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BACTERIOPHAGE-BASED WOUND CARE FORMULATION FOR DIABETIC ULCERS AS A GROWTH INHIBITOR OF *Alcaligenes faecalis*

BACTERIOPHAGE-BASED WOUND CARE FORMULATION FOR DIABETIC ULCERS AS A GROWTH INHIBITOR OF *Alcaligenes faecalis*

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Abstract

Diabetic ulcers are frequently complicated by infections from multidrug-resistant bacteria such as *Alcaligenes faecalis*, necessitating the development of alternative therapeutic strategies. Bacteriophage therapy presents a promising approach due to its specificity and efficacy against antibiotic-resistant pathogens. This study aimed to formulate and evaluate a bacteriophage-based topical cleanser using the phage ϕAFaV1 for treating *A. faecalis* infections, focusing on high antibacterial efficacy and low dermal toxicity. Three suspension formulas (F1, F2, F3) were prepared with varying chitosan concentrations (0.5; 0.75, and 1.0%) as a suspending agent. These formulations underwent physical stability testing over 28 days, including assessments of viscosity, pH, zeta potential, and homogeneity. Antibacterial efficacy was determined through inhibition zone assays. All three formulations effectively inhibited bacterial growth, forming clear zones. The F2 formula (0.75% chitosan), containing 200 µL of active phage ingredient, was identified as the optimum formulation. It demonstrated superior physical stability and the most effective bactericidal activity against *A. faecalis*, as confirmed by statistical analysis. These results indicate that a bacteriophage suspension stabilized with 0.75% chitosan is a highly promising and stable topical agent for combating multidrug-resistant *A. faecalis* in diabetic wound care.

Keywords: *Alcaligenes faecalis*; Bacteriophage; Chitosan; Diabetic ulcers

Abstrak

Ulkus diabetes sering kali merupakan komplikasi oleh infeksi bakteri yang resisten terhadap berbagai obat, seperti Alcaligenes faecalis, sehingga diperlukan pengembangan strategi terapi alternatif. Terapi bakteriofag menawarkan pendekatan yang menjanjikan karena spesifisitas dan kemanjurannya terhadap patogen yang resisten terhadap antibiotik. Penelitian ini bertujuan untuk memformulasi dan mengevaluasi pembersih topikal berbasis bakteriofag menggunakan bakteriofag ϕAFaV1 untuk mengobati infeksi A. faecalis, dengan fokus pada efikasi antibakteri yang tinggi dan toksisitas dermal yang rendah. Tiga formula suspensi (F1, F2, F3) disiapkan dengan variasi konsentrasi kitosan (0,5; 0,75; dan 1,0%) sebagai bahan penyalut. Formula-formula ini menjalani pengujian stabilitas fisik selama 28 hari, yang meliputi penilaian viskositas, pH, zeta potential, dan homogenitas. Efikasi antibakteri ditentukan melalui uji zona hambat. Ketiga formula secara efektif menghambat pertumbuhan bakteri dengan membentuk zona bening. Formula F2 (kitosan 0,75%), yang mengandung 200 µL bahan aktif fag, diidentifikasi sebagai formula yang paling optimum. Formula ini menunjukkan stabilitas fisik yang unggul dan aktivitas bakterisida yang paling efektif terhadap A. faecalis, sebagaimana dikonfirmasi oleh analisis statistik. Hasil ini menunjukkan bahwa suspensi bakteriofag yang distabilkan dengan kitosan 0,75% merupakan agen topikal yang sangat menjanjikan dan stabil untuk memerangi A. faecalis yang multidrug-resistant dalam perawatan luka diabetik

***Kata Kunci**: Alcaligenes faecalis; Bakteriofag; Kitosan; Ulkus diabetes*

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INTRODUCTION

Diabetes is a non-contagious disease that can cause various complications. Patients with diabetes have a 25% chance of developing foot ulcers (Huang, 2020). The most abundant microbes in diabetic ulcers are Gram-negative and Gram-positive bacteria. Based on the results of a previous study, it was found that the results of identifying bacterial in diabetic ulcers, the most bacterial isolates were *Alcaligenes faecalis*, with a percentage of 44% of the total sample of patients with diabetic ulcers in Jember Regency (Risqiyah et al., 2022). The presence of *A. faecalis* bacteria in diabetic ulcers was also reported by Huang (2020). These pathogenic bacteria will potentially emerge and cause opportunistic infections in humans.

