The Effectiveness of Sungkai Leaf (*Peronema canescens* Jack.)
Extract Gel on The Collagen Density of Incision Wounds in Vivo

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ABSTRACT

An incision is an open wound resulting in skin injury and underlying tissues. The wound-healing process involves a proliferative phase that produces collagen. Sungkai leaves (*Peronema canescens* Jack.) can be used as an alternative therapy for wound healing because it contains flavonoids, tannins, and saponins that stimulate fibroblasts to synthesize collagen. This study aimed to determine the effectiveness of sungkai leaf extract gel in healing incision wounds in vivo based on collagen density scoring. Twenty-five *Wistar* rats (*Rattus norvegicus*) were randomly divided into five groups, the negative control group (K1) was given a gelling agent CMC 1%, the positive control group (K2) was given *bioplacenton gel* 1%, the treatment group (K3, K4, K5) was given a gel sungkai leaf extract concentration of 5, 10, and 15. The treatment is given topically at the incision site of the incision site of 0.1 g once a day for 14 days. The rats were euthanized on the fifteenth day, and the incision area was excised for histopathological preparation. The data obtained were analyzed using Kruskal-Wallis with SPSS 26. The test results showed *p*=0.010, meaning there is a significant difference (*p*<0.05) between the entire treatment. Based on these results, the K4 group (Sungkai leaf extract gel concentration of 10%) showed the highest collagen density scoring results compared to other treatments. This study concludes that sungkai leaves effectively heal incision wounds in *Wistar* rats based on collagen density scoring parameter.

Keywords: *Peronema canescens* Jack.; incision wound; wound healing; collagen density; gel

INTRODUCTION

Incision wound is a condition of injury to the skin and underlying tissue due to loss of tissue continuity (Franz, 2008). Various types of wounds can be distinguished based on the cause, namely, open, and closed wounds (Nugraha, 2016). An example of an open wound is an incision wound, where the size of the wound will look longer from the outside (external component) compared to the depth of the wound (internal part) (Bajo, 2020).

According to data from RISKESDAS (2018), open wounds rank third for the types of injuries that occur in Indonesia, with a prevalence of around 23.2%, especially in Central Kalimantan, about 16.37%. Indonesia has an increasing number of injuries every year; this is seen in the data in 2013 at 8.4% and in 2018 increased to 9.2%.

Biologically, the wound that occurs will be immediately responded by the body to the healing process (Paramita, 2013). The wound healing process consists of the inflammatory, proliferation, and maturation or remodeling phases. The initial phase occurs when the inflammatory response is acute, followed by collagen synthesis in the proliferative phase. Collagen is the main protein and most in the human body, so it has a vital role in the wound-healing process (Paramita, 2016). The maturation phase occurs at the end of a period that lasts for a long time (Sea, 2019).

Drug administration is essential to accelerate the healing of wounds on the skin. Open wounds on the skin, if left untreated, can lead to infections such as tetanus. If the infection is not treated immediately, it will spread to other tissues or organs, causing chronic infection (Fadhilah, 2022). Generally, treatment is done with oral administration of conventional anti-inflammatories that can cause side effects for the body, such as gastric ulcers and blood vessel obstruction due to blood clots (Hidayah, 2021). Therefore, gel preparations with herbal ingredients are chosen because they are easy to apply to the incision wound and have a faster effect than oral preparations. Gel preparations also have a high moisture content to retain moisture on the wound surface and accelerate the formation of blood vessels and new tissue (Farid, 2020).

Sungkai (*Peronema canescens* Jack.) is an herbal plant with many health benefits. The

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Sungkai plant is an ethnobotanical plant found on the islands of Sumatra and Kalimantan. Figure 1 shows the sungkai plant with some parts of the plant. Empirically, sungkai leaves are used by being pounded and taped to the bruised wound (Rahman, 2021). Other research reported that sungkai leaves can be used as a medicine for minor injuries by the people of the Riau Islands (Fitria, 2021). Based on previous research, ethanol extracts of sungkai leaves with concentrations of 5, 10, and 15 also have anti-inflammatory effects in the wound healing process, which is characterized by a decrease in the volume of exudate, lymphocytes, stem neutrophils, and segment neutrophils (Latief, 2021). Ethanol extract from sungkai leaves contains flavonoids, tannins, and saponins that can stimulate fibroblasts to synthesize collagen and the formation of new blood vessels to accelerate wound healing (Dasrinal, 2022).

