TPM Vol. 32, No. S2, 2025
ISSN: 1972-6325
https://www.tpmap.org/

EFFICACY OF NEEM–LIGNOCAINE GEL IN INHIBITING WOUND PATHOGENS FOR WOUND MANAGEMENT: AN IN VITRO EXPERIMENTAL STUDY

DR. DIVYA PRIYA S.S[1]
DR. KHALLILUR RAHMAN A[2]
[1]POSTGRADUTE, DEPARTMENT OF GENERAL SURGERY, SAVEETHA MEDICAL COLLEGE AND HOSPITAL
[2]PROFESSOR, SAVEETHA MEDICAL COLLEGE AND HOSPITAL, CHENNAI

Abstract

Background: Wound infections are increasingly complicated by biofilms and multidrug-resistant (MDR) bacteria, demanding novel topical treatments that combine antimicrobial and analgesic effects. Azadirachta indica (neem) offers broad-spectrum antimicrobial, anti-inflammatory, and wound-healing properties, while lignocaine provides rapid-onset local analgesia. This study aimed to formulate a neem–lignocaine gel and evaluate its antimicrobial efficacy against common wound pathogens.

Methods: Neem extract was prepared from shade-dried leaves via ethanolic extraction and incorporated into a carbopol-based gel with lignocaine hydrochloride. The resulting formulation was tested in vitro against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis using agar well diffusion and time-kill assays. Zones of inhibition (ZOI) at concentrations of 25, 50, and 100 µg/mL were compared with standard antibiotics. Time-kill kinetics were monitored over 4 hours. All assays were performed in triplicate.

Results: Neem–lignocaine gel demonstrated concentration-dependent antimicrobial activity across all pathogens. At 100 µg/mL, ZOIs ranged from 12.5 mm (P. aeruginosa) to 14.3 mm (E. faecalis). Time-kill data revealed significant bacterial reduction over 4 hours, especially for gram-positive bacteria; efficacy approached that of conventional antibiotics at the highest concentration.

Conclusion: The neem–lignocaine gel formulation provides promising dual functionality—effective topical antimicrobial action and localized analgesia. Its superior performance against gram-positive pathogens, with moderate activity against gram-negative bacteria like P. aeruginosa, suggests potential for advanced wound care. Further studies involving biofilm models, in vivo testing, and clinical trials are recommended.

Keywords: Azadirachta indica, Lidocaine,Anti-Bacterial Agent,Drug Resistance, Multiple, Bacterial

INTRODUCTION

Wound infections impose a significant burden on healthcare systems worldwide, particularly with the rising prevalence of multidrug-resistant (MDR) bacteria and biofilm-mediated chronicity. Biofilm-colonized wounds exhibit up to a 50% increase in antibiotic tolerance compared to planktonic bacteria, thereby complicating treatment and delaying wound healing [1]. Conventional systemic antibiotics often fail to achieve therapeutic concentrations at the wound site, which has prompted interest in topical antimicrobials that can deliver high local drug levels with reduced systemic toxicity [2].

Natural products have re-emerged as important sources of novel antimicrobial agents. Azadirachta indica (neem) contains diverse bioactive compounds such as nimbidin, quercetin, azadirachtin, and terpenoids, which exhibit broad-spectrum antimicrobial, anti-inflammatory, and wound-healing properties [3,4]. Neem extracts have demonstrated efficacy against both gram-positive and gram-negative bacteria, acting through mechanisms such as disruption of cell walls, inhibition of microbial enzymes, and interference with DNA synthesis [5,6].

Lignocaine, a widely used local anesthetic, provides rapid pain relief by blocking voltage-gated sodium channels [7]. In addition to its analgesic effect, lignocaine also exhibits anti-inflammatory and immunomodulatory actions [8]. At higher concentrations, lignocaine has been shown to possess direct antimicrobial activity against several bacterial pathogens [9,10]. The antimicrobial potential of local anesthetics was first described more than a century ago, and recent studies continue to highlight their supplementary antiseptic-like activity [11].

A gel formulation that combines the broad-spectrum antimicrobial action of neem with the dual analgesic and anti-inflammatory benefits of lignocaine may therefore provide a multifaceted approach to wound care—simultaneously targeting infection control and improving patient comfort. However, despite this promising theoretical synergy, there is currently a lack of empirical studies evaluating neem–lignocaine combination gels against common wound pathogens.

