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ANTIMICROBIAL ACTIVITY OF GREEN TEA FROM GAMBUNG, WEST JAVA, INDONESIA

Daud Abdurrahman [1*], Shabarni Gaffar [2], Ani Riyani [3]

[1]Biotechnology Study Program, Graduate School, Universitas Padjadjaran, Bandung, Indonesia

[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia

[3]Department of Medical Laboratory Technology, Bandung Health Polytechnic, Ministry of Health, Indonesia

*E-mail: *d.abdrn18002@mail.unpad.ac.id*

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ABSTRACT

Gambung green tea (*Camellia sinensis var. assamica*), cultivated in the highlands of West Java, Indonesia, is renowned for its unique sensory qualities and potential health benefits. Given the growing global concern over antibiotic resistance, there is an urgent need to explore natural alternatives with antimicrobial properties. This study investigated the antimicrobial and antioxidant properties of Gambung green tea extracts against clinically relevant pathogens, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Candida albicans*. Crude extracts were prepared using water, ethanol, and ethyl acetate, and phytochemical screening revealed the presence of flavonoids, polyphenols, tannins, and anthocyanins. Ethanol extraction yielded the highest extract mass (37.6%) and demonstrated the most potent antimicrobial activity, particularly against *S. aureus* (IC_{50} 5.0 mg/mL) and *S. pyogenes* (IC_{50} 7.5 mg/mL). The ethanol extract also exhibited strong antioxidant activity, as measured by the DPPH assay. *E. coli* showed moderate resistance (IC_{50} 24.34 mg/mL), while *K. pneumoniae* and *C. albicans* were less susceptible. The results highlight the potential of Gambung green tea, particularly its ethanolic extract, as a natural antimicrobial and antioxidant agent. Further research is needed to identify specific bioactive compounds and their mechanisms of action. The localized origins and unique environment contribute to the distinctive phytochemical composition and biological activities observed. Further investigation into the specific bioactive compounds and structure-activity relationships is warranted to facilitate the development of Gambung green tea as a natural antimicrobial resource.

Keywords: *Gambung green tea, Camellia sinensis, antimicrobial activity, antioxidant activity, pathogenic microorganisms.*

INTRODUCTION

Gambung green tea (*Camellia sinensis var. assamica*) is closely associated with its unique geographic location and environment in the highlands of West Java, Indonesia. It has a long history and reputation that has established it as a distinctive variety of tea. Given these unique qualities, it holds potential as a natural antimicrobial agent in addressing the growing challenge of antibiotic resistance. The emergence of antibiotic-resistant pathogens has become a critical global health concern, necessitating the exploration of alternative antimicrobial sources. Natural compounds from plants like Gambung green tea offer promising alternatives due to their complex phytochemical profiles, which can target multiple bacterial mechanisms simultaneously, potentially reducing the likelihood of resistance development. Furthermore, these natural compounds often exhibit fewer side effects compared to synthetic antibiotics and can potentially work synergistically with existing antimicrobial drugs.

Indonesia faces challenges from both Gram-positive and Gram-negative bacteria, distinguished by their cell structure. Gram-positive bacteria have more peptidoglycan layers and maintain violet-iodine staining, whereas Gram-negative bacteria have fewer layers and lose staining when alcohol is added (changing from purple to red) (Fisher & Mobashery, 2020). *Escherichia coli* (*E. coli*), a Gram-negative bacterium, challenges healthcare due to its resistance to antibiotics, attributed to low cell wall permeability. *E. coli* causes various illnesses, including diarrhea, urinary tract infections, respiratory infections, and pneumonia (Ameer *et al*., 2024; La Combe *et al*., 2019; Todar, 2020).

Conversely, Gram-positive *Staphylococcus aureus* is highly susceptible, causing diseases such as abscesses, skin infections, septicemia, and bacteremia. In severe cases, infections can lead to pneumonia, mastitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, and endocarditis. *Staphylococcus aureus* infections are characterized by tissue damage and pus-filled abscesses, accompanied by toxins and enzymes such as catalase, coagulase, hemolysins, leukocidin, enterotoxins, and toxic shock syndrome toxin (TSST) (Balaban & Rasooly, 2000; Silversides *et al*., 2010). Additionally, two other significant bacteria, *Streptococcus pyogenes* and *Klebsiella pneumoniae*, merit attention due to their clinical relevance. *Streptococcus pyogenes* is associated with various infections, while *Klebsiella pneumoniae* causes pneumonia and other

serious healthcare-associated infections (Joseph *et al*., 2021; Xu & Zhang, 2024).

