

FORMULATION AND EVALUATION OF WOUND HEALING GEL OF WHITE LEADTREE (*LEUCAENA LEUCOCEPHALA* (LAM.) DE WIT.) LEAVES EXTRACT

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ABSTRACT

Objective: The aim of this research is to formulate and evaluate the wound healing gel of *Leucaena leucocephala* leaves extract.

Methods: In this research, the extract of *Leucaena* leaves was formulated into wound healing gel by using a variation of the concentration of Carbopol as a gelling agent and Propylene glycol as a humectant. Afterward, the gel's physical properties (pH, viscosity, spreadability), stability, and sterility were tested. The wound healing activity was evaluated by making excision wounds on the Wistar Albino rat's back, and then the gels were applied to the wound every day. The wound's size was measured and counted as the percentage of wound closure.

Results: The result showed that Formula 4 (contains 1.5% of Carbopol and 12% of Propylene glycol) has the best physical characteristics and wound healing activity. Formula 4 showed 100% wound closure on the 11th day of the treatment, while the negative control only reached 49.12%. The statistical parameter with the p-value < 0.05 stated that they are significantly different.

Conclusion: This research demonstrated that gel with *Leucaena* leaf extract has good physical characteristics, and it can significantly improve the wound healing process.

Keywords: *Leucaena* leaves, Gel, Wound healing, Gelling agent, Humectant

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INTRODUCTION

Wound, an incident often experienced by humans in daily activities, is simply defined as the broken of skin tissue [1]. Many factors can cause the damage of skin tissue, such as physical friction or high temperature that disrupts the arrangement of epithelial or mucosa cells on the skin [2]. The wound healing process is an essential process with which the skin's function as the body protection means can be restored immediately. The wound healing process consists of several stages started from inflammation, a proliferation that includes epithelialization, angiogenesis, and skin remodeling [3]. During those complex processes, it is necessary to maintain the wound's sterility, protect the wound from contamination that can cause infection, prevent dehydration (moisture is needed for the new tissue arrangement), and also absorb or eliminate the wound exudate [4].

According to the Food and Drug Administration (FDA), preparations for wound healing, or also known as wound dressing combined with drugs, are divided into three categories: the wound dressing in solid forms, preparation of semisolid (ointments, creams, gels), and wound cleaning fluid [5]. The application of appropriate dosage forms and formulations in the wound therapeutic is required to accelerate the wound healing process. One of the suitable dosage forms for this purpose is a gel. A gel is a rigid structure formed by a cross-linking network of three dimensions that swells in an appropriate solvent; when the solvent is water, it is known as hydrogel [6]. The high water content in the gel formula is suitable for the environment needed for wound healing. It creates a moist environment and absorbs wound exudates to accelerate the wound healing process [5]. Besides, the gel can provide a cool sensation when applied to the injured skin so that it can reduce the pain experienced by the patient. Further, it is also easily applied and removed from the skin [7].

There are so many topical wound healing products on the market. Most of them contain active pharmaceutical ingredients that belong to antiseptics and antimicrobials categories, such as Neomycin, Mupirocin, Povidone Iodine, Hydrogen Peroxide, and Bacitracin [7]. The use of antimicrobials in the medical world lately starts to cause debates due to the significant risk of the bacteria resistance event [8]. It triggers many kinds of research that focus on searching for

new drug materials that can be used as wound healing agents. One section of the searches involves the use of natural wealth. Spread in nature, many plants have wound healing activity. One of the plants is the White Leadtree (*Leucaena leucocephala*), or also known as *Petai Cina* in Indonesia. *Leucaena* leaf is traditionally used to heal wounds by being chewed or chopped, then placed on the injured skin until it is healed [9]. *Leucaena* leaf has some pharmacology effects, such as an anti-inflammatory and antibacterial activity that relates to the wound healing process [10]. Several studies have proven the effectiveness of this plant in the wound healing process. The ethanol extract of *Leucaena* leaves has shown its ability to accelerate wound closure [11]. Research by Fitriani (2018) proves that the gel of *Leucaena* leaves extract at 15–45% concentration can heal the incision wound in rats. In other research, *Leucaena* leaves extract that is formulated in ointment preparation at 20% concentration is proven to accelerate wound healing in rats [12].

