IJPST
Indonesian Journal of Pharmaceutical Science and Technology

Research Article
Vol. 12 Suppl. 3 (2025): 208-217
https://doi.org/10.24198/ijpst.v12s3.63060

Piper betle Leaf Extract Spray Gel for Diabetic Wound Causes by Bacterial Infections

Ririn L. Wulandari[1*], Achmad Q. Aljufri[1], Safira Chafiffa[2], and Geges A. Andani[2]
[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia
[2]Undergraduate Pharmacy Study Program, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia

Abstract
Antibiotic resistance in the treatment of diabetic wound infections is common. *Piper betle* leaf extract has antibacterial, anti-inflammatory, and antioxidant activities. This study aims to formulate a spray gel from *Piper betle* leaf extract and test its effect on diabetic wounds infected with Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. The gel was made with 10% (F1), 15% (F2), and 20% (F3) extracts. The organoleptic physical characteristics, pH, homogeneity, viscosity, spray pattern, adhesive spreadability, drying time, and physical stability were tested. All rats were given alloxan 170 mg/kgBW, fasting glucose levels were measured, and then grouped into control, MRSA-exposed, and *P. aeruginosa*-exposed groups. The negative and positive controls were also included in the test. A 2 cm wound with a depth of 0.3 cm was given bacteria, except for the normal control. The length of the wound was measured, and the wound reduction was calculated as a percentage of wound closure and statistically analyzed. All the formulas met the physical criteria, significantly closing MRSA wounds by 100%. F2 and F3 significantly closed *P. aeruginosa* wounds by 89.88% and 93.13%, respectively. Collectively, the *Piper betle* leaf extract spray gel had an effect that could relieve diabetic wound infections.

Keywords: diabetic wounds, methicillin resistant *Staphylococcus aureus*, *Piper betle* L., *Pseudomonas aeruginosa*, spray gel

Article History:
Submitted 30 April 2025
Revised 18 October 2025
Accepted 22 October 2025
Published 23 December 2025

*Corresponding author:
ririnlispita@unwahas.ac.id

Citation:
Wulandari RL, Aljufri QA, Chafiffa S, Andani GA. *Piper betle* Leaf Extract Spray Gel for Diabetic Wound Causes by Bacterial Infections. Indonesian Journal of Pharmaceutical Science and Technology. 2025 : 12 (3), 208-217.

Gel Semprot Ekstrak Daun Sirih Hijau (*Piper betle*) Untuk Infeksi Bakteri Penyebab Luka Diabetes

Abstrak
Resistensi antibiotik pada terapi infeksi luka diabetes banyak terjadi. Ekstrak daun *Piper betle* memiliki aktivitas antibakteri, antiinflamasi, antioksidan. Tujuan penelitian untuk memformulasi gel semprot ekstrak daun *Piper betle* dan menguji efeknya pada luka diabetes terinfeksi *Methicillin Resistant Staphylococcus aureus* (MRSA) dan *Pseudomonas aeruginosa*. Metode penelitian dengan desain *pre* and *post test control*. Gel dibuat dengan ekstrak 10% (F1), 15% (F2), 20% (F3). Uji karakteristik fisik organoleptik, pH, homogenitas, viskositas, pola semprot, daya sebar lekat, waktu kering, dan stabilitas fisik. Semua tikus diberi aloksan 170 mg/kgBB, kadar glukosa puasa diukur lalu dikelompokkan menjadi kontrol normal, terpapar MRSA, dan *P.aeruginosa*. Kelompok terpapar bakteri meliputi kontrol negatif, kontrol positif, F1, F2, F3. Luka 2 cm dengan kedalaman 0,3 cm diberi bakteri, kecuali kontrol normal. Panjang luka diukur, dihitung pengurangan luka, kemudian dinyatakan sebagai persentase penutupan luka dan dianalisis statistik. Hasil F1, F2, F3 memenuhi kriteria fisik, menutup luka MRSA secara signifikan 100%. F2 dan F3 menutup luka *P.aeruginosa* signifikan 89.88%; 93.13%, sehingga disimpulkan bahwa gel semprot ekstrak daun *Piper betle* memiliki efek dapat meredakan infeksi luka diabetes.

Kata Kunci: luka diabetes, *methicillin resistant Staphylococcus aureus*, sirih hijau, *Pseudomonas aeruginosa*, gel semprot

1. Introduction

Diabetic wounds are open wounds resulting from damage to skin epithelial tissue caused by invasion of microorganisms and disorders of peripheral autonomic nerves. The prevalence of diabetic wounds in Indonesia reaches 15%, amputation cases 30%, and deaths 35%.[1] Diabetic wounds are the most common cause of hospitalization for DM patients, accounting for 80%.[2] These wounds take a long time to heal because the skin's elasticity is reduced in diabetes and it is susceptible to bacteria, leading to infection.[3]

Bacteria that often cause infections in diabetic wounds include *Methicillin-Resistant Staphylococcus aureus* (MRSA) and Pseudomonas aeruginosa.[4] Management of diabetic wounds can be carried out using systemic antibiotics or topical antibiotics.[5] However, MRSA and *P. aeruginosa* have become highly resistant to systemic therapies such as ampicillin, amoxicillin, cefotaxime, oxacillin, gentamicin, tetracycline, moxifloxacin, levofloxacin, ciprofloxacin, clindamycin, trimethoprim/sulfamethoxazole, and erythromycin.[6,7] Topical therapies are also used to treat diabetic wounds, such as antiseptics, silver, honey, bacteriophage therapy, or NPWT (negative pressure wound therapy). However, these are not recommended for treating diabetic ulcers because they have not been proven to significantly improve wound healing.[8] In addition, silver-type antimicrobials should not be used for more than 2 weeks because they can cause resistance.[9] Based on this background, the discovery of natural medicines needs to be developed as an alternative effort to overcome the problem of wound care. Natural medicines are generally considered safer and less risky than synthetic medicines.[10]

