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Potential of *Mentha spicata* L. Leaf Extract Spray as an Antibacterial and Antiseptic Agent in an Infected Incision Wound Model: In Vitro and In Vivo Studies

Nabilla Putri Auliyah[1], Candra Eka Saputra[2], Aqilla Farhanadila[3], Riswanda Irsadina Najiha[4], Fadilla Citra Suci[5], Ani Florida Ngete[6]

[1,2,3)] Pharmacy Study Program, Faculty of Health Sciences, Kusuma Husada University Surakarta, Indonesia
[4,5)] Three-year diploma nursing Study Program, Faculty of Health Sciences, Kusuma Husada University Surakarta, Indonesia
[6)] Directorate of Learning and Student Affairs (SIMBelmawa), Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology, Indonesia

e-mail:

nabillaputriauliyah@gmail.com

ABSTRACT

Bacterial infections in incision wounds, predominantly caused by pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, present a critical challenge in post operative care. *Mentha spicata* L. leaves, rich in carvone and limonene, possess substantial potential as natural antibacterial and wound healing agents. This study aimed to evaluate the physical stability of a 70% ethanol fraction spray formulation of *Mentha spicata* L. and its in vivo healing efficacy on a *Staphylococcus aureus* infected incision wound model. Initially, an in vitro antibacterial screening against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was conducted using a 5% concentration of n-hexane, chloroform, and 70% ethanol fractions. The 70% ethanol fraction was determined as the optimal fraction against *Staphylococcus aureus*, exhibiting the highest diameter of zone of inhibition (DZH: 16.32 ± 0.31 mm), while *Pseudomonas aeruginosa* showed low sensitivity (5.26 ± 0.41mm) due to its robust biofilm barrier. This optimal fraction was formulated into a spray preparation at three concentrations: 5% (F1), 10% (F2), and 20% (F3). Stability testing through 3 consecutive cycles of a cycling test confirmed that all formulations remained physically and chemically stable. For the in vivo efficacy test, threee male *New Zealand White* rabbits received a 2 cm incision wound inoculated with 10^8 CFU/mL of *Staphylococcus aureus*. The treatment efficacy was evaluated by measuring the relative wound area on Days 0, 7, and 14. Data were analyzed using Two Way ANOVA followed by a Post Hoc LSD test ($p < 0.05$). In vivo results indicated that F3 (20%) significantly accelerated incision wound closure compared to the Negative Control ($p < 0.05$), showing an identical healing progression to the commercial antiseptic Positive Control ($p > 0.05$). In conclusion, the 70% ethanol fraction spray of *Mentha spicata L.* leaves at 20% is stable and effective, making it a promising phytopharmaceutical candidate for treating infected incision wounds.

Keywords: *Mentha spicata* L., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Infected Incision wounds, Spray Formulation.

Introduction

Incision wounds are acute injuries created intentionally using surgical instruments during medical procedures to access deeper tissues or organs (Dougherty & Lister, 2015). During the post operative phase, incision wounds are highly vulnerable to bacterial contamination from the surrounding environment or skin microbiota, which frequently triggers localized infections. Consequently, the clinical management of infected incision wounds remains a monumental challenge in modern healthcare systems (Sura et al., 2023). Globally, incision wounds exhibit the highest prevalence among all acute wound types, accounting for approximately 48% of total recorded cases (Jayanti et al., 2025). In Indonesia, the 2018 National Basic Health Research (Kementerian Kesehatan RI, 2018) report highlighted that incision or laceration type injuries constitute up to 20.1% of all reported trauma cases (Wardani & Nugroho, 2022). If improperly managed, bacterial colonization in an incision wound delays the proliferative phase of healing, prolongs the painful inflammatory response, and exponentially raises the risk of life-threatening systemic complications such as sepsis.

The most formidable complication in incision wound care is the multi species bacterial invasion, predominantly driven by Gram positive and Gram negative pathogens. *Staphylococcus aureus* represents the primary Gram positive opportunist that quickly colonizes open incisions, disrupting early tissue reconstruction. Simultaneously, *Pseudomonas aeruginosa*, a notorious Gram negative nosocomial pathogen, frequently contaminates surgical sites in hospital environments (Zuliana et al., 2023). *Pseudomonas aeruginosa* is particularly dangerous due to its rapid production of an exopolysaccharide matrix (biofilm), which shields the wound from topical treatments and host immune responses. Current golden standards in incision wound management heavily rely on synthetic chemical antiseptics and topical antibiotics. However, prolonged utilization of these chemical agents often exerts cytotoxic effects on surrounding healthy tissues, thereby paradoxically delaying fibroblast proliferation and tissue remodeling (Boyko et al., 2018). Furthermore, the alarming rise of antibiotic resistant strains in both *Staphylococcus aureus* and *Pseudomonas aeruginosa* necessitates an urgent exploration into alternative, biologically active plant derived therapeutics that offer both potent antimicrobial properties and tissue regenerative compatibility (Pereira & Bártolo, 2016).

