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Nanogel Formulations of Kratom (*Mitragyna speciosa*) Extract: A Promising Antibiofilm Agent for Diabetic Ulcer Infections

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ABSTRACT:

Diabetic ulcer infections present a substantial obstacle to wound management due to the prevalence of persistent bacterial biofilms that are impervious to conventional antibiotic therapies. Nanomaterials have emerged as a revolutionary platform for addressing the challenges of antibiotic resistance and biofilm-associated infections. Researchers have the potential to employ Kratom in the development of antibiofilm medicines specifically designed to combat diabetic ulcer infections. This is due to its capacity to impede bacterial quorum sensing and disrupt the process of biofilm development. Objective of this study is to determine the antibiofilm properties of the nanogel derived from kratom extract in diabetic ulcer infections. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (1%, 0.5%, 0.25%, 0.125% w/v) to identify their effects on *S. aureus*. The concentration of extract in nanogel used was 10, 20, and 30% to test antibiofilm in the mid-phase, maturation, and eradication phases. In the mid-phase biofilm, Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v exhibited significant antibiofilm efficacy against *Staphylococcus aureus* (SA), with an inhibition percentage of $79.21\% \pm 0.08$; maturation phase of $74.12\% \pm 0.101$; and eradication of $67.25\% \pm 0.046$. KDNE compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial and antibiofilm agents.

KEYWORDS: Nanogel, Kratom, Biofilm, Diabetic ulcers, Infection

INTRODUCTION:

Diabetic ulcer infections pose a significant challenge in wound management, as they are often associated with persistent bacterial biofilms that are resistant to conventional antibiotic treatments¹. Biofilms are intricate and ever-changing microbial colonies that flourish at the boundary layer between a solid surface and a liquid medium². The complex formations consist of microbial cells that are tightly integrated with one another and with the underlying surface, enclosed inside a self-generated matrix of extracellular polymeric compounds. Biofilm formation is a fundamental survival strategy used by many microorganisms to acclimatise to their habitat, especially under unfavourable circumstances³. Because of the rise of bacteria and viruses that are resistant to antibiotics, choosing the right empirical antibiotic coverage for diabetic ulcer infection therapy has become more challenging in recent years⁴.

To successfully address severe diseases caused by biofilms, new and innovative therapeutic techniques are required. Utilising nanogel formulations of naturally occurring substances with intrinsic antibiofilm characteristics⁵, like kratom extract, is one approach that shows promise. Mitragynine and 7-hydroxymitragynine are two of the active components found in kratom. These molecules target different types of bacteria, including those that cause infections in diabetic ulcers. Regardless, more in vitro and in vivo investigations are needed⁶. There has been a lot of buzz in the ever-evolving world of antimicrobial strategies about targeted and effective approaches to combat biofilm-related diseases. Biofilms are naturally resistant to conventional methods, such as antibiotic use. As a result, researchers are actively seeking novel approaches to this persistent issue, and they have turned their focus to the fascinating realm of nanotechnology⁷.

In their battle against bacterial infections, researchers have shown that nanomaterials and carriers on the nanoscale may kill bacterial cells, slow down quorum sensing, and prevent or eliminate biofilms⁸. Nanomaterials are a game-changer in the fight against biofilm-associated illnesses and antibiotic resistance. In instance, there are unique benefits to delivering natural chemicals with antibiofilm action via nanogel formulations⁹. There are a number of benefits to using nanogels, which are hydrogel nanoparticles, to reduce biofilms. It is easy for the hydrophilic nanocarriers to penetrate bacterial biofilms and disrupt their extracellular matrix. Because of this, antibiofilm medications may be delivered directly to bacterial cells¹⁰. Nanogels' unique properties, such as their scalability, surface charge, and sensitivity to environmental stimuli, might potentially enhance the efficacy of the encapsulated kratom extract¹¹.

Studies have demonstrated that nanogel formulations may prevent biofilm production, break existing biofilms, and improve the efficacy of conventional antibiotics in killing bacteria trapped in biofilms¹². A very promising treatment approach for diabetic ulcer infections, these nanogel formulations effectively target and dissolve bacterial biofilms¹³. In addition to increasing local antibiotic concentrations, nanogels' ability to transport active drugs straight to the infection site aids in the fight against antibiotic resistance by penetrating the protective biofilm matrix¹⁴. This focused strategy may help improve patient outcomes, lessen the likelihood of adverse effects, and lower the requirement for systemic antibiotics, which is particularly important in the treatment of diabetic foot

ulcers caused by biofilm-associated infections¹⁵. The objective of this investigation is to ascertain the antibiofilm properties of the nanogel derived from kratom extract in the context of diabetic ulcer infections.

MATERIALS AND METHODS:

Sampling Place:

We collected samples of kratom leaves (*Mitragyna speciosa*) in the forest of Kuranji District, Tanah Bumbu Regency, South Kalimantan, Indonesia. The process of identifying plants was carried out at Universitas Lambung Mangkurat's Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Banjarmasin, Indonesia.

Materials:

Materials used were ethanol 95 % (Merck, Germany), ethyl acetate (Merck, Germany), chloramphenicol (Sigma-Aldrich, Germany), phosphate-buffered saline (PBS; Sigma—Aldrich), McFarland (HiMedia, India), CMC-Na (Merck, Germany), Oxoid (Brain Heart infusion) (Merck, Germany), crystal violet (Merck, Germany), Carbopol (Merck, Germany), Trietanolamine (Merck, Germany), Gliserol (Merck, Germany), Propilenglikol (Merck, Germany).

Equipment:

Micropipette (Gilson, France), microtiter plate reader (OIS 2100, Spain), tube (Socorex, Swiss), LAF (Sakura, Japan), incubator 2B (Sakura, Japan), Microplate flat-bottom polystyrene 96 well (Iwaki, Japan), spektro genesys (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), analytical balances (Ohaus, USA).

Bacterial strain:

ATCC 25923 strains of standard *Staphylococcus aureus* were cultivated in tryptic soy broth (TSB) medium and subjected to a 72-hour incubation period at 37°C.

Extraction and identification:

The Kratom leaf samples were cleansed by the process of rinsing with flowing water and meticulously inspecting for any contaminants. After washing, we sliced and desiccated the plant samples in an oven at 40°C for 3 hours. Sample standardisation is conducted using precise criteria. We accomplished the Simplicia extraction by fully immersing 300 g of powder in 96% ethanol. We agitate the mixture every 6 hours and replace the solvent every 24 hours, resulting in a total of 5 repetitions or changes of solvent. We subjected the liquid extract to evaporation in a water bath maintained at a temperature of 50°C until the weight reached a steady state. The levels of quinones, terpenoids, phenolics, saponins, steroids, alkaloids, and flavonoids were assessed using the tube technique.

Antibacterial effect of kratom extract against *staphylococcus aureus*:

The antibacterial efficacy was assessed by the use of microdilution and well techniques. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (0,125%, 0,25%, 0,5%, 0,1% w/v) to identify their effects on *S. aureus*. The positive control used was chloramphenicol. In order to get a concentration of 5x10⁶ colony forming units per millilitre (CFU/mL), the bacterial solution in BHI was first adjusted to 0.5 McFarland units and then diluted. The microplate was filled with a solution of ethanol with a concentration of 96% and subjected to incubation for a duration of 15 minutes. An ELISA Reader was employed to conduct optical density measurements at a wavelength of 570 nm.

Formulation of nanogel:

Carbopol and CMC were the two nanogel ingredients used as a standard gel base.

Table 1. Formulation of nanogel extract

No	Ingredients	F1 (g)	F2 (g)	F3 (g)
1	Carbopol 940	0,8	0,8	0,8
2	CMC-Na	0,6	0,6	0,6
3	Gliserol	1,25	1,25	1,25
4	Propilenglikol	1,75	1,75	1,75
5	Trietanolamin	1,5	1,5	1,5
6	Nanoemulsion extract	0,75	0,75	0,75
7	Aquadest ad	20	20	20
	Ingredient nanoemulsion			
1	Ekstrakt	10%	20%	30%
2	Etanol 96%	9 mL	9 mL	9 mL

3	Tween 80	27,5 mL	27,5 mL	27,5 mL
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Inhibitory activity of kratom extract against *staphylococcus aureus* biofilms:

Biofilm formation was assessed during 24 hours (intermediate phase), 48 hours (maturation phase), and 72 hours (eradication) using the Microbroth Dilution Technique (MDT). The microtiter plates were rinsed with distilled water to remove detachable cells, and then allowed to desiccate in the surrounding air at room temperature for a period of 5 minutes. Each well was thereafter stained with a 125 µL portion of a 1% solution of crystal violet to identify living and non-viable cells, as well as any other components of the biofilm. Following a 15-minute incubation period at ambient temperature, we proceeded to rinse the plate with flowing water in order to eliminate the purple hue. The optical density (OD) was determined by adding 200 µL of 96% ethanol to each well and using a microplate reader with a 595 nm wavelength. The bacterial test culture, was deposited onto a microtiter plate including coverslips. In order to promote the biofilm development process, Subsequently, the plate was placed in an incubator that was maintained at a temperature of 37°C for a duration of 24-48 hours.

In vivo biofilm study

Adult rats (*Rattus norvegicus*) weighing 150–200 g were employed to conduct in vivo antibiofilm investigations. The animals were given unfettered access to water and nutritious food and were kept inside a controlled setting. In order to induce type II diabetes, a combination of streptozocin and nicotinamide was administered. The rats were administered a 240 mg/kg intraperitoneal dose of nicotinamide after a 12-hour fasting period. 15 minutes later, I.P. administration of streptozocin at a dosage of 100 mg/kg occurred. The animals were subjected to a 12-hour fast following the streptozocin treatment, and their fasting blood glucose levels were assessed 72 hours later. The investigation categorised animals as diabetic only if their fasting blood glucose level exceeded 150 mg/dL¹⁶. Positive, negative, and the maximum concentration of gel were the three categories into which the mice were divided. To prevent infection, the researchers made an open incision on the rodent with a diameter of 1 cm and administered bacteria. To serve as a positive control, chloramphenicol was administered daily to the lesion until it had fully healed.

SEM study:

Following a thorough washing with sterile distilled water, the coverslip was then subjected to glutaraldehyde during fixation. Methanol was used as a means of decreasing the water content, while the examination was conducted utilising a SEM (scanning electron microscope) functioned at a voltage of 10 kilovolts (kV).

RESULT:

Phytochemical screening

The extraction yielded a filtrate, which was further evaporated using a water bath at 50°C to give a concentrated extract with a consistent weight. The leaf yield was 18.8% with some phytochemical compounds in **Table 2**.

Table 2: Phytochemical compound of kratom extract.

No	Secondary metabolites	Result
1	Alkaloids (Dragendorff)	-
2	Alkaloids (Mayer)	-
3	Alkaloids (Wagner)	-
4	Steroids	-
5	Terpenoids	+
6	Tannin	-
7	Phenolic	+
8	Saponin	-
9	Flavonoids	+
10	Quinones	+

Antibacterial effect of kratom leave extract against *Staphylococcus aureus*:

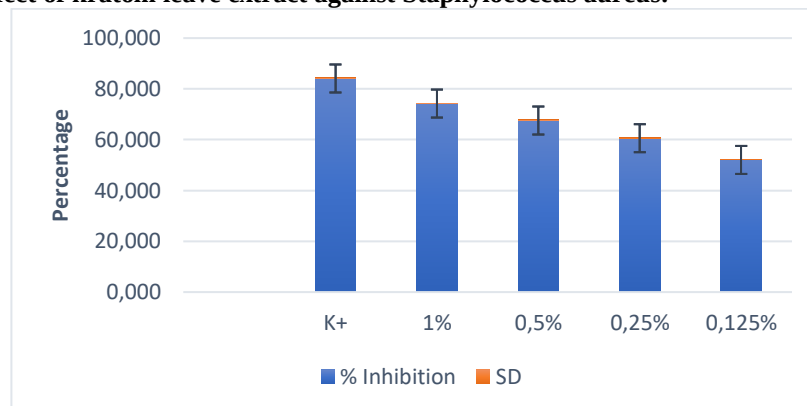


Figure 1: Kratom extract's antibacterial efficacy against *Staphylococcus aureus*

The results demonstrated that the concentration of lollipop flower extract affected its efficacy. At a 1% w/v concentration, the extract successfully suppressed SA growth, showing a substantial ($p < 0.05$) inhibition percentage of $74.226\% \pm 0.002$, notably different from the drug control's $84.097\% \pm 0.001$.

KDNE's inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms:

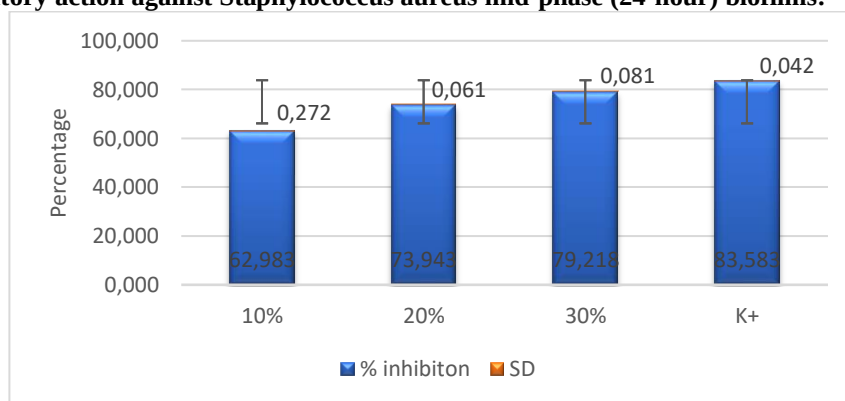


Figure 2. Percentage of inhibitory action against *staphylococcus aureus* mid-phase (24-hour) biofilms

Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v showed strong antibiofilm activity against *Staphylococcus aureus* (SA) during the mid-phase biofilm, with an inhibition percentage of $79.21\% \pm 0.08$. Notably, this inhibition was not significantly different ($p > 0.05$) from the control drug at a concentration of 1% w/v, which showed an inhibition percentage of $83.58\% \pm 0.042$, as depicted in **Figure 2**.

KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms:



Figure 3. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms

During the biofilm maturation phase, KDNE showed an inhibitory activity of $74.12\% \pm 0.101$ at a concentration of 30% w/v. Interestingly, this result did not differ significantly ($p > 0.05$) from the inhibitory effectiveness of the pharmacological control, which showed a higher inhibitory effectiveness of $77.79\% \pm 0.083$. These data provide support for the idea that as the biofilm development duration increases, the arrangement of the matrix rises, resulting in a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms.

KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms:

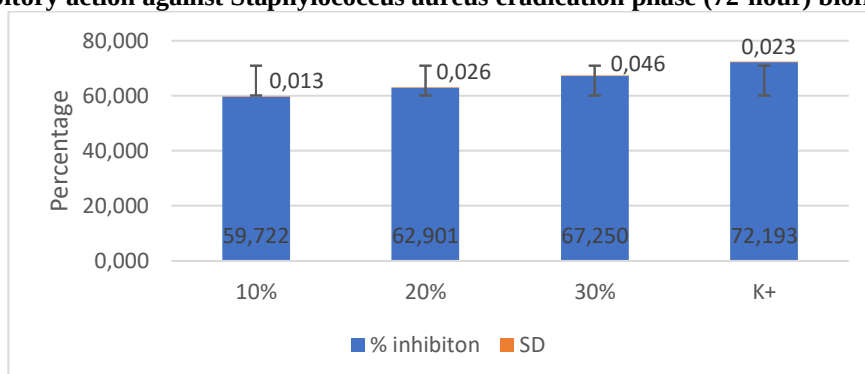


Figure 4. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms

The eradication-phase biofilm of *Staphylococcus aureus* (SA) was effectively inhibited by KDNE at a concentration of 30% w/v, exhibiting an inhibition percentage of $67.25\% \pm 0.046$. Figure 4 shows that at a dose of 1% w/v, the control medication exhibited an inhibition percentage of $72.912\% \pm 0.023$, and this inhibition was not substantially different ($p > 0.05$).

In vivo biofilm study



Figure 5. 5a. untreated group; 5b. Occurrence of Ulcers; 5c. the wound begins to close.

Diabetes is known to hinder the host's immune response and create an environment that encourages the growth of microorganisms. Numerous studies have highlighted the elevated risk of infections in diabetics, including rhinocerebral mucormycosis, malignant external otitis, and foot infections. There is evidence that diabetes-induced alterations in the microcirculatory system and disruption of wound healing processes contribute to this increased susceptibility. This study shows that nanogel treatment can improve wound healing in diabetic mice by increasing angiogenesis and reducing biofilm formation, which is not significantly different from the control group. The ulcers treated with nanogel closed and healed within 11 days, and the healing diameter was almost the same as the control group.

SEM Study:

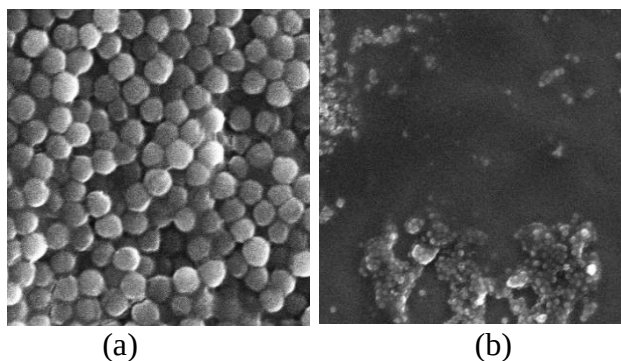


Figure 6: Figure 6a Result of SEM Biofilm with No Treatment, Figure 6b Result of SEM Biofilm with Administration of Kratom Extract 30% b/v.