One of the natural ingredients that can be developed as a medicine is *Piper betle* L. leaves because they have antibacterial properties. *Piper betle* leaf extract at a concentration of 20% has been proven to be effective in inhibiting the growth of MRSA,[11] and a concentration of 15% against *Pseudomonas aeruginosa* in vitro.[12] This antibacterial ability is related to the content of active compounds.[13] Ethanol extract of Piper betle leaves contains flavonoids, tannins, alkaloids, saponins, essential oils, vitamin C, polyphenols, and terpenoids which have wound healing activities.[14]

All of these chemical compounds have functions that support the wound healing process, so that wounds heal more quickly. All of these compounds synergize to accelerate wound healing through antimicrobial activity that can clean wounds, and stimulate fibroblast proliferation and collagen formation to rebuild tissue.[15,16,17]

The development of the extract in topical dosage forms has been carried out in the form of ointment preparations. *Piper betle* leaf extract ointment with a concentration of 5% can increase epidermal thickness and reduce IL-6 expression in MRSA-infected incisions. However, ointment preparations have a high risk of contamination due to direct hand contact.[18] Meanwhile, spray gel preparations have advantages over other topical preparations due to their relatively low level of microbial contamination and their ability to increase drug penetration into the skin,[19] and is more practical to use.[20]

Based on the description above, this research involved a spray gel formulation with varying concentrations of *Piper betle* leaf ethanol extract at 10%, 15%, and 20%. The ethanol extract of *P. betle* has been shown to possess antibacterial activity and contains flavonoids, triterpenoids, alkaloids, and tannins, which are believed to contribute to this activity.[16] The study aimed to investigate the effect of spray gel on wound healing in diabetic rats infected with *MRSA* and *P. aeruginosa.*

2. Materials and Method

2.1. Tools

Oven, moisture balance, simplicia electric scale, blender, rotary evaporator, Buchner flask and funnel, analytical balance, glassware (IWAKI), pH meter, Brookfield viscometer, autoclave, electric stove, micropipette, Laminar Air Flow, Bunsen, round tube, measuring flask syringe, blood glucose measuring instrument (Easy Touch), glucose test strips, sterile surgical knife and scissors, tweezers.

2.2. Materials

Research materials include *Piper betle* leaves, 70% ethanol, TEA, carbopol 940, propylene glycol, propyl paraben, distilled water, Methicillin Resistant *Staphylococcus aureus*, methyl paraben, *Pseudomonas aeruginosa* FNCC 0063, Mc. Farland standard, H_2SO_4, BaCl, NaCl. 0.9%, Brain Heart Infusion media, Aloxan, Lidocaine 2%, and Aqua pro injection.

2.3. Methods

2.3.1. Sample Collection

The *Piper betle* leaves used are fresh, green, and not affected by pests. The leaves are obtained from plantations or cultivation of betel plants in the Kendal area, Central Java.

2.3.2. Extraction

Piper betle leaves were cleaned with running water and dried using an oven (temperature 50°C). The dried leaves are ground into powder, and the moisture content is measured to be less than 10% using a moisture balance tool. Extraction is carried out using the maceration method. Dry leaf powder was added to 70% ethanol, soaked for 3 days, and stirred twice a day. Then, it was macerated for 2 days. The filtrate was filtered and concentrated until a thick extract is obtained.[21] It was a higher polar solvent compared to ethanol at higher concentrations.

2.3.3. Spray Gel Formulation

The spray gel preparation formula refers to research by with a modified composition. The test formula was prepared in three variants, with extract contents of 10%, 15%, and 20% respectively (Table 1). The initial step was to develop Carbopol 940 with hot distilled water, stirring it little by little until a gel base is formed. Then, methyl paraben and propyl paraben were stirred again until a homogeneous mixture is achieved. After that, TEA was added and stirred until a homogeneous mixture was formed (mixture A). In another container, the extract was dissolved in propylene glycol gradually while stirring constantly until a homogeneous mixture was formed (mixture B). Mixture B was added to Mixture A and then stirred until the mixture was homogeneous. Next, distilled water was added to a 100 mL volume.[22]

2.3.4. Physical Evaluation of Piper betle Leaf Extract Spray Gel

Organoleptic Test

Testing of the physical properties of Piper betle leaf extract spray gel included color, aroma and texture.[23]

Homogeneity Test

The preparation was sprayed on a glass object. A gel was said to be homogeneous if there were no granules or solid particles.[23]

pH Test

pH measurement was conduted using a pH meter. The pH requirement for topical preparations was 4.5-7 so as not to cause irritation to the skin surface.[20]

Viscosity test

Viscosity measurement was carried out using a Brookfield viscometer. The preparation was put into a glass beaker and spindle number 63 was installed. the rotor rotates and waited until a stable viscosity value appears on the screen. The viscosity of a good spray gel preparation is 500-5000 cPs.[24]

Test Adhesion

The test was carried out by spraying the gel once onto the skin of the upper arm at a distance of 3 cm. After that, wait for 10 seconds to determine how long the gel sticks to the skin. The test was carried out three times. Good adhesive spreadability means that the gel can stick to the skin or not drip when sprayed.[20]

Test the Spray Pattern

This test was carried out by spraying Piper betle leaf extract gel spray on mica sheets at a distance of 3, 5, 10, 15, 20 cm. After that, the spray pattern was measured accordingly. The farther the spray distance, the more spread the spray pattern.[23]

