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The effectiveness test of film forming hydrogel (FFH) of Yellow Root Extract (*Arcangelisia flava* (L.) Merr) on healing burns of male white mice (*Mus Musculus*)

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ABSTRACT: The Burns are conditions where skin tissue is lost due to direct contact with heat energy. Therefore, a preparation was developed as film forming hydrogel (FFH) from yellow root extract. Yellow roots contain alkaloids, flavonoids, and saponins, which can improve wound healing. This study aims to determine the effect of the concentration of yellow root extract on wound diameter percentage and increasing the number of fibroblast cells and epithelial thickness. This research uses a true experimental laboratory with a design pre-post test study method on mice aged 2-3 months weighing 20-35 g. Samples were taken using a random sampling technique and divided into five treatment groups, namely formula 1 (F1) with 0.5% yellow root extract, formula 2 (F2) with 1% yellow root extract (F2), formula 3 (F3) with 1.5% yellow root extract, bioplacenton® (positive control), normal saline 0.9% (negative control). Treatment of mice for 14 days. The observation parameters were measuring the diameter of the burn wound using the Morton method and the percentage of burn wound healing. The number of fibroblast cells and epithelial thickness were observed at 400x magnification by taking five fields of view in a zig-zag manner. This research shows that the film forming hydrogel (FFH) preparations from yellow root extract with a 1.5% yellow root extract (F3) are the most effective for healing burn wounds. This can be seen from the diameter of the wound, the percentage of wound healing, the increase in fibroblasts, and the thickness of the epithelium.

KEYWORDS: Film Forming Hydrogel (FFH); *Arcangelisia flava* (L.) Merr; healing burns; fibroblasts; epithelial thickness

1. INTRODUCTION

Indonesia is a country that is rich in biodiversity, including flora and fauna; extraordinary biodiversity is spread throughout many regions in Indonesia. Indonesia's biodiversity makes it a country with an abundance of medicinal plants. After Brazil, Indonesia is ranked second in the world with the highest biodiversity [1]. Medicinal plants refer to plants in whole or in part that contain active substances and have the potential to be an alternative medicine that is beneficial for health. Plant parts, namely fruit, roots, flowers, leaves, bark on stems, rhizomes, or sap, can be used for various purposes [2]. One traditional medicinal plant not studied in depth is yellow root. This plant can be found in abundance in the forests of Kalimantan and represents Indonesia's natural wealth. The people of Kalimantan have long used yellow root for generations as a treatment option for various conditions, such as itchy skin, wounds, and ulcers [3]. Yellow root plants are also often used to treat ailments like hepatitis, fever, infections, digestive disorders, worms, and stomach ulcers. Yellow root has several pharmacological effects, including antimalarial, antibacterial, antioxidant, antifungal, antidiabetic, and antidepressant, and can even be used as an anticancer agent [4]. According to the results of phytochemical screening, yellow roots contain alkaloids, flavonoids, tannins, and saponins [5].

Yellow root contains various chemicals, such as hydroxy arcangelisia compounds, a type of flavonoid with great antibacterial activity [4]. Yellow root plant flavonoids also increase fibroblast secretion; increased fibroblast secretion will also increase collagen production in the tissue. Therefore, these two compounds can accelerate the healing process of burn wounds [6]. Yellow roots also contain protoberberine alkaloids, including berberine, palmatin, and jatorrhizine. These compounds have the potential as antimicrobial agents capable of fighting various types of microorganisms. One of the marker compounds in the yellow root is berberine. Berberine functions as an antibacterial agent by stopping peptidoglycan synthesis in cells. This disrupts the formation of the cell wall layers, which ultimately causes cell death [5]. The alkaloid content

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found in yellow roots can increase the number of fibroblasts and collagen density to speed up the healing of burn wounds [7].

Burns occur when the body's surface is exposed to heat or other objects that produce heat through direct heat sources, heat conductor materials, scorching sunlight, electricity, chemicals, and water. This results in damage and tissue loss, with the skin burning [8]. In many cases, burns are a problem that causes relatively high levels of pain and death. The effects of high levels of sunlight or burns are included in the NCDs (Non-Communicable Disease) category. NCDs are a group of non-communicable diseases that are the highest cause of death in the world; the death rate due to NCDs is higher compared to the total number of deaths due to other causes. Globally, the estimated data on deaths caused by burns is 265,000 people every year, with the most victims in countries with low or middle economic levels. Most burns are caused by high temperatures or heat, accounting for 95% of incidents. The types of heat injuries are grouped into three categories, including blisters (50%), injuries due to direct contact with fire (24%), and burns (26%).

The preparations commonly used to treat burns are ointments and gels. The speed at which the active substance can be absorbed can be seen from the formulation of each preparation. The active ingredients contained in the ointment are part of the base or conductor that delivers the drug to the surface of the skin. The selection of appropriate delivery materials for topical formulations can provide beneficial effects. Gel is widely used because it has moisturizing benefits, provides a cooling sensation, is easy to use, has a high water content, and is easily absorbed into the skin [7]. However, conventional preparations also have disadvantages, such as feeling sticky and uncomfortable when applied to the skin, causing skin irritation, and having a short contact time [9].