Spearmint (*Mentha spicata L.*) represents a highly viable phytopharmaceutical candidate due to its rich secondary metabolite profile. The essential oil fractions of *M. spicata* contain substantial amounts of bioactive volatile terpenoids, primarily carvone (55.02%–59.72%) and limonene (15.31%–18.57%) (Hosnaroodi & Ghavam, 2025). These major compounds exhibit potent antimicrobial activity by disrupting bacterial cell membrane permeability, inducing intracellular leakage, and disrupting bacterial energy metabolism (Jiwintarum et al., 2022). Previous studies have reported that *M. spicata* extracts can achieve a Minimum Inhibitory Concentration (MIC) as low as 0.005 µg/mL with an inhibition zone diameter reaching up to 24.5 mm against various pathogenic strains (Tourabi et al., 2023).

While the crude extract of *M. spicata* has been widely scrutinized for its antimicrobial activities, research focusing on specific solvent fractions to concentrate its bioactive compounds against distinct bacterial classes (Gram positive vs. Gram negative) remains scarce. The novelty of this study lies in utilizing a liquid-liquid fractionation approach (n-hexane, chloroform, and 70% ethanol) to isolate the most optimal antibacterial fraction of *M. spicata*, which is subsequently developed into a

topical antiseptic spray formulation. A spray delivery system was selected over conventional ointments or gels to provide a hygienic, touch-free application that minimizes mechanical friction and secondary trauma over newly formed granulation tissue. Therefore, this study aims to evaluate the in vitro antibacterial capacity of various *M. spicata* fractions against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, establish the physical stability of the formulated spray (5%, 10%, and 20% concentrations), and validate its in vivo therapeutic efficacy in treating infected incision wounds.

Methodology

Research Design and Variables

This study utilized a preclinical laboratory experimental design consisting of three sequential phases: in vitro antibacterial screening, stability evaluation of the spray formulations, and an in vivo wound healing assay. The variables established in this research are as follows:

1. Independent Variable: The concentration of the 70% ethanol fraction of *M. spicata* leaves in the spray formulation (5%, 10%, and 20%) and the duration of observation (Day 0, Day 7, and Day 14).
2. Dependent Variable: The Relative Wound Area, representing the progressive contraction and closure of the incision wound over time.

Hypothesis Formulation

Quantitative data were statistically evaluated using a Two Way Analysis of Variance (ANOVA) followed by a Post Hoc Least Significant Difference (LSD) test at a confidence level of 95% (= 0.05). The statistical hypotheses are formulated as follows:

1. H_0 (Null Hypothesis): There is no significant difference in the Relative Wound Area across different concentrations of the *Mentha spicata L.* leaf 70% ethanol fraction spray formulations and observation days.
2. H_α (Alternative Hypothesis): There is a significant difference in the Relative Wound Area based on the concentration variations of the *Mentha spicata L.* leaf 70% ethanol fraction spray formulations and the duration of observation.

Research Procedure

Tools and Materials

The materials used in this study included dried *Mentha spicata L*. leaves, 96% ethanol, n-hexane, chloroform, 70% ethanol, propylene glycol, methylparaben, distilled water, Nutrient Agar (NA) media, and pure cultures of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853.

The laboratory equipment utilized consisted of a rotary evaporator, digital calipers, a freeze-thaw chamber, sterile well-punches, an incubator, moisture balance, and analytical balances.

The experimental subjects used were three male *New Zealand White* rabbits (*Oryctolagus cuniculus*), weighing 2.0–2.5 kg, which had been acclimated under standardized laboratory conditions with certified ethical clearance.

