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Antimicrobial Properties of *Nelumbo nucifera* from Unisel Lake

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Abstract

The increasing prevalence of antibiotic-resistant bacteria poses a significant global public health challenge and necessitates the exploration of alternative antimicrobial agents from natural sources. Although the antimicrobial properties of *Nelumbo nucifera* have been reported previously, comparative evaluations of different plant parts, particularly leaves, flowers, and stems, under standardized experimental conditions remain limited. Therefore, this study aimed to compare the antimicrobial activities of *N. nucifera* leaves, flowers, and stems against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative), two common foodborne pathogens. Ethanolic extracts of the selected plant parts were prepared and evaluated using the disc diffusion method, followed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The results demonstrated that all three plant parts exhibited antimicrobial activity against *S. aureus*, with leaf extracts producing the largest zone of inhibition (23.3 mm), followed by flower extracts (17.7 mm) and stem extracts (16.0 mm) at a concentration of 500 mg/mL. In contrast, none of the extracts showed inhibitory activity against *E. coli*. MIC and MBC analyses further confirmed that a concentration of 500 mg/mL was required to inhibit and eliminate the growth of *S. aureus*. The observed differences in antimicrobial susceptibility between the two bacterial species may be attributed to variations in cell wall structure, particularly the presence of an outer lipopolysaccharide membrane in Gram-negative bacteria, which can limit the penetration of plant-derived bioactive compounds. These findings highlight the potential of *N. nucifera*, particularly its leaves, as a promising source of plant-based antimicrobial agents against Gram-positive bacteria. This study contributes to the growing body of knowledge on medicinal plants as sustainable alternatives for combating antibiotic resistance. Further investigations involving optimized extraction techniques, phytochemical characterization, toxicity assessment, and broader-spectrum antimicrobial testing are recommended to support the development of *N. nucifera*-derived antimicrobial products.

Keywords: Antibiotic resistance; Antimicrobial activity; *Escherichia coli*; *Nelumbo nucifera*; *Staphylococcus aureus*

INTRODUCTION

The emergence and rapid dissemination of antimicrobial resistance have become one of the most pressing global public health challenges of the twenty-first century. The excessive and inappropriate use of antibiotics in clinical, agricultural, and veterinary settings has accelerated the evolution of resistant bacterial strains, resulting in reduced treatment efficacy, prolonged hospitalisation, increased healthcare costs, and elevated mortality rates (Muteeb et al., 2023; Alara and Alara, 2024). Pathogens such as *Staphylococcus aureus* and *Escherichia coli* are among the most common causative agents of foodborne illnesses and opportunistic infections, with numerous strains demonstrating resistance to multiple classes of antibiotics (Grudlewska-Buda et al., 2023). Consequently, there is an urgent need to identify novel, safe, and effective antimicrobial agents capable of combating resistant microorganisms and reducing dependence on conventional antibiotics.

Natural products derived from plants have attracted considerable attention as alternative sources of antimicrobial compounds. Historically, medicinal plants have served as the foundation of traditional healthcare systems and have contributed significantly to the discovery of modern pharmaceuticals (Izah et al., 2024). Plant-derived antimicrobial agents offer several advantages over synthetic antibiotics, including lower toxicity, greater biocompatibility, environmental sustainability, affordability, and the presence of diverse bioactive compounds that may act synergistically against pathogens (Abdallah et al., 2023; El-Saadony et al., 2025). Unlike synthetic antimicrobial agents that often target specific cellular pathways, phytochemicals frequently exhibit multiple mechanisms of action, reducing the likelihood of resistance development (Jadimurthy et al., 2023). Consequently, medicinal plants represent a valuable reservoir for the discovery of novel antimicrobial compounds.

The antimicrobial potential of medicinal plants has been widely documented in the literature. Numerous plant species, including *Azadirachta indica* (Neem), *Moringa oleifera*, *Curcuma longa* (Turmeric), *Allium sativum* (Garlic), and *Camellia sinensis* (Green Tea), have demonstrated significant inhibitory effects against Gram-positive and Gram-negative bacteria (Chew et al., 2022; Naz et al., 2024; Sarkar et al., 2024). Several plant-derived compounds have successfully progressed into pharmaceutical and healthcare applications. For example, berberine, allicin, thymol, and eugenol have exhibited broad-spectrum antimicrobial activities and have been incorporated into various medicinal and therapeutic formulations (Ali and Mishra, 2024). These success stories highlight the immense potential of medicinal plants as sustainable sources of bioactive compounds for antimicrobial drug discovery.