Test Dry Time

Spray gel was applied to the volunteer's lower arm and then the time needed for it to dry was calculated. A good spray gel preparation has a dry time of less than 5 minutes.[25]

Physical Stability Test

Spray gel extract of Piper betle leaf as much as 10 mL in a tube was centrifuged at a speed of 3800 rpm for 30 minutes. Physical changes were observed which were characterized by separation or deposition.[23]

Table 1. The Piper betle leaf ethanol extract spray gel formula

Materials	Function	Concentration (%)		
		F1	F2	F3
Piper betle leaf ethanol extract	active substance	10	15	20
Carbopol 940	gelling agent	1.5	1.5	1.5
Trietanolamin	alkalizing agent	1	1	1
Propilen glikol	humectant	10	10	10
Metil paraben	preservative	0.18	0.18	0.18
Propil paraben	preservative	0.02	0.02	0.02
Aquadest ad	solvent	100	100	100

2.3.5. Animal Preparation

The test animals used were Wistar rats, male, weighing 100-200 grams, 2-3 months old, and healthy. Male rats were adapted for 7 days in the Pharmacology Laboratory, Faculty of Pharmacy, Wahid Hasyim University, Semarang. After the animal is adapted, weighing is carried out to determine the dose or volume of administration. This study consisted of two types of testing: diabetic wound testing using MRSA and *P. aeruginosa* infections. Experimental animals were grouped into normal controls, MRSA-exposed, and P. aeruginosa-exposed controls. The bacterial exposure subgroups consisted of negative controls, positive controls, and F1, F2, and F3 spray gels. The normal control (uninfected) was a diabetic rat with a wound that was not infected with bacteria and was exposed to spray gel (not containing extract) twice a day.

The negative control was a diabetic rat with a wound infected with bacteria and was given active spray gel basis (not containing extract) twice a day. The positive control was a diabetic rat with a wound infected with bacteria and was given Silver gel (Star Ag) twice a day. Groups F1, F2, and F3 were diabetic rats given F1, F2, and F3 spray gel, respectively. Animal treatment procedures have met research ethics requirements based on Ethical Clearance No. 384/X/2024/Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University, Semarang. Test animals were placed in a special room at room temperature and adequate lighting (automatically dark and bright), and were given sufficient food and water every day, with one cage per animal. Researchers used personal protective equipment when treating test animals.

All rat that had completed the test were euthanized. The dead mice were wrapped in two layers of plastic bags, then sealed and disposed of in an appropriate infectious waste container before being incinerated. Prior to testing, the mice were acclimatized for 1 week and fasted for 12 hours before being induced with alloxan. Next, alloxan was given at a dose of 170 mg/kg BW intraperitoneally. On the 4th day after alloxan induction, all rats had their blood glucose levels measured again.[26] Blood glucose levels were measured using a glucometer. Rats were declared diabetic if blood glucose levels were ≥ 200 mg/dL.[28]

All diabetic mice were wounded on day 5 after alloxan administration. This was due to the stable increase in glucose levels after 4 days of induction.[26] To create the wound, the mouse's fur in the dorsal area was shaved and cleaned, then anesthetized subcutaneously with 2% lidocaine (0.03 mL/200 g BW) until the mouse experienced a loss of feeling.[29] After that, the dorsal area was injured using a sterile scalpel 2 cm long with a depth of 0.3 cm, reaching the dermis, marked by bleeding. After the bleeding stopped, the rats were infected with a suspension of 50 µL (1 × 108 CFU/mL) of MRSA and *P. aeruginosa* bacteria intradermally.[29]

The condition of the wound was observed after 24 hours of administration of the bacteria, to determine the success of the infection. The characteristic of MRSA infection is the presence of pus or fluid in the wound[30], while *P. aeruginosa* infection has bluish-green pus.[31]

2.3.6. Diabetic wound infection healing test

Infected rats were given spray gel F1, F2, and F3 by spraying it on the wound area. The normal control group (non-infected) was only given the spray gel base. The negative control group was given spray gel base; the positive control group was given silver gel (Star Ag). All preparations were given 2 times a day for 14 days.

Macroscopic observations and measurements of wound length were carried out every day. The condition of the wound observed is signs of wound healing, namely the color changes to white, no scabs, and the formation of new skin. The percentage of wound closure was calculated using the formula of subtracting the initial wound length from the final wound length then dividing the initial wound length and multiplying by 100%.[32,33]

2.3.7. Data analysis

The results of the evaluation of the physical characteristics of the *Piper betle* leaf extract spray gel, blood glucose levels, morphological conditions were analyzed descriptively. The percentage of wound closure was statistically analyzed using the Kruskal-Wallis and Mann-Whitney tests, and a significant difference was reported if $p < 0.05$.

3. Result

3.1. Physical evaluation

Physical evaluation of the preparation is a test carried out to determine the quality of the spray gel preparation produced. This evaluation includes organoleptic tests, homogeneity, pH, viscosity, adhesion, spray pattern, dry time, stability. Spray gel preparations are said to be good if they meet the specified requirements. The evaluation results of all formulas are in Table 2.

Organoleptic test results show that all formulas have the same color and aroma. The aroma of the gel preparation is the characteristic smell of *Piper betle* leaves. The difference occurs in the texture of F1,

Table 2. Evaluation Results of Piper betle Leaf Extract Spray Gel

Type of testing	F1	F2	F3
Organoleptic	Color: blackish green Aroma: typical Piper betle Texture: thick	Color: blackish green Aroma: typical Piper betle Texture: a little thick	Color: blackish green Aroma: typical Piper betle Texture: a little thick
Homogeneity	homogeneous	homogeneous	Homogeneous
pH	5.31	5.27	5.25
Viscosity (cps)	4992	3597.3	1522.6
Sticking power (second)	10	10	10
Dry time (minute)	4.37	3.54	3.09
Stability	No separation occurred	No separation occurred	No separation occurred
Spray Pattern			
3 cm	Clot	Clot	Clot
5 cm	Clot	Clot	Clot
10 cm	Clot	Clot	Spread
15 cm	Spread	Spread	Spread
20 cm	spread	Spread	Spread

which is thick. F1 contains less active substance than F2 and F3. The viscosity of the preparation decreases with increasing extract concentration.