The method of treating *A. faecalis* with antibiotics is apparently unable to treat optimally, due to the high resistance of *A. faecalis* to antibiotics (Risqiyah et al., 2022). Based on these findings, an alternative solution is needed that can infect bacteria in diabetic ulcers, namely by using bacteriophages. Bacteriophages are bacterial parasites that live depending on bacteria as their host (Lin et al., 2017). Bacteriophages that infect specific groups of bacteria can lyse bacteria which is characterized by the formation of a lysis zone (Zhang et al., 2017). The use of bacteriophages as antibacterials is more beneficial than the use of antibiotics because bacteriophages work specifically to only attack target pathogens, while antibiotics can attack all microorganisms, including normal microflora in the body, so that it will disrupt the normal microflora that benefits the body (Hardanti et al., 2018).

Treatment of diabetic ulcers can be handled optimally if wound management is done properly and efficiently. Several methods are used in the treatment of diabetic ulcers, including wound cleansing, debridement, and selecting the right dressing (Farida et al., 2021). Wound cleansers in general use antiseptics such as hydrogen peroxide, povidone iodine, acetic acid, and chlorohexidine which can interfere with the healing process of the body because these antiseptic ingredients not only kill germs, but also damage leukocytes which can kill pathogenic bacteria and fibroblast tissue which forms new skin tissue so that the risk of infection is greater and wound healing takes longer (Kristiyaningrum et al., 2013).

The risk that comes from existing diabetic wound cleansers comes from the ingredients of the drug, whereas the selection of ingredients for the preparation of wound cleansing drugs must be based on the effectiveness and lack of toxicity of the cleaning solution (Narulita et al., 2023). Therefore, it is necessary to prepare alternative formulations in making diabetic wound cleansing drugs by adding active ingredients that have high effectiveness in killing bacteria and low levels of toxicity to the skin.

Preparation of formulations in the form of colloidal suspension, with suspended materials, namely bacteriophages and using a suspending agent in the form of chitosan. The choice of suspension formulation is based on its aqueous and easily lost wound cleansing properties; it is not suitable for the work of bacteriophages, where bacteriophages must contact bacteria so that they are assisted with a suspending agent in the form of chitosan to prolong contact time and increase the effectiveness of bacteria. Chitosan can be used as a gelling agent in hydrogel preparations or gel preparations (Imtihani et al., 2020). Another function of chitosan is also as a carrier in the process of making nanoparticles, which are very compatible with various kinds of active ingredients, as tablet excipients, disintegrants, tablet coatings, and antimicrobials, and anti-cholesterols. Chitosan helps interact with the cell wall of the negatively charged sialic acid (mucin) bacteria interacts with the positive charge of chitosan resulting in electrostatic interactions that can help phage contact with bacteria not wasted when applied to diabetic ulcers. This study examined the characteristics of bacteriophage-based wound care for diabetic ulcers as well as its effectiveness in inhibiting *A. faecalis* growth.

MATERIALS AND METHODS

Alcaligenes faecalis isolate rejuvenated in Luria Bertani (LB) media with a composition of 1% peptone, 0.5% yeast, and 1% sodium chloride, and incubated at 37 °C for 24 hours (Narulita et al., 2023). The bacteriophage propagation method was carried out based on Narulita et al. (2023) with modifications. The medium used was LB double layer agar with a bottom of 1% and top agar of 0.5%.

The warm top agar (±50 °C) was vortexed with 300 µL 4-hour-old *A. faecalis* isolate (log phase) in LB. The suspension of top agar and *A. faecalis* was poured over the bottom layer of the compacted media. The spot test was carried out by dripping 2 µL of ϕAFaV1 suspension on the solidified media. Incubation was carried out for 24–72 hours at 37 °C. The spot test results were shaken using SM buffer and then incubated at 4 °C overnight. The suspension obtained was centrifuged at 12,000 rpm at 4 °C for 10 minutes. The supernatant obtained was filtered using a 0.2 µm membrane filter.