Figure 6b shows that the SEM test showed that the KDNE effectively stopped and removed the *Staphylococcus aureus* (SA) biofilm. Figure 6a shows the results of the scanning electron microscopy investigation into the untreated SA biofilm, which reveals a compact cellular structure inside the extracellular polymeric substance (EPS) matrix and hence a safe habitat for the organism. Limitation, cell death, and EPS matrix damage were the outcomes of treating biofilm with a 30% w/v concentration of KDNE.

DISCUSSION:

Polyphenols also quinones, natural plant-derived compounds, have garnered significant attention for their remarkable antibiofilm properties. These molecules work in different ways to stop different stages of biofilm formation. For example, they stop cells from attaching at the start, stop the production of extracellular matrix, and mess up the signalling pathways for quorum sensing¹⁷. Flavonoids, tannins, and other phenolic compounds have been shown to be able to get into the biofilm matrix, cause oxidative stress, and damage the integrity of the microbiome cell membrane, which makes it harder for cells that are embedded in biofilm to stay alive. Similarly, studies have shown that quinones, a class of aromatic compounds, interfere with bacterial quorum sensing systems, crucial for coordinating biofilm development and maturation¹⁸. Because of their strong antibacterial and antibiofilm properties, flavonoids and quinones have attracted a lot of interest from antimicrobial researchers. Flavonoids have antibacterial properties because they disrupt bacterial membranes, inhibit bacterial nucleic acid replication, reduce bacterial efflux pump function, and block bacterial adenosine triphosphate synthase¹⁹. Offlavonoids are effective against bacteria because they may bind to extracellular proteins to create complex molecules that compromise bacterial cell membrane integrity. Quinones have antibacterial properties because they can create amino acid compounds that negatively impact protein function. When terpenoids bind to carbs and lipids in bacteria, they make their cell membranes less permeable²⁰.

Recent research has shown that kratom extracts may effectively hinder the development and disturb the structure of biofilms created by several harmful bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*²¹. Kratom has the potential to be used in the development of antibiofilm drugs that specifically target diabetic ulcer infections such as its ability to impede bacterial quorum sensing and disrupt biofilm formation²². By incorporating these bioactive molecules into nanogel formulations, their stability, solubility, and targeted delivery to the infection site may be improved²³. Attachment of microbial cells to a surface is the first step in the multi-stage process that culminates in the detachment of cells from the biofilm after microcolonies have developed and the biofilm structure has matured²⁴. This data implies that it is more problematic to suppress bacteria during biofilm development, since the inhibition percentage is lower compared to bacteria in planktonic form. For bacteria in planktonic form, KDNE had a substantial inhibitory effect of $83.58\% \pm 0.41$; for bacteria that had developed a biofilm during the mid-phase, the inhibitory effect was somewhat lower, at $79.21\% \pm 0.8$. These data support the idea that as the duration of biofilm development increases, there is a rise in the arrangement of the matrix, leading to a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms²⁵.

The physicochemical properties of nanomaterials, including hydrophobicity and pore size, can be regulated to optimise their antimicrobial properties. Researchers have reported that increasing the nanoroughness of the surface reduces bacterial adhesion to medical devices, implying the development of nanogel formulations to curb biofilm formation and bacterial attachment²⁶. Nanogel formulations can also work as nanovehicles, making it easier to deliver naturally occurring compounds that can break down biofilms. Nanogel forms of kratom extract have been shown in new research to effectively stop the growth of biofilms made by many bacteria that are clinically important, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As a result, the active chemicals might be more effectively administered locally at the site of infection, which could lessen the likelihood of systemic bad effects associated with traditional antibiotic treatments²⁷.

CONCLUSION:

The results indicate that the activity of KDNE in inhibiting the growth of *Staphylococcus aureus* biofilms varied with its concentration. At a concentration of 30%, the extract exhibited equivalent antibacterial, mid-phase, maturation, and eradication efficacy to the control. Plant-derived compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial agents.

CONFLICT OF INTEREST:

Regarding this study, the writers do not have any competing interests.

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REFERENCES:

1. Jang, E. J., Patel, R. & Patel, M. Electrospinning Nanofibers as a Dressing to Treat Diabetic Wounds. *Pharmaceutics* **12**, 1 (2023).
2. Li, H. *et al.* Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics* **11**, 1–45 (2022).
3. Rather, M. A., Gupta, K. & Mandal, M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology* **1**, 1701–718 (2021).
4. Pessoa e Costa, T. *et al.* Multidrug-resistant bacteria in diabetic foot infections: Experience from a portuguese tertiary centre. *Int Wound J* **17**, 1835–1839 (2020).
5. Kumar Bari, A., Sandeep Belalekar, T., Poojary, A. & Rohra, S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. *Front Cell Infect Microbiol* **7** (2023) doi:10.3389/fcimb.2023.1155699.
6. Syahida, N. & Ma, N. . Indonesian Journal of Chemical Science Kratom (*Mitragyna speciosa*): Medicinal Marvel or Menace? Assessing Potency, Risk, and Future Prospect of Herbal Medicine. *J. Chem. Sci* **13**, (2024).
7. Kishore, M. Y. *et al.* Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol* **1**, 1–30 (2023).
8. Mukherjee, A., Bose, S., Shao, A. & Das, S. K. Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. *Royal Society of Chemistry* **4**, 2544–2572 (2023).
9. Ramadan, Y. F. ; N. ; *et al.* Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicine* **413**, (2023).
10. Sookkhee, S. *et al.* Synergistic Effects of Some Methoxyflavones Extracted from Rhizome of *Kaempferia parviflora* Combined with Gentamicin against Carbapenem-Resistant Strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *MDPI Plants* **11**, 16 (2022).
11. Kishore Mohanta, Y. *et al.* Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol* **1–30** (2023) doi:10.3389/fmicb.2022.1028086.
12. Varma, A. *et al.* The role of nanocomposites against biofilm infections in humans. *Front Cell Infect Microbiol* **1–16** (2023) doi:10.3389/fcimb.2023.1104615.
13. Arunachalam, K. *et al.* Exploring Possible Ways to Enhance the Potential and Use of Natural Products through Nanotechnology in the Battle against Biofilms of Foodborne Bacterial Pathogens. *MDPI Pathogens* **1** (2023) doi:10.3390/pathogens12020270.
14. Mirghani, R. *et al.* Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology* vol. 8 240–278 Preprint at <https://doi.org/10.3934/microbiol.2022019> (2022).
15. Tuon, F. F. *et al.* Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics* vol. 12 Preprint at <https://doi.org/10.3390/antibiotics12010087> (2023).
16. Wu, X. *et al.* *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm* **7**, 100175 (2024).
17. Raffaelli, S., Abreo, E., Altier, N., Vázquez, Á. & Alborés, S. molecules Bioprospecting the Antibiofilm and Antimicrobial Activity of Soil and Insect Gut Bacteria. *MDPI Molecules* **27**, 1–13 (2022).
18. Ivanov, M. *et al.* Polyphenols as Inhibitors of Antibiotic Resistant Bacteria-Mechanisms Underlying Rutin Interference with Bacterial Virulence. *MDPI Pharmaceuticals* **15**, 1–21 (2022).
19. Majnooni, M. B. *et al.* and Antioxidant Activities of Flavonoids Isolated from *Allium colchicifolium* Leaves. *J Food Biochem* **1**, 1–14 (2023).
20. Cloudya Panjaitan, C., Sari Widyarman, A., Amtha, R., Erri Astoeti, T. & Widyarman, S. Antimicrobial and Antibiofilm Activity of Cinnamon (*Cinnamomum burmanii*) Extract on Periodontal Pathogens-An in vitro study. *Eur J Dent* **16**, (2022).
21. Shamim, A. *et al.* Natural Medicine a Promising Candidate in Combating Microbial Biofilm. *Antibiotics* **12**, 2–26 (2023).
22. Tasmia Asma, S. *et al.* Natural Strategies as Potential Weapons against Bacterial Biofilms. *Life MDPI* **12**, 1–35 (2022).
23. Liu, M. *et al.* Recent Advances in Nano-Drug Delivery Systems for the Treatment of Diabetic Wound Healing. *Int J Nanomedicine* 1537–1560 (2023) doi:10.2147/IJN.S395438.
24. Rather, M. A., Gupta, K. & Mandal, M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology* **1**, 1701–718 (2021).
25. Tasmia Asma, S. *et al.* An Overview of Biofilm Formation-Combating Strategies and Mechanisms of Action of Antibiofilm Agents. *MDPI Life* **1**, (2022).
26. Kreve, S. & Reis, A. C. D. Bacterial adhesion to biomaterials: What regulates this attachment? A review. *Japanese Dental Science Review* vol. 57 85–96 Preprint at <https://doi.org/10.1016/j.jdsr.2021.05.003> (2021).
27. Letek, M. *et al.* Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics on behalf of the Global AMR Insights Ambassador Network. *MDPI Antibiotics* **11**,.

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Type of Manuscript:
Research

Nanogel Formulations of Kratom (*Mitragyna speciosa*) Extract: A Promising Antibiofilm Agent for Diabetic Ulcer Infections

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ABSTRACT:

Diabetic ulcer infections present a substantial obstacle to wound management due to the prevalence of persistent bacterial biofilms that are impervious to conventional antibiotic therapies. Nanomaterials have emerged as a revolutionary platform for addressing the challenges of antibiotic resistance and biofilm-associated infections. Researchers have the potential to employ Kratom in the development of antibiofilm medicines specifically designed to combat diabetic ulcer infections. This is due to its capacity to impede bacterial quorum sensing and disrupt the process of biofilm development. Objective of this study is to determine the antibiofilm properties of the nanogel derived from kratom extract in diabetic ulcer infections. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (1%, 0.5%, 0.25%, 0.125% w/v) to identify their effects on *S. aureus*. The concentration of extract in nanogel used was 10, 20, and 30% to test antibiofilm in the mid-phase, maturation, and eradication phases. In the mid-phase biofilm, Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v exhibited significant antibiofilm efficacy against *Staphylococcus aureus* (SA), with an inhibition percentage of $79.21\% \pm 0.08$; maturation phase of $74.12\% \pm 0.101$; and eradication of $67.25\% \pm 0.046$. KDNE compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial and antibiofilm agents.

KEYWORDS: Nanogel, Kratom, Biofilm, Diabetic ulcers, Infection

INTRODUCTION:

Diabetic ulcer infections pose a significant challenge in wound management, as they are often associated with persistent bacterial biofilms that are resistant to conventional antibiotic treatments¹. Biofilms are intricate and ever-changing microbial colonies that flourish at the boundary layer between a solid surface and a liquid medium². The complex formations consist of microbial cells that are tightly integrated with one another and with the underlying surface, enclosed inside a self-generated matrix of extracellular polymeric compounds. Biofilm formation is a fundamental survival strategy used by many microorganisms to acclimatise to their habitat, especially under unfavourable circumstances³. Because of the rise of bacteria and viruses that are resistant to antibiotics, choosing the right empirical antibiotic coverage for diabetic ulcer infection therapy has become more challenging in recent years⁴.

To successfully address severe diseases caused by biofilms, new and innovative therapeutic techniques are required. Utilising nanogel formulations of naturally occurring substances with intrinsic antibiofilm characteristics⁵, like kratom extract, is one approach that shows promise. Mitragynine and 7-hydroxymitragynine are two of the active components found in kratom. These molecules target different types of bacteria, including those that cause infections in diabetic ulcers. Regardless, more *in vitro* and *in vivo* investigations are needed⁶. There has been a lot of buzz in the ever-evolving world of antimicrobial strategies about targeted and effective approaches to combat biofilm-related diseases. Biofilms are naturally resistant to conventional methods, such as antibiotic use. As a result, researchers are actively seeking novel approaches to this persistent issue, and they have turned their focus to the fascinating realm of nanotechnology⁷.

In their battle against bacterial infections, researchers have shown that nanomaterials and carriers on the nanoscale may kill bacterial cells, slow down quorum sensing, and prevent or eliminate biofilms⁸. Nanomaterials are a game-changer in the fight against biofilm-associated illnesses and antibiotic resistance. In instance, there are unique benefits to delivering natural chemicals with antibiofilm action via nanogel formulations⁹. There are a number of benefits to using nanogels, which are hydrogel nanoparticles, to reduce biofilms. It is easy for the hydrophilic nanocarriers to penetrate bacterial biofilms and disrupt their extracellular matrix. Because of this, antibiofilm medications may be delivered directly to bacterial cells¹⁰. Nanogels' unique properties, such as their scalability, surface charge, and sensitivity to environmental stimuli, might potentially enhance the efficacy of the encapsulated kratom extract¹¹.

Studies have demonstrated that nanogel formulations may prevent biofilm production, break existing biofilms, and improve the efficacy of conventional antibiotics in killing bacteria trapped in biofilms¹². A very promising treatment approach for diabetic ulcer infections, these nanogel formulations effectively target and dissolve bacterial biofilms¹³. In addition to increasing local antibiotic concentrations, nanogels' ability to transport active drugs straight to the infection site aids in the fight against antibiotic resistance by penetrating the protective biofilm matrix¹⁴. This focused strategy may help improve patient outcomes, lessen the likelihood of adverse effects, and lower the requirement for systemic antibiotics, which is particularly important in the treatment of diabetic foot

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ulcers caused by biofilm-associated infections¹⁵. The objective of this investigation is to ascertain the antibiofilm properties of the nanogel derived from kratom extract in the context of diabetic ulcer infections.

MATERIALS AND METHODS:

Sampling Place:

We collected samples of kratom leaves (*Mitragyna speciosa*) in the forest of Kuranji District, Tanah Bumbu Regency, South Kalimantan, Indonesia. The process of identifying plants was carried out at Universitas Lambung Mangkurat's Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Banjarmasin, Indonesia.

Materials:

Materials used were ethanol 95 % (Merck, Germany), ethyl acetate (Merck, Germany), chloramphenicol (Sigma-Aldrich, Germany), phosphate-buffered saline (PBS; Sigma—Aldrich), McFarland (HiMedia, India), CMC-Na (Merck, Germany), Oxoid (Brain Heart infusion) (Merck, Germany), crystal violet (Merck, Germany), Carbopol (Merck, Germany), Trietanolamine (Merck, Germany), Gliserol (Merck, Germany), Propilenglikol (Merck, Germany).

Equipment:

Micropipette (Gilson, France), microtiter plate reader (OIS 2100, Spain), tube (Socorex, Swiss), LAF (Sakura, Japan), incubator 2B (Sakura, Japan), Microplate flat-bottom polystyrene 96 well (Iwaki, Japan), spektro genesys (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), analytical balances (Ohaus, USA).

Bacterial strain:

ATCC 25923 strains of standard *Staphylococcus aureus* were cultivated in tryptic soy broth (TSB) medium and subjected to a 72-hour incubation period at 37°C.

Extraction and identification:

The Kratom leaf samples were cleansed by the process of rinsing with flowing water and meticulously inspecting for any contaminants. After washing, we sliced and desiccated the plant samples in an oven at 40°C for 3 hours. Sample standardisation is conducted using precise criteria. We accomplished the *Simplicia* extraction by fully immersing 300 g of powder in 96% ethanol. We agitate the mixture every 6 hours and replace the solvent every 24 hours, resulting in a total of 5 repetitions or changes of solvent. We subjected the liquid extract to evaporation in a water bath maintained at a temperature of 50°C until the weight reached a steady state. The levels of quinones, terpenoids, phenolics, saponins, steroids, alkaloids, and flavonoids were assessed using the tube technique.

Antibacterial effect of kratom extract against *staphylococcus aureus*:

The antibacterial efficacy was assessed by the use of microdilution and well techniques. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (0,125%, 0.25%, 0.5%, 0.1% w/v) to identify their effects on *S. aureus*. The positive control used was chloramphenicol. In order to get a concentration of 5x10⁶ colony forming units per millilitre (CFU/mL), the bacterial solution in BHI was first adjusted to 0.5 McFarland units and then diluted. The microplate was filled with a solution of ethanol with a concentration of 96% and subjected to incubation for a duration of 15 minutes. An ELISA Reader was employed to conduct optical density measurements at a wavelength of 570 nm.

Formulation of nanogel:

Carbopol and CMC were the two nanogel ingredients used as a standard gel base.

Table 1. Formulation of nanogel extract

No	Ingredients	F1 (g)	F2 (g)	F3 (g)
1	Carbopol 940	0,8	0,8	0,8
2	CMC-Na	0,6	0,6	0,6
3	Gliserol	1,25	1,25	1,25
4	Propilenglikol	1,75	1,75	1,75
5	Trietanolamin	1,5	1,5	1,5
6	Nanoemulsion extract	0,75	0,75	0,75
7	Aquadest ad	20	20	20
	Ingredient nanoemulsion			
1	Ekstrakt	10%	20%	30%
2	Etanol 96%	9 mL	9 mL	9 mL

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3	Tween 80	27,5 mL	27,5 mL	27,5 mL
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Inhibitory activity of kratom extract against *staphylococcus aureus* biofilms:

Biofilm formation was assessed during 24 hours (intermediate phase), 48 hours (maturation phase), and 72 hours (eradication) using the Microbroth Dilution Technique (MDT). The microtiter plates were rinsed with distilled water to remove detachable cells, and then allowed to desiccate in the surrounding air at room temperature for a period of 5 minutes. Each well was thereafter stained with a 125 µL portion of a 1% solution of crystal violet to identify living and non-viable cells, as well as any other components of the biofilm. Following a 15-minute incubation period at ambient temperature, we proceeded to rinse the plate with flowing water in order to eliminate the purple hue. The optical density (OD) was determined by adding 200 µL of 96% ethanol to each well and using a microplate reader with a 595 nm wavelength. The bacterial test culture, was deposited onto a microtiter plate including coverslips. In order to promote the biofilm development process, Subsequently, the plate was placed in an incubator that was maintained at a temperature of 37°C for a duration of 24-48 hours.