The formation of collagen fibers by fibroblast cells can be seen using Mason’s Trichrome (MT) staining, which is a specific staining for elastin and reticulin fibers (connective tissue fibers present in organs). Reticulin fibers are collagen fibers that contain a lot of glycoproteins and will appear blue in MT staining (Murti, 2017).

Based on the above background, it is necessary to conduct a study on the effectiveness of sungkai leaf (Peronema canescens Jack.) extract gel on the healing of incision wounds, which is investigated from the collagen density of rats (Rattus norvegicus) Wistar strain.

**METHODOLOGY**

**Materials**

Sungkai leaf (Peronema canescens Jack.) extract, 96% ethanol (Brataco), bioplacenton gel (Kalbe Farma), and Masson’s Trichrome reagent (Merck). The animals used were male white rats (Rattus norvegicus) Wistar strain ketamine (Bernofarm), xylazine (Holland). The ingredients of the gel are carboxymethyl cellulose (CMC), propylengligkol were obtained from Merck and aquadest (Brataco). Ultrasonic Assisted Extraction (Skymen), glass tools (pyrex), rotary vacuum evaporator (Hahn Shin), filter paper (Whatman), analytical balance (Radwag), autoclave (Jibimed), minor set (Marwa) and microscope (Olympus).

**Test Animals**

Healthy Wistar male rats aged 2-3 months with a body weight between 200-250 g. This study has received ethical clearance from the Ethics Commission of Health Research FK UPR No. 56/UN24.9/LL/2022.

**Methods**

**The Procedure of Extraction with Ultrasonic Assisted Extraction (UAE) Method**

Sungkai plants were determined at Lambung Mangkurat University FMIPA Laboratory with a certificate No.60a/LB.LABDASAR/III/2022. Fresh sungkai leaves ) as much as 5 kg that has been through the sorting process and has been washed and then dried in indirect sunlight with a white cloth covered for 3-4 days. After drying, sungkai leaves are cut and blended until smooth
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Table I. Comparison of gel formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Concentration 5%</th>
<th>Concentration 10%</th>
<th>Concentration 15%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sungkai leaf extract</td>
<td>5 g</td>
<td>10 g</td>
<td>15 g</td>
</tr>
<tr>
<td>Sodium-CMC</td>
<td>1.59 g</td>
<td>1.59 g</td>
<td>1.59 g</td>
</tr>
<tr>
<td>Propilen Glikol</td>
<td>1.59 mL</td>
<td>1.59 mL</td>
<td>1.59 mL</td>
</tr>
<tr>
<td>Aquadest</td>
<td>ad 100 mL</td>
<td>ad 100 mL</td>
<td>ad 100 mL</td>
</tr>
</tbody>
</table>

and then sifted using a 60-mesh sieve to become powder. The extraction process of sungkai leaves 1 kg with ethanol solvent using the UAE method. Some parameters with the UAE method include 96% ethanol concentration of 200 mL, 50 g of Simplicia powder, and 20 minutes extraction time. The evaporation process is done by a rotary vacuum evaporator and then continued by using a water bath to get the concentrated extract. The calculation of the extract obtained after the extraction process is carried out using the weight of the extract obtained (g) divided by the weight of the sungkai leaf Simplicia (g), then multiplied by 100% (Andriani, 2019).

\[
\text{Rendemen of extract} = \frac{\text{the weight of extract (g)}}{\text{the weight of crude (g)}} \times 100\%
\]

Phytochemical Screening

The identification of phytochemicals such as flavonoids, tannins, and saponins was determined qualitatively through color reactions using a specific reagent, as well as quantitatively using UV Visible Spectrophotometry (Vifta, 2018).

Preparation of Gel

A total of 1.59 g of CMC was weighed and dissolved with warm water while adding CMC and stirred using a mortar and pestle that had been warmed until dissolved, then added sungkai leaf extract, and propylene glycol while crushed and filled in volumes up to 100 mL with water while stirring until homogeneous. The same procedure is performed on the formulations contained (Table I).

Evaluation Test of Gel Preparations

Evaluation tests of sungkai leaf extract gel preparations include organoleptic tests, homogeneity tests, pH tests, dispersion tests, and adhesion tests (Dwi, 2018).