In the pharmaceutical formulation design, the compatibility between the active ingredients and other excipients in the dosage form should be considered. Ethanolic extract has good compatibility with water; so in this research, the ethanolic extract of *Leucaena* leaf is formulated as hydrogel. The urgency of this research is to find a natural ingredient that can be used as wound healing medicine and to formulate the ingredient into the dosage form that fulfills the pharmaceutical aspects, such as efficacy, safety, and quality. In this study, the formulations of wound healing gel preparation using *Leucaena* leaves extract with various concentrations of gelling agent (Carbopol 940) and humectant (Propylene glycol) were done. The evaluation was carried out on the physical properties of the gel, the stability of the gel, and the wound healing activity of each formula, in order to study the characteristics of the gel-based on the formulation consideration.

MATERIALS AND METHODS

Materials

Leucaena leucocephala leaves, Ethanol 96%, Propylene glycol (Pharmaceutical grade), Carbopol®940 (Pharmaceutical grade), Triethanolamine (Pharmaceutical grade), Methylparaben (Pharmaceutical grade), distilled water, 12 albino adult male Wistar rats (weight 200–300 g), and Bioplacenton®gel.

Preparation of *Leucaena* leaves extract

The *Leucaena* leaves were harvested from the Botanical Garden of the Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia. The determination of the plant from which the harvest was done had been conducted by the legal determinator of the Faculty of Pharmacy, Sanata Dharma University, with specimen number 003/IKTO/far-USD/II/13. The harvested leaves were washed under flowing water to remove all the dirt. Then, the leaves were dried naturally until they were thoroughly dried up and easily crushed when squeezed. The dried *Simplicia* was crushed using a blender and sieved with a mesh 40 sieve to obtain powders with a uniform size. A total of 25 grams of *Leucaena* leaves powder was macerated with 500 ml of a mixture of distilled water: ethanol 96% (1:1) for three days at room temperature. The extract was then filtered with the help of a vacuum pump, and the filtrate was macerated again with 500 ml 96% ethanol for 1 d, then filtered again. Both filtrate liquids were mixed and concentrated with

Vacuum Rotary Evaporator, and then the extract was stored in tightly-sealed containers at room temperature for the next step.

Formulation of gel

The gel was prepared using the formula written in table 1; Carbopol as a gelling agent, propylene glycol as a humectant, triethanolamine as a pH neutralizer, and methylparaben as a preservative are the commonly used excipients in pharmaceutical gel formulations, especially for topical usage. Carbopol 940 was swelled in the distilled water, and then left overnight until it was perfectly swollen. Propylene glycol and methylparaben were added to the mixture and then stirred using a mixer. Triethanolamine was added slowly until the pH of the gel base reached the neutral point (± 7.0), then it was stirred until it was forming a homogeneous base [13]. The gel base, *Leucaena* leaves extract, and distilled water were then sterilized using an autoclave at a temperature of 121 °C for 15 min. Under the LAF (Laminar Air Flow), *Leucaena* leaves extract and the rest of the distilled water were added into the gel base and stirred with a mixer for 1 min.

Table 1: Gel formula

Materials	F1 (%w/w)	F2 (%w/w)	F3 (%w/w)	F4 (%w/w)
<i>Leucaena</i> leaves extract	6	6	6	6
Carbopol 940	1	1.5	1	1.5
Propylene glycol	10	10	12	12
Methyl paraben	0.1	0.1	0.1	0.1
Triethanolamine (TEA)	Until the pH of the gel base is neutral			
Distilled water	Ad 100%	Ad 100%	Ad 100%	Ad 100%

Physical properties and physical stability test of gel

Organoleptic and pH

An organoleptic test was carried out by observing the odor and color of the gel 48 h after the preparation. The measurement of pH was done using universal pH indicators (pH stick) by putting the stick into the preparations and then comparing the color appearing in the indicator with standard color [14].

