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Research Article

Benefits of Wild Plant: *Camellia sinensis* extract usage in medicine

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Abstract

There is an increasing interest in the beneficial effects of green tea extracts, which are effective against various diseases due to the bioactive compounds they contain. Researchers have investigated the ability of green tea extract to inhibit microbial growth due to its polyphenolic compounds, particularly catechins. This study examines the antimicrobial effect of *Camellia sinensis* extract on clinical bacterial isolates obtained from wound and burn infections, as well as its impact on the gene expression of efflux pump regulator (EMR), penicillin-binding protein (PBP), metal-β-lactamase (MPH), and AmpC β-lactamase (AMP). Samples were collected from clinical cases of wounds and burns; the bacterial isolates were cultured and characterized using conventional methods. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of *C. sinensis* extract against the isolates were also determined. The bioactive composition of *C. sinensis* extract was analyzed, and real-time PCR was used to assess gene expression, with relative quantification of EMR, PBP, MPH, and AMP genes in the presence and absence of *C. sinensis* extract.

Keywords: *Camellia sinensis*, antibacterial activity, wound, burn

Introduction

Wound infection significantly exacerbates chronic wounds and impairs wound healing (Liu et al., 2020). Effective wound care is crucial for public health, as it improves community well-being by reducing the incidence of illness and enhancing functionality and improving quality of life. Best practices in wound care minimize the recurrence of chronic wounds (Zhang et al., 2022). However,

every injury, regardless of its cause or severity, becomes colonized by bacteria from skin flora. The composition and quantity of these microorganisms vary between wounds, complicating the understanding of wound healing and microbial colonization (Mulder et al., 2024). Several factors influence wound colonization and infection, including the wound source, location, size, and duration (Xiang et al., 2024). Chronic wounds, which take a long time to heal, affect approximately 1-2% of the population in developing countries (Y. Zhao et al., 2024). Wound healing is complex. Tightly regulated processes are susceptible to endogenous and exogenous factors, including microorganisms. Acute and chronic wound openings inevitably lead to microbial colonization, hindering healing (Turkoglu, Pekmezci, & Kilic, 2020). The severity of wound infection depends on pathogen virulence and the host`s immune status. Clinically, wound infection is characterized by changes in color, hardness, irritation, warmth, swelling, and the formation of pus or crusts (Turkoglu et al., 2020). Excessive microbial growth increases the risk of adverse wound healing outcomes in both acute and chronic wounds (T. Zhao, Li, Wang, & Song, 2022). Antibiotic resistance, driven by antibiotic use, poses a major global health threat, increasing morbidity, mortality, and healthcare costs. This is particularly problematic in skin and soft tissue infections (SSTIs), including those associated with wounds and burns, given the rising prevalence of multidrug-resistant (MDR) pathogens; effective antimicrobial stewardship is therefore paramount. The identification, along with the development of medicinal plants as therapeutic agents, has shown effectiveness in combating multidrug-resistant patterns observed in both clinical and environmental samples. The primary benefits of employing plant-based medicines are their superior safety relative to synthetic alternatives, substantial therapeutic efficacy, and economic viability in treatment. Researchers believed that certain plant extracts might have a significant impact on harmful bacteria, potentially aiding in the development of antimicrobial supplements.

The extract of the leaves of *Camellia sinensis* (green tea) demonstrates a wide range of antimicrobial properties, owing to its elevated concentration of catechins, especially epi gallate (EGCG One promising approach involves investigating plant-derived compounds with antimicrobial and biofilm activity (Xia et al., 2021). This study investigates the antimicrobial properties of green tea extract as well as EGCG in relation to clinically significant wounds and burn isolates, and its effect on relevant gene expression.

Martial and methods

Sample collection and identification

This study involved 120 wound infection swab samples collected from Al-Yarmouk Teaching Hospital, Al-Karkh Hospital, and Al-Kindi Teaching Hospital in Baghdad, Iraq, within 48 hours of hospital admission. Samples were transported in a suitable medium to the microbiology laboratory. All specimens were cultured on MacConkey as well as blood agar (assuming HIV-negative patients). Aerobic incubation at 37°C for 24 hours followed, and the isolates were identified through standard biochemical methods (Patrone et al., 2016; Raivio, Laird, Joly, & Silhavy, 2000).