The suspension of bacteriophage formulation was prepared using the dispersion method, which involved adding phage to the mucilage formed and then diluting it. Low molecular weight chitosan powder was dissolved as much as 0.25, 0.375, and 0.5 g in 50 mL of 1% acetic acid solution (300 ppm) (Table 1). Chitosan solution in a magnetic stirrer at 40 °C for 60 minutes (Trisnawati et al., 2023). After that, 10^6 CFU/mL (200 µL) bacteriophage was added, then the combination was stirred in a magnetic stirrer until it was homogeneous, and the pH was measured according to the skin pH (4.5–6.5), and incubated at 28 °C.

Table 1. Bacteriophage suspension formulas

Composition	Formulation		
	F1 (0.5%)	F2 (0.75%)	F3 (1%)
Bacteriophage (µL)	200	200	200
Acetic acid 1%	50	50	50
Chitosan (g)	0.25	0.375	0.5
Aquadest (mL)	50	50	50

Formulation evaluation consisted of potential zeta, pH, viscosity, homogeneity as well as bacteriophage stability. Measurement of the zeta potential of suspension preparations using a Particle Size Analyzer (PSA). Microparticles with zeta potential values close to +30 mV/ -30 mV are said to be stable. pH testing is carried out using a calibrated pH meter. The pH value of the preparation meets the skin pH criteria and does not irritate, namely pH 4.5–6.5 (Nikam, 2017; Okuma et al., 2015). Viscosity measurement was carried out with a Brookfield viscometer with a rotation of 30 rpm. According to SNI, the viscosity value of the suspension is 37–396 cPs. The homogeneity test was based on the principle of observing the particles formed from the preparation, from which samples were taken from various places, and after the suspension was shaken, the phage level of the sample was calculated.

Testing the stability of bacteriophages against the suspension solution was carried out by conducting a bacterial inhibition test using the disc paper diffusion method. Observation of the bacterial inhibition zone was carried out 7 times (day 0, 1, 3, 7, 14, 21, 28) for 28 days of storage.

RESULTS

Potential zeta testing result (Table 2) shows the stability of the colloid. Interactions between particles have a role in the stability of a colloid. The pH test was carried out to determine the stability of the pH in the suspension against the effect of the addition of active ingredients and the suspending agent used. The pH test also aims to make the preparation have a pH value that is adjusted to the pH of the skin (Table 2). Meanwhile, viscosity (Table 2) testing aimed to determine the effect of the amount of chitosan much consistency and shows the thickness of the bacteriophage suspension that has been made. The homogeneity determines the homogeneity of the suspension prepared by comparing the phage content (Table 2). The uniformity (homogeneity) of a suspension-specifically a suspension containing phages (viruses that infect bacteria)-is assessed or verified by measuring and comparing the amount of phage present in different samples or parts of the suspension. If the phage content is consistent throughout the suspension, then the suspension is considered homogeneous. The stability of bacteriophages against suspension solutions for 28 days of observation was shown by inhibition test (Table 3; Figure 1). The 0.75% concentration of chitosan had the largest inhibition zone among the tested concentrations, indicating the strongest antibacterial effect (Table 3). Early dpi (e.g., 1 dpi) might show smaller inhibition zones, while later dpi (e.g., 14+ dpi) could indicate a stronger or diminishing effect (Figure 1).

Table 2. Evaluation of bacteriophage suspension formulas

Bacteriophage suspension formulas	Potential zeta	Viscosity	pH	Homogeneity
F1 (0.5%)	-0.8 mV	49.70 ± 7.25	5.84 ± 0.10	0.27 ± 0.007 (homogenous)
F2 (0.75%)	-0.5 mV	95.34 ± 16.00	5.88 ± 0.04	0.29 ± 0.005 (homogenous)
F3 (1%)	0.0 mV	178.20 ± 74.68	5.88 ± 0.04	0.28 ± 0 (homogenous)

Table 3. Diameter of bacterial inhibition zone

Concentration of bacteriophage	Diameter of inhibition zone (cm)	Explanation
0.5 %	4.48 ± 0.56	Weak
0.75 %	7.95 ± 0.52	Moderate
1 %	4.86 ± 0.61	Weak
Control (+)	10.38 ± 0.14	Strong
Control (-)	0.00 ± 0	Weak

