Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Synthesis, Characterization, and Antibacterial Activity Test of Chitosan–Nanosilver–Black Cumin Seed Oil (*Nigella sativa*) Gel Preparation against *Staphylococcus aureus* Bacteria

Mohammad Nurul Mustofa, Sari Edi Cahyaningrum*

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Surabaya, Surabaya, Indonesia
*e-mail: saricahyaningrum@unesa.ac.id

Received: January 7, 2026. Accepted: January 30, 2026. Published: February 19, 2026

Abstract: The skin is the outermost organ of the human body and functions as a protective barrier against environmental factors, making it susceptible to wounds caused by physical trauma and microbial infections. One pathogenic bacterium frequently associated with skin disorders such as boils, acne, and wound infections is *Staphylococcus aureus*. Therefore, topical formulations are required to prevent and manage infection. The combination of chitosan, nanosilver, and black cumin oil exhibits antibacterial activity and biocompatibility. This study aimed to evaluate the characteristics and antibacterial activity of a chitosan–nanosilver–black cumin oil gel against *Staphylococcus aureus*. The gel was formulated in five variations containing black cumin oil concentrations of 3%, 4%, 5%, 6%, and 7%. Chemical characterization was performed using Fourier Transform Infrared spectroscopy to identify functional groups, while nanosilver particle size was analyzed using a Particle Size Analyzer. Physical evaluation included a pH measurement to assess compatibility with the skin's pH. Antibacterial activity was determined by the disc diffusion method, measuring inhibition zone diameters. The Particle Size Analyzer results showed an average nanosilver particle size of 31.54 nm. FTIR analysis of the chitosan–nanosilver system confirmed the presence of O–H, N–H, C–H, and C=O functional groups, while FTIR characterization of black cumin oil confirmed the presence of functional groups associated with C–H stretching, C=O, C–O, and =C–H bending. The formulated gel exhibited a pH of 5.12–5.72, within the physiological pH range of human skin. Antibacterial testing demonstrated that inhibitory activity increased with increasing black cumin oil concentration, with the largest inhibition zone observed in the 7% formulation (21.67 mm). These findings indicate that chitosan–nanosilver–black cumin oil gel has potential for development as an antibacterial formulation.

Keywords: Antibacterial; Black Cumin Seed Oil; Chitosan; Nanosilver; *Staphylococcus aureus*.

Introduction

The skin is the body's outermost layer and serves as the primary protective barrier against environmental factors. It is the largest organ in the human body and receives approximately one-third of the total blood flow. The skin is highly susceptible to damage in the form of wounds. A wound is defined as the loss of a portion of body tissue caused by various factors such as sharp or blunt trauma, extreme temperature changes, chemical exposure, explosions, electric shocks, or animal bites. Several physiological responses may occur following a wound, including partial or complete impairment of organ function, bleeding and blood clotting, bacterial invasion, and cell death. According to the 2013 report from the Ministry of Health of the Republic of Indonesia, the incidence of wounds in Indonesia reached 8.2% [1].

The wound healing process plays a crucial role in restoring organ function as quickly as possible. Wound healing is a complex biological process in which the body repairs damaged cells and tissues through cytokines, chemokines, and various growth factors. If healing progresses slowly, it can cause discomfort and increase the risk of infection from microbial invasion. Infection occurs when pathogenic microbes enter and proliferate within body tissues. One of the bacteria frequently associated with various skin disorders, such as boils, acne, and wound infections, is *Staphylococcus aureus* [2].

One commonly used approach for wound treatment is the administration of topical formulations. Topical therapy is considered more effective because active ingredients act directly on the wound site, thereby accelerating tissue closure. Among various topical dosage forms, gels are the most widely used. Gel stability is influenced by several physical characteristics, including pH, homogeneity, spreadability, and organoleptic properties. The selection of an appropriate gelling agent greatly determines the quality of the resulting gel [3]. Topical formulations are usually developed using ingredients with antibacterial activity, one of which is chitosan, a material widely utilized in wound healing [4].