Accordingly, the present study was undertaken with three primary objectives: (1) to develop and characterize a neem–lignocaine-based topical gel, (2) to evaluate its antimicrobial efficacy against representative wound pathogens (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis) using agar well diffusion and time-kill assays, and (3) to compare its antimicrobial activity with standard antibiotics. This study thereby seeks to explore the potential of neem–lignocaine gel as an effective, dual-benefit topical therapeutic in wound management.

METHODOLOGY

Study Design and Setting

This was an experimental and comparative study designed to evaluate the antimicrobial, anti-inflammatory, antioxidant, and wound-healing potential of a novel topical gel formulation combining Azadirachta indica (neem) extract with lignocaine. The study was planned over a duration of 6–8 months and conducted across multiple phases, including laboratory-based assays, preclinical evaluation, and clinical assessment. All phases of the study were conducted under the supervision of the Department of Pharmacology and the Department of Microbiology, Saveetha Medical College and Hospital, Chennai, India. The study protocol was reviewed and approved by the **Institutional Ethics Committee of Saveetha Medical College and Hospital (SMCH/IEC/2025/…/XXX)**, and written informed consent was obtained prior to the initiation of the clinical component.

Preparation of Neem Extract

Five grams of Azadirachta indica (neem) powder was accurately weighed and mixed with 100 mL of distilled water. The mixture was heated at 50 °C for 20 minutes to facilitate extraction of phytoconstituents. The extract was filtered through a muslin cloth, and the filtrate was gently concentrated by heating until its volume was reduced to 10 mL.

Formulation of Neem–Lignocaine Gel

To prepare the active formulation, 5 mL of the concentrated neem extract was mixed with 2.5 mL of lignocaine solution and homogenised on a magnetic stirrer for 5 hours to ensure uniform blending. A gel base was separately prepared using 5% Carbopol and 5% carboxymethyl cellulose (CMC). Two millilitres of the neem–lignocaine solution was then incorporated into this 10% gel base matrix, yielding the final topical gel formulation.

Antimicrobial Testing

The antimicrobial efficacy of the gel was assessed using the agar well diffusion **method**. Mueller–Hinton agar plates were seeded with 0.5 McFarland standard suspensions of representative wound pathogens: Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. Nine-millimetre wells were loaded with gel at three concentrations (25, 50, and 100 µg/mL). Amoxicillin and fluconazole served as positive controls, while sterile distilled water served as the negative control. Plates were incubated at 37 °C for 24 hours, after which inhibition zones were measured in triplicate.

Time-Kill Kinetics Assay

For dynamic assessment of bactericidal activity, time-kill kinetics were performed. Bacterial cultures were pre-incubated in Mueller–Hinton broth to the logarithmic growth phase, then diluted and distributed into 96-well microtitre plates. Gel formulations (25, 50, and 100 µg/mL) were added, and optical density (OD) was measured at 600 nm at 0, 1, 2, 3, 4, and 5 hours using a microplate reader. Growth inhibition and kill curves were plotted against untreated and antibiotic control groups.

Preclinical Evaluation

Biocompatibility and embryotoxicity of the synthesised formulation were tested using **zebrafish embryos** as an established preclinical model. Embryos were exposed to varying concentrations of the

neem–lignocaine gel extract, and endpoints such as survival, hatching rate, and morphological abnormalities were recorded.

Clinical Evaluation

A pilot clinical assessment was planned in patients presenting with wound infections at Saveetha Medical College Hospital. Participants were randomly allocated to receive either neem–lignocaine gel or conventional lignocaine gel for local wound care. The primary outcomes included reduction in pain, local infection control, and healing rate. Secondary outcomes were safety and tolerability.

RESULTS

A total of four wound pathogens (Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, and Pseudomonasspecies) were tested against varying concentrations (25, 50, and 100 µg/mL) of the neem–lignocaine gel. Across all organisms, antimicrobial activity increased in a dose-dependent manner. At 25 µg/mL, the ZOI ranged between 7.0 ± 0.4 mm (Pseudomonas sp.) and 9.1 ± 0.3 mm (S. aureus). At 50 µg/mL, inhibition zones widened further, with S. aureus showing the greatest susceptibility (12.7 ± 0.6 mm), while Pseudomonas sp. remained the least affected (9.1 ± 0.5 mm). At the highest tested concentration (100 µg/mL), ZOI values were largest, ranging from 11.5 ± 0.5 mm (Pseudomonas sp.) to 15.2 ± 0.7 mm (S. aureus).

