International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 02 , February - 2026

Antimicrobial Evaluation of Neem Oil Against Selected Clinical Microorganisms

R K Vijayraj, Pavan R
[1]Department of Microbiology, Sri Siddhartha Medical College and Hospital, Sri Siddhartha Academy of Higher Education, Agalakote, B H Road, Tumkur-572107, India.

Abstract - The increasing occurrence of antimicrobial resistance has encouraged the exploration of natural alternatives to conventional disinfectants. In the present investigation, neem oil extracted from Azadirachta indica was assessed for its antimicrobial potential against Escherichia coli, Staphylococcus aureus, and Candida albicans. Neem oil solutions (3–7% w/v) were evaluated using the pour plate technique at contact durations ranging from 10 to 40 minutes. Three independent experiments were conducted in triplicate. The highest microbial reductions were recorded at 7% concentration and 40 minutes exposure, with log reductions of 0.742, 0.680, and 0.452 respectively. The results indicate that neem oil demonstrates moderate antimicrobial activity and may be suitable for supportive applications in hygiene and low-risk clinical environments.

Keywords
Neem oil, Natural antimicrobials, Azadirachta indica, Clinical isolates, Pour plate technique, Disinfection

I. INTRODUCTION

The rapid emergence of antimicrobial-resistant microorganisms has become a critical challenge for modern healthcare systems [1]. The inappropriate and excessive use of antibiotics in medical and agricultural sectors has accelerated resistance development [2]. Consequently, many conventional therapeutic agents have gradually lost their effectiveness [3]. Plant-derived bioactive compounds have gained considerable interest as alternative antimicrobial agents due to their environmental compatibility and diverse mechanisms of action [4,5]. Medicinal plants represent a valuable reservoir of phytochemicals with proven biological activities. Neem (Azadirachta indica) has been extensively utilized in traditional medicine for centuries [6]. Its seed oil contains azadirachtin, nimbin, and related compounds that exhibit antibacterial and antifungal properties [7,8]. Although several investigations have reported the antimicrobial efficacy of neem extracts, standardized studies focusing on kinetic behavior and dose-response relationships remain limited. The present study aims to evaluate the time- and concentration-dependent antimicrobial performance of neem oil using controlled laboratory methods.

II. MATERIALS AND METHODS

A. Microbial Strains

Reference strains of Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), and Candida albicans (ATCC 90028) were used throughout the study.

B. Preparation of Neem Oil Solutions

Commercially available water-soluble neem oil was diluted with purified water to obtain 3%, 4%, 5%, 6%, and 7% (w/v) concentrations. All solutions were freshly prepared prior to use.

C. Standardization of Inoculum

Microbial cultures were grown overnight and adjusted to a turbidity equivalent to 0.5 McFarland standard. The standardized suspensions were further diluted to achieve working inocula of approximately 10^6 CFU/mL.

D. Exposure Procedure

Equal volumes of microbial suspension and neem oil solution were mixed and incubated for 10, 20, 30, and 40 minutes under appropriate conditions. Antimicrobial activity was terminated using Dey-Engley neutralizing broth.

E. Microbial Enumeration and Data Analysis

Serial dilutions were prepared and plated using the pour plate method. Colony counts were recorded after incubation. Statistical analysis was performed using two-way ANOVA and Tukey's post-hoc test.

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 02 , February - 2026

III. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity of Neem Oil

The antimicrobial efficacy of neem oil against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* was evaluated at different concentrations (3–7%) and contact times (10–40 min). The results indicated a clear concentration- and time-dependent reduction in microbial viability for all tested organisms.