The homogeneity test aims to determine the presence of insoluble particles in the preparation, because this indicates incomplete mixing of the substances. The test results show that all the resulting formulas are homogeneous. The homogeneity of the preparation is related to the uniformity of the active substance dispersed in the spray gel mass.[34]

The pH test was carried out to determine the degree of acidity of the spray gel. The preparation must meet the pH range that matches the skin's pH so as not to irritate the skin (pH 4.5-7). The test results showed that the pH of the Piper betle leaf extract spray gel preparation was included in this range. A low pH can pose a risk of skin irritation, while a high pH can cause the skin to become slippery and dry quickly.[35] Viscosity evaluation aims to determine the viscosity of the spray gel. The test results show different values for each formula (Table 2). The higher the concentration of the active substance, the lower the viscosity. Evaluation of the drying time for spray gel preparations is carried out to determine how long it takes for the preparation to dry after being applied to the skin. The ideal dry time is less than 5 minutes. The test results show that the dry time for the three formulas is less than 5 minutes. Spray gel that has a fast drying time creates a comfortable feeling because it does not stick to the skin.[23]

Evaluation of the spray pattern aims to determine the condition of the spray coming out of the applicator. There are several factors that influence the spraying pattern of the preparation, namely the characteristics of the formula, viscosity, and spray distance. The farther the spraying distance, the wider the spray pattern can be produced.[35] Based on the research results (Table 2), the spraying patterns of the three formulas varied. F1 starts to spread at 20 cm, F2 starts to spread at a distance of 15 cm, while F3 at a distance of 10 cm.

Adhesion evaluation aims to determine how long the preparation remains attached to the skin. The good durability of the spray gel is that it doesn't drip after 10 seconds. Based on test results, the three formulas did not drip after 10 seconds.[25] Physical evaluation of the spray was also carried out to determine any instability of the preparation. In the research, a stability test was carried out using the centrifugation method. The test results showed that the three formulas were stable, indicated by the absence of separation after centrifugation.

3.2. Measurement of blood glucose levels

Blood glucose levels are checked first on test animals to ensure that all animals used are healthy and do not have diabetes. Measurements were also carried out after administering alloxan to ensure that all animals had diabetes. The results from blood glucose levels (Table 3) showed that the administration of alloxan as a diabetes inducing agent had succeeded in making all test animals suffer from diabetes, indicated by blood glucose levels ≥200 mg/dL on the 4th day. The administration of a dose of 170 mg/kg body weight can maintain diabetic conditions in rats. All the rats were then infected with bacteria for the subsequent analysis.

Table 3. The average blood glucose levels in test animals

Groups	Fasting blood glucose levels (mg/dL)	
	Before induction	After induction (fourth day)
Normal (uninfected+ base of spray gel)	123.40 ± 22.47	392.60 ± 41.89*
Infected by MRSA		
Negatif control (base of spray gel)	81.00 ± 11.86	454.00 ± 55.01*
Positive control (Silver gel)	138.80 ± 22.02	506.40 ± 24.99*
F1	105.00 ± 14.36	457.80 ± 49.79*
F2	102.60 ± 17.39	438.80 ± 53.43*
F3	99.40 ± 18.08	354.80 ± 19.78*
Infected by *P.aeruginosa*		
Negatif control (base of spray gel)	112.40 ± 16.88	500.20 ± 34.03*
Positive control (Silver gel)	113.00 ± 10.40	427.00 ± 11.30*
F1	94.20 ± 7.57	480.80 ± 33.88*
F2	119.60 ± 8.42	496.40 ± 16.90*
F3	105.20 ± 13.64	549.20 ± 10.73*

All values were presented in the table as mean ± SD (n=4). *, $p < 0.05$ from the Wilcoxon test

3.3. Test the healing effect of diabetic wound infection

This test aimed to determine the effect of *Piper betle* leaf ethanol extract gel spray on wound healing in diabetic rats infected with *Pseudomonas aeruginosa* and MRSA. Both bacteria are opportunistic pathogens that attack individuals with weakened immune systems, such as patients with diabetes mellitus. Infections caused by these bacteria can slow the wound healing process and even worsen the wound condition, requiring amputation. Wound healing testing was conducted by treating infected diabetic rats with wounds by spraying the preparation onto the wound area twice daily for 14 days. The spray gel preparation tested contained ethanol extract of Piper betle leaf at concentrations of 10%, 15%, and 20%. Negative and non-infected controls were given spray gel without active ingredients.

The negative control was used as a comparison to the *Piper betle* leaf ethanol extract spray gel formula to determine its healing activity in diabetic wounds infected with MRSA. The non-infected control served to validate the success of the infected wound creation process, aiming to ensure there was a difference between infected and uninfected wounds. The positive control group was given Star Ag gel containing silver, which has been clinically proven to be effective in healing diabetic wounds.[27] The positive control in this study aimed to validate the results. Observations on the length of the wound were carried out for 14 days to see the physical changes that occurred in the injured area. Visual observations include changes in wound color, scab formation as an early sign of the proliferation phase, and the formation of new skin. Wound color changes as the wound heals. The reduction in wound length over 14 days was used as a parameter to assess the activity of the spray gel.