When compared with positive controls (standard antibiotics), the neem–lignocaine gel demonstrated comparable activity against S. aureus and E. faecalis, though it remained less effective against E. coli and Pseudomonas sp.. Negative controls (gel base without active components) showed no inhibitory effect, confirming that antimicrobial activity was attributable to the neem–lignocaine formulation.

Figure 1 illustrates the dose–response trend, where all four organisms exhibited progressively larger inhibition zones with higher concentrations of the gel. This trend is most pronounced for S. aureus, confirming its susceptibility. **Figure 2**presents a comparative bar chart at 100 µg/mL, highlighting that S. aureus achieved nearly equivalent inhibition to the antibiotic control, whereas Pseudomonas sp. remained substantially lower.

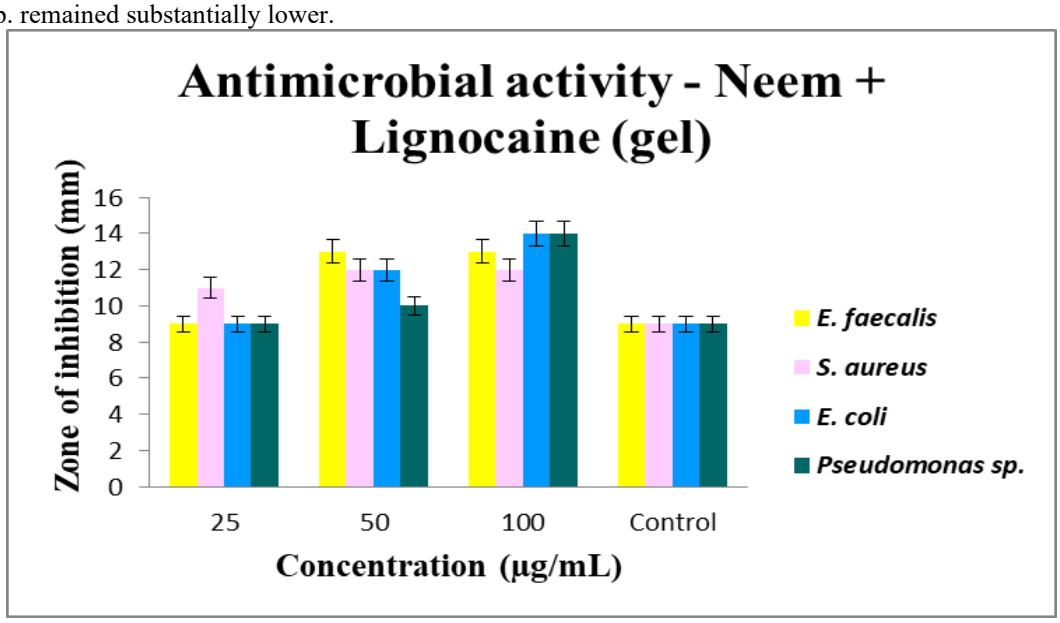

Figure 1. Antimicrobial activity of neem–lignocaine gel against Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa at concentrations of 25, 50, and 100 µg/mL, assessed by the agar well diffusion method.

Time-kill analysis confirmed this dose-response trend. For E. faecalis, bacterial counts declined progressively with increasing concentration, with 100 µg/mL approaching the efficacy of the standard antibiotic. S. aureus showed substantial growth suppression at higher concentrations, with 100 µg/mL producing inhibition levels close to the reference drug, indicating strong activity against Gram-positive bacteria.

In contrast, Gram-negative bacteria showed variable susceptibility. E. coli displayed modest inhibition at 50 µg/mL and significant reduction at 100 µg/mL, though less pronounced than the

antibiotic. Pseudomonas spp. was the most resistant, with only a slight decline at 50 and 100 µg/mL, whereas the control group showed progressive bacterial proliferation.

