The Effectiveness of Combination of *Piper betle* L. Ethanol Extract and Manuka Honey Spray Gel to Accelerating Acute Wound Healing

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Abstract. Acute wounds are injuries that occur suddenly to tissues that are prone to the risk of being infected. Therefore, acute wound care must be considered carefully. *Piper betle* leaf contains flavonoids that have a function in the wound healing process, Manuka honey also contains vitamins that can support the healing phase. The combination of *Piper betle* L. and manuka honey is made into a spray gel preparation because it has advantages over other topical preparations. The purpose of this study is to see how well a combination wound healing spray gel of *Piper betle* L. and Manuka honey works on an acute wound in mice. This study uses experimental research with a randomized, matched post-test-only control group design. The formulation taken was maceration extraction from *Piper betle* L. Simplicia powder with 70% ethanol then evaporated to acquire a concentrated concentrate. Spray gel preparations of a combination of *Piper betle* L extract and manuka honey were made with a concentration of 1% *Piper betle* L and Manuka honey, 3% *Piper betle* L and Manuka honey, and 5% *Piper betle* L and Manuka honey. Balb/c mice that had full-thickness acute wounds were divided into 7 treatment groups, namely SM1, SM2, SM3, M, KP, B, and D. Mice were treated for 14 days and the wound area ratio was calculated and analyzed by ANOVA. The test results showed a significant difference between groups SM3-M, SM5-M, M-SM3, M-SM5, KP-M, B-SM3, and BM with a significance value below 0.05 (p≤0.05) which proves that the spray gel combination of *Piper betle* L extract and Manuka honey, oxoferin, and base both have activity in accelerating acute wound healing.

1 Introduction

A wound refers to a state wherein there is harm or injury to the body’s tissues due to disruption of tissue continuity caused by injury or surgery. Wounds often occur on the skin, causing damage to the epithelium or damage to the normal anatomical structures of these tissues [1]. The incidence of injuries in Indonesia every year continues to increase,
reaching millions of cases per year. Every year the number of injuries both acute and chronic rises. In Indonesia, based on available data, the number of patients with wounds is 350 per 1000 people. Wounds can be defined as an interruption in cellular continuity, which is subsequently succeeded by the process of wound healing [2].

Acute wounds are injuries that occur suddenly to tissues that are prone to the risk of being infected. Therefore, the treatment of acute wounds must be considered carefully. Based on the healing time, there are two types of wounds, namely acute wounds and chronic wounds. Basically, all wounds are acute wounds can become chronic if the wound healing time exceeds the physiological wound healing time [3]. Most people experience acute wounds, both large and small wounds, and the depth and severity of these wounds are still considered acute wounds during the physiological wound-healing process, so it is very important to treat and prevent acute wounds from turning into chronic wounds [4] [5]. The consequences triggered by a wound include organ dysfunction or dysfunctionality of certain organ parts, an involuntary sympathetic response, hemorrhaging, thrombus formation, microbial infection, and necrosis. The administration of manufactured pharmaceutical substances poses detrimental hazards like the onset of superimposed inflammation, which pertains to an escalation in the number of bacterial cohorts in the incision when compromised resistance transpires, thereby inducing discomfort [6], [7].

The high price of modern medicine on the market is one reason to explore the use of traditional medicine. Various medicinal flora in Indonesia have been employed as primary components, several of which have undergone clinical trials to assess their phytochemical composition, effectiveness, and noted tolerability [8]. The utilization of medicinal plants as a remedy is a customary practice to address inherited maladies, whereas Indonesia's heritage entails the use of conventional herbs embedded within the culture and adopted for centuries. Materials that are easily available and low in cost make traditional medicine often used. Herbal plant-based treatment is considered safer when used in accordance with the correct ingredients, accurate dosage, timeliness of use, accuracy of how to use, the accuracy of information review, and the accuracy of drug selection for certain indications [8–10].