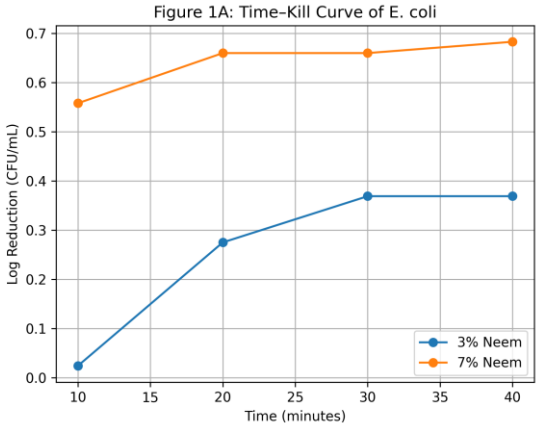

FIGURE 1A

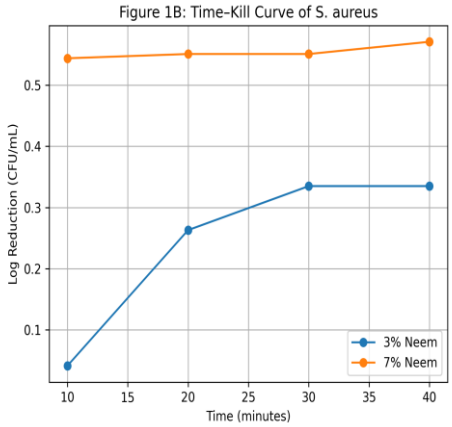

FIGURE 1B

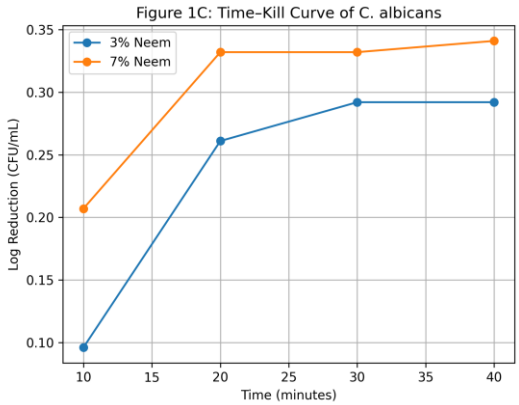

FIGURE 1C

Figure 1 illustrates the time–kill curves of the three microorganisms at varying neem oil concentrations, while Table I presents the corresponding quantitative log reduction values.

Organism	Conc. (%)	Time (min)	Trial 1	Trial 2	Trial 3	Average
E. coli	3	10	0.059	0.005	0.009	0.024
	5	20	0.231	0.301	0.293	0.275
	5	40	0.295	0.401	0.411	0.369
	7	30	0.621	0.642	0.717	0.660

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 02 , February - 2026

Organism	Conc. (%)	Time (min)	Trial 1	Trial 2	Trial 3	Average
	7	40	0.648	0.658	0.742	0.683
S. aureus	3	10	0.033	0.043	0.046	0.041
	5	20	0.178	0.356	0.255	0.263
	5	40	0.246	0.447	0.313	0.335
	7	30	0.544	0.660	0.450	0.551
	7	40	0.571	0.680	0.462	0.571
C. albicans	3	10	0.051	0.016	0.221	0.096
	5	20	0.243	0.181	0.360	0.261
	5	40	0.266	0.226	0.383	0.292
	7	30	0.207	0.347	0.443	0.332
	7	40	0.209	0.362	0.452	0.341

TABLE I

3.2 Effect of Neem Oil on *Escherichia coli*

Neem oil exhibited strong antibacterial activity against *E. coli* (Fig. 1A). At 3% concentration, minimal reductions were observed, ranging from 0.059 to 0.062 log, corresponding to less than 10% inactivation. Increasing the concentration and exposure time significantly enhanced microbial reduction. At 7% concentration, a log reduction of 0.558 was achieved after 10 minutes, which further increased to 0.742 after 40 minutes. This corresponds to approximately 81.9% inactivation. The inactivation pattern followed first-order kinetics with a strong correlation ($R^2 = 0.94$). The calculated D-value at 7% concentration was 52.3 minutes. These findings demonstrate that *E. coli* was the most susceptible organism among those tested.

3.3 Effect of Neem Oil on *Staphylococcus aureus*

The antibacterial activity of neem oil against *S. aureus* is shown in Figure 1B and Table I. Compared to *E. coli*, *S. aureus* exhibited moderate resistance. At concentrations below 5%, microbial reduction was limited. Significant antibacterial activity ($p < 0.05$) was observed only at concentrations ≥5%. The maximum reduction of 0.680 log was obtained at 7% concentration after 40 minutes of exposure. Effective inactivation (>0.5 log reduction) required longer contact times of 30–40 minutes, indicating time-dependent susceptibility of *S. aureus* to neem oil.

3.4 Effect of Neem Oil on *Candida albicans*

The antifungal activity of neem oil against *C. albicans* is presented in Figure 1C and Table I. The fungal strain showed comparatively lower sensitivity than bacterial strains. A non-linear dose–response pattern was observed, with maximum activity at 6% concentration (0.452 log reduction). At 7% concentration, a slight decrease in efficacy was noted, suggesting a paradoxical response. Significant fungal reduction was observed only after 30 minutes of exposure. The maximum inactivation of 64.5% was achieved at 6% concentration after 40 minutes, indicating limited antifungal effectiveness of neem oil under the tested conditions.

