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Ethanol Extract Burn Gel Formulation of Bay Leaves (Syzygium polyanthum (Wight.) Walp.)

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ABSTRACT

Burns are skin tissue damage caused by hot objects or hot liquids. One of the potentially healing plants for burns is the bay leaves (*Syzygium polyanthum* (Wight.) Walp.). *Bay leaves ethanol extract contains flavonoids known for their anti-inflammatory properties, which can aid in wound healing. This study aimed to determine the formulation and effectiveness of a gel made from ethanol bay leaves extract. Bay leaves were extracted using the maceration method with a 70% ethanol solvent; then, its extract was formulated into gel preparations. Following the formulation,the researchers conducted physicochemical evaluations and burn effectiveness trials using New Zealand albino rabbits. The research results indicated that the burn gel formulations with 3% and 6% concentration variations exhibited favourable physical properties, including organoleptic, homogeneity, and pH from 5.33 to 5.8, spreadability from 4.77 to 5.55 cm, adhesion strength from 5.47 to 11.6 seconds, and viscosity ranged from 12.507 to 16.080 cPs. The 15-day burn effectiveness test revealed no significant differences, indicating that the reduction in wound diameter was relatively consistent across all test groups. The percentages of wound healing in the negative control, bioplacentone, F1, and F2 were 42.48%, 54.73%, 43.84%, and 65.89%, respectively. Therefore, F2 demonstrated the best wound-healing performance.*

Keywords: Bay Leaves, Gel, Physicochemical Properties, Effectiveness of burn gel.

ABSTRAK

Luka bakar adalah kejadian rusaknya jaringan kulit akibat benda panas atau cairan panas. Salah satu tanaman yang berpotensi menyembuhkan luka bakar adalah daun salam (*Syzygium polyanthum* (Wight.) Walp.). Ekstrak etanol daun salam memiliki kandungan flavonoid yang memiliki aktivitas sebagai anti inflamasi sehingga dapat membantu menyembuhkan luka. Tujuan dari penelitian ini adalah untuk mengetahui formulasi dan efektivitas gel dari ekstrak etanol daun salam. Daun salam diekstraksi menggunakan metode maserasi dengan pelarut etanol 70%, kemudian ekstrak etanol daun salam diformulasikan menjadi sediaan gel. Setelah dilakukan formulasi kemudian dilakukan evaluasi fisikokimia dan uji efektivitas luka bakar menggunakan hewan uji kelinci albino galur *New Zealand*. Hasil penelitian menunjukkan formula gel luka bakar dengan variasi konsentrasi 3% dan 6% memiliki sifat fisik yang baik seperti organoleptik, homogenitas, pH sediaan 5,33-5,8, daya sebar 4,77-5,55 cm, daya lekat 5,47-11,6 detik, dan viskositas 12.507-16.080 cPs. Hasil pengujian efektivitas luka bakar selama 15 hari menunjukkan tidak berbeda signifikan yang berarti penurunan diameter luka pada tiap kelompok uji relatif sama. Persentase penyembuhan luka pada kontrol negatif, bioplacentone, F1, dan F2 secara berurutan yaitu sebesar 42,48%, 54,73%, 43,84% dan 65,89%. Sehingga, F2 memiliki efek paling baik dalam menyembuhkan luka.

Kata kunci: Daun Salam, Gel, Sifat Fisikokimia, Efektivitas gel luka bakar

INTRODUCTION

Tissue damage in the body due to trauma, which can be caused by various factors such as physical trauma, chemicals, extreme temperature increases, or animal bites, is the definition of a wound (Wintoko, R., & Yadika, A. D. N., 2020). It can be categorized into acute and chronic wounds based on their healing time. Those conditions that heal quickly are called acute wounds, while wounds that take a long time to heal are called chronic wounds (Abdullah et al., 2022). For example, chronic wounds include foot ulcers due to diabetes and pressure ulcers, while acute wounds include wounds resulting from surgery and burns (Lindholm, C., & Searle, R., 2016).

