toxicity categories [34]. Then the EC and EGC compounds fall into toxicity class VI, meaning both are. The positive comparator ligand (chlorhexidine) and the negative comparator ligand (chloramphenicol) fall into toxicity class IV, meaning the compound may be harmful if ingested. Then, for the prediction of hepatotoxicity based on the results, it is known that all ligands except the native ligand are predicted to be safe and unlikely to cause toxicity to the liver. Based on the results, it is known that all tested ligands are not carcinogenic.

Based on the PASS Online or way2drug website, the Pa value means the probability for a compound's "active" type of activity. Pi implies the likelihood of an "inactive" activity in a compound. These Pa and Pi probabilities are used to evaluate the possibility of a compound exhibiting a particular biological activity based on the predictions provided by PASS Online. The higher the Pa value and the lower the Pi value, the more likely it is that the compound will show the predicted activity [35].

Based on the results of predictions of biological activities (Table 5) in the form of antibacterial and antibiotic properties, it is known that the native ligand has the highest pa value for antibacterial activity, which is 0.703, and an antibiotic pa value of 0.544. The second highest pa value for antibacterial activity is found in the negative control ligand (chloramphenicol), which is 0.507. This is because the negative control (chloramphenicol) is an antibiotic. Antibiotics represent a frequently employed therapeutic modality for managing bacterial infections across diverse domains, including human health, agriculture, livestock breeding, and fish farming [36]. The reason for using chloramphenicol as a negative control ligand is that, according to Yardha et al. (2024), chloramphenicol has experienced 100% resistance against Streptococcus mutans [8].

The following results in sequence show that the highest pa values for antibacterial agents are found in compounds EGCG, EGC, ECG, and EC, while the last is the positive control ligand (chlorhexidine). Chlorhexidine has no pa or pi values for antibiotics because chlorhexidine is a drug that is not classified as an antibiotic. Chlorhexidine is a cationic polibiguanide, one of the first antiseptic agents proposed for dental caries. To this day, chlorhexidine remains the 'gold standard' for antiplaque agents [37]. Chlorhexidine is recommended at concentrations of 0.12% to 0.2%; any concentration above 0.2% will increase unwanted side effects. The difference between antibiotics and regular medications is that antibiotics are a specific type of medication used solely for bacterial infections, while regular medications encompass all kinds of drugs for various medical conditions that are not always related to infection, and the use of non-antibiotic medications can help reduce the risk of antibiotic resistance [38].

Table 5. Ligand Activity Against Antibacterials and Antibiotics

Ligand	Antibacterial (Pa)	Antibacterial (Pi)	Antibiotics (Pa)	Antibiotics (Pi)
Native Ligand	0.703	0.004	0.544	0.004
ECG	0.379	0.035	0.154	0.046
EGCG	0.397	0.031	0.171	0.038
EC	0.320	0.053	0.149	0.049
EGC	0.387	0.033	0.173	0.037
Control p	0.171	0.032	-	-
Control n	0.507	0.016	0.246	0.020

Based on the results, it is known that all active compounds from green tea leaves have good enough pa and pi values against antibacterials, but not good enough for pa and pi values against antibiotics, because the pa value against antibiotics is still < 3. The compound with the best pa and pi values against antibacterials and pa and pi values against antibiotics is the EGCG compound, with a pa value for antibacterials higher than its pi value and a pa value for antibiotics higher than its pi value. However, the pa value of antibiotics is so small that it does not fulfill the criteria for a good antibiotic candidate. Based on previous research, according to Renzetti et al. (2020), compounds from green tea catechins, such as EGCG and ECG, show high antibacterial activity against antibacterial action mechanisms, including inhibition of virulence factors, cell membrane damage, and oxidative stress [39]. Meanwhile, according to research by Han et al. (2023), based on in silico tests, it is known that green tea catechins, especially epigallocatechin gallate (EGCG) and epicatechin gallate (ECG), can inhibit the growth and production of acid by Streptococcus mutans [12].

Conclusion

Based on the research results, it can be concluded that the interaction between active compounds in green tea leaves (Camellia sinensis) and the Glucosucrase receptor (3AIC) of Streptococcus mutans demonstrates the formation of chemical bonds. These bonds include hydrogen bonds, hydrophobic interactions, and electrostatic interactions, all of which contribute to the stability of the complex between the active compound and the target protein. This indicates that compounds from green tea (Camellia sinensis) have potential as antibacterial agents against Streptococcus mutans. Active compounds from green tea (Camellia sinensis) exhibit varying affinity energies for the target protein. The ECG compound is known to have the highest binding affinity of -9.5 kcal/mol, followed by EGCG at -9.3 kcal/mol, EC at -8.2 kcal/mol, and EGC at -8.1 kcal/mol. In comparison, the original ligand has a binding affinity of -8.8 kcal/mol, the positive control chlorhexidine has -7.6 kcal/mol, and the negative control chloramphenicol has -6.6 kcal/mol. Of all the compounds tested, epicatechin gallate (ECG) demonstrated a higher binding affinity than the original and reference ligands. This compound adheres to Lipinski's five rules, is categorized in the class IV toxicity group, and is neither hepatotoxic nor carcinogenic. Therefore, this study concludes that compounds from green tea, particularly epicatechin gallate (ECG), have potential as antibacterials against Streptococcus mutans.

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Author Contributions

V. K. S assisted in conducting the research, and D. D. R. T assisted in supervising it.

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