*Piper betle* L. is among the phytomedicines cultivated in Indonesia that possess numerous advantages in curing injuries. In several studies, *Piper betle* L. has anticancer, antiallergic, antimalarial, antibacterial, antifungal, insecticide, antioxidant, antidiabetic, gastro-protectant, and wound healing activity. The content of *Piper betle* L. consists of tannins, phenols, saponins, and flavonoids that have a function in the wound healing process through antimicrobial and anti-inflammatory activities that can accelerate epithelization and wound grafting [11–13]. The flavonoid content in *Piper betle* leaf is 25.31 /mL [14].

Research conducted by Jain (2017) said that honey also contains vitamins that are believed to heal wounds. These vitamins include vitamin C and vitamin E as well as vitamins B1, B2, and B6 which can help with nutrition in the wound healing process [15]. A study conducted by Wahyuningtyas (2018), said that honey possesses essential qualities in the wound healing mechanism, for instance, anti-inflammatory, antioxidant, and antibacterial potency, the competency to instill the process of discharging damaged tissue or debridement, preventing fetor in the wounds, and preserving wound humidity. These
attributes ultimately expedite the process of wound healing [16]. According to research by Nasruddin (2017), the optimum concentration of honey for maximum wound healing is 15% [17].

The combination of Piper betel and manuka honey in this study will be made into a spray gel preparation. The spray gel form was chosen because it has advantages over other topical preparations, including being safer, more practical to use, and easier to wash [18]. Spray gel consists of several components, namely preservatives, humectants, and gelling agents [19]. This research has the following objectives: to know the effectiveness of a spray gel combination of Piper betle L. and Manuka honey to accelerate acute wound healing.

2 Method

2.1 Preparation of spray gel combination of piper betle leaf extract and manuka honey

Spray gel is made using a carbopol base with various concentrations of Piper betle extract 1%, 3%, and 5% (Fig. 1). The first step is to put carbopol 940 and aquadest into the mortar and stir until homogeneous (A). In addition, the methylparaben and propylparaben are combined in a beaker glass with propylene glycol and stirred until homogeneous (B). Using a different vessel, the Piper betle leaf extract was dissolved in a DMSO solution. Mixture B was added to mixture A, and both were homogenized until completely mixed then the extract, which had been dissolved in DMSO solution and honey, was added little by little, stirring until homogeneous. Next, add triethanolamine, and stir until homogeneous, then the mixture is mixed with aquadest and homogenized (Table 1). The result is put into a spray bottle.

![Image](https://example.com/image.png)

**Fig. 1.** Spray Gel Formulation Combination of *Piper betle* L. Extract and Manuka Honey
Table 1. Formulation of *Piper betle* Leaf Extract and Manuka Honey Spray Gel

<table>
<thead>
<tr>
<th>Material Name</th>
<th>Function</th>
<th>Basis</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Piper betle Extract (gr)</td>
<td>Active substance</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Manuka Honey (gr)</td>
<td>Active substance</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Carbopol 940 (gr)</td>
<td>Polimer</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>TEA (gr)</td>
<td>base</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Propilen glikol (gr)</td>
<td>Solubilizer</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Metil paraben (gr)</td>
<td>preservative</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Propil paraben (gr)</td>
<td>preservative</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>DMSO (gr)</td>
<td>Co-solvent</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Aquaest (gr)</td>
<td>Solvent</td>
<td>Ad 100</td>
<td>Ad 100</td>
</tr>
</tbody>
</table>

2.2 Preparation for making acute wounds for experiment procedure

As many as 21 test animals male Balb/c (Mus musculus) mice, 5 months old, with body weight 20-35 grams that have been acclimatized were divided into 7 groups randomly. The grouping of mice can be seen in Table 2.