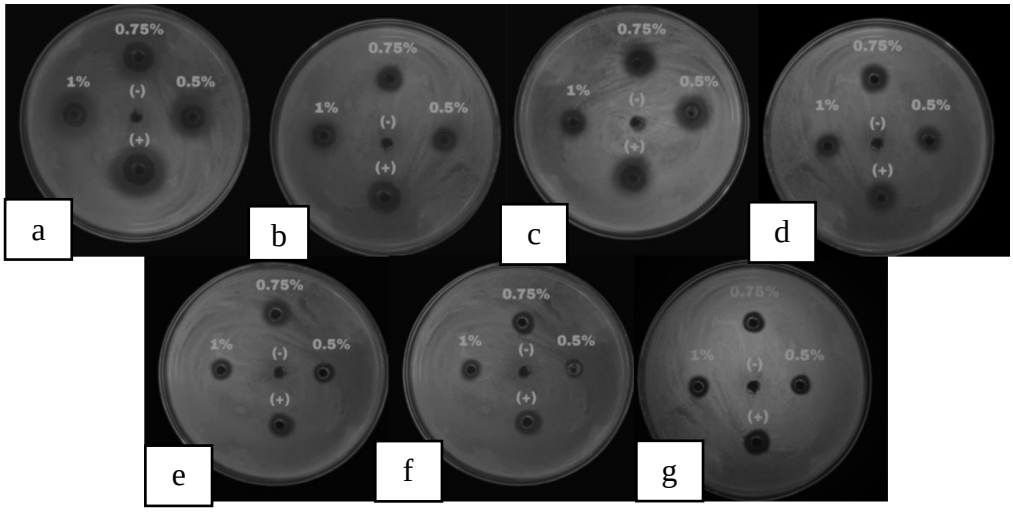

Figure 1. Inhibition zone of bacteriophage suspension, 0 dpi (a), 1 dpi (b), 3 dpi (c), 7 dpi (d), 14 dpi (e), 21 dpi (f), and 28 dpi (g). dpi= days post infection. Figure title was written one space if more than one line, left flat, without using the dots at the end

DISCUSSION

A suspension is a liquid preparation that contains insoluble solid particles dispersed in the liquid phase. Achieving physical stability in suspensions can be challenging during their preparation. Therefore, it is necessary to use a suspending agent to enhance their stability. In this study, chitosan was used as the suspending agent. The three formulations contained the same amount of active ingredient but differed in their concentrations of chitosan. This variation in formulation aims to compare the antibacterial activity of each formula in inhibiting the growth of *A. faecalis* in vitro.

The results of the bacteriophage suspension viscosity test (Table 2) revealed that the lowest viscosity value was observed in Figure 1 (37.4 cP to 54.8 cP) when using the suspending agent, namely 0.5% chitosan. Conversely, the highest viscosity was recorded for F3 (99.95 cP to 258.1 cP) with a 1% suspending agent. F2 exhibited intermediate viscosity (70.8 cP to 109.5 cP) with a concentration of 0.75% chitosan. According to the Indonesian National Standard (SNI), the acceptable viscosity range for suspensions is 37 cP to 396 cP. Notably, all three formulas fell within this range, indicating compliance with the SNI. Interestingly, as the concentration of chitosan increased in each formula, so did the viscosity value.

Excessively high viscosity is undesirable because it can hinder pouring the suspension from the container and make redispersion challenging (Liu et al., 2006). Insufficient viscosity may compromise the homogeneity of the unstable mixture, potentially affecting the accuracy of the administered doses. At the time of measuring the viscosity value for four weeks of observation, the three preparations experienced a decrease in viscosity. The decrease in viscosity can be influenced by an increase in temperature. High temperatures tend to reduce interfacial tension and viscosity (Ben et al., 2013).

Despite the decrease in viscosity observed in all three preparations, the viscosity values remained within the acceptable range according to the viscosity criteria. This suggests that the formulation was stable. Among the preparations, F2 exhibited the smallest change in viscosity, indicating that it was the most stable preparation compared to the others.

The pH values of the three preparations decreased to 5.7 and 5.8 during the 3rd and 4th weeks (data not shown), indicating instability during storage. Various factors can influence pH changes in suspensions, including the use of a solvent such as 1% acetic acid derived from chitosan. Despite the decrease in pH observed during the stability test, the pH of the three suspension formulas remained relatively stable over the four-week observation period (Table 2). This suggests that the preparations are chemically stable, with no significant chemical reactions or interactions occurring among the ingredients (Kim et al., 2008).