Extract Preparation, Phytochemical screening, Fractionation, and In vitro Antibacterial Screening

Dried leaves of *Mentha spicata L*. were extracted via maceration using 96% ethanol for 72 hours, and the filtrate was concentrated using a rotary evaporator to obtain the crude extract. Phytocemical screening tests were immediately conducted on the crude extract to identify groups of active compounds qualitatively based on

color reactions or the formation of specific precipitates. In the flavonoid test using magnesium powder, amyl alcohol, and concentrated HCl, a brick red color was formed as a result of the reduction of flavonoid groups by magnesium metal and the formation of flavilium ion complexes (Suharyanto & Prima, 2020). The tannin test was carried out by adding $FeCl_3$ solution to the extract that had been diluted with distilled water, producing a blackish green color due to the formation of a complex between Fe^{3+} ions and phenolic groups in tannins (Pratama et al., 2019).

Meanwhile, the saponin test was conducted by shaking the extract using distilled water that had been heated until a stable foam 1–10 cm high appeared, indicating the surfactant properties of saponin. The foam stability was enhanced by the addition of 2N HCl (Ngginak et al., 2017). The essential oil test was conducted by mixing the extract and 70% ethanol, where the formation of turbidity or layers indicated the presence of essential oil components that were not completely soluble in the polar solvent (Junita et al., 2021).

Following the screening, this crude extract was then dissolved in distilled water and transferred into a separating funnel for liquid-liquid fractionation. The separation was performed progressively using n-hexane, chloroform, and 70% ethanol in a 1:1 volume ratio. The mixture was shaken vigorously and allowed to stand until two distinct layers separated clearly. Each solvent layer was collected and concentrated again using a rotary evaporator to yield the dry fractions.

The antibacterial activity of each fraction (at a screening concentration of 5% w/v) was evaluated against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 using the well diffusion method on Nutrient Agar (NA) media. Each well was filled with 50 µL of the fraction, a commercial antiseptic spray as the Positive Control, or sterile distilled water as the Negative Control. After incubation at 35°C for 24 hours, the Diameter of Zone Inhibition (DZH) was measured using a digital caliper (Purwantiningsih et al., 2019). This assay was performed with five replicates (n=5) to determine the most optimal fraction for the spray formulation.

Spray Formulation and Physical Stability Evaluation (Cycling Test)

The 70% ethanol fraction of *M. spicata L*. leaves was formulated into a topical liquid spray preparation at three distinct concentrations: 5% (F1), 10% (F2), and 20% (F3). The spray base consisted of propylene glycol as a humectant, methylparaben as a preservative, and distilled water as the solvent.

The procedure for preparing the antibacterial and antiseptic spray from the optimal fraction of *Mentha spicata L*. leaves was conducted as follows: all required ingredients were accurately weighed according to the predefined formulas (F0, F1, F2, F3). The optimal fraction, nipagin, and nipasol were completely dissolved in preheated distilled water, after which glycerin was added and the mixture was thoroughly stirred until completely homogeneous. A 0.9% physiological saline solution was then added to adjust the total volume to 100 mL. Finally, the resulting solution was homogenized and transferred into sterile spray bottles for subsequent evaluations.

Physical quality evaluation of preparations includes organoleptic testing, homogeneity, pH, clarity, drying time, and stability. Organoleptic testing is performed by observing the color, smell, and shape of the preparation. Homogeneity is tested by observing 0.5 mL of the preparation on a glass slide to ensure that there are no coarse particles (Novrita et al., 2024). Clarity tests are performed using visual methods to ensure that the liquid is clear and free of sediment or suspended particles. The pH value is measured using a pH meter to ensure compatibility with skin pH (4.5–6.5)

(Stevani et al., 2019). Drying time is measured by calculating the drying time of the spray on the skin of the hand (<5 minutes) (Zubaydah et al., 2022).

The physical stability of the formulated sprays was evaluated using a accelerated stability test (cycling test) consisting of 3 consecutive cycles. Each cycle involved storing the preparations at a low temperature (4°C) for 24 hours, followed by immediate transfer to a high temperature (40°C) for another 24 hours. Physical and chemical parameters, including organoleptic properties (color, odor, clarity), pH, homogeneity, drying time, spray pattern, and weight per spray, were critically assessed at baseline (Cycle 0) and after the completion of the 3rd cycle to ensure formulation integrity.

