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ORIGINAL ARTICLE

Potential of gambier leaf ethanol extract cream for incised wound healing

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Abstract

Incised wounds are highly susceptible to bacterial infection, which requires stimulation of healing and restoration of normal function of the injured body part. This study aimed to test the effect of gambier leaf ethanol extract cream administration on the acceleration of wound healing on the skin surface of male Wistar rats. The research used a pre-test post-test with a control group design. Twenty male white rats were divided into four groups: control group (P0), treatment group 1 (P1), treatment 2 (P2), and treatment 3 (P3). The incision wounds in white rats were treated twice a day (in the morning and evening) for 14 days. Wound healing was observed by measuring the average length of the wound every day, from the first day of wounding until day 14. Statistical tests used were one-way ANOVA and post-hoc LSD test ($p < 0.05$). In this study, it was found that the 15% gambier leaf extract cream was more effective in wound healing in white rats than the 5% and 7.5% gambier leaf extract creams. However, in the group treated with 7.5% gambier leaf extract cream, the situation was already close to that in the treatment group treated with 15% gambier leaf extract cream. This is because, at a concentration of 7.5%, the secondary metabolite compounds in the gambier leaf extract had an effect on wounds, but at a concentration of 15% gambier leaf extract cream, the effect was similar to that of 15% gambier leaf extract cream.

Keywords: extract cream, gambier leaf, cut wound

Introduction

A wound is a disruption or injury caused by the structure and function of the anatomy, resulting from severe damage to body organs, such as the skin. This condition is associated with an increase in morbidity and mortality.[1,2] A systematic review reported that the prevalence of chronic wounds with mixed etiology is 2.21 cases per 1000 populations.[3] The estimated prevalence rate of chronic wounds ranges from 1% to 2% of the general population in developed countries, making it a significant burden for patients, nurses, and the medical system.[4,5] One common type of wound is an incised wound caused by a cut on a rough surface, such as a knife or other sharp objects. Although it does not damage tissues too deeply, it is essential to clean and care for incised wounds properly to prevent infection.[6,7] If infected, the healing process will slow, resulting in exudate fluid and toxins. Infection can also lead to the death of regenerative cells.[8,9] Therefore, it is crucial to stimulate healing and restore normal function of the injured area to reduce the risk of infection.[10] The wound healing process on the skin involves various cellular substrates and physiological processes, including the hemostasis phase, which includes vasoconstriction, primary hemostasis, and secondary hemostasis. The ongoing challenges related to wound healing, especially incised wounds, are significant due to the high number of cases and the variety of evolving treatment methods in the current era of globalization.[11–13]

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The use of plants and natural materials in traditional medicine has rapidly increased in Indonesia, and some natural materials have been produced on a large scale. Traditional medicines are considered to have fewer side effects than chemical-derived drugs. Traditionally, raw materials are easily accessible, and their cost is relatively low.[14] Many studies related to wound healing have used natural ingredients such as herbal plants and fruits formulated as preparations to assist in wound healing.[15,16] Around 80% of developing countries still rely on the benefits of traditional plant-based medicine. More than 20,000 species of medicinal plants have been documented by WHO and recognized as potential sources for the development of new drugs.[17,18] There are more than 1340 plants known to have antimicrobial activity, with over 30,000 antimicrobial compounds successfully isolated from plants.[19] In addition, estimates show that 14-28% of high-level plant species have medicinal properties, with about 74% of bioactive plant-derived compounds discovered through ethnomedicine knowledge.[20]