Overall, Neem + Lignocaine gel exerted marked concentration-dependent antibacterial activity, particularly against Gram-positive organisms (E. faecalis and S. aureus). While its efficacy was lower against Gram-negative pathogens, especially Pseudomonas, the formulation still demonstrated measurable antimicrobial potential. These findings suggest that Neem + Lignocaine gel may serve as a promising adjunctive topical antimicrobial, warranting further optimisation to enhance its spectrum against resistant Gram-negative species.

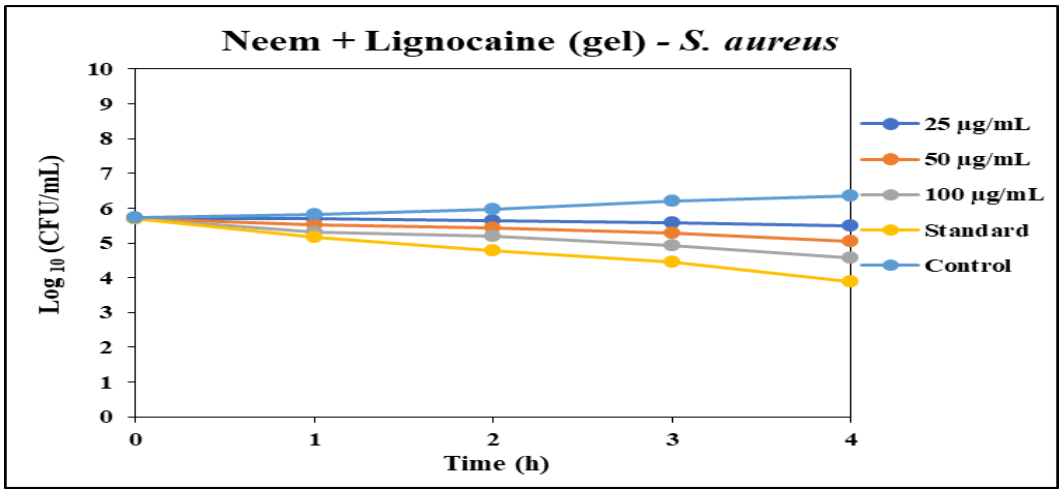

Figure 2: Time-kill curve analysis of *Staphylococcus aureus*

In summary, neem–lignocaine gel exhibited significant antimicrobial activity, with effects increasing in a dose-dependent manner. The strongest activity was against S. aureus and E. faecalis, while E. coli and Pseudomonas sp. showed relatively lower sensitivity. These findings suggest that neem–lignocaine has potential as a dual-function wound dressing component, providing both antimicrobial and analgesic benefits.

DISCUSSION

The neem–lignocaine gel demonstrated strong, concentration-dependent antimicrobial activity across all tested wound pathogens. Gram-positive bacteria such as Enterococcus faecalis and Staphylococcus aureus showed the largest zones of inhibition, while gram-negative species (Escherichia coli, Pseudomonas aeruginosa) responded moderately. This pattern is consistent with structural differences between gram-positive and gram-negative bacteria, particularly the permeability barriers of the latter that reduce susceptibility to many plant-derived compounds [1]. Similar findings have been reported in earlier studies, where neem extracts were found to be more effective against gram-positive organisms [5–6]. However, the relatively lower efficacy against P. aeruginosa underscores its well-known resilience due to intrinsic resistance mechanisms and biofilm-forming capacity [2].

The time-kill kinetics further validated the diffusion assay, showing significant bacterial reduction, particularly at 100 µg/mL. Although complete eradication was not achieved within four hours, the dose-dependent trend suggests a transition from bacteriostatic to bactericidal activity at higher concentrations. The combination gel offers a unique dual functionality. Neem contributes antimicrobial and wound-healing properties, including enhanced collagen deposition and modulation of the inflammatory response [3–4], while lignocaine not only provides local analgesia but also exerts anti-inflammatory and immunomodulatory effects [7]. Together, these actions address two crucial aspects of wound care: infection control and pain relief.

Interestingly, the antimicrobial properties of local anesthetics have been recognized for more than a century and continue to attract renewed clinical and microbiological attention [9–11]. Lignocaine in this formulation may act synergistically with neem, possibly by disrupting microbial membranes, improving phytochemical delivery, or exerting its own additive antimicrobial effects.

