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Research Article

Phytochemical Profiling, Antibacterial Properties and Toxicity of Amla Fruit Tea (*Phyllanthus emblica* L.): An *In Vitro* and *In Silico* Study

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Abstract
Antimicrobial resistance represents a critical global health challenge, necessitating the exploration of alternative therapeutic agents. This study investigated the antimicrobial potential of amla fruit tea (*Phyllanthus emblica* L.) through comprehensive phytochemical characterization, antibacterial assessment, and computational modeling to identify potential mechanisms of action. LC-HRMS analysis was employed for phytochemical profiling, antibacterial activity was evaluated via disk diffusion method against *Staphylococcus aureus* and *Escherichia coli*, and molecular docking studies were conducted against tyrosyl-tRNA synthetase and FimH adhesin proteins. Analysis identified 89 bioactive compounds, with oxidized hydroxytetrahydrofuranyl acetate, L-α-palmitin, and ellagic acid predominating. Antibacterial activity against *S. aureus* and *E. coli* was evaluated via the disk diffusion method, revealing that moderate inhibition increased at higher concentrations (25%) and with extended exposure, with *E. coli* exhibiting greater susceptibility than *S. aureus*. Molecular docking studies against tyrosyl-tRNA synthetase (*S. aureus*) and FimH adhesin protein (*E. coli*) identified the W-18 benzenesulfonamide derivative as the most promising compound, which demonstrated strong binding affinities of -11.01 and -7.48 kcal/mol, respectively. While all five principal compounds met Lipinski's drug-likeness criteria, toxicological assessment revealed varying safety profiles, with two compounds classified as "possibly hazardous" and two as "toxic when swallowed." These findings suggest that amla fruit tea has antibacterial properties through two mechanisms: disruption of protein synthesis and bacterial adhesion. However, its efficacy remains considerably lower than that of conventional antibiotics, suggesting potential applications as complementary therapy rather than antibiotic replacement.

Keywords: amla fruit tea, antimicrobial, phytochemicals, molecular docking, toxicity, LC-HRMS, tyrosyl-tRNA synthetase, FimH adhesin

1. INTRODUCTION

The global health crisis of antimicrobial resistance (AMR) represents one of the most pressing public health challenges of the 21st century, threatening the efficacy of conventional antibiotics and potentially returning humanity to a pre-antibiotic era [1][2]. Current projections estimate that AMR could cause 10 million deaths annually by 2050, with economic consequences exceeding $100 trillion globally [3]. The rapid emergence of multidrug-resistant pathogens, coupled with a declining pipeline of novel antimicrobial agents, creates an urgent need for alternative therapeutic approaches with distinct mechanisms of action. This emerging crisis necessitates the urgent exploration of alternative antimicrobial, particularly those derived from plant sources that offer reduced side effects and novel mechanisms to combat resistant pathogens [4]-[7].

The development of plant-based traditional medicines into standardized formulations addresses the growing preference for natural therapeutic agents perceived as safer, with reduced side effects and a lower potential for developing resistance with prolonged use [8]. This trend coincides with the increasing prevalence of infectious diseases and associated complications exacerbated by antimicrobial resistance [9]. Conventional antibiotics, while effective, often present complications, including allergic reactions, organ toxicity, and disruption of the gut microbiota, leading to secondary infections such as *Clostridioides difficile*-associated diarrhea [10]. Recent reviews indicate that plant-derived antimicrobials can maintain efficacy against resistant strains while demonstrating favorable safety profiles, with up to a 60% reduction in adverse events compared with synthetic alternatives [11]-[13].

Amla fruit (*Phyllanthus emblica* L.) has emerged

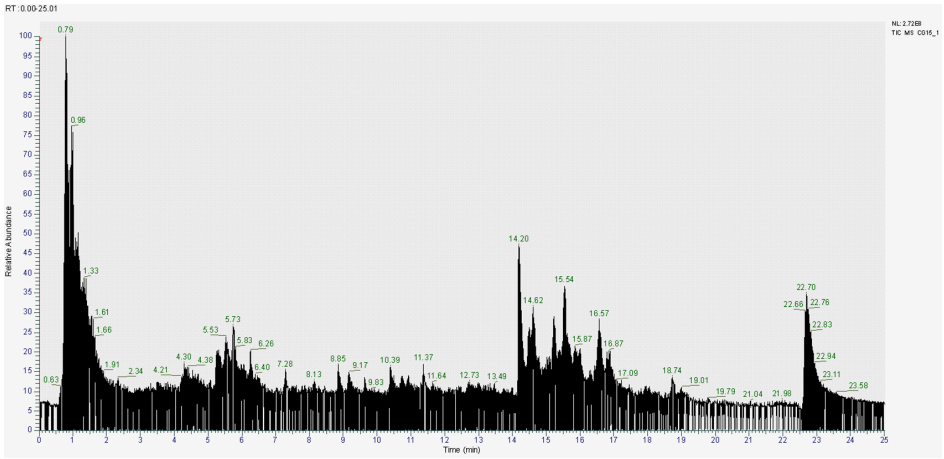

Figure 1. LC-HRMS chromatogram of amla fruit tea infusion.

as a particularly promising candidate for antimicrobial applications because of its rich phytochemical profile [14][15]. Traditional applications of amla in Ayurvedic, Chinese, and various Southeast Asian medical systems have documented its use in treating infectious conditions, suggesting that its inherent antimicrobial properties are worthy of scientific investigation [12][16]. Previous research has identified numerous bioactive compounds in amla, including emblicanin, punigluconin, pedunculagin, geranin, chebulagic acid, corilagin, gallic acid, and various flavonoids, that contribute to its therapeutic potential [17]-[20]. These compounds have demonstrated preliminary antibacterial activity through various mechanisms, including membrane disruption, enzyme inhibition, and quorum sensing interference [21]. Recent investigations have reported minimum inhibitory concentrations (MICs) of amla extracts ranging from 62.5–500 µg/mL against common pathogens, with synergistic effects observed when these extracts are combined with conventional antibiotics [22]. This synergism suggests potential applications in combination therapies to combat resistant infections and reduce the required antibiotic dosages.

Despite extensive traditional use, the scientific validation of amla's antimicrobial properties has been predominantly limited to crude extracts or isolated compounds rather than consumer-ready formulations that facilitate practical application [23]. This research gap exists alongside inconsistent standardization practices that complicate the translation of traditional knowledge into evidence-based applications. Traditional preparation methods often involve complex procedures that create barriers to consistent dosing and therapeutic outcomes [24][25]. While previous studies have identified specific antimicrobial compounds in amla, they have rarely addressed critical factors for clinical translation, including bioavailability, stability, and delivery optimization. Furthermore, comprehensive mechanistic investigations linking phytochemical profiles to specific molecular targets remain scarce, limiting rational formulation design [26]. Therefore, developing standardized, accessible formulations that preserve amla's bioactive profile while meeting modern consumption preferences represents a significant advancement in natural product research.

Our previous investigations characterized the antioxidant capacities of various amla preparations and reported that decoctions demonstrated an antioxidant capacity of 164 mg/100 mL with an IC_{50} of 691.1 g/mL, whereas expressed juice preparations presented values of 100 mg/100 mL and 706 g/mL [25]. On the basis of these findings, we hypothesized that a tea formulation could effectively extract and stabilize amla's bioactive compounds while providing a convenient delivery system. This approach addresses a fundamental question in natural product development: can traditional medicinal plants be effectively translated into standardized, evidence-based therapeutic agents while maintaining their complex bioactivity profiles? The novelty of this research lies in the development of amla fruit tea as a potential phytopharmaceutical candidate through comprehensive analysis of its phytochemical constitution, antibacterial properties, and structure–

activity relationships via computational modeling. This research applies an integrated analytical approach combining advanced chromatographic techniques with *in vitro* antibacterial assessment and *in silico* molecular docking to elucidate potential mechanisms of action against representative pathogenic bacteria.

This study aimed to investigate the antibacterial potential of amla fruit tea through phytochemical characterization, *in vitro* antimicrobial assessment against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria, and *in silico* molecular docking analysis to identify potential mechanisms of action. By providing comprehensive chemical and biological characterization of amla fruit tea, this research contributes to the growing field of evidence-based natural product development for combating antimicrobial resistance while addressing the need for accessible, standardized phytopharmaceutical formulations with reduced side effect profiles compared with those of conventional antibiotics. These findings may provide a foundation for the development of novel plant-based antimicrobial agents that address the dual challenges of increasing resistance and adverse effects associated with conventional antibiotics, potentially offering new therapeutic options for infectious disease management in an era of diminishing antimicrobial efficacy.

