Phytochemical Profile, Antioxidants, Bioactive Compounds, and Antibacterial Activity of a Bangladeshi Commercial Green Tea (*Camellia sinensis* L.) Extract: A Correlative Investigation Using *in vitro* and *in silico* Approaches

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Article

Phytochemical Profile, Antioxidants, Bioactive Compounds, and Antibacterial Activity of a Bangladeshi Commercial Green Tea (*Camellia sinensis* L.) Extract: A Correlative Investigation Using *in vitro* and *in silico* Approaches

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Abstract

Tea (*Camellia sinensis*), a widely consumed beverage, reduces oxidative stress and has antimicrobial properties due to its phytochemicals. Ethanolic extracts from Bangladeshi green tea were analyzed for phytochemicals, antioxidants, bioactive compounds, and antibacterial activity using in vitro and in silico methods. Qualitative screening detected alkaloids, phenolics, flavonoids, and tannins, with total phenolic content (TPC) at 35.95 ± 0.24 mg GAE/g and total flavonoid content (TFC) at 34.61 ± 1.53 mg QE/g. Antioxidant tests showed strong total antioxidant capacity (301.01 ± 14.32 mg AAE/g) and DPPH radical scavenging (IC50 2.70 μg/mL vs. ascorbic acid's 3.75 μg/mL). HPLC identified gallic acid and vanillic acid as key compounds. Agar well diffusion assays revealed dose-dependent zones of inhibition against *Staphylococcus aureus* (12–22 mm) and *Escherichia coli* (10–20 mm) at concentrations of 25–200 mg/mL. In silico docking showed gallic and vanillic acids binding to *S. aureus* (PBP2a, SrtA) and *E. coli* (AmpC β-lactamase, GyrB) targets with affinities of -4.9 to -6.0 kcal/mol, stabilized by hydrogen bonds and π-interactions. ADME profiles indicated high gastrointestinal absorption, Lipinski compliance, and bioavailability (0.56–0.85). Toxicity predictions suggested minimal risks, mainly nephrotoxicity. PASS analysis predicted antibacterial, β-lactamase, and DNA gyrase inhibition activities. These findings underscore Bangladeshi green tea as a promising source of antioxidants and antibacterials for oxidative stress and infections.

Keywords: tea; oxidative stress; bioactive compounds; antibacterial activity

1. Introduction

Tea, derived from the leaves of *Camellia sinensis*, is recognized as the second-most-consumed beverage globally, after water [1,2]. The preparation of tea involves infusing hot or cold water with extracts from the leaves, leaf buds, or internodes of the tea plant, or using pre-packaged tea bags [3]. Recently, tea has gained international acclaim for its refreshing flavor profile, aromatic qualities, and numerous health-promoting properties [4].

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Tea can be categorized into three primary types based on fermentation: fermented, non-fermented, and semi-fermented [5]. The characteristics of tea include its color, sensory properties (including overall acceptability), active chemical constituents, and aromatic or flavor attributes, all of which are influenced by the stage of fermentation [6]. Specifically, *Camellia sinensis* L., belonging to the *Theaceae* family, yields green tea, a variety characterized by unoxidized, non-fermented leaves [7]. In contemporary society, green tea has surged in popularity, largely attributed to its wide array of health benefits [8]. It is estimated that approximately two-thirds of the global population actively consumes green tea, which is generally deemed safe, non-toxic, and devoid of significant adverse effects [9]. Nevertheless, excessive consumption may lead to health complications, particularly hepatotoxicity [10].

Oxidative stress is a biological phenomenon characterized by the excessive accumulation of reactive oxygen species (ROS) that exceeds the capacity of antioxidant defenses, leading to detrimental effects on lipids, proteins, and DNA, thereby compromising cellular homeostasis [11]. The ensuing disruptions promote inflammatory responses, mitochondrial dysfunction, and genomic instability, mechanisms increasingly recognized by health authorities as critical links between contemporary lifestyle factors and the emergence of chronic diseases [12–14]. In cardiovascular pathology, oxidative stress is implicated in the development of endothelial dysfunction, atherosclerosis, and heart failure, primarily through mechanisms involving plaque formation [15]. Similarly, neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease, demonstrate neuronal injury attributable to elevated levels of ROS [16]. Furthermore, in oncological contexts, cancer progression is often associated with ROS-induced cellular proliferation and metastasis, facilitated by genomic instability [17]. In metabolic disorders such as diabetes, oxidative stress contributes to insulin resistance and β-cell apoptosis, thereby exacerbating the disease state [18].

Bacterial infections, including pneumonia, tuberculosis, sepsis, and urinary tract infections, continue to represent significant contributors to global morbidity and mortality [19]. Data released by the World Health Organization (WHO) in 2023 indicate that approximately one in six laboratory-confirmed bacterial infections exhibited resistance to antibiotic treatment [20]. Alarmingly, the rates of resistance are escalating at a rate of 5-15% annually across more than 40% of assessed pathogen-antibiotic pairs which is particularly pronounced in Gram-negative bacteria, notably *Escherichia coli* and *Klebsiella pneumoniae* [21,22]. Global surveillance reveals that over 40% of *E. coli* strains and more than 55% of *K. pneumoniae* isolates demonstrate resistance to third-generation cephalosporins, with resistance rates surpassing 70% in regions such as Africa [19,21,23]. Antimicrobial resistance (AMR) was attributed to approximately 1.27 million fatalities in 2019, and current projections suggest that without effective intervention, the annual death toll could reach as high as 10 million by the year 2050 [24].

Natural compounds are widely recognized for their capacity to mitigate oxidative stress, primarily attributable to their abundant content of polyphenols and phenolic constituents [25]. These bioactive substances engage in the neutralization of ROS and enhance the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Moreover, they activate critical protective signaling pathways such as Nrf2/HO-1 and PI3K/Akt, thereby safeguarding cellular integrity against damage induced by ROS imbalance [26,27]. In addition to their antioxidant properties, natural compounds may also exhibit antimicrobial activity through various mechanisms [28]. Among these, green tea stands out as a widely consumed natural product, renowned for its potential therapeutic effects against a range of diseases. Despite its global popularity as a beverage, there remains a paucity of comprehensive knowledge of the antioxidant and antimicrobial activities of commercially available green tea, particularly regarding its bioactive constituents. Accordingly, this study conducted a qualitative screening and identification of phytochemicals and bioactive compounds in green tea, along with an evaluation of their antioxidant and antimicrobial properties. Furthermore, it investigated the specific bioactive compounds believed to underlie the antimicrobial activity, utilizing both in vitro and in silico methodologies.

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2. Materials and Methods

2.1. Sample Collection and Extraction

A commercial Bangladeshi green tea packet was obtained from a supermarket in Savar District, Dhaka, Bangladesh. To prepare the ethanolic extract, 100.0 grams of green tea dry powder were combined with 100 ml of 95% ethanol and left at room temperature in a shaker for seven days. After this period, the mixture was first filtered through a muslin cloth to remove coarse residues, followed by a final filtration using Whatman No.1 filter paper. The extract was then stored in an airtight bottle at 4 °C.