Staphylococcus aureus and *Escherichia coli* serve as indicators for assessing the effectiveness of natural antimicrobials. Overuse and misuse of antibiotics have led to multidrug resistance organisms (MDROs), prompting efforts to develop broad-spectrum natural antimicrobials (Munita & Arias, 2017; Osińska *et al*., 2017). This involves isolating compounds from diverse Indonesian plants.

This study aims to address local antimicrobial challenges by leveraging the natural attributes of Gambung tea, a unique variety of green tea (*Camellia sinensis*) cultivated in the highlands of West Java, Indonesia. While the antimicrobial properties of green tea have been widely studied, the specific chemical constituents and bioactivity of Gambung green tea, which is influenced by its distinct terroir (soil, climate, and altitude), have not been thoroughly investigated. Previous research on green tea has primarily focused on common varieties, such as *Camellia sinensis var. sinensis*, and their major bioactive compounds, including catechins (e.g., epigallocatechin gallate, EGCG), flavonoids, and polyphenols, which are known for their antimicrobial and antioxidant properties (Koch *et al*., 2020; Liu *et al*., 2022). However, the unique environmental conditions of the Gambung region may result in a distinct phytochemical profile, potentially leading to enhanced or novel bioactivity.

The primary objective of this study was to investigate the in vitro antimicrobial properties of Gambung green tea extracts (ethanol, ethyl acetate, and water) against clinically relevant pathogens, including Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*), and the fungal pathogen *Candida albicans*. The study employed a disc diffusion method with varying extract concentrations to elucidate their antimicrobial potential (Balouiri *et al*., 2016)

METHODS

Materials

The following are the main materials and tools used in the study:

1. Tea Leaves: Dried, loose leaf Gambung green tea (*Camellia sinensis var. assamica*) was sourced directly from the Indonesia Research Institute for Tea and Cinchona (PPTK) in West Java. This ensured an authenticated Gambung cultivar with minimal processing.

2. Solvents: Demineralized water, ethanol (96%), and ethyl acetate.
3. Microbial Strains: Clinically relevant local strains of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae*, and *Candida albicans* from the Microbiology Laboratory collection of Bandung Health Polytechnic.
4. Chemicals and Reagents: Folin Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl), ferric chloride, ammonium, sulfuric acid and other reagents used for phytochemical tests.
5. Tools and Equipment: Rotary evaporator, microscope, UV-Vis spectrophotometer, and Whatman No. 1 filter paper.
6. Standards: McFarland standard for microbial suspension calibration.

Research Path

1. Acquisition of Green Tea Leaves

Dried, loose leaf Gambung green tea was obtained directly from the Indonesia Research Institute for Tea and Cinchona (PPTK) in West Java. The leaves were sealed and promptly delivered to the research laboratory to maintain phytochemical integrity.

2. Preparation of Crude Extracts

Three separate glass jars, each containing 1 L of demineralized water, ethanol 96%, and ethyl acetate, were prepared. Into each jar, 100 g of tea leaves was added. Maceration was conducted over 24 hours with intermittent hand shaking every 2 hours at room temperature (25 ± 2°C). The solvents were subsequently separated by filtration using Whatman No. 1 filter paper and concentrated using a rotary evaporator (Buchi R-200) under reduced pressure at optimized temperatures: water extract at 70°C, ethanol extract at 60°C, and ethyl acetate extract at 50°C. The resulting crude extracts were further diluted with sterile water to create suspensions with a concentration of 10 mg/mL.

3. Preparing Microbial Suspensions

Bacterial suspension stocks were derived from clinically relevant local strains of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae,* and *Candida albicans* from the Microbiology Laboratory collection of Bandung Health Polytechnic. Following a 24-hour incubation at 37°C on nutrient agar, colonies were selectively picked and diluted in PBS to achieve a standardized McFarland 0.5 suspension. For Candida albicans, a 48-hour incubation at 28°C on Sabouraud agar was employed.