Wounds caused by hot liquids, hot objects, radiation, electricity or chemicals are called burns (Putri et al., 2021). All burns cause tissue damage due to energy transfer, but the physiological response differs depending on the cause of the burn. For example, wound from flames or hot oil can cause deep burns, while burns that produce more superficial blisters can result from hot liquids or hot steam. Blisters that appear shallow are caused by the rapid dilution of the heat source and energy (Jeschke et al., 2020). Women are prone to burns because of their work involving electricity, hot objects and hot liquids due to cooking. Apart from that, older adults aged over 50 years are susceptible to burn injuries (Ramdani, 2019). Basic Health Research Data from the Ministry of Health of the

Republic of Indonesia in 2018 in Lampung shows that the incidence of burn injuries occurs more frequently in the elderly, aged 65 to 74 years. The percentage of burn injuries is 3.90%. The incidence of burns for the elderly is 75 years to 75 years and over is 3.74%. Then, the percentage of burns occurring is also higher in women, with a percentage of 1.57%; while in men, it is 1.21% (Ministry of Health of the Republic of Indonesia, 2019).

Burns must be treated to prevent complications such as infection, bleeding or swelling (Purwanti et al., 2018). Wound treatment and care generally depend on the type and the cause. The wound healing process also depends on the wound care process, where the better people treat the wound, the shorter the wound healing time will be. Generally, its care process is carried out by cleaning the wound with an antiseptic and maintaining the wound so that infection does not occur, worsening it (Oktaviani et al., 2019). One of the topical dosage forms available on the market and used to treat burns is the gel dosage form. Gel has the advantages of spreading well on the skin, being well absorbed, relaxed, and easy to apply when applied. It produces a transparent film or layer, does not flow easily when used, is not sticky, has thixotropic properties, is clear, elegant, easy to wash, and is stable in storage (Sidiq et al., 2018), (Putri, W. E., & Anindhita, M. A., 2022).

The current trend in society, which is starting to return to nature, influences pharmaceutical preparations on the market, where preparations containing natural ingredients are more popular because they are considered relatively safer [Mirza et al., 20170. One of the plants that have the potential to heal burnt wounds is bay leaves (Syzygium et al..). The ethanol extract of bay leaves has secondary metabolites with a flavonoid content of 0.51%, alkaloids 0.34%, tannins 0.16%, phenols 0.12% and essential oils consisting of citral and eugenol have a content of 0.2% (Rivai et al., 2019). It is known that the compounds found in bay leaves have pharmacological activity as antioxidants, anti-inflammatory, analgesic, antibacterial, anticonvulsant and immunostimulant (Batool et al., 2019). The flavonoids in bay leaves have anti-inflammatory and antioxidant properties, which can reduce excess ROS and increase wound contraction so that the wound cover quickly. Apart from that, flavonoids also have antibacterial properties, which can prevent bacterial infections, thereby preventing wounds from getting worse and taking longer to heal. Tannin compounds also have antibacterial and antioxidant properties, which can help heal wounds by accelerating the epithelialization process (Lallo et al., 2020). Previous research shows that bay leaves extract can heal burns in an average of 15.4 days, faster than the negative control results, namely 20.2 days (Riyadi et al., 2021). Therefore, based on this information, researchers were interested in formulating bay leaves ethanol extract into a gel preparation, which is expected to be an alternative for treating burns.

METHOD

Tools and Materials

The tools used are glass jars, blenders, petri dishes, stirring rods, test tubes (pyrex), beakers (pyrex), measuring cups (pyrex), analytical scales (OHAUS), rotary evaporator (IKA), Brookfield Hi-Vi viscometer (IKA), pH meter (Hanna), rabbit cage, oven, refrigerator, filter paper, porcelain cup, dropper pipette, test tube rack, slide, water bath, calliper, hot plate (Thermo), hair shaver, gloves, thermometer, magnetic stirrer, and metal plates.

The materials used in this research were bay leaves (*Syzygium polyanthum* (Wight) Walp.) obtained from the village of Sukoharjo Barat 3, Pringsewu, 70% ethanol, $FeCl_3$, HCl, Mg powder, Dragendorff reagent (KI, bismuth subnitrate, acetic acid glacial and distilled water), Mayer's reagent ($HgCl_2$, distilled water, KI), HPMC, propylene glycol, methylparaben, propylparaben, distilled water, 1% gelatin solution, ether, Lieberman Burchad (anhydrous acetic acid and concentrated sulfuric acid) (Pharmapreneurstore), male rabbit (Bunny House Lampung), rabbit food (Vital Rabbit), Lidocaine HCl 2% injection, 1 cc syringe, and bioplacentone gel (Placenta extract 10% and Neomycin sulphate 0.5%).

