SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2026.v07i04.008

OPEN ACCESS

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

Antibacterial Effect of *Camellia sinensis* Extract on *Pseudomonas aeruginosa*

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Article History: | Received: 23.06.2026 | Accepted: 15.08.2026 | Published: 20.08.2026 |

Abstract: ***Objective***: The development of bacterial pathogens that are resistant to many drugs poses a major threat to global health care. Some of the bacteria are *Pseudomonas aeruginosa* that are considered very difficult opportunist because of their drug-resistance nature and ability to form biofilms. With this situation, the identification of new antimicrobial compounds from natural sources takes an important role. Therefore, in this study, the purpose was to find out the effect of *Camellia sinensis* extract on *Pseudomonas aeruginosa* bacteria. ***Methods of Work***: The study used ten isolates obtained from patients with different diseases. These included the tests for antibacterial activity using the agar well diffusion test, MIC test, and MBC test. Various concentrations of green tea extract were used, namely 25, 50, 75, and 100 mg/mL. Ciprofloxacin acted as positive control while distilled water was the negative control. ***Results***: The inhibition zone was between 8 to 17 mm. The highest inhibition zone was recorded for ciprofloxacin (22 mm). MIC ranged from 0.8–2 mg/mL, whereas MBC was within the range of 2–4 mg/mL**.** Statistical significance through one-way ANOVA analysis indicated extremely significant differences among the treatments ($p < 0.001$). Antibacterial activity can be explained by the presence of catechins and polyphenols found in green tea. This means that green tea extract could be a promising alternative for fighting bacteria that have developed resistance.

Keywords: *Pseudomonas aeruginosa*, *Camellia sinensis*, Antibacterial Activity.

Introduction

Pseudomonas aeruginosa is a Gram-negative bacteria that causes several infections such as respiratory infections, urinary tract infections, infections from burns, wound infections, and hospital infections [1]. This bacterium exhibits many virulence factors including toxins, enzymes, biofilm formation, and pigment production, among others, which play a critical role in its pathogenesis and resistance to antimicrobials [2].

There have been many cases involving the resistant nature of *Pseudomonas aeruginosa* to antimicrobial drugs. The bacteria develop resistance to antibiotics through the use of many processes such as efflux pump activity, low membrane permeability, enzymes production, and biofilm formation [3]. This makes biofilm formation a vital aspect of resisting antibiotics and host immune systems [4].

Because of the rise in antimicrobial resistance, plants have become popular scientifically because of their potential use as alternate sources of treatment. The bioactive components present in plant extracts include alkaloids, tannins, flavonoids, and polyphenols, among others, that act against microbes [5].

In the case of various medicinal herbs, Camellia sinensis, which is also known as green tea, is one of the most widely consumed beverages globally, and this herbal plant is rich in medicinal properties [6]. The antioxidants in green tea are present in components such as EGCG, ECG, and EC [7]. It is noted in several studies that the extract of green tea possesses antibacterial action against various bacteria like S. aureus, E. coli, and P. aeruginosa [8, 9]. Green tea extract possesses antibacterial action depending upon the presence of catechins which destroy the bacteria by destroying the membrane of bacteria, enzyme inhibition, metabolism inhibition, and preventing biofilm formation [10].

Also, some researches have shown that there is a synergy between green tea polyphenols and traditional antibiotics due to their antibacterial activity against antibiotic-resistant bacteria [11]. This study seeks to investigate the antibacterial activity of *Camellia sinensis* extract against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

1- Plant Material and Extraction

The green tea leaves (*Camellia sinensis*) were obtained from a local market. These leaves were rinsed using distilled water and then dried in open air. Dried leaves were grounded to fine powder with the help of an electric grinder.

Twenty grams of powder were taken in distilled water or 70% ethanol and allowed to stand overnight under constant stirring conditions [8]. The mixture was filtered by Whatman No.1 filter paper, and then the filtrate was concentrated with the help of a rotary evaporator.

2- Bacterial Isolates

The clinical strains of *Pseudomonas aeruginosa* were collected from the microbiology laboratory. In total, ten bacteria strains were tested in this experiment. Bacteria identification was done based on the colonial appearance, gram stain, pigment production, and biochemical testing following the guidelines of CLSI [12].

The bacteria were grown on nutrient agar medium at 37°C for 24 hours. The bacterial suspension was prepared to have 0.5 McFarland standard equivalent to 1.5×10^8 colony-forming unit/mL (CFU/mL). All of the experiments were done in triplicate.

3- Antibacterial Activity Assay

The antibacterial potential of the green tea extract was determined by means of the well diffusion technique [8]. Mueller Hinton Agar plates were first swabbed with standardized inoculum using cotton swabs. Wells (of diameter 6 mm) were aseptically made on the agar medium.

Different concentrations of green tea extract (25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) were then introduced to each well (100 µl per well). Ciprofloxacin antibiotic was used as a positive control, and sterilized distilled water as a negative control.

4- Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of Green Tea Extract against *Pseudomonas aeruginosa* was performed via broth dilution technique, according to CLSI standards [12].

Serial dilutions of the extract in range of 0.25 to 4 mg/mL in tubes containing nutrient broth were carried out. Subsequently, the tubes were inoculated with standardized suspension of the bacteria and incubated for 24 hours at 37°C.

5- Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was calculated after determining the minimum inhibitory concentration (MIC). The absence of bacterial growth was observed in the tubes and was sub-cultured in Mueller Hinton Agar plates and incubated for 24 hours at 37°C. The lowest concentration at which there was no bacterial growth was considered the bactericidal concentration.

6- Statistical Analysis

All tests were repeated three times, and results were recorded as Mean ± SD. Statistical significance of results was done using One-way ANOVA, and Tukey's post-hoc test was used to determine the difference between means.

The experiments for each test, that is, Agar well diffusion, MIC, and MBC tests, were performed in three replicates to increase consistency and accuracy. The recorded data were expressed in terms of mean ± standard deviation (mean ± SD).

RESULTS DISCUSSION

Results

1- Antibacterial Activity

The results demonstrated that *Camellia sinensis* extract exhibited concentration-dependent antibacterial activity against *Pseudomonas aeruginosa.* As Table 1 and Figure 2,3.

There was an increment of the size of the inhibition zone with increasing concentrations of the extracts. The maximum antibacterial activities were observed at 100 mg/ml for green tea extract, whereas Ciprofloxacin had the largest inhibition zone.

Table 1: Inhibition zones of green tea extract against *Pseudomonas aeruginosa* compared with ciprofloxacin and distilled water controls. Values represent mean inhibition zones (mm) ± SD (n = 3)

Treatment	Concentration (mg/mL)	Inhibition Zone (mm)
Green tea extract	25	8 ± 0.5
Green tea extract	50	11 ± 0.7
Green tea extract	75	14 ± 0.6
Green tea extract	100	17 ± 0.8
Ciprofloxacin (Positive control)	5µg	22 ± 0.9
Distilled water (Negative control)	—	0

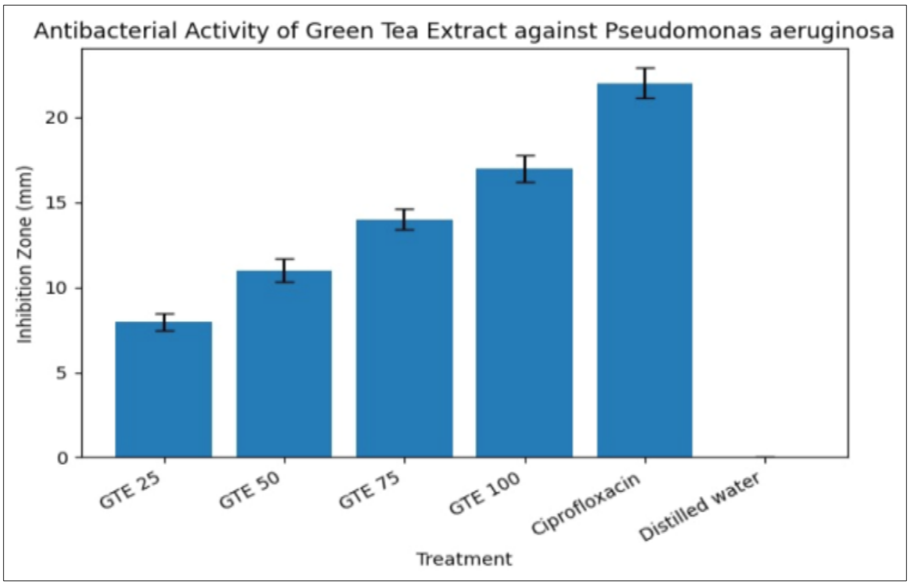

Figure 1: Antibacterial activity of different concentrations of green tea extract against *Pseudomonas aeruginosa*

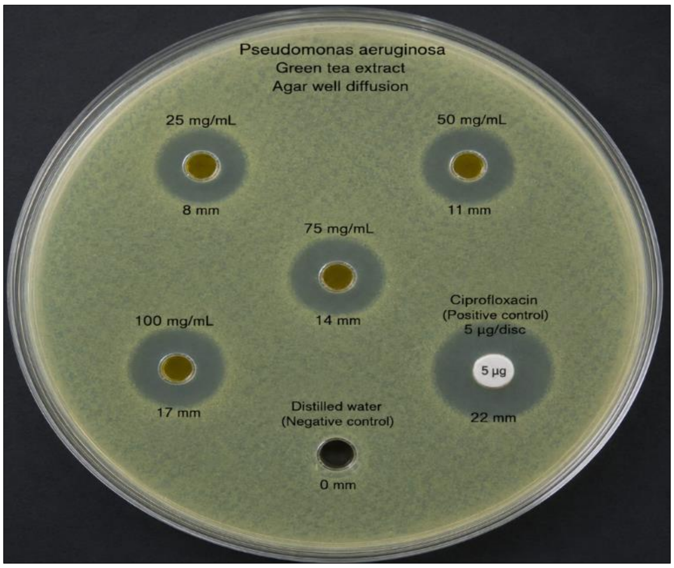

Figure 2: Antibacterial activity of different concentrations of green tea extract against *Pseudomonas aeruginosa* compared with ciprofloxacin and distilled water controls

2- Minimum Inhibitory Concentration (MIC)

The MIC and MBC of green tea extract against *Pseudomonas aeruginosa* was in the range of 0.8-2 mg/mL, while that of MBC was between 2-4 mg/mL. As Table 2. The obtained results indicated moderate antibacterial activity of the extract against the tested isolates.