2. MATERIALS AND METHODS

2.1. Study Design and Setting

This study used an experimental research design with a complete randomized design [27]. This research was divided into two stages: the first stage included screening the profile of chemical

Table 1. Bioactive compounds found in amla fruit tea infusions.

Chemical Name	Molecular Formula	Molecular Weight (g/mol)	Retention Time (min)
Ellagic acid	$C_{14}H_6O_8$	302.19	5.2810
L-alfa-Palmitin	$C_{19}H_{38}O_4$	330.50	14.502
1-Stearoylglycerol	$C_{21}H_{42}O_4$	358.60	15.228
Stearamide	$C_{18}H_{37}NO$	283.50	
Betaine	$C_5H_{11}NO_2$	117.15	0.8660
Choline	$C_5H_{13}NO$	104.17	0.7570
Quercetin	$C_{15}H_{10}O_7$	302.23	6.2510
Bis (4-ethylbenzylidene) sorbitol	$C_{24}H_{30}O_6$	414.50	11.370
(2*E*,4*Z*,12*E*)-1-(1-Piperidinyl)- 2,4,12-octadecatrien-1-one	$C_{23}H_{39}NO$	345.56	16.004
Gallic acid	$C_7H_6O_5$	170.12	0.8000
Melamine	$C_3H_6N_6$	126.12	0.7880
D-(+)-Pyroglutamic acid	$C_5H_7NO_3$	129.11	0.9890
Capsiamide	$C_{17}H_{35}NO$	269.50	14.638
Curcumin	$C_{21}H_{20}O_6$	368.40	10.932
Phytosphingosine	$C_{18}H_{39}NO_3$	317.50	10.953
Indane	C_9H_{10}	118.18	12.707
Bis(methylbenzylidene) sorbitol	$C_{22}H_{26}O_6$	386.40	10.425
α-Eleostearic acid	$C_{18}H_{30}O_2$	278.40	12.420
1-(14-Methylhexadecanoyl) pyrrolidine	$C_{21}H_{41}NO$	323.60	
Adenine	$C_5H_5N_5$	135.13	0.7980
Nicotinamide	$C_6H_6N_2O$	122.12	0.9690
Benzaldehyde	C_7H_6O	106.12	0.7930

Note: Compounds are shown on the basis of the largest area.

Table 2. Phytochemical screening of amla fruit tea infusion.

Compound	Reagents	Sample (Amla Fruit Tea)	
		Color	Description
Flavonoids	NaOH 10%	Brownish yellow	+
Phenol	$FeCl_3$	Brownish-brown	+
Tannins	$FeCl_3$	Dark brown–black	+++
Terpenoids	Lieberan-Burchard	Yellow–brown	+

(+)= relatively low; (+++)= relatively high content

compounds contained in amla fruit tea, and the second stage included testing antibacterial activity and toxicity *in vitro* and *in silico*. The research was conducted at the Regional Technical Implementation Unit Kerthi Bali Sadhajiwa Health Laboratory Center of the Bali Provincial Health Office and the Medical Biology Laboratory, Universitas Hindu Indonesia, Denpasar, Indonesia. This research was approved and declared ethical by the Ethics Committees of the Faculty of Veterinary Medicine, Udayana University, with registration number B/146/UN14.2.9/PT.01.04/2024.

2.2. Specimens and Determination

Amla fruit samples were collected from collectors located in Mendoyo Village, Jembrana Regency, Bali Province, Indonesia. Amla fruits were then identified at the Botany Laboratory, Faculty of Information Technology and Science, Universitas Hindu Indonesia, Denpasar, Indonesia. Postidentification amla fruits were dried by aeration and mashed for further use. The samples were processed within 24 h of harvesting to minimize metabolite degradation.

2.3. Preparation of Amla Fruit Tea

The preparation of amla fruit tea involved processing 3 kg of selected amla fruits that were ready for harvest, washing thoroughly under running water, and thinly slicing them using a stainless steel commercial fruit cutter (Model FS-350, precision thickness capability 2–3 mm). The sliced amla was then arranged in a single layer on a food-grade perforated tray and subjected to controlled dehydration in a laboratory convection oven (Memmert UF110, temperature accuracy ±0.5 °C) maintained at 40 °C for 45 min. After dehydration, the moisture content was verified via a digital moisture analyzer (Shimadzu MOC-120H, accuracy ±0.01%) to ensure that it reached the target moisture content of 8–10%. The dried amla slices were then pulverized into a coarse powder via a high-speed stainless-steel mechanical grinder. The resulting powder was carefully measured (±0.01 g) via an analytical balance (Sartorius Entris II BCE224i-1S) and packed in heat-sealable, food-safe nonwoven filter paper tea bags (6×4 cm, 2 g capacity per bag), followed by heat sealing via a semiautomatic tea bag sealing machine. The sealed tea bags were subjected to quality assessment for uniform weight distribution and structural stability before final packaging in moisture-proof aluminum-coated bags via a vacuum sealer (FoodSaver V4400) to maintain product freshness.

2.4. Phytochemical Profiling with Liquid Chromatography-High-Resolution Mass Spectrometry

Phytochemical analysis of amla fruit tea was conducted via liquid chromatography-high-resolution mass spectrometry (LC-HRMS) with an untargeted metabolomic approach. Tea infusion was prepared by adding 100 mL of double-distilled water (80 °C) to 2 g of dried amla fruit and allowing it to steep for 10 min, followed by filtration through a 0.22 µm membrane filter. Sample preparation involved dilution with methanol (1:1 v/v) and centrifugation at 12,000 rpm for 15 min to remove particulates. The instrumentation consisted of an UltiMate liquid chromatograph (Thermo Scientific™ Vanquish™ UHPLC Binary Pump) coupled with a Q Exactive HF mass spectrometer (Thermo Fisher Scientific). Separation was performed on an Accucore Phenyl-Hexyl column (100 mm × 2.1 mm × 2.6 µm) at 40°C.

The mobile phases consisted of 0.1% formic acid

in water (A) and 0.1% formic acid in methanol (B) at 0.3 mL/min. The gradient program ran from 5% to 95% B (25 min), increased to 90% B (16 min), decreased to 5% B (5 min), and underwent brief re-equilibration (0.01 min). The ESI parameters included 3.30 kV (negative mode) and 4.2 kV (positive mode) with a capillary temperature of 320°C. Full-scan data-dependent acquisition was performed at 70,000 FWHM resolution (m/z 200) across the 66.7–1000 m/z range. Fragmentation was performed using a collision energy of 40 eV, with targeted MS/MS performed at 15,000 FWHM in both ionization modes. The acquired data were subsequently processed and analyzed via Compound Discoverer 3.2 for compound identification and relative quantification of the metabolites present in the amla fruit tea infusion.

2.5. Qualitative Phytochemical Screening of Amla Fruit Tea

Qualitative phytochemical analysis of amla fruit tea was performed following standardized methodologies. Alkaloid determination employed Mayer's and Dragendorff's reagents, where 2 g of amla fruit tea steeped in hot water was tested with 2 drops of Mayer's reagent (1 mL infusion), with a white/yellow precipitate indicating positive results, whereas an orange–brown precipitate confirmed the presence of alkaloids with Dragendorff's reagent. Terpenoid assessment was performed via 5 mL of ether extraction followed by evaporation, and the residue was treated with two drops of acetic anhydride and one drop of concentrated sulfuric acid, where red–green coloration indicated steroids, while the blue–violet hue confirmed terpenoids. Flavonoid identification involved adding 0.5 g of zinc powder and 2 mL of 2 M HCl and allowing the mixture to stand for 1 min before introducing 10 drops of concentrated HCl, with intense red coloration within 2–5 min, verifying the presence of flavonoids. Tannin detection requires the tea infusion to be heated for 5 min, followed by the addition of 2–5 drops of 1% $FeCl_3$, with green–violet coloration indicating tannins. Saponin evaluation involved heating 2 mL of tea infusion, cooling, and vigorously agitating for 10 s, and persistent foam formation confirmed the presence of saponin.

2.6. Antibacterial Assay

The in vitro antibacterial activity of amla fruit tea was assessed via the disk diffusion method on Mueller–Hinton agar (MHA) medium. The bacterial strains employed in this study were antibiotic-sensitive reference strains (*E. coli* ATCC 25922 and *S. aureus* ATCC 25923) rather than antimicrobial-

Table 3. Antibacterial activity of amla fruit tea infusion.