Bay Leaf Extraction

The maceration method is used in the bay leaves extraction process. The extraction was done by weighing 200 grams of powder and placing it in a glass jar. Next, with a solvent ratio of 1:10, 2L of 70% ethanol was added. The maceration container is then placed in a place protected from light and stirred every 6 hours. The process lasts 24 hours; after that, it is filtered and re-macerated twice. After the filtrate from the maceration and remaceration processes is collected, the solvent is evaporated from the liquid extract at a temperature of 40°C using a rotary evaporator (Sani et al., 2021) (Indonesian Ministry of Health, 2017).

Phytochemical Screening

Phytochemical screening uses a colour reaction using specific reagents according to the compound the researchers want to observe. It includes the alkaloid, flavonoid, tannin, steroid, triterpenoid, saponin, and phenol tests (Farnsworth, 1966).

a. Alkaloid Test

The test was carried out by taking two grams of extract, adding 5ml of 2N HCl to the extract, then dividing it into 3 test tubes. In the first reaction tube, 3 drops of 2N HCl were given as a control. In the second test tube, 3 drops of Dragendorff's reagent are given. If there is an orange precipitate, this indicates an alkaloid. Then, 3 drops of Mayer's reagent are given in the third tube. If there is a yellow precipitate, it indicates the presence of alkaloids.

b. Flavonoid Test

Flavonoid testing was done by adding 0.5 grams of thick bay leaves extract into a test tube. Then, add a little magnesium powder and 2 N HCl to the test tube. 2 ml of amyl alcohol is added to the test tube and shaken vigorously. The colour change in the amyl alcohol layer to red, orange, or yellow indicates the presence of flavonoids.

c. Tannin Test

Tannin testing was done by adding 0.5 grams of extract and 2 mL of distilled water into a test tube. Then, 3 - 4 drops of 1% gelatin solution were added. If there is a white precipitate, it proves the presence of tannin.

d. Steroid and Triterpenoid Test

Testing for steroids and triterpenoids is carried out by taking one gram of thick extract, then adding ether and filtering it. The filtrate was collected in a porcelain cup and evaporated until dry. 3 - 4 drops of Liebermann Burchard reagent solution were added. If it is positive for triterpenoids, the colour will change to red-purple; meanwhile, if it is positive for steroids, the colour will change to green-blue.

e. Saponin Test

Saponin is tested by placing 1 gram of thick extract into a test tube and adding 10 ml of hot water. The mixture is then filtered, and the filtrate is taken. The filtrate was collected in another test tube and shaken quickly for 10 seconds; then, the foam was allowed to stand for 10 minutes. Next, a few drops of 2N HCl are added; if the foam remains and the height is ± 1 cm, it indicates that it is positive for saponin.

f. Phenol Test

Phenol testing was done by taking 0.5 grams of thick extract, placing it in a test tube, and adding 2 ml of water and filtering. The filtrate was given $FeCl_3$. Colour changes to green, blue-green, red-purple, blue-black, and dark black will occur when positive for phenolate. If a brown precipitate appears, then it is positive for polyphenols.

Formulation and Manufacturing

Table 1. Bay Leaves Ethanol Extract Gel Formulation

Material	Function	Formula 1 (%)	Formula 2 (%)
Bay leaves ethanol extract	Active substance	3	6
HPMC	*Gelling agent*	1,5	1,5
Propylene glycol	*Enchanter*, humectant	5	5
Methylparaben	Preservative	0,18	0,18
Propylparaben	Preservative	0,02	0,02
Peppermint essence	Fragrance	0,1	0,1
Aquadest	Solvent	ad 100	ad 100

Process :

First, All ingredients listed in Table 1 are weighed. The first stage was begun by developing the HPMC in distilled water at 80-90ºC, then stirring at 150 rpm using a magnetic stirrer until homogeneous (Rowe et al., 2009). Then, methylparaben and propylparaben were dissolved in propylene glycol in a different container. The ethanol extract of bay leaves was added to the mixture, and peppermint essence was added. The mixture of excipients and extracts that have been made is then added slowly to the gel base, which has begun to swell, and stirred until homogeneous (Yati et al., 2019). The speed is reduced to 100 rpm during the mixing process.

Evaluation of Bay Leaf Ethanol Extract Gel Preparation

1. Organoleptic Test

Organoleptic testing is done by monitoring aroma, colour and shape through the five senses to know changes during storage (Yati et al., 2019). The absence of changes during the storage period in terms of aroma, shape, and colour is a reason for the gel to be categorized as good (Tandolambung, 2021).