Spreadability test

The measurement of the gel's spreadability was carried out 48 h after preparation. For this test, 1 gram of the gel was placed in the center of a round scaled glass plate. On top of the gel, a round glass and a weight with the combined weight of 125 grams were placed. The condition was preserved for 1 minute, and then the diameter of the gel's spread was recorded [15].

Viscosity test

The viscosity test was done twice: 48 h after gel preparation and 1 mo after the gel was put in the storage. The viscosity of each formula was tested using Viscotester Rion series VT 04. The size of the rotor used was the number 2 [16].

Sterility test

The sterility test was carried out using the spread plate technique. A small amount of the gel was taken and placed in the Nutrient Agar (NA) medium, which was put on a petri dish and flattened with the help of an aseptic spreader rod. The petri dish was incubated for ± 24 h. After that, it was observed to indicate whether colonies of bacteria in the medium were present [17].

Wound healing activity test

This procedure has been granted the Ethical Clearance approval from the Medical and Health Research Ethics Committee (MHREC) Gadjah Mada University, Yogyakarta, Indonesia (Approval Number: KE/FK/378/EC). The wound healing activity test was done using a model of wound excision. Six albino adult male Wistar rats (weight 200–300 g) were divided randomly into six groups; one rat in each group. The back of each rat was injured and prepared after being cleaned. The skin area with a diameter of ± 1 cm on the back of each rat was cut using a sterile scalpel and scissors after the rats were

anesthetized. Group 1 was given Bioplacenton® gel (positive control), group 2 was not given any treatment (negative control), while groups 3, 4, 5, and 6 were given *Leucaena* leaves extract formula 1–4 respectively, once daily. The wounds that were treated with gel were dressed with sterile gauze and bandage. On each day after the treatment, the wound diameter was measured and the gel was reapplied until the wound was closed entirely. The repetitions were carried out three times by giving three wounds to each rat. Contraction of the wound was measured and presented in the daily percentage of wound reduction [18, 19].

$$\text{Percentage of wound closure} = \frac{\text{area of wound on 0 day} - \text{area of wound on the } N^{\text{th}} \text{ day}}{\text{area of wound on 0 day}} \times 100\% \quad [20].$$

RESULTS AND DISCUSSION

Formulation consideration

The selection of each material and excipient used in the formula should be carefully considered to ensure that all of them can produce viable efficacy of the drug. In this research, ethanol and distilled water are used as the diluents in the extraction process. The election is taken with the consideration of the solubility of the active constituents having wound healing activity existed in *Leucaena* leaf (tannin, saponin, and flavonoid) [21]. Tannin and flavonoid of the *Leucaena* leaf were found in the non-polar extract solvent (ethanol), as the saponin was found in the polar solvent (water) [22].

Wound healing is a complex process, and along that process, it is important to keep the wound area sterile, free from contamination and bacteria that can cause infection, and hydrated [4]. The recent development in wound management found that a moist environment would accelerate the wound healing process, especially in the reepithelization process [23]. Carbopol, when dispersed into water and alkalized, will form a rigid three-dimensional network that has the ability to absorb water and to retain the water content absorbed in its network [24]. Propylene glycol is a humectant that has a vital role in topical preparations by maintaining skin moisture and increasing water absorption from the epidermis to the deeper parts of the skin (the dermis) [25]. As water considerably facilitate microbial growth, in order to prevent this happening in the formulation of the hydrogel that contains more than 80% of water, methylparaben as a preservative is added in the formula. Thus, the wound will not be contaminated or infected, and the infection will not inhibit the wound healing process.

Physical properties and physical stability test of gel

Organoleptic and pH

The physical appearance (organoleptic) of a wound-healing preparation is an important factor that needs to be considered. Specifically, the organoleptic is closely related to the acceptance of the preparation of the patient, which is associated with the patients' comfort in taking the preparations. The organoleptic test for the four formulas of wound healing gel generated green colored gel that exudes characteristic herb odor, which was acceptable. The preparation's pH is one of the physical properties that affect its efficacy because it concerns the drug release process at the site of action.