The preparations were developed to overcome the shortcomings of the above preparations, namely film forming hydrogel (FFH). FFH is a type of non-solid preparation that can form a film on the skin or other body surfaces directly when applied to the body surface or in situ. Forming Hydrogel (FFH) preparations makes the permease process easier or allows for transferring drugs to the skin, gives longer contact time, and provides comfort for users. This thin film penetrates the skin more easily than patches, unlike conventional drugs such as gels and ointments [9]. The formula for making FFH consists of various active substances, polymers, and excipient substances, such as plasticizers, penetration enhancers, and volatile solvents. Once applied, this liquid can form a film on the skin. The FFH dosage form has been considered an attractive transdermal delivery method due to its potential advantages over conventional products [10]. FFH preparations have the advantage of being easy to remove drug residue left on the skin, and compared to conventional gel preparations, FFH preparations make it easier for drugs to penetrate the skin because of the thin film formed after being applied to the skin. Therefore, choosing FFH depends on the formulation that produces good results. Even though there are many conventional medicines for treating burns, such as gels and ointments, these preparations have shortcomings because when applied to the skin, they feel sticky and uncomfortable, can cause skin irritation, and the contact time is relatively short [8].

This study aims to determine the effect of the concentration of yellow root extract on wound diameter to determine the best percentage of yellow root extract in FFH preparations on healing speed. Burn wounds are used to determine the optimum extract concentration that increases the number of fibroblast cells and thickness of epithelial tissue and to determine the effectiveness of the FFH preparations of yellow root extract in healing burn wounds.

2. RESULT

2.1 Diameter for Burn Wounds Healing on the 3, 7, and 14 Days

On days 3, 7, and 14, the diameter of the burn wound on each mice was calculated using a screw micrometer (mm). The results of observations of burn wound diameter on days 3, 7, and 14 and the average diameter of burn wound healing (mm) can be seen in Table 1. The table above shows a decrease in the average healing diameter of burn wounds from the 3, 7, and 14 days in the treatment given bioplacenton®, FFH (F1), (F2), and (F3). The average healing diameter of the positive control treatment using a bioplacenton® on the three days was 9.14 mm; on the seven days, it was 8.12 mm, and on the 14 days, it was 5.92 mm. The average healing diameter of treatment using F1 preparations on the third day was 9.56 mm; on the seventh day, it was 7.97 mm. Moreover, on the 14 day it was 5.80 mm. The average healing diameter of treatment using F2 preparations on the third day was 9.06 mm; on the seventh day, it was 7.89 mm. On the 14th day, it was 5.78 mm. The average healing diameter of treatment using FFH F3 preparations on the third day was 8.99 mm; on the seventh day, it was 7.78 mm. Furthermore, on the 14 day it was 5.22 mm.

Table 1. Average diameter of burn wound healing (mm)

Days to-	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
3	9.75 ± 0.14	9.14 ± 0.10	9.56 ± 0.05	9.06 ± 0.21	8.99 ± 0.11
7	8.65 ± 0.16	8.12 ± 0.12	7.97 ± 0.05	7.89 ± 0.11	7.78 ± 0.06
14	6.89 ± 0.26	5.92 ± 0.11	5.80 ± 0.14	5.78 ± 0.17	5.22 ± 0.13

*Data are expressed as mean±SD, n=5

Based on the data analysis, treating second-degree burns using FFH preparations (F1, F2, F3) can improve burn wound healing. This is seen from the decrease in the average wound diameter compared to the group that was not given any treatment for burns. This can be seen in the negative control, which had a higher average healing diameter than all other treatment groups. The initial step used in the analysis is a normality test for each treatment and control group on the 3, 7, and 14 days. The results showed that all data on day three was normally distributed but not homogeneously distributed, data on day seven was not normally distributed but was homogeneously distributed, and on day 14, the data was not normally distributed, but data was homogeneously distributed. Therefore, observation data on days 3, 7, and 14 can be analyzed further using the non-parametric Kruskal Wallis test, and further tests can be carried out. The results of follow-up tests are used to determine the probability of each group so that the differences between one group and another can be seen on the 3-day (Table 2), 7-day (Table 3), and 14-day (Table 4).

The positive control in this study used bioplacenton® gel containing 10% placenta extract, triggering the formation of new tissue and accelerating wound healing [11]. The negative control used in this research was 0.9% NaCl. Normal saline was used as a negative control in this study because it is an isotonic fluid, so in the burn wound healing process, it only affects superficial burn healing [12].

Table 2. Results of statistical analysis of follow-up tests measuring burn wound diameter on day 3

Group	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Negative control		0.006*	0.344	0.002*	0,000*
Positive control	0.006*		0.071	0.763	0.355
Formula 1 (F1)	0.344	0.071		0.035*	0.006*
Formula 2 (F2)	0.002*	0.763	0.035*		0.532
Formula 3 (F3)	0,000*	0.355	0.006*	0.532	

* Significantly different

Data were analyzed to measure the diameter of healing burn wounds on the third day using the Kruskal-Wallis non-parametric test using a significance value of $p < 0.05$. Testing in the treatment group resulted in $p < 0.001$, meaning the data differed significantly. Next, the test was carried out and continued to determine any significant differences in each group. Further test results can be seen in the following Table 2.

Based on the results of the follow-up test on day 3, it showed that there were no significant differences, namely, the negative control towards F1 with a p-value > 0.344, the positive control towards F1 with a p-value> 0.071, the positive control towards F2 with a p-value > 0.763, the control positive towards formula 3 with a p-value > 0.355. Further tests showed a significant difference in the negative control group against the positive control group, the F2 group, and the formula 3 group. There was a significant difference in F1 against F2 and formula 3, and there was a significant difference in formula 3 against F1.

The data for measuring the diameter of healing burn wounds on the seventh day were analyzed using the non-parametric Kruskal-Wallis test using a significance value of $p < 0.05$. Testing in the treatment group resulted in $p < 0.000$, meaning the data differed significantly. Next, the test was carried out and continued to determine any significant differences in each group. Further test results can be seen in the following Table 3.