Natural materials that are beneficial in traditional medicine and can be used in the wound healing process include *gambier* leaf (*Uncaria gambier Raxb*). Gambier is an industrial plant with high economic value and can be used as a component in medicines for various purposes such as burns, wounds, headaches, diarrhea, mouthwash, canker sores, skin conditions, and to facilitate the digestion process.[2122] Gambier plant contains functional compounds that belong to the group of polyphenolic compounds, primarily catechins. The largest chemical compound groups in gambier leaf include flavonoids, pyrocatechol, and quercetin. Traditionally, gambier has been widely used as a tanning agent, dye, component in betel quid, and in traditional medicine.[23] Gambier contains two main components: catechin and catechuic acid tannin. Tannins in gambier leaf belong to the proanthocyanidin family. Gambier leaf contain catechins that are slightly soluble in cold water but readily soluble in hot water. These tannins have properties similar to algicides, antibacterials, and antifungals. Young gambier leaf have higher catechin content than mature leaves. The gambier plant also contains wax located on the leaf surface, which is a monoester of a fatty acid and alcohol.[24]

A study by Thaib et al[25] demonstrated that gambier leaf extract using ethyl acetate solvent can be formulated as a cream for burn wound healing. The gambier leaf extract cream formulation effectively healed burn wounds in rabbits, with the 10% extract concentration showing the fastest healing time, specifically within 17 days. Another study by Sulasmi[26] indicated that a 70% ethanol gel of gambier latex extract can heal incised wounds in male rats. The ethanol extract of gambier latex was superior to gel-based treatments, but not superior to betadine treatment. Therefore, this study aimed to test the ability of ethanol extract cream from gambier leaf to accelerate the healing of incised wounds on the skin surface of male Wistar rats.

Method

This study falls under the category of laboratory experimental research or true experiments. The research employed a pre-test post-test with a control group design. Twenty male Wistar rats were divided into 4 (four) groups: control group (P0), treatment group 1 (P1), treatment group 2 (P2), and treatment group 3 (P3). The variables in this study included independent variables, namely the gambier leaf extract cream (concentrations of 0% or blank without gambier leaf extract; concentrations of 5%, 7.5%, and 15%), and dependent variables, namely the acceleration of the incised wound-healing process in Wistar rats.

The tools used in this study included minor surgical instruments (stainless-steel tray, scalpel, blade, scissors, and forceps), a scale, sterile gloves, cotton swabs, gauze, rat cages, rat food containers, writing tools, markers, and calipers. The materials used included gambier leaf, glycerin, triethanolamine (TEA), ethyl alcohol, acetic acid, methyl paraben, propyl paraben, distilled water (aquades), sterile tampons, anesthesia (ketamine), xylazine, Wistar white rats, and rat food and beverages.

Before treatment, all white rat animals were acclimated in the Animal House of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, for seven days, with living space, a new environment, and food and beverages in accordance with their needs. Freshly picked gambier leaf were cleaned to remove impurities, washed with running water until clean, drained, cut into pieces, and then dried in an oven at a temperature–50-60°C. The dried gambier leaf were blended into a powder (simplicia). Simplicia was then soaked in a maceration vessel separately, then given 90% ethanol solvent until the simplicia was completely submerged. The maceration vessel was tightly closed and left for ± 5 (five) days

with stirring once a day. The obtained results were filtered, repeated three times, and then collected in a bottle. The extract was then evaporated using a rotary evaporator at 70°C to remove ethanol, resulting in a concentrated extract. The gambier leaf extract cream was prepared using an oil-in-water (O/W) emulsion. All cream-making ingredients, including gambier leaf extract, were weighed according to the desired concentration or formula, as shown in Table 1, and distilled water was added until the total weight of the ingredients reached 100 g.

Table 1. Formula for gambier leaf extract cream (O/W) preparation

Ingredients	Formula/Cream dose (gram)			
	F0	F1	F2	F3
Gambier leaf extract	0	5	7,5	15
Ethyl alcohol	4	4	4	4
Gliserin	15	15	15	15
TEA (trietanolamin)	3	3	3	3
Stearic Acid	12	12	12	12
Methyl Paraben	0,2	0,2	0,2	0,2
Propyl Paraben	0,02	0,02	0,02	0,02
Distilled Water	100	100	100	100