Table 2. Grouping of test animals

<table>
<thead>
<tr>
<th>No</th>
<th>Groups</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intervention 1 code: SM 1</td>
<td>mice + acute wound + Hydrocoloid Dressing + Formulation of Piper Betel Leaf 1 % Extract and Manuka Honey Spray Gel</td>
</tr>
<tr>
<td>2</td>
<td>Intervention 2 code: SM 3</td>
<td>mice + acute wound + Hydrocoloid Dressing + Formulation of Piper Betel Leaf 3 % Extract and Manuka Honey Spray Gel</td>
</tr>
<tr>
<td>3</td>
<td>Intervention 3 code: SM 5</td>
<td>mice + acute wound + Hydrocoloid Dressing + Formulation of Piper Betel Leaf 5 % Extract and Manuka Honey Spray Gel</td>
</tr>
<tr>
<td>4</td>
<td>Intervention 4 code: M</td>
<td>mice + acute wound + Hydrocoloid Dressing + Manuka Honey</td>
</tr>
<tr>
<td>5</td>
<td>Control (+) code: KP</td>
<td>mice + acute wound + Hydrocoloid Dressing + Oxoferin</td>
</tr>
<tr>
<td>6</td>
<td>Control (-) code: B</td>
<td>mice + acute wound + Hydrocolloid Dressing + Spray Gel base</td>
</tr>
<tr>
<td>7</td>
<td>Control (-) code: D</td>
<td>mice + acute wound + Hydrocolloid Dressing</td>
</tr>
</tbody>
</table>

According to investigation carried out by Nasruddin et al (2014), full-thickness acute wounds are manufactured by anesthetizing mice before making the wound [20]. Anesthesia was performed using a chemical mixture of Ketamine 50 mg/kg and Xylazine at a dose of 5 mg/kg via the intraperitoneal (i.p) injection route. Then the hair of the mice on the back and stomach was shaved using an animal shaver until it was smooth and the surface of the skin was visible. In addition, an aseptic disposable punch biopsy, measuring 4 mm in diameter, was employed to create circular wounds on the dorsal surfaces of male Balb/c mice’s back.
2.3 Macroscopic observation

Evaluation of wound healing in mice was performed by macroscopic observation on day 0, followed by daily observation until day 14 post-wounding to determine the progress of the healing process. The peri-wound area was subjected to irrigation with a sterile physiological solution of sodium chloride (NaCl) with a concentration of 0.9%, prior to its observation and the wounds in mice were documented using a digital camera. Every day, the wound edges of mice are traced on mica plastic with a permanent marker. The results of the imitation of the wound during the observation will be observed using an observer device (scanner) which is then transferred to a computer. The dimensions of the injury site were computed by means of image processing techniques, software Scion Image Beta 4.02 (Scion Corporation, Frederick, Maryland, USA). The wound ratio was calculated by comparing the area of the wound on the right and left dorsal (back) of mice. The area of the wound is calculated by the Eq. (1) [20].

\[
\text{Wound area ratio} = \frac{\text{Wound area day-n}}{\text{Wound area on day 0}}
\]

2.4 Data analysis

This study was conducted to see the ratio of wound area on the skin of all treatment groups. The information acquired will be analysed via IBM SPSS Statistics 25 software. The statistical prerequisites of the research data will be evaluated by conducting both normality and homogeneity tests. The normality of the data will be determined using the Shapiro-Wilk test and if the p-value is greater than 0.05, it is considered to be normal. Homogeneity test was also performed using Levene's test: if the p-value was greater than \(>0.05\) the data was said to be homogeneous. If the tested data has met the requirements, then the wound area ratio analysis is continued with the Anova Repeated Measure test. The variability in the wound area ratio results is observed across all groups whenever the p-value is less than 0.05, which is then confirmed by applying the Tukey-Cramer Post Hoc test at a confidence level of 95%.

3 Results and discussion

The treatment for each group was carried out for 14 days using the diameter of the wound in each treatment group. Wound observations were carried out for 14 days because after 14 days the wound would enter a remodeling phase, wherein the resulting graph tends to be straight, making it difficult to observe because of the high potential for biased data. On day 0 the wound experienced an inflammatory phase marked by an enlarged wound size resulting in exudate, and gradually the wound entered a proliferative phase that was characterized by the size of the wound starting to shrink until the 14th day, which was the end of the observation period. On day 14, it appears that the area of the wound in the Piper Betel Leaf 5 % Extract and Manuka honey Spray Gel and the base is larger than all groups, this can be seen in the graph in Fig. 2.
Fig. 2. Wound contraction percentage from day 0 until 14.