2.2. Phytochemical Analysis

2.2.1. Qualitative Assessment

To detect alkaloids, 10 g of powdered plant material was combined with 50 ml of 4% HCl on a steam bath. Subsequently, 1 ml of the filtrate was mixed with Mayer's reagent. The formation of a white precipitate indicated the presence of alkaloids. For phenol detection, 2 ml of the extract was mixed with a few drops of ferric chloride solution, and the emergence of a greenish-yellow color confirmed phenol presence. To test for flavonoids, 2 ml of the test solution was combined with a small amount of 10% low-acidity lead acetate. The appearance of white precipitates in any sample suggested the extract contained flavonoids. For tannin identification, 2 ml of the extract was treated with a 10% alcoholic ferric chloride solution, and the development of a blue or green color indicated tannins. To identify saponins, 5 ml of plant extract was mixed with 1-3 ml of mercury chloride solution, resulting in a white precipitate that signified the presence of saponins [29].

2.2.2. Quantitative Assessment

2.2.2.1. Determination of Total Phenolic Content

The total phenolic content was assessed using gallic acid as a standard reference. The Folin-Ciocalteau colorimetric method, with slight modifications from the protocol by Turkmen et al., was employed to determine the total phenolic content (TPC) [30]. Various concentrations of gallic acid (4, 6, 8, 10, 12, 14 μg/mL) were prepared from a stock solution. The sample was initially prepared by dissolving 10 mg in 10 mL of ethanol, followed by further dilution to 200 μg/mL. In brief, 100 μL of both the standard and samples were mixed with 1 mL of 10% Folin-Ciocalteu reagent and allowed to react for three minutes. Subsequently, 3 mL of 7.5% Na_2HCO_3 was added to the mixture, which was then shaken for five minutes and incubated at 37 °C for 15 minutes, followed by a one-hour incubation in the dark. The absorbance was measured at 759.75 nm using UV-Visible Spectrophotometry, with distilled water serving as a blank. A standard curve was constructed using gallic acid, and the total phenolic content was expressed as mg/g gallic acid equivalent (GAE).

2.2.2.2. Determination of Total Flavonoid Content

The total flavonoid content in the extract was determined using a modified version of the method by Chang et al. [31]. Quercetin standards were prepared at concentrations of 6, 8, 10, 12, and 14 μg/mL from a 1000 μg/mL stock solution. In short, 1 mL of each standard and sample was placed into separate centrifuge tubes, followed by the addition of 0.2 mL of 10% $AlCl_3$ and 0.1 mL of 1M CH_3COOK. The mixture in each tube was thoroughly mixed using a vortex mixer for two to three minutes and then left to stand at room temperature for 30 minutes. Absorbance was measured at 435 nm using UV-Visible spectrophotometry. Quercetin served as the standard reference for quantifying total flavonoids, and the total flavonoid content is expressed as grams of quercetin equivalent (QE) per extract.

2.2.3. Antioxidant Activity

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2.2.3.1. Total Antioxidant Capacity Assay

The total antioxidant capacity (TAC) was evaluated using a method that had been previously established [32]. Extracts of 0.1 ml were combined with ethanol, while ascorbic acid was used separately as a standard in concentrations ranging from 5 to 200 g/mL, along with 1 ml of a reagent mixture in ethanol. The test tubes were then covered with aluminum foil and placed in a water bath at 95 °C for 90 minutes. After incubation, the extracts were allowed to cool to room temperature. Once cooled, the sample's absorption was measured at 695 nm. Ascorbic acid served as the standard. The antioxidant activity is represented as the equivalent amount of ascorbic acid and is calculated using the following equation: The antioxidant capacity was determined using the following formula:

$$\text{Antioxidant effect (\%)} = \left[\left(\text{control absorbance} - \text{sample absorbance}\right)/\left(\text{control absorbance}\right)\right] \times 100$$

2.2.3.2. DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was assessed using a method based on Turkmen et al., with minor adjustments [30]. The assay for radical scavenging activity was conducted using 1,1-diphenyl-2-picrylhydrazyl (DPPH) with slight modifications. A DPPH solution (0.004% w/v) was created by dissolving the DPPH radical in ethanol. Sample stock solutions at a concentration of 1 mg/ml were diluted to final concentrations of 800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/ml in 95% ethanol. To 1.6 ml of these sample solutions at varying concentrations, 0.5 ml of the DPPH ethanol solution (0.2 mM) was added, mixed thoroughly using a vortex, and left to react at room temperature in the dark. After 30 minutes, the absorbance was measured at 517 nm using a UV/Vis spectrophotometer.

The free radical scavenging activity of the samples was measured using the following formula:

$$\text{DPPH Inhibition}(\%) = [1 - A_1/A_0] \times 100$$

(A_1= Absorbance of sample, A_0= Absorbance of control)

2.2.4. Bioactive Compound Analysis Using HPLC

High-performance liquid chromatography (HPLC) analysis was performed following previously established methods, with some adjustments, to identify the bioactive compounds [33]. Initially, all standards and sample extracts were dissolved in a small volume of ultrapure water. These solutions were then filtered using a solid phase extraction (SPE) filter (0.45 µm) and stored separately. The analysis was carried out using an HPLC system (SPD-20AV, Serial no.: L20144701414AE, Shimadzu Corporation, Kyoto, Japan) equipped with a UV detector (SPD-20AV, Serial no.: L20144701414AE, Shimadzu Corporation, Kyoto, Japan). The method employed a Luna Phenomenex, C18 100A (150×3×4.60 mm, 5 µm) HPLC column. The binary mobile phase was composed of solvent A (ultrapure water with 0.1% phosphoric acid) and solvent B (pure methanol with 0.1% phosphoric acid). A linear gradient flow was established, with a flow rate ranging from 0.5 to 1.0 mL/min. The detection wavelength spanned from 200 to 450 nm, with specific monitoring at 265 nm. Identification of phenolic and flavonoid compounds was achieved by comparing the retention times of the analytes with those of the reference standards. Pure standards of phenolic and flavonoid compounds, such as gallic acid, vanillic acid, rutin, tannic acid, and quercetin, were utilized.

2.2.5. Antibacterial Activity Analysis

2.2.5.1. Collection, Subculture, and Inoculum of the Bacterial Strains

The microbial test samples were obtained from the Department of Microbiology at Gono Bishwabidyalay, Bangladesh, to investigate the potential antibacterial properties of the extracts. Both gram-positive bacteria, such as *Staphylococcus aureus*, and gram-negative bacteria, like *E. coli*, were

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chosen for the study. The bacterial isolates were cultured on nutrient agar media at 37 °C for 24 hours. A loopful of isolated colonies of *S. aureus* and *E. coli* was introduced into 10 ml of 0.9% saline solution and incubated at 37 °C for 4 hours. The concentration of the bacterial suspension was then adjusted through serial dilution in saline to achieve 2×10^8 colony-forming units per ml (CFU/ml). This activated culture was subsequently used as inoculums for further experimentation.