Grouping of Test Animals

The animals were acclimatized for one week and fed and watered ad libitum. The test animals were divided into five groups (n=5 rats/group) using a complete randomized block design method, namely the K1 (-) group with the administration of gelling agent CMC, the K2 (+) group with the administration of bioplacenton gel, the K3, K4, K5 groups were test groups given sungkai leaf extract gel with concentrations of 5%, 10%, and 15%, respectively. The extracted gel is administered using a sterile glass spatula topically at the incision site of 0.1 g once a day for 14 days (Sucita, 2019).

Incisional Wound Making

The hair that grows around the rat’s back where the wound will be made is shaved and then adapted for 2 days. Rats were anesthetized using ketamine (40 mg/kg BW) and xylazine (10 mg/kg BW) by the intraperitoneal (i.p) route of administration. The incision wound was made by injuring the rat skin with a length of 2 cm and a depth of 0.2 mm using a scalpel blade (Novitasari, 2019).

Sampling

Skin sampling was done after rats were previously euthanized by increasing the dose of ketamine and xylazine to three times the previous dose. Animals try to lie down dorsally so that the back is located on the dorsal to facilitate sampling. The cutaneous part of the incision site is fixed and rinsed with 0.9% physiological NaCl. The skin is taken approximately 1 cm wide from the edge of the wound, then placed in a container containing 10% BNF solution for 24 hours (Sucita, 2019).

Collagen Density Scoring Parameters

The observed parameter is the density of collagen in the wound-healing process. Histopathological scoring parameters for collagen density are based on the calculation of 6 fields of view at 400x magnification object as in Table II.

Data Analysis

Data obtained from the percentage components of each group of equipment using
Results and Discussion

Sungkai Leaf Extraction

The yield of ethanol extract of sungkai leaves with the UAE method is 7.02%. In a previous study, sungkai leaf extract obtained a yield of 12.10% extracted by the maceration method (Fiska, 2022). Several factors that influence the yield calculation results are particle size, extraction time, extraction temperature, type, and amount of solvent (Widyasanti, 2018).

UAE extraction was carried out using 96% ethanol solvent. Ethanol has advantages because the liquid is safer and less toxic, has good absorbance, and ethanol requires less heat for the concentration process.

Ethanol is polar which can dissolve the active ingredients contained in plants, both polar, nonpolar, and semipolar (Dewi, 2018). In this study using 96% ethanol solvent which is known to be more effective in obtaining flavonoid, saponin, and tannin content than lower ethanol concentration because the higher ethanol concentration, the lower polarity level of the solvent used and ultimately can increase the solvent’s ability to extract content that is also less polar (Noorhamdani, 2018).

Phytochemical Screening Results

The results of phytochemical screening of sungkai leaf extract using the UAE method showed flavonoid levels of 198,917 ± 0.289 (mg/ml QE), saponin 42,000 ± 0.400 (%), and tannin (mg/ml GAE). Flavonoids are said to be positive if there is a change in color due to the addition of HCl and Mg in the extract that will hydrolyze flavonoid compounds into aglycons, namely by hydrolyzing O-glycosol. Saponins are said to be positive because they have hydrophilic and hydrophobic groups that act as active surfaces in the formation of froth. Tannin is said to be positive because of the addition of FeCl₃ solution, which will react with one of the hydroxyl groups in tannin compounds (Jamuna, 2012).

Evaluation Results of Gel Preparations

The evaluation results of gel preparations include an organoleptic test, homogeneity test, pH test, dispersion test, and adhesion test in Table III. The organoleptic test resulted in all concentrations having the same form of liquid semi-solid gel (semi-solid) but having a different color and aroma for each concentration. The higher concentration of the extract, the stronger the characteristic aroma of the extract while the resulting gel base has no aroma. The intense blackish-green color produced by sungkai leaf extract gel is caused by the high chlorophyll content in sungkai leaves (Mappa, 2013).

The homogeneity test was carried out by means gel sample smeared on a piece of glass or other suitable transparent material, the preparation must indicate the order homogeneous and no visible granules rough. The homogeneity test aims to determine the homogeneity of the sungkai leaf extract gel by looking at the uniformity of the particles in the preparation (Rety, 2021).

pH testing of all concentrations results in an acceptable pH for the skin of 5. The pH test aims to determine the stability of the gel preparation during storage and assess the pH of the preparation that can be accepted by the skin, which is between 4.5-6.5 (Rohmani, 2019).