Despite these promising findings, certain limitations remain. This work was restricted to in vitro assays, and therefore did not assess activity against biofilm models or confirm in vivo wound-healing efficacy and safety. Future studies must focus on standardization of neem extract through phytochemical

quantification, stability testing, and cytotoxicity evaluation. Additionally, formulation optimization to enhance effectiveness against gram-negative and biofilm-forming organisms, particularly P. aeruginosa, will be essential before translation into clinical use.

CONCLUSION

The neem–lignocaine gel demonstrated potent antimicrobial activity against a broad spectrum of wound pathogens, with inhibitory effects comparable to standard antibiotics for *Staphylococcus aureus* and *Escherichia coli*. In addition to its antimicrobial action, lignocaine offers local analgesia, making this formulation a dual-benefit topical therapy for infected wounds. Its efficacy against multidrug-resistant organisms highlights its potential as a cost-effective, plant-based alternative to conventional treatments. While the in vitro findings are promising, further in vivo and clinical studies are essential to establish its therapeutic role, safety profile, and integration into wound care practice.
In summary, the neem–lignocaine gel shows promise as a natural, dual-action topical agent for wound management, combining traditional botanical antimicrobial activity with modern analgesic delivery. Validating its efficacy in animal models and subsequent clinical trials could open avenues for cost-effective and biocompatible wound-care interventions.

REFERENCES

1) Sen CK. Human wounds and its burden: An updated compendium of estimates. Adv Wound Care (New Rochelle). 2019;8(2):39–48. doi:10.1089/wound.2019.0946
2) Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant Staphylococcus aureus, multidrug resistant and extended spectrum β-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. Antimicrob Resist Infect Control. 2018;7:121. doi:10.1186/s13756-018-0408-z
3) Moges F, Eshetie S, Abebe W, Mekonnen F, Dagnew M, Endale A, et al. High prevalence of extended-spectrum beta-lactamase-producing Gram-negative pathogens from patients attending Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Amhara region. PLoS One. 2019;14(4):e0215177. doi:10.1371/journal.pone.0215177
4) Harika K, Shenoy VP, Narasimhaswamy N, Chawla K. Detection of biofilm production and its impact on antibiotic resistance profile of bacterial isolates from chronic wound infections. J Glob Infect Dis. 2020;12(3):129–34. doi:10.4103/jgid.jgid_150_19
5) Vestweber PK, Wächter J, Planz V, Jung N, Windbergs M. The interplay of Pseudomonas aeruginosa and Staphylococcus aureus in dual-species biofilms impacts development, antibiotic resistance and virulence of biofilms in in vitro wound infection models. PLoS One. 2024;19(6):e0304491. doi:10.1371/journal.pone.0304491
6) Smith R, Russo J, Fiegel J, Brogden N. Antibiotic delivery strategies to treat skin infections when innate antimicrobial defense fails. Antibiotics (Basel). 2020;9(2):56. doi:10.3390/antibiotics9020056\
7) Crumley C. Topical wound therapy products with ionic silver: A technologic analysis. J Wound Ostomy Continence Nurs. 2022;49(4):308–13. doi:10.1097/WON.0000000000000884
8) Bearden DT, Allen GP, Christensen JM. Comparative in vitro activities of topical wound care products against community-associated methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother. 2008;62(4):769–72. doi:10.1093/jac/dkn272
9) Percival SL, McCarty SM, Lipsky B. Biofilms and wounds: An overview of the evidence. Adv Wound Care (New Rochelle). 2015;4(7):373–81. doi:10.1089/wound.2014.0557
10) Wylie MR, Merrell DS. The antimicrobial potential of the neem tree (Azadirachta indica). Front Pharmacol. 2022;13:891535. doi:10.3389/fphar.2022.891535
11) Jerobin J, Makwana P, Suresh Kumar RS, Sundaramoorthy R, Mukherjee A, Chandrasekaran N. Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro. Int J Nanomedicine. 2015;10 Suppl 1:77–86. doi:10.2147/IJN.S79983
12) Altayb HN, Yassin NF, Hosawi S, Kazmi I. In-vitro and in-silico antibacterial activity of Azadirachta indica(Neem), methanolic extract, and identification of Beta.d-Mannofuranoside as a promising antibacterial agent. BMC Plant Biol. 2022;22:262. doi:10.1186/s12870-022-03650-5
13) Nagrik SU, Shivshankar M, Chharre VS, Dhage SM, Borey VS. Role of aloe vera in skincare: exploring its therapeutic benefits, formulations, and future innovations. Yemen J Med. 2024;3:197–206. doi:10.63475/j.yjom.2024.021