Chitosan is a natural polymer derived from animal sources and is widely used in the pharmaceutical field. It possesses adhesive properties that enhance the quality of gel formulations by improving their adhesion to the skin surface [3]. Chitosan also offers several advantages, including bioactivity, biocompatibility, metal-ion chelating ability, antibacterial properties, and biodegradability [5]. Its antibacterial activity occurs through the formation of complexes with essential bacterial nutrients and through the interaction between chitosan's positive charge and the negatively charged bacterial surface, leading to intracellular

How to Cite:
M. N. Mustofa and S. E. Cahyaningrum, "Synthesis, Characterization, and Antibacterial Activity Test of Chitosan–Nanosilver–Black Cumin Seed Oil (Nigella sativa) Gel Preparation against Staphylococcus aureus Bacteria", *J. Pijar.MIPA*, vol. 21, no. 1, pp. 130–135, Feb. 2026. https://doi.org/10.29303/jpm.v21i1.11306

leakage and cell death. Another mechanism involves the inhibition of mRNA formation and protein synthesis due to chitosan's interaction with microbial DNA hydrolysis products [6]. Studies have shown that chitosan is effective in inhibiting, and in some cases killing, S. aureus through its lysozyme activity and aminopolysaccharide groups, which enhance its antibacterial activity [7].

In addition to chitosan, nanosilver is also known as a highly effective antimicrobial agent that is widely used in healthcare [8]. Topical formulations containing nanosilver are commonly applied to treat infected wounds [9]. Nanosilver possesses strong antibacterial, antiviral, and antifungal activities and is beneficial in preventing infections in chronic wounds [10]. Its antibacterial mechanism includes pore formation in bacterial cell walls, disruption of membrane permeability, increased reactive oxygen species (ROS) formation, and DNA damage that leads to mutation or cell death [11]. Nanosilver has been shown to exhibit antibacterial activity against both gram-negative and gram-positive bacteria, including *S. aureus*, with an effectiveness comparable to certain antibiotics [9]. The combination of nanosilver and chitosan has also been proven effective against various harmful pathogens and exhibits anti-inflammatory effects [12].

In addition to synthetic agents, herbal ingredients are widely utilized for their antibacterial properties. One of the well-known herbal plants with strong antibacterial activity is black seed (*Nigella sativa*). Black seed oil has long been used in traditional medicine to treat various ailments [13]. The plant contains numerous bioactive compounds such as thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, t-anethole, and thymol [14]. Thymoquinone, a major component belonging to the terpenoid group, acts as the primary antibacterial compound. It functions by permanently damaging the cell membrane and inhibiting the growth of bacteria, viruses, and parasites through the destruction of porins in bacterial cell walls [15]. The combination of black seed oil and chitosan exhibits anti-inflammatory, immunomodulatory, antioxidant, and antibacterial effects, all of which support the wound healing process [13]. Several studies have demonstrated that *Nigella sativa* oil is highly effective in inhibiting the growth of *Staphylococcus aureus* [14][15].

Based on the background above, this study aims to formulate a gel containing chitosan–nanosilver–black seed oil as an antibacterial agent against *Staphylococcus aureus*. The resulting formulation is then characterized using FTIR (Fourier-Transform Infrared) and PSA (Particle Size Analyzer) instruments, followed by antibacterial activity testing to determine the effectiveness of the developed gel.

Research Methods

Tools and Materials

The tools used in this study included test tubes, analytical balance, burettes, beakers, spatulas, measuring cylinders, dropper pipettes, hot plate, pH meter, viscometer, inoculating loop, tweezers, autoclave, incubator, Petri dishes, volumetric pipette, stopwatch, and magnetic stirrer. The instruments used were a Fourier Transform InfraRed Spectrophotometer (FT-IR) and a Particle Size Analyzer (PSA). The materials used in this study included distilled water, chitosan, 1% glacial acetic acid, $AgNO_3$, sodium borohydride, black cumin oil, propylene glycol, xanthan gum, Staphylococcus aureus bacterial culture, Nutrient Agar (NA), Nutrient Broth (NB), and disc paper.

Procedure for Research

Preparation of Chitosan Solution

A total of 0.3 g of chitosan powder was dissolved in 100 mL of 2% glacial acetic acid, yielding a 0.3 M chitosan solution.