Bacteria	Group	Mean ± SD	R^2	F	*p*-value
	CT	0.000±0.000			
S. aureus (1 hour)	CP	31.30±0.000	0.9998	19.317	<0.0001 ****
	T	1.125±0.4425			
	CT	0.000±0.000			
S. aureus (25% concentration)	CP	31.30±0.000	1.000	229.145	<0.0001 ****
	T	2.525±0.1258			
	CT	0.000±0.000			
E. coli (1 hour)	CP	25.50±0.000	0.9997	9.030	<0.0001 ****
	T	1.050±0.5260			
	CT	0.000 ±0.000			
E. coli (25% concentration)	CP	25.50 ±0.000	1.000	138.251	<0.0001 ****
	T	3.350 ±0.129			

CP: positive control group (streptomycin); CT: negative control group (10% DMSO); T: treatment (Amla fruit tea infusion). All groups met the normality assumption (normal distribution, $p>0.05$), as verified via the Shapiro–Wilk test. The probability level was set at 99% ($p<0.01$). *p*-value significance: 0.1234 (ns); * *p*-value significance at 0.0332; ** *p*-value significance at 0.0021; *** *p*-value significance at 0.0002; **** *p*-value significance at <0.0001.

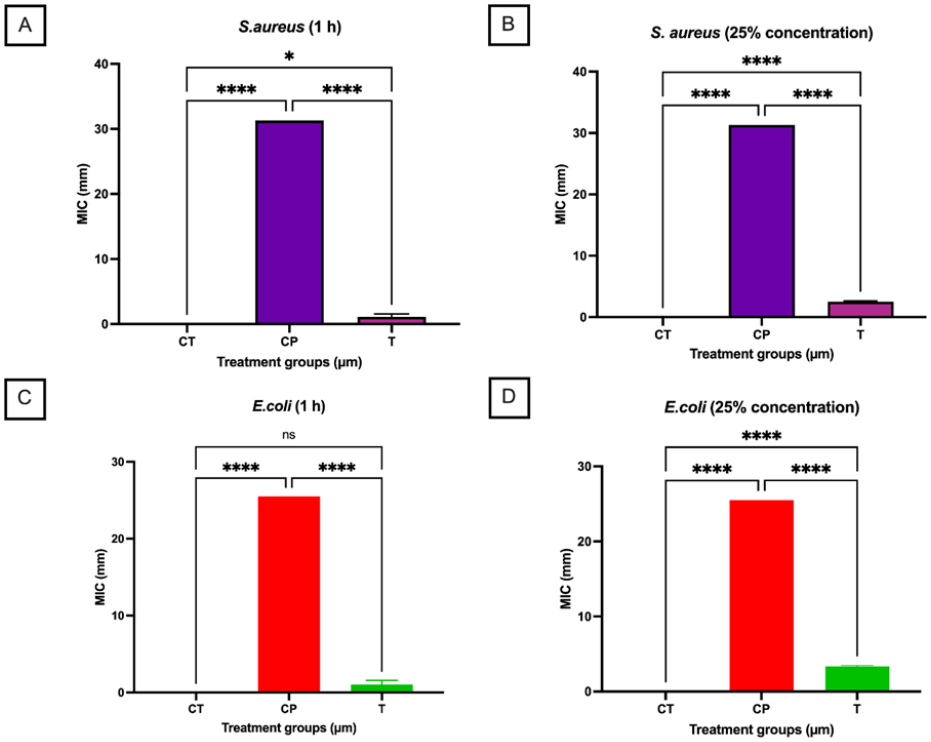

Figure 2. Tukey's multiple comparisons test showing differences in the antibacterial activity of Amla fruit tea infusion. CP: positive control group; CT: negative control group; T: Amla fruit tea treatment group. The probability level was set at 99% ($p < 0.01$). *p*-value significance: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), < 0.0001 (****).

resistant isolates, as recommended by Clinical and Laboratory Standards Institute guidelines for initial antimicrobial activity screening. The medium was prepared by dissolving 19.08 g of MHA powder in 250 mL of distilled water, followed by sterilization in an autoclave at 121 °C for 15 min. The sterile medium was subsequently distributed into sterile Petri dishes at a volume of approximately 15 mL per dish and allowed to solidify at room temperature. Bacterial inocula were prepared from pure cultures of *E. coli* and *S. aureus* with a cell density equivalent to the 0.5 McFarland standard. The bacterial suspensions were inoculated onto the MHA surface via sterile swabs to achieve uniform growth. Amla fruit tea infusion was applied to sterile paper disks at specified volumes, which were subsequently placed on the inoculated media surface. The incubation was performed at 37 °C for 24 h in an inverted position. The antibacterial activity was evaluated by measuring the inhibition zone diameter via a digital caliper with 0.01 mm precision. All tests were performed in triplicate and were accompanied by positive and negative controls for method validation, according to the Clinical and Laboratory Standards Institute (CLSI) standards.

2.7. Molecular Docking Analysis

Bioactive compounds in amla tea were identified via LC-HRMS. A systematic *in silico* approach was used to evaluate their inhibitory potential against bacterial targets. Molecular docking was performed against tyrosyl-tRNA synthetase from *S. aureus* (PDB ID: 1JIJ, with native ligands ([2-amino-3-(4-hydroxy-phenyl)-propionylamino]-(1, 3, 4, 5-tetrahydroxy- 4 -hydroxymethyl-piperidin- 2 -yl)-acetic acid) and FimH protein from *E. coli* (PDB ID: 4X5P, with native ligands 4-{[3 -chloro- 4 -(alpha-D-mannopyranosyloxy) phenyl]carbamoyl} benzoic acid), with three-dimensional structures extracted from their respective protein complexes and redocked for protocol validation, with RMSD values below 2.0 Å considered acceptable. Protein preparation included the removal of water molecules, the addition of hydrogen atoms, and the assignment of Gasteiger charges via AutoDock Tools 1.5.6. The 2D structures of the bioactive compounds identified via LC–HRMS analysis were retrieved from PubChem, optimized, and converted

to 3D conformations via ChemDraw Professional, with energy minimization performed via the MMFF94 force field.

The binding site was defined on the basis of the cocrystallized ligand position and appropriate grid dimensions. Docking simulations were performed via AutoDock Vina with an exhaustiveness set to 8, and the binding interactions were analyzed via Discovery Studio Visualizer. Binding interactions were characterized on the basis of hydrogen bonds (distance threshold of 3.5 Å, minimum angle of 120°), hydrophobic interactions, π–π stacking, cation–π interactions, and salt bridges. The compounds were ranked on the basis of their binding affinity scores, with those comparable to or better than the native ligands prioritized for further analysis. The drug-likeness and ADME properties of the selected compounds were evaluated via SwissADME, which assesses parameters such as molecular weight, topological polar surface area, hydrogen bond donors/acceptors, and the calculated octanol–water partition coefficient. Compliance with Lipinski's Rule of Five was evaluated to predict oral bioavailability. Toxicity profiling was conducted via ProTox-II-predicted acute toxicity (LD_{50}), organ toxicity (hepatotoxicity, cardiotoxicity, and nephrotoxicity), and systemic toxicity (immunotoxicity and cytotoxicity). The integrated results identified amla tea compounds with optimal bacterial target binding, favorable drug-like properties, and acceptable safety profiles for future evaluation.

2.8. Statistical and Data Analysis

The experimental data were analyzed via one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test at the 99% confidence level ($p < 0.01$). The primary outcome measured was the antibacterial inhibition capacity on MHA medium after treatment with Amla fruit tea infusion. All the statistical analyses were performed via GraphPad Prism Pro Version 10.3.1 LLC. In the USA, molecular docking was conducted via established web-based standard tools following conventional protocols.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical Profiling of Amla Fruit Tea Infusions via LC–HRMS

LC-HRMS analysis of amla fruit tea infusion revealed a complex chromatographic profile with