Table 3. Results of statistical analysis of follow-up tests measuring burn wound diameter on day 7

Group	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Negative control		0.204	0.024*	0.003*	0,000*
Positive control	0.204		0.322	0.089	0.003*
Formula 1 (F1)	0.024*	0.322		0.478	0.045*
Formula 2 (F2)	0.003*	0.089	0.478		0.197

Formula 3 (F3)	0,000*	0.003*	0.045*	0.197	

* Significantly different

Based on the results of the follow-up test on day 7, it showed that there were no significant differences, namely, the negative control group against the positive control with a p-value > 0.204, the positive control against F1 with a p-value > 0.322, the positive control against F2 with a p-value > 0.089, the formula one against F2 with p-value > 0.478, F2 group against formula 3 with p-value > 0.197. The results of further tests showed that there was a significant difference between the negative control group and the F1, formula two, and formula three groups, there was a significant difference between the positive control group and formula 3, and there was a significant difference in the F1 against formula 3.

Table 4. Results of statistical analysis of follow-up tests measuring burn wound diameter on day 14

Group	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Negative control		0.102	0.024*	0.010*	0,000*
Positive control	0.102*		0.533	0.355	0.008*
Formula 1 (F1)	0.024*	0.533		0.763	0.041*
Formula 2 (F2)	0.010*	0.355	0.763		0.081
Formula 3 (F3)	0,000*	0.008*	0.041*	0.081	

* Significantly different

Data were analyzed to measure the diameter of healing burn wounds on the 14th day using the Kruskal-Wallis non-parametric test using a significance value of $p < 0.05$. Testing in the treatment group resulted in $p < 0.001$, meaning the data differed significantly. Next, the test was carried out and continued to determine any significant differences in each group. Further test results can be seen in the following Table 4.

Based on the results of the follow-up test on the 14th day, it showed that there were no significant differences, namely, the negative control group against the positive control with a p-value > 0.102, the positive control against F1 with a p-value > 0.533, the positive control against F2 with a p-value> 0.355, the formula one against F2 with a p-value > 0.763, F2 group against formula 3 with a p-value > 0.081. The results of further tests showed a significant difference between the negative control group and the F1, F2, and F3 groups, a significant difference between the positive control group and formula 3, and a significant difference in the F1 group against formula 3.

Data analysis shows that preparing FFH yellow root extract influences the healing process of burn wounds in mice. This can be shown in the results of data analysis according to the parameters in the burn wound healing process, including measuring the diameter of the burn wound. Observations of the healing process of burn wounds were carried out for 14 consecutive days using macroscopic observations. Healing of burn wounds on the back skin of mice on the 14th day showed improvement, as indicated by the wound closing. At first, the burn wound is reddish and slightly swollen, and this is due to the initial process of the inflammatory phase. Then, the burn wound is covered by clotted blood and forms a crust. Crust formation is the initial proliferation phase in the burn wound healing process. The crust will fall off by itself, and the diameter of the wound will become smaller. The substances also influence the speed of the wound healing process when the medication is given. Wounds will heal if the medication can increase healing by stimulating faster growth of new cells in the skin [13].

2.2 Percentage of Healing of Burn Wounds on 14-Day

For 14 days, the wound diameter in mice was measured using a screw micrometer using the Morton method. The wound diameter data was then searched for the average of each measurement, and the percentage of healing on the 14th day was calculated. The results of observations of the average percentage of wound healing can be seen in Table 5.

Table 5. The average percentage of burn wound healing in the 14 days (%)

Group	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Mice 1	63	71	71	75	78
Mice 2	63	72	71	70	77
Mice 3	59	69	74	72	77
Mice 4	57	72	72	72	79

Mice 5	61	71	73	72	77
Mean ± SD	61 ± 0.03	71 ± 0.01	72 ± 0.01	72 ± 0.02	78 ± 0.01

*Data are expressed as mean±SD, n=5

The percentage of burn wound healing showed that the highest healing data was obtained from treatment using the FFH formula three preparations, where the healing percentage was 78%, followed by the F2 group with a healing percentage of 72 % and F1 with a percentage of 72%. This indicates that treatment using FFH preparations with the addition of yellow root extract is better in accelerating the healing of burn wounds compared to the positive control, with a percentage of 71%, and the negative control, with a percentage of 61%.

Statistical analysis of burn wound healing percentage data was analyzed using normality and homogeneity tests. Data on the percentage of burn wound healing has been tested for normality, and it is known that the results of the percentage of burn wounds are typically and homogeneously distributed. Data analysis then continued using the parametric one-way ANOVA test and post hoc test (LSD). The results of the LSD test are used to determine the probability of each group so that the difference between one group and another group can be seen on the 14th day. The results of the LSD test for the percentage of healing of burn wounds on the 14th day can be seen in the following Table 6.

Table 6. Results of statistical analysis of the LSD test for the percentage of healing of burn wounds on the 14-day

Group	Negative control	Positive control	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)
Negative control		0,000*	0,000*	0,000*	0,000*
Positive control	0,000*		0.331	0.242	0,000*
Formula 1 (F1)	0,000*	0.331		0.836	0,000*
Formula 2 (F2)	0,000*	0.242	0.836		0,000*
Formula 3 (F3)	0,000*	0,000*	0,000*	0,000*	

*Significantly different

Based on the LSD test results in the table shows that there is no significant difference in the positive control group to F1 with a p-value > 0.331, the positive control group to F2 with a p-value > 0.242, the F1 group to F2 with a p-value > 0.836, The LSD test results show that there is a significant difference in the negative control group compared to the positive control group, F1, F2, F3. The F3 group also significantly differs from the negative control group, positive control, F1, and F2. Based on these data, it can be concluded that the difference in the concentration of yellow roots in the FFH preparation significantly differs in the percentage of burn wound healing. Based on the percentage of healing of grade IIA burns in male white mice, all treatments in Table 5 show varying results. The treatment group showed a higher percentage of burn wound healing than the positive control. Based on these data, the difference in the concentration of yellow roots in the FFH preparation significantly affects the percentage of burn wound healing.