4. Antimicrobial Susceptibility Testing

Sterile paper discs (Whatman No. 1, 6 mm diameter) were impregnated with 20 µL of extract at concentrations of 10, 20, and 30 µg/disc. Bacterial suspensions (0.5 McFarland standard, ~1.5 × 10^8 CFU/mL) were spread on Mueller-Hinton agar plates, while Sabouraud Dextrose agar was used for C. albicans. The discs were placed 24 mm apart on the inoculated plates, including positive controls (tetracycline 30 µg for bacteria, fluconazole 25 µg for C. albicans) and negative controls (solvent only). Plates were incubated at 37°C for 24 hours (bacteria) or 28°C for 48 hours (C. albicans). Inhibition zones were measured in triplicate using a digital caliper, with zones ≥ 7 mm considered positive for antimicrobial activity. Results were expressed as mean ± standard deviation.

5. Phytochemical Analysis

Phytochemical tests were conducted on ethanol, ethyl acetate, and water extracts from Gambung green tea leaves to assess the presence of bioactive compounds. Flavonoids were identified using the Shinoda and sodium hydroxide tests, whereas total polyphenols were quantified through the Folin Ciocalteu method. Tannins were detected via reactions with ferric chloride, saponins were screened using the frothing test, and anthocyanins were assessed with acidified ethanol. Triterpenoids were identified through the Liebermann–Burchard reaction.

6. Antioxidant Activity

In the antioxidant assay, three samples of green tea leaf extracts were prepared using ethanol, ethyl acetate, and water as solvents. Each extract was initially dissolved in its respective solvent to create stock solutions at a concentration of 100 ppm. These stock solutions were further diluted to generate various concentrations, ranging from 5 to 9 ppm. To assess the antioxidant activity, a stock solution of 50 ppm DPPH was prepared. For testing, 2 ml of each sample solution and 2 ml of the DPPH solution were mixed and incubated at 27°C for 30 minutes. The resulting color change was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.

7. Quantification of Total Polyphenol

In the quantitative total polyphenol assay, the green tea leaf extract was first dissolved in methanol and then transferred into reaction tubes, each containing 50 µL of the extract. Following this, 2.5 mL of Folin Ciocalteu reagent (diluted to 1/10) and 2 mL of a 7.5% Na2CO3 solution were added to each tube. The mixture was incubated at 45°C, and the absorbance was measured at 753 nm. The obtained

absorbance values were plotted against a standard curve for gallic acid.

8. Statistical Data Analysis

Descriptive statistics, including mean, standard deviation, and percentage of inhibition, were computed using IBM SPSS Statistics 23. For dose-response evaluation and IC_{50} determination, probit regression analysis was conducted using MedCalc.

RESULTS AND DISCUSSION

Result

1. Extract Yield

The extraction of Gambung green tea was performed using 24-hour maceration at room termperature (25 ± 2°C) with three different solvents, followed by concentration using a rotary evaporator under reduced pressure at optimized temperatures (water extract at 70°C, ethanol extract at 60°C, and ethyl acetate extract at 50°C). The process yielded varying amounts of extract. The use of ethanol resulted in the highest extract mass of 75.2 g and a percentage yield of 37.6%, producing a dark brown extract. Ethyl acetate yielded an extract mass of 16.3 g and a percentage yield of 8.2%, resulting in a dark green extract. Water extraction resulted in an extract mass of 15.9 g and a percentage yield of 8.0%, with the extracted substance being brown.

2. Phytochemical Analysis

The qualitative phytochemical analysis aimed to identify the presence of various bioactive compounds in the Gambung green tea extracts obtained from different solvents. The results of this screening are presented in Table 1. This analysis provided insights into the potential contribution of these phytochemicals to the observed biological activities, such as antimicrobial and antioxidant effects.

Table 1. Qualitative Phytochemical Analysis

No.	Compound	Solvent		
		Water	**Ethanol 96%**	**Ethyl Acetate**
1	Flavonoid	+	+ +	+
2	Polyphenol	+	+	+
3	Tannin	+	+	+
4	Saponin	+	+	+
5	Anthocyanin	+	+	+
6	Triterpenoid	-	-	-

The findings indicate that the effectiveness of extracting various phytochemicals differs depending on the solvent used. Ethanol 96% appears to be more effective in extracting flavonoids and anthocyanins, while the presence of polyphenols, saponins and tannins is consistent across all solvents. Triterpenoids are absent in all tested solvents.