In vivo Effectiveness Test

Three male rabbits of the *Oryctolagus cuniculus* New Zealand *White* breed underwent acclimatization in the laboratory environment for seven days. After the acclimatization period, the rabbits' back skin was shaved. A total of 15 incisions were made on the rabbits' back area, distributed equally across the animals (five incisions per rabbit). The wounds were made after local anesthesia using lidocaine cream. Paravertebral linear incisions of ±1 cm were made using a scalpel, with a depth of ±0.2 mm. The wounds were inoculated with *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria 24 hours after the wounds were made. The wounds showed erythema as a sign of infection.

The treatments tested consisted of five treatment groups with three replicates (n=3), including: positive control (commercial antiseptic), negative control (distilled water), and optimum fraction spray preparations with concentrations of 5% (F1), 10% (F2), and 20% (F3). Observation and measurement of wound closure were conducted periodically over 14 days, namely on Day 0, Day 7, and Day 14. Effectiveness was measured by observing and recording the percentage of wound closure area (planimetry method) periodically (Day 0, 7, 14) (Mufidah et al., 2023).

Data Analysis

1. In vitro Test Data Analysis: The antibacterial inhibition zone data from the three fractions were analyzed using the Two Way ANOVA statistical test. If there was a significant difference ($p<0.05$), it was followed by the LSD Post Hoc Test to compare which fraction had the most superior inhibition.
2. In vivo Test Data Analysis: Wound closure percentage data were analyzed using a Two-Way ANOVA statistical test to examine the effects of concentration and time. Significant results ($p < 0.05$) were followed by a Post Hoc LSD Test to determine the comparison of healing effectiveness between each treatment group and the control group.

Results and Discussion

1.1 Phytochemical Screening and Fractination Results

1.1.1 Phytochemical Screening

The phytochemical screening was initially conducted to identify the secondary metabolites present in *Mentha spicata L*. Extracts. The qualitative profiles of these chemical constituents are summarized in Table 1.

Table 1. Results of Phytochemical Screening *of Mentha spicata L.* Extracts

No.	Chemical compound	Test results
1.	Essential oil	+
2.	Flavonoids	+
3.	Tannin	+
4.	Saponin	+

Note: (+) positive: contains the compound group
The test results presented in Table 1 show that *Mentha spicata L.* leaf extract is positive for essential oils, flavonoids, tannins, and saponins. These findings are consistent with the literature (İsfendiyaroğlu et al., 2024).

1.1.2 Fractination

The concentrated extract was then fractionated to separate the active compounds based on the principles of affinity and polarity differences. Non-polar (n-hexane), semi-polar (chloroform), and polar (70% ethanol) solvents were used sequentially. The antibacterial compounds from phenolic derivatives of carvone in *Mentha spicata L.* leaves tend to be semi-polar to polar; thus, the 70% ethanol fraction is expected to produce the most optimal biological activity (Menyiy et al., 2022).

1.2 In vitro Antibacterial Activity Test

The initial phase of this study evaluated the in vitro antibacterial capacity of n-hexane, chloroform, and 70% ethanol fractions of *Mentha spicata L*. leaves against both Gram positive (*Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa*) pathogens. The tracking of antibacterial efficacy through the well diffusion method revealed a distinct variance in sensitivity profiles between the two bacterial strains, as compiled in Table 2.

Table 2. Antibacterial Activity of *Mentha spicata L.* Leaf Fractions (5% w/v) Against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (n=5)

Fraction	*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
	Concentration	Diameter (mm)	Concentration	Diameter (mm)
n-hexane	5%	1.04 ± 0.20	5%	8.42 ± 0.19
Chloroform	5%	3.16 ± 0.24	5%	10.16 ± 0.27
70% ethanol	5%	5.26 ± 0.41	5%	16.32 ± 0.31
Positive control	5%	17.26 ± 0.30	5%	17.28 ± 0.48
Negative control	5%	0.00±0.00	5%	0.00 ± 0.00

Note: The values presented are the mean ± standard deviation (SD) of the test results with 5 replicates (n=5) per treatment group and bacterial type.

The Two Way ANOVA analysis demonstrated that bacterial type significantly affected the inhibition zone diameter ($F = 4021.164$; $p < 0.001$). Likewise, fraction type significantly influenced the antibacterial activity ($F = 5277.450$; $p < 0.001$). A significant interaction between bacterial type and fraction type was also observed ($F = 745.550$; $p < 0.001$), indicating that the antibacterial effectiveness of each fraction varied depending on the bacterial species tested. ±

Further analysis using the Post Hoc Least Significant Difference (LSD) test was performed to identify differences among treatment groups. As presented in Table 3:

Table 3. Average Inhibition Zone Diameter for Each Treatment Group

Treatment Group	Average Inhibitory Zone Diameter (mm)	Significance Notation
Positive Control	17.27±0.38	a
70% Ethanol Fraction	10,79±5,83	b
Chloroform fraction	6,66±3,69	c
n-Hexane fraction	4,73±3,89	d
Negative control	0,00±0,00	e

Note: Different letters indicate significant differences based on the LSD test ($p < 0.05$).