2.3 Calculation of the Number of Fibroblasts

Images of preparations with hematoxylin-eosin staining were observed with a microscope at 400x magnification. Figure 1 shows the fibroblast cells resulting from the treatment, and Table 6 calculates the number of fibroblast cells.

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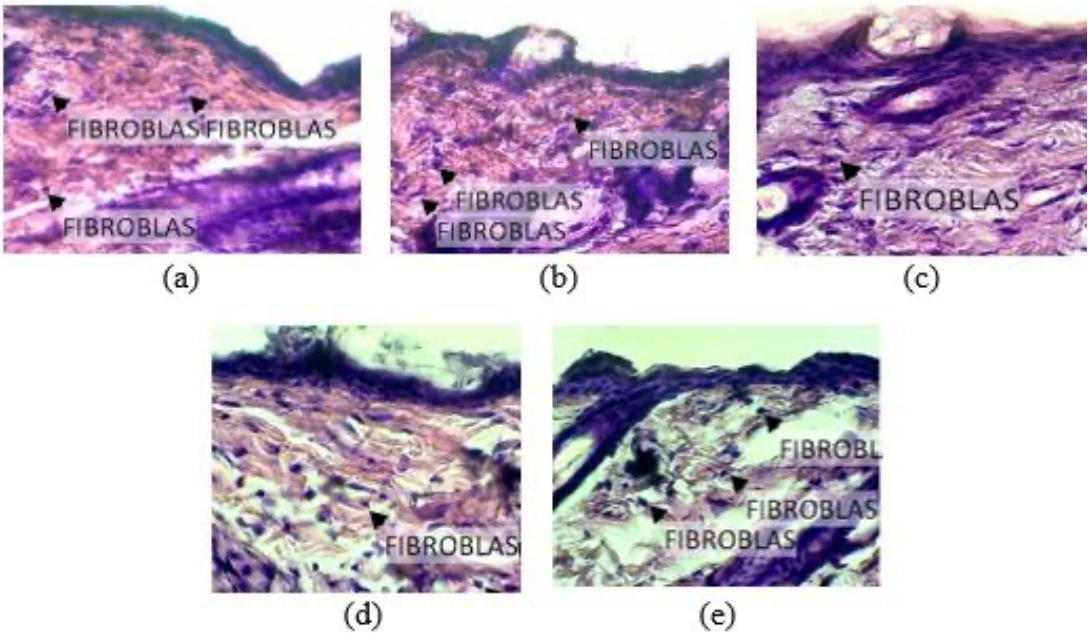

Figure 1. Histological image of several fibroblasts: (a) Formula 1 (F1); (b) Formula 2 (F2); (c) Formula 3 (F3); (d) positive control; (e) negative control

Table 7. Average number of fibroblasts in burn wounds

Treatment Group	Mean number of fibroblasts ± SD
Formula 1 (F1)	25.9 ± 7.37
Formula 2 (F2)	27.9 ± 4.51
Formula 3 (F3)	29.9 ± 3.78
Positive control	33.5 ± 0.70
Negative control	25.3 ± 3.52

*Data are expressed as mean±SD, n=5

Statistical data analysis used the parametric one-way ANOVA test and the LSD test. The data obtained was normally distributed and homogeneous. Next, an LSD test is carried out; the following are the LSD test results in Table 8.

Table 8. Results of statistical analysis of the LSD test for the number of fibroblasts

Group	Formula 1 (F1)	Formula 2 (F2)	Formula 3 (F3)	Positive control	Negative control
Formula 1 (F1)		0.611	0.311	0.066	0.860
Formula 2 (F2)	0.611		0.599	0.155	0.496
Formula 3 (F3)	0.311	0.599		0.343	0.240
Positive control	0.066	0.155	0.343		0.049*
Negative control	0.860	0.496	0.240	0.049*	

* Significantly different

Based on the table above, there is a significant difference between the positive and negative control groups. This is indicated by a significance value of less than 0.05, namely 0.049. Significant values from the opposing group and positive group indicate the existence of a pharmacological effect on bioplacenton® treatment. An insignificant significance value was obtained in the LSD test results of the treatment groups (F1, F2, F3) with the favorable treatment group. Apart from that, all LSD test results between treatment groups had a significance value greater than 0.05, so there were no significant differences between treatment groups F1, F2, and F3.

2.4 Epithelial Thickness Measurement

Figure 2 shows the results of epithelial thickness images, and Table 8 shows the results of epithelial thickness measurements.

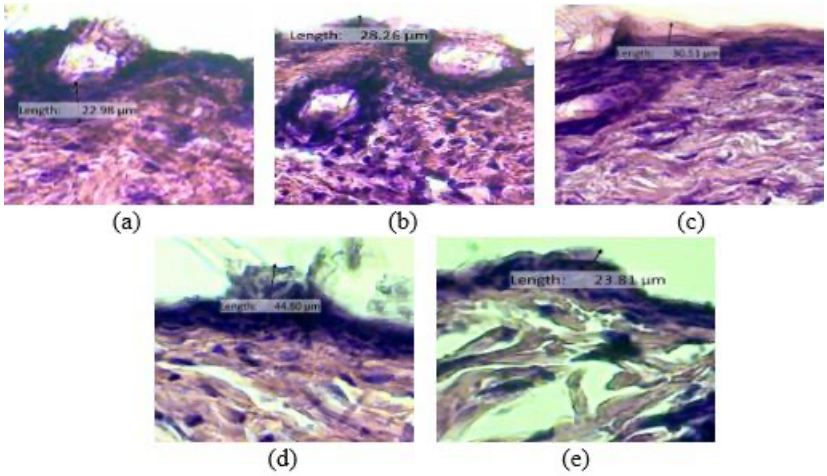

Figure 2. Histological image of several fibroblasts: (a) Formula 1 (F1); (b) Formula 2 (F2); (c) Formula 3 (F3); (d) positive control; (e) negative control.