3. Total Polyphenol

In addition to the qualitative analysis, the quantification of total polyphenols was conducted to determine their relative abundance in the extracts. Polyphenols are

known for their potent antioxidant and antimicrobial properties, making their quantification crucial for understanding the bioactivity of the extracts. The results of the total polyphenol content analysis are presented in Table 2.

Table 2. Percentage of Polyphenol in Extract Yield

Sample concentration (mg/L)	Total polyphenol (%)		
	Ethanol	**Ethyl acetate**	**Water**
10	23,2	24,5	20,11
5	26,4	26,3	17,30
2,5	28,9	20,9	31,90
Average	**25,87**	**23,9**	**23,10**

Table 2 shows the percentage of total polyphenols present in the extract yields obtained from different solvents and at varying concentrations. The ethanolic extract exhibited the highest average polyphenol content of 25.87%, followed by the ethyl acetate (23.9%) and water (23.10%) extracts. These results corroborate the qualitative findings, indicating the superior extraction efficiency of ethanol for polyphenolic compounds from Gambung green tea leaves.

4. Antioxidant Activity

The method used to assess the antioxidant capacity was DPPH (2,2-diphenyl-1-picrylhydrazyl), which is a common spectrophotometric technique for this purpose. Vitamin C (ascorbic acid) was utilized as a reference standard to construct a concentration-response curve by measuring absorbance at different concentrations. This vitamin C standard curve enables quantification of the antioxidant capacity of test samples in relation to the vitamin C standard. Three green tea extracts were prepared using different solvents - water, ethanol, and ethyl acetate - and each extract was tested in 5 replications. BHT (butylated hydroxytoluene) served as a positive control standard for comparison. The antioxidant activities of the different green tea extracts are compared in Figure 1.

The IC_{50} values were determined by constructing dose-response curves for each extract, plotting concentration versus percentage inhibition of DPPH. The concentration of extract causing 50% inhibition of DPPH absorbance was interpolated from the linear portion of the curve and reported as the IC_{50} value for each extract. These IC_{50} values, representing the concentrations that caused 50% inhibition of DPPH, were calculated for the extracts and presented in a bar chart to allow comparison. The results showed that the ethanol extract exhibited the lowest IC_{50}, indicating that it possessed the highest antioxidant potency among the three extracts tested.

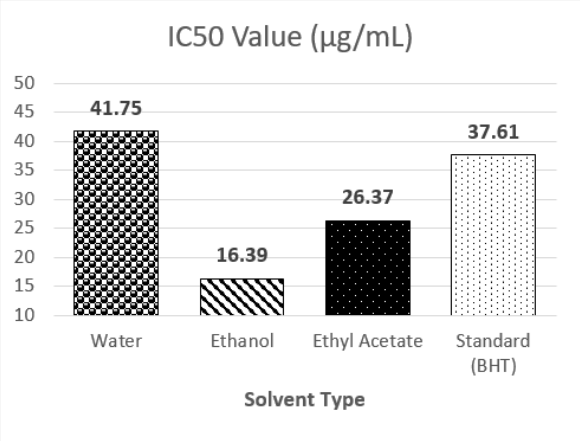

Figure 1. Comparison of Green Tea Extract Solvent Antioxidant Activity

5. Microbial Susceptibility to Extracts

The ethanol extract of green tea was tested for antimicrobial activity against several pathogens using the disc diffusion method. Discs containing different concentrations of the extract were placed on agar plates inoculated with the test microorganisms. After incubation, the zones of inhibition around the discs were measured.

Table 3 presents the quantitative data on the zones of inhibition observed when testing the antimicrobial effects of the ethanolic green tea extract at different concentrations. The ethanol extract exhibited strong inhibition against Staphylococcus aureus and Streptococcus pyogenes, moderate inhibition against Candida albicans, and weak or no inhibition against Escherichia coli and Klebsiella pneumoniae. Increasing the extract concentration resulted in larger inhibition zones.