2.2.5.2. Antibacterial Activity

The microbiological analysis utilized Muller-Hinton agar (Merck, Germany). The agars were prepared following the manufacturer's guidelines. The antibacterial properties of the ethanolic green tea extract (1.0 g/ml) were assessed against each strain using the agar well diffusion technique, with volumes of 50, 100, 150, and 200 µL per well. The bacterial inoculum was spread on the Muller-Hinton agar medium using a sterile cotton swab in both horizontal and vertical directions to ensure even microbial growth. Wells measuring 6 mm were created in the agar and filled with extracts. Control plates were prepared using Ampicillin (10 mg/ml) as a positive control and ethanol as a negative control. The plates were incubated at 37 °C for 18-24 hours. The antimicrobial activity was determined by measuring the inhibition zones around the tested bacteria. This method was derived from the study by Turkmen et al. [34].

2.2.6. In Silico Analysis

2.2.6.1. Molecular Docking Analysis of Extracted Molecules

The molecular docking, which means receptor-ligand binding, is an essential tool for computational drug and vaccine development, and the more negative score indicates the more stable binding capacity [35–37]. The extracted gallic acid and vanillic acid molecules 3D structure were retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database in SDF format [38]. On the other hand, the target proteins: Penicillin-Binding Protein 2a (PBP2a), Sortase A (SrtA) for *S. aureus* and AmpC β-lactamase, DNA Gyrase B (Gyr-B) for *E. coli* were selected for this study based on the literature review and their activity for the infections [39–42]. Their 3D structure was downloaded from the RCSB PDB database (https://www.rcsb.org/) with the PDB ID: 1VQQ (PBP2a), 6R1V (SrtA), 3BLS (β-lactamase), and 6KZV (Gyr-B) [43]. Subsequently, molecular docking of extracted gallic acid and vanillic acid molecules with the selected target proteins was conducted with AutoDock techniques inside PyRx software [44]. Initially, we minimized the energy of all ligands and transformed the SDF ligand format into PDBQT. Afterwards, a grid box was established with the coordinates for the center (X = 18.0807, Y = 28.2054, and Z = 51.6334) and dimensions (Å) (X = 69.1567, Y = 57.1468, and Z = 129.4400) for PBP2a, center (X = -0.3650, Y = 10.2535, and Z = 16.1502) and dimensions (Å) (X = 38.9708, Y = 41.4497, and Z = 52.1391) for SrtA, center (X = 31.2760, Y = -0.3896, and Z = 18.9596) and dimensions (Å) (X = 56.5650, Y = 53.2838, and Z = 54.1409) for β-lactamase, and center (X = 0.9881, Y = 21.7224, and Z = -0.2180) and dimensions (Å) (X = 48.4103, Y = 52.7810, and Z = 43.4929) for Gyr-B. The docking scores (binding affinity in kcal/mol) between ligands and the target proteins were acquired as a CSV file using the molecular docking procedure. Lastly, the different types of bond interactions between ligand and protein were visualized and analyzed by BIOVIA Discovery Studio Visualizer v20.1.0.19295 software [45].

2.2.6.2. Pharmacokinetics (ADME) Analysis of Extracted Molecules

Pharmacokinetics (ADME) encompasses the four phases of absorption, distribution, metabolism, and excretion is essential for drug development, since it influences the efficacy and quality of pharmaceuticals. Consequently, the ADME properties of extracted molecules: gallic acid and vanillic acid were assessed using the SwissADME server (http://www.swissadme.ch/), which predicts various characteristics of small molecules, including physicochemical properties, aqueous solubility, lipophilicity, gastrointestinal absorption, blood-brain barrier permeability, liver metabolic

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enzyme CYP inhibition, and other therapeutic potentials (drug-likeness and medicinal chemistry) [46].

2.2.6.3. Toxicity Analysis of Extracted Molecules

Toxicity prediction of drug molecules is crucial in medication development. Consequently, we were utilized the online ProTox 3.0 (https://tox.charite.de/protox3/) server to assess the toxicity profiles, including organ (liver, brain, kidney, lung, heart) toxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity [47].

2.2.6.4. PASS Prediction of Extracted Molecules

The PASS (Prediction of Activity Spectra for Substances) prediction is used for the estimation of the biological activity spectrum for compounds. To predict the biological activities of the extracted molecules: gallic acid and vanillic acid, we using the Prediction of Activity Spectra for Substances; PASS-Way2Drug web server (https://www.way2drug.com/passonline/) [48]. Using MNA (multilevelneighbors of atoms) descriptors, it helps to determine the effects of a compound based solely on its molecular formula, which implies that biological behavior is solely a function of its chemical structure.

3. Results

3.1. Phytochemical Screening

The secondary metabolites tested were alkaloids, saponins, phenolics, and flavonoids. The results showed that alkaloids, phenolics, tannins, and flavonoids were present in green tea, whereas no saponins were observed (Table 1).

Table 1. Phytochemical screening of the green tea extract.

Phytochemical test	Tea Leaves extract
Alkaloids	Positive
Flavonoids	Positive
Phenols	Positive
Tannins	Positive
Saponins	Negative

3.2. Phytochemical Quantification

The TPC and TFC of green tea was found to be similar in quantity where the values were 35.95±0.24 mg GAE/g and 34.61±1.53 mg QE/g respectively (Table 2).

Table 2. Total phenolic and total flavonoid content for green tea extracts.

	Total Phenolic Content (TPC) (mg GAE/g)	Total Flavonoids Content (TFC) (mg QE/g)
Green tea extract	35.95±0.24	34.61±1.53

3.3. Antioxidant Activity

The antioxidant activity of green tea was observed in both the Total Antioxidant Capacity (TAC) and DPPH radical scavenging assays. The TAC was determined to be 301.01±14.32 mg AAE/g, whereas the IC_{50} value of green tea was found to be 2.70 µg/mL as compared to the IC_{50} value of ascorbic acid (3.75 µg/mL) in the percent (%) scavenging activity in DPPH Radical Scavenging Assay (Table 3).

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Table 3. Total antioxidant capacity and DPPH radical scavenging assay.

Total Antioxidant Capacity	Total antioxidant activity assay (mg AAE/g)	301.01±14.32
DPPH Radical Scavenging Assay	IC_{50} value of Ascorbic Acid	3.75
	IC_{50} value of green tea	2.70

3.4. Identification of the Bioactive Compounds

More than one phenolic compound was identified among all the samples. Figure 1 and Table 4 represent the detailed list of the identified compounds.