Human skin is known to have a normal pH between 4.0 and 6.0 [26]. When an acute wound occurs, the skin's pH changes to a more alkaline level of about 7.4. Following that, naturally, the body will respond by returning the acidity of the skin to restore the wound. During this, the environment surrounding the wounds will turn acidic. This acidic environment will inhibit the growth of bacteria, so infection does not occur. This is one of the activities in wound healing [27]. In order for a wound-healing preparation to assist this process, its pH must not be more than 6.0. Carbopol as the gelling agent, naturally has acidic pH, so it needs to be neutralized to reach the targeted pH of formulation [28]. Triethanolamine (TEA) as the pH adjuster plays the important role to alkalize the gel's pH. When the TEA is added into the formulation, its pH increases. The pH test on all four formulas of *Leucaena* leaves extract gel generated the results of 6.0. These results indicate that the organoleptic and the pH of the preparation meet the standard requirements.

Spreadability and viscosity test

The other important aspects affecting the quality of topical preparations are spreadability and viscosity. These two factors affect the retention time of the preparation and its ease of application over the skin surface, which will finally have an impact on the effectiveness of the therapy [29]. Smaller spreadability means higher viscosity, which will make the preparation more difficult to apply on the skin. Too high spreadability, which means too low viscosity, is also undesirable because it will shorten the retention time on the skin surface. Thus, it is necessary to have the ideal dispersibility and viscosity values. The results of the spreadability and viscosity tests for the four wound healing gel formulas from *Leucaena* leaf extract, after 48 h of storage, are presented in table 2. The spreadability ranges from 3.783–4.650 cm and the viscosity from 2433.333–3000 cps. The test results show that the gel has good dispersibility and viscosity properties and is in accordance with the initial target, i.e., the spreadability of 3–5 cm and a viscosity of 2000–3000 cps.

As shown in table 2, Formula 1 and 3 have greater spreadability than other formulas. It's because Formula 1 and 3 contain less gelling agent (1.0% of Carbopol). It is known that the lower concentration of Carbopol will increase the gel capability to spread [28]. On the other side, the viscosity of Formula 1 and 3 are lower than Formula 2 and 4. So, it can be said that when the concentration of the gelling agent is getting lower, its viscosity is decreasing, and its spreadability is increasing.

A viscosity shift test was performed to evaluate the gel's physical stability in a storage environment. The viscosity of the gel that had been stored for 1 mo was compared with the viscosity of the gel after 48 h of storage. The smaller the percentage shift in viscosity, the better the physical stability of the gel is.

Table 2: Test result of spreadability and viscosity

Formula	Spreadability*(cm)	Viscosity after 48 h of storage (cps)*
1	4.650±0.265	2433.333±57.735
2	3.983±0.416	3000.000±0.000
3	4.100±0.350	2466.667±57.735
4	3.783±0.029	2750.000±0.000

*All values are mentioned in mean±SD (n=3)

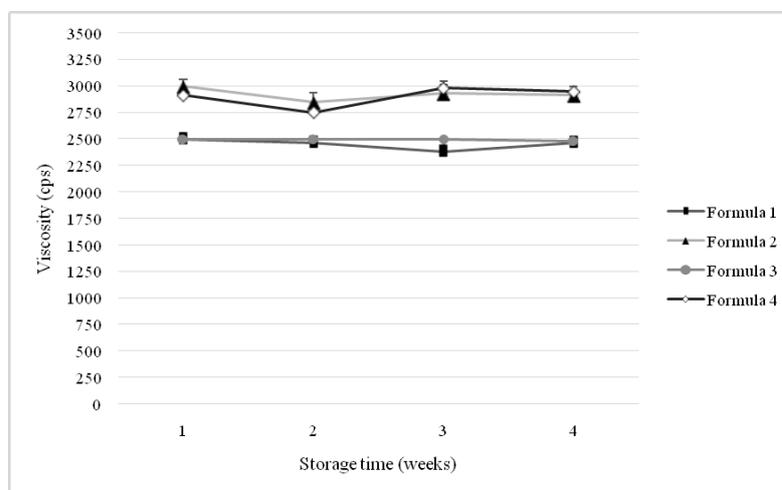


Fig. 1: Graphic of viscosity shift (all of the results are calculated as mean±SD; n=3)