Table 3. Zones of Inhibition (mm) of Various microorganisms against Different Concentrations of Ethanolic Green Tea Extract

Microbe	Inhibition zone (mm)			
	Ethanol Extract			Tetra-cycline 30 µg
	10 µg	20 µg	30 µg	
S. aureus	17,55	21,85	29,05	30,00
S. pyogenes	18,05	22,65	24,65	32,50
E. coli	8,15	10,65	15,00	25,00
K. pneumoniae	0	0	0	25,33
C. albicans	13,37	14,40	14,73	38,33

The figure illustrates the differential inhibition patterns observed in the disc diffusion assay, where darker zones indicate stronger antimicrobial activity. The largest zones of inhibition were observed with Gram-positive bacteria (*S. aureus* and *S. pyogenes*), while Gram-negative bacteria (*E. coli* and *K. pneumoniae*) showed limited susceptibility to the extract. This visual representation correlates with the quantitative measurements presented in Table 3.

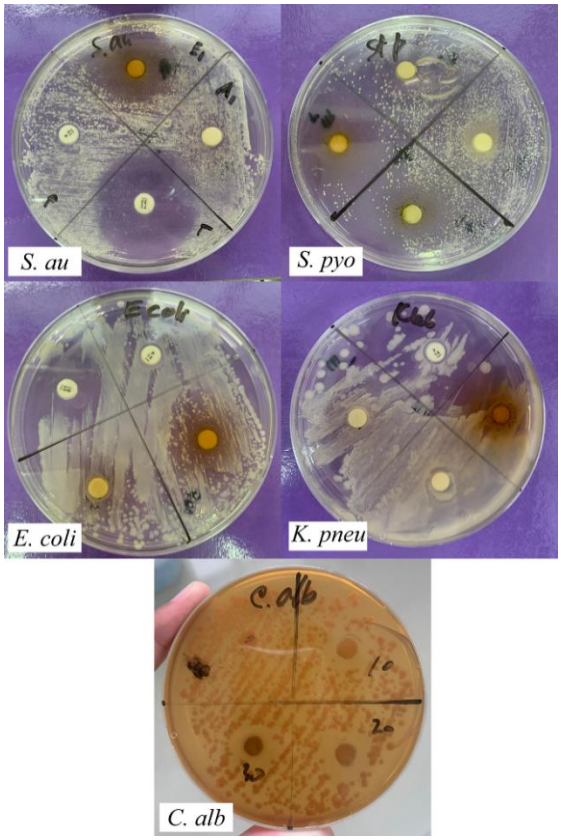

Figure 2. Green Tea Extract Against Various Microbe Using Disc Diffusion Method

The IC_{50} values determined through probit regression modeling are presented in Table 4. Probit regression modeling was utilized to evaluate the dose-response relationships between green tea extract concentration and growth inhibition of three bacterial strains: *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*.

The analysis yielded the half maximal inhibitory concentration (IC_{50}) and quantitative dose-response coefficients for each organism. *S. aureus* exhibited the lowest IC_{50} of 5,0 mg/mL, indicating high sensitivity to the antimicrobial effects of the green tea extract. *S. pyogenes* showed moderate sensitivity with an IC_{50} of 7.5 mg/mL. *E. coli* was the most resistant strain, with an IC_{50} of 24.34 mg/mL. The dose coefficients for all three bacteria were statistically significant ($p<0.05$), confirming a quantitative relationship between green tea extract dose and growth inhibition. However, the wide confidence intervals indicate uncertainty in the precise dose-response curves based on these preliminary data. Overall, the results demonstrate that green tea extract has broad spectrum antimicrobial activity, with potency depending on the bacterial species.