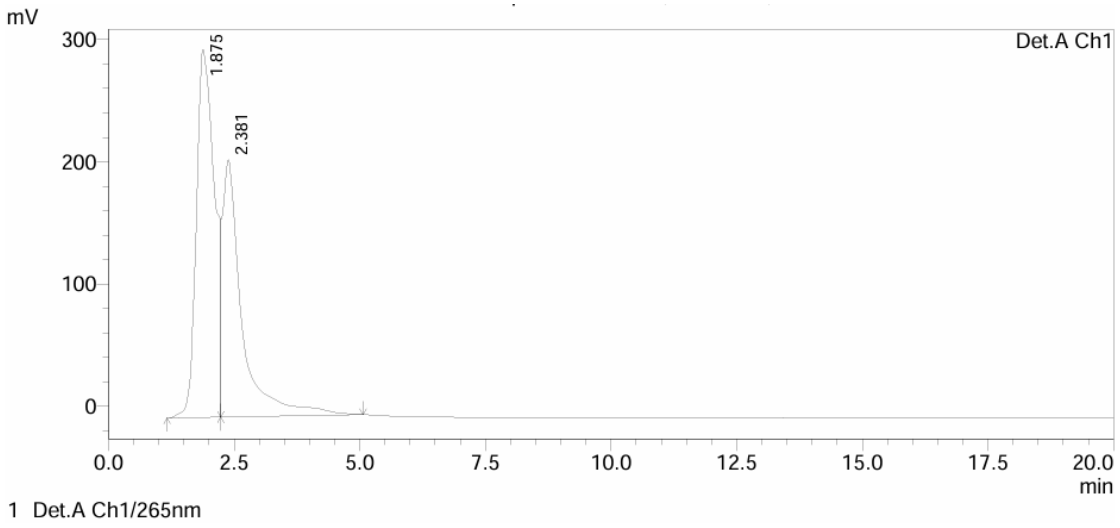

Figure 1. HPLC chromatogram analyzing bioactive compounds from the green tea extract.

Table 4. List of identified compounds in green tea compared to the standard.

Standards	**Retention time**	**Identified bioactive compounds in sample**	**Retention time**
Gallic acid	1.83	Gallic acid	1.86
Vanillic acid	2.42	Vanillic acid	2.38
Rutin	3.19	ND	-
Tannic acid	3.92	ND	-
Quercetin	6.63	ND	-

Here, retention time was expressed in minutes. ND: Not detected.

3.5. Antibacterial Effects

The zone of inhibition was observed against both the bacteria (i.e., *S. aureus* and *E. coli*) for each of the concentrations used of green tea extract. The lowest diameter of zone was observed as 12 mm and 10mm for *S. aureus* and *E. coli,* respectively, for the 25 mg/ml extract of green tea; whereas the highest diameter of zone was observed as 22 mm and 20 mm for *S. aureus* and *E. coli*, respectively, for the 200 mg/ml extract of green tea (Figure 2).

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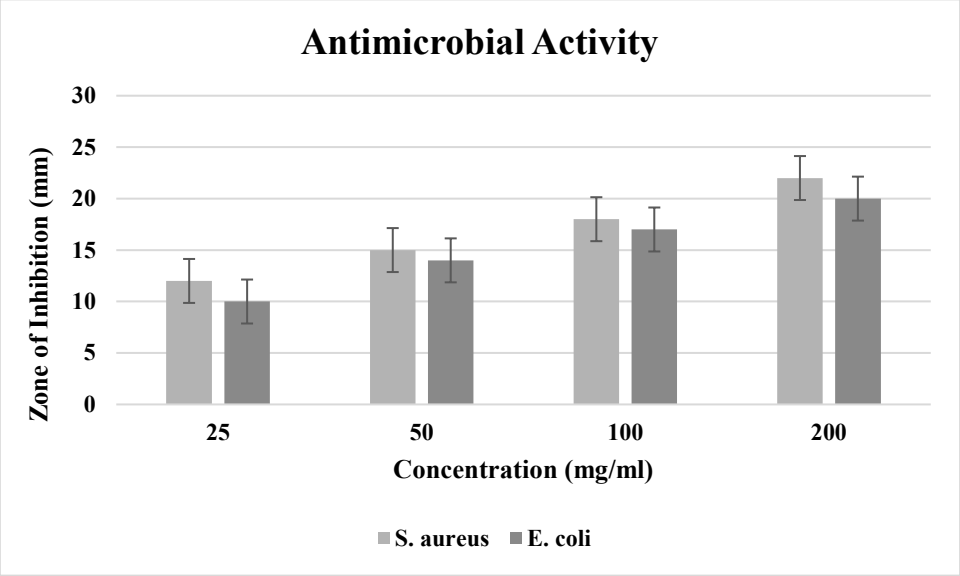

Figure 2. Assessment of the diameter of the zone of inhibition using different concentrations of green tea extract for *S. aureus* and *E. coli*.

3.6. In Silico Analyses

3.6.1. Molecular Docking Analysis of Extracted Molecules

Following the molecular docking analysis using PyRx software, we found the favorable negative binding affinity in kcal/mol and different bond interactions of ligand-protein complexes, which is shown in Table 5.

Table 5. The docking scores and bond interactions of ligand-protein complexes.

Compound Name and PubChem CID	Target Protein	Docking score (kcal/mol)	Bond interactions name and binding residues of protein	
Gallic Acid (CID370)	PBP2a	-6.0	Conventional Hydrogen Bond	GLY282, TYR366, ASN393, TYR496, GLN396, LEU391
			Carbon Hydrogen Bond	HIS251
			Pi-Donor Hydrogen Bond	ASN393
			Pi-Alkyl	LYS281
	SrtA	-5.1	Conventional Hydrogen Bond	GLU47
Vanillic Acid (CID8468)	PBP2a	-5.8	Conventional Hydrogen Bond	THR399, LYS604, SER400
			Alkyl	LEU603, LYS604
			Pi-Alkyl	ILE614
	SrtA	-4.9	Conventional Hydrogen Bond	ARG101, GLN120
			Carbon Hydrogen Bond	VAL110
			Pi-Sigma	VAL143
Gallic Acid (CID370)	β-lactamase	-5.7	Conventional Hydrogen Bond	ARG148, ALA292
			Carbon Hydrogen Bond	HIS314
			Pi-Anion	GLU272
			Van Der Waals	SER287
			Amide-Pi Stacked	GLY286
			Pi-Alkyl	MET265, ALA292
	Gyr-B	-5.9	Conventional Hydrogen Bond	ASP73, GLU50, GLY77

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Vanillic Acid (CID8468)	β-lactamase	-5.8	Pi-Anion	GLU50
			Van Der Waals	ALA47
			Amide-Pi Stacked	ASN46
			Pi-Alkyl	ILE78
			Conventional Hydrogen Bond	ARG148, HIS314, ALA292
			Carbon Hydrogen Bond	HIS314, GLU272
			Pi-Sulfur	MET265
			Alkyl	ALA292
			Pi-Alkyl	TYR150, ALA292
			Conventional Hydrogen Bond	ASN46
			Carbon Hydrogen Bond	ASN46
	Gyr-B	-6.0	Pi-Sigma	THR165
			Van Der Waals	ALA47
			Amide-Pi Stacked	ASN46
			Pi-Alkyl	VAL120, VAL167

Additionally, Figures 3–6 illustrated the 2D and 3D representations of bond interactions between ligands and amino acids in proteins.