In vivo biofilm study

Adult rats (*Rattus norvegicus*) weighing 150–200 g were employed to conduct in vivo antibiofilm investigations. The animals were given unfettered access to water and nutritious food and were kept inside a controlled setting. In order to induce type II diabetes, a combination of streptozocin and nicotinamide was administered. The rats were administered a 240 mg/kg intraperitoneal dose of nicotinamide after a 12-hour fasting period. 15 minutes later, I.P. administration of streptozocin at a dosage of 100 mg/kg occurred. The animals were subjected to a 12-hour fast following the streptozocin treatment, and their fasting blood glucose levels were assessed 72 hours later. The investigation categorised animals as diabetic only if their fasting blood glucose level exceeded 150 mg/dL¹⁶. Positive, negative, and the maximum concentration of gel were the three categories into which the mice were divided. To prevent infection, the researchers made an open incision on the rodent with a diameter of 1 cm and administered bacteria. To serve as a positive control, chloramphenicol was administered daily to the lesion until it had fully healed.

SEM study:

Following a thorough washing with sterile distilled water, the coverslip was then subjected to glutaraldehyde during fixation. Methanol was used as a means of decreasing the water content, while the examination was conducted utilising a SEM (scanning electron microscope) functioned at a voltage of 10 kilovolts (kV).

RESULT:

Phytochemical screening

The extraction yielded a filtrate, which was further evaporated using a water bath at 50°C to give a concentrated extract with a consistent weight. The leaf yield was 18.8% with some phytochemical compounds in **Table 2**.

Table 2: Phytochemical compound of kratom extract.

No	Secondary metabolites	Result
1	Alkaloids (Dragendorff)	-
2	Alkaloids (Mayer)	-
3	Alkaloids (Wagner)	-
4	Steroids	-
5	Terpenoids	+
6	Tannin	-
7	Phenolic	+
8	Saponin	-
9	Flavonoids	+
10	Quinones	+

Antibacterial effect of kratom leave extract against *Staphylococcus aureus*:

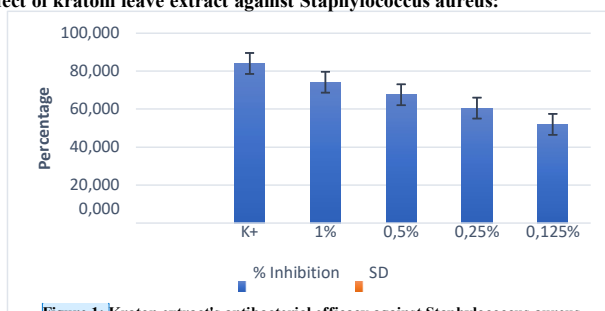


Figure 1: Kratom extract's antibacterial efficacy against *Staphylococcus aureus*

The results demonstrated that the concentration of lollipop flower extract affected its efficacy. At a 1% w/v concentration, the extract successfully suppressed SA growth, showing a substantial ($p < 0.05$) inhibition percentage of $74.226\% \pm 0.002$, notably different from the drug control's $84.097\% \pm 0.001$.

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KDNE's inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms:

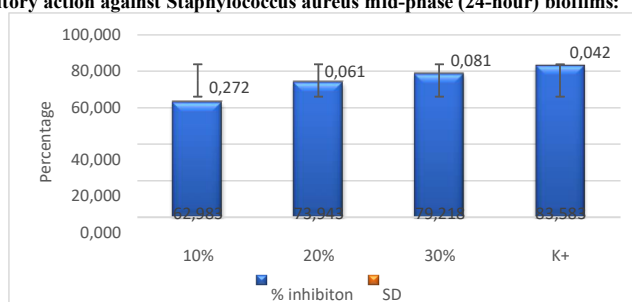


Figure 2. Percentage of inhibitory action against *staphylococcus aureus* mid-phase (24-hour) biofilms

Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v showed strong antibiofilm activity against *Staphylococcus aureus* (SA) during the mid-phase biofilm, with an inhibition percentage of $79.21\% \pm 0.08$. Notably, this inhibition was not significantly different ($p > 0.05$) from the control drug at a concentration of 1% w/v, which showed an inhibition percentage of $83.58\% \pm 0.042$, as depicted in Figure 2.

KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms:

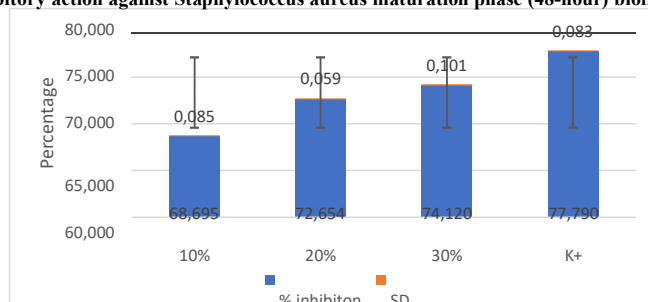


Figure 3. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms

During the biofilm maturation phase, KDNE showed an inhibitory activity of $74.12\% \pm 0.101$ at a concentration of 30% w/v. Interestingly, this result did not differ significantly ($p > 0.05$) from the inhibitory effectiveness of the pharmacological control, which showed a higher inhibitory effectiveness of $77.79\% \pm 0.083$. These data provide support for the idea that as the biofilm development duration increases, the arrangement of the matrix rises, resulting in a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms.

KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms:

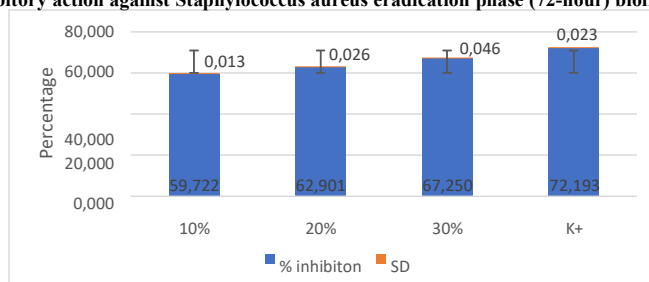


Figure 4. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms

The eradication-phase biofilm of *Staphylococcus aureus* (SA) was effectively inhibited by KDNE at a concentration of 30% w/v, exhibiting an inhibition percentage of $67.25\% \pm 0.046$. Figure 4 shows that at a dose of 1% w/v, the control medication exhibited an inhibition percentage of $72.912\% \pm 0.023$, and this inhibition was not substantially different ($p > 0.05$).

In vivo biofilm study



Figure 5. 5a. untreated group; 5b. Occurrence of Ulcers; 5c. the wound begins to close.

Diabetes is known to hinder the host's immune response and create an environment that encourages the growth of microorganisms. Numerous studies have highlighted the elevated risk of infections in diabetics, including rhinocerebral mucormycosis, malignant external otitis, and foot infections. There is evidence that diabetes-induced alterations in the microcirculatory system and disruption of wound healing processes contribute to this increased susceptibility. This study shows that nanogel treatment can improve wound healing in diabetic mice by increasing angiogenesis and reducing biofilm formation, which is not significantly different from the control group. The ulcers treated with nanogel closed and healed within 11 days, and the healing diameter was almost the same as the control group.

SEM Study:

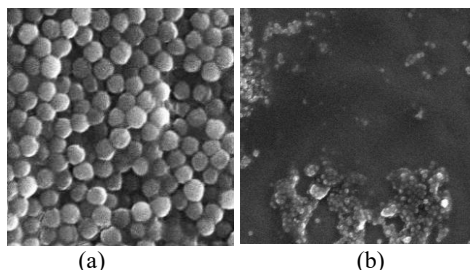


Figure 6: Figure 6a Result of SEM Biofilm with No Treatment, Figure 6b Result of SEM Biofilm with Administration of Kratom Extract 30% b/v.

Figure 6b shows that the SEM test showed that the KDNE effectively stopped and removed the *Staphylococcus aureus* (SA) biofilm. Figure 6a shows the results of the scanning electron microscopy investigation into the untreated SA biofilm, which reveals a compact cellular structure inside the extracellular polymeric substance (EPS) matrix and hence a safe habitat for the organism. Limitation, cell death, and EPS matrix damage were the outcomes of treating biofilm with a 30% w/v concentration of KDNE.

DISCUSSION:

Polyphenols also quinones, natural plant-derived compounds, have garnered significant attention for their remarkable antibiofilm properties. These molecules work in different ways to stop different stages of biofilm formation. For example, they stop cells from attaching at the start, stop the production of extracellular matrix, and mess up the signalling pathways for quorum sensing¹⁷. Flavonoids, tannins, and other phenolic compounds have been shown to be able to get into the biofilm matrix, cause oxidative stress, and damage the integrity of the microbiome cell membrane, which makes it harder for cells that are embedded in biofilm to stay alive. Similarly, studies have shown that quinones, a class of aromatic compounds, interfere with bacterial quorum sensing systems, crucial for coordinating biofilm development and maturation¹⁸. Because of their strong antibacterial and antibiofilm properties, flavonoids and quinones have attracted a lot of interest from antimicrobial researchers. Flavonoids have antibacterial properties because they disrupt bacterial membranes, inhibit bacterial nucleic acid replication, reduce bacterial efflux pump function, and block bacterial adenosine triphosphate synthase¹⁹. Offlavonoids are effective against bacteria because they may bind to extracellular proteins to create complex molecules that compromise bacterial cell membrane integrity. Quinones have antibacterial properties because they can create amino acid compounds that negatively impact protein function. When terpenoids bind to carbs and lipids in bacteria, they make their cell membranes less permeable²⁰.

Recent research has shown that kratom extracts may effectively hinder the development and disturb the structure of biofilms created by several harmful bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*²¹. Kratom has the potential to be used in the development of antibiofilm drugs that specifically target diabetic ulcer infections such as its ability to impede bacterial quorum sensing and disrupt biofilm formation²². By incorporating these bioactive molecules into nanogel formulations, their stability, solubility, and targeted delivery to the infection site may be improved²³. Attachment of microbial cells to a surface is the first step in the multi-stage process that culminates in the detachment of cells from the biofilm after microcolonies have developed and the biofilm structure has matured²⁴. This data implies that it is more problematic to suppress bacteria during biofilm development, since the inhibition percentage is lower compared to bacteria in planktonic form. For bacteria in planktonic form, KDNE had a substantial inhibitory effect of $83.58\% \pm 0.41$; for bacteria that had developed a biofilm during the mid-phase, the inhibitory effect was somewhat lower, at $79.21\% \pm 0.8$. These data support the idea that as the duration of biofilm development increases, there is a rise in the arrangement of the matrix, leading to a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms²⁵.

The physicochemical properties of nanomaterials, including hydrophobicity and pore size, can be regulated to optimise their antimicrobial properties. Researchers have reported that increasing the nanoroughness of the surface reduces bacterial adhesion to medical devices, implying the development of nanogel formulations to curb biofilm formation and bacterial attachment²⁶. Nanogel formulations can also work as nanovehicles, making it easier to deliver naturally occurring compounds that can break down biofilms. Nanogel forms of kratom extract have been shown in new research to effectively stop the growth of biofilms made by many bacteria that are clinically important, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As a result, the active chemicals might be more effectively administered locally at the site of infection, which could lessen the likelihood of systemic bad effects associated with traditional antibiotic treatments²⁷.

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CONCLUSION:

The results indicate that the activity of KDNE in inhibiting the growth of *Staphylococcus aureus* biofilms varied with its concentration. At a concentration of 30%, the extract exhibited equivalent antibacterial, mid-phase, maturation, and eradication efficacy to the control. Plant-derived compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial agents.

CONFLICT OF INTEREST:

Regarding this study, the writers do not have any competing interests.

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REFERENCES:

1. Jang, E. J., Patel, R. & Patel, M. Electrospinning Nanofibers as a Dressing to Treat Diabetic Wounds. *Pharmaceutics* **12**, 1 (2023).
2. Li, H. *et al.* Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics* **11**, 1–45 (2022).
3. Rather, M. A., Gupta, K. & Mandal, M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology* **1**, 1701–1718 (2021).
4. Pessoa e Costa, T. *et al.* Multidrug-resistant bacteria in diabetic foot infections: Experience from a portuguese tertiary centre. *Int Wound J* **17**, 1835–1839 (2020).
5. Kumar Bari, A., Sandeep Belalekar, T., Poojary, A. & Rohra, S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. *Front Cell Infect Microbiol* **7** (2023) doi:10.3389/fcimb.2023.1155699.
6. Syahida, N. & Ma, N. '. Indonesian Journal of Chemical Science Kratom (*Mitragyna speciosa*): Medicinal Marvel or Menace? Assessing Potency, Risk, and Future Prospect of Herbal Medicine. *J. Chem. Sci* **13**, (2024).
7. Kishore, M. Y. *et al.* Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol* **1**, 1–30 (2023).
8. Mukherjee, A., Bose, S., Shaoo, A. & Das, S. K. Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. *Royal Society of Chemistry* **4**, 2544–2572 (2023).
9. Ramadan, Y. F. ; N. ; *et al.* Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicine* **413**, (2023).
10. Sookkhee, S. *et al.* Synergistic Effects of Some Methoxyflavones Extracted from Rhizome of *Kaempferia parviflora* Combined with Gentamicin against Carbapenem-Resistant Strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *MDPI Plants* **11**, 16 (2022).
11. Kishore Mohanta, Y. *et al.* Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol* **1**–30 (2023) doi:10.3389/fmicb.2022.1028086.
12. Varma, A. *et al.* The role of nanocomposites against biofilm infections in humans. *Front Cell Infect Microbiol* **1**–16 (2023) doi:10.3389/fcimb.2023.1104615.
13. Arunachalam, K. *et al.* Exploring Possible Ways to Enhance the Potential and Use of Natural Products through Nanotechnology in the Battle against Biofilms of Foodborne Bacterial Pathogens. *MDPI Pathogens* **1** (2023) doi:10.3390/pathogens12020270.
14. Mirghani, R. *et al.* Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology* vol. 8 240–278 Preprint at <https://doi.org/10.3934/microbiol.2022019> (2022).
15. Tuon, F. F. *et al.* Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics* vol. 12 Preprint at <https://doi.org/10.3390/antibiotics12010087> (2023).
16. Wu, X. *et al.* *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm* **7**, 100175 (2024).
17. Raffaelli, S., Abreo, E., Altier, N., Vázquez, Á. & Alborés, S. molecules Bioprospecting the Antibiofilm and Antimicrobial Activity of Soil and Insect Gut Bacteria. *MDPI Molecules* **27**, 1–13 (2022).
18. Ivanov, M. *et al.* Polyphenols as Inhibitors of Antibiotic Resistant Bacteria-Mechanisms Underlying Rutin Interference with Bacterial Virulence. *MDPI Pharmaceutics* **15**, 1–21 (2022).
19. Majnooni, M. B. *et al.* and Antioxidant Activities of Flavonoids Isolated from *Allium colchicifolium* Leaves. *J Food Biochem* **1**, 1–14 (2023).
20. Cloudya Panjaitan, C., Sari Widyarman, A., Amtha, R., Erri Astoeti, T. & Widyarman, S. Antimicrobial and Antibiofilm Activity of Cinnamon (*Cinnamomum burmanii*) Extract on Periodontal Pathogens-An in vitro study. *Eur J Dent* **16**, (2022).
21. Shamim, A. *et al.* Natural Medicine a Promising Candidate in Combating Microbial Biofilm. *Antibiotics* **12**, 2–26 (2023).
22. Tasmia Asma, S. *et al.* Natural Strategies as Potential Weapons against Bacterial Biofilms. *Life MDPI* **12**, 1–35 (2022).
23. Liu, M. *et al.* Recent Advances in Nano-Drug Delivery Systems for the Treatment of Diabetic Wound Healing. *Int J Nanomedicine* 1537–1560 (2023) doi:10.2147/IJN.S395438.
24. Rather, M. A., Gupta, K. & Mandal, M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology* **1**, 1701–1718 (2021).
25. Tasmia Asma, S. *et al.* An Overview of Biofilm Formation-Combating Strategies and Mechanisms of Action of Antibiofilm Agents. *MDPI Life* **1**, (2022).
26. Kreve, S. & Reis, A. C. D. Bacterial adhesion to biomaterials: What regulates this attachment? A review. *Japanese Dental Science Review* vol. 57 85–96 Preprint at <https://doi.org/10.1016/j.jdsr.2021.05.003> (2021).
27. Letek, M. *et al.* Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics on behalf of the Global AMR Insights Ambassador Network. *MDPI Antibiotics* **11**,.

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Table 1	Left	Formulation of nanogel	-	Original table format from submitted manuscript	Table layout adjusted to journal formatting requirements
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RESEARCH ARTICLE

Nanogel Formulations of Kratom (*Mitragyna speciosa*) Extract: A Promising Antibiofilm agent for Diabetic Ulcer Infections

**Deni Setiawan^{1*}, Samsul Hadi¹, Nurul Mardiaty¹, Nur Mahdi¹, Amalia Khairunnisa¹,
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ABSTRACT:

Diabetic ulcer infections present a substantial obstacle to wound management due to the prevalence of persistent bacterial biofilms that are impervious to conventional antibiotic therapies. Nanomaterials have emerged as a revolutionary platform for addressing the challenges of antibiotic resistance and biofilm-associated infections. Researchers have the potential to employ Kratom in the development of antibiofilm medicines specifically designed to combat diabetic ulcer infections. This is due to its capacity to impede bacterial quorum sensing and disrupt the process of biofilm development. Objective of this study is to determine the antibiofilm properties of the nanogel derived from kratom extract in diabetic ulcer infections. The study was carried out on a 96-well flatbottom polystyrene microtiter plate using various doses of test chemicals (1%, 0.5%, 0.25%, 0.125% w/v) to identify their effects on *S. aureus*. The concentration of extract in nanogel used was 10, 20, and 30% to test antibiofilm in the mid-phase, maturation, and eradication phases. In the mid-phase biofilm, Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v exhibited significant antibiofilm efficacy against *Staphylococcus aureus* (SA), with an inhibition percentage of $79.21\% \pm 0.08$; maturation phase of $74.12\% \pm 0.101$; and eradication of $67.25\% \pm 0.046$. KDNE compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial and antibiofilm agents.