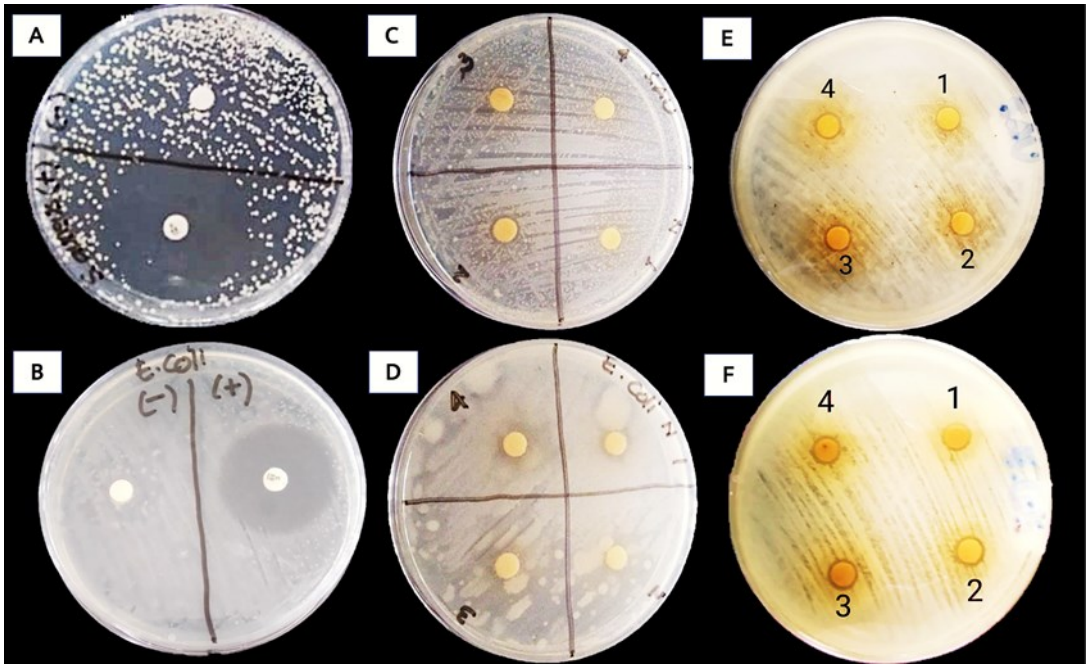

Figure 3. Antibacterial activity testing of amla fruit tea infusion. The upper panel represents the *S. aureus* groups, which include (a) colonies, (c) 100% amla fruit extract application, and (e) 25% amla extract concentration, whereas the lower panel represents the *E. coli* groups, which include (b) colonies, (d) 100% amla fruit extract application, and (f) 25% amla fruit extract concentration.

Table 4. Physicochemical and drug-likeness assessment of compounds from amla tea infusion.

Compound	Molecular weight (g/mol)	Consensus Log P	H-bond donor	H-bond Acceptor	Violation of Lipinski's rule
Standard	< 500	≤ 5	<10	<5	≤ 1
Ethyl iso-allocholate	436.62	3.46	3	5	Yes; 0 violation
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine	322.44	4.05	0	1	Yes; 0 violation
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)	421.90	3.11	0	5	Yes; 0 violation
Carbamic acid, *N*-[10, (moricizine)	427.52	2.54	1	5	Yes; 0 violation
p-Menth-1-en-3-one, (piperitone)	152.23	2.20	0	1	Yes; 0 violation

multiple peaks detected across the 0–25 min retention time range. Principal peaks were observed at approximately 0.79, 0.96, 14.20, 15.54, and 22.70 min, indicating the presence of dominant compounds in the sample. The chromatogram demonstrated a broad distribution of compounds spanning from polar molecules eluting early (0–5 min) to nonpolar compounds in the later elution phases (> 15 min). The highest relative intensity was observed at the 0.79-min peak, indicating an elevated concentration of this compound. The diversity of the detected peaks indicates the chemical complexity of the amla fruit tea infusion, potentially encompassing various compound classes, including polyphenols, organic acids, glycosides, and other secondary metabolites (Figure 1).

Characterization of the amla fruit tea infusion revealed 89 distinct compounds. The detected metabolite profile revealed diverse bioactive compounds, including phenolic acids, flavonoids, fatty acids, amides, and various other secondary metabolites. On the basis of the chromatographic peak areas, the predominant compounds were oxidized hydroxytetrahydrofuranyl acetate ($C_{13}H_{12}O_{11}$), with peak areas of 632,556,968.2, followed by L-α-palmitin ($C_{19}H_{38}O_4$), with peak areas of 583,478,284.3, and ellagic acid ($C_{14}H_6O_8$), with peak areas of 418,343,397.7. The significant presence of ellagic acid alongside gallic acid indicates a high tannin content, which is consistent with the characteristic phytochemical profile of amla fruit. Additional bioactive compounds detected include quercetin, α-linolenic acid, and phytosphingosine, which contribute to the antioxidant capacity and potential health benefits of amla fruit tea infusions. The complex metabolite profile reflects the phytochemical richness of amla fruit, supporting its application in traditional medicine and its potential for phytopharmaceutical development. The compounds identified by LC–HRMS are listed in Table 1.

3.2. Qualitative Phytochemical Screening of Amla Fruit Tea

Phytochemical screening results from amla fruit tea infusions conducted via colorimetric methods demonstrated the presence of several secondary metabolites. The color formation observed in the

test tubes indicated that amla fruit tea contains flavonoids, phenols, tannins, and terpenoids (Table 2). These qualitative findings align with the results of the quantitative LC–HRMS characterization, particularly with respect to the significant presence of tannins, which presented relatively high contents, whereas flavonoids, phenols, and terpenoids presented relatively low relative abundances. The predominance of tannins detected through the $FeCl_3$ reaction, which results in dark brown–black coloration, corroborates the LC-HRMS findings, where ellagic acid and gallic acid, both key components of hydrolyzable tannins, were identified among the major compounds. The complementary nature of both analytical approaches provides comprehensive evidence of the phytochemical composition of amla fruit tea, with preliminary colorimetric screening confirming the broad range of compound classes and LC-HRMS offering detailed molecular identification and relative quantification. This phytochemical profile supports the traditional medicinal applications of amla and offers insight into the bioactive components potentially responsible for its therapeutic properties.

3.3. Antibacterial Properties of Amla Fruit Tea Infusions

The antibacterial properties of Amla fruit tea were evaluated against both *S. aureus* and *E. coli* bacteria via repeated-measures ANOVA. The experimental design included three treatment groups: a negative control (10% DMSO, designated CT), a positive control (streptomycin, designated CP), and Amla fruit tea treatment (designated T). Measurements were conducted under two conditions: 1-h exposure and 25% concentration enhancement. Statistical analysis revealed highly significant differences across all treatment groups ($p < 0.0001$) with robust model fitting ($R^2 \geq 0.9997$), as presented in Table 3.

For *S. aureus* after 1 h of exposure, the positive control (streptomycin) exhibited maximum inhibition (31.30±0.000 mm), while Amla fruit tea demonstrated moderate antibacterial activity (1.125±0.4425 mm). The effect was more pronounced at the 25% concentration, where Amla fruit tea showed enhanced inhibition (2.525±0.1258 mm), although it was still significantly lower than

Table 5. Predicted toxicity levels of the compounds from amla fruit tea infusion.

Prediction accuracy	Average similarity	Predicted toxicity class	Predicted LD_{50}	Compound
72.90	91.85	Five (Possibly Hazardous)	5.000	Ethyl iso-allocholate
5.00	15.93	Three (toxic swallowed)	300	1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine
67.38	50.57	Three (toxic swallowed)	300	W-18 ((E)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzenesulfonamide)
100	100	Four (harmful)	1.000	Carbamic acid, *N*-[10, (moricizine)
100	100	Five (Possibly Hazardous)	2.450	*p*-Menth-1-en-3-one, (piperitone)

Table 6. Predicted organ toxicity of the compounds from amla fruit tea infusion.

Compounds	Organ Toxicity				
	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity	Cyto-toxicity
Ethyl iso-allocholate	0.60*	0.75*	0.57**	0.73*	0.75*
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine	0.71*	0.69*	0.97*	0.71*	0.72*
W-18((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl)piperidin-2-ylidene)benzenesulfonamide)	0.61*	0.51*	0.97*	0.50**	0.71*
Carbamic acid, *N*-[10, (moricizine)	0.61*	0.61*	0.91*	0.68*	0.59*
p-Menth-1-en-3-one, (piperitone)	0.64*	0.79*	0.92*	0.93*	0.87*

that of the positive control ($p < 0.0001$). Similarly, for *E. coli*, streptomycin administration demonstrated superior inhibition (25.50±0.000 mm) at both time points. Amla fruit tea exhibited moderate activity against *E. coli* after 1 hour of exposure (1.050±0.5260 mm), with increased efficacy at a 25% concentration (3.350±0.129 mm). A Geisser–Greenhouse epsilon value of 0.5000 across all the treatments indicated sphericity in the repeated-measures design.