The fur around the wound area (back part) of the rats was shaved, and then the rats were anesthetized using a combination of ketamine (80 ml/kg BW) and xylazine (5 ml/kg BW). Subsequently, the rats were incised with a scalpel-blade along 2 cm with a depth of ± 0.2 mm to the dermis layer. After creating the incision wound, treatment was administered based on wound care protocols and continued according to the predetermined treatment groups. In the control group (P0), the incision wound on the rats was given a base or blank cream (without gambier leaf extract) and covered with gauze. In treatment group 1 (P1), the incision wound on the rats was treated with 5% gambier leaf extract cream and covered with gauze. Meanwhile, in treatment group 2 (P2), the incision wound on the rats was treated with 7.5% gambier leaf extract cream and covered with gauze. In treatment group 3 (P3), the incision wound on the rats was treated with 15% gambier leaf extract cream and covered with gauze. White rats were treated with incision wounds twice a day (morning and evening) for 14 days. Wound healing was observed by measuring the average length of the wound every day from the first day of wound creation to the 14th day. After 14 days, all white rats were euthanized using excess technical chloroform inhalation.

Histopathological examination was performed at the macroscopic level by observing fibroblast cell growth. The procedure started with a biopsy of the rat skin measuring 2 × 2 cm on days 5th and 14th days. The skin tissue was cut crosswise to observe the number of fibroblasts. Wistar rat skin tissue was stored in urine pots, soaked in 10% formalin, and stained with Hematoxylin Eosin in the Microbiology Laboratory of Universitas Sumatera Utara. Crust formation, re-epithelialization, collagen fibers, angiogenesis, inflammatory cells, and fibroblast cells were observed and counted using digital analysis methods, photographed with a camera and microscope at a magnification of 100x, photographed four times, and stored. The number of fibroblast cells in the photo was analyzed using Image Raster 3 software. Fibroblast cell tissue observed from the histological examination results was recorded as red-colored pixels by the software. Tissues with different colors were selected and recorded as pixels of other tissues. The hypothesis was tested using one-way Anova test ($p < 0.05$). Further tests were carried out with a Post Hoc test to determine which treatment group was most effective among the experimental groups.

Results

A study on the phytochemical content and analysis of gambier leaf extract was conducted at the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The samples used in this research were gambier leaf originating from the Berastagi Region, Karo Regency, cultivated by farmers around Gurusinga Village. Based on the phytochemical testing results of the ethyl acetate fraction, n-hexane fraction, and alcohol/ethanol 90% fraction of gambier leaf extract, the types of secondary metabolite compounds were identified. The test results demonstrated that gambier leaf extract contains flavonoids, saponins, tannins, and alkaloids. Consequently, it can be concluded that gambier leaf extract possesses phytochemical content that can be utilized as a medicinal ingredient because of its high antioxidant compound concentration.

Table 2. Phytochemical testing results of gambier leaf extract

Content	Reagent	Color Result	Description
Flavonoid	Mg, Concentrated HCL	Yellow	Positive
Saponin	Aquadest (Distilled Water)	Presence of foam	Positive
Tanin	$FeCl_3$	Greenish-black	Positive
Alkaloid	Reagen Warner	Brownish-colored precipitate	Positive

Observation of incision wound healing was conducted every 2 days for 14 days in four treatment groups: those treat-