KEYWORDS: Nanogel, Kratom, Biofilm, Diabetic ulcers, Infection.

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INTRODUCTION:

Diabetic ulcer infections pose a significant challenge in wound management, as they are often associated with persistent bacterial biofilms that are resistant to conventional antibiotic treatments¹. Biofilms are intricate and ever-changing microbial colonies that flourish at the boundary layer between a solid surface and a liquid medium². The complex formations consist of microbial cells that are tightly integrated with one another and with the underlying surface, enclosed inside a self-generated matrix of extracellular polymeric compounds³. Biofilm formation is a fundamental survival strategy used by many microorganisms to acclimatise to their habitat, especially under unfavourable circumstances⁴. Because of the rise of bacteria and viruses that are resistant to antibiotics, choosing the right empirical antibiotic coverage for diabetic ulcer infection therapy has become more challenging in recent years⁵.

To successfully address severe diseases caused by biofilms, new and innovative therapeutic techniques are required⁶. Utilising nanogel formulations of naturally occurring substances with intrinsic antibiofilm characteristics⁷, like kratom extract, is one approach that shows promise. Mitragynine and 7-hydroxymitragynine are two of the active components found in kratom. These molecules target different types of bacteria, including those that cause infections in diabetic ulcers. Regardless, more in vitro and in vivo investigations are needed⁸. There has been a lot of buzz in the everevolving world of antimicrobial strategies about targeted and effective approaches to combat biofilm-related diseases⁹. Biofilms are naturally resistant to conventional methods, such as antibiotic use. As a result, researchers are actively seeking novel approaches to this persistent issue, and they have turned their focus to the fascinating realm of nanotechnology¹⁰.

In their battle against bacterial infections, researchers have shown that nanomaterials and carriers on the nanoscale may kill bacterial cells, slow down quorum sensing, and prevent or eliminate biofilms¹¹. Nanomaterials are a game-changer in the fight against biofilm-associated illnesses and antibiotic resistance. In instance, there are unique benefits to delivering natural chemicals with antibiofilm action via nanogel formulations¹². There are a number of benefits to using nanogels, which are hydrogel nanoparticles, to reduce biofilms¹³. It is easy for the hydrophilic nanocarriers to

penetrate bacterial biofilms and disrupt their extracellular matrix. Because of this, antibiofilm medications may be delivered directly to bacterial cells¹⁴. Nanogels' unique properties, such as their scalability, surface charge, and sensitivity to environmental stimuli, might potentially enhance the efficacy of the encapsulated kratom extract¹⁵.

Studies have demonstrated that nanogel formulations may prevent biofilm production, break existing biofilms, and improve the efficacy of conventional antibiotics in killing bacteria trapped in biofilms¹⁶. A very promising treatment approach for diabetic ulcer infections, these nanogel formulations effectively target and dissolve bacterial biofilms¹⁷. In addition to increasing local antibiotic concentrations, nanogels' ability to transport active drugs straight to the infection site aids in the fight against antibiotic resistance by penetrating the protective biofilm matrix¹⁸. This focused strategy may help improve patient outcomes, lessen the likelihood of adverse effects, and lower the requirement for systemic antibiotics, which is particularly important in the treatment of diabetic foot ulcers caused by biofilm-associated infections¹⁹. The objective of this investigation is to ascertain the antibiofilm properties of the nanogel derived from kratom extract in the context of diabetic ulcer infections.

MATERIALS AND METHODS:

Sampling Place:

We collected samples of kratom leaves (*Mitragyna speciosa*) in the forest of Kuranji District, Tanah Bumbu Regency, South Kalimantan, Indonesia. The process of identifying plants was carried out at Universitas Lambung Mangkurat's Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Banjarmasin, Indonesia.

Materials:

Materials used were ethanol 95% (Merck, Germany), ethyl acetate (Merck, Germany), chloramphenicol (Sigma-Aldrich, Germany), phosphate-buffered saline (PBS; Sigma—Aldrich), McFarland (HiMedia, India), CMC-Na (Merck, Germany), Oxoid (Brain Heart infusion) (Merck, Germany), crystal violet (Merck, Germany), Carbopol (Merck, Germany), Trietanolamine (Merck, Germany), Gliserol (Merck, Germany), Propilenglikol (Merck, Germany).

Equipment:

Micropipette (Gilson, France), microtiter plate reader

(OIS 2100, Spain), tube (Socorex, Swiss), LAF (Sakura, Japan), incubator 2B (Sakura, Japan), Microplate flatbottom polystyrene 96 well (Iwaki, Japan), spektro genesys (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), analytical balances (Ohaus, USA).

Bacterial strain:

ATCC 25923 strains of standard *Staphylococcus aureus* were cultivated in tryptic soy broth (TSB) medium and subjected to a 72-hour incubation period at 37°C.

Extraction and identification:

The Kratom leaf samples were cleansed by the process of rinsing with flowing water and meticulously inspecting for any contaminants. After washing, we sliced and desiccated the plant samples in an oven at 40°C for 3 hours. Sample standardisation is conducted using precise criteria. We accomplished the Simplicia extraction by fully immersing 300g of powder in 96% ethanol. We agitate the mixture every 6 hours and replace the solvent every 24 hours, resulting in a total of 5 repetitions or changes of solvent. We subjected the liquid extract to evaporation in a water bath maintained at a temperature of 50°C until the weight reached a steady state. The levels of quinones, terpenoids, phenolics, saponins, steroids, alkaloids, and flavonoids were assessed using the tube technique.

Antibacterial effect of kratom extract against *staphylococcus aureus*:

The antibacterial efficacy was assessed by the use of microdilution and well techniques. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (0,125%, 0.25%, 0.5%, 0.1% w/v) to identify their effects on *S. aureus*. The positive control used was chloramphenicol. In order to get a concentration of 5×10^6 colony forming units per millilitre (CFU/mL), the bacterial solution in BHI was first adjusted to 0.5 McFarland units and then diluted. The microplate was filled with a solution of ethanol with a concentration of 96% and subjected to incubation for a duration of 15 minutes. An ELISA Reader was employed to conduct optical density measurements at a wavelength of 570 nm.

Formulation of nanogel:

Carbopol and CMC were the two nanogel ingredients used as a standard gel base.

Table 1. Formulation of nanogel extract

No	Ingredients	F1 (g)	F2 (g)	F3 (g)
1	Carbopol 940	0,8	0,8	0,8
2	CMC-Na	0,6	0,6	0,6
3	Gliserol	1,25	1,25	1,25
4	Propilenglikol	1,75	1,75	1,75
5	Trietanolamin	1,5	1,5	1,5
6	Nanoemulsion extract	0,75	0,75	0,75
7	Aquadest ad	20	20	20
	Ingredient nanoemulsion			
1	Ekstrakt	10%	20%	30%
2	Etanol 96%	9 mL	9 mL	9 mL
3	Tween 80	27,5 mL	27,5 mL	27,5 mL

Inhibitory activity of kratom extract against *staphylococcus aureus* biofilms:

Biofilm formation was assessed during 24 hours (intermediate phase), 48 hours (maturation phase), and 72 hours (eradication) using the Microbroth Dilution Technique (MDT). The microtiter plates were rinsed with distilled water to remove detachable cells, and then allowed to desiccate in the surrounding air at room temperature for a period of 5 minutes. Each well was thereafter stained with a 125 µL portion of a 1% solution of crystal violet to identify living and non-viable cells, as well

as any other components of the biofilm. Following a 15-minute incubation period at ambient temperature, we proceeded to rinse the plate with flowing water in order to eliminate the purple hue. The optical density (OD) was determined by adding 200µL of 96% ethanol to each well and using a microplate reader with a 595nm wavelength. The bacterial test culture, was deposited onto a microtiter plate including coverslips. In order to promote the biofilm development process, Subsequently, the plate was placed in an incubator that was maintained at a temperature of 37°C for a duration of 24-48hours.

***In vivo* biofilm study:**

Adult rats (*Rattus norvegicus*) weighing 150–200g were employed to conduct in vivo antibiofilm investigations. The animals were given unfettered access to water and nutritious food and were kept inside a controlled setting. In order to induce type II diabetes, a combination of streptozocin and nicotinamide was administered. The rats were administered a 240mg/kg intraperitoneal dose of nicotinamide after a 12hour fasting period. 15 minutes later, I.P. administration of streptozocin at a dosage of 100mg/kg occurred. The animals were subjected to a 12hour fast following the streptozocin treatment, and their fasting blood glucose levels were assessed 72hours later. The investigation categorised animals as diabetic only if their fasting blood glucose level exceeded 150mg/dL²⁰. Positive, negative, and the maximum concentration of gel were the three categories into which the mice were divided. To prevent infection, the researchers made an open incision on the rodent with a diameter of 1cm and administered bacteria. To serve as a positive control, chloramphenicol was administered daily to the lesion until it had fully healed.

SEM study:

Following a thorough washing with sterile distilled water, the coverslip was then subjected to glutaraldehyde during fixation. Methanol was used as a means of decreasing the water content, while the examination was conducted utilising a SEM (scanning electron microscope) functioned at a voltage of 10 kilovolts (kV).

RESULT:

Phytochemical screening

The extraction yielded a filtrate, which was further evaporated using a water bath at 50°C to give a concentrated extract with a consistent weight. The leaf yield was 18.8% with some phytochemical compounds in Table 2.

Table 2: Phytochemical compound of kratom extract.

S. No.	Secondary metabolites	Result
1	Alkaloids (Dragendorff)	-
2	Alkaloids (Mayer)	-
3	Alkaloids (Wagner)	-
4	Steroids	-
5	Terpenoids	+
6	Tannin	-
7	Phenolic	+
8	Saponin	-

9	Flavonoids	+
10	Quinones	+

Antibacterial effect of kratom leave extract against *Staphylococcus aureus*:

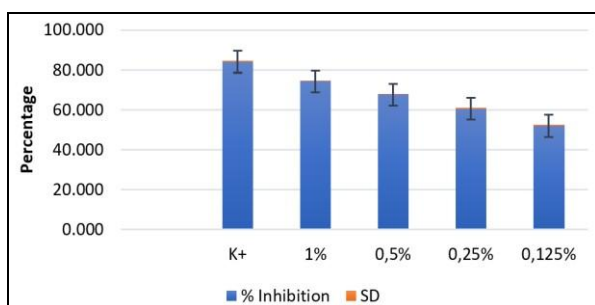


Figure 1: Kraton extract's antibacterial efficacy against *Staphylococcus aureus*

Figure 2. Percentage of inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms

Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v showed strong antibiofilm activity against *Staphylococcus aureus* (SA) during the mid-phase biofilm, with an inhibition percentage of $79.21\% \pm 0.08$. Notably, this inhibition was not significantly different ($p > 0.05$) from the control drug at a concentration of 1% w/v, which showed an inhibition percentage of $83.58\% \pm 0.042$, as depicted in Figure 2.

KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms:

Figure 3. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms

During the biofilm maturation phase, KDNE showed an inhibitory activity of $74.12\% \pm 0.101$ at a concentration of 30% w/v. Interestingly, this result did not differ significantly ($p > 0.05$) from the inhibitory effectiveness of the pharmacological control, which showed a higher inhibitory effectiveness of $77.79\% \pm 0.083$. These data provide support for the idea that as the biofilm development duration increases, the arrangement of the matrix rises, resulting in a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms²¹.

The results demonstrated that the concentration of lollipop flower extract affected its efficacy. At a 1% w/v concentration, the extract successfully suppressed SA growth, showing a substantial ($p < 0.05$) inhibition percentage of $74.226\% \pm 0.002$, notably different from the drug control's $84.097\% \pm 0.01$.

KDNE's inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms:

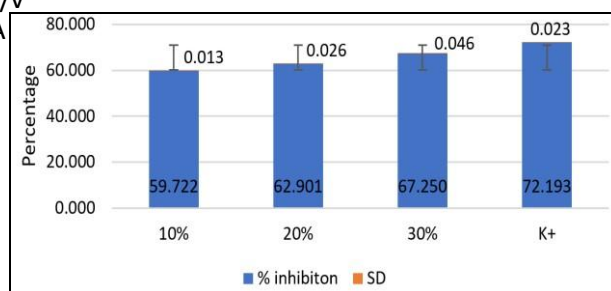
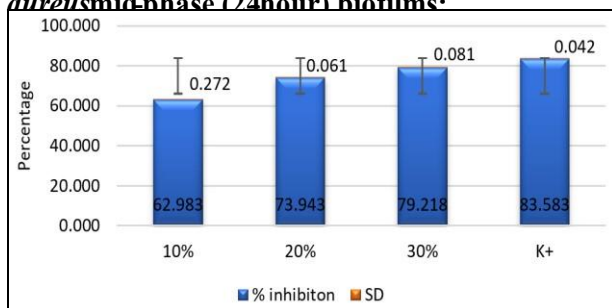


Figure 4. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms

The eradication phase biofilm of *Staphylococcus aureus* (SA) was effectively inhibited by KDNE at a concentration of 30% w/v, exhibiting an inhibition percentage of $67.25\% \pm 0.046$. Figure 4 shows that at a dose of 1% w/v, the control medication exhibited an inhibition percentage of $72.912\% \pm 0.023$, and this inhibition was not substantially different ($p > 0.05$).

In vivo biofilm study:

KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms:

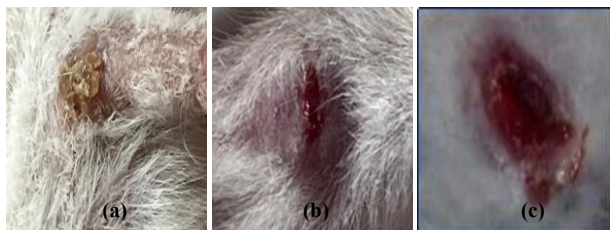
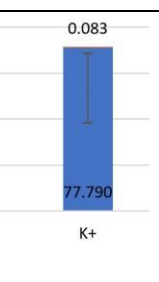


Figure 5. 5a. untreated group; 5b. Occurrence of Ulcers; 5c. the wound begins to close.



Diabetes is known to hinder the host's immune response and create an environment that encourages the growth of microorganisms. Numerous studies have highlighted the elevated risk of infections in diabetics, including rhinocerebral mucormycosis, malignant external otitis, and foot infections. There is evidence that diabetes-induced alterations in the microcirculatory system and disruption of wound healing processes contribute to this increased susceptibility²². This study shows that nanogel treatment can improve wound healing in diabetic mice by increasing angiogenesis and reducing biofilm formation, which is not significantly different from the control group. The ulcers treated with nanogel closed and healed within 11 days, and the healing diameter was almost the same as the control group.

SEM Study:

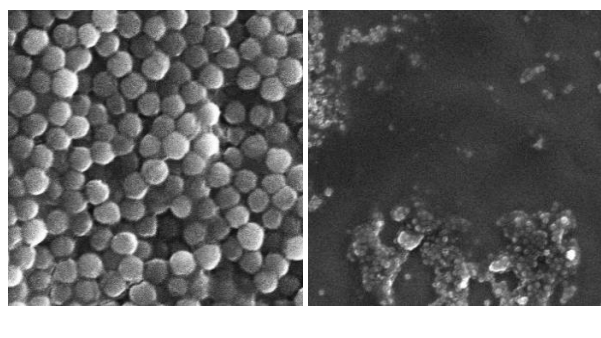


Figure 6: Figure 6a Result of SEM Biofilm with No Treatment, Figure 6b Result of SEM Biofilm with Administration of Kratom Extract 30% b/v.

Figure 6b shows that the SEM test showed that the KDNE effectively stopped and removed the *Staphylococcus aureus* (SA) biofilm. Figure 6a shows the results of the scanning electron microscopy investigation into the untreated SA biofilm, which reveals a compact cellular structure inside the extracellular polymeric substance (EPS) matrix and hence a safe habitat for the organism. Limitation, cell death, and EPS matrix damage were the outcomes of treating biofilm with a 30% w/v concentration of KDNE.

DISCUSSION:

Polyphenols also quinones, natural plant-derived compounds, have garnered significant attention for their remarkable antibiofilm properties. These molecules work in different ways to stop different stages of biofilm formation²³. For example, they stop cells from attaching at the start, stop the production of extracellular matrix, and mess up the signalling pathways for quorum sensing²⁴. Flavonoids, tannins, and other phenolic compounds have been shown to be able to get into the biofilm matrix, cause oxidative stress, and damage the integrity of the microbiome cell membrane, which makes it harder for cells that are embedded in biofilm to stay alive²⁵. Similarly, studies have shown that quinones, a class of aromatic compounds, interfere with bacterial quorum sensing systems, crucial for coordinating biofilm development and maturation²⁶. Because of their strong

antibacterial and antibiofilm properties, flavonoids and quinones have attracted a lot of interest from antimicrobial researchers. Flavonoids have antibacterial properties because they disrupt bacterial membranes, inhibit bacterial nucleic acid replication, reduce bacterial efflux pump function, and block bacterial adenosine triphosphate synthase²⁷.

Offlavonoids are effective against bacteria because they may bind to extracellular proteins to create complex molecules that compromise bacterial cell membrane integrity²⁸. Quinones have antibacterial properties because they can create amino acid compounds that negatively impact protein function. When terpenoids bind to carbs and lipids in bacteria, they make their cell membranes less permeable²⁹.