The results of the histopathology of the skin show that each treatment group has different numbers of fibroblast cells and epithelial thicknesses, as seen in Figure 1 and Figure 2. Fibroblast cells are spread along collagen fiber bundles and are spindle-shaped with tapered ends, have one or more nuclei, are basophilic, and stain purple with hematoxylin-eosin staining [14]. Tables 6 and 8 show that the number of fibroblast cells is directly proportional to the thickness of the epithelium obtained. The more fibroblast cells there are, the greater the thickness of the epithelium will be. In the treatment of giving FFH preparations with concentrations of 0.5 %, 1 %, and 1.5 %, it showed that the preparations were effective in healing wounds by seeing an increase in the number of fibroblast cells and epithelial thickness better than the negative control treatment. This is because FFH preparations create a supersaturation system or saturated condition immediately after being applied to the skin. They overcome the problem of preparation instability by increasing drug penetration through the skin better than other transdermal dosage forms. From the research results obtained, the extract concentration influences the increase in the number of fibroblast cells and epithelial thickness, where the higher the extract concentration, the higher the number of fibroblast cells and epithelial thickness. This is related to the contents of yellow roots, namely alkaloids, flavonoids, and saponins. Alkaloid compounds in the early phase of wound healing play a role in stimulating the formation of fibroblast precursors. Fibroblast stimulation will increase fibroblast secretion; increased fibroblast secretion will also increase collagen production in the tissue. The formation of fibroblasts is directly proportional to collagen synthesis in wound tissue [6].

Statistical data analysis used the parametric one-way ANOVA test and the LSD test. The data obtained was normally distributed and homogeneous. Next, an LSD test was carried out; the results are in Table 10.

Based on the table above, there is a significant difference between the positive and negative control groups. This is indicated by a significance value of less than 0.05, namely 0.011. All LSD test results between treatment groups had a significance value greater than 0.05, so there were no significant differences between treatment groups F1, F2, and F3. Apart from that, there was an insignificant difference shown in the LSD test results between the positive control group and the treatment group given F3.

The FFH yellow root extract preparations on the number of fibroblasts and epithelial thickness. This was done to determine the relationship between one variable and another, which means that another variable can influence it when one variable occurs. This study determined the relationship between the influence of the extract concentration level and the number of cells and thickness of the epithelium. The correlation test results obtained are shown in Table 11.

Table 9. Average epithelial thickness in burn wounds

Treatment group	Mean epithelial thickness (µm) ± SD
Formula 1 (F1)	26.7 ± 3.26
Formula 2 (F2)	28.5 ± 4.94
Formula 3 (F3)	35.2 ± 10.2
Positive control	44.5 ± 4.85
Negative control	26.6 ± 8.80

*Data are expressed as mean±SD, n=5

Table 10. Results of statistical analysis of the LSD test for epithelial thickness

Group	F1	F2	F3	Positive control	Negative control
Formula 1 (F1)		0.767	0.171	0.011*	0.986
Formula 2 (F2)	0.767		0.269	0.019*	0.754
Formula 3 (F3)	0.171	0.269		0.133	0.166
Positive control	0.011*	0.019*	0.133		0.011*
Negative control	0.986	0.754	0.166	0.011*	

* Significantly different

Table 11. Correlation test results

		Number fibroblasts	Epithelial thickness
Extract concentration	Pearson correlation	0.340	0.515
	Sign.	0.371	0.156

Based on the table above, the significant values obtained from the correlation test were more than 0.05, namely 0.371 and 0.156. Thus, the concentration of the extract with the number of fibroblasts and epithelial thickness was insignificant. In this table, the Pearson correlation of extract concentration to the number of fibroblasts is 0.340, which is a sufficient but insignificant correlation. Meanwhile, the correlation value of extract concentration on epithelial thickness, 0.515, can be a solid but insignificant correlation. In the guidelines for the degree of relationship, the closer the correlation value is to 1, the stronger the correlation value. The direction of the relationship between extract concentration and the number of fibroblasts is positive. So, it can be said that the extract level is positively related (in the same direction) to the number of fibroblast cells. The higher the extract level, the higher the number of fibroblast cells.

Conversely, the lower the extract level, the lower the number of fibroblasts. Likewise, the direction of the relationship between extract concentration and epithelial thickness is positive. So, it can be said that the extract level is positively related (in the same direction) to the thickness of the epithelium; the higher the extract level, the higher the epithelial thickness; conversely, the lower the extract level, the lower the epithelial thickness. It can be concluded from the results of the correlation test that increasing the extract concentration can increase the number of fibroblast cells and epithelial thickness.

3. DISCUSSION

The transdermal route delivers drugs to the systemic channel by placing the drug on the skin's surface, such as cream, gel, or patch. The advantages of these preparations include continuous release over a specific time interval, low systemic irritability, and good patient compliance. Despite the advantages of transdermal preparations, there are limitations, namely difficult drug penetration. The presence of a dense and difficult-to-penetration layer of the stratum corneum is a barrier to drug penetration and thus provides a therapeutic effect [15].

The film forming hydrogel (FFH) is a dosage form that can be formed into a film through solvent evaporation after being applied to a wound. FFH is a hydrogel dosage form consisting of a film-forming hydrophilic polymer, plasticizer, and a volatile solvent resistant to skin. FFH applied to the wound area must provide a film layer resistant to physiological stress caused by skin movement and maintain contact between the film layer and the skin for a long time. FFH has the advantages of hydrogel, such as easy application, quick drying on the skin, appropriate hardness and adhesion, and the benefits of the film, namely good flexibility and elasticity [15].