Antibiotic susceptibility test of the bacterial isolates

For checking the efficacy of the antibiotics, we employed the Kirby-Bauer disc diffusion method (Sarwa, Rudrapal, & Debnath, 2013). We had prepared cultures overnight and ensured that turbidity was 0.5 McFarland standards, i.e., 1.5×108 CFU/ml. We applied the antibiotic discs (VA, PB, AM, LV, AZM, CIP, AX, ATM, and GEN) on pre-infected Muller-Hinton agar plates (Himedia/India). Then, the plates were incubated for a complete day at 37°C. We analyzed and measured the zones of inhibition based on the 2020 CLSI guidelines. The isolates were classified into two categories: sensitive (S) and resistant (R).

Biofilm formation of bacterial isolates

The microtiter plate method was employed to evaluate biofilm formation (Babapour, Haddadi, Mirnejad, Angaji, & Amirmozafari, 2016; Rio, Ares, Hannon, & Nilsen, 2010). A bacterial suspension (0.5 McFarland standard) was introduced into microtiter wells filled with BHI broth supplemented with 2% sucrose and incubated at 37°C for 24 hours. Non-adherent cells were eliminated through washing with PBS (pH 7.2), followed by air-drying of the wells. A 1% solution of bound crystal violet was prepared by dissolving it in 95% ethanol, and then the optical density was measured at 630 nm.

Collection of *C. sinensis* and extract preparation

Mature and healthy leaves of *C. sinensis* were harvested from Halabja, Sulaimaniyah province, Iraq 35.1770° N, 45.9864° E). Collected samples were washed, dried at 40°C for 48 hours and ground to a fine powder, and stored at room temperature. Extraction was performed using Soxhlet extraction with methanol (1:10 w/v) at 65°C for 4 hours, with complete solvent cycling twice per hour (Peterson & Freeman, 2009).

Moisture content determination

The moisture content of the powdered sample was assessed using a standard oven-drying method. The sample was accurately measured and placed into a pre-dried and pre-weighed moisture dish. The quantity was approximately 5 g. The sample was subsequently placed in a hot air oven maintained at 105°C and allowed to dry for 4 hours. After drying, it was carefully removed and allowed to cool in a desiccator to prevent it from absorbing moisture from the air again. After the sample had cooled, the container was weighed again. To find the moisture percentage, we used the formula (Ajiboye et al., 2013): Moisture (%) = [(Initial weight − Dry weight) / Initial weight] × 100. This meant finding the difference between the initial and final weights.

Ash Content Determination

The analysis of ash content required the accurate weighing of approximately 2 grams of the powdered tea sample, which was subsequently placed into a pre-weighed crucible. The sample was burned in a muffle furnace at 550°C for 4 to 6 hours, or until a steady weight of white ash was reached. This meant that all of the organic matter had been burned. After incineration, the crucible was allowed to cool in a desiccator to prevent moisture absorption and was subsequently reweighed. We used the formula (Ajiboye et al., 2013) to figure out how much ash was in the sample. To find the ash percentage, you take the weight of the ash, divide it by the weight of the sample, and then multiply that number by 100.

Determination of Total Polysaccharide Content

The powdered sample was initially defatted using petroleum ether and then subjected to extraction with hot water heated between 85 and 90°C for two hours. Following extraction, the mixture was filtered to eliminate solid residues and concentrated to reduce its volume. Polysaccharides were precipitated by adding three volumes of 95% ethanol to the concentrated extract and storing the mixture at 4°C overnight. The precipitated polysaccharides were then collected by centrifugation at 5000 rpm for 15 minutes, washed with ethanol to purify, and dried. The amount of polysaccharides was quantified using the phenol-sulfuric acid method, with glucose as the reference standard, and absorbance was measured at 490 nm to assess concentration (Ajiboye et al., 2013).