Figure 1A showed that the percentage of wound length reduction in the negative control was lower than that in the uninfected control ($p<0.05$). This means that infection can slow down wound healing. The results of testing on diabetic wounds infected with *P. aeruginosa* showed that F1, F2, and F3 were able to reduce the length of the wound by 77.25%, 89.88%, and 91.13%, respectively; however, F1 could not be concluded to affect healing wounds because the results were not significantly different compared to the negative control. All formulas can reduce wound length by 100%, and the reduction was insignificant ($p>0.05$) compared to the positive control. This indicates that the F1, F2, and F3 spray gels have the same effect as the Silver gel (Star Ag).

Furthermore, in the *P. aeruginosa* infected wound test, the uninfected control had a significantly greater percentage of wound length reduction than the negative control ($p<0.05$) (Figure 1B). This indicates that infection affects wound healing. The wound length reduction of F2 and F3 was significantly higher than that of the negative control. This means that F2 and F3 have a wound-healing effect. For F1, the reduction was higher, but not significantly different from the negative control ($p>0.05$). This means that F1 did not have a healing effect. The wound length reduction of F2 and

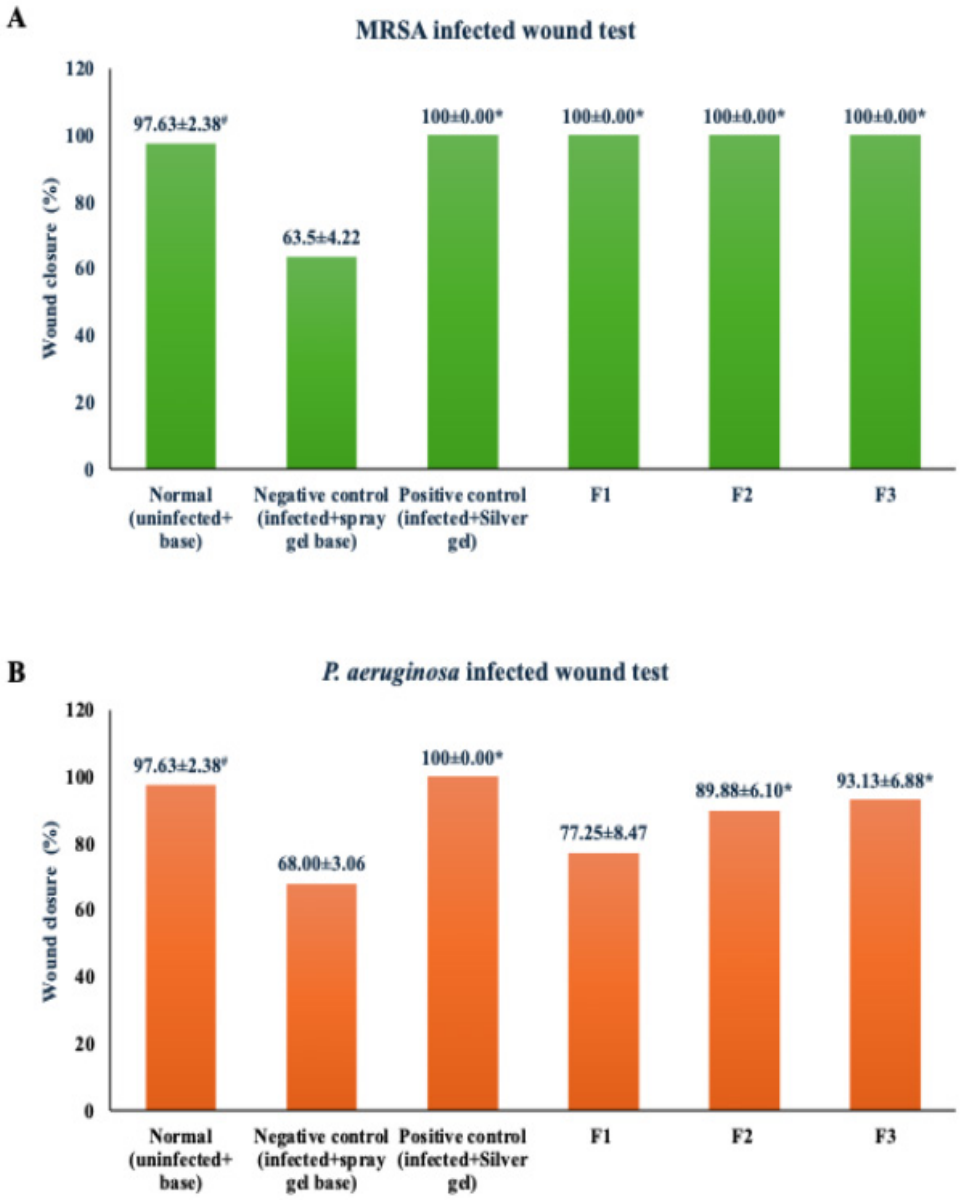

Figure 1. Average percentage of closure of diabetic wounds infected with (A) MRSA and (B) P. aeruginosa. All values were presented as mean ± SEM, n = 4, #p<0.05 when compared to negative control; *p<0.05 when compared to control (infected+gel base).

F3 was lower than that of the positive control, but not significantly different (p>0.05), so it can be said that F2 and F3 have a wound length reduction effect that is almost comparable to that of Silver gel.

4. Discussion

This research aims to formulate *Piper betle* leaf extract into a spray gel preparation and determine its effect in healing infected diabetic wounds. *Piper betle* extract was obtained using the maceration method, which is suitable for extracting active compounds that are easily destroyed by heating. Most of the compounds contained in *Piper betle* extract are heat-resistant. Flavonoids and other phenolic compounds are sensitive to heat (thermolabile) and easily oxidized, so heating during the extraction process can cause degradation or a decrease in their levels.[36,37] In addition to this, this method was chosen because it is easy to perform and does not require complex equipment.[38] The test formula uses three series of different extract concentrations. The gelling agent used in this research is carbopol 940.