Synthesis of Nanosilver

The nanosilver was synthesised via chemical reduction using sodium borohydride as the reducing agent. A $NaBH_4$ solution was prepared by dissolving 0.42 g of $NaBH_4$ in distilled water. Next, 40 mL of 0.001 M $AgNO_3$ solution was placed in an ice bath and stirred using a magnetic stirrer for 20 minutes. The ice bath was used to maintain better control over the size and shape of the silver particles. Then, 5 mL of $NaBH_4$ solution was added dropwise. Stirring was stopped once the solution began to turn yellow.

Formulation of gel Chitosan–Nanosilver–Black Cumin Seed

The chitosan–nanosilver–black cumin seed extract gel formulation was prepared in five variations, as shown in the following table:

Table 1. Formulation of Gel Chitosan–Nanosilver–Black Cumin Oil

Material	Formulation % v/v				
	F_1	F_2	F_3	F_4	F_5
Propylene glycol	5	5	5	5	5
Xanthan gum	0.4	0.4	0.4	0.4	0.4
Benzoate	0.1	0.1	0.1	0.1	0.1
Chitosan	3	3	3	3	3
Nanosilver	3	3	3	3	3
Black cumin oil	3	4	5	6	7
Ad Aquades	100	100	100	100	100

Distilled water was first heated, then propylene glycol, followed by the chitosan solution, nanosilver, black cumin oil, xanthan gum, and sodium benzoate, which were stirred until a gel formed. The resulting gel was stored overnight in a dark, cool place at 10°C–15°C.

pH Measurement

The pH of the gel formulation was measured directly using a pH meter.

FTIR Analysis

Functional group characterization was conducted using FTIR spectroscopy to determine the functional groups present in black cumin oil.

Particle Size Analysis

Particle size distribution of the nanosilver was determined using a Particle Size Analyzer (PSA).

Antibacterial Activity Test

Antibacterial activity was evaluated using the disc diffusion method. All instruments were sterilized prior to use. Nutrient Agar (NA) medium was prepared by dissolving agar powder in distilled water and sterilizing it in an autoclave at 120°C for 15 minutes. The medium was cooled to approximately 80°C, poured into plates, and left to solidify before incubation for 24 hours. The *Staphylococcus aureus* culture was prepared by streaking the bacteria onto NA and incubating for 24 hours. The grown colonies were then suspended in NaCl solution and mixed until homogeneous. The bacterial suspension was spread evenly on Mueller–Hinton (MH) agar. Paper discs were immersed in the sample and placed onto the inoculated MH agar. The plates were incubated for 24 hours, and the inhibition zones formed were measured. Each antibacterial assay was performed in triplicate, and the inhibition zone diameter was reported as the mean.

Results and Discussion

FTIR Characterization

FTIR spectroscopy was performed to identify the functional groups present in black cumin oil within the wavenumber range of 4000–400 cm^{-1}. The FTIR characterization results for each sample are presented in the following figure.

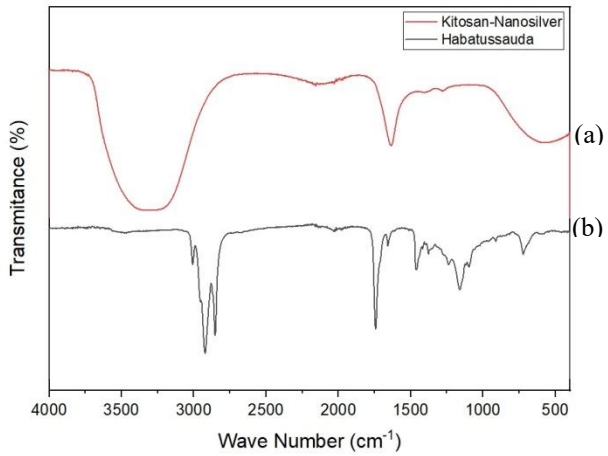

Figure 1. FTIR spectra of (a) chitosan–nanosilver and (b) black cumin (Nigella sativa) seed oil.

Figure 1(a) presents the FTIR spectrum of chitosan–nanosilver. The spectrum shows four main peaks in the wavenumber range of 4000–400 cm^{-1} at 3334.37, 2157.47, 1636.31, and 587.05 cm^{-1}. The absorption band at 3334.37 cm^{-1} corresponds to the stretching vibrations of O–H and N–H groups, which originate from the hydroxyl and amine groups in the chitosan structure. The broadening of this peak indicates the presence of intermolecular hydrogen bonding as well as possible interactions between chitosan amine groups and the nanosilver surface. The presence of $–NH_2$ groups is particularly important, as they play a role in stabilizing nanosilver through coordination interactions with Ag^+ ions.