Protein Content (Bradford Assay)

We mixed one gram of powder with a phosphate buffer solution that had a pH of 7.0. After that, we used a centrifuge to determine the amount of protein in the sample. After processing, the supernatant was used for testing and evaluation. We put Bradford reagent in the supernatant and

let it sit at room temperature for 10 minutes. We made a standard curve with bovine serum albumin (BSA) and then used the 595 nm wavelength to measure the absorbance to find out how much protein was in the sample (Ajiboye et al., 2013).

Total Phenolic Content (TPC)

We mixed 1 mL of tea extract with 5 mL of 10% Folin–Ciocalteu reagent and let them react for 5 minutes. Then, 4 mL of a 7.5% sodium carbonate solution was added, and the mixture was left to sit in the dark at room temperature for 30 minutes. After the incubation period, the absorbance was measured at 765 nm. Gallic acid served as a standard for measuring the total phenolic content (TPC), and the results were expressed in milligrams of gallic acid equivalents (GAE) per gram of dry sample weight (Ajiboye et al., 2013).

Total Flavonoid Content (TFC)

To determine the amount of flavonoids in the extract, one millilitre was mixed with four millilitres of distilled water and 0.3 millilitres of a 5% sodium nitrite solution. After five minutes, 0.3 mL of 10% aluminium chloride was added to the mixture. After another six minutes, 2 milliliters of 1 M sodium hydroxide were added. It was important to get the final volume exactly right at 10 mL with distilled water, and the absorbance at 510 nm was accurately measured. The flavonoid content was measured in milligrams of quercetin equivalents per gram of dry weight, with quercetin being the standard (Parvez et al., 2019).

Preparation and isolation of epigallocatechin from the *Camellia sinensis*

Following the collection of the plant materials, they were washed with tap water two or three times and then once more with distilled water. We used distilled water to extract catechin compounds from green tea. We spun it at 50 °C and 300 rpm for 4 hours. Weigh out 5 grams of dry, ground green tea leaves and place them in a 500 mL triangular flask that already contains 150 mL of distilled water. After that, a rotary evaporator was used to filter and concentrate the extract until it reached a final volume of 30 mL. The extract was mixed with an equal volume of chloroform to remove impurities. To separate catechins from the water phase, the same amount of ethyl acetate was used. To separate epigallocatechin gallate (EGCG) from green tea, a C18 reversed-phase preparative column (250*22 mm) filled with 15 μm materials was used. The mobile phase was a mixture of 0.1% acetic acid in water and acetonitrile at a volume ratio of 87/13%. The injection volume was kept at 15 μL/min, and the flow rate of the mobile phase was kept at 1 mL/min. The effluent was collected from the column outlet and then evaporated until it reached a final volume

of 1 millilitre for HPLC analysis. The volume of the injection was 20 microlitres, and the flow rate and composition of the mobile phase were kept within the limits set for the preparative column. By carefully adjusting the retention times of EGCG, the composition of the mobile phase in preparative HPLC was optimized to achieve better resolution. A stock solution of EGCG was prepared from 5 g of dry ground green tea leaves, utilising 0.01 g of EGCG and 1 mL of distilled water. A solution was prepared utilising 1mL of EGCG combined with 1mL of distilled water. To ensure the accuracy and reliability of our findings, we performed supplementary experiments utilising EGCG sourced from Sigma-Aldrich, USA.

Antimicrobial activity of Camellia sinensis crude extracts and EGCG.