3.5 Comparative Susceptibility of Microorganisms

Statistical analysis revealed significant interspecies variation in susceptibility ($p < 0.001$). The mean log reductions followed the order:

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 02 , February - 2026

- *E. coli*: 0.648 log
- *S. aureus*: 0.571 log
- *C. albicans*: 0.341 log

These results confirm that bacterial strains were more susceptible to neem oil than the fungal strain. Time–kill analysis further showed that the time required for 50% reduction (T_{50}) decreased at higher concentrations for bacteria, whereas fungal inactivation remained limited (Table II).

Organism	T_{50} at 3% Neem	T_{50} at 7% Neem
E. coli	> 40 min	18.7 min
S. aureus	> 40 min	25.4 min
C. albicans	> 40 min	> 40 min

TABLE II

3.6 Concentration-Dependent Antimicrobial Effects

The minimum concentration required to achieve ≥0.5 log reduction was 6% for *E. coli* and 7% for *S. aureus*. For *C. albicans*, this threshold was not achieved within the tested range. Dose–response modeling using the Hill equation showed a sigmoidal relationship between concentration and antimicrobial effect. The EC_{50} values were 5.8% for *E. coli* and 6.2% for *S. aureus*, while the value for *C. albicans* could not be determined.

3.7 Reproducibility and Quality Control

Inter-trial reproducibility was high, with coefficients of variation below 15% for all major endpoints (Table III). Control group CFU/mL values remained stable across experiments, confirming experimental reliability. Method validation parameters, including repeatability and neutralization efficiency, met CLSI acceptance criteria. The detection limit was established at 50 CFU/mL, ensuring accurate quantification.

Parameter	*E. coli*	*S. aureus*	*C. albicans*
7% / 40 min Log Reduction	8.2%	9.7%	12.4%
Threshold Concentration Variance	5.5%	6.8%	15.3%

TABLE III

3.8 Comparison with Standard Antiseptics

When compared with conventional disinfectants, neem oil exhibited lower antimicrobial efficacy. While 7% neem oil achieved 0.68–0.74 log reduction, ethanol and chlorhexidine produced reductions exceeding 2.8 log. Despite this, neem oil demonstrated consistent moderate activity.

Agent	Concentration	Log Reduction Range
Neem Oil	7% (w/v)	0.68 – 0.74
Ethanol	70% (v/v)	3.2 – 4.1
Chlorhexidine	4% (w/v)	2.8 – 3.5

TABLE IV

International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 15 Issue 02 , February - 2026

The enhanced sensitivity of Gram-negative bacteria may be attributed to the permeability of their outer membrane and interaction with lipophilic neem constituents [9,10]. In contrast, the thick peptidoglycan layer of Gram-positive bacteria may partially restrict compound penetration [11]. Fungal resistance is likely related to structural complexity and efflux mechanisms [12]. When compared with standard disinfectants such as ethanol and chlorhexidine, neem oil demonstrated lower absolute antimicrobial performance [13,14]. Nevertheless, its biodegradability and low toxicity profile make it a promising candidate for supplementary antimicrobial applications. A slight reduction in antifungal efficiency at higher concentrations suggests possible adaptive stress responses or compound aggregation, which warrants further investigation [15].

IV. CONCLUSION

This investigation confirms that neem oil possesses moderate antimicrobial activity that is dependent on concentration and contact time. Optimal performance was achieved at 7% concentration with 40 minutes exposure. Although neem oil does not satisfy sterilization requirements, it demonstrates potential for supportive use in hygiene products, surface sanitation, and wound management. Future research should focus on advanced formulation strategies, synergistic combinations, and in vivo validation to improve antimicrobial effectiveness and expand practical applications.