Recent research has shown that kratom extracts may effectively hinder the development and disturb the structure of biofilms created by several harmful bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*³⁰. Kratom has the potential to be used in the development of antibiofilm drugs that specifically target diabetic ulcer infections such as its ability to impede bacterial quorum sensing and disrupt biofilm formation³¹. By incorporating these bioactive molecules into nanogel formulations, their stability, solubility, and targeted delivery to the infection site may be improved³². Attachment of microbial cells to a surface is the first step in the multi-stage process that culminates in the detachment of cells from the biofilm after microcolonies have developed and the biofilm structure has matured³³. This data implies that it is more problematic to suppress bacteria during biofilm development, since the inhibition percentage is lower compared to bacteria in planktonic form³⁴. For bacteria in planktonic form, KDNE had a substantial inhibitory effect of 83.58% $\pm$ 0.41; for bacteria that had developed a biofilm during the mid-phase, the inhibitory effect was somewhat lower, at 79.21% $\pm$ 0.8. These data support the idea that as the duration of biofilm development increases, there is a rise in the arrangement of the matrix, leading to a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms³⁵.

The physicochemical properties of nanomaterials, including hydrophobicity and pore size, can be regulated to optimise their antimicrobial properties. Researchers have reported that increasing the nanoroughness of the surface reduces bacterial adhesion to medical devices, implying the development of nanogel formulations to curb biofilm formation and bacterial attachment³⁶. Nanogel formulations can also work as nanovehicles, making it easier to deliver naturally occurring compounds that can break down biofilms. Nanogel forms of kratom extract have been shown in new research to effectively stop the growth of biofilms made by many bacteria that are clinically important, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As a result, the active chemicals might be more effectively administered locally at the site of infection, which could lessen the likelihood of systemic bad effects associated with traditional antibiotic treatments³⁷.

CONCLUSION:

The results indicate that the activity of KDNE in inhibiting the growth of *Staphylococcus aureus* biofilms varied with its concentration. At a concentration of 30%, the extract exhibited equivalent antibacterial, mid-phase, maturation, and eradication efficacy to the control. Plant-derived

compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial agents.

CONFLICT OF INTEREST:

Regarding this study, the writers do not have any competing interests.

ACKNOWLEDGMENTS:

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REFERENCES:

1. Jang EJ, Patel R, Patel M. Electrospinning Nanofibers as a Dressing to Treat Diabetic Wounds. *Pharmaceutics*. 2023; 12: 1. doi:10.3390/pharmaceutics15041144
2. Li H, Mavumengwana V, Gan RY, et al. Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics*. 2022; 11(108): 1-45. doi:10.3390/antibiotics11010108
3. Deepigaa M. Antibacterial Resistance of Bacteria in Biofilms. *Res J Pharm Technol*. 2017; 10(11): 4019. doi:10.5958/0974-360X.2017.00728.4
4. Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology*. 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
5. Pessoa e Costa T, Duarte B, João AL, et al. Multidrug-resistant bacteria in diabetic foot infections: Experience from a portuguese tertiary centre. *Int Wound J*. 2020; 17(6): 1835-1839. doi:10.1111/iwj.13473
6. Pushpam AC, Karthik R, Chelvan Y, Ramalingam K, Vanitha MC. Evaluation of the Antibiofilm Properties of *Arthrobacter defluvii* AMET1677 Strain Isolated from Shrimp Pond Sediment against Marine Biofilm Forming Bacteria. *Res J Pharm Technol*. 2016; 9(4): 373. doi:10.5958/0974-360X.2016.00067.6
7. Kumar Bari A, Sandeep Belalekar T, Poojary A, Rohra S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. *Front Cell Infect Microbiol*. 2023; (4): 7. doi:10.3389/fcimb.2023.1155699
8. Syahida N, Ma N'. Indonesian Journal of Chemical Science Kratom (*Mitragyna speciosa*): Medicinal Marvel or Menace? Assessing Potency, Risk, and Future Prospect of Herbal Medicine. *J Chem Sci*. 2024; 13(1). <http://journal.unnes.ac.id/sju/index.php/ijcs>
9. Selvi KV, Hemalatha M, Rajeshkumar S. Analysis of bioactive compounds from *Spirulina gessneri* and studying its Antibacterial activity against wound Infection causing pathogens. *Res J Pharm Technol*. 2017; 10(6): 1983. doi:10.5958/0974-360X.2017.00347.X
10. Kishore MY, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol*. 2023; 1(3): 1-30. doi:10.3389/fmicb.2022.1028086
11. Mukherjee A, Bose S, Shao A, Das SK. Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. *Royal Society of Chemistry*. 2023; 4(4): 2544-2572. doi:10.1039/d2ma00846g
12. Ramadan YF; N;, Al-Harbi AI; A, Ahmed E;, et al. Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicine*. 2023; 413(11). doi:10.3390/biomedicine11020413
13. Umamageswari SSM, Manipriya B, Kalyani M. Evaluation of antibacterial activity of zinc oxide nanoparticles against biofilm producing methicillin resistant *Staphylococcus aureus* (MRSA). *Res J Pharm Technol*. 2018; 11(5): 1884. doi:10.5958/0974-360X.2018.00350.5
14. Sookkhee S, Sakonwasun C, Mungkornasawakul P, Khamnoi P, Wikan N, Nimlamool W. Synergistic Effects of Some Methoxyflavones Extracted from Rhizome of *Kaempferia parviflora* Combined with Gentamicin against Carbapenem Resistant Strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *MDPI Plants*. 2022; 11(3128): 16. doi:10.3390/plants11223128

15. Kishore Mohanta Y, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol.* 2023; (3): 1-30. doi:10.3389/fmicb.2022.1028086
16. Varma A, Warghane A, Dhiman NK, et al. The role of nanocomposites against biofilm infections in humans. *Front Cell Infect Microbiol.* 2023; (2): 1-16. doi:10.3389/fcimb.2023.1104615
17. Arunachalam K, Krishnan GP, Sethuraman S, et al. Exploring Possible Ways to Enhance the Potential and Use of Natural Products through Nanotechnology in the Battle against Biofilms of Foodborne Bacterial Pathogens. *MDPI Pathogens.* 2023; (270): 1. doi:10.3390/pathogens12020270
18. Mirghani R, Saba T, Khaliq H, et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiol.* 2022; 8(3): 240-278. doi:10.3934/microbiol.2022019
19. Tuon FF, Suss PH, Telles JP, Dantas LR, Borges NH, Ribeiro VST. Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics.* 2023; 12(1). doi:10.3390/antibiotics12010087
20. Wu X, Wang H, Xiong J, et al. *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm.* 2024; 7: 100175. doi:10.1016/J.BIOFLM.2023.100175
21. Mary RNI, Banu N. Inhibition of Antibiofilm Mediated Virulence Factors in *Pseudomonas aeruginosa* by *Andrographis paniculata*. *Res J Pharm Technol.* 2017; 10(1): 141. doi:10.5958/0974-360X.2017.00031.2
22. Dewangan D, Ajazuddin, Agrawal P, et al. Formulation and Evaluation of oral reconstitutable Azithromycin Suspension for the treatment of Bacterial Infection. *Res J Pharm Technol.* 2018; 11(4): 1351. doi:10.5958/0974-360X.2018.00251.2
23. Karpagam P, Manonmani G, Brindha D. Phytochemical Screening, Antimicrobial Activity and Antimicrobial Finishing of Polyherbal Extract on Nonwoven Wound Dressing. *Res J Pharm Technol.* 2019; 12(2): 632. doi:10.5958/0974-360X.2019.00112.4
24. Raffaelli S, Abreo E, Altier N, Vázquez Á, Alborés S. molecules Bioprospecting the Antibiofilm and Antimicrobial Activity of Soil and Insect Gut Bacteria. *MDPI Molecules.* 2022; 27(2002): 1-13. doi:10.3390/molecules27062002
25. Abdul-Lateef LA, Hassan E, Taha Abd-Alla Bactash E. Effect of Cranberry on biofilm formation by *P. mirabilis* isolated from Patients Suffering from Urinary tract Infections. *Res J Pharm Technol.* 2018; 11(3): 1097. doi:10.5958/0974-360X.2018.00206.8
26. Ivanov M, Novovi'cnovi'c K, Malešević M, et al. Polyphenols as Inhibitors of Antibiotic Resistant Bacteria Mechanisms Underlying Rutin Interference with Bacterial Virulence. *MDPI Pharmaceuticals.* 2022; 15(385): 1-21. doi:10.3390/ph15030385
27. Majnooni MB, Ghanadian SM, Mojarrab M, et al. and Antioxidant Activities of Flavonoids Isolated from *Allium colchicifolium* Leaves. *J Food Biochem.* 2023; 1(14): 1-14. doi:10.1155/2023/5521661
28. Mary RNI, Banu N. Inhibition of biofilm formation in *Serratia marcescens* by *Andrographolide* from *Andrographis paniculata*. *Res J Pharm Technol.* 2017; 10(3): 789. doi:10.5958/0974-360X.2017.00148.2
29. Cloudya Panjaitan C, Sari Widayrman A, Amtha R, Erri Astoeti T, Widayrman S. Antimicrobial and Antibiofilm Activity of Cinnamon (*Cinnamomum burmanii*) Extract on Periodontal Pathogens-An in vitro study. *Eur J Dent.* 2022; 16(4). doi:10.1055/s-0041-1742125
30. Shamim A, Ali A, Iqbal Z, et al. Natural Medicine a Promising Candidate in Combating Microbial Biofilm. *Antibiotics.* 2023; 12(299): 2-26. doi:10.3390/antibiotics12020299
31. Tasmia Asma S, Imre K, Morar A, et al. Natural Strategies as Potential Weapons against Bacterial Biofilms. *Life MDPI.* 2022; 12(1618): 1-35. doi:10.3390/life12101618
32. Liu M, Wei X, Zheng Z, et al. Recent Advances in Nano-Drug Delivery Systems for the Treatment of Diabetic Wound Healing. *Int J Nanomedicine.* 2023; (3): 1537-1560. doi:10.2147/IJN.S395438
33. Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology.* 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
34. Bhavani G, P G. Detection of biofilm among clinical isolates of *Acinetobacter baumannii* by tissue culture plate method (TCP). *Res J Pharm Technol.* 2016; 9(10): 1635. doi:10.5958/0974-

360X.2016.00327.9

35. Tasmia Asma S, Imre K, Morar A, et al. An Overview of Biofilm Formation-Combating Strategies and Mechanisms of Action of Antibiofilm Agents. MDPI Life. 2022; 1(1110). doi:10.3390/life12081110
36. Kreve S, Reis ACD. Bacterial adhesion to biomaterials: What regulates this attachment? A review. Japanese Dental Science Review. 2021; 57: 85-96. doi:10.1016/j.jdsr.2021.05.003
37. Letek M, Behrends V, Murugaiyan J, et al. Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics on behalf of the Global AMR Insights Ambassador Network. MDPI Antibiotics. 11(200). doi:10.3390/antibiotics11020200

RESEARCH ARTICLE

Nanogel Formulations of Kratom (*Mitragyna speciosa*) Extract: A Promising Antibiofilm agent for Diabetic Ulcer Infections

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ABSTRACT:

Diabetic ulcer infections present a substantial obstacle to wound management due to the prevalence of persistent bacterial biofilms that are impervious to conventional antibiotic therapies. Nanomaterials have emerged as a revolutionary platform for addressing the challenges of antibiotic resistance and biofilm-associated infections. Researchers have the potential to employ Kratom in the development of antibiofilm medicines specifically designed to combat diabetic ulcer infections. This is due to its capacity to impede bacterial quorum sensing and disrupt the process of biofilm development. Objective of this study is to determine the antibiofilm properties of the nanogel derived from kratom extract in diabetic ulcer infections. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (1%, 0.5%, 0.25%, 0.125% w/v) to identify their effects on *S. aureus*. The concentration of extract in nanogel used was 10, 20, and 30% to test antibiofilm in the mid-phase, maturation, and eradication phases. In the mid-phase biofilm, Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v exhibited significant antibiofilm efficacy against *Staphylococcus aureus* (SA), with an inhibition percentage of $79.21\% \pm 0.08$; maturation phase of $74.12\% \pm 0.101$; and eradication of $67.25\% \pm 0.046$. KDNE compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial and antibiofilm agents.

KEYWORDS: Nanogel, Kratom, Biofilm, Diabetic ulcers, Infection.

INTRODUCTION:

Diabetic ulcer infections pose a significant challenge in wound management, as they are often associated with persistent bacterial biofilms that are resistant to conventional antibiotic treatments¹.

Biofilms are intricate and ever-changing microbial colonies that flourish at the boundary layer between a solid surface and a liquid medium². The complex formations consist of microbial cells that are tightly integrated with one another and with the underlying surface, enclosed inside a self-generated matrix of extracellular polymeric compounds³. Biofilm formation is a fundamental survival strategy used by many microorganisms to acclimatise to their habitat, especially under unfavourable circumstances⁴. Because of the rise of bacteria and viruses that are resistant to antibiotics, choosing the right empirical antibiotic coverage for diabetic ulcer infection therapy has become more challenging in recent years⁵.



To successfully address severe diseases caused by biofilms, new and innovative therapeutic techniques are required⁶. Utilising nanogel formulations of naturally occurring substances with intrinsic antibiofilm characteristics⁷, like kratom extract, is one approach that shows promise. Mitragynine and 7-hydroxymitragynine are two of the active components found in kratom. These molecules target different types of bacteria, including those that cause infections in diabetic ulcers. Regardless, more in vitro and in vivo investigations are needed⁸. There has been a lot of buzz in the ever-evolving world of antimicrobial strategies about targeted and effective approaches to combat biofilm-related diseases⁹. Biofilms are naturally resistant to conventional methods, such as antibiotic use. As a result, researchers are actively seeking novel approaches to this persistent issue, and they have turned their focus to the fascinating realm of nanotechnology¹⁰.

In their battle against bacterial infections, researchers have shown that nanomaterials and carriers on the nanoscale may kill bacterial cells, slow down quorum sensing, and prevent or eliminate biofilms¹¹. Nanomaterials are a game-changer in the fight against biofilm-associated illnesses and antibiotic resistance. In instance, there are unique benefits to delivering natural chemicals with antibiofilm action via nanogel formulations¹². There are a number of benefits to using nanogels, which are hydrogel nanoparticles, to reduce biofilms¹³. It is easy for the hydrophilic nanocarriers to penetrate bacterial biofilms and disrupt their extracellular matrix. Because of this, antibiofilm medications may be delivered directly to bacterial cells¹⁴. Nanogels' unique properties, such as their scalability, surface charge, and sensitivity to environmental stimuli, might potentially enhance the efficacy of the encapsulated kratom extract¹⁵.

Studies have demonstrated that nanogel formulations may prevent biofilm production, break existing biofilms, and improve the efficacy of conventional antibiotics in killing bacteria trapped in biofilms¹⁶. A very promising treatment approach for diabetic ulcer infections, these nanogel formulations effectively target and dissolve bacterial biofilms¹⁷. In addition to increasing local antibiotic concentrations, nanogels' ability to transport active drugs straight to the infection site aids in the fight against antibiotic resistance by penetrating the protective biofilm matrix¹⁸. This focused strategy may help improve patient outcomes, lessen the likelihood of adverse effects, and lower the requirement for systemic antibiotics, which is particularly important in the treatment of diabetic foot ulcers caused by biofilm-associated infections¹⁹. The objective of this investigation is to ascertain the antibiofilm properties of the nanogel derived from kratom extract in the context of diabetic ulcer infections.

MATERIALS AND METHODS:

Sampling Place:

We collected samples of kratom leaves (*Mitragyna speciosa*) in the forest of Kuranji District, Tanah Bumbu Regency, South Kalimantan, Indonesia. The process of identifying plants was carried out at Universitas Lambung Mangkurat's Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Banjarmasin, Indonesia.

Materials:

Materials used were ethanol 95% (Merck, Germany), ethyl acetate (Merck, Germany), chloramphenicol (Sigma-Aldrich, Germany), phosphate-buffered saline (PBS; Sigma—Aldrich), McFarland (HiMedia, India), CMC-Na (Merck, Germany), Oxoid (Brain Heart infusion) (Merck, Germany), crystal violet (Merck, Germany), Carbopol (Merck, Germany), Trietanolamine (Merck, Germany), Gliserol (Merck, Germany), Propilenglikol (Merck, Germany).

Equipment:

Micropipette (Gilson, France), microtiter plate reader (OIS 2100, Spain), tube (Socorex, Swiss), LAF (Sakura, Japan), incubator 2B (Sakura, Japan), Microplate flat-bottom polystyrene 96 well (Iwaki, Japan), spektro genesys (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), analytical balances (Ohaus, USA).

Bacterial strain:

ATCC 25923 strains of standard *Staphylococcus aureus* were cultivated in tryptic soy broth (TSB) medium and subjected to a 72-hour incubation period at 37°C.

Extraction and identification:

The Kratom leaf samples were cleansed by the process of rinsing with flowing water and meticulously inspecting for any contaminants. After washing, we sliced and desiccated the plant samples in an oven at 40°C for 3 hours. Sample standardisation is conducted using precise criteria. We accomplished the *Simplicia* extraction by fully immersing 300g of powder in 96% ethanol. We agitate the mixture every 6hours and replace the solvent every 24hours, resulting in a total of 5 repetitions or changes of solvent. We subjected the liquid extract to evaporation in a water bath maintained at a temperature of 50°C until the weight reached a steady state. The levels of quinones, terpenoids, phenolics, saponins, steroids, alkaloids, and flavonoids were assessed using the tube technique.

Antibacterial effect of kratom extract against *staphylococcus aureus*:

The antibacterial efficacy was assessed by the use of microdilution and well techniques. The study was carried out on a 96-well flat-bottom polystyrene

microtiter plate using various doses of test chemicals (0,125%, 0,25%, 0,5%, 0,1% w/v) to identify their effects on *S. aureus*. The positive control used was chloramphenicol. In order to get a concentration of 5×10^6 colony forming units per millilitre (CFU/mL), the bacterial solution in BHI was first adjusted to 0.5 McFarland units and then diluted. The microplate was filled with a solution of ethanol with a concentration of 96% and subjected to incubation for a duration of 15 minutes. An ELISA Reader was employed to conduct optical density measurements at a wavelength of 570 nm.