The results of the LSD post hoc test (Table 3) confirm that the positive control showed the highest antibacterial activity and was significantly different ($p < 0.05$) from all fraction groups. Among the tested fractions, the 70% ethanol fraction consistently produced the largest inhibition zone diameter, followed by the chloroform and n-hexane fractions. Meanwhile, the negative control showed no

inhibitory activity. These findings confirm that the 70% ethanol fraction is the optimal fraction and possesses the greatest antibacterial potential for further formulation development..

Further evaluation demonstrated that the antibacterial activity of the 70% ethanol fraction was substantially higher against *Staphylococcus aureus* (16.32 ± 0.31 mm) than against *Pseudomonas aeruginosa* (5.26 ± 0.42 mm). The lower susceptibility of *Pseudomonas aeruginosa* is likely associated with its ability to form biofilms, which function as a protective matrix that limits the penetration of antibacterial compounds into bacterial cells (Agustin et al., 2022; Mahmuda et al., 2025; Scania & Ningsih, 2023). This finding is consistent with previous studies reporting that biofilm formation plays a major role in antimicrobial resistance (Wahyudi & Soetarto, 2021).

Overall, the data indicate that *Staphylococcus aureus* exhibited a significantly greater susceptibility to the optimum fraction than *Pseudomonas aeruginosa*. Considering that *Staphylococcus aureus* is the predominant pathogen associated with infected incision wounds, and to rigorously uphold animal welfare ethics (the 3Rs: Reduction and Refinement), the subsequent *in vivo* phase was rationally focused exclusively on the *Staphylococcus aureus* infected incision model. Utilizing only this susceptible bacterial model avoids unnecessary distress and unviable systemic biohazards in the animal subjects while allowing therapeutic efficacy to be tracked optimally.

1.3 Spray Formulation and Physical Stability Evaluation

Based on the in vitro antibacterial results, the 70% ethanol fraction was selected as the active ingredient for spray formulation because it demonstrated the strongest inhibitory activity against the tested bacteria. The fraction was formulated into antibacterial and antiseptic spray preparations at three concentrations series, namely F1 (5%), (F2) 10%, and (F3) 20%. The comprehensive master formula and composition for each preparation batch are systematically detailed in Table 4.

Table 4. Antibacterial and Antiseptic Spray Formula

Ingredient	F0	F1(5%)	F2(10%)	F3(20%)	Function
Optimum fraction	-	5 gram	10 gram	20 gram	Active ingredient
Glycerin	-	10 ml	10 ml	10 ml	Humectant
Nipagin	-	0.05 gram	0.05 gram	0.05 gram	Preservative
Nipasol	-	0.01 gram	0.01 gram	0.01 gram	Preservative
Distilled water	qs	qs	qs	qs	Carrier
0.9% physiological saline solution	-	ad 100 ml	ad 100 ml	100 ml	Isotonic regulating agent and carrier

The qualitative physical appearance and visual parameters of the fully developed spray series are visually documented in Figure 1.

Figure 1. Results of the formulation of antibacterial and antiseptic spray preparations of 70% ethanol fraction of *Mentha spicata L*. leaves.

The physical quality evaluation demonstrated that all formulations met the required parameters. Organoleptic and homogeneity assessments showed that the

formulations remained completely clear, homogeneous, and free from any detectable phase separation or precipitation. It was noted that the intensity of the yellow color and the characteristic mint odor increased with increasing fraction concentration, with F3 (20%) exhibiting the most pronounced organoleptic characteristics.

Furthermore, the pH values of all batches remained within the acceptable physiological range for topical preparations (4.5–6.5), indicating excellent skin compatibility and a low risk of localized skin irritation upon application. In addition, all developed sprays exhibited a rapid drying time (<5 min) when tested on the skin, which drastically enhances user convenience and facilitates optimal coverage over open wound surfaces.