The absorption band at 2157.47 cm^{-1} is associated with the aliphatic C–H stretching vibrations of methyl and methylene groups in the chitosan polysaccharide chain, indicating that the basic structure of chitosan remains preserved after the formation of the nanosilver nanocomposite. The peak at 1636.31 cm^{-1} corresponds to the C=O (amide I) stretching vibration and N–H bending vibration. This band is attributed to the stretching vibration of the carbonyl (C=O) group of residual N-acetyl groups (amide I) as well as the bending vibration of the N–H group. The absorption band at 587.05 cm^{-1} appears in the fingerprint region. Peaks in the fingerprint region (below 1500 cm^{-1}) are associated with possible Ag–N or Ag–O bond vibrations, indicating interactions between silver atoms and the amine or hydroxyl groups of chitosan. The presence of this peak supports the assumption that nanosilver is successfully bound and dispersed within the chitosan matrix. The FTIR spectrum of chitosan–nanosilver is consistent with previous studies, as indicated by the presence of O–H, N–H, C-H, and C=O functional groups [16][17] .

Figure 1(b) presents the FTIR spectrum of black cumin oil. The spectrum exhibits twelve absorption peaks within the wavenumber range of 4000–400 cm^{-1}, specifically at 3008.17, 2922.22, 2852.80, 2028.34, 1742.71, 1658.95, 1463.49, 1377.40, 1238.99, 1161.36, 1097.55, and 721.66 cm^{-1}. The absorption band at 3008.17 cm^{-1} corresponds to =C–H stretching, which typically appears in aromatic compounds or conjugated alkenes. In black cumin oil, this peak is likely attributed to thymoquinone or thymol, both of which contain aromatic ring structures. The peaks at 2922.22 cm^{-1} and 2852.80 cm^{-1} indicate C–H stretching vibrations of alkanes (CH_2 and CH_3 groups). The absorption at 1742.71 cm^{-1} represents a C=O stretching band characteristic of esters, ketones, or carboxylates, which is significant because thymoquinone contains two carbonyl (C=O) groups. The peak at 1658.95 cm^{-1} corresponds to aromatic C=C or conjugated C=O stretching. This absorption is commonly observed for aromatic C=C bonds in thymol and p-cymene, as well as for the conjugated C=O groups in thymoquinone. The band at 1463.49 cm^{-1} reflects C–H bending vibrations of methylene and methyl groups, while the peak at 1377.40 cm^{-1} indicates CH_3 bending, which is typically present in structures containing isopropyl groups, such as those found in thymoquinone and thymol. This is consistent with known components of black cumin oil. The peak at 1238.99 cm^{-1} corresponds to C–O stretching (ester or phenolic), whereas the absorptions at 1161.36 and 1097.55 cm^{-1} indicate C–O stretching associated with alcohols or ethers. Finally, the band at 721.66 cm^{-1} represents =C–H bending typical of alkenes. The FTIR spectral results of black cumin oil are in agreement with previous studies, as indicated by the presence of C–H stretching, C=O, C–O, and =C–H bending functional groups [18].

PSA Characterization

Particle size characterisation was performed using PSA to determine the particle size of the synthesised nanosilver. The PSA results are presented in Figure 2.

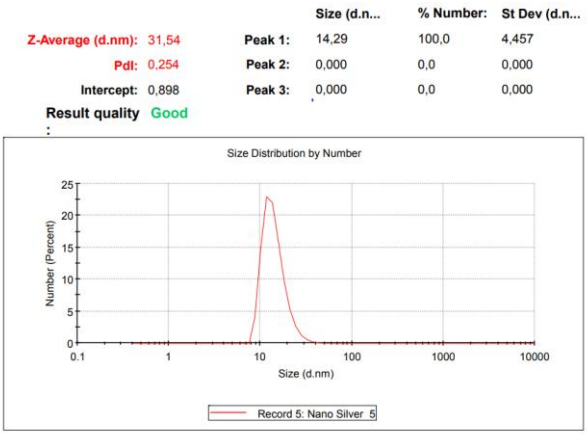

Figure 2. PSA Analysis Results of Nanosilver

The figure shows that the nanosilver used in this study has a particle size of 31.54 nm. This result is consistent with the general characteristics of nanosilver, which is a silver-based (Ag) nanoparticle with a typical size range of 1–100 nm. Nanosilver is well-known for its antibacterial properties and its wide applicability in various daily-life applications[19]. This finding also aligns with previous research, which reported that nanosilver with a particle size range of 7.36–36.68 nm can be effectively applied as an antibacterial agent for treating infected wounds [20].