Formulation of nanogel:

Carbopol and CMC were the two nanogel ingredients used as a standard gel base.

Table 1. Formulation of nanogel extract

No	Ingredients	F1 (g)	F2 (g)	F3 (g)
1	Carbopol 940	0,8	0,8	0,8
2	CMC-Na	0,6	0,6	0,6
3	Gliserol	1,25	1,25	1,25
4	Propilenglikol	1,75	1,75	1,75
5	Trietanolamin	1,5	1,5	1,5
6	Nanoemulsion extract	0,75	0,75	0,75
7	Aquadest ad	20	20	20
Ingredient nanoemulsion				
1	Ekstrakt	10%	20%	30%
2	Etanol 96%	9 mL	9 mL	9 mL
3	Tween 80	27,5 mL	27,5 mL	27,5 mL

Inhibitory activity of kratom extract against *staphylococcus aureus* biofilms:

Biofilm formation was assessed during 24hours (intermediate phase), 48hours (maturation phase), and 72hours (eradication) using the Microbroth Dilution Technique (MDT). The microtiter plates were rinsed with distilled water to remove detachable cells, and then allowed to desiccate in the surrounding air at room temperature for a period of 5minutes. Each well was thereafter stained with a 125µL portion of a 1% solution of crystal violet to identify living and non-viable cells, as well as any other components of the biofilm. Following a 15-minute incubation period at ambient temperature, we proceeded to rinse the plate with flowing water in order to eliminate the purple hue. The optical density (OD) was determined by adding 200µL of 96% ethanol to each well and using a microplate reader with a 595nm wavelength. The bacterial test culture, was deposited onto a microtiter plate including coverslips. In order to promote the biofilm development process, Subsequently, the plate was placed in an incubator that was maintained at a temperature of 37°C for a duration of 24-48hours.

In vivo biofilm study:

Adult rats (*Rattus norvegicus*) weighing 150–200g were

employed to conduct in vivo antibiofilm investigations. The animals were given unfettered access to water and nutritious food and were kept inside a controlled setting. In order to induce type II diabetes, a combination of streptozocin and nicotinamide was administered. The rats were administered a 240mg/kg intraperitoneal dose of nicotinamide after a 12hour fasting period. 15 minutes later, I.P. administration of streptozocin at a dosage of 100mg/kg occurred. The animals were subjected to a 12hour fast following the streptozocin treatment, and their fasting blood glucose levels were assessed 72hours later. The investigation categorised animals as diabetic only if their fasting blood glucose level exceeded 150mg/dL²⁰. Positive, negative, and the maximum concentration of gel were the three categories into which the mice were divided. To prevent infection, the researchers made an open incision on the rodent with a diameter of 1cm and administered bacteria. To serve as a positive control, chloramphenicol was administered daily to the lesion until it had fully healed.

SEM study:

Following a thorough washing with sterile distilled water, the coverslip was then subjected to glutaraldehyde during fixation. Methanol was used as a means of decreasing the water content, while the examination was conducted utilising a SEM (scanning electron microscope) functioned at a voltage of 10 kilovolts (kV).

RESULT:

Phytochemical screening

The extraction yielded a filtrate, which was further evaporated using a water bath at 50°C to give a concentrated extract with a consistent weight. The leaf yield was 18.8% with some phytochemical compounds in Table 2.

Table 2: Phytochemical compound of kratom extract.

S. No.	Secondary metabolites	Result
1	Alkaloids (Dragendorff)	-
2	Alkaloids (Mayer)	-
3	Alkaloids (Wagner)	-
4	Steroids	-
5	Terpenoids	+
6	Tannin	-
7	Phenolic	+
8	Saponin	-
9	Flavonoids	+
10	Quinones	+

Antibacterial effect of kratom leave extract against *Staphylococcus aureus*:

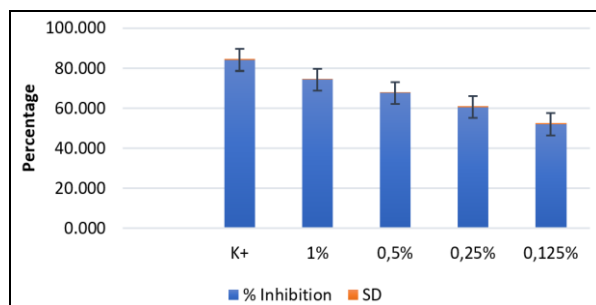


Figure 1: Kraton extract's antibacterial efficacy against *Staphylococcus aureus*

The results demonstrated that the concentration of lollipop flower extract affected its efficacy. At a 1% w/v concentration, the extract successfully suppressed SA growth, showing a substantial ($p < 0.05$) inhibition percentage of $74.226\% \pm 0.002$, notably different from the drug control's $84.097\% \pm 0.001$.

KDNE's inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms:

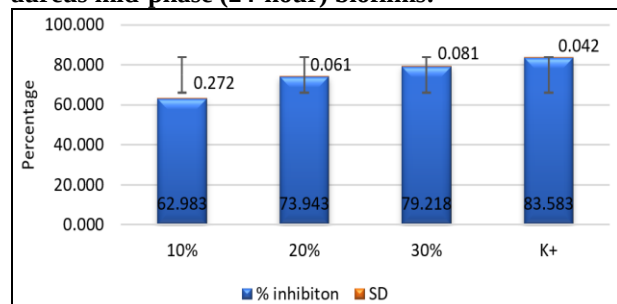


Figure 2. Percentage of inhibitory action against *staphylococcus aureus* mid-phase (24-hour) biofilms

Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v showed strong antibiofilm activity against *Staphylococcus aureus* (SA) during the mid-phase biofilm, with an inhibition percentage of $79.21\% \pm 0.08$. Notably, this inhibition was not significantly different ($p > 0.05$) from the control drug at a concentration of 1% w/v, which showed an inhibition percentage of $83.58\% \pm 0.042$, as depicted in Figure 2.

KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms:

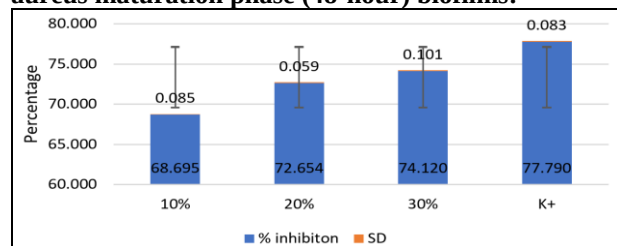


Figure 3. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms

During the biofilm maturation phase, KDNE showed an inhibitory activity of $74.12\% \pm 0.101$ at a concentration of 30% w/v. Interestingly, this result did not differ

significantly ($p > 0.05$) from the inhibitory effectiveness of the pharmacological control, which showed a higher inhibitory effectiveness of $77.79\% \pm 0.083$. These data provide support for the idea that as the biofilm development duration increases, the arrangement of the matrix rises, resulting in a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms²¹.

KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms:

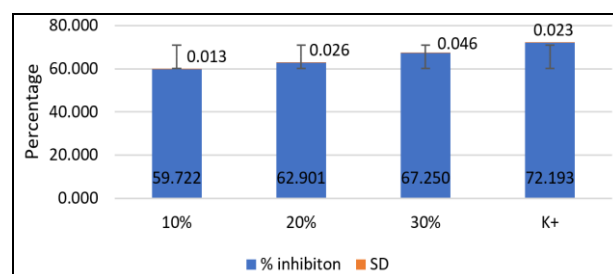


Figure 4. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms

The eradication-phase biofilm of *Staphylococcus aureus* (SA) was effectively inhibited by KDNE at a concentration of 30% w/v, exhibiting an inhibition percentage of $67.25\% \pm 0.046$. Figure 4 shows that at a dose of 1% w/v, the control medication exhibited an inhibition percentage of $72.912\% \pm 0.023$, and this inhibition was not substantially different ($p > 0.05$).

In vivo biofilm study:

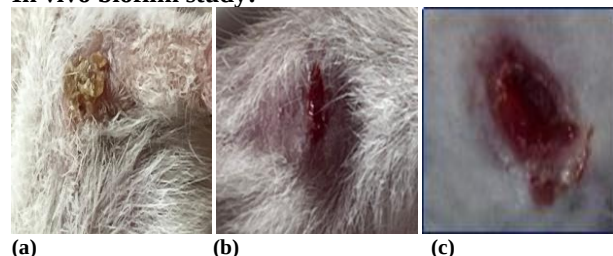


Figure 5. 5a. untreated group; 5b. Occurrence of Ulcers; 5c. the wound begins to close.

Diabetes is known to hinder the host's immune response and create an environment that encourages the growth of microorganisms. Numerous studies have highlighted the elevated risk of infections in diabetics, including rhinocerebral mucormycosis, malignant external otitis, and foot infections. There is evidence that diabetes-induced alterations in the microcirculatory system and disruption of wound healing processes contribute to this increased susceptibility²². This study shows that nanogel treatment can improve wound healing in diabetic mice by increasing angiogenesis and reducing biofilm formation, which is not significantly different from the

control group. The ulcers treated with nanogel closed and healed within 11 days, and the healing diameter was almost the same as the control group.

SEM Study:

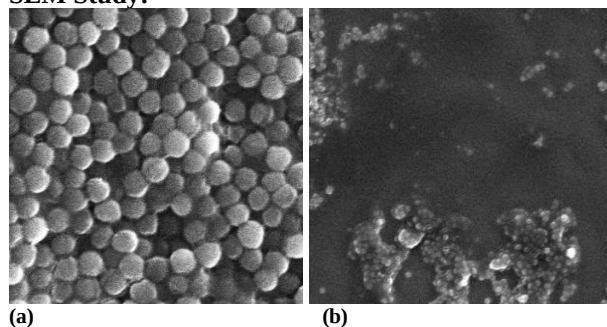


Figure 6: Figure 6a Result of SEM Biofilm with No Treatment, Figure 6b Result of SEM Biofilm with Administration of Kratom Extract 30% w/v.

Figure 6b shows that the SEM test showed that the KDNE effectively stopped and removed the *Staphylococcus aureus* (SA) biofilm. Figure 6a shows the results of the scanning electron microscopy investigation into the untreated SA biofilm, which reveals a compact cellular structure inside the extracellular polymeric substance (EPS) matrix and hence a safe habitat for the organism. Limitation, cell death, and EPS matrix damage were the outcomes of treating biofilm with a 30% w/v concentration of KDNE.

DISCUSSION:

Polyphenols also quinones, natural plant-derived compounds, have garnered significant attention for their remarkable antibiofilm properties. These molecules work in different ways to stop different stages of biofilm formation²³. For example, they stop cells from attaching at the start, stop the production of extracellular matrix, and mess up the signalling pathways for quorum sensing²⁴. Flavonoids, tannins, and other phenolic compounds have been shown to be able to get into the biofilm matrix, cause oxidative stress, and damage the integrity of the microbiome cell membrane, which makes it harder for cells that are embedded in biofilm to stay alive²⁵. Similarly, studies have shown that quinones, a class of aromatic compounds, interfere with bacterial quorum sensing systems, crucial for coordinating biofilm development and maturation²⁶. Because of their strong antibacterial and antibiofilm properties, flavonoids and quinones have attracted a lot of interest from antimicrobial researchers. Flavonoids have antibacterial properties because they disrupt bacterial membranes, inhibit bacterial nucleic acid replication, reduce bacterial efflux pump function, and block bacterial adenosine triphosphate synthase²⁷. Offlavonoids are effective against bacteria because they may bind to extracellular proteins to create complex

molecules that compromise bacterial cell membrane integrity²⁸. Quinones have antibacterial properties because they can create amino acid compounds that negatively impact protein function. When terpenoids bind to carbs and lipids in bacteria, they make their cell membranes less permeable²⁹.

Recent research has shown that kratom extracts may effectively hinder the development and disturb the structure of biofilms created by several harmful bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*³⁰. Kratom has the potential to be used in the development of antibiofilm drugs that specifically target diabetic ulcer infections such as its ability to impede bacterial quorum sensing and disrupt biofilm formation³¹. By incorporating these bioactive molecules into nanogel formulations, their stability, solubility, and targeted delivery to the infection site may be improved³². Attachment of microbial cells to a surface is the first step in the multi-stage process that culminates in the detachment of cells from the biofilm after microcolonies have developed and the biofilm structure has matured³³. This data implies that it is more problematic to suppress bacteria during biofilm development, since the inhibition percentage is lower compared to bacteria in planktonic form³⁴. For bacteria in planktonic form, KDNE had a substantial inhibitory effect of $83.58\% \pm 0.41$; for bacteria that had developed a biofilm during the mid-phase, the inhibitory effect was somewhat lower, at $79.21\% \pm 0.8$. These data support the idea that as the duration of biofilm development increases, there is a rise in the arrangement of the matrix, leading to a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms³⁵.

The physicochemical properties of nanomaterials, including hydrophobicity and pore size, can be regulated to optimise their antimicrobial properties. Researchers have reported that increasing the nanoroughness of the surface reduces bacterial adhesion to medical devices, implying the development of nanogel formulations to curb biofilm formation and bacterial attachment³⁶. Nanogel formulations can also work as nanovehicles, making it easier to deliver naturally occurring compounds that can break down biofilms. Nanogel forms of kratom extract have been shown in new research to effectively stop the growth of biofilms made by many bacteria that are clinically important, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As a result, the active chemicals might be more effectively administered locally at the site of infection, which could lessen the likelihood of systemic bad effects associated with traditional

antibiotic treatments³⁷.

CONCLUSION:

The results indicate that the activity of KDNE in inhibiting the growth of *Staphylococcus aureus* biofilms varied with its concentration. At a concentration of 30%, the extract exhibited equivalent antibacterial, mid-phase, maturation, and eradication efficacy to the control. Plant-derived compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial agents.

CONFLICT OF INTEREST:

Regarding this study, the writers do not have any competing interests.

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REFERENCES:

- Jang EJ, Patel R, Patel M. Electrospinning Nanofibers as a Dressing to Treat Diabetic Wounds. *Pharmaceutics*. 2023; 12: 1. doi:10.3390/pharmaceutics15041144
- Li H, Mavumengwana V, Gan RY, et al. Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics*. 2022; 11(108): 1-45. doi:10.3390/antibiotics11010108
- Deepigaa M. Antibacterial Resistance of Bacteria in Biofilms. *Res J Pharm Technol*. 2017; 10(11): 4019. doi:10.5958/0974-360X.2017.00728.4
- Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology*. 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
- Pessoa e Costa T, Duarte B, João AL, et al. Multidrug-resistant bacteria in diabetic foot infections: Experience from a portuguese tertiary centre. *Int Wound J*. 2020; 17(6): 1835-1839. doi:10.1111/iwj.13473
- Pushpam AC, Karthik R, Chelvan Y, Ramalingam K, Vanitha MC. Evaluation of the Antibiofilm Properties of *Arthrobacter defluvii* AMET1677 Strain Isolated from Shrimp Pond Sediment against Marine Biofilm Forming Bacteria. *Res J Pharm Technol*. 2016; 9(4): 373. doi:10.5958/0974-360X.2016.00067.6
- Kumar Bari A, Sandeep Belalekar T, Poojary A, Rohra S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. *Front Cell Infect Microbiol*. 2023; (4): 7. doi:10.3389/fcimb.2023.1155699
- Syahida N, Ma N'. Indonesian Journal of Chemical Science Kratom (*Mitragyna speciosa*): Medicinal Marvel or Menace? Assessing Potency, Risk, and Future Prospect of Herbal Medicine. *J Chem Sci*. 2024; 13(1). <http://journal.unnes.ac.id/sju/index.php/ijcs>
- Selvi KV, Hemalatha M, Rajeshkumar S. Analysis of bioactive compounds from *Spirulina gessneri* and studying its Antibacterial activity against wound Infection causing pathogens. *Res J Pharm Technol*. 2017; 10(6): 1983. doi:10.5958/0974-360X.2017.00347.X
- Kishore MY, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol*. 2023; 1(3): 1-30. doi:10.3389/fmicb.2022.1028086
- Mukherjee A, Bose S, Shao A, Das SK. Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. *Royal Society of Chemistry*. 2023; 4(4): 2544-2572. doi:10.1039/d2ma00846g
- Ramadan YF; N; Al-Harbi AI; A, Ahmed E; et al. Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicines*. 2023; 413(11). doi:10.3390/biomedicines11020413
- Umamageswari SSM, Manipriya B, Kalyani M. Evaluation of antibacterial activity of zinc oxide nanoparticles against biofilm producing methicillin resistant *Staphylococcus aureus* (MRSA). *Res J Pharm Technol*. 2018; 11(5): 1884. doi:10.5958/0974-360X.2018.00350.5
- Sookkhee S, Sakonwasun C, Mungkornasawakul P, Khamnoi P, Wikan N, Nimlamool W. Synergistic Effects of Some Methoxyflavones Extracted from *Rhizome of Kaempferia parviflora* Combined with Gentamicin against Carbapenem-Resistant Strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *MDPI Plants*. 2022; 11(3128): 16. doi:10.3390/plants11223128
- Kishore Mohanta Y, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol*. 2023; (3): 1-30. doi:10.3389/fmicb.2022.1028086
- Varma A, Warghane A, Dhiman NK, et al. The role of nanocomposites against biofilm infections in humans. *Front Cell Infect Microbiol*. 2023; (2): 1-16. doi:10.3389/fcimb.2023.1104615
- Arunachalam K, Krishnan GP, Sethuraman S, et al. Exploring Possible Ways to Enhance the Potential and Use of Natural Products through Nanotechnology in the Battle against Biofilms of Foodborne Bacterial Pathogens. *MDPI Pathogens*. 2023; (270): 1. doi:10.3390/pathogens12020270
- Mirghani R, Saba T, Khaliq H, et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiol*. 2022; 8(3): 240-278. doi:10.3934/microbiol.2022019
- Tuon FF, Suss PH, Telles JP, Dantas LR, Borges NH, Ribeiro VST. Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics*. 2023; 12(1). doi:10.3390/antibiotics12010087
- Wu X, Wang H, Xiong J, et al. *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm*. 2024; 7: 100175. doi:10.1016/J.BIOFLM.2023.100175
- Mary RNI, Banu N. Inhibition of Antibiofilm Mediated Virulence Factors in *Pseudomonas aeruginosa* by *Andrographis paniculata*. *Res J Pharm Technol*. 2017; 10(1): 141. doi:10.5958/0974-360X.2017.00031.2
- Dewangan D, Ajazuddin, Agrawal P, et al. Formulation and Evaluation of oral reconstitutable Azithromycin Suspension for the treatment of Bacterial Infection. *Res J Pharm Technol*. 2018; 11(4): 1351. doi:10.5958/0974-360X.2018.00251.2
- Karpagam P, Manonmani G, Brindha D. Phytochemical Screening, Antimicrobial Activity and Antimicrobial Finishing of Polyherbal Extract on Nonwoven Wound Dressing. *Res J Pharm Technol*. 2019; 12(2): 632. doi:10.5958/0974-360X.2019.00112.4
- Raffaelli S, Abreo E, Altier N, Vázquez Á, Alborés S. molecules Bioprospecting the Antibiofilm and Antimicrobial Activity of Soil and Insect Gut Bacteria. *MDPI Molecules*. 2022; 27(2002): 1-13. doi:10.3390/molecules27062002
- Abdul-Lateef LA, Hassan E, Taha Abd-Alla Bactash E. Effect of Cranberry on biofilm formation by *P. mirabilis* isolated from Patients Suffering from Urinary tract Infections. *Res J Pharm Technol*. 2018; 11(3): 1097. doi:10.5958/0974-360X.2018.00206.8