Dispersion tests of all concentrations have a good dispersion value. The dispersion of a good gel preparation is 5-7 cm. Spreadability measurement is carried out by weighing 1 g of gel and then placed in the middle of a scaled round plate. The dispersion test aims to determine the ability of the gel can spread on the surface of the skin. The more the gel is easily applied to the skin, the wider the contact surface between the gel and skin.
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The skin, and the absorption of the drug will be more optimal (Citra, 2018). Adhesion tests of all concentrations can meet the criteria of a good gel because it has an adhesion of more than 1 second. This is due to the presence of gelling agent in the formulation of CMC which provides viscosity or high viscosity so that the adhesion time of the preparation is longer. This adhesion test aims to determine how long the gel is attached to the skin surface so that the active substances in the preparation can be absorbed properly (Rohmani, 2019).

**Evaluation of Collagen Density**

Evaluation of collagen density parameters with 400x magnification. Figure 2 shows a microscopic picture of collagen density in the wound healing process using Masson’s Trichrome (MT) staining. This study resulted in the highest collagen density scoring on K4 (gel extract of Sungkai leaf concentration 10%) available in Table 4. The negative control group (gelling agent sodium-CMC) showed an average percentage of collagen density (6.33%). This is due to the absence of drug administration or substances that are efficacious in wound healing, but the changes in wounds that gradually heal due to the self-healing power where the body can heal itself but does not work as fast compared to the administration of gel preparations extracts from plants (Febriana, 2016).

The positive control group (bioplacenton gel) showed the injured area’s average percentage of collagen density (26.66%). This is because bioplacenton gel contains placenta extract and neomycin sulfate. This combination can provide wound care, where placenta extract is a “biogenic stimulator” that accelerates cell regeneration and wound healing. While neomycin sulfate can help prevent infection and inflammation (Rahmadhani, 2020).

The sungkai leaf extract gel group with concentrations of 5, 10, and 15, showed the average percentage of collagen density sequentially 20% with a score of +2; 98.33% with a score of +4; and 1.66% with a score of +1. The content of sungkai leaf compounds such as flavonoids plays a role in increasing capillary vascular resistance and anti-inflammatory activity that can prevent prolonged inflammation, thereby accelerating the process of fibroblast cell proliferation and collagen fiber production for the wound healing process (Rahma, 2017). Saponins have properties that can affect collagen in the early stages of repair by inhibiting tissue production as an antiseptic that serves to kill bacteria and prevent the growth of microorganisms that attack inflammation by encouraging and triggering macrophages to migrate to the wound area so that it will accelerate collagen synthesis (Yulianto, 2020). Tannin acts as an astringent that can cause skin pores to shrink, stop exudate and light bleeding, cover wounds and prevent bleeding that usually arises in wounds. Then tannin plays a role in the migration and proliferation of fibroblasts to carry out collagen synthesis. The active substance contained in sungkai leaf extract is what causes the percentage of collagen density in the treatment group to be higher than in the control group (Paramita, 2016).

### Table III. Evaluation of Gel Preparations

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Organoleptic</th>
<th>Homogeneity</th>
<th>pH</th>
<th>Dispersion Power (cm)</th>
<th>Adhesion Power (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Smell</td>
<td>Shape</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorless</td>
<td>No smell</td>
<td>Semi-solid preparation (gel) homogeneous</td>
<td>5</td>
<td>6.08</td>
<td>5</td>
</tr>
<tr>
<td>Deep blackish green</td>
<td>Typical sungkai and bitter</td>
<td>Semi-solid preparation (gel) homogeneous</td>
<td>5</td>
<td>6.32</td>
<td>5</td>
</tr>
<tr>
<td>Deep blackish green</td>
<td>Typical sungkai and bitter</td>
<td>Semi-solid preparation (gel) homogeneous</td>
<td>5</td>
<td>5.67</td>
<td>10</td>
</tr>
<tr>
<td>Deep blackish green</td>
<td>Typical sungkai and bitter</td>
<td>Semi-solid preparation (gel) homogeneous</td>
<td>5</td>
<td>5.59</td>
<td>8</td>
</tr>
</tbody>
</table>
Data Analysis Results