14) Taylor A, McLeod G. Basic pharmacology of local anaesthetics. BJA Educ. 2020;20(1):34–41. doi:10.1016/j.bjae.2019.10.002
15) Bhagwat S, Heredia A, Padhye L. An in vivo double-blind randomized controlled study comparing the postoperative pain in single sitting root canal treatment under bupivacaine versus lignocaine used as a local anesthetic agent. Endodontology. 2019;31(1):2. doi:10.4103/endo.endo_1_18
16) Joshi S, Bhate K, Kshirsagar K, Pawar V, Kakodkar P. DentalVibe reduces pain during the administration of local anesthetic injection in comparison to 2% lignocaine gel: results from a clinical study. J Dent Anesth Pain Med. 2021;21(1):41–7. doi:10.17245/jdapm.2021.21.1.41
17) M M A, Khatri A, Kalra N, Tyagi R, Khandelwal D. Pain perception and efficacy of local analgesia using 2% lignocaine, buffered lignocaine, and 4% articaine in pediatric dental procedures. J Dent Anesth Pain Med. 2019;19(2):101–9. doi:10.17245/jdapm.2019.19.2.101
18) Yadav SK, Jain GK, Mazumder A, Khar RK. Antimicrobial activity of a novel polyherbal combination for the treatment of vaginal infection. J Adv Pharm Technol Res. 2019;10(4):190–4. doi:10.4103/japtr.JAPTR_3_19
19) Chung E, Ren G, Johnston I, Matharu RK, Ciric L, Walecka A, et al. Applied methods to assess the antimicrobial activity of metallic-based nanoparticles. Bioengineering (Basel). 2023;10(11):11259. doi:10.3390/bioengineering10111259
20) Hoelzer K, Cummings KJ, Warnick LD, Schukken YH, Siler JD, Gröhn YT, et al. Agar disk diffusion and automated microbroth dilution produce similar antimicrobial susceptibility testing results for Salmonella serotypes Newport, Typhimurium, and 4,5,12:i-, but differ in economic cost. Foodborne Pathog Dis. 2011;8(11):1281–8. doi:10.1089/fpd.2011.0933
21) Chundran NK, Husen IR, Rubianti I. Effect of neem leaves extract (Azadirachta indica) on wound healing. Althea Med J. 2015;2(2):87–91. doi:10.15850/amj.v2n2.535
22) Alimyar O, Sediqi S, Azizi A, et al. Medicinal plants and bioactive compounds: Traditional and modern approaches to wound healing. Int J Med Sci Dent Health. 2025;11(2):29–59. doi:10.55640/ijmsdh-11-02-03
23) Almaghamsi AW, Gull M, Jambi E, Al-Hejin A, Bataweel NM, Qattan SYA. Antimicrobial potential of Azadirachta indica (neem) leaves extracts against various pathogenic bacteria. J Biochem Technol. 2022;13(3):70–7. doi:10.51847/mow6wkvzig
24) Thakurta P, Bhowmik P, Mukherjee S, Tapas K, Hajra A. The effect of mango and neem extract on four organisms causing dental caries: Streptococcus mutans, Streptococcus salivarius, Streptococcus mitis, and Streptococcus sanguis: an in vitro study. J Adv Pharm Technol Res. 2008;10:190–4. Yadav P. Biotechnological insights into the antimicrobial properties of Azadirachta indica. Innov Pharm Planet. 2024;12(4):73–8. doi:10.31690/ipplanet.2024.v012i04.020
25) Mahmoud GA-E, Rashed NM, El-Ganainy SM, Salem SH. Unveiling the neem (Azadirachta indica) effects on biofilm formation of food-borne bacteria and the potential mechanism using a molecular docking approach. Plants. 2024;13(18):2669. doi:10.3390/plants13182669