Based on the results of the pH test, the pH of the preparation remained within the acceptable range for skin pH, despite the decrease observed. The suspension preparations maintained a pH between 4.5 and 6.5, which is non-irritating for the skin (Nikam, 2017; Okuma et al., 2015). F2 and F3 exhibited slight pH changes over the four weeks. Lower pH values can potentially lead to skin irritation. Conversely, excessively high pH values can cause skin dryness and, if persistent, may result in itching, rash, redness, and scaling.

The zeta potential results for the three formulations (Table 2) indicate negative values, falling within the range of ±5 mV. This suggests a tendency toward fast aggregation. However, it is important to note that zeta potential alone is not the sole determinant of microemulsion stability. Zeta potential is just one parameter among several that influence stability in microemulsions (Shah et al., 2014). Therefore, while negative zeta potential values may raise concerns, they do not necessarily indicate instability. The nano-sized particle size of the bacteriophage suspension preparation is a positive sign. This small particle size ensures stability because it prevents settling or aggregation due to Brownian motion. Brownian motion refers to the continuous random movement of colloidal particles within the dispersion medium. It occurs because of unequal collisions between medium molecules and colloidal particles. Interestingly, larger colloidal particles experience slower Brownian motion, while smaller particles exhibit faster motion. Additionally, higher temperatures enhance Brownian motion due to increased energy in the medium molecules, leading to stronger collisions (Sinko, 2006).

The homogeneity test for the three suspension preparations indicated uniformity because the total phage content in each preparation was nearly identical (Table 2) and did not exhibit significant differences. Homogeneity is crucial because it ensures consistent drug particle distribution within each volume of administration. If a preparation lacks homogeneity, the number of drug particles may vary, leading to inconsistent pharmacological effects and potentially missing the desired therapeutic target (Kartikasari et al., 2021). A stable suspension should prevent rapid settling of the suspended substance. It must be easily redispersed into a homogeneous mixture with a suitable viscosity, allowing for easy pouring from the container (Naveed et al., 2017).

Based on antibacterial activity test of bacteriophage suspension with various concentrations of chitosan affected the growth of *A. faecalis* (Table 3; Figure 1). The 0.75% concentration of chitosan had the largest inhibition zone among the tested concentrations, indicating the strongest antibacterial effect. Surprisingly, the 1% concentration had a smaller inhibition zone than 0.75%, suggesting that increasing the chitosan concentration beyond a certain level does not always improve antibacterial effectiveness (Table 3). The diameter of inhibition zones appears to change over time, indicating the effectiveness of the bacteriophage suspension at different stages. Early dpi (e.g., 1 dpi) might show smaller inhibition zones, while later dpi (e.g., 14+ dpi) could indicate a stronger or diminishing effect. The differences in bacteriophage concentration (1; 0.75; 0.5%) might play a role in the size of the inhibition zones (Figure 1). The results showed that each treatment had a significantly different effectiveness on the growth of *A. faecalis*. The higher the concentration of chitosan (1%) used, the lower the antibacterial activity of the bacteriophages, so that the diameter of the inhibition zone produced is smaller. The 1% concentration had a smaller inhibition zone than 0.75%, suggesting that increasing the chitosan concentration beyond a certain level does not always improve antibacterial effectiveness. This can happen because the increase in chitosan concentration leads to higher viscosity

in the preparation. As viscosity rises, resistance increases, potentially hindering the release of active substances to their intended target, such as the bacterial growth medium (Suhesti et al., 2021).

According to Suhesti et al. (2021) and Ness et al. (2018) the decrease in viscosity during storage can impact the spreadability of the suspension. As the storage period lengthens, changes in the suspension's spreadability occur, which in turn affect the bacterial inhibition zone. Among the formulations, F2 (with 0.75% chitosan) demonstrated the most effective stability. Although its value closely approached that of the positive control, it remained significantly different. Therefore, F2 cannot be considered equivalent to the positive control. Notably, in this study, the bacteriophage suspension formula with a chitosan concentration of 0.75% exhibited the highest effectiveness in killing *A. faecalis*.