We used the microdilution technique to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EGCG, crude green tea extracts, and the antibiotic Gentamicin. This was done to find out how well they worked against bacteria. Gentamicin was chosen because it works well as an antibiotic for infections caused by both Gram-positive and Gram-negative bacteria. Also, people are apprehensive that this antibiotic will become resistant to other drugs. In each well of the 96-well plate, we put 100 µL of nutrient broth. The first row of the plate got 200 µL of the stock solution of test extracts. After that, two sets of serial dilutions were made. We grew bacteria in nutrient broth and then compared its density to that of a 0.5 McFarland solution. In each well, 100 µL of the bacterial suspension was added. We created a negative control by using only the media, as well as a positive control by mixing the media with the bacterial culture. The test plates were subsequently maintained at 37 °C for 18 hours. The MIC is the lowest concentration at which growth or turbidity is not observed. To find the minimum bactericidal concentration, a nutrient agar plate was treated with the minimum inhibitory concentration and at least two concentrated dilutions (10 or 7 µL). After the incubation period, the minimum concentration at which no visible growth occurred was recorded as the MBC (Parvez et al., 2019).

RNA extraction by TRIzol and cDNA synthesis

Total RNA was extracted from bacterial cells using TRIzol reagent (iNtRON, Korea) (Parvez et al., 2019). Cells were harvested by centrifugation, suspended in TRIzol, and incubated. Chloroform was added, and phases were separated by centrifugation. RNA was precipitated with ethanol, washed, and eluted.

cDNA synthesis

Complementary DNA (cDNA) was synthesized using a cDNA ready-to-use kit (Bioneer, Korea). RNA extract was mixed with hexamer primer and incubated in a PCR thermocycler (Applied Biosystems, USA) at 37°C for 10 minutes, 42°C for 1 hour, and 95°C for 5-10 minutes (Bessa, Fazii, Di Giulio, & Cellini, 2015).

Quantitative reverse transcription-PCR (qRT-PCR)

Real-time PCR primers for *Staphylococcus aureus* and *Klebsiella pneumoniae* gene expression were designed using the NCBI GenBank database and provided by Macrogen (Korea) (Table 1). cDNA was added to PCR tubes with primers and water to a final volume of 20 µl. The mixture was subjected to a qPCR machine (Bioneer, South Korea).

Table 1. *Staphylococcus aureus* and *Klebsiella pneumoniae* qPCR detection gene primers with their nucleotide sequence

ıcteria	Primer	Sequence (5'-3') – Forward	Sequence (5'-3') – Reverse	Product Size (bp)	GenBank Code
Staphylococcus aureus	ermAS	AAGCGGTAAACCCTCTGA	TTCGCAAATCCCTTCTCAAC	190	LT549456.1
	Pbp1S	AATGGCAATTTTGCATCACA	CCACGTTTAGGCTGCTTCTC	194	MF070946.1
	recAS	TTAGGAGGTCTCGCTTTGGA	CCACCTACACCTAGCGCATT	176	AF317802.1
Klebsiella pneumonia	MphAK	AATGAGCTTGGGCTCGACTA	GTCTTCGAGCATGGGATAGG	210	MN310374.1
	ampCK	GGTTCGGTCAGCAAAACATT	CAGCAGTGTAGGTTGCGAGA	157	JQ235790.1
	recAK	GAAAATCGGCGACTCTCACAT	ACGCACAGAGGCGTAGAACT	195	DQ859859.1

Calculation of the fold of gene expression

The Levak equation was applied for the assessment of the fold expression of the housekeeping gene and the control sample (Stepanović et al., 2007).

Analysis of Variance (ANOVA) conducted using IBM SPSS Statistics 21.0 was used to assess the influence of various variables on research parameters, while means ± standard deviations were utilised to quantify the bioactive components in the *Camellia sinensis* plant.

Results

Isolation and Identification of Bacteria

Out of 120 samples collected and processed, 70 (58.33%) samples were from surgical wound infection patients and 50 (41.66%) from burn. Among the collected pus samples, 67 (55.83%) were

from male patients and 53 (44.16%) from female patients. A maximum 8 (6.66%) of samples were collected from patients of the age group of 2-5years, (male=6 and female=2), followed by the age group of more than 5 years, which had 112 samples. Among the 120 samples collected, 33 (27.5%) did not detect any growth, while 87 (72.5%) showed positive growth for bacteria (Table 2). All isolates were catalase-positive, and only *P. aeruginosa* isolates were oxidase-positive. These results are consistent with those reported in Choi, Yoon, Kim, & Han (2023) and Hameed, Hussain, Mahmood, Deeba, & Riaz. Similarly, among 87 isolates, Gram-negative bacteria were predominating, constituting 39 (44.82%), and Gram-positive bacteria constituted 37 (42.52%). Among the total bacterial isolates, S. aureus was the predominant species, accounting for 37 (42.52%), followed by *P. aeruginosa* (22, 25.28%) and *K. pneumoniae* (17, 19.54%) in number.