The graphic illustration in Fig. 2 exhibits alterations in the wound area throughout the monitoring duration. The plot is an outcome of evaluating the proportion of wound area detected on observation day relative to the wound area at the outset, spanning day 0 to day 14. The data indicates that all test groups underwent a parallel healing pattern: an initial increase in wound area proportion within approximately the first 4 days, followed by gradual reduction until the conclusion of the end observation.

The average wound area ratio on day 14 (Table 3) showed that the smallest wound area ratio was oxoferin as a positive control, followed by the honey spray gel group, the dressing group, the spray gel group with a combination of *Piper betle* leaf extract, and Manuka honey with a concentration of 1%, 3%, base, and spray gel combination of *Piper betle* leaf extract and 5% concentration of Manuka honey did not have that much difference in the wound ratio, but the resulting SD (Deviation Standart) values were very different. In spray gel, the combination of *Piper betle* extract and 5% Manuka honey had the largest wound area which was probably caused by less-than-optimal preparations. This can be seen from the presence of coarse particles in the preparation because the residue filtering process is carried away, so the preparation is less than optimal, which causes the test to be inappropriate. The combination of *Piper betle* and Manuka honey can affect antioxidant activity because each element exhibits Secondary metabolites, which may react with one another upon amalgamation, resulting in certain cases, in the development of extensive exudative lesions [3], [21].

<table>
<thead>
<tr>
<th>Group</th>
<th>Wound area ratio on day 14 ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray Gel combination Formulation of <em>Piper Betel</em> Leaf 1 % Extract and Manuka Honey Spray Gel (SM 1)</td>
<td>0.31 ± 0.14</td>
</tr>
<tr>
<td>Spray Gel combination Formulation of <em>Piper Betel</em> Leaf 3 % Extract and Manuka Honey Spray Gel (SM 3)</td>
<td>0.32 ± 0.09</td>
</tr>
<tr>
<td>Spray Gel combination Formulation of <em>Piper Betel</em> Leaf 5 % Extract and Manuka Honey Spray Gel (SM 5)</td>
<td>0.37 ± 0.07</td>
</tr>
<tr>
<td><em>Spray Gel</em> Manuka Honey (M)</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>
Based on the results of research that have been carried out, a spray gel combination of *Piper betle* leaf extract and manuka honey has the potential to be developed as a wound-healing spray gel preparation. However, it is necessary to test the toxicity of the spray gel of *Piper betle* L. leaf extract and Manuka honey to ensure the safety of the spray gel.

The variation in antioxidant efficacy is attributed to the allocation and composition of secondary metabolite compounds possessing antioxidant qualities, present in the formula under consideration. According to Aftab and Vieira (2010), the synergy between various phytochemical compounds can augment the antioxidative capacity through the replenishment of redundant antioxidant molecules [22]. The act of offering protons by the antioxidant compounds to stabilize radical compounds makes them deficient in their antioxidant capacity. The synthesis of phytochemical compounds, however, enables the antioxidants to rejuvenate even when an ingredient has a low antioxidant potential [23].

Honey spray gel has the smallest wound area, this shows that honey activity in accelerating wound healing in mice because honey has a high osmotic pressure and prevents bacterial growth [15,24]. Not only useful as an antibacterial, honey is also useful in the body’s defense system which is also related to the wound healing process. It is also known that honey can remove glutathione from wounds, thereby accelerating wound healing or infection, and has methylgligoxil substances where this substance as the main cause has an antibacterial effect that can treat wounds [17,25,26].