The use of bacteriophage active ingredients in the preparation of this diabetic ulcer wound cleansing suspension is because bacteriophages have a very narrow spectrum of activity so that they will not affect normal microflora in the body, while antibiotics attack almost all microorganisms, including normal microflora in the body (Principi et al., 2019). In addition, the mechanism of bacteriophages that attack host cells by lysing the host cell wall so that they can kill bacteria quickly is also a consideration for the use of bacteriophages for treatment (Hardanti et al., 2018).

Lytic bacteriophages in topical solutions such as ointments, creams, cleansers, and lotions can improve the wound healing process (Harada et al., 2018; Brown et al., 2018). These types of bacteriophage-containing solutions are easy to apply and remove (with just soap and water) and are stable during treatment, avoiding frequent application and use of bacteriostatic agents. In addition to these characteristics, bacteriophages in topical solutions have reduced side effects and very low toxicity for patients (El-Shibiny & El-Sahhar, 2017). In drug delivery systems, it is critical to ensure proper incorporation of bacteriophages into the product so that bacteriophage distribution is homogeneous and consistent during wound application (El-Shibiny & El-Sahhar, 2017).

CONCLUSION

The three bacteriophage suspension formulas effectively inhibit bacterial growth by forming clear zones on the bacterial observation medium. This demonstrates their ability to combat Alcaligenes faecalis. Among the formulations, F2 (with 0.75% chitosan) and 200 µL of bacteriophage active ingredient emerge as the optimal combination. Formula F2 meets the criteria for optimal suspension based on stability test data and effectiveness. The utilization of bacteriophages as active ingredients in the manufacture of diabetic ulcer wound care suspensions is based on their exceptionally narrow spectrum of activity. Unlike antibiotics, which can indiscriminately target a wide range of microorganisms (including normal flora), bacteriophages specifically focus on harmful bacteria without disrupting the body's normal microflora. In addition, the mechanism by which bacteriophages attack host cells (by lysing the host cell wall) allows them to swiftly eliminate bacteria. This efficient action is a crucial factor in considering the use of bacteriophages for treatment.

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REFERENCES

Ben, E. S., Suardi, M., Chalid, T. J., & Yulianto. (2013). Optimasi nanoemulsi minyak kelapa sawit (palm oil) menggunakan sukrosa monoester. *Pelayanan Kefarmasian & Herbal Medicine.* Prosiding Seminar Nasional Perkembangan Terkini Sains Farmasi dan Klinik III. Padang, Indonesia. Retrieved from https://core.ac.uk/reader/269027220.

Brown, T. L., Petrovski, S., Chan, H. T., Angove, M. J., & Tucci, J. (2018). Semi-Solid and solid dosage forms for the delivery of phage therapy to epithelia. *Pharmaceuticals (Basel, Switzerland), 11*(1), 26. doi: 10.3390/ph11010026.

El-Shibiny, A., & El-Sahhar, S. (2017). Bacteriophages: The possible solution to treat infections caused by pathogenic bacteria. *Canadian Journal of Microbiology, 63*, 865-879.

Farida, I., Widyastuti, M., Wicaksono, M., Nurhayati, C., & Muhaji, I. (2021). Efektifitas sabun antiseptik untuk mencuci luka pada *diabetik foot ulcer* di Rumah Luka Surabaya. *Jurnal Ilmu Kesehatan, 9*(2), 131-136. doi: 10.32831/jik.v9i2.315.

Harada, L. K., Silva, E. C., Campos, W. F., Del Fiol, F. S., Vila, M., D ˛abrowska, K., … Balcão, V. M. (2018). Biotechnological applications of bacteriophages: State of the art. *Microbiology, 212*, 38-58.

Hardanti, S., Wardani, A. K., & Putri, W. D. (2018). Isolasi dan karakterisasi spesifik *Salmonella typhi* dari kulit ayam. *Jurnal Teknologi Pertanian, 19*(2), 107-116.

Huang, C. (2020). Diabetic foot ulcer with *A. faecalis* infection. *Dubai Diabetes and Endocrinology Journal*, *26*(3), 128-133. doi: 10.1159/000508094.

Imtihani, H. N., Ruri, A. W., & Silfiana, N. P. (2020). *Biopolimer kitosan dan penggunaannya dalam formulasi obat*. Gresik: Graniti.