Carbopol 940 is a biodegradable, biocompatible, and bioadhesive gelling agent that is not absorbed into the body and does not irritate the skin. However, a limitation of this study is that toxicity testing has not been conducted to ensure safety for skin use. Additionally, the gelling agent is very stable and

resistant to microbes.[25] Based on the results of the physical test evaluation, it was found that all *Piper betle* leaf extract spray gel formulas met the physical criteria for the preparation.

Our findings showed that all alloxan-induced rats showed blood glucose levels >200 mg/dL, indicating hyperglycemia. Alloxan induction in rats causes an increase in blood glucose. Alloxan is a chemical compound commonly used to induce diabetes in laboratory animals. Its mechanism involves the degradation of pancreatic β-cells, resulting in decreased quality and quantity of insulin production, leading to hyperglycemia.[39]

Long-term high blood glucose levels can cause pathological changes in blood vessels, nerves, and the immune system, which can increase the risk of diabetic wounds. Furthermore, wound healing in patients with diabetes mellitus takes longer due to impaired skin tissue regeneration, prolonged inflammation, and infection.[40] This is consistent with research by Riana and Jeffrey (2021), which found that 57% of respondents with abnormal blood glucose levels (≥ 200 mg/dL) experienced impaired post-operative wound healing. Post-operative wounds in DM patients are at risk of infection due to abnormalities in blood glucose levels, which lead to decreased immune functions, such as chemotaxis and oxidative killing potential of neutrophil cells.[41]

Therefore, all spray gel formulas were further tested on test animals. it was also found that the higher the concentration of Piper betle leaf extract in the spray gel preparation, the greater the effect in reducing wound length. The results of this study are in line with Za'rah's (2021) research, which proved that *Piper betle* L. leaf extract can heal wounds in mice (*Mus musculus* L.).[15] This effect is related to the presence of active chemical compounds in the extract, namely tannins, saponins, alkaloids, flavonoids, essential oils, vitamin C, polyphenols, and terpenoids.[42] Each of these active compounds has a function that can accelerate the wound healing process. Flavonoids have antioxidant, antibacterial, and anti-inflammatory effects.[16]

Flavonoids trigger re-epithelialization and tissue repair by stimulating the growth and movement of fibroblasts and keratinocytes to the wound area, and they reduce inflammation by modulating macrophage cells and reducing inflammatory cytokines. Flavonoids as antioxidants can help inhibit oxidative stress, which can interfere with wound healing.[16] Flavonoids can damage bacterial cell walls and DNA, inhibit the formation of extracellular proteins and bacterial cell motility. Phenol can inactivate and denature bacterial cell proteins. *Piper betle* leaf essential oil contains phenolic compounds (carvacrol) and phenylpropanoids (eugenol and kavikol), which are effective as very strong bactericides and fungicides.[43] Essential oils, flavonoids, tannins, phenolic saponins, alkaloids, coumarins, emodins, steroids, and most contain betephenol. Essential oils can inhibit the formation of cell walls, nucleic acids and bacterial cell proteins. Betel leaves contain high levels of vitamin C and polyphenols, which can neutralize harmful free radicals produced during injury. Saponins increase collagen production, thus helping to improve wound healing. Saponins are also able to interfere with bacterial permeability.[44] Tannins damage polypeptides and inhibit the activity of bacterial extracellular enzymes. Alkaloids can accelerate wound healing through their antibacterial mechanism by destroying cell membranes and interfering with bacterial nucleic acid synthesis.[45]

In this study, observations and measurements of wound length were conducted over a 14-day period. This period was taken to obtain representative measurement results. Wound healing is a complex and time-consuming process, so long-term monitoring is crucial to obtain accurate data on the spray gel's effectiveness over time. Longer follow-up can yield more convincing results and identify potential long-term side effects that may not be apparent in a short period of time.[46] A limitation of this study is that histopathological testing was not performed. Histopathological testing is helpful in determining the level of wound healing. This preparation also needs to be tested for toxicity to ensure its safety for use on the skin.

5. Conclusion

Piper betle extract spray gel of 10% (F1), 15% (F2), and 20% (F3) can significantly close wounds infected with MRSA. In *P. aeruginosa* infections, wounds closed substantially by 89.88% (F2) and 93.13% (F3). Thus, Piper betle leaf extract spray gel has the effect of reducing bacterially infected diabetic wounds.

Acknowledgment

The author would like to thank Wahid Hasyim University for funding the research through DIPA (LP2M) and all parties who have assisted in the research process.

Conflict of Interest

The authors declare no conflicts of interest.

Reference

1. Rassi YET, Efendy MA. Pola Diet Dengan Proses Penyembuhan Luka Diabetik. Journal of Health and Community Sciences. 2023;3(3):113-7.