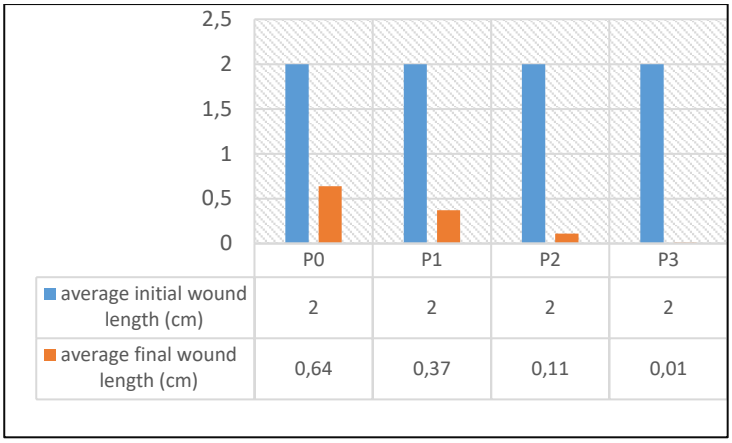


Figure 1. Comparison of incision wound healing

ed with *5%* gambier leaf extract (P1), 7.5% gambier leaf extract (P2), 15% gambier leaf extract (P3), and the control group (P0). In treatment group P3, there was a better average percentage of incision wound healing, which was 0.99 cm, calculated as the average healing from the first day to the 14th day. In groups P0, P1, and P2, the average incision wound healing was 1.32 cm, 1.21 cm, and 1.08 cm, respectively. Furthermore, it can also be explained that in group P3, there was an average reduction in the length of the incision wound by 1.1 cm (Figure 1), calculated from the initial length of 2 cm minus the average on the 14th day, which is 0.10 cm. In groups P0, P1, and P2, the average reduction in the length of the wound in each group from the initial condition was 1.36 cm, 1.63 cm, and 1.89 cm, respectively.

Based on the average incision wound healing in each group, it can be concluded that the healing of incision wounds in group P3 or the group administered gambier leaf extract ointment at a concentration of 15% occurred more quickly, followed by groups P2 and P1. The slowest incision wound healing occurred in the control group (P0) or the control group administered only the base cream (0% extract).

Table 3. Results of Post Hoc Bonferroni test

(I) Group	(J) Group	Mean diff. (I-J)	p
Group P0	Group P1	.09400*	0.000
	Group P2	.20400*	0.000
	Group P3	.28600*	0.000
Group P1	Group P0	-.09400*	0.000
	Group P2	.11000*	0.163
	Group P3	.19200*	0.000
Group P2	Group P0	-.20400*	0.000
	Group P1	-.11000*	0.163
	Group P3	.08200*	0.000
Group P3	Group P0	-.28600*	0.000
	Group P1	-.19200*	0.000
	Group P2	-.08200*	0.000

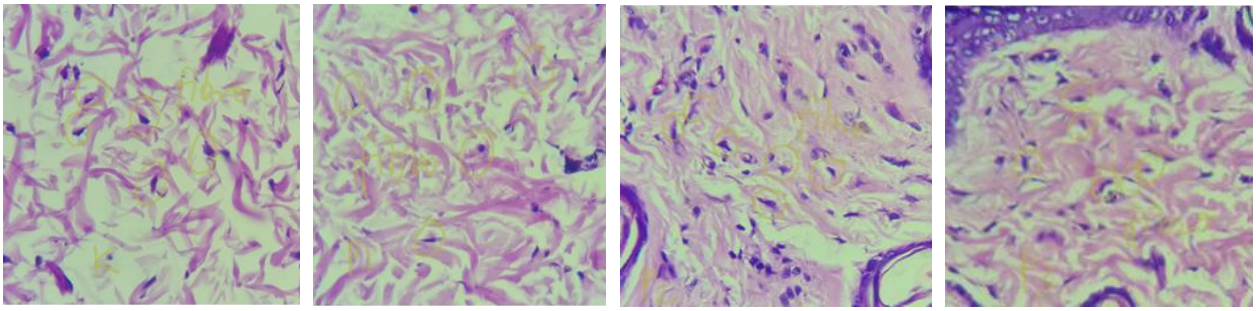
*) The mean difference is significant at the 0.05 level

The results of the Shapiro-Wilk test showed p-values greater than 0.05 in the control group (P0=0.899) and treatment groups (P1=0.928; P2=0.373; P3=0.980), indicating that the data were normally distributed. Meanwhile, the test of homogeneity of variance results indicated that the variance of the research data in the control group (P0) and groups P1, P2, and P3 was homogeneous or originated from a population with the same variance ($p>0.05$). From the one-way ANOVA results, it was concluded that there was a significant difference in the percentage of burn wound healing across all the observation groups ($p<0.05$). Therefore, a post-hoc test must be conducted to identify which groups differ. The test results indicated that the comparison between all the groups showed differences in the average length of incision wound healing.