Tukey's multiple comparisons test confirmed significant differences between all treatment groups compared with the negative control ($p < 0.0001$), except for the Amla tea treatment against *E. coli* after 1 h of exposure ($p = 0.0562$). This analysis revealed substantial mean differences between the positive control and Amla tea treatments, with higher q values observed at a 25% concentration for both bacterial strains (Figure 2). The inhibitory effects were visually confirmed through the zone of inhibition measurements, as illustrated in Figure 3, where clear differences in bacterial growth suppression were observed between the treatment conditions. The inhibition zones corresponded to the quantitative measurements reported in Table 3. These findings indicate that Amla fruit tea has measurable antibacterial properties against both *S. aureus* and *E. coli* and that its efficacy is significantly lower than that of standard antibiotic controls, with slightly better performance against *E. coli* at higher concentrations.

3.4. Molecular Docking Analysis

3.4.1. Physicochemical Analysis and Drug-Likeness Assessment

A comprehensive analysis of the physicochemical properties of the compounds isolated from Amla Tea infusion revealed complete compliance with Lipinski's rule for the five parameters (Table 4). All five selected compounds demonstrated favorable drug-like properties with zero violations, exhibiting appropriate molecular weights (< 500 g/mol), consensus log P values (≤ 5), H-bond donors (< 10), and acceptors (≤ 5). The structural diversity of these compounds, ranging from steroidal (ethyl isoallocholate) to terpene (*p*-menth-1-en-3-one) structures, enhances the therapeutic versatility of Amla Tea as a

phytopharmaceutical resource.

The alignment of these parameters suggests advantageous absorption, distribution, metabolism, and excretion (ADME) profiles, which are critical for successful drug development. The molecular weights ranged from 152.23 to 436.62 g/mol, with log P values between 2.20 and 4.05, indicating appropriate lipophilicity for membrane permeation. The limited number of hydrogen bond donors (0–3) and acceptors (1–5) further supports the potential for optimal oral bioavailability. This evidence highlights the pharmaceutical value of Amla Tea as a source of drug-like compounds with promising pharmacokinetic properties. The presence of compounds with unique structural features, such as W-18 ((E)-4-chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene)benzene sulfonamide), suggests novel mechanisms of action warranting investigation for targeted therapeutic applications, particularly in metabolic and inflammatory conditions where amla has demonstrated traditional efficacy.

3.4.2. Predicted Toxicity Levels of the Compounds from Amla Fruit Tea Infusion

The toxicological profile of amla fruit tea infusion reveals safety concerns on the basis of an *in-silico* analysis of its phytochemical constituents. Table 5 presents the predicted toxicity levels of the five principal compounds isolated from the Amla fruit tea infusion. These compounds demonstrated varying degrees of toxicity, with LD_{50} values ranging from 300 to 5,000 mg/kg body weight. Notably, two compounds were classified as "possibly hazardous" (Class Five): ethyl isoallocholate (LD_{50} 5,000 mg/kg) and p-menth-1-en-3-one (piperitone) (LD_{50} 2,450 mg/kg), both of which exhibited perfect prediction accuracy (72.9% and 100%, respectively). Two compounds fell into Class Three ("toxic when swallowed"): the 1-isopropoxy-5-propyl derivative and the W-18 benzenesulfonamide derivative, both with LD_{50} values of 300 mg/kg, suggesting significant toxicity potential despite their disparity in prediction accuracy (5% versus 67.38%). Carbamic acid, *N*-[10, (morizicine), was classified as class four ("harmful") with an LD_{50} of 1,000 mg/kg and perfect similarity and prediction accuracy scores (100%). These findings suggest that caution should be exercised with respect to the regular consumption of Amla fruit tea, particularly among vulnerable populations such as pregnant women, children, and individuals with compromised hepatic function. The prediction models demonstrated robust performance, with generally high similarity indices (15.93–100) and prediction accuracies (5–100), although the marked variability in these parameters for certain compounds warrants further investigation.

3.4.3. Predicted Organ Toxicity of the Compounds from Amla Fruit Tea Infusion

A comprehensive *in silico* toxicological assessment of five compounds isolated from Amla fruit tea infusion revealed diverse organ-specific toxicity profiles, as presented in Table 6. The compounds predominantly demonstrated an inactive status across multiple toxicological endpoints, with only two compounds exhibiting active properties in specific organs. A detailed analysis of the hepatotoxicity potential revealed values ranging from 0.6–0.71, with the 1-Isopropoxy-5-propyl-2,3-

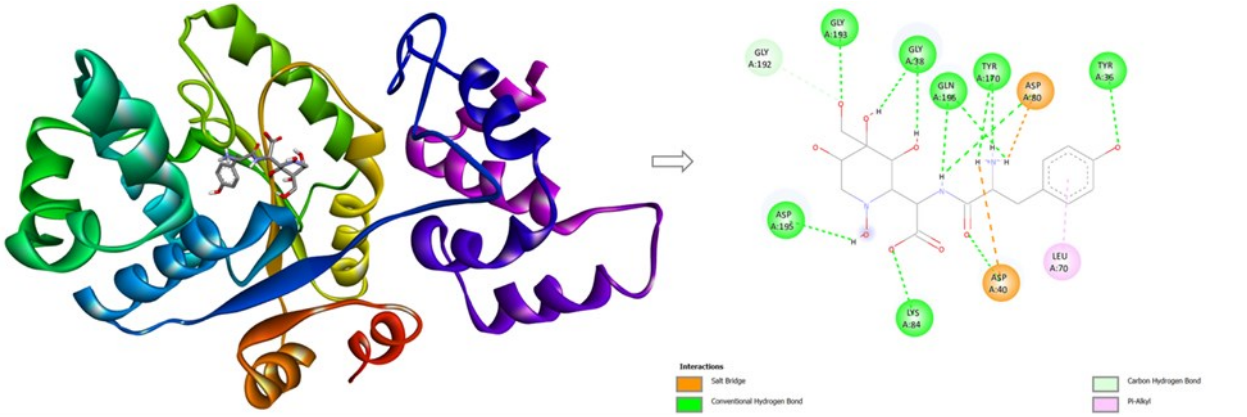

Figure 4. Interaction visualization between the native ligand and tyrosyl-tRNA synthetase protein.

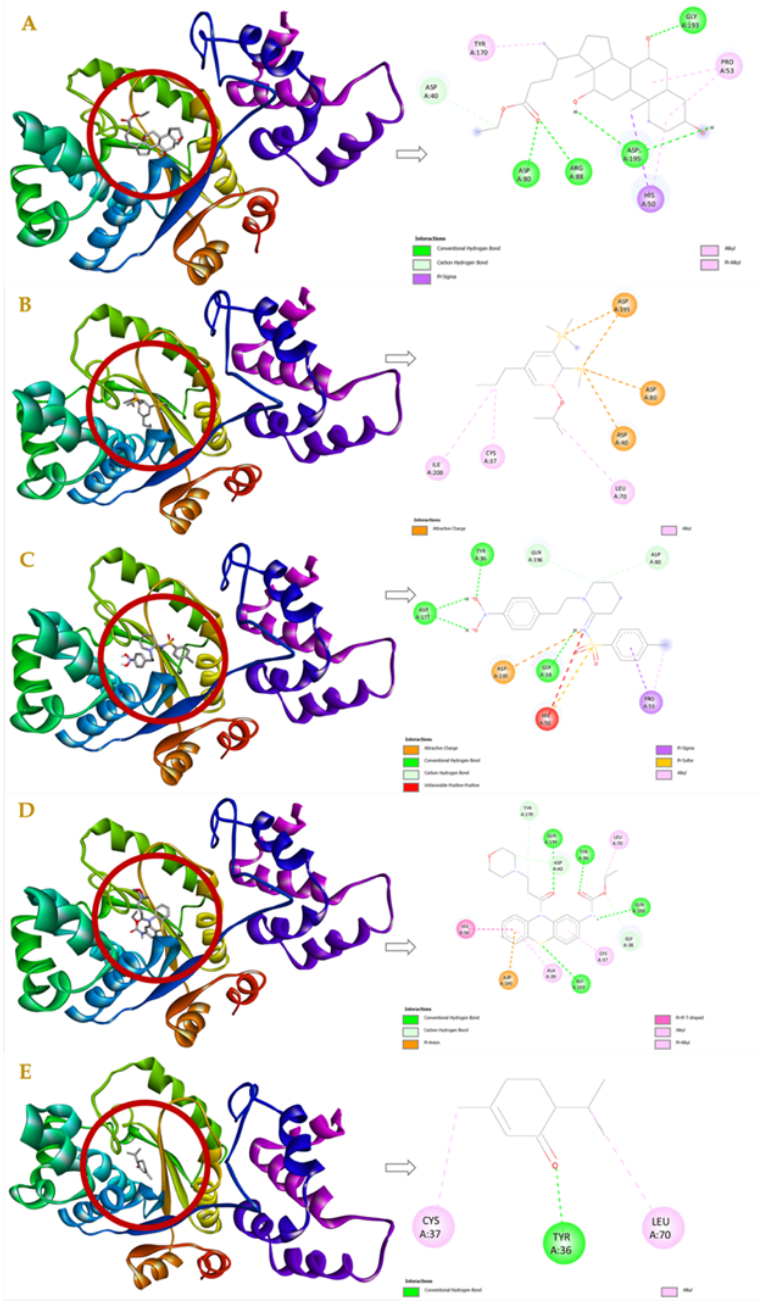

Figure 5. 3D (left) and 2D (right) visualization and binding sites (red circles) of the amla fruit tea infusion compounds with tyrosyl-tRNA synthetase from the *S. aureus* protein. (a) Ethyl isoallocholate, (b) 1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine, (c) W-18 ((E)-4-chloro-*N*-(1-(4-nitrophenethyl)piperidin-2-ylidene)benzenesulfonamide), (d) carbamic acid, and (e) *N*-[10, (moricizine).