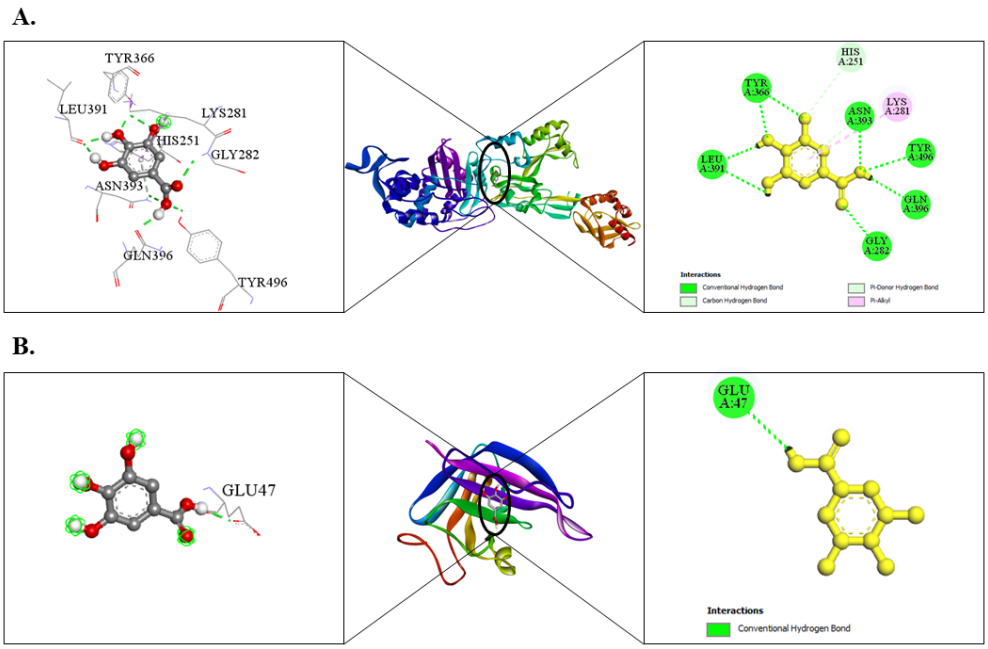

Figure 3. 3D (left) and 2D (right) representation of ligand-protein complexes. (A) Gallic acid with PBP2a and (B) Gallic acid with SrtA protein.

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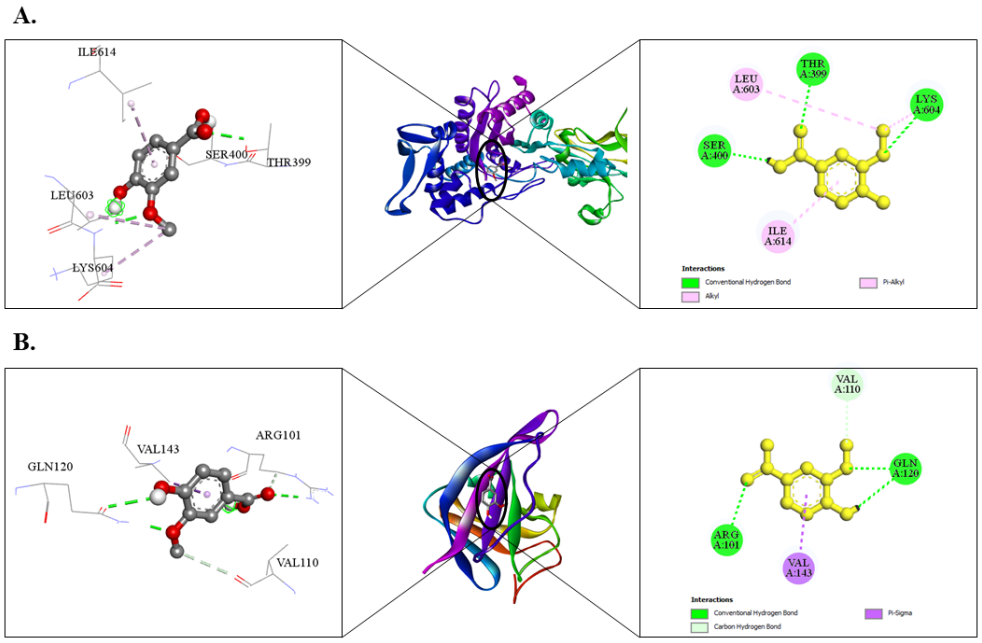

Figure 4. 3D (left) and 2D (right) representation of ligand-protein complexes. (A) Vanillic acid with PBP2a and (B) Vanillic acid with SrtA protein.

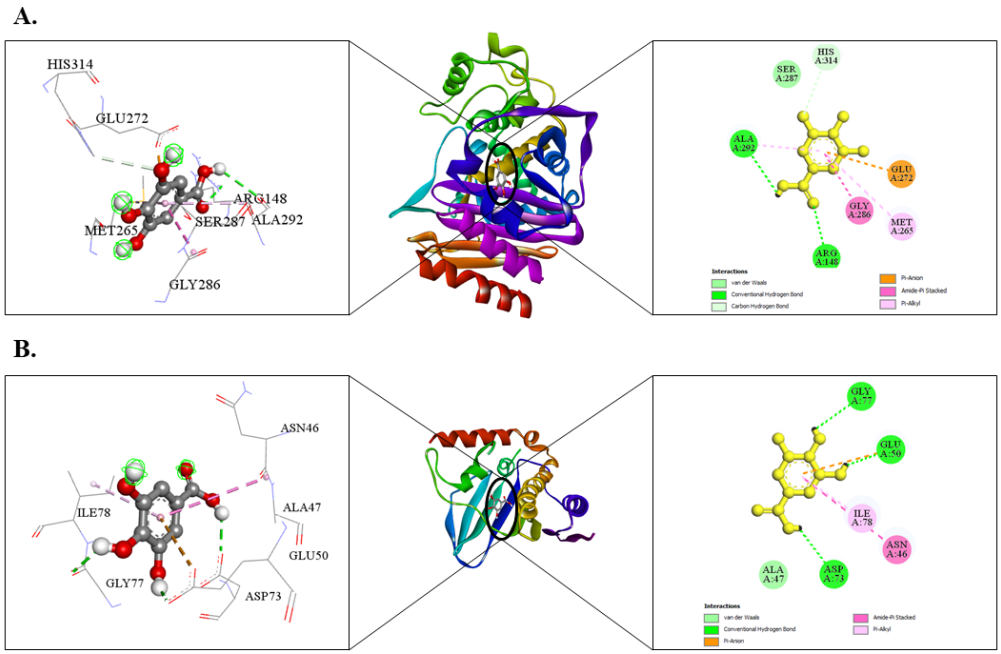

Figure 5. 3D (left) and 2D (right) representation of ligand-protein complexes. (A) Gallic acid with AmpC β-lactamase and (B) Gallic acid with Gyr-B protein.