2. Mugiyanto E, Irham LM. Manajemen ulkus diabetikum: Sebuah kajian. Yogyakarta: Deepublish Digital; 2022.
3. Risnawati R, Yusuf S, Syam Y. Identifikasi jenis bakteri pada luka kaki diabetik (LKD) berdasarkan lama menderita luka. Jurnal Kesehatan Manarang. 2018;4(2):87-92.
4. Ramirez-Acuña JM, Cardenas-Cadena SA, Marquez-Salas PA, Garza-Veloz I, Perez-Favila A, Cid-Baez MA, et al. Diabetic foot ulcers: Current advances in antimicrobial therapies and emerging treatments. Antibiotics. 2019;8(4):193.
5. Ministry of Health Malaysia. Management of diabetic foot. 2nd ed. Putrajaya: Malaysia Health Technology Assessment Section (MaHTAS); 2018.
6. Akbar GT, Karimi J, Anggraini D. Pola bakteri dan resistensi antibiotik pada ulkus diabetik grade dua di RSUD Arifin Achmad periode 2012. Jurnal Online Mahasiswa. 2014;1(2):1-15.
7. Setianingsih Y, Fadraersada J, Ibrahim A, Ramadhan AM. Pola resistensi bakteri terhadap antibiotik pada pasien diabetic foot di RSUD Abdul Wahab Sjahranie Samarinda periode Agustus-Oktober 2016. Proceeding Mulawarman Pharmaceuticals Conference. 2016; 4:407–14.
8. Senneville É, Albalawi Z, van Asten SA, Abbas ZG, Allison G, Aragón-Sánchez J, et al. IWGDF/IDSA guidelines on the diagnosis and treatment of diabetes-related foot infections (IWGDF/IDSA 2023). Diabetes Metabolism Research and Reviews. 2024;40(3):e3687.
9. Bagenda TD, Munir NW, Agustini T. Pengaruh pemberian antimicrobial terhadap penyembuhan luka pada pasien ulkus diabetikum. Window of Nursing Journal. 2021;2(1): 101-6.
10. Sumayyah S, Salsabila N. Obat tradisional: Antara khasiat dan efek sampingnya. Majalah Farmasetika. 2017;2(5):1-4.
11. Raudah S, Wahid RSA, Huzaimah. Uji Aktivitas Antibakteri Ekstrak Daun Sirih Hijau (Piper betle L.) dan Daun Sirih Merah (Piper crocatum Ruiz & Pav) terhadap Bakteri pada Luka Diabetes Mellitus Secara invitro. Proceeding 2nd SETIABUDI-CIHAMS 2022. 2022:59-69.
12. Manarisip GE, Fatimawati F, Rotinsulu H. Standarisasi Ekstrak Daun Sirih Hijau (Piper betle L.) dan Uji Antibakteri Terhadap Bakteri Pseudomonas aeruginosa. Pharmacon. 2020;9(4):533-41.
13. Suhesti, Rusmalina S. Kandungan Senyawa Metabolit Sekunder Berkhasiat Pada Penyembuhan Luka Diabetes. RISTEK Jurnal Riset Inovasi dan Teknologi Kabupaten Batang. 2021;5(2):35-40.
14. Nisyak K, Hisbiyah A, Haqqo A. Aktivitas Antibakteri Ekstrak Etanol dan Minyak Atsiri Sirih Hijau Terhadap Methicillin Resistant Staphylococcus aureus. Journal of Pharmaceutical-Care Anwar Medika. 2022;5(1): 1-14.
15. Zar'ah NA, Syachruddin, Kusmiyati. The Effect of Green Betel Leaves (Piper betle L.) Extract on Wounding Healing in Mice (Mus musculus L.). Jurnal Biologi Tropis. 2021;21(1):103-11.
16. Carvalho MTB, Araújo-Filho HG, Barreto AS, Quintans-Júnior LJ, Quintans JSS, Barreto RSS. Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action. Phytomedicine. 2021;90: 153636.
17. Pradhan D, Suri DKA, Pradhan DDK, Biswasroy P. Golden Heart of the Nature: Piper betle L. Journal of Pharmacognosy and Phytochemistry. 2013. 1(6):147-67
18. Al-Kindy DW. Pengaruh Pemberian Salep Ekstrak Daun Sirih Hijau (Piper betle L.) Terhadap Ekspresi IL-6 dan Ketebalan Epidermis pada Tikus (Rattus norvegicus) Model Luka Insisi dengan Infeksi Methicillin-Resistance Staphylococcus Aureus (MRSA). Tesis. Universitas Brawijaya; 2018.
19. Estikomah SA, Amal ASS, Safaatsih SF. Uji Daya Hambat Terhadap Bakteri Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes Gel Semprot Ekstrak Etanol Daun Kersen (Muntingia calabura L.) Karbopol 940. Journal of Islamic Pharmacy. 2021;5(1):36-53.
20. Rizal R, Salman, Maharani V. Formulasi Sediaan Spray Gel Ekstrak Etanol Pegagan (Centella asiatica (L.) Urban) dan Uji Daya Tabir Surya. Jurnal Sains Farmasi dan Kesehatan. 2023;1(1):48-59.
21. Hidayat R, Wulandari P. Methods of Extraction: Maceration, Percolation and Decoction. Eureka Herba Indonesia. 2021;2(1):73-9.
22. Anggoro N, Endriyatno NC. Formulasi gel ekstrak daun sirih cina (Peperomia pellucida L. Kunth) variasi carbopol 940 serta uji fisik dan stabilitasnya. Sasambo Journal of Pharmacy. 2024;5(1):46-54.
23. Indalifiany A, Zubaydah WS, Kasim ER. Formulasi Spray Gel Ekstrak Etanol Batang Etlingera rubroloba Menggunakan HPMC sebagai Gelling Agent. Jurnal Sains dan Kesehatan. 2023;5(2):140-8.
24. Kamishita T, Miyaaki T, Okunp Y. Spray Gel Base and Spray Gel Preparation Using Thereof; US Patent US5158761A. 1992, 27 Oktober
25. Karlina DW, Noval N, Yuwindry I. Formulasi dan Evaluasi Nano Spray Gel dengan Ekstrak Daun Sirih Merah (Piper crocatum Ruiz & Pav) sebagai Antioksidan dengan Variasi Konsentrasi Carbopol 940. Jurnal Surya Medika. 2024;10(2): 289-301
26. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (Kaunas). 2017;53(6):365-74.
27. Candra S, Susilawati E, Adnyana IK. Pengaruh Gel Ekstrak Daun Kerehau (Callicarpa longifolia Lam.) Terhadap Penyembuhan Luka Pada Model Tikus Diabetes. Kartika Jurnal Ilmu Farmasi. 2019;6(2):70-80.
28. Rao PS, Subramanayam G. Induction of Diabetes by Alloxan in Male Wistar Albino Rats-A Simplified Methodology. European Journal of Biomedical and Pharmaceutical Science. 2017;4(11):68-75.
29. Purba MNB, Meutia R, Karo RMB. Efektivitas Gel Ekstrak Etanol Daun Insulin (Tithonia diversifolia Hemsl) A. Gray) Sebagai Penyembuh Luka pada Tikus Diabetes. Jurnal Kesehatan Tambusai. 2024;5(3): 9889-98.
30. Nismawati RS, Sjahril R, Agus R. Deteksi Methicillin Resistant Staphylococcus aureus (MRSA) pada Pasien Rumah Sakit Universitas Hasanuddin dengan Metode Kultur. Prosiding Seminar Nasional Megabiodiversitas Indonesia. 2018;4(1):15-21.
31. Sekhi RJ. Pseudomonas aeruginosa: A Review Article. European Scholar Journal. 2022;3(3):78-84.