pH Characterization

The pH of the formulated chitosan–nanosilver–black cumin oil gel was evaluated to determine the acidity of the preparation containing chitosan, nanosilver, and black cumin oil. pH is an essential parameter that influences the safety and comfort of topical formulations, as it must fall within the physiological skin pH range of 4.5–6.5. A pH value outside this range may potentially cause irritation, disrupt the skin barrier function, and affect the stability of active compounds within the formulation [21]. The pH characterization results are presented in Table 2

Table 2. pH Characterization Results

Formulation	pH
F_1	5.12
F_2	5.24
F_3	5.44
F_4	5.58
F_5	5.72

Based on Table 2, the chitosan–nanosilver–black cumin oil gel formulation exhibited pH values ranging from 5.12 to 5.72, which remain within the acceptable and safe range for topical application and are consistent with the physiological pH of human skin. The pH values obtained also meet the requirements of the Indonesian National Standard (SNI No. 06-2588), which specifies that topical formulations should have a pH range of 4.5–6.5 to minimize the risk of skin irritation and to maintain formulation stability. An appropriate pH range is crucial for preserving the stability of active ingredients in the formulation, particularly chitosan and nanosilver. The antimicrobial activity of chitosan is influenced by environmental pH through the protonation of its amino groups, which enhances electrostatic interactions with bacterial cell membranes, whereas nanosilver maintains its effectiveness under stable physiological pH conditions [22]. Furthermore, formulations with a pH close to that of the skin are known to improve user comfort and reduce the potential for irritation during long-term topical application, as maintaining the physiological condition of the skin and the integrity of the acid mantle plays an important role in skin homeostasis [23].

Antibacterial Activity

In this stage, the antibacterial activity of the formulated chitosan–nanosilver–black cumin oil gel was evaluated against *Staphylococcus aureus*. The gel samples were tested at varying concentrations of black cumin oil (3 mL, 4 mL, 5 mL, 6 mL, and 7 mL), along with a positive control (Betadine ointment) and a negative control (distilled water). Observations were conducted after an incubation period of 24 hours, followed by measurement of the inhibition zones using a caliper. The antibacterial activity results are presented in Table 3.

Table 3. Antibacterial Test Results of Chitosan-Nanosilver-Black Cumin Oil

Formulation	Clear Zona (mm) Repetition			Average
	I	II	III	
K^-	0	0	0	0
K^+	17.4	17.5	17.6	17.5
F_1	15.5	15.6	15.8	15.63
F_2	16.2	16.3	16.4	16.31
F_3	18.3	18.4	18.6	18.42
F_4	21.0	21.1	21.1	21.07
F_5	21.6	21.7	21.7	21.67

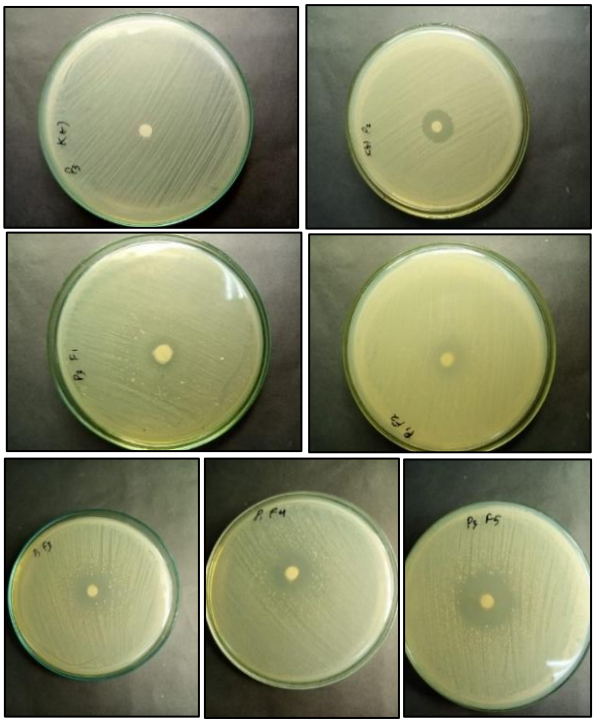

Figure 3. The clear zone formed from the results of the antibacterial test

Based on Table 3, the results indicate that the chitosan–nanosilver–black cumin oil gel formulation