Fig. 1 presents the general physical stability of the tested gel. Formula 2 and 4 have the same graphic profile; their viscosities tend to be higher than Formula 1 and 3. This is because Formula 2 and 4 contain higher Carbopol as their gelling agent (1.5%) than Formula 1 and 3 (1.0%). Therefore, according to the characteristics of the gelling agent: the greater the gelling agent concentration, the more

viscous the preparation will be. Likewise, the viscosity profile of Formula 1 and 3 contains less gelling agent than the other two formulas, so that the viscosity is lower. In addition, from the graph, it can be seen that after the 3rd week, all formulas have a relatively stable viscosity. From table 2, it is known that the four gel formulas have good physical stability, with the value of the viscosity shift of

less than 10%. The smallest shift in viscosity is shown by Formula 3, and the largest shift in viscosity is shown by Formula 4.

Sterility test

Topical preparations used for wound healing must meet certain sterility requirements [30]. It is related to product safety toward the

exposed skin tissue because the wound is susceptible to infection by bacteria and other microorganisms. Therefore, it is necessary to ensure the product sterility in order not to harm the patients further. From table 3, it can be seen that, in general, the preparations meet the sterility requirements, even though non-sterile gels also exist, i.e., Formula 1 replication 3 and Formula 4 replication 3.

Table 3: Test result of gel's sterility

Replication	Media control	F1	F2	F3	F4
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	+	-	+

(-): the absence of bacteria growth, (+): the presence of bacteria growth

Wound healing activity

This test was conducted to determine the *Leucaena* leaf extract gel's ability to heal wounds in male Wistar rats. The test was carried out by applying the *Leucaena* leaf extract gel on the backs of the rats that had been injured. The rats were divided into six treatment groups, one rat each for Formula 1-4, one treatment group for the negative

control (rats were injured without treatment), and one treatment group for the positive control (Bioplacenton® gel). The procedure was carried out in three repetitions that is by giving three wounds to each rat. Every day, the wound diameter was measured and given the gel until the wound was completely closed. Wound contraction was measured in the percentage reduction in wound area for each day.

Table 4: Wound closure percentage

Day	Wound closure (%)					
	Positive control	Negative control	F.1	F.2	F.3	F.4
0	0±0.000	0±0.000	0±0.000	0±0.000	0±0.000	0±0.000
1	2.011±1.040	8.303±1.748	4.125±1.274	2.887±3.046	1.516±1.219	1.870±3.221
3	20.341±9.178	16.98±8.703	28.785±3.006	22.404±5.248	17.378±11.780	12.217±1.927
5	29.766±6.032	24.677±1.353	34.309±4.502	32.818±1.588	31.639±8.867	27.768±7.959
7	42.816±3.484	38.208±7.040	48.638±1.518	42.887±6.991	41.731±9.394	40.494±12.632
9	67.955±4.683	44.068±5.138	68.141±1.901	63.030±4.266	58.476±6.655	60.878±7.435
11	83.295±9.717	49.124±5.104	95.611±7.602	82.206±7.650	88.536±19.857	100.000±0.000

*All values are mentioned in mean±SD (n=3)

Table 4 shows that the negative control gave the lowest percentage of wound closure; it only reaches 49.124% on the 11th day. Meanwhile, the biggest percentage of wound closure is shown by Formula 4, reaching 100% on the 11th day. In addition, Formula 1, 3, and 4 even had a better percentage of wound closure than the positive control.

A statistical test was conducted to determine whether there was a significant difference in the percent value of wound closure between treatments. The normality test was carried out with the Shapiro Wilk test. It was known that abnormal data (*p<0.05) existed, namely Formula 1, 3, and 4. Therefore, a non-parametric test, which the Kruskal Wallis test, was also conducted. This test aims to see the significant difference between the negative controls and the formula. The result obtained is *p-value<0.05, so it can be said that there are at least two groups that have significant mean differences. The test was continued with the two samples of the Wilcoxon test, and the results of the p-value were presented in table 5.