bis-trimethylsilyl-1,2-dihydroborinine derivative exhibiting the highest hepatotoxic prediction. Carcinogenicity predictions indicated that piperitone (p-menth-1-en-3-one) presented the highest carcinogenic potential, whereas the W-18 benzenesulfonamide derivative presented the lowest. Notably, immunotoxicity predictions were consistently elevated across all the compounds, with two compounds 1-Isopropoxy-5-propyl-2,3-bis -trimethylsilyl-1,2-dihydroborinine and W-18 benzenesulfonamide derivatives approaching the activity threshold. Only two compounds were active: the ethyl isoallocholate demonstrated immunotoxic activity, and the W-18 benzenesulfonamide derivative showed mutagenic activity. Piperitone exhibited the highest cytotoxicity prediction and mutagenicity potential. These findings suggest that most compounds remain below the activity threshold for direct organ toxicity, and their collective presence in Amla fruit

tea warrants cautious consumption. The predictive toxicology approach employed provides valuable preliminary insights into organ-specific safety profiles.

3.4.4. Molecular Docking Compounds Targeted by Tyrosyl-Trna Synthetase

Molecular docking analysis of five principal compounds isolated from Amla fruit tea infusion against tyrosyl-tRNA synthetase from *S. aureus* revealed promising inhibitory potential with differential binding affinities. The docking protocol demonstrated robust validation parameters, as evidenced by the reference ligand having an RMSD value of 1.512 Å (< 2 Å), a binding free energy (ΔG) of -10.01 kcal/mol, and an inhibition constant (Ki) of 46.16 nM. The results of the 2D interaction analysis revealed that four interactions occurred between the native ligand and the tyrosyl-tRNA synthetase protein of *S. aureus*. These interactions included two salt bridge interactions (Asp40 and Asp80), seven conventional hydrogen bond interactions (Tyr36, Gly38, Lys84, Gly193, Asp195, Gln196, dan Tyr170), one carbon hydrogen bond interaction (Gly192), and one Pi–alkyl interaction (Leu70), as shown in Figure 4.

Among the five docked compounds (Table 7 and Figure 5), W-18 ((E)-4-chloro-*N*-(1-(4-nitrophenylethyl) piperidin-2-ylidene) benzenesulfonamide) exhibited the most favorable interaction profile, with the lowest binding free energy (-11.01 kcal/mol) and highest binding affinity (Ki = 8.47 nM), suggesting its potent inhibitory activity against *S. aureus*. Two-dimensional interaction analysis revealed seven interactions between this compound and the tyrosyl-tRNA synthetase protein complex of *S. aureus*. These interactions included one attractive charge (ASP195), three conventional hydrogen bond interactions (Tyr36, Gly38, and Asp177), two carbon hydrogen bond interactions (Asp80 and Gln196), one unfavorable positive-positive and pi-sulfur interaction (His50), and one pi-sigma and alkyl interaction (Pro53). In addition, the carbamic acid derivative (morizicine) and ethyl isoallocholate also demonstrated substantial binding affinities, with ΔG values of -10.23 and -9.37 kcal/mol, corresponding to Ki values of 31.60 and 134.50 nM, respectively.

Table 7. Summary of the molecular docking compounds against tyrosyl-tRNA synthetase.

Compound	Binding Energy (ΔG, kcal/mol)	Inhibition Constant (Ki)
Ethyl iso-allocholate	-9.37	134.50 nM
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine	-7.76	2.04 uM
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl)piperidin-2-ylidene)benzenesulfonamide)	-11.01	8.47 nM
Carbamic acid, *N*-[10, (moricizine)	-10.23	31.60 nM
p-Menth-1-en-3-one, (piperitone)	-6.33	22.73 uM

The 1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl -1,2-dihydroborinine derivative and piperitone displayed relatively modest interactions (ΔG = -7.76 and -6.33 kcal/mol; Ki = 2.04 and 22.73 μM, respectively). Molecular interaction analysis revealed multiple binding modalities involving hydrogen bonds, salt bridges, π–π stacking, and hydrophobic interactions with key amino acid residues in the active site, thereby disrupting the aminoacylation function essential for bacterial protein synthesis. The correlation between the binding energy and inhibition constant provides valuable structure–activity relationship insights for the future optimization of these phytochemicals as potential antibacterial agents that target tyrosyl–tRNA synthetase in *S. aureus*. These findings suggest that specific compounds in Amla fruit tea exert bacteriostatic effects by inhibiting the essential protein synthesis machinery.

3.4.5. Molecular Docking Compounds Targeting the Fimh Protein

Molecular docking analysis of five bioactive compounds from Amla fruit tea infusion against the FimH adhesin protein from *E. coli* revealed moderate to strong binding interactions with potential antiadhesive properties. The computational docking protocol was effectively validated using a mannose-derived reference ligand (4-{[3 -chloro- 4 -(alpha-D-mannopyranosyloxy) phenyl]carbamoyl}benzoic acid) , which exhibited an RMSD value of 1.361 Å (< 2 Å), a ΔG of -8.87 kcal/mol, and a Ki of 316.40 nM. Four interactions were formed in this compound. These interactions include six conventional hydrogen bond interactions (Phe1, Asp47, Asp54, Gln133, Asn135, dan Asp140), a stacked π–π interaction (Tyr48), and one alkyl (Tyr137) and π–alkyl (Ile52) interaction, as shown in Figure 6.

Among the tested compounds (Table 8 and Figure 7), W-18 ((*E*)-4-chloro-*N*-(1-(4-nitrophenyl) piperidin-2-ylidene) benzenesulfonamide) demonstrated the strongest binding affinity, with the lowest binding free energy (-7.48 kcal/mol) and inhibition constant (3.30 μM), suggesting potential disruption of bacterial adhesion mechanisms. Five interactions were formed in this compound. These interactions included one attractive charge interaction (ASP47), two conventional hydrogen bond interactions (PHE1 and ASP 54), two Pi–sigma interactions (TYR48 and ILE52), one stacked Pi–Pi interaction (TYR 48), and an alkyl interaction (ILE13). Ethyl isoallocholate and carbamic acid (morizicine) exhibited comparable binding energies (-6.10 and -6.01 kcal/mol, respectively) with moderate inhibition constants (33.60 and 39.23 μM).

The remaining compounds, piperitone and the 1-Isopropoxy- 5 -propyl- 2,3 -bis-trimethylsilyl- 1,2-dihydroborinine derivative, showed weaker interactions (-5.72 and -5.04 kcal/mol; 64.07 and 203.63 μM, respectively). Interaction analysis revealed that these compounds bind primarily to the mannose recognition pocket of FimH through hydrogen bonding networks with polar residues and hydrophobic interactions with aromatic amino acids, potentially competing with natural glycosylated receptors on epithelial cells. Although the binding affinities were notably lower than those observed against *S. aureus* targets, these findings suggest that Amla fruit tea compounds possess moderate antiadhesive properties against uropathogenic *E. coli* by targeting the FimH lectin domain, potentially contributing to prevention of urinary tract infection.