Table 4 shows the disappearance of erythema (redness) in the control group (P0) from day 9 to day 11, with the fastest disappearance observed in experimental mice 1, 4, and 5. In treatment group 1 (P1), it occurred from day 8 to day 9, with the fastest rate observed in experimental mice 1, 2, and 3. In treatment group 2 (P2), it occurred from day 6 to day 8, with the fastest rate observed in experimental mouse 1. In treatment group 3 (P3), it occurred from day 6 to day 7, with the fastest rate observed in experimental mouse 5.

The healing of an incision wound consists of several phases: the inflammatory, proliferative, and maturation phases. In the proliferative phase, fibroblasts play a crucial role in producing proteins for wound healing, including collagen. The inflammatory phase is characterized by vascular and cellular responses that occur due to skin tissue injury. Collagen scar tissue continues to undergo reorganization and strengthens over several months. The goal of this maturation phase is to perfect the formation of new tissues into high-quality healing tissues. The aspects observed in this study were the proliferation of fibroblast cells and collagen density, both of which are part of the proliferative phase occurring from days 7 to 14.

In Figure 2, it is evident that the treatment groups receiving 7.5% gambier leaf extract cream (P2) and 15% gambier leaf extract cream (P3) exhibited a higher density of fibroblast cells than the control group (P0) and the group treated with 5% gambier leaf extract cream (P1). However, the histopathological com-



P0 P1 P2 P3

Figure 2. Fibroblast cell proliferation overview on day 14 (magnification 400x)

parison between the P3 and P2 groups revealed a denser collagen fiber network in the P3 group. Furthermore, when comparing the control group P0 with the group treated with 5% gambier leaf extract cream (P1), it was apparent that the fibroblast cells in the P0 group were more numerous and somewhat densely packed, whereas the treatment group receiving 5% gambier leaf extract cream (P1) displayed fewer fibroblast cells with a sparse distribution. However, the collagen fibers in the P1 group appeared thicker and denser than those in the P0 group. This phase indicates fibroblast proliferation.

Fibroblasts play a pivotal role in the repair process as they are responsible for preparing and producing structural protein products used during tissue reconstruction. The ability of flavonoids to increase the fibroblast count is supported by research that concluded that an increase in the number of fibroblasts is attributed to flavonoid compounds.

Table 4. Results of physiological observations on incision wounds

Group	Rats Number	Condition of the wound on day-													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control (P0)	1	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt
	2	Mb	Mb	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt
	3	Mb	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt
	4	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt
	5	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km
Treatment	1	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km
Dose 5%	2	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km
(P1)	3	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt	Km
	4	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km
	5	Mb	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km
Treatment	1	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km	-	-
Dose 7,5%	2	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt	Km	Km
(P2)	3	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km
	4	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt	Km
	5	Mb	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km
Treatment	1	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt	Km	Km
Dose 15%	2	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Kt	Km	Km
(P3)	3	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km	-
	4	Mb	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km	-
	5	Mb	Mb	Mb	M	M	Kt	Kt	Kt	Kt	Kt	Km	Km	-	-

Explanation: Mb (Swollen red), M (Red), Kt (Dry open), Km (Dry closed)

Discussion

In this study, the assessment of wound healing effects was based on a reduction in the length of the incision wounds. The extract used in this study was derived from gambier leaf. The formulation of the extract was in the form of a cream because of its excellent physical stability during storage at room temperature for 14 days, as evidenced by the absence of organoleptic changes, pH alteration, adhesiveness, spread ability, and viscosity, meeting the requirements for cream formulations. Additionally, cream formulations are more convenient for application and are generally preferred.[27] A total of 27 rats, each weighing an average of 108.87 grams, were used. Prior to treatment, the rats were acclimatized for