Table 2: MIC and MBC values of green tea extract against *Pseudomonas aeruginosa* isolates

Isolate Number	MIC (mg/mL)	MBC (mg/mL)
1	0.8	2.0
2	1.0	2.0
3	1.0	2.0
4	1.2	2.5
5	1.2	2.5
6	1.5	3.0
7	1.5	3.0
8	1.8	3.5
9	2.0	4.0
10	2.0	4.0

DISCUSSION

In the current study, it was found that *Camellia sinensis* extract had substantial antibacterial activity against *Pseudomonas aeruginosa*. The higher the concentration of the extract used, the larger the inhibition zones were, thus confirming the dose-dependent antibacterial property of the extract.

The current observation of antibacterial activity may be explained by the catechin and polyphenolic substances in the extract, especially EGCG, which can alter the integrity of the bacterial cell membranes, making them more permeable [10]. The mentioned substances may also act by inhibiting bacterial enzymes and metabolic pathways [13].

The outcomes of the present experiment are similar to the findings from previous experiments which emphasize the antibiotic properties of *Camellia sinensis* against pathogenic microorganisms like *Pseudomonas aeruginosa*. As mentioned by Radji *et al*., [8], green tea extracts exhibit excellent inhibitory effects on Gram-negative bacteria. This process of inhibition is proven to be related to the concentration of green tea extract, which is a reason for the dose-related antimicrobial effects observed in this study. Moreover, it was reported by Hosseini Jazani *et al.,* [9], that the presence of phenols in green tea is vital for membrane disruption of bacteria [14].

Further, Zhang *et al*., [10], proved the activity of epigallocatechin gallate (EGCG), an ingredient of green tea, against the formation of the biofilm in *Ps. aeruginosa*, thus increasing the sensitivity of bacteria to antibiotics. The above-mentioned proves the results of the research on antibacterial activity of the extracts, but they have been less efficient than ciprofloxacin. Such discrepancies may be well correlated with the results by Cushnie and Lamb [15]. These imply that natural products have shown moderate efficacy as compared to conventional antibiotics but can be used as an alternate anti-microbial agent. Anyway, the present findings do not conflict with the earlier literature rather support the fact that Camellia sinensis is a good natural source of antibacterial agents [16, 17].

The findings of the present study are in line with previous studies, which demonstrated the high antibacterial effect of the green tea extract on the multidrug-resistant Pseudomonas aeruginosa strains [8, 9]. In addition, it has been indicated by Reygaert [7], that green tea catechins exert broad-spectrum antibacterial effect via disrupting membranes and inhibiting biofilm formation. The MIC values found in the current experiment (0.8–2 mg/mL) represent moderate antibacterial effects, in accordance with other published data regarding the antimicrobial susceptibility of Gram-negative bacteria to green tea polyphenolic compounds [9-18]. Some MIC values being lower can denote greater sensitivity of these isolates to catechin substances.

MBC values found were in the range of 2–4 mg/mL; thus, a higher concentration was needed for the complete eradication of bacteria rather than only for their growth inhibition. Such differences between MIC and MBC can be due to the protective role of the outer membrane in *Pseudomonas aeruginosa*, making the entry of antibacterial substances more difficult [13-19].

In addition, the polyphenols present in the tea have been found to be able to inhibit the formation of biofilm, a very crucial factor in terms of virulence in case of *Pseudomonas aeruginosa* [4-20].

While the antibacterial activity in the extract was found to be less effective than Ciprofloxacin, it still shows that there is a lot of potential in green tea extract. The statistical difference among different treatments has validated the result obtained.

CONCLUSION

It was revealed that the *Camellia sinensis* extract is endowed with a pronounced antibacterial effect against *Pseudomonas aeruginosa* bacteria. There was an enhancement of antibacterial effect depending on the concentration of the extract, which was maximal in case of 100 mg/ml. The MIC of the extract was between 0.8-2 mg/ml, and MBC values were between 2-4 mg/ml, indicating moderate antibacterial and bactericidal properties against the selected isolates. Although ciprofloxacin was found to have a more pronounced antibacterial effect, the extract still showed promising inhibitory properties towards *Pseudomonas aeruginosa* isolates. Therefore, the results obtained from the experiment can be considered as evidence of the possible use of the green tea extract for treatment of resistant bacteria strains.

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