Table 4. Probit Regression & IC_{50}

Types of Microbe	Dose Coeffi-cient	Std Error	P-value	IC_{50}
S. aureus	1,1648	272,96	0.0712	5,00
S. pyogenes	2,3699	515,78	0.0027	7,50
E. coli	1.1739	220,73	0.0027	24,33
K. pneu	-	-	-	-
C. albicans	-	-	-	-

Discussion

The ethanol extract exhibited potent antimicrobial activity, aligning with its high extraction efficiency of beneficial phytochemicals from the Gambung green tea leaves. In comparison to ethyl acetate and water, ethanol extracted a far greater

yield from an equivalent starting quantity of leaves, resulting in 75.2 g of extract from 100 g of dried leaves - more than double the yields of the other solvents. These findings are consistent with those of Koch *et al*., who found that among various solvents tested for extracting catechins and polyphenols from green tea leaves, ethyl acetate was by far the least effective extractant. The ethyl acetate extracts contained only small amounts of catechins and had the lowest total phenolic content compared to solvents like ethanol, water, methanol, etc (Koch *et al*., 2020).

Ethanol's superior extraction capacity can be attributed to its intermediate polarity. This enables it to effectively solubilize the diverse array of polar and non-polar bioactive components inherent to the leaves. These likely incorporate polyphenols, flavonoids, alkaloids and other antimicrobial phytochemicals. Higher ethanol concentrations increased polyphenol extraction compared to lower percentages, aligning with ethanol's efficacy at solubilizing these antioxidants (Evitasari & Susanti, 2021). This ability to efficiently extract a broad spectrum of compounds contributed to the dark brown pigmentation of the ethanol extract, in addition to its potent antimicrobial effects against *Staphylococcus aureus* and *Streptococcus pyogenes*. The swelling effect of the leaf matrix in ethanol and high solubility of tea components like polyphenols and catechins likely contributed to the excellent extraction efficiency (Hu *et al*., 2016). Furthermore, the darker hue of the ethanol extract compared to ethyl acetate and water provides further evidence of higher tannin levels, given that tannins confer darker colors in plant extracts. The dried leaf extract exhibited marginally higher tannin content, indicating the potential impact of the drying process on phytochemical extraction (Samadi & Raouf Fard, 2020).

The phytochemical screening demonstrated variations in the extraction efficiency of different bioactive compounds based on solvent polarity. While polyphenols and tannins were consistently present across all solvents, ethanol appeared more effective at extracting flavonoids and anthocyanins compared to water or ethyl acetate. This aligns with existing literature indicating that the major polyphenolic compounds in green tea include catechins like epigallocatechin gallate (EGCG), which are most soluble in moderately polar solvents like ethanol. In addition, highly polar saponins were present in all solvents tested, contributing to the broad spectrum of extracted bioactive compounds. The presence of these saponins, along with flavonoids and anthocyanins, likely

contributed to the enhanced antimicrobial effects exhibited by the ethanol extract (Fan *et al*., 2021; Yu & He, 2018). Further identification and characterization of the phytochemical profile merits investigation to determine the specific compounds responsible for the observed biological activities. Overall, the results agree with previous studies demonstrating ethanol as an optimal solvent for extracting antioxidative and antimicrobial polyphenols from green tea leaves.

The antibacterial effects of green tea catechins can be attributed to inhibition of bacterial dihydrofolate reductase (DHFR), an enzyme critical for folate metabolism and growth (He *et al*., 2020). The tea catechin EGCG potently blocks DHFR activity in pathogens like *S. aureus*, disrupting biosynthesis of nucleotides needed for proliferation. By targeting both the bacterial membrane and pivotal enzymatic pathways, bioactive compounds in green tea exert broad-spectrum antimicrobial action, overcoming potential resistance mechanisms (Li *et al*., 2015). The antifolate properties of tea catechins substantiate their therapeutic potential against Gram-positive bacteria like *S. aureus* and *S. pyogenes* (Navarro-Perán *et al*., 2005).

The reduced susceptibility of Gram-negative bacteria like *E. coli* and *Klebsiella* to the green tea extracts may be attributed to three key differences compared to Gram-positives. Firstly, the outer membrane surrounding the peptidoglycan cell wall in Gram-negatives likely impedes extract penetration to intracellular target sites. Secondly, Gram-negatives possess more active efflux pumps that can expel the extracts before they exert antimicrobial effects. Finally, variability in cell surface binding sites between Gram-negative and -positive bacteria may account for differences in extract binding and activity. Thus, the combination of the Gram-negative outer membrane barrier, multidrug efflux pumps, and variability in cell surface binding sites helps explain the overall lower susceptibility of *E. coli* and *Klebsiella* to the green tea extracts compared to the more susceptible Gram-positive species (Ibrahim Alghamdi, 2023).