2. Homogeneity Test

Homogeneity testing was done by taking 0.5 grams of gel and placing it on a glass object, after which the object was overlaid with another. The gel was then observed to see whether there were granules or not. When observed or touched, the absence of granules in the gel indicates a homogeneous gel (Sani et al., 2021), (Murniyati, 2021).

3. pH Test

pH testing was done by dipping a calibrated pH meter into the gel and looking at the pH reading. The range of 4.5-8.0 is a safe skin pH criterion for topical preparations (Sani et al., 2021), (Ermawati et al., 2022).

4. Spreadability Test

Spreadability testing was carried out using petri dishes. The gel was taken and weighed 0.5 grams, then placed in the middle of a petri dish, which was placed upside down and covered with another petri dish. Next, leave it for 1 minute and put the weights weighing 50, 100, 150, 200 and 250 grams every 1 minute and measure the diameter. The 5 - 7 cm range is a criterion for good spreadability for gel preparations (Tandolambung, 2021), (Ermawati et al., 2022).

5. Adhesion Test

The adhesion testing was carried out by taking 0.5 grams of gel and placing it on a glass plate tied using a rope to hold the glass plate, then closed using another glass plate tied with a rope and connected to a pendulum weighing 80 grams. After that, 1 kg of the load was placed on the adhesive

device and left to rest for 5 minutes. Last, the glass plate was removed by releasing the pendulum attached to the glass plate (Putri, W. E., & Anindhita, M. A., 2022). The time for the two plates to come off must be recorded. At least 4 seconds is a requirement for good adhesion (Tandolambung, 2021).

6. Viscosity Test

Gel viscosity testing uses a Brookfield Hi-Vi viscometer by dipping the spindle into a 200 ml base at the appropriate speed [Anggun, B. D., & Pambudi, D. B., 2020]. The range of 3,000 – 50,000 cPs is the criterion for suitable gel viscosity according to SNI 16-4380-1996 (National Standardization Agency, 1996), (Pertiwi et al., 2016), (Ermawati et al., 2022).

Test of Bay Leaves Ethanol Extract Gel Preparation

The experimental animals used in this research were New Zealand male albino rabbits with healthy criteria, 8-9 months old and weighing 2-3 kg [BPOM, 2017]. Before use, rabbits must be acclimatized or adapted to the experimental environment for five days [Manapode, Y. Y., 2016]. The testing was divided into five treatment groups for each rabbit (Table 2).

Table 2. Treatment Group

Group	Treatments
Normal group	Healthy skin that is not subjected to hot metal induction
Negative control	Hot metal induces the skin to form burns, and the preparation is not applied.
Positive control	Hot metal induces the skin to form burns and is given bioplacentone gel.
F1	Hot metal induces the skin to form burns, and a gel preparation of 3% bay leaf ethanol extract is applied.
F2	Hot metal induces the skin to form burns, and a gel preparation of 6% bay leaf ethanol extract is applied.

The day before the burn induction treatment, the rabbit's fur is shaved first on the back with an area of around 10 x 15 cm. Before induction, it was anesthetized using subcutaneous injection of 2% Lidocaine HCl. The rabbit's back that has been shaved is cleaned with alcohol cotton, after which it can be directly induced using iron metal heated over a stove for 3 minutes and placed on the rabbit's back for about 10 seconds without pressing to get 2nd-degree burns (Hilaria et al., 2016), (Ihsan et al., 2022), (Hosseini et al., 2020), ((Bintarti & Widyaswari, 2022). The burn rabbit's skin was treated using 3% and 6% bay leaves ethanol extract gel and bioplacentone gel (Placenta extract 10% and Neomycin sulphate 0.5%) evenly and applied once a day. After applying the preparation, the wound is covered using gauze to avoid contamination (Elmitra, D. L., Herlina , & Rikomah, S. E., 2017). Before the re-application is carried out the next day, the diameter of the wound is measured first and cleaned with alcohol before being re-applied with the preparation.

The parameters observed are edema and burn wound diameter. Edema is observed daily for 15 days using the Draize Scoring System and the percentage of wound healing (Djerrou et al., 2013), (Sari, 2022).