Antibiotic Susceptibility Pattern of Isolates

S. aureus bacteria showed complete resistance to Vancomycin, while their resistance to other antibiotics varied, with 16.6% resistance to Aztreonam, 33.3% to Azithromycin, 50% to Ciprofloxacin and Levofloxacin, and 66.6% to Amoxicillin and Polymyxin (Table 3). While *P. aeruginosa* is characterized by its resistance to Amoxicillin and vancomycin, it is fully sensitive to Amikacin, Ciprofloxacin, and Levofloxacin, 83.3% sensitive to Aztreonam, and 66.6% resistant to Polymyxin. In the case of *K. pneumonia,* Ciprofloxacin, Amoxicillin, Levofloxacin, and Polymyxin were the most effective drugs, showing 100% sensitivity, followed by Gentamicin (66.6%), whereas Azithromycin (50%) and Aztreonam (33.3%) were less effective. While Ciprofloxacin and Vancomycin were not effective, exhibiting 100% resistance. The sensitivity pattern was statistically found to be significant with $p<0.001$).

Table 2. Percentage of antibiotic resistance of *K. pneumoniae, S. aureus,* and *P. aeruginisa* isolates

Antibiotic	***K. pneumonia***		***S. aureus***		*P. aeruginosa*	
	Sensitive N. (%)	Resistance N. (%)	Sensitive N. (%)	Resistance N. (%)	Sensitive N. (%)	Resistance N. (%)
Amikacin AM 10mg	6(100%)	0	6(100%)	0	6(100%)	0
Ciprofloxacin CIP 10mg	0	6(100%)	3(50%)	3(50%)	6(100%)	0
Levofloxacin LV 5mg	6(100%)	0	3(50%)	3(50%)	6(100%)	0
Amoxicillin AMX 25mg	0	6 (100%)	2(33.3%)	4(66.6%)	0	6(100%)

Azithromycin 15mg	AZM	3 (50%)	3 (50%)	4(66.6%)	2(33.3%)	4(66.6%)	2(33.3%)
Aztreonam 30mg	ATM	2 (33.3%)	4 (66.6%)	5(83.3%)	1(16.6%)	5(83.3%)	1(16.6%)
Polymyxin 30mg	PB	6 (100%)	0	2(33.3%)	4(66.6%)	2(33.3%)	4(66.6%)
Vancomycin 30mg	VA	0	6 (100%)	0	6(100%)	0	6(100%)
Gentamicin 10mg	GEN	4(66.6%)	2 (33.3%)	5(83.3%)	1(16.6%)	6(100%)	0

Biofilm formation

Biofilm formation was assessed using the microtiter plate method. *S. aureus* isolates 1,2,3, and 6 formed strong biofilms, while isolates 2 and 4 formed moderate biofilms (Fig. 2A). All *P. aeruginosa* isolates formed strong biofilms (Fig. 2B), *K. pneumoniae* isolates 2 and 3 formed moderate biofilms, while isolates 1,4,5, and 6 formed strong biofilms (Fig. 2C).