26. Ivanov M, Novovi'cnovovi'c K, Maleševi'cmaleševi'c M, et al. Polyphenols as Inhibitors of Antibiotic Resistant Bacteria-Mechanisms Underlying Rutin Interference with Bacterial Virulence. MDPI Pharmaceuticals. 2022; 15(385): 1-21. doi:10.3390/ph15030385
27. Majnooni MB, Ghanadian SM, Mojarrab M, et al. and Antioxidant Activities of Flavonoids Isolated from Allium colchicifolium Leaves. J Food Biochem. 2023; 1(14): 1-14. doi:10.1155/2023/5521661
28. Mary RNI, Banu N. Inhibition of biofilm formation in Serratia marcescens by Andrographolide from Andrographis paniculata. Res J Pharm Technol. 2017; 10(3): 789. doi:10.5958/0974-360X.2017.00148.2
29. Cloudya Panjaitan C, Sari Widyarman A, Amtha R, Erri Astoeti T, Widyarman S. Antimicrobial and Antibiofilm Activity of Cinnamon (Cinnamomum burmanii) Extract on Periodontal Pathogens-An in vitro study. Eur J Dent. 2022; 16(4). doi:10.1055/s-0041-1742125
30. Shamim A, Ali A, Iqbal Z, et al. Natural Medicine a Promising Candidate in Combating Microbial Biofilm. Antibiotics. 2023; 12(299): 2-26. doi:10.3390/antibiotics12020299
31. Tasmia Asma S, Imre K, Morar A, et al. Natural Strategies as Potential Weapons against Bacterial Biofilms. Life MDPI. 2022; 12(1618): 1-35. doi:10.3390/life12101618
32. Liu M, Wei X, Zheng Z, et al. Recent Advances in Nano-Drug Delivery Systems for the Treatment of Diabetic Wound Healing. Int J Nanomedicine. 2023; (3): 1537-1560. doi:10.2147/IJN.S395438
33. Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. Brazilian Journal of Microbiology. 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
34. Bhavani G, P G. Detection of biofilm among clinical isolates of Acinetobacter baumannii by tissue culture plate method (TCP). Res J Pharm Technol. 2016; 9(10): 1635. doi:10.5958/0974-360X.2016.00327.9
35. Tasmia Asma S, Imre K, Morar A, et al. An Overview of Biofilm Formation-Combating Strategies and Mechanisms of Action of Antibiofilm Agents. MDPI Life. 2022; 1(1110). doi:10.3390/life12081110
36. Kreve S, Reis ACD. Bacterial adhesion to biomaterials: What regulates this attachment? A review. Japanese Dental Science Review. 2021; 57: 85-96. doi:10.1016/j.jdsr.2021.05.003
37. Letek M, Behrends V, Murugaiyan J, et al. Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics on behalf of the Global AMR Insights Ambassador Network. MDPI Antibiotics. 11(200). doi:10.3390/antibiotics11020200

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RESEARCH ARTICLE

Nanogel Formulations of Kratom (*Mitragyna speciosa*) Extract: A Promising Antibiofilm agent for Diabetic Ulcer Infections

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ABSTRACT:

Diabetic ulcer infections present a substantial obstacle to wound management due to the prevalence of persistent bacterial biofilms that are impervious to conventional antibiotic therapies. Nanomaterials have emerged as a revolutionary platform for addressing the challenges of antibiotic resistance and biofilm-associated infections. Researchers have the potential to employ Kratom in the development of antibiofilm medicines specifically designed to combat diabetic ulcer infections. This is due to its capacity to impede bacterial quorum sensing and disrupt the process of biofilm development. Objective of this study is to determine the antibiofilm properties of the nanogel derived from kratom extract in diabetic ulcer infections. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (1%, 0.5%, 0.25%, 0.125% w/v) to identify their effects on *S. aureus*. The concentration of extract in nanogel used was 10, 20, and 30% to test antibiofilm in the mid-phase, maturation, and eradication phases. In the mid-phase biofilm, Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v exhibited significant antibiofilm efficacy against *Staphylococcus aureus* (SA), with an inhibition percentage of $79.21\% \pm 0.08$; maturation phase of $74.12\% \pm 0.101$; and eradication of $67.25\% \pm 0.046$. KDNE compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial and antibiofilm agents.

KEYWORDS: Nanogel, Kratom, Biofilm, Diabetic ulcers, Infection.

INTRODUCTION:

Diabetic ulcer infections pose a significant challenge in wound management, as they are often associated with persistent bacterial biofilms that are resistant to conventional antibiotic treatments¹. Biofilms are intricate and ever-changing microbial colonies that flourish at the boundary layer between a solid surface and a liquid medium². The complex formations consist of microbial cells that are tightly integrated with one another and with the underlying surface, enclosed inside a self-generated matrix of extracellular polymeric compounds³. Biofilm formation is a fundamental survival strategy used by many microorganisms to acclimatise to their habitat, especially under unfavourable circumstances⁴. Because of the rise of bacteria and viruses that are resistant to antibiotics,

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choosing the right empirical antibiotic coverage for diabetic ulcer infection therapy has become more challenging in recent years⁵.

To successfully address severe diseases caused by biofilms, new and innovative therapeutic techniques are required⁶. Utilising nanogel formulations of naturally occurring substances with intrinsic antibiofilm characteristics⁷, like kratom extract, is one approach that shows promise. Mitragynine and 7-hydroxymitragynine are two of the active components found in kratom. These molecules target different types of bacteria, including those that cause infections in diabetic ulcers. Regardless, more in vitro and in vivo investigations are needed⁸. There has been a lot of buzz in the ever-evolving world of antimicrobial strategies about targeted and effective approaches to combat biofilm-related diseases⁹. Biofilms are naturally resistant to conventional methods, such as antibiotic use. As a result, researchers are actively seeking novel approaches to this persistent issue, and they have turned their focus to the fascinating realm of nanotechnology¹⁰.

In their battle against bacterial infections, researchers have shown that nanomaterials and carriers on the nanoscale may kill bacterial cells, slow down quorum sensing, and prevent or eliminate biofilms¹¹. Nanomaterials are a game-changer in the fight against biofilm-associated illnesses and antibiotic resistance. In instance, there are unique benefits to delivering natural chemicals with antibiofilm action via nanogel formulations¹². There are a number of benefits to using nanogels, which are hydrogel nanoparticles, to reduce biofilms¹³. It is easy for the hydrophilic nanocarriers to penetrate bacterial biofilms and disrupt their extracellular matrix. Because of this, antibiofilm medications may be delivered directly to bacterial cells¹⁴. Nanogels' unique properties, such as their scalability, surface charge, and sensitivity to environmental stimuli, might potentially enhance the efficacy of the encapsulated kratom extract¹⁵.

Studies have demonstrated that nanogel formulations may prevent biofilm production, break existing biofilms, and improve the efficacy of conventional antibiotics in killing bacteria trapped in biofilms¹⁶. A very promising treatment approach for diabetic ulcer infections, these nanogel formulations effectively target and dissolve bacterial biofilms¹⁷. In addition to increasing local antibiotic concentrations, nanogels' ability to transport active drugs straight to the infection site aids in the fight against antibiotic resistance by penetrating the protective biofilm matrix¹⁸. This focused strategy may help improve patient outcomes, lessen the likelihood of adverse effects, and lower the requirement for systemic antibiotics, which is particularly important in the treatment of diabetic foot ulcers caused by biofilm-

associated infections¹⁹. The objective of this investigation is to ascertain the antibiofilm properties of the nanogel derived from kratom extract in the context of diabetic ulcer infections.

MATERIALS AND METHODS:

Sampling Place:

We collected samples of kratom leaves (*Mitragyna speciosa*) in the forest of Kuranji District, Tanah Bumbu Regency, South Kalimantan, Indonesia. The process of identifying plants was carried out at Universitas Lambung Mangkurat's Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Banjarmasin, Indonesia.

Materials:

Materials used were ethanol 95% (Merck, Germany), ethyl acetate (Merck, Germany), chloramphenicol (Sigma-Aldrich, Germany), phosphate-buffered saline (PBS; Sigma—Aldrich), McFarland (HiMedia, India), CMC-Na (Merck, Germany), Oxoid (Brain Heart infusion) (Merck, Germany), crystal violet (Merck, Germany), Carbopol (Merck, Germany), Trietanolamine (Merck, Germany), Gliserol (Merck, Germany), Propilenglikol (Merck, Germany).

Equipment:

Micropipette (Gilson, France), microtiter plate reader (OIS 2100, Spain), tube (Socorex, Swiss), LAF (Sakura, Japan), incubator 2B (Sakura, Japan), Microplate flat-bottom polystyrene 96 well (Iwaki, Japan), spektro genesys (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), analytical balances (Ohaus, USA).

Bacterial strain:

ATCC 25923 strains of standard *Staphylococcus aureus* were cultivated in tryptic soy broth (TSB) medium and subjected to a 72-hour incubation period at 37°C.

Extraction and identification:

The Kratom leaf samples were cleansed by the process of rinsing with flowing water and meticulously inspecting for any contaminants. After washing, we sliced and desiccated the plant samples in an oven at 40°C for 3 hours. Sample standardisation is conducted using precise criteria. We accomplished the Simplicia extraction by fully immersing 300g of powder in 96% ethanol. We agitate the mixture every 6hours and replace the solvent every 24hours, resulting in a total of 5 repetitions or changes of solvent. We subjected the liquid extract to evaporation in a water bath maintained at a temperature of 50°C until the weight reached a steady state. The levels of quinones, terpenoids, phenolics, saponins, steroids, alkaloids, and flavonoids were assessed using the tube technique.

Antibacterial effect of kratom extract against *staphylococcus aureus*:

The antibacterial efficacy was assessed by the use of microdilution and well techniques. The study was carried out on a 96-well flat-bottom polystyrene microtiter plate using various doses of test chemicals (0,125%, 0,25%, 0,5%, 0,1% w/v) to identify their effects on *S. aureus*. The positive control used was chloramphenicol. In order to get a concentration of 5×10^6 colony forming units per millilitre (CFU/mL), the bacterial solution in BHI was first adjusted to 0.5 McFarland units and then diluted. The microplate was filled with a solution of ethanol with a concentration of 96% and subjected to incubation for a duration of 15 minutes. An ELISA Reader was employed to conduct optical density measurements at a wavelength of 570 nm.

Formulation of nanogel:

Carbopol and CMC were the two nanogel ingredients used as a standard gel base.

Table 1. Formulation of nanogel extract

No	Ingredients	F1 (g)	F2 (g)	F3 (g)
1	Carbopol 940	0,8	0,8	0,8
2	CMC-Na	0,6	0,6	0,6
3	Glycerol	1,25	1,25	1,25
4	Propylenglykol	1,75	1,75	1,75
5	Trietanolamin	1,5	1,5	1,5
6	Nanoemulsion extract	0,75	0,75	0,75
7	Aquadest ad	20	20	20
Ingredient nanoemulsion				
1	Ekstrakt	10%	20%	30%
2	Ethanol 96%	9 mL	9 mL	9 mL
3	Tween 80	27,5 mL	27,5 mL	27,5 mL

Inhibitory activity of kratom extract against *staphylococcus aureus* biofilms:

Biofilm formation was assessed during 24hours (intermediate phase), 48hours (maturation phase), and 72hours (eradication) using the Microbroth Dilution Technique (MDT). The microtiter plates were rinsed with distilled water to remove detachable cells, and then allowed to desiccate in the surrounding air at room temperature for a period of 5minutes. Each well was thereafter stained with a 125µL portion of a 1% solution of crystal violet to identify living and non-viable cells, as well as any other components of the biofilm. Following a 15-minute incubation period at ambient temperature, we proceeded to rinse the plate with flowing water in order to eliminate the purple hue. The optical density (OD) was determined by adding 200µL of 96% ethanol to each well and using a microplate reader with a 595nm wavelength. The bacterial test culture, was deposited onto a microtiter plate including coverslips. In order to promote the biofilm development process, Subsequently, the plate was placed in an

incubator that was maintained at a temperature of 37°C for a duration of 24-48hours.

In vivo biofilm study:

Adult rats (*Rattus norvegicus*) weighing 150–200g were employed to conduct in vivo antibiofilm investigations. The animals were given unfettered access to water and nutritious food and were kept inside a controlled setting. In order to induce type II diabetes, a combination of streptozocin and nicotinamide was administered. The rats were administered a 240mg/kg intraperitoneal dose of nicotinamide after a 12hour fasting period. 15 minutes later, I.P. administration of streptozocin at a dosage of 100mg/kg occurred. The animals were subjected to a 12hour fast following the streptozocin treatment, and their fasting blood glucose levels were assessed 72hours later. The investigation categorised animals as diabetic only if their fasting blood glucose level exceeded 150mg/dL²⁰. Positive, negative, and the maximum concentration of gel were the three categories into which the mice were divided. To prevent infection, the researchers made an open incision on the rodent with a diameter of 1cm and administered bacteria. To serve as a positive control, chloramphenicol was administered daily to the lesion until it had fully healed.

SEM study:

Following a thorough washing with sterile distilled water, the coverslip was then subjected to glutaraldehyde during fixation. Methanol was used as a means of decreasing the water content, while the examination was conducted utilising a SEM (scanning electron microscope) functioned at a voltage of 10 kilovolts (kV).

RESULT:

Phytochemical screening

The extraction yielded a filtrate, which was further evaporated using a water bath at 50°C to give a concentrated extract with a consistent weight. The leaf yield was 18.8% with some phytochemical compounds in Table 2.

Table 2: Phytochemical compound of kratom extract.

S. No.	Secondary metabolites	Result
1	Alkaloids (Dragendorff)	-
2	Alkaloids (Mayer)	-
3	Alkaloids (Wagner)	-
4	Steroids	-
5	Terpenoids	+
6	Tannin	-
7	Phenolic	+
8	Saponin	-
9	Flavonoids	+
10	Quinones	+

Antibacterial effect of kratom leave extract against *Staphylococcus aureus*:

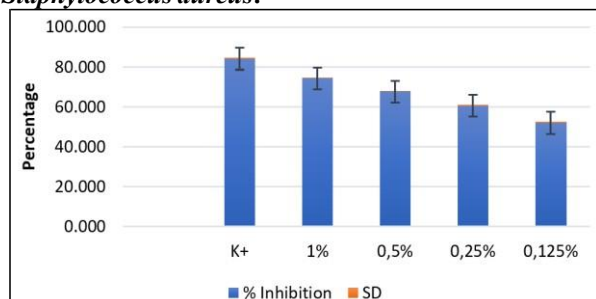


Figure 1: Kratom extract's antibacterial efficacy against *Staphylococcus aureus*

The results demonstrated that the concentration of lollipop flower extract affected its efficacy. At a 1% w/v concentration, the extract successfully suppressed SA growth, showing a substantial ($p < 0.05$) inhibition percentage of $74.226\% \pm 0.002$, notably different from the drug control's $84.097\% \pm 0.001$.

KDNE's inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms:

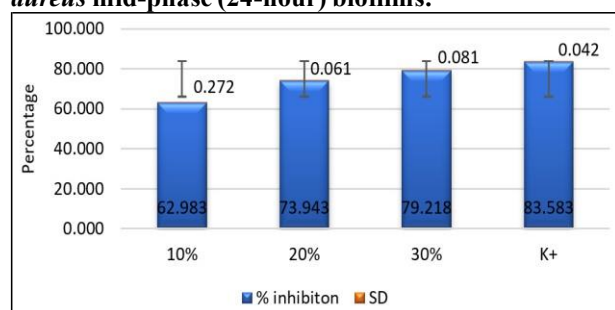


Figure 2. Percentage of inhibitory action against *Staphylococcus aureus* mid-phase (24-hour) biofilms

Kratom-derived nanogel extract (KDNE) at a concentration of 30% w/v showed strong antibiofilm activity against *Staphylococcus aureus* (SA) during the mid-phase biofilm, with an inhibition percentage of $79.21\% \pm 0.08$. Notably, this inhibition was not significantly different ($p > 0.05$) from the control drug at a concentration of 1% w/v, which showed an inhibition percentage of $83.58\% \pm 0.042$, as depicted in Figure 2.

KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms:

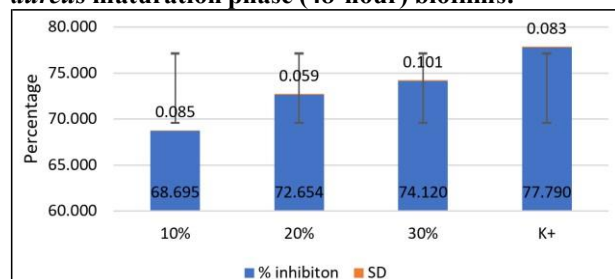


Figure 3. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* maturation phase (48-hour) biofilms

During the biofilm maturation phase, KDNE showed an inhibitory activity of $74.12\% \pm 0.101$ at a concentration of 30% w/v. Interestingly, this result did not differ significantly ($p > 0.05$) from the inhibitory effectiveness of the pharmacological control, which showed a higher inhibitory effectiveness of $77.79\% \pm 0.083$. These data provide support for the idea that as the biofilm development duration increases, the arrangement of the matrix rises, resulting in a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms²¹.

KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms:

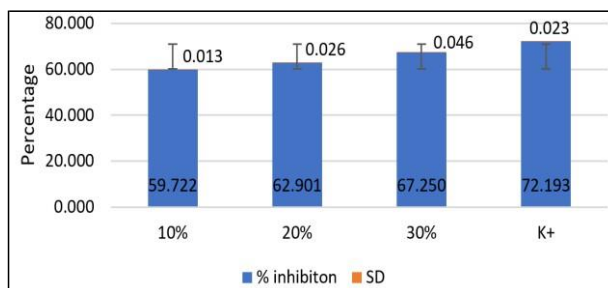


Figure 4. Percentage of KDNE's inhibitory action against *Staphylococcus aureus* eradication phase (72-hour) biofilms

The eradication-phase biofilm of *Staphylococcus aureus* (SA) was effectively inhibited by KDNE at a concentration of 30% w/v, exhibiting an inhibition percentage of $67.25\% \pm 0.046$. Figure 4 shows that at a dose of 1% w/v, the control medication exhibited an inhibition percentage of $72.912\% \pm 0.023$, and this inhibition was not substantially different ($p > 0.05$).

In vivo biofilm study:

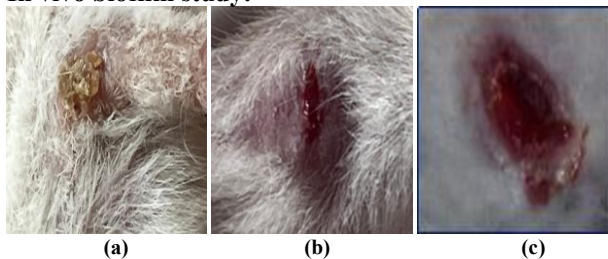


Figure 5. 5a. untreated group; 5b. Occurrence of Ulcers; 5c. the wound begins to close.

Diabetes is known to hinder the host's immune response and create an environment that encourages the growth of microorganisms. Numerous studies have highlighted the elevated risk of infections in diabetics, including rhinocerebral mucormycosis, malignant external otitis, and foot infections. There is evidence that diabetes-induced alterations in the microcirculatory system and disruption of wound healing processes contribute to this increased susceptibility²². This study shows that nanogel

treatment can improve wound healing in diabetic mice by increasing angiogenesis and reducing biofilm formation, which is not significantly different from the control group. The ulcers treated with nanogel closed and healed within 11 days, and the healing diameter was almost the same as the control group.

SEM Study:

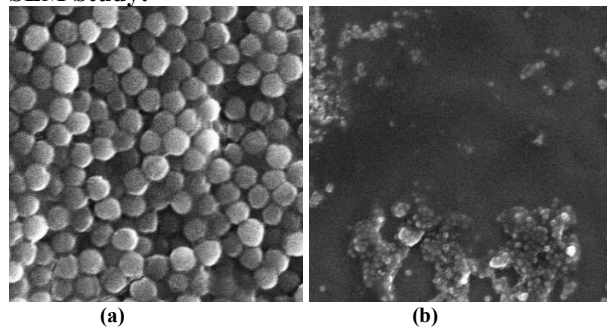


Figure 6: Figure 6a Result of SEM Biofilm with No Treatment, Figure 6b Result of SEM Biofilm with Administration of Kratom Extract 30% w/v.

Figure 6b shows that the SEM test showed that the KDNE effectively stopped and removed the *Staphylococcus aureus* (SA) biofilm. Figure 6a shows the results of the scanning electron microscopy investigation into the untreated SA biofilm, which reveals a compact cellular structure inside the extracellular polymeric substance (EPS) matrix and hence a safe habitat for the organism. Limitation, cell death, and EPS matrix damage were the outcomes of treating biofilm with a 30% w/v concentration of KDNE.

DISCUSSION:

Polyphenols also quinones, natural plant-derived compounds, have garnered significant attention for their remarkable antibiofilm properties. These molecules work in different ways to stop different stages of biofilm formation²³. For example, they stop cells from attaching at the start, stop the production of extracellular matrix, and mess up the signalling pathways for quorum sensing²⁴. Flavonoids, tannins, and other phenolic compounds have been shown to be able to get into the biofilm matrix, cause oxidative stress, and damage the integrity of the microbiome cell membrane, which makes it harder for cells that are embedded in biofilm to stay alive²⁵. Similarly, studies have shown that quinones, a class of aromatic compounds, interfere with bacterial quorum sensing systems, crucial for coordinating biofilm development and maturation²⁶. Because of their strong antibacterial and antibiofilm properties, flavonoids and quinones have attracted a lot of interest from antimicrobial researchers. Flavonoids have antibacterial properties because they disrupt bacterial membranes, inhibit bacterial nucleic acid replication, reduce bacterial efflux pump function, and block

bacterial adenosine triphosphate synthase²⁷. Offlavonoids are effective against bacteria because they may bind to extracellular proteins to create complex molecules that compromise bacterial cell membrane integrity²⁸. Quinones have antibacterial properties because they can create amino acid compounds that negatively impact protein function. When terpenoids bind to carbs and lipids in bacteria, they make their cell membranes less permeable²⁹.

Recent research has shown that kratom extracts may effectively hinder the development and disturb the structure of biofilms created by several harmful bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*³⁰. Kratom has the potential to be used in the development of antibiofilm drugs that specifically target diabetic ulcer infections such as its ability to impede bacterial quorum sensing and disrupt biofilm formation³¹. By incorporating these bioactive molecules into nanogel formulations, their stability, solubility, and targeted delivery to the infection site may be improved³². Attachment of microbial cells to a surface is the first step in the multi-stage process that culminates in the detachment of cells from the biofilm after microcolonies have developed and the biofilm structure has matured³³. This data implies that it is more problematic to suppress bacteria during biofilm development, since the inhibition percentage is lower compared to bacteria in planktonic form³⁴. For bacteria in planktonic form, KDNE had a substantial inhibitory effect of 83.58% $\pm$ 0.41; for bacteria that had developed a biofilm during the mid-phase, the inhibitory effect was somewhat lower, at 79.21% $\pm$ 0.8. These data support the idea that as the duration of biofilm development increases, there is a rise in the arrangement of the matrix, leading to a stronger and more complex biofilm structure. The intricate nature of this structure reduces the efficacy of both the test substance and pharmacological control in preventing the growth of biofilms³⁵.

The physicochemical properties of nanomaterials, including hydrophobicity and pore size, can be regulated to optimise their antimicrobial properties. Researchers have reported that increasing the nanoroughness of the surface reduces bacterial adhesion to medical devices, implying the development of nanogel formulations to curb biofilm formation and bacterial attachment³⁶. Nanogel formulations can also work as nanovehicles, making it easier to deliver naturally occurring compounds that can break down biofilms. Nanogel forms of kratom extract have been shown in new research to effectively stop the growth of biofilms made by many bacteria that are clinically important, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. As a result, the active chemicals

might be more effectively administered locally at the site of infection, which could lessen the likelihood of systemic bad effects associated with traditional antibiotic treatments³⁷.

CONCLUSION:

The results indicate that the activity of KDNE in inhibiting the growth of *Staphylococcus aureus* biofilms varied with its concentration. At a concentration of 30%, the extract exhibited equivalent antibacterial, mid-phase, maturation, and eradication efficacy to the control. Plant-derived compounds have shown efficacy against *Staphylococcus aureus*, suggesting their potential as natural antibacterial agents.

CONFLICT OF INTEREST:

Regarding this study, the writers do not have any competing interests.

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REFERENCES:

- Jang EJ, Patel R, Patel M. Electrospinning Nanofibers as a Dressing to Treat Diabetic Wounds. *Pharmaceutics*. 2023; 12: 1. doi:10.3390/pharmaceutics15041144
- Li H, Mavumengwana V, Gan RY, et al. Nanoparticles-Attractive Carriers of Antimicrobial Essential Oils. *Antibiotics*. 2022; 11(108): 1-45. doi:10.3390/antibiotics11010108
- Deepigaa M. Antibacterial Resistance of Bacteria in Biofilms. *Res J Pharm Technol*. 2017; 10(11): 4019. doi:10.5958/0974-360X.2017.00728.4
- Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology*. 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
- Pessoa e Costa T, Duarte B, João AL, et al. Multidrug-resistant bacteria in diabetic foot infections: Experience from a portuguese tertiary centre. *Int Wound J*. 2020; 17(6): 1835-1839. doi:10.1111/iwj.13473
- Pushpam AC, Karthik R, Chelvan Y, Ramalingam K, Vanitha MC. Evaluation of the Antibiofilm Properties of *Arthrobacter defluvii* AMET1677 Strain Isolated from Shrimp Pond Sediment against Marine Biofilm Forming Bacteria. *Res J Pharm Technol*. 2016; 9(4): 373. doi:10.5958/0974-360X.2016.00067.6
- Kumar Bari A, Sandeep Belalekar T, Poojary A, Rohra S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. *Front Cell Infect Microbiol*. 2023; 4(7): 7. doi:10.3389/fcimb.2023.1155699
- Syahida N, Ma N'. Indonesian Journal of Chemical Science Kratom (*Mitragyna speciosa*): Medicinal Marvel or Menace? Assessing Potency, Risk, and Future Prospect of Herbal Medicine. *J Chem Sci*. 2024; 13(1). <http://journal.unnes.ac.id/sju/index.php/ijcs>
- Selvi KV, Hemalatha M, Rajeshkumar S. Analysis of bioactive compounds from *Spirulina gessneri* and studying its Antibacterial activity against wound Infection causing pathogens. *Res J Pharm Technol*. 2017; 10(6): 1983. doi:10.5958/0974-360X.2017.00347.X
- Kishore MY, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol*. 2023; 1(3): 1-30. doi:10.3389/fmicb.2022.1028086
- Mukherjee A, Bose S, Shao A, Das SK. Nanotechnology based therapeutic approaches: an advanced strategy to target the biofilm of ESKAPE pathogens. *Royal Society of Chemistry*. 2023; 4(4): 2544-2572. doi:10.1039/d2ma00846g
- Ramadan YF; N.; Al-Harbi AI; A, Ahmed E.; et al. Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicine*. 2023; 413(11). doi:10.3390/biomedicine11020413
- Umamageswari SSM, Manipriya B, Kalyani M. Evaluation of antibacterial activity of zinc oxide nanoparticles against biofilm producing methicillin resistant *Staphylococcus aureus* (MRSA). *Res J Pharm Technol*. 2018; 11(5): 1884. doi:10.5958/0974-360X.2018.00350.5
- Sookkhee S, Sakonwasun C, Mungkornasawakul P, Khamnoi P, Wikan N, Nimlamool W. Synergistic Effects of Some Methoxyflavones Extracted from Rhizome of *Kaempferia parviflora* Combined with Gentamicin against Carbapenem-Resistant Strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. *MDPI Plants*. 2022; 11(3128): 16. doi:10.3390/plants11223128
- Kishore Mohanta Y, Chakrabartty I, Kumar Mishra A, et al. Nanotechnology in combating biofilm: A smart and promising therapeutic strategy. *Front Microbiol*. 2023; (3): 1-30. doi:10.3389/fmicb.2022.1028086
- Varma A, Warghane A, Dhiman NK, et al. The role of nanocomposites against biofilm infections in humans. *Front Cell Infect Microbiol*. 2023; (2): 1-16. doi:10.3389/fcimb.2023.1104615
- Arunachalam K, Krishnan GP, Sethuraman S, et al. Exploring Possible Ways to Enhance the Potential and Use of Natural Products through Nanotechnology in the Battle against Biofilms of Foodborne Bacterial Pathogens. *MDPI Pathogens*. 2023; (270): 1. doi:10.3390/pathogens12020270
- Mirghani R, Saba T, Khaliq H, et al. Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiol*. 2022; 8(3): 240-278. doi:10.3934/microbiol.2022019
- Tuon FF, Suss PH, Telles JP, Dantas LR, Borges NH, Ribeiro VST. Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics*. 2023; 12(1). doi:10.3390/antibiotics12010087
- Wu X, Wang H, Xiong J, et al. *Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics. *Biofilm*. 2024; 7: 100175. doi:10.1016/J.BIOFLM.2023.100175
- Mary RNI, Banu N. Inhibition of Antibiofilm Mediated Virulence Factors in *Pseudomonas aeruginosa* by *Andrographis paniculata*. *Res J Pharm Technol*. 2017; 10(1): 141. doi:10.5958/0974-360X.2017.00031.2
- Dewangan D, Ajazuddin, Agrawal P, et al. Formulation and Evaluation of oral reconstitutable Azithromycin Suspension for the treatment of Bacterial Infection. *Res J Pharm Technol*. 2018; 11(4): 1351. doi:10.5958/0974-360X.2018.00251.2
- Karpagam P, Manonmani G, Brindha D. Phytochemical Screening, Antimicrobial Activity and Antimicrobial Finishing of Polyherbal Extract on Nonwoven Wound Dressing. *Res J Pharm Technol*. 2019; 12(2): 632. doi:10.5958/0974-360X.2019.00112.4
- Raffaelli S, Abreo E, Altier N, Vázquez Á, Alborés S. molecules Bioprospecting the Antibiofilm and Antimicrobial Activity of Soil and Insect Gut Bacteria. *MDPI Molecules*. 2022; 27(2002): 1-13. doi:10.3390/molecules27062002
- Abdul-Lateef LA, Hassan E, Taha Abd-Alla Bactash E. Effect of Cranberry on biofilm formation by *P. mirabilis* isolated from

- Patients Suffering from Urinary tract Infections. *Res J Pharm Technol.* 2018; 11(3): 1097. doi:10.5958/0974-360X.2018.00206.8
26. Ivanov M, Novovićnovović K, Maleševićmalešević M, et al. Polyphenols as Inhibitors of Antibiotic Resistant Bacteria-Mechanisms Underlying Rutin Interference with Bacterial Virulence. *MDPI Pharmaceuticals.* 2022; 15(385): 1-21. doi:10.3390/ph15030385
27. Majnooni MB, Ghanadian SM, Mojarab M, et al. and Antioxidant Activities of Flavonoids Isolated from *Allium colchicifolium* Leaves. *J Food Biochem.* 2023; 1(14): 1-14. doi:10.1155/2023/5521661
28. Mary RNI, Banu N. Inhibition of biofilm formation in *Serratia marcescens* by Andrographolide from *Andrographis paniculata*. *Res J Pharm Technol.* 2017; 10(3): 789. doi:10.5958/0974-360X.2017.00148.2
29. Cloudya Panjaitan C, Sari Widyanman A, Amtha R, Erri Astoeti T, Widyanman S. Antimicrobial and Antibiofilm Activity of Cinnamon (*Cinnamomum burmanii*) Extract on Periodontal Pathogens-An in vitro study. *Eur J Dent.* 2022; 16(4). doi:10.1055/s-0041-1742125
30. Shamim A, Ali A, Iqbal Z, et al. Natural Medicine a Promising Candidate in Combating Microbial Biofilm. *Antibiotics.* 2023; 12(299): 2-26. doi:10.3390/antibiotics12020299
31. Tasmia Asma S, Imre K, Morar A, et al. Natural Strategies as Potential Weapons against Bacterial Biofilms. *Life MDPI.* 2022; 12(1618): 1-35. doi:10.3390/life12101618
32. Liu M, Wei X, Zheng Z, et al. Recent Advances in Nano-Drug Delivery Systems for the Treatment of Diabetic Wound Healing. *Int J Nanomedicine.* 2023; (3): 1537-1560. doi:10.2147/IJN.S395438
33. Rather MA, Gupta K, Mandal M. Microbial biofilm: formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology.* 2021; 1(52): 1701-1718. doi:10.1007/s42770-021-00624-x
34. Bhavani G, P G. Detection of biofilm among clinical isolates of *Acinetobacter baumannii* by tissue culture plate method (TCP). *Res J Pharm Technol.* 2016; 9(10): 1635. doi:10.5958/0974-360X.2016.00327.9
35. Tasmia Asma S, Imre K, Morar A, et al. An Overview of Biofilm Formation-Combating Strategies and Mechanisms of Action of Antibiofilm Agents. *MDPI Life.* 2022; 1(1110). doi:10.3390/life12081110
36. Kreve S, Reis ACD. Bacterial adhesion to biomaterials: What regulates this attachment? A review. *Japanese Dental Science Review.* 2021; 57: 85-96. doi:10.1016/j.jdsr.2021.05.003
37. Letek M, Behrends V, Murugaiyan J, et al. Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics on behalf of the Global AMR Insights Ambassador Network. *MDPI Antibiotics.* 11(200). doi:10.3390/antibiotics11020200