Data Analysis

Quantitative data results were analyzed using SPSS 26.0 software. Less than 50 data points were subjected to statistical analysis using a normality test using Shapiro Wilk. In the normality test, if $p>0.05$, H_0 is accepted, and H_1 is rejected. It means the data is usually distributed. If $p<0.05$, H_0 is rejected, and H_1 is accepted; which means the data is not normally distributed (Riyadi et al., 2021). Homogeneity was tested using the Levene test. Suppose the data is regular and homogeneous. In that case, it can be continued with the One Way Anova test to see whether the differences between each test are significantly different and continued with the Post Hoc Tukey test to see significant differences in the tested formula (Sari, 2022). In the Anova test, the data are said to be significantly

different if $p<0.05$. If the data is not normal, then the data is tested using the Kruskal-Wallis test and continued with the Pairwise test.

RESULTS AND DISCUSSION

Phytochemical Yield and Screening

From 200 grams of simplicia, 37.28 grams of thick extract or a yield of 18.64% were obtained. The yield of the thick extract produced is required in the Indonesian Herbal Pharmacopoeia; the thick extract of bay leaves is at least 18.2% (Ministry of Health of the Republic of Indonesia, 2017).

Based on the results of the phytochemical screening, it was found that secondary metabolite compounds, namely flavonoids, tannins, saponins, and phenols, were found in the ethanol extract of bay leaves. The results of the flavonoid test showed a red colour in the amyl alcohol layer. The red colour indicates the presence of flavilium salts. Flavonoids with a benzopyrone core will be reduced by adding Mg powder. Then, the free flavonoids will be attracted due to the addition of amyl alcohol, resulting in a red colour, which indicates the presence of flavonoid compounds (Setyawaty et al., 2020), (Asmara et al., 2017). The tannin test using the gelatin method resulted in a positive, indicating the presence of a white precipitate. The white precipitate appears due to the formation of hydrogen bonds in the tannin hydroxy groups and protein carbonyl groups in gelatin (Asmara et al., 2017).

Furthermore, the results of the saponin test showed that the ethanol extract of bay leaves was positive for saponin with stable foam as high as 1.4 cm from the initial foam height of 3 cm. The foam formed during saponin testing is caused by glycosides, which become foamy in the water and hydrolyze into glucose when shaken (Agustina et al., 2017). The results of the phenol test on the ethanol extract of bay leaves showed a change in colour to pitch black. This black colour is formed due to the hydroxyl groups in phenol compounds reacting with FeCl3 to form a dark black colour (Putri et al., 2018). The difference in compound content in bay leaves ethanol extract with the literature is influenced by several factors; the age of the sample, the location where the plant grows, and the time of harvest (Supriatna et al., 2019).

Evaluation of Bay Leaf Ethanol Extract Gel Preparation

1. Organoleptic Test

The results of organoleptic testing on formula 1 (3% extract) and formula 2 (6% extract) showed that the gel preparation was greenish brown with peppermint aroma and was in gel form. In F2, the extract's distinctive aroma is more pronounced than in F1, even though it has been given a peppermint fragrance with the same concentration. The difference in aroma between formulas 1 and 2 is related to the concentration of bay leaves ethanol extract, where a higher extract concentration will affect the distinctive aroma of the extract being more pronounced.

2. Homogeneity Test

Mixing ingredients in a formulation can be determined by conducting a homogeneity test (Afianti & Murrukmihadi, 2015). Not finding coarse particles or grains during homogeneity testing is a condition for good homogeneity. Based on the homogeneity test results, formula 0 (0% extract), formula 1 (3% extract) and formula 2 (6% extract) showed homogeneous results. Therefore, all gel formulas meet the homogeneity requirements.

3. pH Test

The safety of gel preparations can be determined by carrying out pH testing. Topical preparations must follow the skin's pH requirements to be safe. 4.5-8.0 is the skin pH range required in SNI 16-4380-1996. The pH results obtained at F0, F1, and F2 were 5.8 ± 0.00, 5.46 ± 0.00, 5.34 ± 0.00. The pH values of F0, F1, and F2 gels fall into the skin pH range, namely 4.5 – 8.0. In order to avoid skin irritation or making the skin dry and scaly, the pH of the topical preparation must match the skin's pH range (Resti et al., 2018).

The Pearson correlation test results indicate a relationship between the extract concentration and the pH value because the significance is 0.00, which is less than 0.05. After that, the Pearson

correlation coefficient value was obtained - 0.965, which shows a robust and contradictory correlation (-) between the extract concentration in each formula and the pH value. So, based on these results, it is known that using a higher extract concentration will cause a decrease in the pH of a preparation (Wulansari, 2016).