Kartikasari, D., Rahman, I. R., & Hairunisa, R. A. (2021). Potensi suspensi cangkang telur ayam (*Gallus domesticus*) sebagai antasida. *Jurnal Insan Farmasi Indonesia*, *4*(2), 168-175.

Kim, B. S., Won, M., Yang., Lee, K. M., & Kim, C. S. (2008). In vitro permeation studies of nanoemulsions containing ketoprofen as a model drug. *Drug Delivery*, *15*(7), 465-469. doi: 10.1080/10717540802328599.

Kristiyaningrum., Indanah., & Suwarto. (2013). Efektivitas penggunaan larutan NaCl dibandingkan dengan d40% terhadap proses penyembuhan luka ulkus DM di RSUD Kudus. *Jurnal Ilmu Keperawatan dan Kebidana*n, *4*(02), 52-58.

Lin, D. M., Koskella, B., & Lin, H.C. (2017). Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics*, *8*(3), 162-173.

Liu, N., Chen, X. G., Park, H. J., Liu, C. G., Liu, C. S., Meng, X. H., & Yu, L. J. (2006). Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydrate Polymers*, *64*(1), 60-65. doi: 10.1016/j.carbpol.2005.10.028.

Narulita, E., Cahyati, V. I. N., Febrianti, R. A., & Iqbal, M. (2023). Potential bacteriophages to overcome bacterial infection of *A. faecalis* in diabetic ulcer. *Pediatric and Endocrinology Diabetes Metabolism*, *29*(2), 61-66. doi: 10.5114/pedm.2023.125363.

Naveed, S., Akhtar, F., & Khan, S. (2017). An overview of the stability of extemporaneously prepared pharmaceutical suspension. *Journal of Bioequivalence & Bioavailability*, *9*(4), 452-454. doi: 10.4172/jbb.1000343.

Ness, C., Mari, R., & Cates, M. E. (2018). Shaken and stirred: Random organization reduces viscosity and dissipation in granular suspensions. *Science advances*, *4*(3), eaar3296. doi: 10.1126/sciadv.aar3296.

Nikam, S. (2017). Anti-acne gel of isotretinoin: Formulation and evaluation. *Asian Journal of Pharmaceutical and Clinical Research, 10*(11), 257-266. doi: 10.22159/ajpcr.2017.v10i11.19614.

Okuma, C. H., Andrade, T. M., Caetano, G. F., Finci, L. I., Maciel, N. R., Topan, J. F., … Rocha-Filho, P. A. (2015). Development of lamellar gel phase emulsion containing marigold oil (*Calendula officinalis*) as a potential modern wound dressing. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences*, *71*, 62-72.

Principi, N., Silvestri, E., & Esposito, S. (2019). Advantages and limitations of bacteriophages for the treatment of bacterial infections. *Frontier in Pharmacology*, *10*(513), 1-9.

Risqiyah, W., Narulita, E., Rofiqoh, A., Ludfi, A. S., & Iqbal, M. (2022). Morphological and molecular identification of multi-antibiotic resistant bacteria in the wound site of diabetic ulcers. *Biodiversitas*, *23*(2), 663-670. doi: 10.13057/biodiv/d230207.

Shah, R., Daniel, E., Enzo, P., & Ian, H. (2014). Optimisation and stability assessment of solid lipid nanoparticles using particle size and zeta potential. *Journal of Physical Science, 25*(1), 59-75.

Sinko, P. J. (2006). *Martin farmasi fisika dan ilmu farmestika edisi 5*. Jakarta: EGC.

Suhesti, T. S., Rohman, M. M. H., & Sunarto. (2021). Formulation of gel hand sanitizer of nagasari leaf extract (*Mesua ferrea* L.). *Indonesian Journal of Pharmaceutical Science and Technology, 1*(1), 31-38.

Trisnawati, E., Andesti, D., & Saleh, A. (2023). Pembuatan kitosan dari limbah cangkang kepiting sebagai bahan pengawet buah duku dengan variasi lama pengawetan. *Teknik Kimia, 19*(2), 17-27.

Zhang, H. H., Wang, Y., Hao, C., Wang, J., & Zhang, X. L. (2017). Biodegradation of ochratoxin a by *A. faecalis* isolated from soil. *Journal of Applied Microbiology, 123*(3), 661-668. doi: 10.1111/jam.13537.