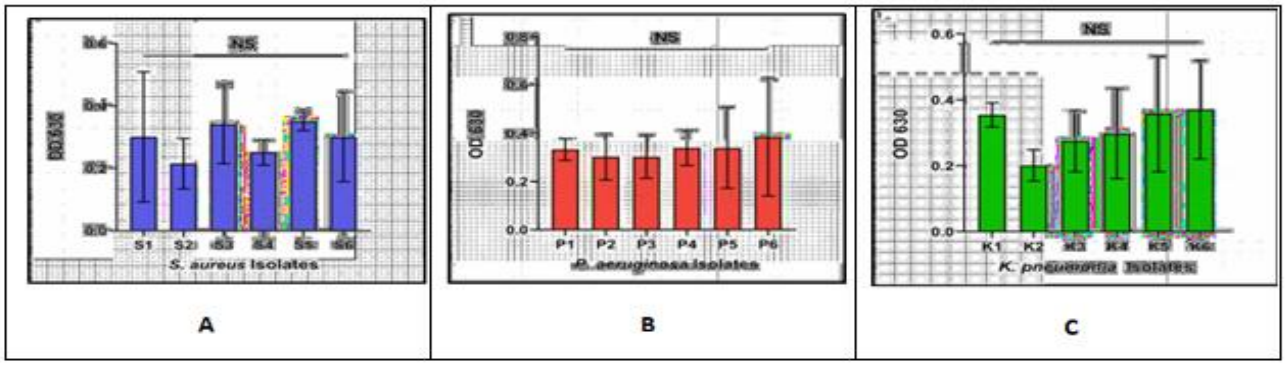

Figure 2. Biofilm formation by (A) *S. aureus* isolates, (B) *P. aeruginosa* isolates, and (C) *K. pneumoniae* isolates.

Bioactive composition analysis

The main bioactive components in the *Camellia sinensis* plant are listed in Table 4.

Table 3. Bioactive components in the *Camellia sinensis* plant

Components	Content (%)
Moisture	5.20 ± 0.5 % per w
Ash	7.20 ± 1.0 % per w
total polyphenolic	170 ± 34 mg GAE/g
Polysaccharides	14.4 ± 30 mg/g dry leaf

total flavonoid content	27.30±5.25 QE/g (quercetin equivalents per g dry extract)

(The data are presented as the means ± SDs)

Determination of MIC for Camellia sinensis extract

The MIC values of the *Camellia sinensis* extract are shown in Table 4. The MIC for *S. aureus* isolates S2 and S5 was 256 µg (sub-MIC 128 mg/ml), and for *K. pneumoniae* isolates K2 and K6, it was 128 mg/ml (sub-MIC 64 mg/ml). Variations in MIC values among bacterial species are attributed to factors such as cell wall structure, natural resistance mechanisms, and environmental conditions.

Table 4. MIC of alcoholic extract of *Camellia sinensis*

	***C. sinensis* alcoholic extract**		EGCG		Gentamicin	
Pathogenic bacteria	MBC (µg/L)	MIC (µg/L)	MBC (µg/L)	MIC (µg/L)	MBC (µg/L)	MIC (µg/L)
S2	512	256	128	128	64	16
S5	512	256	256	128	64	16
K2	512	128	384	256	128	64
K6	512	128	384	256	128	64

Effect of sub-MIC of alcoholic extract of *Camellia sinensis* on EMR gene expression in *S. aureus*
As Figure 3. Figure A shows the MIC values of the *Camellia sinensis* extract, amoxicillin, and azithromycin on EMR gene expression in *S. aureus*, indicating that gene expression was reduced when the treatments were used separately, but significantly increased after combination treatment with *C. sinensis* extract and AZM(expression) expression ≈ 0.097 (9.74% of control). Effect of sub-MIC of alcoholic extract of *Camellia sinensis* on pbp gene expression in *S. aureus*

The overall ANOVA indicates that there are differences in the Expression Fold Change across different treatment groups, where gene expression was significantly reduced after treating with *C. sinensis* extract and amoxicillin, whereas gene expression was at its peak when using amoxicillin only. The one-way ANOVA yielded an F-statistic of 4.7343 compared to the control, as shown in Figure 3.B.