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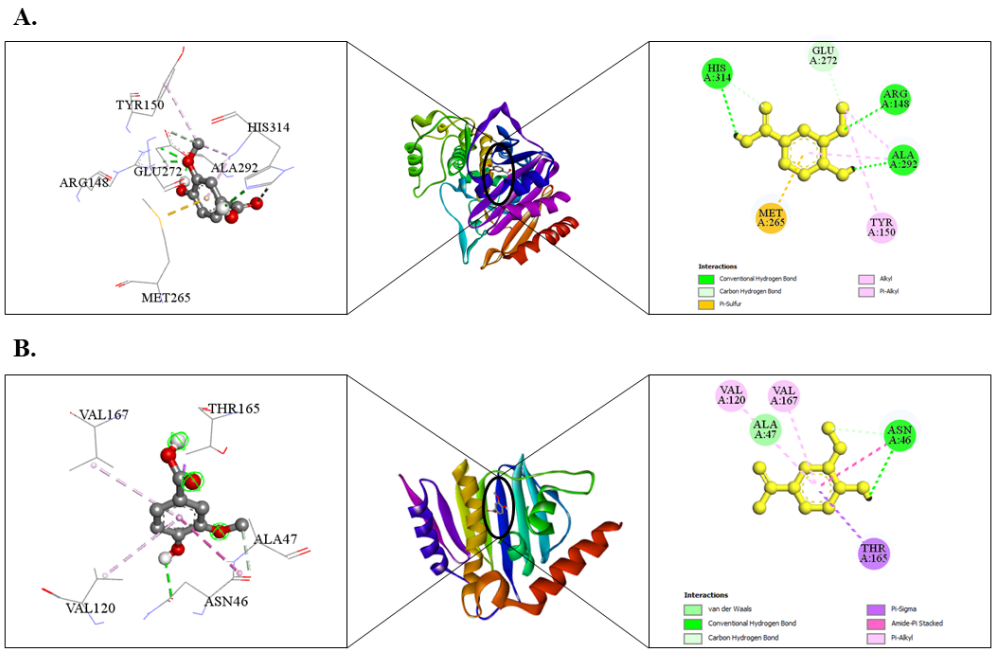

Figure 6. 3D (left) and 2D (right) representation of ligand-protein complexes. (A) Vanillic acid with AmpC β-lactamase and (B) Vanillic acid with Gyr-B protein.

3.6.2. Pharmacokinetics (ADME) Analysis of Extracted Molecules

In pharmacokinetics (ADME) analysis, we discovered the extracted molecules exhibiting enhanced pharmacokinetics profiles, such as outstanding physicochemical properties, good solubility in water and lipid, high GI absorption, no violations in Lipinski rule of 5 and etc. shown in Table 6.

Table 6. Pharmacokinetics (ADME) profiles of gallic acid and vanillic acid.

Properties		Gallic Acid	Vanillic Acid
Physico-chemical properties	Formula	$C_7H_6O_5$	$C_8H_8O_4$
	Molecular weight (g/mol)	170.12	168.15
	Number of Heavy atoms	12	12
	Number of Aromatic heavy atoms	6	6
	Number of Rotatable bonds	1	2
	Number of H-bond acceptors	5	4
	Number of H-bond donors	4	2
	Water solubility (Log S (ESOL))	-1.64 (Very soluble)	-2.02 (Soluble)
	Lipophilicity (Consensus Log Po/w)	0.21	1.08
Pharmacokinetics	GI absorption	High	High
	BBB permeability	No	No
	P-gp substrate	No	No
	CYP1A2 inhibitor	No	No
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	Yes	No
	Skin Permeability (Log Kp) (cm/s)	-6.84	-6.31
Drug Likeness	Lipinski rule of 5	Yes; 0 violation	Yes; 0 violation
	Bioavailability Score	0.56	0.85
Medicinal Chemistry	Synthetic accessibility	1.22	1.42

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3.6.3. Toxicity Analysis of Extracted Molecules

We analyzed the toxicity properties of gallic acid and vanillic acid, and we identified both compounds has outstanding safer profiles for medications, while gallic acid has few active toxicity properties and vanillic acid shows only nephrotoxicity. Table 7 demonstrated the toxicity profiles of these compounds.

Table 7. Toxicity profiles of gallic acid and vanillic acid.

Toxicity Profiles	Gallic Acid	Vanillic Acid
Hepatotoxicity	Inactive	Inactive
Neurotoxicity	Inactive	Inactive
Nephrotoxicity	Active	Active
Respiratory toxicity	Active	Inactive
Cardiotoxicity	Inactive	Inactive
Carcinogenicity	Active	Inactive
Immunotoxicity	Inactive	Inactive
Mutagenicity	Inactive	Inactive
Cytotoxicity	Inactive	Inactive

3.6.4. PASS Prediction of Extracted Molecules

The biological activity of gallic acid and vanillic acid predicted by using the PASS-Way2Drug webserver, which having identical biological activities. Both compounds were found to have antibacterial, Beta lactamase, DNA gyrase inhibitor properties. When Pa>Pi, both molecules showed strong antibacterial properties, with higher Pa range than Pi. The predicted activities of gallic acid and vanillic acid with the highest Pa values are shown in Table 8.

Table 8. Biological activity prediction of gallic acid and vanillic acid.

Compound	Pa	Pi	Biological Activity
Gallic Acid	0,418	0,026	Antibacterial
	0,266	0,008	Beta lactamase inhibitor
	0,085	0,004	DNA gyrase inhibitor
Vanillic Acid	0,376	0,036	Antibacterial
	0,214	0,015	Beta lactamase inhibitor
	0,066	0,004	DNA gyrase inhibitor

4. Discussion

This study presents a comprehensive analysis of the phytochemical constituents present in the ethanolic extract of a commercial green tea sourced from Bangladesh. The investigation involved assessing its antioxidant capacity using both total antioxidant capacity (TAC) and DPPH radical scavenging assays. Notably, bioactive compounds, including gallic and vanillic acids, were identified in the extract. Furthermore, the antimicrobial efficacy of the extract was evaluated against a range of pathogenic microorganisms, including *S. aureus* and *E. coli*. In addition to these investigations, in silico analyses were conducted, including molecular docking, pharmacokinetic evaluations, toxicity profiling, and bioactivity predictions using the PASS prediction methodology for the identified compounds.