Film formation will occur immediately after the solvent evaporates, producing a flexible and transparent layer on the skin's surface. The principle of this drug delivery is that after being applied to the skin, it will leave a thin film layer on the skin surface due to the loss of volatile components in the FFH preparations formula. The solvent evaporation rate influences the release of active substances in FFH. The faster the solvent evaporates, the faster the active substance can penetrate. The evaporation of the solvent in the formulation is influenced by the formulation ingredients, namely the plasticizer in this formulation, propylene glycol. In FFH preparations, plasticizers play a role in reducing the glass transition temperature (Tg) of the polymer film formed and play a role in increasing drug diffusion [18].

This research was done by making FFH gel preparations from yellow root extract. This study used three formulas with different concentrations: F1, F2, and F3, each with an extract concentration of 0.5, 1, and 1.5 %. The gel bases used are PVA and PVP. In this study, burn wound healing was based on reducing the diameter of the burn wound and increasing the percentage of burn wound healing. In addition, the healing of burn wounds is seen in an increase in the number of fibroblast cells and epithelial thickness. The burns given to mice were classified as grade II, characterized by the pink and white wound surface and higher above the normal skin surface [19].

Treating FFH preparations with a concentration of 0.5, 1, 1.5% effectively heal wounds by seeing an increase in fibroblast cells and epithelial thickness better than the adverse control treatment. This is because the FFH preparations create a supersaturation system or saturated condition immediately after being applied to the skin. They overcome the problem of preparation instability by increasing drug penetration through the skin better than other transdermal dosage forms. The FFH preparations have good drug penetration in the body so that the desired therapeutic effect is maximized in the speed of healing of burn wounds [20].

FFH is commonly used in the medical field for healing as a physical barrier over wounds that helps prevent inflammation, control the wound environment, and accelerate healing [23]. FFH is gaining popularity due to its non-invasive nature, ease of application, biocompatibility, and potential inclusion of antimicrobial treatments. Additionally, FFH offers horror, satisfaction, gas exchange capability, and transparency [24]. The development of FFH has been widely investigated as a primary or secondary dressing for hydrogel or foam to treat partial-thickness wounds without (or minimal) exudate, necrosis, and infection [24]. As already mentioned, the film can be used in superficial burns (first and second degree), stage 1 and 2 decubitus ulcers, skin grafts, prevention of skin damage, and post-surgical wounds because this film makes it easier to monitor the wound [25]. In addition, FFH has drug delivery applications commonly used in the pharmaceutical field to penetrate certain drugs into the skin [26].

From the research results obtained, the extract concentration influences the increase in the number of fibroblast cells and epithelial thickness, where the higher the extract concentration, the higher the number of fibroblast cells and epithelial thickness. This is related to the contents of yellow roots, namely alkaloids, flavonoids, and saponins. Alkaloid compounds in the early phase of wound healing play a role in stimulating the formation of fibroblast precursors. Fibroblast stimulation will increase fibroblast secretion; increased fibroblast secretion will also increase collagen production in the tissue. The formation of fibroblasts is directly proportional to collagen synthesis in wound tissue [6].

The healing phase of burn wounds is divided into three: the inflammatory phase, the proliferation phase, and the maturation (remodeling) phase. At the beginning of the inflammatory phase, macrophages play an essential role in wound healing, including scar tissue formation. Platelet distribution occurs more evenly in the fibrin matrix than in leukocyte cells. Platelets in the fibrin matrix are essential in wound healing by releasing cytokines, chemokines, and growth factors. Platelets have a significant role in 31 stages of wound healing, including initial coagulation, inflammation, angiogenesis, and the maturation (remodeling) stage [10]. In the proliferation phase, the wound is filled with fibroblast and collagen inflammatory cells, which work together to form tissue that bleeds easily with a red color and smooth lumps on the surface. Fibers are formed during the proliferative phase and then re-decomposed to compensate for the tension in wounds that tend to shrink. This characteristic produces traction on the wound edges and is supported by the ability of myofibroblasts to contract [9]. Next, the wound will enter a new phase, namely the maturation (remodeling) phase.

Berberine alkaloids in yellow root plants have the potential to act as antimicrobial agents that can fight various types of microorganisms. One marker compound in the yellow root is berberine. Berberine functions as an antibacterial agent by stopping peptidoglycan synthesis in cells. This disrupts the formation of the cell wall layers, which ultimately causes cell death [5]. The alkaloid content found in yellow roots can increase the number of fibroblasts and collagen density to speed up the healing of burn wounds [6]. Alkaloid compounds in the early phase of wound healing play a role in stimulating the formation of fibroblast

precursors. Fibroblast stimulation will increase fibroblast secretion; increased fibroblast secretion will increase collagen production in the tissue. The formation of fibroblasts will be directly proportional to collagen synthesis in wound tissue [6].

From the research results obtained, increasing the concentration of the extract affects the speed of healing of burn wounds by reducing the diameter and increasing the healing percentage in the number of fibroblast cells and epithelial thickness. This is related to the contents of yellow roots, namely alkaloids, flavonoids, and saponins. Berberine alkaloids in yellow root plants have the potential to act as antimicrobial agents that can fight various types of microorganisms. One marker compound in the yellow root is berberine. Berberine functions as an antibacterial agent by stopping peptidoglycan synthesis in cells. This disrupts the formation of the cell wall layers, which ultimately causes cell death [5]. The alkaloid content found in yellow roots can increase the number of fibroblasts and collagen density to speed up the healing of burn wounds [6]. Alkaloid compounds in the early phase of wound healing play a role in stimulating the formation of fibroblast precursors. Fibroblast stimulation will increase fibroblast secretion; increased fibroblast secretion will increase collagen production in the tissue. The formation of fibroblasts will be directly proportional to collagen synthesis in wound tissue [6].