4. Spreadability Test

The spreading ability of the gel applied to the skin can be determined by conducting a spreadability test (Afianti & Murrukmihadi, 2015). A 5-7 cm range is required for a good spreadability test (Tandolambung et al., 2021). The results of testing the spreadability of bay leaves ethanol extract gel preparations from F0, F1, and F2 were 5.53 ± 0.00, 5.25 ± 0.00, and 4.84 ± 0.00. These results show that the spreadability of gels F0, F1, and F2 is by the requirements, namely 5-7 cm, so all formulas can be said to have met the requirements for a good gel.

The Pearson correlation test results indicated a relationship between the extract concentration in each formula and the spreadability value because the significance was 0.00, which was less than 0.05. Pearson correlation coefficient was obtained - 0.979, showing a robust and contradictory correlation (-) between the extract concentration in each formula and the spreadability value. So, it can be seen that the decrease in spreadability test results is related to the use of higher extract concentrations (Wulansari, 2016).

5. Adhesion Test

The ability of the gel to stick well to the skin can be seen by testing the adhesive force with the condition that a good test is more than 4 seconds (Afianti & Murrukmihadi, 2015), good (Tandolambung et al., 2021). The results of testing the adhesion of the bay leaves ethanol extract gel preparation showed the average results sequentially from F0, F1, and F2, namely, 14.82 ± 0.01, 5.65 ± 0.15, and 10.36 ± 0.11. The adhesion of gels F0, F1, and F2 falls into the required range, not less than 4 seconds.

The Pearson correlation test results indicate no relationship between extract concentration and adhesive strength, known from the significance result of 0.328, which exceeds 0.05 (Wulansari, 2016).

6. Viscosity Test

Viscosity testing is important to determine the gel preparation's viscosity, which affects the gel preparation's texture and appearance and is related to spreadability and stickiness. The 3,000 – 50,000 cPs range is the viscosity range required by SNI 16-4380-1996 for gel preparations. The results of the average viscosity values respectively from F0, F1, and F2 are 5,001.66 ± 5.36 cPs, 12,658 ± 84.70 cPs, and 15,964.33 ± 58.35 cPs. The viscosity of gels F0, F1, and F2 falls into the range required by SNI, namely 3,000 – 50,000 cPs, so it can be stated that it meets the requirements for good gel viscosity.

The Pearson correlation test results indicate a relationship between the extract concentration and the viscosity value, known from a significance of less than 0.05, namely 0.00. Pearson correlation coefficient of 0.975 was obtained, indicating a powerful and continuous correlation (+) between the extract concentration in each formula and the viscosity value. Based on the results of correlation testing, it is known that using a higher extract concentration will affect the increase in the viscosity value (Wulansari, 2016).

Ethical Clearance

The animal code of ethics test is conducted at the Faculty of Veterinary Medicine, Gadjah Mada University (UGM), by filling out a formula and sending a research proposal online. The code of ethics test was carried out before the research took place. This research has received research ethics approval from Gadjah Mada University with number KE/FK/1150/EC/2023.

Effectiveness Test of Bay Leaves Ethanol Extract Gel Preparation

Induction of rabbit burns was carried out using an iron axle with a 2 cm diameter cut to a thickness of 1 cm. Before induction, the rabbit's back was cleaned with 70% ethanol. Iron metal is heated over a stove for approximately 3 minutes and then placed on the rabbit's back for approximately 10 seconds without pressing to obtain 2nd-degree burns (Hilaria et al., 2016), (Ihsan et al., 2022), (Hosseini et al., 2020), (Bintarti & Widyaswari, 2022). The induction results can be seen in Figure 1, and the results of measuring the wound diameter can be seen in Table 3.

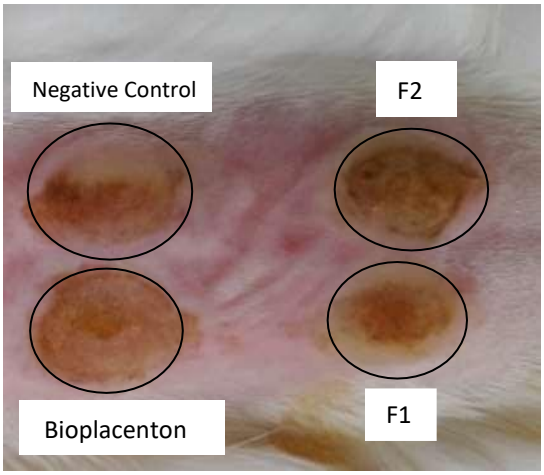

Figure 1. Burn Wound Test Results on Experimental Animals

Table 3. Results of Measurement of Burn Wound Diameter and % Healing on Experimental Animals