Among the numerous medicinal plants investigated, *Nelumbo nucifera*, commonly known as the sacred lotus, has emerged as a particularly promising candidate due to its rich phytochemical profile and extensive ethnomedicinal applications. Widely distributed throughout Asia, *N. nucifera* has been traditionally used for the treatment of diarrhoea, inflammation, fever, skin disorders, and microbial infections (Sahu et al., 2024). Different plant parts, including leaves, flowers, seeds, rhizomes, and stems, contain a diverse array of bioactive compounds such as flavonoids, alkaloids, tannins, phenolic acids, and glycosides, many of which possess potent antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Nguyen et al., 2024; Joshi et al., 2026). Compared with many other medicinal plants, *N. nucifera* offers the advantage of containing high concentrations of multifunctional phytochemicals across multiple plant tissues, thereby maximising its potential as a renewable source of therapeutic compounds (Bishayee et al., 2022). Previous studies have reported that ethanol extracts of *N. nucifera* leaves and flowers exhibited substantial antibacterial activity against *S. aureus*, producing inhibition zones of approximately 25 mm and 20 mm, respectively (Niranjan et al., 2023). These findings suggest that *N. nucifera* possesses strong antimicrobial efficacy comparable to or exceeding that of several commonly studied medicinal plants.

Despite the growing body of literature on *N. nucifera*, significant research gaps remain. Most existing studies have focused primarily on individual plant parts, particularly leaves, flowers, seeds, or rhizomes, while the antimicrobial potential of stems has received limited attention. Furthermore, variations in extraction methods, solvents, bacterial strains, and experimental conditions across studies make direct comparisons between plant parts difficult. To date, systematic side-by-side evaluations of leaves, flowers, and stems under identical extraction and antimicrobial testing conditions remain scarce. This lack of comparative data limits the identification of the most effective plant part for antimicrobial applications and hinders the optimal utilisation of *N. nucifera* as a natural antimicrobial resource.

The novelty of the present study lies in the inclusion of *N. nucifera* stem extracts, which remain relatively underexplored in antimicrobial research, and in the direct comparative evaluation of leaves, flowers, and stems using standardised extraction and testing protocols within a single experimental framework. By eliminating methodological variations, this study provides a more reliable assessment of the relative antimicrobial efficacy of different plant parts.

Therefore, this study aims to address the existing research gaps by systematically evaluating and comparing the antimicrobial activities of *N. nucifera* leaf, flower, and stem extracts against *S. aureus* and *E. coli*. Specifically, the objectives of this study are to (i) determine the antimicrobial activity of extracts obtained from different parts of *N. nucifera*, (ii) compare their relative effectiveness against selected Gram-positive and Gram-negative bacterial pathogens, and (iii) identify the plant part with the greatest potential for future development as a natural antimicrobial agent. The findings are expected to contribute to the growing body of knowledge on plant-based antimicrobials and support the development of sustainable alternatives to conventional antibiotics in addressing the global challenge of antimicrobial resistance.

METHODOLOGY

Plant Material Collection and Extraction

Fresh *N. nucifera* plant was collected from the UNISEL lake. Then, it was dried in air blast oven at 60 °C until constant weight for five days and ground into powder using a blender. Ethanolic extraction was performed by soaking the 16 g plant powder in 400 mL of 95% ethanol for three days, followed by filtration and evaporation using an air-blast oven at 60 °C. Ethanol was selected as the solvent due to its high efficiency in extracting a broad range of polar phytochemicals such as flavonoids, alkaloids, and phenolic compounds, and its compatibility with antimicrobial assays.

Disc Diffusion Method

The disc diffusion method was performed to assess antimicrobial activity. Mueller-Hinton agar (MHA) plates were used as the growth medium. Sterile discs were impregnated with 40 µL of 500 mg/mL *N. nucifera* extract; positive control (Ciprofloxacin, CIP 5) and negative control (20% DMSO) controls were included. Bacterial inoculum was standardized with an absorbance of 0.5 at OD_{600} using a spectrophotometer before swabbing onto the agar surface. After incubation at 37 °C for 24 hours, zones of inhibition diameters were measured in millimetres using a ruler. The experiment was conducted in triplicate to ensure consistency.

MIC and MBC Determination

MIC and MBC were determined using the broth microdilution technique. A 96-well microtiter plate was prepared with serial two-fold dilutions of *N. nucifera* extracts ranging from 500 mg/mL to 3.9 mg/mL in 100 µL of Mueller-Hinton broth (MHB). Wells were inoculated with 100 µL of standardized bacterial suspensions (0.5 McFarland standard). MIC was defined as the lowest concentration without visible growth after 24 hours of incubation. For MBC, 100 µL from first four wells (500 mg/mL to 62.5 mg/mL) showing no visible growth were subcultured onto Mueller-Hinton agar plates, and the lowest concentration with no bacterial growth was recorded as the MBC.