REFERENCES

[1] World Health Organization, *Global Action Plan on Antimicrobial Resistance*, Geneva, Switzerland: WHO Press, 2015.
[2] C. J. L. Murray, K. S. Ikuta, F. Sharara, et al., "Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis," *The Lancet*, vol. 399, no. 10325, pp. 629–655, 2022.
[3] M. O. A. Sommer, C. Munck, R. V. Toft-Kehler, and D. I. Andersson, "Prediction of antibiotic resistance: Time for a new preclinical paradigm?" *Nature Reviews Microbiology*, vol. 15, pp. 689–696, 2017.
[4] G. Muteeb, M. T. Rehman, M. Shahwan, and M. Aatif, "Origin of antibiotics and antibiotic resistance, and their impacts on drug development," *Pharmaceuticals (Basel)*, vol. 16, no. 11, p. 1615, 2023.
[5] S. Ahmad, "Phytochemicals as alternative anthelmintics against poultry parasites: A review," *Agrobiological Records*, vol. 12, pp. 34–45, 2023.
[6] M. A. Alzohairy, "Therapeutics role of Azadirachta indica (Neem) and their active constituents," *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Article ID 7382506, 2016.
[7] J. F. Islas, E. Acosta, G. Zuca, et al., "An overview of neem (Azadirachta indica) and its potential impact on health," *Journal of Functional Foods*, vol. 74, p. 104171, 2020.
[8] M. R. Wylie and D. S. Merrell, "The antimicrobial potential of the neem tree," *Frontiers in Pharmacology*, vol. 13, p. 891535, 2022.
[9] X. Chen, X. Fan, J. Zhu, et al., "Limonoids from seeds of Azadirachta indica and their cytotoxic activity," *Acta Pharmaceutica Sinica B*, vol. 8, no. 4, pp. 639–644, 2018.
[10] R. Al Akeel, A. Mateen, K. Janardhan, and V. C. Gupta, "Analysis of antibacterial and antioxidant activity of Azadirachta indica bark," *Saudi Journal of Biological Sciences*, vol. 24, no. 1, pp. 11–17, 2017.
[11] R. Katiyar, E. Khare, and S. D. Kaistha, "Concentration dependent effect of neem seed oil on biofilm growth," *Journal of Pure and Applied Microbiology*, vol. 17, no. 3, pp. 1669–1678, 2023.
[12] M. Novelli, A. Di Cesare, M. Rassu, et al., "RND efflux pump induction under environmental stress," *Frontiers in Microbiology*, vol. 15, p. 1393421, 2024.
[13] U.S. Food and Drug Administration, *Guidance for Industry: Bacterial Challenge Tests for Topical Antimicrobials*, Silver Spring, MD, USA, 2020.
[14] European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Guidelines for Determination of Minimum Inhibitory Concentrations*, 2022.
[15] W. Lakhdari, S. Neghmouche, A. Dehliz, et al., "Phytochemical composition and antimicrobial activity of neem," *International Journal of Secondary Metabolite*, vol. 12, no. 3, pp. 615–629, 2025.
[16] Centers for Disease Control and Prevention, *Antibiotic Resistance Threats in the United States*, Atlanta, GA, USA, 2019.
[17] FAO, OIE, and WHO, *Technical Brief on Antimicrobial Resistance and the Agri-Food Sector*, Rome, Italy, 2021.
[18] Organisation for Economic Co-operation and Development, *Test No. 429: Skin Sensitization – Local Lymph Node Assay*, Paris, France, 2019.
[19] Government of India, Ministry of Health and Family Welfare, *The Ayurvedic Pharmacopoeia of India, Part I, Vol. IX*, New Delhi, India, 2020.
[20] N. O. Bolaji, N. Y. A. Abolade, N. S. Aduwa, et al., "Phytochemical screening of neem leaves," *GSC Biological and Pharmaceutical Sciences*, vol. 26, no. 3, pp. 068–083, 2024.
[21] J. M. Álvarez-Caballero and E. Coy-Barrera, "Chemical and antifungal variability of Azadirachta indica," *Plants*, vol. 8, no. 12, p. 555, 2019.
[22] A. Eid, N. Jaradat, and N. Elmarzugi, "Chemical constituents and traditional usage of neem," *Palestinian Medical and Pharmaceutical Journal*, vol. 2, no. 2, pp. 75–81, 2017.
[23] Z. S. S. Al-Hashemi and M. A. Hossain, "Biological activities of neem extracts," *Pacific Science Review A*, vol. 18, no. 2, pp. 128–131, 2016.
[24] R Core Team, *R: A Language and Environment for Statistical Computing*, Vienna, Austria: R Foundation, 2022.
[25] GraphPad Software, *Prism 9 Statistics Guide*, San Diego, CA, USA, 2023.
[26] Clinical and Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing: M100*, 31st ed., Wayne, PA, USA, 2021.
[27] World Health Organization, *Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report*, Geneva, Switzerland, 2021.
[28] World Health Organization, *WHO Traditional Medicine Strategy 2025*, Geneva, Switzerland, 2023.
[29] M. Ahmed, D. A. Marrez, N. M. Abdelmoeen, et al., "Antioxidant and antimicrobial activities of neem extracts," *Processes*, vol. 11, no. 6, p. 1644, 2023.
[30] R. Katiyar, E. Khare, and S. D. Kaistha, "Neem seed oil activity against biofilms," *Journal of Pure and Applied Microbiology*, vol. 17, no. 3, pp. 1669–1678, 2023.