A qualitative analysis of our green tea extract indicated the presence of various bioactive compounds, including alkaloids, flavonoids, phenols, and tannins (Table 1). Notably, tea extracts, particularly those derived from black, white, and oolong varieties, are abundant in these compounds, which are associated with enhanced antioxidant properties and potential health benefits. Our findings are consistent with existing literature. For example, previous studies have documented significant concentrations of alkaloids, including caffeine, as well as tannins and phenols, alongside

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flavonoids such as catechins in oxidized teas, particularly black tea [25,49]. Similarly, less processed and partially oxidized teas, such as white and oolong teas, exhibit considerable levels of these beneficial compounds. Furthermore, Tan et al. conducted a quantitative analysis of 58 Chinese white tea samples using HPLC, identifying major catechins, hydrolysable tannins, phenolic acids, flavonol glycosides, alkaloids, and theanine [50]. Concurrently, studies by Wang and Dou et al. have corroborated the presence of these bioactive compounds in oolong tea, reinforcing the significance of tea varieties for health and wellness [51,52].

This study quantitatively assessed the total phenolic content (TPC) in the study sample, yielding a measurement of 35.95 ± 0.24 mg gallic acid equivalents per gram (GAE/g) (Table 2). The existing literature presents a spectrum of TPC values across various tea types, thereby facilitating comparative analysis. For instance, a comprehensive study of eight Ethiopian tea brands identified TPC values ranging from 21.3 ± 0.24 mg GAE/g in Eirmon tea to 31.6 ± 0.31 mg GAE/g in certain green tea varieties [53]. Notably, Unachukwu et al. recorded TPC values of 1.17 mg GAE/g for Silver Needle green tea and 0.96 mg GAE/g for Jasmine Silver Needle white tea, suggesting that local harvesting practices and minimal fermentation contribute to reduced phenolic concentrations [54]. Furthermore, white teas, characterized by lower levels of oxidation, had a TPC of approximately 0.96 mg GAE/g. In contrast, chamomile herbal blends exhibited even lower TPC values, measuring 0.84 mg GAE/g, while berry herbal teas recorded a significantly low concentration of 0.099 ± 0.015 mg GAE/g, and hibiscus blends presented a TPC of 0.65 mg GAE/g. These discrepancies underscore the impact of gentle processing and blending on the dilution of polyphenolic compounds in herbal infusions [54–57]. Additionally, an analysis of black tea powders revealed that TPC varies by grade, with broken orange pekoe (BOP) at 14.16 mg GAE/g and super red dust (SRD) at 17.27 mg GAE/g, a variation attributable to factors such as leaf fragmentation and oxidation [58]. Some Korean green teas showed particularly low TPC levels, down to 7.69 mg GAE/g [55,59]. The observed variations in TPC across different tea types can be attributed to multiple factors, including cultivar traits, processing methodologies, extraction techniques, and regional cultivation practices, highlighting the need for standardized methodologies in future studies to ensure consistent and comparable results [30,56,60].

This study also quantitatively assessed the total flavonoid content (TFC) in green tea extract at 61 ± 53 mg quercetin equivalents per gram (mg QE/g) (Table 2). The TFC across various tea types exhibits considerable variability, attributable to factors such as cultivar selection, genetic differences, and extraction methodologies. For example, Darjeeling teas exhibit higher catechin concentrations than Ceylon teas [61]. The processing technique employed significantly affects TFC levels; non-fermented teas, such as white and green varieties, retain higher levels of native flavonoids, particularly catechins, whereas fully fermented teas, exemplified by black tea, undergo a transformation in which the majority of flavonoids are converted into thearubigins, leading to a reduction in TFC [60–62]. Recent investigations have systematically quantified flavonoid levels across different tea types, e.g., black, white, green, oolong, among others, expressed in mg QE/g [62]. For instance, research by Fawwaz et al. on green tea extracts reported moderate TFC levels, with crude extracts at approximately 2.77 mg QE/g and purified extracts at roughly 3.14 mg QE/g, indicating a slight enhancement post-purification [63]. Similarly, Cong-Hau et al. documented TFC values for oolong tea in the range of 42.0-59.8 mg QE/g through a spectrophotometric method [62]. Additionally, Tartary buckwheat teas exhibited TFC levels ranging from 8 to 24 mg QE/g, with heightened levels observed in scented varieties attributed to the presence of rutin [64].

In this investigation, the use of polar solvents, notably ethanol, yielded the highest crude extract yields and the most varied phytochemical composition. The ethanolic extract showed a high flavonoid content, a class of bioactive compounds known for their antioxidant properties.

The IC_{50} value indicates the concentration of antioxidants (in µg/mL) required to neutralize 50% of free radicals, with a lower IC50 reflecting stronger antioxidant activity. All extracts showed a dose-dependent increase in their scavenging ability, which we compared directly to ascorbic acid as a standard (Table 3). Literature reviews highlight differences in antioxidant capacity among tea types. For instance, green tea exhibits a significantly higher DPPH free-radical scavenging activity (90.8%)

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than black tea (81.7%), due to differences in processing and polyphenol content [65]. Green tea also has a higher TPC at 2083 mg gallic acid equivalents per liter, compared to 1844 mg in black tea, aligning with its superior scavenging effects reported in many studies [27,56,66,67]. Quantitative data further confirm these findings: green tea achieved a DPPH scavenging capacity of 12,390 µg ascorbic acid equivalents (AAE) per gram, whereas black tea reached 9890 µg AAE/g [67]. White tea recorded 1900 µg AAE/g, and herbal teas like chamomile and hibiscus had lower values at 110 µg AAE/g and 639 µg AAE/g, respectively [68–70]. Black tea's total antioxidant capacity (TAC) was measured at 2.034 ± 1.39 mg AAE/g, ranking it as a moderate antioxidant among teas [71,72].

This study identified the presence of two bioactive compounds—gallic acid and vanillic acid in the analyzed green tea sample (Figure 1) (Table 4). Gallic acid (3,4,5-trihydroxybenzoic acid) and vanillic acid (4-hydroxy-3-methoxybenzoic acid) are phytogenic phenolic compounds that are frequently found in various teas and are recognized for their antibacterial properties [73]. Gallic acid primarily generates from the hydrolysis of tannins, including gallotannins and ellagitannins, and is naturally occurring in a range of foods such as tea leaves, blackberries, bearberries, gallnuts, vinegar, cloves, hot chocolate, red wine, and green tea [74,75]. In contrast, vanillic acid is a product of lignin degradation and is found in the roots of *Angelica sinensis*, tea leaves, cereals, whole grains, and several fruits, as well as in fermented beverages [76–78]. These phenolic compounds combat bacteria through diverse mechanisms, including disrupting cell membranes via lipid peroxidation and pore formation, inhibiting key enzymes like β-galactosidase and alkaline phosphatase, binding essential divalent metal ions such as calcium (Ca^{2+}) and iron (Fe^{2+}), which are crucial for bacterial growth, and reducing biofilm formation by downregulating genes like *icaAD* and *sarA* in pathogenic strains such as methicillin-resistant *S. aureus* (MRSA) [73,79–81].