inhibits the growth of *Staphylococcus aureus*. The inhibition zones formed varied across treatments, with a trend toward larger diameters at higher concentrations of black cumin oil. The largest inhibition zone was observed at the 7% concentration, measuring 21.67 mm.

The antibacterial activity observed in the chitosan–nanosilver–black cumin oil gel is attributed to the synergistic mechanisms of chitosan, nanosilver, and thymoquinone. Chitosan exhibits antimicrobial activity through electrostatic interactions between its protonated amino groups ($–NH_3^+$) and the negatively charged bacterial cell membrane, leading to increased membrane permeability, leakage of intracellular components, and disruption of essential bacterial metabolic pathways [24]. Nanosilver enhances this antibacterial effect by adhering to the bacterial cell surface and releasing Ag^+ ions, which subsequently penetrate the cell, interact with thiol-containing enzymes, induce the generation of reactive oxygen species (ROS), and cause structural and functional damage to bacterial proteins, membranes, and DNA [25]. Meanwhile, thymoquinone, the major bioactive compound in black cumin oil, contributes to the antibacterial activity through its lipophilic properties, enabling its integration into the lipid bilayer of the bacterial membrane and resulting in decreased membrane stability and integrity [26]. In addition, thymoquinone has been reported to inhibit enzymatic activity, disrupt membrane transport systems, and induce oxidative stress that ultimately leads to bacterial DNA fragmentation [27]. The combined action of these three components produces a multi-target antibacterial mechanism that effectively inhibits the growth of *Staphylococcus aureus*, which explains the observed increase in inhibition zone diameter with increasing concentrations of black cumin oil in the gel formulation.

Conclusion

The chitosan–nanosilver–black cumin oil gel was successfully formulated and exhibited favorable physicochemical characteristics. PSA characterization indicated that the nanosilver used had a particle size of 31.54 nm. FTIR analysis of the chitosan–nanosilver system confirmed the presence of O–H, N–H, C–H, and C=O functional groups, while FTIR characterization of black cumin oil confirmed the presence of functional groups associated with C–H stretching, C=O, C–O, and =C–H bending. The chitosan–nanosilver–black cumin oil gel exhibited a pH range of 5.12–5.72, indicating its suitability for topical application. Antibacterial testing demonstrated that the gel inhibited the growth of *Staphylococcus aureus*, with the highest antibacterial activity observed at a 7% concentration of black cumin oil, producing an inhibition zone diameter of 21.67 mm. These results suggest that the chitosan–nanosilver–black cumin oil gel has promising potential as a topical antibacterial formulation for the management of *S. aureus*-infected wounds and may serve as a basis for further development in pharmaceutical or biomedical applications.

Author's Contribution

M. N. Mustofa: Conceptualization, Methodology, Investigation, Synthesis and Formulation, Characterization, Data Curation, Formal Analysis, Writing-Original Draft. S. E. Cahyaningrum: Supervision, Project Administration, Validation, Resources, Funding Acquisition, Writing-Review & Editing.

Acknowledgements

The authors would like to express their sincere gratitude to the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, for providing laboratory facilities and technical support during the research process. Appreciation is also extended to all laboratory staff who assisted in sample preparation, characterization, and antibacterial testing. This research was carried out as part of an undergraduate thesis and was supported by institutional resources of Universitas Negeri Surabaya. The authors also thank all parties who contributed, directly or indirectly, to the completion of this study.

References

[1] R. Wintoko, A. Dwi, and N. Yadika, "Manajemen Terkini Perawatan Luka Update Wound Care Management," vol. 4, pp. 183–189, 2020.