The observation of the area of the wound was carried out using IBM SPSS Statistics 25.0 software and passed the prerequisite test, namely the normality test using Shapiro Wilk which showed that there were several groups that were the distribution deviates from normality (p<0.05), and Levene’s test has been employed to assess the homogeneity of variances. was carried out on the normally distributed group. All groups were found to be homogeneous (p<0.05). In the group that was not normally distributed, the Kruskal-Wallis H test was utilized in conducting the method (p<0.05) while the normally distributed group was tested using the Post Hoc Tukey-Cramer method (p<0.05). Characterized by a decrease in wound diameter. From the test results, there were significant differences between the groups SM3-M, SM5-M, M-SM3, M-SM5, KP-M, B-SM3, and BM with a significance value below 0.05 (p<0.05) which indicates proves that the spray gel combination of *Piper betle* leaf extract and Manuka honey, oxoferin, and base both have activity in accelerating wound healing.

Because of the *Piper betle* content, namely saponins, which are useful for stimulating the growth of collagen, which plays a role in wound healing, the area of the wound in Manuka honey, spray gel combination of *Piper betle* leaf extract and Manuka honey (1% and 3%), shrinks faster. Another benefit of saponin compounds in the wound healing process is the beneficial biological effect that increases the immune system (immunomodulator). Progressive collagen synthesis will cause the formation of connective tissue to be faster and more optimal. This connective tissue will strengthen

<table>
<thead>
<tr>
<th>Group</th>
<th>Wound area ratio on day 14 + SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoferin (KP)</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Control negative Base (B)</td>
<td>0.34 ± 0.10</td>
</tr>
<tr>
<td>Control negative Dressing (D)</td>
<td>0.28 ± 0.10</td>
</tr>
</tbody>
</table>
the wound-healing process and make it more perfect. Collagen has a function in the wound-healing phase. Collagen is the main protein that makes up the extracellular matrix component and the protein that occurs in the highest amount in the human body. Collagen synthesis in fibroblasts is a process that requires oxygen. Collagen formation in relation to the wound healing process is influenced by several factors such as infection, partial oxygen pressure of the tissue around the wound, oxygen diffusion, phagocytosis, stress, pain, nutrition, and genetic factors in each individual (host factor). Acute bleeding that causes acute anemia affects the formation of collagen due to decreased oxygen diffusion in the wound area. Collagen is the main structural component of connective tissue fibers, is white in color and is present in all animal tissues and organs, and plays an important role in building body shape. Collagen is present in the integumentary system, tendons, articular cartilage, and several other types of connective tissue in mammals [27]. Compared to the broad base negative group, the wound began to appear smaller on the 10th day. The base group showed high inflammation and low remodeling. This is influenced by the base spray gel carbopol and propylene glycol as humectants and also by the lack of bioactive compounds that assist in the reparative process of the wound led to a prolonged phase of healing, whereas, the cohort that received dressing experienced the least amount of inflammation on the fifth day, the main goal of the dressing. The goal of the inflammatory phase is to stop bleeding, prevent infection, and get rid of necrotic tissue, foreign bodies, and bacteria that arise. It appears to be low after the honey during the remodeling phase. In the wound dressing group, the wound quickly shrinks because it has a low evaporation rate which will cause high humidity above the wound surface, and the oxygen pressure in the wound tissue is quite high, so the wound healing stage will be faster [26], [28]. Then at the time of testing on the mice’s wounds, the plaster was released, and due to the air entering the back wounds of the mice, the wounds quickly shrank.

The amount of exudate varies from very little to moderate, a lot, and very much [3], [21]. According to the findings of the conducted investigation, a spray gel combination of Piper betle leaf extract and manuka honey has the potential to be developed as a wound-healing spray gel preparation. However, it is necessary to test the toxicity of the spray gel of Piper betle L. extract and Manuka honey to guarantee the protection of the spray gel.

4 Conclusion

The combination of Piper betle L. and manuka honey can affect antioxidant activity because each ingredient has secondary metabolites that have the potential to interact with each other when combined. The best wound healing activity was in the honey spray gel group, and in the treatment group, there was a spray gel combination of manuka honey and Piper betle L. extract with a concentration of 1%. In this future research, it is necessary to carry out further research on wound healing activities in the formulation of spray gel preparations, a combination of Piper betle leaf extract and Manuka honey, observed microscopically by histological testing.
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References