The compounds contained in flavonoids are kaempferol and quercetin, which have an anti-inflammatory effect that prevents increased inflammation. A prolonged inflammatory phase can inhibit the acceleration of wound healing. This is because, during the inflammatory phase, it is characterized by B endorphin being secreted by the pituitary gland, which can reduce macrophage activity, causing the activation of cytokines released by macrophages such as TNF-a, IL-1, IL-6, IL-8, TGF to decrease. So when growth factor cytokines decrease, they will inhibit collagen growth. So, if substances can increase cytokine secretion, which functions as a growth factor, collagen production in wound tissue will increase [6].

The yellow root FFH preparations can also increase macrophage activation because they contain flavonoids. Activation of macrophages will increase the secretion of TGF-β (Transforming Growth Factor-β), which triggers fibroblast proliferation, extracellular matrix storage, and stimulation of endothelial cells to form new blood vessels. Increased TGF-β secretion causes increased fibroblast proliferation, which leads to an increase in the number of fibroblasts in the tissue. An increase in fibroblasts will produce large amounts of collagen for remodeling. Apart from that, saponins are also known to increase monocyte proliferation, which causes an increase in the number of macrophages and secretes growth factors to produce fibroblasts. The formation of new fibroblasts stimulated by saponin also increases the collagen synthesized by fibroblasts [6].

Two essential processes co-occur in wound healing: closure and angiogenesis (formation of new capillaries). Reepithelialization, granulation tissue formation, and collagen deposition in the wound area are part of the wound closure process. Reepithelialization greatly influences wound healing because the faster the reepithelialization process, the faster the wound will close, so the faster the wound will heal. The thicker the epithelium, the faster the reepithelialization process, so the faster the wound healing process.

A decrease in wound diameter can be considered as a parameter for wound healing because the more minor and closed the wound indicates that the wound healing is more effective, whereas in calculating the percentage of wound healing, which is indicated by a more significant percentage indicating that it is more efficacious [12]. In addition, the number of fibroblasts can also be considered a wound-healing parameter. In the proliferation phase, we will see an increase in the number of cells and wound-healing factors, including fibroblast proliferation. The proliferation of fibroblasts determines the outcome of wound healing. Fibroblasts will produce collagen, binding the wound, and fibroblasts will also influence the reepithelialization process, which will close the wound. Fibroblast proliferation so that the number of fibroblasts in the wound area will be more significant, and the wound will close quickly [17].

4. CONCLUSION

Based on the results of research regarding the effectiveness of FFH gel preparations from yellow root extract on burn wound healing, it can be concluded that the concentration of yellow root extract significantly affects the diameter of burn wound healing on the 3, 7, and 14 days. The yellow root extract concentration significantly influences the burn wound healing percentage. The optimum extract concentration that increases the number of fibroblast cells and epithelial tissue thickness is formula 3 (F3), with an extract concentration of 1.5%. The FFH preparations from yellow root extract are effective in healing burn wounds, which is characterized by decreasing the diameter of the burn wound, increasing the percentage of burn healing, and increasing the number of fibroblast cells and epithelial thickness.

5. MATERIALS AND METHODS

5.1 Research design

This research uses a true experimental laboratory with a design pre-post test study method.

5.2 Material

The ingredients needed are distilled water, 70% alcohol (Brataco), FFH preparations, bioplacenton® (PT Kalbe Farma), normal saline (NaCl 0.9%) (Merck), formalin (Merck), ketamine (OGB Dexa), and xylazine (Xyla Halland). The yellow root extract samples were purchased from vendors in East Kalimantan and identified at the Materia Medica in Batu, East Java, Indonesia, with the accession number 067/543/102.20/2023. The specimens were then stored in the pharmacognosy Laboratory of the Pharmacy Department, Maulana Malik Ibrahim, State Islamic University of Malang.

5.3 Population and Sample

The samples used in this research were male mice (*Mus musculus*) with a body weight of 20-35 g. The sample size used was 25 individuals from the calculation results of the replication formula from Steel and Torrie [22]. However, to avoid the possibility of dead samples, replication is used with the formula 1/ (1 – f) x number of samples per group, f value = 25%, so: 1/ (1-0.25) x 5 = 6.67 samples. So, the number of mice needed in this study was 35 mice [13]. This research has complied with the research ethics clearance of the Health Research Ethics Commission, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim, with Number: 35/02/EC/KEPK-FKIK/09/2023.

5.4 Yellow Root Extract

Ultrasonic-assisted extraction (UAE) is used in the extraction process. The yellow root was extracted thrice with 500 mL ethanol at ambient temperature through sonication Q2400 (Sonica, USA) at 10-minute intervals. About 25 g of the sample was dissolved in 500 mL of 70 % ethanol at 1:20. The filtrate was then separated from the solvent using a rotary evaporator at 40 ◦ C until a thick extract was obtained. Next, evaporate the extraction solvent, followed by an oven at a temperature of 50˚C so that the extract obtained is dry and free from solvents (Heidolph, Germany [14].

5.5 Film Forming Hydrogel (FFH) Preparations from Yellow Root Extract

The initial stage is to dissolve the PVA in distilled water at 80°C. Then, PVP is poured evenly over the surface of the hot distilled water for 15 minutes and stirred until homogeneous. Next, mix PVA and PVP until homogeneous. In the next step, the homogeneous mixture of PVA and PVP was added to propylene glycol, nipagin, and nipasol and stirred until homogeneous. Finally, pour the yellow root extract and distilled water into the mixture and stir until homogeneous with the help of a homogenizer. The extract dose used in the FFH formulation is 0.5%, 1%, and 1.5%, as seen in Table 11.