Statistical Analysis

The experimental data were analysed using one-way ANOVA in IBM SPSS (Statistical Package for the Social Sciences) and Post-hoc with Duncan tests. The value of $p < 0.05$ was considered statistically significant. The experiment was conducted in triplicate, and the result is reported as the mean ± SD (standard deviation).

RESULTS AND DISCUSSION

All *N. nucifera* extracts exhibited antimicrobial activity against *S. aureus*. The ethanol extract of leaves showed the largest inhibition zone (23.3 ± 0.58 mm), followed by flowers (17.7 ± 0.58 mm) and stems (16 ± 0 mm). No inhibition was observed against *E. coli*. Table 1 demonstrates that leaves extract exhibited significantly higher antimicrobial activity compared to flowers and stems extracts ($p < 0.05$), indicating variation in antimicrobial efficacy among different plant parts.

Table 1 Antimicrobial activity of *Nelumbo nucifera* extracts from various parts, leaves, flowers and stems

	Zone of inhibition diameter in mm				
Bacteria	**500 mg/mL of leaves extract**	**500 mg/mL of flowers extract**	**500 mg/mL of stems extract**	**Positive control (Ciprofloxacin)**	**Negative control (DMSO)**
Staphylococcus aureus	23.3 ± 0.58^{c}	17.7 ± 0.58^{b}	16.0 ± 0^{a}	33.7 ± 0.58^{d}	-
Escherichia coli	-	-	-	33.7±0.58	-

Results are the means of values ± standard deviation, DMSO = dimethyl sulphoxide, - = no inhibition zone, the values in the different columns with the different alphabet differ significantly at $p < 0.05$.

This indicates that the antimicrobial efficacy of *N. nucifera* varies significantly depending on the plant part, with leaves demonstrating the highest activity. Previous studies reported ethanol leaves extract against *S. aureus* as 25 mm ± 10 at 40 mg/mL (Niranjan et al., 2023) and flowers as 20 mm at 300 µg/mL (Rajput et al., 2022). The findings of this study are generally consistent with previous studies reporting antimicrobial activity of *N. nucifera* against Gram-positive bacteria. Although the inhibition zones observed in this study are comparable, the higher concentration required (500 mg/mL) suggests a relatively lower potency of the extracts, which may be influenced by extraction efficiency, solvent concentration, or variation in phytochemical composition. No previous studies were found reporting ethanol stem extracts against *S. aureus* and *E. coli*, highlighting the novelty of including stem extracts in this comparative study. The presence of polyphenols, flavonoids, and alkaloids likely contributes to the antimicrobial activity by disrupting bacterial membranes and inhibiting protein synthesis (Bishayee et al., 2022). The stronger activity observed in leaves extracts may be attributed to a higher concentration or synergistic interaction of these bioactive compounds compared to other plant parts. The lack of activity against *E. coli* suggests limitations due to the protective lipopolysaccharide layer in Gram-negative bacteria (Breijyeh et al., 2020). Additionally, intrinsic resistance mechanisms such as efflux pumps may further reduce susceptibility to plant-derived compounds. Statistically, the results differed significantly across plant parts ($p < 0.05$) (Table 1), confirming that the variation in antimicrobial activity is not due to random variation but reflects true differences in extract efficacy.

Figures 1 to 3 provide visual support for the antimicrobial susceptibility testing, MIC, and MBC findings observed in this study. MIC and MBC for *S. aureus* were both 500 mg/mL for all plant parts, with no detectable activity against *E. coli*.

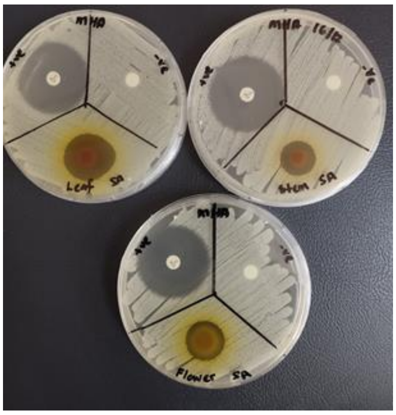

Figure 1 Antimicrobial susceptibility testing of leaves, flowers and stems Extract, positive control (CIP 5), negative control (DMSO) on *Staphylococcus aureus*