Effect of sub-MIC of alcoholic extract of *Camellia sinensis* on MPH A gene expression in *K. pneumoniae*

Treating with the combination of the extract of *C. sinensis* and azithromycin gave the strongest effect on gene expression compared to the control, whereas using azithromycin only showed the

weakest effect on gene expression, as shown in (Fig. 3.C) .The ANOVA Results: F-statistic: 1.08 and p-value: 0.45

Effect of sub-MIC of alcoholic extract of *Camellia sinensis* on AMP gene expression in *K. pneumoniae*

C. sinensis extract showed the most effect on gene expression by reducing the gene expression compared to the control, as (Fig. 3.D) shows, where the strongest gene expression was by using the AZM alone. The ANOVA result indicates an F-statistic = 2.33 and a p-value = 0.16

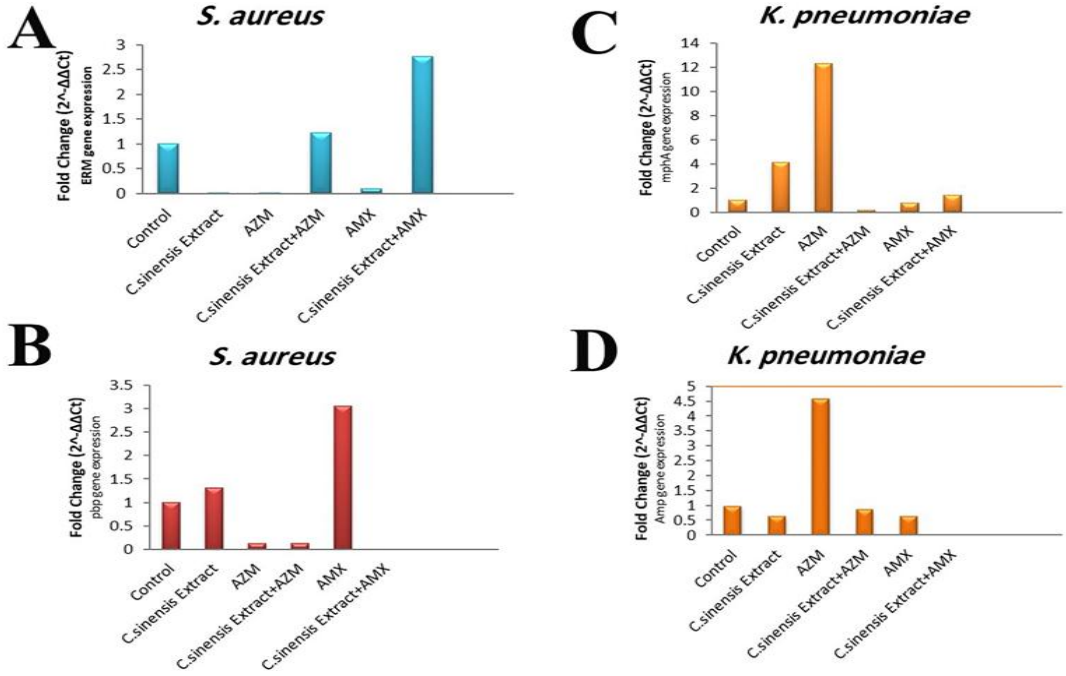

Figure 3A. *ERM* gene expression in *S. aureus; (*B) PBP gene expression in *S. aureus; (*C) *MPH A* gene expression in *K. pneumoniae*; (D) *AMP* gene expression in *K. pneumoniae*

Discussion

A variety of plants, herbs, and spices contain naturally occurring compounds that possess antimicrobial properties and serve as effective agents against clinically isolated bacteria. Bacterial infectious diseases are a major cause of high death and illness rates around the world. As a result, there is a growing interest in creating new antibacterial agents to treat infections caused by bacteria. This investigation assessed the in vitro antibacterial properties of methanol extracts from *C. sinensis* (Green tea) targeting pathogenic bacteria associated with wound infections. It highlighted, also, antimicrobial resistance, biofilm formation capability of multidrug-resistant bacteria isolated from clinical samples, and the effects of the *Camellia sinensis* extract on gene expression. The prevalence of bacteria in this study was aligned with the previous studies (Abed, Abdulmajeed, &

Najm, 2021; Eisaa, 2022; Mahmood, Luaibi, Al-Janabi, & Gaaz, 2024; Schmittgen, 2001). A study performed in Iraq reported that the prevalence of *Staphylococcus aureus* was 25%, that of Pseudomonas aeruginosa was 31%, and that of *Klebsiella pneumonia* was 30%, which differs from this study (Abed et al., 2021; Jasim, Alzubaidi, & Al-Rubaye, 2022). This variation among the isolates could refer to the conditions of sample collection or the time from the patient.