The physicochemical characteristics of the FFH formula used in testing the effectiveness of wound healing are formulas with F1 characteristics that meet the physicochemical properties test, including a semi-solid form with a characteristic odor. Yellow root extract is yellow to light brown, pH 5.58 ± 0.026, spreadability 5.45 ± 0.01, adhesion (seconds) 1.02 ± 0.25, drying time (seconds) 3.95 ± 0, 67, sticky properties show no film attached to the fiber, viscosity (Cps) 7120 ± 9078.58, elongation (Mpa) 3.53 ± 0.65, tensile strength (%) 44.44 ± 15.03. F2 characteristics of pH 5.88 ± 0.36, spreadability 5.05 ± 0.01, adhesion (seconds) 1.08 ± 0.12, drying time (seconds) 3.85 ± 0,07, sticky properties show no film attached to the fiber, viscosity (Cps) 7520 ± 9178.12, elongation (Mpa) 3.63 ± 0.65, tensile strength (%) 46.74 ± 13.03. F3 characteristics of pH 5.98 ± 0.06, spreadability 5.52 ± 0.02, adhesion (seconds) 1.28 ± 0.02, drying time (seconds) 3.95 ± 0,05, sticky properties show no film attached to the fiber, viscosity (Cps) 7932 ± 108.12, elongation (Mpa) 3.91 ± 0.05, tensile strength (%) 49.61 ± 0.03.

Table 11. Yellow Root FFH Formulation

Ingredient	Function	Concentration % (w/w)		
		F1	F2	F3
Extract	Active substance	0.5	1	1.5
PVP	Polymer	2.8	2.8	2.8
PVA	Polymer	15.2	15.2	15.2
Propylene glykol	Plasticizers	3	3	3
Nipagin	preservative	0.18	0.18	0.18
Nipasol	preservative	0.02	0.02	0.02
Aquadest	Solvent	ad 100	ad 100	ad 100

5.6 Creation of Degree IIA Burns

The initial step was to carry out intraperitoneal anesthesia on the mice's thighs using ketamine and xylazine in a 2:1 ratio of 0.03 ml so that the experimental animals did not feel pain when induced by second-degree burns [16]. After that, clean and shave the upper back area with a 1-3 cm diameter. Then, disinfect the back area where the burn will be made with 70% alcohol [21]. Next, degree IIA burns were induced using a circular iron with a diameter of 1.1 cm, and then for 5 seconds, the iron was first burned with fire for 10 seconds [16]. Induction of burns is carried out to the dermis and the underlying tissue, resulting in blistering and peeling of the skin in certain areas [9]. Then, the wound is compressed with normal saline to prevent the burn from spreading or worsening.

5.7 Treatment of Grade IIA Burns

Burn wound treatment is carried out openly the day after the burn wound has been induced for 14 days. Wound care was carried out differently according to the treatment group, which was carried out once a day. The treatment group consisted of five treatment groups, namely formula 1 (F1) with 0.5% yellow root extract, formula 2 (F2) with 1% yellow root extract (F2), formula 3 (F3) with 1.5% yellow root extract, bioplacenton® (positive control), normal saline 0.9% (negative control).

5.8 Wound Diameter Measurement

Wound diameter measurements can be made using a screw micrometer (mm) using the Morton method. Observations were made by looking macroscopically at the development of healing from burn wounds on the backs of test animals and measuring the diameter of the burn wounds formed using a screw micrometer. The way to measure the diameter of a burn wound is to measure four wound diameters regularly and then calculate the average diameter value for each measurement [15]. The data used for statistical analysis was the final diameter of the burn wound at the time of measurement on day 3 (inflammatory phase), day 7 (transition from inflammatory phase to proliferative phase), and day 14 (proliferative phase) [17]. Next, calculations are carried out using the following formula [21].

$$Dx = \frac{dx1+dx2+dx3+dx4}{4}.$$

Information
dx = Average diameter on day x (mm)
Dx (1,2,3 and 4) = wound diameter is measured from 4 directions

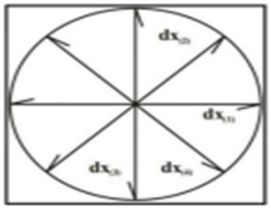

Figure 3. Wound Diameter Measurement [21]

5.9 Calculation of Burn Wound Healing Percentage

The percentage of burn wound healing that was observed was measuring the initial wound area by measuring the area of the wound on the 14th day, where a high percentage indicated effective burn healing with the size of the wound getting more minor from day to day [12]. Data were taken to measure the diameter of the burn wound on each treatment on the first day and the day when one of the treatments was thought to have experienced complete healing (day 14), and the difference was calculated. The wound diameter measurement is converted into a healing percentage (%) using the following percentage conversion formula.

$$Px = \frac{(d1)\ 2 - (dx)\ 2 \times 100\%}{(d1)\ 2}$$

Information
Px = percentage of healing on day x (in%)
d1 = First-day wound diameter (mm)
dx = wound diameter on day x (mm)

5.10 Identification of Fibroblast Cell Number and Epithelial Thickness

On the 15th day, surgery was performed to remove the skin from the area previously caused by the burn. Before surgery, euthanasia was carried out and cleaned first with 0.9% NaCl. The next step was to fix the mice's skin by placing it in 10% formalin. This aims to make the skin more durable and last longer so that it will not change shape or size. It is also used to inhibit the process of decay, autolysis, and tissue hardening, which will affect coloring [6]. After that, preparations were made with Hematoxylin-eosin staining. This identification was done using a microscope with 400x magnification in five fields of view. The cell number was calculated manually, with the results obtained by adding and averaging. Meanwhile, epithelial thickness measurements were carried out with the help of an image raster application in µm units.

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