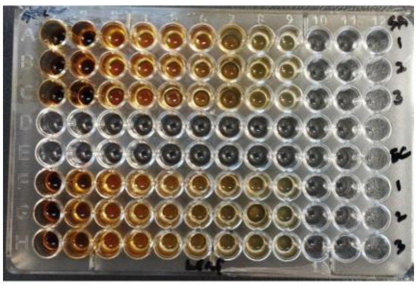

Figure 2 Minimum inhibition concentration of *Nelumbo nucifera* leaves extract against *S.aureus* and *E.coli*

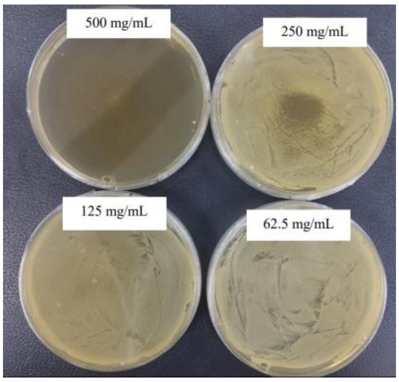

Figure 3 Minimum bactericidal concentration of *Nelumbo nucifera* leaves extract against *S.aureus*

Tables 2 and 3 indicate that all extracts required relatively high concentrations to inhibit and kill *S. aureus,* suggesting moderate antimicrobial potency of the crude extracts. Previous studies reported the MIC and MBC of flower extract against *S. aureus* as 100 mg/mL (Pengon et al., 2020), which is lower than in this study. This discrepancy may be due to differences in experimental conditions, extraction methods, or plant origin, which are known to influence phytochemical yield and antimicrobial activity.

Table 2 Minimum inhibitory concentration (mg/ml) of *Nelumbo nucifera* extracts from various parts, leaves, flowers and stems

	Minimum inhibitory concentration (mg/mL) *Nelumbo nucifera* extract		
Bacteria	**500 mg/mL of leaves extract**	**500 mg/mL of flowers extract**	**500 mg/mL of stems extract**
Staphylococcus aureus	500 ± 0	500 ± 0	500 ± 0
Escherichia coli	ND	ND	ND

Results are the means of values ± standard deviation, ND=not detected

Table 3 Minimum inhibitory concentration (mg/ml) of *Nelumbo nucifera* extracts from various parts, leaves, flowers and stems

	Minimum bactericidal concentration (mg/mL) *Nelumbo nucifera* extract		
Bacteria	**500 mg/mL of leaves extract**	**500 mg/mL of flowers extract**	**500 mg/mL of stems extract**
Staphylococcus aureus	500 ± 0	500 ± 0	500 ± 0
Escherichia coli	ND	ND	ND

Results are the means of values ± standard deviation, ND=not detected

The use of only ethanol as a solvent may have excluded bioactive compounds soluble in other solvents, and the drying temperature of 60 °C could have degraded thermolabile compounds. Expanding solvent types and optimizing drying conditions could improve both yield and antimicrobial activity. The lack of activity against *E.coli* suggests the need for exploring combinations with other plant extracts or chemical enhancers to combat resistance in gram-negative bacteria. Further phytochemical analysis is necessary to identify the bioactive compounds responsible for the observed effects.

CONCLUSION

This study demonstrates the potential of *N. nucifera* as a promising source of plant-based antimicrobial agents, particularly against *S. aureus*. The findings indicate that different plant parts exhibit varying levels of antibacterial activity, with leaves extracts showing the greatest efficacy. At 500 mg/mL, leaves extract exhibited the largest inhibition zone against *S. aureus* (23.3 ± 0.58 mm), followed by flowers (17.7 ± 0.58 mm) and stems (16.0 ± 0 mm), whereas no inhibitory activity was observed against *E. coli*. MIC and MBC values of 500 mg/mL were recorded for all extracts against *S. aureus*, with no detectable activity against *E. coli*. The selective activity observed against Gram-positive bacteria highlights the importance of bacterial cell structure in determining susceptibility to plant-derived compounds. These findings support the growing interest in medicinal plants as sustainable alternatives to conventional antibiotics in combating antimicrobial resistance. The comparative evaluation of leaves, flowers, and stems also highlights the broader antimicrobial potential of different *N. nucifera* plant parts under standardized conditions. The study supports the potential application of *N. nucifera* in the development of novel antimicrobial agents, particularly for targeting Gram-positive pathogens. Further research focusing on optimized extraction techniques, phytochemical characterization, and broader antimicrobial screening is necessary to enhance its efficacy and expand its therapeutic potential.

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