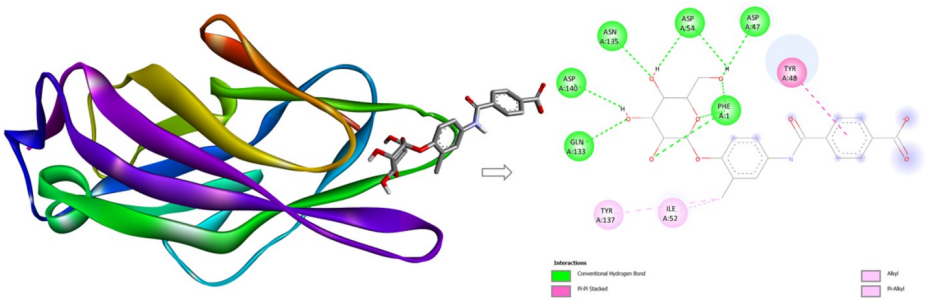

Figure 6. Interaction visualization between the native ligand and the FimH protein from *E. coli*.

Table 8. Summary of the molecular docking compounds against the FimH protein.

Compound	Binding Energy (ΔG) (kcal/mol)	Inhibition Constant (Ki)
Ethyl iso-allocholate	-6.10	33.60 uM
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine	-5.04	203.63 uM
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene)benzenesulfonamide)	-7.48	3.30 uM
Carbamic acid, *N*-[10, (moricizine)	-6.01	39.23 uM
p-Menth-1-en-3-one, (piperitone)	-5.72	64.07 uM

3.4.6. Binding Sites of Amla Fruit Tea Infusion Compounds

A comprehensive molecular interaction analysis of five bioactive compounds from Amla fruit tea infusion revealed characteristic binding site profiles against two significant bacterial targets: tyrosyl-tRNA synthetase from *S. aureus* and the FimH adhesin protein from *E. coli*. As shown in Table 9, these compounds exhibited diverse interaction patterns characterized by multiple binding modalities. The active site binding interactions of Amla tea compounds with tyrosyl-tRNA synthetase proteins were characterized by four primary bonds serving as recurring interaction partners: two conventional hydrogen bonds (Tyr36 and Gly193), one carbon hydrogen bond (Asp40), one attractive charge bond (Asp195), and two alkyl bonds (Leu70). In contrast, the active site binding of amla tea compounds against the FimH protein features four primary bonds serving as recurring interaction partners: one conventional hydrogen bond (Asn135), two alkyl bonds (Ile13 and Ile52), two pi –alkyl bonds (Tyr137 and Phe142), and one pi–sigma bond (Ile52). These distinctive binding profiles demonstrate the potential antimicrobial mechanisms of Amla fruit tea compounds, suggesting their ability to interfere with protein synthesis in *S. aureus* through tyrosyl-tRNA synthetase inhibition and with bacterial adhesion in *E. coli* through FimH targeting, which could contribute to their antibacterial properties against infections.

Phytochemical characterization of amla fruit (*Phyllanthus emblica* L.) tea infusion via LC-HRMS revealed a complex profile of 89 bioactive compounds, including phenolic acids, flavonoids, fatty acids, and various secondary metabolites. The predominant compounds identified included oxidized hydroxytetrahydrofuranyl-acetate ($C_{13}H_{12}O_{11}$), L-α-palmitin ($C_{19}H_{38}O_4$), and ellagic acid ($C_{14}H_6O_8$), with a significant presence of gallic acid, indicating a high tannin content [28]. These findings align with those of previous studies by Ganesh et al. [29], Orabi et al. [30], and Sun et al. [20], who reported that ellagic acid has diverse biological activities, including anticancer, antimicrobial, antioxidant, antimutagenic, antinflamation, anticholinesterase and antiviral properties. The presence of quercetin further

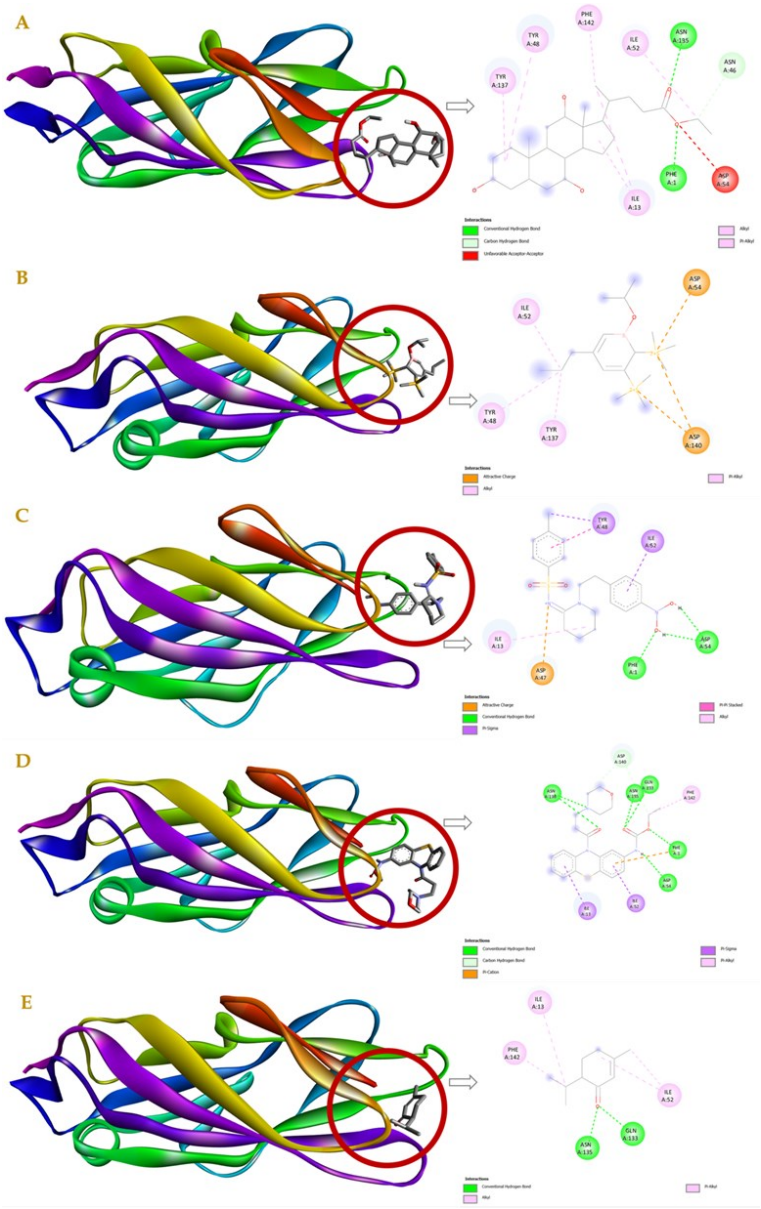

Figure 9. 3D (left) and 2D (right) visualization and binding sites (red circles) of the amla fruit tea infusion compounds with the FimH protein from *E. coli*. (a) Ethyl isoallocholate, (b) 1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine, (c) W-18 ((*E*)-4-chloro-*N*-(1-(4-nitrophenethyl)piperidin-2-ylidene) benzenesulfonamide), (d) carbamic acid, *N*-[10, (moricizine), and (e) *p*-menth-1-en-3-one, (piperitone).

enhances the therapeutic profile through its anti-inflammatory, cardioprotective, and antidiabetic effects [31]. Additional compounds, such as phytosphingosine and curcumin, although present in relatively small quantities, contribute to the immunomodulatory and anti-inflammatory properties of amla fruit tea.

The antibacterial activity assessment demonstrated differential effects against *S. aureus* and *E. coli* bacteria, with more pronounced inhibition against *E. coli* at a 25% concentration. This enhanced activity against gram-negative bacteria may be attributed to amla's unique phytochemical composition, particularly its high content of hydrolyzable tannins and flavonoids, which have shown selective permeability across bacterial cell membranes [14][21][26][32][33]. The time-dependent increase in antibacterial activity, evidenced by improved inhibition zones during extended exposure periods, suggests a cumulative mechanism of action, which is consistent with findings from similar plant-based antimicrobial studies [7][34]-[36]. However, the antibacterial efficacy remained significantly lower than that of

Table 9. The binding sites of compounds from amla fruit tea infusion.