In this study, bacterial isolates demonstrated high resistance to various antibiotics. *K. pneumoniae* isolates exhibited 100% resistance to ciprofloxacin, amoxicillin, and vancomycin, consistent with (Khosravi et al., 2015). Resistance rates were 66.6% for aztreonam, 50% for azithromycin, and 33.3% for gentamicin, in agreement with (Khoramrooz et al., 2017). *S. aureus* isolates showed 100% resistance to vancomycin and 66.6% resistance to amoxicillin and polymyxin, consistent with. Resistance rates were 50% for ciprofloxacin and levofloxacin, 33.3% for azithromycin, and 16.6% for azetronam and gentamicin, differing from (Mahmmod & AlHadban, 2022). *P. aeruginosa* isolates showed 100% resistance to amoxicillin and vancomycin, 66.6% resistance to polymyxin, 33.3% resistance to azithromycin, and 16.6% resistance to azetronam and gentamicin, partially agreeing with (Al-Asady, Al-Saray, & Al-Araji, 2022) and disagreeing with (Mahmood et al., 2024) and also disagrees with a study performed in Karachi, Pakistan, that showed 72.22% of *K. pneumoniae* were resistant to ciprofloxacin (Ali et al., 2010) Another study in Kirkuk-Iraq showed that *P. aeruginosa* was 54% resistant to amoxicillin (Rashid Mahmood & Mansour Hussein, 2022). The prevalence of biofilm-producing bacteria showed that *K. pneumoniae* and *Staphylococcus aureus* were 35% and 16%, respectively.

Incorporation of epicatechin gallate (ECg) into the phospholipid bilayer of methicillin-resistant *Staphylococcus aureus* (MRSA) has been shown to partially disrupt the association of penicillin-binding protein 2a (PBP2a) with the PBP2/PBP2a and FtsZ/PBP2/PBP2a complexes. This disruption is indicative of a compromised replication apparatus, which may contribute to the enhanced susceptibility of MRSA to β-lactam antibiotics. The findings suggest that combining ECg, functioning as an antibiotic resistance-modifying agent, with β-lactam antibiotics previously ineffective against MRSA represents a novel and potentially effective therapeutic strategy for overcoming antibiotic resistance.

The increasing prevalence of multidrug-resistant bacteria necessitates alternative therapies. *C. sinensis* extract showed potent antimicrobial activity against the isolates. The downregulation of EMR, PBP, MPH, AND AMP genes suggests that *C. sinensis* may inhibit efflux pump, cell wall

synthesis, and β-lactamase production. Further research is needed to determine optimal dosage, safety, efficacy, and potential interaction with conventional antibiotics. To date, there is no evidence from peer-reviewed literature indicating that polyphenolic compounds found in green tea (specifically epigallocatechin gallate (EGCG) or epicatechin gallate (ECG))influence the transcriptional activity of *erm* genes, including *ermA*, *ermB*, and *ermC*, in *Staphylococcus aureus*. These genes are known to mediate resistance to macrolide-lincosamide-streptogramin B (MLSB) antibiotics through methylation of the 23S rRNA component of the bacterial ribosome. Furthermore, the AMPc and MPHA genes. However, Betts et al suggested that EGCG can disturb the cell membrane and interfere with the resistance mechanism through erm gene expression was not the primary focus (Kono K, 1994).

Conclusion

This study demonstrates that methanol extracts of *C. sinensis* exhibit antibacterial properties against clinically significant wound and burn pathogens, in addition to influencing relevant gene expression. This study enhances the existing knowledge regarding the potential health benefits associated with green tea. These findings may encourage additional investigation into the antimicrobial properties of commercially available green tea and other plant-based products, including black tea and coffee.

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