[2] M. C. Muntiaha, P. V. Y. Yamlean, and A. Lolo, "uji efektivitas sediaan krim getah jarak cina (Jatropha multifida L .) Untuk pengobatan luka sayat yang terinfeksi bakteri Staphylococcus aureus PADA KELINCI (Orytolagus cuniculus)," vol. 3, no. 3, pp. 294–302, 2014.

[3] Mensie Martha Lovianie, Susi Nurmanila, and Mustika, "pengaruh pemberian sediaan emulgel kitosan-ekstrak daun tapak dara (catharantus roseus (l.) G. Don.) Dan emulgel kitosan-ekstrak kulit pisang ambon (*Musa paradisiaca* L.) Untuk penyembuhan luka bakar pada kelinci," *J. Borneo Cendekia Med.*, vol. 2, no. 2, pp. 223–234, 2018.

[4] M. A. Matica, F. L. Aachmann, A. Tøndervik, H. Sletta, and V. Ostafe, "Chitosan as a Wound Dressing Starting Material : Antimicrobial Properties and Mode of Action," no. 1, pp. 1–33, 2019.

[5] D. Kurniasari and S. Atun, "pembuatan dan karakterisasi nanopartikel ekstrak etanol temu kunci (Boesenbergia pandurata) pada berbagai variasi komposisi kitosan the preparation and characterization of fingerroot (Boesenbergia pandurata) etanol extract nanoparticles with various C," vol. 6, no. 1, pp. 31–35, 2017.

[6] P. Kulawik, E. Jamróz, and F. Özogul, *Chitosan for Seafood Processing and Preservation*. 2019. doi: 10.1007/978-3-030-16581-9.

[7] U. dan Surahmaida, "Isolasi, Identifikasi, dan Uji Antibakteri Kitosan Cangkang Bekicot (Achatina fulica) Terhadap Staphylococcus aureus dari Penderita Ulkus Diabetikum," *simbiosa J.*, vol. 8, no. 1, pp. 37–49, 2019, doi: 10.33373/sim-bio.v8i1.1894.

[8] Aimmatul Jannah, "aktivitas antibakteri sintesis nanopartikel perak (Ag-NP) dan gel nanopartikel perak (Ag-NP) terhadap bakteri Staphylococcus aureus aktivitas antibakteri sintesis nanopartikel perak (Ag-NP) dan gel nanopartikel perak (Ag-NP) terhadap bakteri Staph," *Univ. Islam negeri*

maulana malik ibrahim, 2019.

[9] Komang Tri Aksari Dewi, Kartini, Johan Sukweenadhi, and Christina Avanti, "Karakter Fisik dan Aktivitas Antibakteri Nanopartikel Perak Hasil Green Synthesis Menggunakan Ekstrak Air Daun Sendok (Plantago major L.) Physical," *Pharm. Sci. Res.*, 2019.

[10] N. Masood *et al.*, "Silver Nanoparticle Impregnated Chitosan-PEG Hydrogel Enhances Wound Healing in Diabetes induced Rabbits," *Int. J. Pharm.*, 2019.

[11] K. G. Kaiser *et al.*, "Nanosilver : An Old Antibacterial Agent with Great Promise in the Fight against Antibiotic Resistance," *Antibiotics*, vol. 12, 2023.

[12] C. S. Karthik, H. M. Manukumar, A. P. Ananda, S. Nagashree, and K. P. Rakesh, "International Journal of Biological Macromolecules Synthesis of novel benzodioxane midst piperazine moiety decorated chitosan silver nanoparticle against biohazard pathogens and as potential anti-inflammatory candidate : A molecular docking studies," *Int. J. Biol. Macromol.*, vol. 108, pp. 489–502, 2018, doi: 10.1016/j.ijbiomac.2017.12.045.

[13] F. Bagheri, S. Darakhshan, S. Mazloomi, B. Shiri, and R. Tahvilian, "Dual loading of Nigella sativa oil-atorvastatin in chitosan – carboxymethyl cellulose nanogel as a transdermal delivery system nanogel as a transdermal delivery system," *Drug Dev. Ind. Pharm.*, vol. 0, no. 0, pp. 1–10, 2021, doi: 10.1080/03639045.2021.1892742.

[14] P. Balyan, S. Shinde, and A. Ali, "Potential activities of nanoparticles synthesized from Nigella sativa L. and its phytoconstituents : An overview," *J. Phytonanotechnology Pharm. Sci.*, vol. 1, no. 2, pp. 1–9, 2021.