Compound	Type of protein/ Bacteria	Binding site of compounds (interaction and amino acid residues)	
Ethyl iso-allocholate	tyrosyl-tRNA synthetase/ *S. aureus*	Conventional hydrogen bond	Asp80, Arg88, **Gly193,** Asp195
		Carbon hydrogen bond	**Asp40**
		Pi-Sigma	His50
		Alkyl	Tyr170
		Pi-alkyl	His50, Pro53
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine		Attractive charge	Asp40, Asp80, **Asp195**
		Alkyl	Cys37, **Leu70,** Ile200
W-18 ((*E*)-4-Chloro-*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzene sulfonamide)		Attractive charge	**Asp195**,
		Conventional hydrogen bond	**Tyr36**, Gly38, Asp177,
		Carbon hydrogen bond	Asp80, Gln196
		Unfavorable Positive-positive	His50
		Pi- Sigma	Pro53
		Pi-Sulfur	His50
		Alkyl	Pro53
Carbamic acid, *N*-[10, (moricizine)		Conventional hydrogen bond	**Tyr36**, Gln174, Gln190, **Gly193**
		Carbon hydrogen bond	Gly38, **Asp40**, Tyr170
		Pi Anion	Asp195
		Pi-Pi T-shaped	His50
		Alkyl	**Leu70**
		Pi- Alkyl	Cys37, Ala39
p-Menth-1-en-3-one, (piperitone)		Conventional hydrogen bond	**Tyr36**
		Alkyl	Cys37, **Leu70**
Ethyl iso-allocholate	FimH/*E.coli*	Conventional hydrogen bond	Phe1, **Asn135**
		Carbon hydrogen bond	Asn46
		Unfavorable acceptor-acceptor	Asp54
		Alkyl	**Ile13**, Tyr48, Phe142
		Pi- Alkyl	Ile13, Ile52, **Tyr137**
1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine		Attractive charge	Asp54, Asp140
		Alkyl	Tyr48, **Ile52**
		Pi- Alkyl	**Tyr137**
W-18 ((*E*)-4-Chloro -*N*-(1-(4-nitrophenethyl) piperidin-2-ylidene) benzene sulfonamide)		Attractive charge	Asp47
		Conventional hydrogen bond	Phe1, Asp54
		Pi-Sigma	Tyr48, **Ile152**
		Pi-Pi stacked	Tyr48
		Alkyl	**Ile13**

Table 9. *Cont.*

Compound	Type of protein/ Bacteria	Binding site of compounds (interaction and amino acid residues)	
Carbamic acid, *N*-[10, (moricizine)	FimH/*E.coli*	Conventional hydrogen bond	Gln133, **Asn135,** Asn138
		Carbon hydrogen bond	Asp140
		Pi-Cation	Phe1
		Pi-Sigma	Ile13, **Ile52**
		Pi-Alkyl	**Phe142**
p-Menth-1-en-3-one, (piperitone)		Conventional hydrogen bond	Gln133, **Asn135**
		Alkyl	**Ile13, Ile52**
		Pi- Alkyl	**Phe142**

Note: Residues with interaction poses similar to those of the control are in bold.

the positive control (streptomycin®), indicating its potential application as a complementary therapy rather than as a primary antibiotic replacement.

The concentration-dependent response observed in both bacterial strains aligns with established pharmacodynamic principles of natural antimicrobial compounds, with enhanced activity at a 25% concentration suggesting a threshold effect [12][15][30][37]. The mechanistic actions of amla fruit tea compounds as antibacterial agents operate through multiple pathways, particularly those involving membrane disruption, enzyme inhibition, and adhesion interference [22][37]. The high tannin content (evidenced by ellagic acid and gallic acid) likely contributes to bacterial membrane destabilization by binding to membrane proteins and disrupting structural integrity [23][38]-[40]. The phenolic compounds identified via LC-HRMS analysis, including quercetin and curcumin, have been shown to interfere with bacterial cell wall synthesis and increase membrane permeability, leading to leakage of the contents of the bacteria and eventual cell death [35][41]-[43].

Molecular docking analysis provided valuable insights into the potential mechanisms underlying the antibacterial activity of amla fruit tea compounds. Five principal compounds were assessed for their drug-likeness parameters according to Lipinski's rule of five, all of which demonstrated favorable physicochemical properties for oral bioavailability with zero violations [44]. The in silico toxicological assessment revealed varying degrees of predicted toxicity, with two compounds classified as "possibly hazardous" (ethyl isoallocholate and *p*-menth-1-en-3-one) and two compounds categorized as "toxic when swallowed" (1-Isopropoxy-5-propyl-2,3-bis-trimethylsilyl-1,2-dihydroborinine derivative and W -18 benzenesulfonamide derivative). Among the five docked compounds, W-18 exhibited the most favorable interaction profile with both bacterial targets, with the lowest binding free energy (-11.01 kcal/mol) and highest binding affinity (Ki = 8.47 nM) for tyrosyl-tRNA synthetase from *S. aureus*. This compound forms multiple interactions, including hydrogen bonds, hydrophobic bonds, and electrostatic interactions with key amino acid residues, potentially disrupting the aminoacylation function essential for bacterial protein synthesis [45].

Molecular interaction analysis of the FimH adhesin protein from *E. coli* revealed moderate to strong binding interactions with potential antiadhesive properties. W-18 demonstrated the strongest binding affinity with the lowest binding free energy (-7.48 kcal/mol) and inhibition constant (3.30 μM), suggesting potential disruption of bacterial adhesion mechanisms [46]-[48]. Interaction analysis revealed that these compounds bind primarily to the mannose-recognizing pocket of FimH through hydrogen bonding networks with polar residues and hydrophobic interactions with aromatic amino acids [49]-[51]. The molecular docking analysis further revealed specific mechanisms through which amla compounds exhibit antibacterial activity: (1) W-18 and

carbamic acid (Moricizine) derivatives demonstrate high-affinity binding to tyrosyl-tRNA synthetase, potentially inhibiting protein synthesis in *S. aureus* by preventing aminoacylation of tRNA, a mechanism similar to that of many clinically used antibiotics [45][52]; (2) interactions with the FimH adhesin protein suggest antivirulence activity through prevention of bacterial attachment to host epithelial cells, a critical initial step in infection establishment, particularly for urinary tract infections caused by *E. coli* [49].

The distinctive binding profiles demonstrate the potential antimicrobial mechanism of amla fruit tea compounds through dual inhibition pathways: interference with protein synthesis in *S. aureus* via tyrosyl-tRNA synthetase inhibition and disruption of bacterial adhesion in *E. coli* through FimH targeting [49][51][53][54]. These findings suggest that specific compounds from amla fruit tea possess bacteriostatic properties by inhibiting essential bacterial machinery, although further preclinical and clinical research is needed to develop these compounds as antibiotic drug candidates. The concentration-dependent efficacy observed in the antibacterial assays aligns with these mechanistic explanations, as higher concentrations increase the availability of active compounds for target engagement [55][56]. The research implications extend beyond direct antibacterial applications to the potential development of antivirulence strategies that do not exert selective pressure for resistance development. This approach, which utilizes phytochemical characterization coupled with in silico modeling, represents a promising strategy for identifying novel antimicrobial agents from natural sources to address the growing challenge of antibiotic resistance.

This study has several limitations, including the antibacterial assessment was conducted using antibiotic-sensitive reference strains rather than clinically relevant multidrug-resistant isolates, which may not accurately reflect the therapeutic potential of resistant pathogens. The lack of *in vivo* efficacy and safety assessments limits the translation of these findings for clinical applications. The absence of resistance development evaluation during prolonged exposure to amla compounds represents a critical gap in the understanding of potential selective pressure. The limited investigation of synergistic effects with conventional antibiotics restricts the exploration of combination therapy. Additionally, toxicological assessments have relied solely on in silico predictions, necessitating comprehensive experimental toxicological studies to validate safety profiles. Finally, this study did not examine the bioavailability and pharmacokinetic properties of the identified compounds, which are crucial for determining therapeutic efficacy. Future research should focus on the fractionation and isolation of key compounds (particularly W-18 derivatives), assessment of pharmacokinetic properties, investigation of potential synergistic combinations with conventional antibiotics to overcome resistance, and evaluation of safety profiles through comprehensive toxicological studies.

4. CONCLUSIONS

In conclusion, the phytochemical analysis of amla fruit tea infusions revealed 89 bioactive compounds, with oxidized hydroxytetrahydrofuranyl acetate, L-α-palmitin, and ellagic acid being the predominant compounds. Amla tea has moderate antibacterial activity against *S. aureus* and *E. coli*, with enhanced efficacy at high concentrations (25%); however, it is substantially less effective than conventional antibiotics. Molecular docking revealed that the W-18 benzenesulfonamide derivative exhibited the strongest binding affinity against tyrosyl-tRNA synthetase in *S. aureus* (ΔG = -11.01 kcal/mol) and FimH adhesin in *E. coli* (ΔG = -7.48 kcal/mol), indicating dual inhibition mechanisms through protein synthesis disruption and bacterial adhesion interference. Future investigations should prioritize the isolation of key bioactive compounds, comprehensive pharmacokinetic profiling, and *in vivo* efficacy and safety validation.

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Conflicts of Interest

The authors declare no conflict of interest.

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