To evaluate the thermodynamic stability profile of the preparations, an accelerated stability testing protocol was performed using a freeze thaw cycling method. The formulations were alternately stored at 4°C and 40°C every 24 hours for a total of 3 consecutive cycles. The results revealed no significant alterations in organoleptic properties, clarity, or pH values. These findings verify that the developed spray formulations possess resilient physical integrity against extreme temperature fluctuations, fully supporting their subsequent application in the in vivo wound healing study.

1.4 In vivo Wound Healing Efficacy Test

The efficacy of the spray formulation was tested on an incision wound infected with *Staphylococcus aureus* in rabbits. Observations were conducted over 14 days, with the average reduction in wound length presented in Table 5:

Table 5. Average Wound Length (cm) Test Animals in Various Treatment Groups

Treatment Group	Day 0	Day 7	Day 14
Positive Control	1.00 ± 0.00	0.15 ± 0.05	0.02 ± 0.03
Negative Control	1.00 ± 0.00	0.63 ± 0.15	0.43 ± 0.15
Treatment Concentration 5%	1.00 ± 0.00	0.59 ± 0.08	0.15 ± 0.05
Treatment Concentration 10%	1.00 ± 0.00	0.30 ± 0.10	0.05 ± 0.05
Treatment Concentration 20%	1.00 ± 0.00	0.08 ± 0.08	0.05 ± 0.05

Note: Data are presented as mean standard deviation (n=3). Day 0 indicates the wound after incision, while days 7 and 14 indicate wound healing progress after administration of the preparation.

The average wound length data for each treatment group are presented in Table 5. To provide a clearer visualization of wound healing progression during the 14-day observation period, the data are also presented in Figure 2.

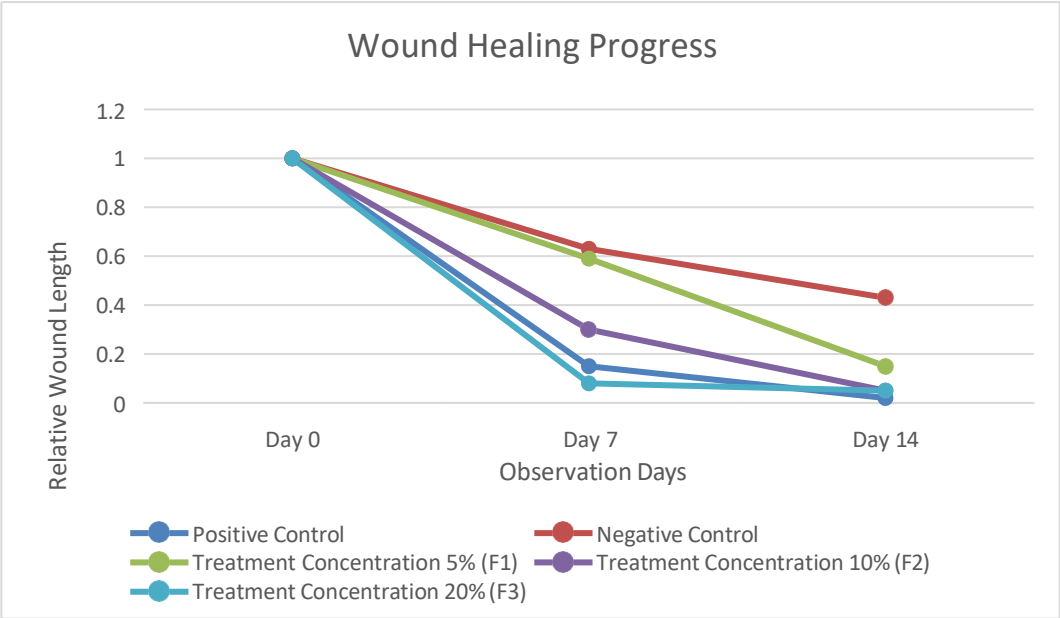

Figure 2. Changes in wound length during the 14 day treatment period in different treatment groups

The wound length reduction in each treatment group is presented in Figure 2. As shown in the figure, all treatment groups demonstrated a progressive decrease in wound length over the observation period. The F3 group exhibited the greatest reduction compared to the other groups, particularly on day 14, indicating a faster wound healing process. In addition to the graphical data, macroscopic changes in the wound area were also documented photographically at each observation time point, as shown in Figure 3.