Treatment Group	Experimental Animals	Wound Diameter Size Day 0 (cm)	Average Wound Diameter on Day 15 (cm)	% of Healing
Negative Control	Rabbit 1	2,22		
	Rabbit 2	2,25	1,26 ± 0,04	42,48
	Rabbit 3	2,12		
Positive Control	Rabbit 1	2,00		
	Rabbit 2	2,05	0,92 ± 0,29	54,73
	Rabbit 3	2,07		
F1	Rabbit 1	2,02		
	Rabbit 2	2,07	1,14 ± 0,15	43,84
	Rabbit 3	2,00		
F2	Rabbit 1	2,02		
	Rabbit 2	2,02	0,68 ± 0,34	65,89
	Rabbit 3	2,00		

Information :
F1: Gel preparation containing 3% bay leaves ethanol extract
F2: Gel preparation containing 6% bay leaves ethanol extract

Table 4. Edema Observation Results

Edema			
Day	**Rabbit 1**	**Rabbit 2**	**Rabbit 3**
1	3	4	4
2	1	3	3
3	1	1	1
4	0	0	0

5	0	0	0
6	0	0	0
7	0	0	0
8	0	0	0
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0
13	0	0	0
14	0	0	0
15	0	0	0

After measuring the diameter, the wound is treated with preparations according to the specified location. Rabbits are smeared with the preparation once a day at 16.00 WIB (Indonesia Western Time). Wound diameter and edema were measured for 15 days and were carried out every day before the wound was treated. The results of measuring the diameter of the burn wound for 15 days and the percentage of wound healing can be seen in Table 3, showing that F2 had the minor diameter of the burn wound after 15 days and had the highest percentage of healing at 65.89%, followed by the positive control, namely bioplacentone (54.73%), F1 (43.84%) and negative control (42.48%).

Based on these results, it is known that F2 has more potential in treating burns than positive control (bioplacentone). In the F2 test group, there was more potential in healing wounds if paired with positive control groups due to using bay leaves ethanol extract by 6%. Compounds such as flavonoids, tannins, saponins, and phenols contained in bay leaves ethanol extract play a role in helping wound healing. Anti-inflammatory activity in flavonoids, working with the mechanism of inhibiting cyclooxygenase and lipoxygenase enzymes during inflammation, reduces leukotriene and prostaglandin production. Decreased prostaglandin, an inflammatory mediator, will limit inflammatory cells in the wound area so that the inflammatory process can run quickly (Riyadi et al., 2021), (Bintarti & Widyaswari, 2022). In addition, saponins can help wound healing by repairing damaged endothelial cells by increasing the proliferation of epidermal cells and increasing keratin migration so that the wound can be covered quickly (Anggraini et al., 2019).

Bay leaves ethanol extract gel can help heal burns due to flavonoids and saponins. Data from the percentage of burns healing shows that the use of extract concentrations in gel preparations will affect the effectiveness of gel preparations. Therefore, from this study, the increase in the concentration of extracts in bay leaves ethanol extract gel will increase the percentage of burns healing.

CONCLUSIONS AND SUGGESTIONS

The formulation of burn gel preparations with various concentrations of bay leaf ethanol extract showed good physicochemical properties such as organoleptic, homogeneity, pH, viscosity and adhesion by the requirements. However, only Formula 1 meets the requirements for the spreadability test results, while Formula 2 does not meet the requirements. The stability test results on formula 1 and formula 2 show that the statistical analysis results are unstable due to changes in temperature, light conditions and air humidity conditions in the storage area. The results of the effectiveness test of the ethanol extract of bay leaves burn gel using rabbit experimental animals showed no significant difference between the positive control (Bioplacentone), F1, and F2. However, the wound healing percentage results show that F2 has the highest healing percentage. Based on the

test results, it is known that using an extract with a higher concentration will increase the healing percentage.

ETHICAL CONSIDERATIONS

This research has received ethical permission from the Faculty of Veterinary Medicine, Gadjah Mada University (UGM), with number KE/FK/1150/EC/2023, obtained before conducting the research.

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