one week to adapt to their environment. The rats were divided into groups: Group I (P0) served as the control and received a base cream (0% extract), Group II (P1) received topical application of gambier leaf extract cream with a concentration of 5%, Group III (P2) received topical application of gambier leaf extract cream with a concentration of 7.5%, and Group IV (P3) received topical application of gambier leaf extract cream with a concentration of 15%. Two rats were housed together in a cage separated by a divider to minimize movement, ensure that it does not affect the incision wound healing process, and avoid any interaction between rats. Ideally, wound care should involve the use of plasters and gauze to prevent infection. However, during wound care, a challenge arose, as the plasters used could not adhere to the rat skin, leading to detachment, and rats often bit the plasters and gauze. Consequently, wound care in this study was conducted without using plasters or gauze. Incision wounds were made with a length of 2 cm and a depth of 0.2 cm, but the widening of the wounds exceeded 0.2 cm. This was due to the movement of the rats during induction, even though they were locally anesthetized with chloroform. The incision wounds were treated daily, and wound measurements were performed daily using calipers during the 14-day healing period.

Redness (erythema) is the first noticeable sign in the inflamed area. According to Qomariah et al.,[28] redness in rat wounds is caused by inflammation. Visual observations during the study of 20 rats with incision wounds revealed erythema and swelling from day 1 to day 3 after treatment with 5%, 7.5%, 10%, and the base cream. This indicates the inflammation phase, which involves blood clot formation as a reaction to activated platelets and the release of fibrinogen by blood vessels. However, on day 4, Groups P2 and P3 entered the proliferation phase, characterized by tissue regeneration, and did not display erythema, while Groups P1 and the control group (P0) still exhibited erythema. The incision wounds in the treatment groups treated with 7.5% extract (P2) and 15% extract (P3) experienced the fastest maturation phase, with all five rats closing their wounds completely by day 10, leaving only scars. In contrast, Groups P1 and P0 had wounds that were partially closed by day 10, forming scabs, indicating the proliferation phase. This indicated the initiation of new cell growth with the edges of the wounds coming together. The formation of scabs indicates the end of the inflammatory phase and marks the beginning of the proliferation phase, leading to perfect skin formation.

The wound healing process consists of three phases: inflammation, proliferation, and maturation.[29] The average duration of the wound healing phases from day 1 to day 4 in the groups treated with gambier leaf extract cream, specifically Group P2 (7.5%) and P3 (15%), showed a quicker reduction in wound length compared to Groups P1 (5%) and the control group (C), which experienced inflammation until day 6. This is attributed to the bioactive compounds in lemongrass, namely, flavonoids, which can halt bleeding and act as anti-inflammatory agents that affect the production of inflammatory cells during the inflammation phase of wound healing. As astringents, tannins can reduce mucosal permeability and strengthen mucosal binding, thereby preventing irritation. Tannins indirectly affect mucosal and bacterial wall permeability, causing bacteria to contract and die. Phenolic acid in gambier prevents cell damage caused by free radicals and prevents inflammation and swelling.

The study results indicated that the group receiving 15% gambier leaf extract cream (P3) was more effective than the other groups. However, the treatment group with 7.5% gambier leaf extract cream (P2) also exhibited significant healing effects after the treatment period compared with the group receiving 5% gambier leaf extract cream and the base cream (P0). This is because at a concentration of 15%, secondary metabolites in the gambier leaf extract had a pronounced effect on wound healing. Conversely, at lower concentrations, they only inhibited microorganisms, making them less effective for wound healing. This aligns with Purbowati et al.,[30] who stated that at low concentrations, antibacterial agents only inhibit (bacteriostatic), but at high concentrations, they are bactericidal and kill microorganisms.

Conclusion

In this study, it was found that the group receiving 15% gambier leaf extract cream was more effective in the healing of incision wounds in white rats than the groups receiving 5% and 7.5% gambier leaf extract cream. However, the condition of the group receiving 7.5% gambier leaf extract cream approached that of the group receiving 15% gambier leaf extract cream. This was attributed to the fact that at a concentration of 7.5%, the secondary metabolites in the gambier leaf extract had already begun to

exert an effect on wound healing. However, at lower concentrations, these compounds only inhibit microorganisms, making them less effective in wound healing.

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