32. Muttaqien YV, Purnama ER. Kadar Glukosa Darah dan Penyembuhan Ulkus Mencit Diabetes Setelah Perlakuan Ekstrak Daun Bakau Bruguiera gymnorrhiza. Lentera Bio. 2024;13(1):55-64.
33. Amfotis ML, Suarni NMR, Arpiwi NL. Wound Healing of Cuts in the Skin of White Rat (Rattus norvegicus) is Given Kirinyuh (Chromolaena odorata) Leaf Extract. Metamorfosa Journal of Biological Sciences. 2022;9(1):139.
34. Cendana Y, Adrianta KA, Suena NMDS. Formulasi Spray Gel Minyak Atsiri Kayu Cendana (Santalum album L.) sebagai Salah Satu Kandidat Sediaan Anti Inflamasi. Jurnal Ilmiah Medicamento. 2021;7(2):84-9.
35. Puspita W, Puspasari H, Shabrina A. Formulasi dan Stabilitas Fisik Gel Semprot Ekstrak Daun Buas-buas (Premna serratifolia L.). Pharmacon Jurnal Farmasi Indonesia. 2021;(2020):1-8.
36. Samudra AG, Ramadhani N, Fitriani D, Putri D. Perbandingan Metode Ekstraksi Maserasi dan Sokletasi. Seminar Nasional Hasil Penelitian dan Pengabdian Kepada Masyarakat LP2M UST Jogja. 2022.
37. Jaya FK, Hasnah S. Comparative Analysis of Maceration and Soxhlation Extraction for The Total Flavonoid Content of Sungkai Leaves (Peronema canescens Jack.). Jurnal Ilmiah Berkala Sains dan Terapan Kimia. 2024;18(1): 19-30.
38. Sankeshwari R, Ankola A, Bhat K, Hullatti K. Soxhlet versus cold maceration: Which method gives better antimicrobial activity to licorice extract against Streptococcus mutans?. Journal of Scientific Society. 2018;45(2):67.
39. Yasaroh S, Christijanti W, Lisdiana L, Iswari RS. Efek Ekstrak Daun Kelor (Moringa oleifera) Terhadap Kadar Glukosa Darah Tikus Diabetes Induksi Aloksan. Prosiding Seminar Nasional Biologi. 2021;9: 224-9.
40. Nurlina, Hasanuddin F, Rahmawati, Harmawati, Aryuninda M. Analisis Perawatan dan Penyembuhan Luka Pasien Diabetes Meliitus Tipe 2. Jurnal Mitrasehat. 2025;15(1):810-4.
41. Riana A, Jeffrey J. Hubungan Glukosa Darah Terhadap Proses Penyembuhan Luka Pasca Operasi di Rumah Sakit Sumber Waras di Jakarta. Ebers Papyrus. 2021;27(2):50-6.
42. Suharto IPS, Ramayanti ED, Yunalia EM, Ulfa N. Betel Leaf Extract's Effect (Piper Batle Linn) on The Healing Process of Incision Wounds in Rats (Rattus Norvegicus). Jurnal Pendidikan Keperawatan Indonesia. 2021;7(2):123-8.
43. Kurniawati D, Rukmi I, Lunggani AT. Aktivitas Antimikroba Kombinasi Rebusan Daun Sirih Hijau (Piper betle) dan Daun Sirih Merah (Piper crocatum) Terhadap Candida albicans. Jurnal Biologi. 2014;3(1):55-61.
44. Wahyuningtyas ES, Wijayatri R, Handayani E. The Effectiveness of Combination of Piper betle L. ethanol Extract and Manuka Honey Spray Gel to Accelerating Acute Wound Healing. E3S Web Conference. 2024;500:04003.
45. Dubey DV, Yadav S, Jaiswal JK, Devi A. Piper Betle: Ethnopharmacology, Phytochemistry, and Therapeutic Applications. International Journal of Pharmaceutical Research and Applications. 2023;8(5):964-72.
46. Adiprama GSP, Me H. Perbandingan Penyembuhan Luka Superfisial Pasca Eksisi Shave Dengan Kontak Larutan NaCL 0.9% dan Kelompok Tidak Diberi Larutan NaCL 0.9%. Jurnal Inovasi Global. 2023;3(3): 608-23.