[15] B. Erguden, "Phenol group of terpenoids is crucial for antibacterial activity upon ion leakage," *Lett. Appl. Microbiol.*, pp. 1–8, 2021, doi: 10.1111/lam.13529.

[16] G. Vanti *et al.*, "Synthesis and Characterization of Multifunctional Chitosan – Silver Nanoparticles : An In-Vitro Approach for Biomedical Applications," *Pharmaceuticals*, pp. 1–14, 2024.

[17] S. Sekhar *et al.*, "Green synthesis of silver-chitosan nanocomposite exhibits promising antibiofilm properties against pathogenic bacteria Escherichia coli and Staphylococcus aureus," *The Microbe*, vol. 6, no. October 2024, p. 100264, 2025, doi: 10.1016/j.microb.2025.100264.

[18] Y. Nurchollifah, "Literature Study Of Pharmacological Effects Of Black Seed (Nigella Sativa) Based On Active Seeds Studi Literatur Efek Farmakologi Biji Habbatussauda / Jintan Hitam (Nigella Sativa) Berdasarkan Zat Aktif," *Univ. Res. Colloqium*, pp. 815–832, 2021.

[19] A W Wahab, A Karim, N La Nafie, P Satrimafitrah, Triana, and and I W Sutapa, "Production of the nanoparticles using leaf of Muntingia calabura L . as bioreductor and potential as a blood sugar nanosensor Production of the nanoparticles using leaf of Muntingia calabura L . as bioreductor and potential as a blood sugar nanosensor," *J. Phys.*, 2019, doi: 10.1088/1742-6596/1242/1/012004.

[20] V. A. Fabiani, M. A. Putri, M. E. Saputra, and P. Indriyani, "sintesis nanosilver menggunakan bioreduktor ekstrak daun pelawan (Tristaniopsis merguensis) dan uji aktivitas antibakteri Synthesis of Nano Silver using Bioreductor of Tristaniopsis merguensis Leaf Extracts and Its Antibacterial Activity Test," *JKPK (JURNAL Kim. DAN Pendidik. Kim.*, vol. 4, no. 3, pp. 172–178, 2019.

[21] M. Ayu, M. Ebit, I. Nur, S. Musiam, and V. Andre, "Hand Sanitizer Ekstrak Daun Pucuk Idat (Cratoxylum glaucum) sebagai Antibakteri Staphylococcus aureus dan Escherichia coli," *ALCHEMY J. Penelit. Kim.*, vol. 16, no. 2, pp. 227–231, 2020, doi: 10.20961/alchemy.16.2.32208.227-231.

[22] C. L. Ke, F. S. Deng, C. Y. Chuang, and C. H. Lin, "Antimicrobial actions and applications of Chitosan," Mar. 02, 2021, *MDPI AG*. doi: 10.3390/polym13060904.

[23] M. Lukić, I. Pantelić, and S. D. Savić, "Towards optimal ph of the skin and topical formulations: From the current state of the art to tailored products," Aug. 01, 2021, *MDPI AG*. doi: 10.3390/cosmetics8030069.

[24] S. Ahmed and S. Ikram, "Chitosan Based Scaffolds and Their Applications in Wound Healing," *Achiev. Life Sci.*, vol. 10, no. 1, pp. 27–37, Jun. 2016, doi: 10.1016/j.als.2016.04.001.

[25] Y. N. Slavin, J. Asnis, U. O. Häfeli, and H. Bach, "Metal nanoparticles: Understanding the mechanisms behind antibacterial activity," Oct. 03, 2017, *BioMed Central Ltd.* doi: 10.1186/s12951-017-0308-z.

[26] M. A. Hannan, M. S. Zahan, P. P. Sarker, A. Moni, H. Ha, and M. J. Uddin, "Protective effects of black cumin (Nigella sativa) and its bioactive constituent, thymoquinone against kidney injury: An aspect on pharmacological insights," Aug. 02, 2021, *MDPI*. doi: 10.3390/ijms22169078.

[27] Z. Gholamnezhad, S. Havakhah, and M. H. Boskabady, "Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review," Aug. 22, 2016, *Elsevier Ireland Ltd*. doi: 10.1016/j.jep.2016.06.061.