Incision Wound	**Infected Wound**	**Day 0**	**Day 7**	**Day 14**

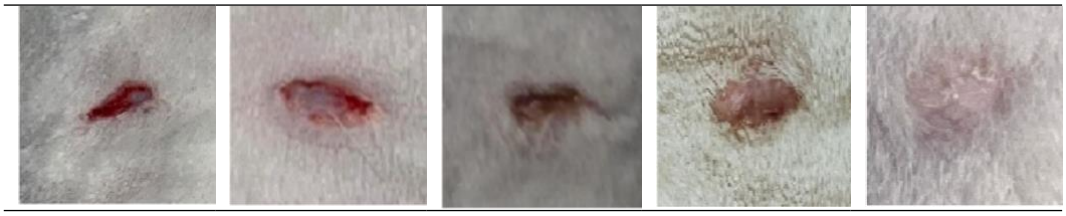

Figure 3. Representative Images of *Staphylococcus aureus* Infected Incision Wound Healing in *New Zealand White* Rabbits From Each Treatment Group on Days 0, 7, and 14 After Treatment.

Descriptively (Table 5), the 20% Concentration Treatment group showed the best results among the treatment groups with a remaining wound length of 0.05 ± 0.05 cm on Day 14. These results indicate a very significant acceleration in wound closure, approaching the results of the Positive Control (0.02 ± 0.03 cm). In contrast, the Negative Control showed the slowest healing (0.43 ± 0.15 cm).

Statistical analysis using a Two-Way ANOVA test showed that the treatment factor (concentration) and observation day (time) independently had a significant effect ($p < 0.05$) on the acceleration of wound healing. The Post Hoc LSD test confirmed that: The 20% Concentration Formula was significantly different ($p < 0.05$) from the Negative Control.

The 20% Concentration Formula did not show a significant difference ($p < 0.05$) with the Positive Control group (commercial antiseptic). These results prove that the spray preparation with 70% ethanol fraction of *Mentha spicata L*. leaves at a concentration of 20% is equivalent in effectiveness to commercial antiseptics in accelerating the closure of incision wounds infected with *Staphylococcus aureus*. This mechanism of effectiveness is thought to be due to the compound carvone in the 70% ethanol fraction directly controlling *Staphylococcus aureus* infection (which is the main inhibitor of wound healing). Controlling the infection allows the proliferation phase (wound closure) to proceed optimally.

As shown in Table 6, the representative image illustrates the progression of infected incision wounds in rabbits. The wound condition is shown starting from the post incision stage, followed by bacterial infection leading to wound deterioration, and continued observation during the treatment period on days 0, 7, and 14. The image demonstrates gradual improvement of the wound after treatment application.

Conclusion

The 70% ethanol fraction of *Mentha spicata L*. leaves exhibits the strongest antibacterial activity against *Staphylococcus aureus* compared to the chloroform and n-hexane fractions, making it the optimal fraction for antibacterial and antiseptic spray formulations. The spray formulations with concentrations of 5%, 10%, and 20% exhibited good physical quality, stability during storage, and safety for the skin. In vivo test results showed that the 20% concentration was able to accelerate the healing of Staphylococcus aureus-infected incision wounds with efficacy comparable to the positive control (commercial antiseptic). Thus, the spray formulation based on the 70% ethanol fraction of *Mentha spicata L*. leaves has the potential to be developed as an effective, safe, and stable natural antiseptic for the treatment of infected incision wounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Recommendations for Further Research:

1. Further research is recommended to compare extraction methods focused on isolating essential oils, such as distillation, to obtain higher and purer concentrations of the active compound (carvone).
2. Further research is recommended to conduct a quantitative assessment of the

compounds contained in *Mentha spicata L*. leaves in each fraction.
3. Further in vivo studies are recommended using an infected pressure ulcer model with diabetes induction in experimental animals to enhance clinical relevance and better simulate actual chronic wound conditions in patients.
4. The potential of the fractions should be developed into other topical formulations relevant to wound therapy, in addition to sprays, to optimize the release of active substances.

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Author's Contribution

The authors' roles in the preparation of this scientific article are as follows: Nabilla Putri Auliyah directed the team in conducting research; Candra Eka Saputra conducted experiments and prepared the manuscript; Riswanda Irsadina Najiha conducted experiments and data analysis; Aqilla Farhanadila conducted experiments and compiled research administrative activities; Fadilla Citra Suci conducted experiments; Ani Florida Ngete provided research guidance, research design, and manuscript completion.

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