Table 5: The result of wilcoxon test of the gels' wound healing activity

Treatments	p-value
Formula 1: (-) control	0.0463
Formula 2: (-) control	0.04953
Formula 3: (-) control	0.1212
Formula 4: (-) control	0.0369
Formula 1: (+) control	0.2683
Formula 2: (+) control	0.8273
Formula 3: (+) control	0.5066
Formula 4: (+) control	0.0369

The Wilcoxon test data show that Formula 1, 2, and 4 have wound healing activity when compared to negative controls because they

produce a *p-value<0.05, while Formula 3 does not have wound healing activity. The comparison between the wound closure by each formula and the positive control shows that Formula 4 has a better percentage of wound closure because it had a *p-value<0.05 (0.0369), while the other three formulas have the same percentage of wound closure as the positive control (*p-value>0.05). This is because Formula 4 contains the highest amount of Carbopol as the gelling agent and Propylene glycol as the humectant. The development in wound management found that a moist environment would accelerate the wound healing process, especially in the reepithelization process [23]. Carbopol, when dispersed into water and its pH is neutralized, will form a rigid three-dimensional network that has the ability to absorb water, even to retain the water content that is absorbed in that network [24]. Propylene glycol is a humectant that has a vital role in topical preparations by maintaining skin moisture and increasing water absorption from the epidermis to the deeper parts of the skin (the dermis) [25]. Thus, the high concentration of Carbopol and Propylene glycol in Formula 4 can support the creation of a moist environment in the injured skin. This condition makes the fastest healing process compared to the other formulas. In addition, the high viscosity of Formula 4 is also considered to play a role in this. With its high viscosity, the retention time of the preparation on the skin surface is longer so that the absorption process of the drug into the skin tissue becomes more optimal.

A study that evaluated the phytochemical contents of *Leucaena* leaves proved that *Leucaena* leaves contain tannins, saponins, and flavonoids [22]. Tannins, saponins, and flavonoids are the ingredients of *Leucaena* leaves, which are believed to have wound healing activities that especially play a role in the process of forming new blood vessels [21]. Tannins are reported to have anti-inflammatory and antibacterial activities that highly contribute to the wound healing process [31]. Flavonoid is one of the most common secondary metabolites found in *Leucaena* leaves [32]. Flavonoids have been shown to have activity in accelerating wound

healing [33]. Saponins are reported to support the proliferation and formation of new blood vessels in the wound healing process [34]. Formula 4 is proven to have the best wound healing activity, because Formula 4 also contains the most Carbopol as a gelling agent and Propylene glycol as a humectant; compared to the other formulas, they can improve the humidity of the formula and maintain it. *Leucaena* leaf extract gel can heal wounds, possibly because of its antibacterial properties. Besides, it can also reduce pain, stimulate the formation of new blood vessels, and accelerate reepithelialization because it can provide a moist environment around the injured skin.

The formulation consideration plays an important role in wound healing preparation. The efficacy of the *Leucaena* leaf extract as a wound-healing agent is tightly associated with the selection of every material and excipient used in the formulation. Carbopol as the gelling agent, propylene glycol as the humectant, TEA as the alkalizing agent, methylparaben as the preservative, and distilled water as the vehicle can produce a gel with good characteristic that fulfills the pharmaceutical preparation specifications.

CONCLUSION

The formulation of *Leucaena* leaf extract in a gel preparation for wound healing has been proven to produce preparations that meet various quality requirements of pharmaceutical products, including pH, viscosity, sterility, and wound healing activity. The gelling agent (Carbopol) and humectant (propylene glycol) used in this work have a significant role in the formulation; they can improve the humidity of the formula and maintain it. The moist environment can improve the wound healing process. Formula 4, with the greatest amount of gelling agent and humectant, has the best wound healing activity, even better than the positive control, while its physical and stability test results meet the requirements. Therefore, it can be concluded that Formula 4 is the best formula.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally to this research article.

CONFLICT OF INTERESTS

The authors declared that no conflict of interest should arise concerning the authorship of this research article.

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