Various studies investigated the antibacterial properties of polyphenolic catechins and various phenolic acids, including gallic and vanillic acids, present in different types of tea, revealing antibacterial activity that corroborates our findings. For instance, Naderi et al. reported minimum inhibitory concentrations of 150 mg/mL for Iranian green tea and 50 mg/mL for black tea extracts against *Streptococcus mutans*, with inhibition zones of 9.5 mm and 10.9 mm, respectively [82]. Another investigation assessed the antibacterial efficacy of Lipton black tea in Nigeria against *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis*, and *S. aureus*. This study utilized six concentrations of both aqueous and methanolic extracts, revealing that *E. coli* exhibited moderate sensitivity to 6%, 8%, and 10% aqueous extracts, as well as to 2% to 10% methanolic extracts, while *S. aureus* displayed moderate sensitivity to 4% to 10% aqueous and 2% to 10% methanolic extracts [28] (Figure 2). Additionally, a comparative analysis by Liu et al. evaluated the antibacterial effects of four tea varieties—green, oolong, black, and Fuzhuan against two Gram-positive bacteria (*Enterococcus faecalis* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *Salmonella typhimurium*). The results demonstrated that all four teas exhibited dose-dependent antibacterial activity, with green tea demonstrating the most significant effects, followed by oolong, Fuzhuan, and black tea [83].

In the antimicrobial activity evaluation, the sample exhibited greater effectiveness against *S. aureus* than against *E. coli* at all examined extract concentrations (Figure 2). This disparity can be attributed to the inherent differences in susceptibility between Gram-positive and Gram-negative bacteria. Specifically, Gram-negative bacteria are typically less sensitive to antimicrobial agents due to various structural and physiological barriers. These include a lipopolysaccharide-rich outer membrane, upregulation of multidrug efflux pumps, periplasmic enzymes that hydrolyze antimicrobial compounds, and mutations in outer membrane porins [84–86]. Such factors collectively contribute to the enhanced resistance of Gram-negative bacteria compared to the Gram-positive bacterial species.

Most research in this area has predominantly focused on the in vitro antimicrobial properties of polyphenolic compounds such as catechin. In contrast, the present study identified two key bioactive compounds, gallic acid and vanillic acid, and evaluated their antimicrobial effects against pathogenic bacteria through in silico analysis using the PASS prediction method, complemented by in vitro testing of the antibacterial activity of tea extracts (Table 5) (Figures 3–6). Furthermore, PASS analysis

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suggests that both gallic acid and vanillic acid may inhibit DNA gyrase, an enzyme critical for DNA supercoiling during replication and transcription, similar to the action of quinolone antibiotics such as ciprofloxacin, which target this enzyme (Table 8) [87,88]. Additionally, the predictions indicated that these compounds possess β-lactamase inhibitory activity, thereby highlighting their potential utility against pathogenic bacteria, including but not limited to *E. coli*, *Klebsiella* spp., methicillin-resistant *S. aureus* (MRSA), *Enterobacter* spp., and other extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* that exhibit resistance to β-lactam antibiotics such as penicillins, cephalosporins, carbapenems, and monobactams [89]. The results confirmed that green tea extract demonstrates significant antibacterial effects, thereby supporting its potential as a valuable source for the development of novel therapeutic agents against pathogenic bacterial strains.

Analysis of the toxicity profiles of the two bioactive compounds, gallic acid and vanillic acid, identified by HPLC, indicated that both are safe for use in medications, confirming the safety of the studied green tea sample. Although gallic acid exhibited low-level toxic properties, such as nephrotoxicity, respiratory toxicity, and carcinogenicity, previous studies have shown that it has a favorable safety profile, with no nephrotoxicity, respiratory toxicity, or carcinogenicity at typical dietary levels associated with tea consumption (Table 7) [90–92]. Multiple studies emphasize its protective effects against various types of cellular damage. Specifically, isolated gallic acid has been shown to possess significant renoprotective properties, effectively reducing nickel-induced kidney injury and cisplatin-related nephrotoxicity through its antioxidant, anti-inflammatory, and anti-apoptotic actions [92,93].

Current research shows no definitive evidence connecting gallic acid from tea extracts to respiratory toxicity. Instead, the findings emphasize its favorable safety profile and potential anti-inflammatory properties, which may confer indirect benefits for respiratory health [91,94,95]. Furthermore, gallic acid is well known for its anticarcinogenic and antimutagenic properties. Meta-analyses examining tea consumption suggest a correlation with reduced incidence of certain cancers, including prostate and oral cancers, likely due to its ability to reduce oxidative stress and inhibit cancer progression [91,96].

In contrast, vanillic acid has demonstrated adverse effects on renal function, despite the absence of any evidence suggesting such toxicity within tea extract. Studies consistently indicate that vanillic acid possesses renoprotective properties in various experimental models of kidney injury. These benefits are attributed to its multifaceted mechanisms, which include antioxidant, anti-inflammatory, and anti-apoptotic actions [97–99]. In a model of methotrexate-induced kidney damage, vanillic acid doses ranging from 50 to 100 mg/kg markedly reduced serum creatinine and urea nitrogen levels, concurrently decreasing malondialdehyde (MDA) concentrations while elevating TAC level and increasing Nrf-2 expression in rodent subjects [97]. Furthermore, vanillic acid has been shown to alleviate kidney toxicity induced by sodium arsenite and cisplatin, primarily by restoring antioxidant enzyme activity and reducing pro-inflammatory markers [98,100].

This study highlights the remarkable properties of the ethanolic extract of Bangladeshi green tea, emphasizing its elevated levels of TPC and TFC. The extract exhibited significant antioxidant activity, as demonstrated by TAC and DPPH radical scavenging assays. Furthermore, it revealed notable antibacterial activity against *S. aureus* and *E. coli*, attributed to the presence of key phytochemicals, including gallic and vanillic acids. In silico analyses further substantiate its therapeutic potential by elucidating mechanisms such as gyrase inhibition and modulation of β-lactamases. Toxicity evaluations suggest a favorable safety profile, thereby indicating the potential of this green tea extract as a promising candidate for the development of novel antioxidant and antimicrobial agents.

5. Limitations of the Study

This study did not determine any protective properties of the extract or the identified bioactive compounds in vivo. This study did not use any confirmed antibiotic-resistant bacteria from human samples to further confirm the effect of the extract against MDR or XDR.

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6. Conclusions

Green tea leaves have dual benefits, such as medicinal values and food values by antioxidant properties. In this study, we found that leaf extracts were found to be potential antibacterial agents against the various bacteria causing infection. Thus, *Camellia sinensis* leaves can be used as an alternative medicine against bacterial infection. The research showed that green tea extract possessed a significantly high antioxidant activity due to the greatest amount of biologically active substances. As the green tea exhibited better antioxidant activity, this study will help to promote the consumption of green tea in Bangladesh.

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