Green tea extract, particularly its active compound epigallocatechin gallate (EGCG), exhibits a multifaceted antibacterial mechanism. The primary action involves disrupting the bacterial cell membrane, resulting in increased permeability, which is measured by changes in electrical conductivity. This disruption compromises the membrane's integrity, causing leakage of intracellular components such as nucleic acids, proteins, and solute

sugars, leading to cell lysis and damage. Catechins, rich in phenolic hydroxyl groups and polycyclic structures, have a high affinity for biomacromolecules like lipids, proteins, hydrocarbons, and nucleic acids. This affinity leads to reactions with the bacterial cell membrane, making the cell structure unstable, altering membrane fluidity, and ultimately destroying its integrity. Furthermore, EGCG interferes with essential cellular processes by preventing DNA supercoiling, which inhibits bacterial replication and transcription (Liu *et al*., 2022).

Additionally, EGCG generates reactive oxygen species (ROS) and hydrogen peroxide, contributing to oxidative stress within bacterial cells, causing significant damage to cellular components and ultimately leading to cell death. Beyond direct bactericidal actions, green tea extract inhibits biofilm formation and quorum sensing (QS) in bacteria such as *Morganella morganii*. The extract significantly reduces biofilm formation at concentrations as low as 62.5 μg/mL by interfering with the QS system, which relies on short-chain acyl homoserine lactones (AHLs) for signaling. This disruption prevents bacteria from coordinating biofilm formation and virulence factor expression, thereby reducing pathogenicity without directly killing the bacteria. Furthermore, EGCG shows a synergistic effect when combined with antibiotics like gentamicin, enhancing overall antibacterial activity against multi-drug resistant pathogens such as *S. aureus* and *E. coli* (Guzman *et al*., 2020; Parvez *et al*., 2019).

Probit regression modeling provided quantitative insights into the concentration-dependent antimicrobial effects of the green tea extracts. Determination of IC_{50} values enabled direct comparisons of potency, with *S. aureus* exhibiting the lowest IC_{50} of 5.0 mg/mL indicating high sensitivity. In contrast, *E. coli* displayed an IC_{50} of 24.34 mg/mL, reflecting its relative resistance to the extracts compared to *S. aureus*. While wide confidence intervals indicate uncertainty in the exact dose-response curves based on this preliminary data, the quantitative differences in IC_{50} values between species reveal valuable structure-activity insights that can guide further characterization and development of green tea extracts as broad-spectrum antimicrobials (Chen *et al*., 2013).

Notably, the green tea extracts displayed a broad spectrum of activity encompassing both Gram-positive and Gram-negative bacteria. However, the quantitative variations in potency (IC_{50}) between species highlights that

antimicrobial efficacy is nuanced and dependent on the pathogen. While preliminary, the dose-response modeling provides a robust platform to systematically delineate these fine distinctions in spectrum and potency. Follow-up time-kill kinetic studies monitoring growth inhibition over time would serve as an complementary approach to further confirm the concentration-dependent antimicrobial effects and quantitative differences predicted by the dose-response analysis. Integrating multiple quantitative techniques strengthens the elucidation of subtle structure-activity relationships governing the broad but variable spectrum of the green tea extracts against bacterial pathogens.

CONCLUSIONS

Ethanol extracts of Gambung green tea exhibited potent broad-spectrum inhibition against Gram-positives like *S. aureus* (IC_{50} 5 mg/mL) and *S. pyogenes* (IC_{50} 7.5 mg/mL). Moderate effects were observed against *C. albicans* and the Gram-negative *E. coli* (IC_{50} 24.34 mg/mL). The unique terroir likely contributes to the distinctive antimicrobial phytochemical profile. Further identification of bioactive compounds using bioassay-guided fractionation is warranted to uncover novel antimicrobial agents. Pharmacokinetic and durability studies would provide additional insights to guide translational development. Overall, the quantitative dose-response analyses substantiate the antimicrobial potential of Gambung green tea extracts against pathogenic bacteria and fungi. Further phytochemical characterization and preclinical optimization are required to translate these promising preliminary findings into viable clinical applications.

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