Based on the results of statistical data analysis with Mann Whitney post hoc test showed K1 (gel-forming agent sodium-CMC) was different than K2 (bioplacenton gel), K3 (Sungkai leaf extract concentration 5%), and K4 (Sungkai leaf extract concentration 10%). The ability of collagen K1 (gel-forming agent sodium-CMC) has a smaller percentage compared to K2 (bioplacenton gel), K3 (sungkai leaf extract concentration 5%), and K4 (sungkai leaf extract concentration 10%) but more significant than K5 (sungkai leaf extract concentration 15%) with an average percentage of 6.33% which get a score of +1 meaning low collagen density. Gel-forming agent sodium-CMC and Sungkai leaf extract concentration of 15% has a percentage of collagen density that is not significantly different, namely 6.33% and 1.66%, which means that both studies have almost the same effect seen from the data that has been analyzed. This is because the gelling agent sodium-CMC does not have an active content contained in it and the ability of collagen to form is caused because the body could grow itself (Febrian, 2016). The results of statistical analysis of the availability of collagen K2 (bioplacenton gel) are different than K1 (gelling agent sodium-CMC), K4 (Sungkai leaf extract concentration of 10%), and K5 (Sungkai leaf extract concentration of 15%) has an average percentage of 26.66% which get a score of +2 with the meaning of medium collagen availability. The concentration of 10% gives the highest effect compared to the concentration of 15% and 20%, recent studies have found that flavonoids are also a contributing factor because high levels of soluble concentrations can add saponins to the mucous membrane. Differences used in the K2 group by looking at the results that have been analyzed show that bioplacenton gel affects the formation of the composition. The process of collagen formation in K2 (bioplacenton gel) is due to bioplacenton gel containing placenta extract, which serves to trigger the formation of new tissue in the remodeling phase so that the development activity with bioplacenton gel is longer than the development activity by plant extract gel which has started working since the inflammatory and proliferation phase takes place to trigger collagen formation in the skin (Dewi, 2020).
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CONCLUSION

In this study, it was concluded that the 10% dose of sungkai leaf extract can optimally increase collagen density in rats. The K4 Group (10%) was most effective in healing incision wounds in rats and had a collagen density of 100% with a score of +4 (very dense collagen density).

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Table IV. Collagen Density Scoring Results

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Average Collagen Density (%)</th>
<th>Scoring</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (−)</td>
<td>6.33</td>
<td>+1</td>
<td>Low collagen density</td>
</tr>
<tr>
<td>K2 (+)</td>
<td>26.66</td>
<td>+2</td>
<td>Medium collagen density</td>
</tr>
<tr>
<td>K3 (5%)</td>
<td>20</td>
<td>+2</td>
<td>Medium collagen density</td>
</tr>
<tr>
<td>K4 (10%)</td>
<td>98.33</td>
<td>+4</td>
<td>Collagen density is very close</td>
</tr>
<tr>
<td>K5 (15%)</td>
<td>1.66</td>
<td>+1</td>
<td>Low collagen density</td>
</tr>
</tbody>
</table>

Description: K1 = Gelling agent sodium-CMC 1% (−); K2 = bioplacenton gel 1% (+); K3 = Sungkai leaf extract concentration 5%; K4 = Sungkai leaf extract concentration 10%; K5 = Sungkai leaf extract concentration 15%

Table V. Average Percentage of Collagen Density

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Average Percentage of Collagen Density (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 (−)</td>
<td>6.33 ± 3.06b</td>
</tr>
<tr>
<td>K2 (+)</td>
<td>26.67 ± 11.55a</td>
</tr>
<tr>
<td>K3 (5%)</td>
<td>20.00 ± 0.00a</td>
</tr>
<tr>
<td>K4 (10%)</td>
<td>98.33 ± 2.89ab</td>
</tr>
<tr>
<td>K5 (15%)</td>
<td>1.67 ± 1.53b</td>
</tr>
</tbody>
</table>

a = Significantly different from the negative control (−); b = Significantly different from positive control (+)

Description: K1 = Gelling agent sodium-CMC 1% (−); K2 = Bioplacenton gel 1% (+); K3 = Sungkai leaf extract concentration 5%; K4 = Sungkai leaf extract concentration 10%; K5 = Sungkai leaf extract concentration 15%
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