

Effectiveness Of Incision Wound Healing Of *Pandanus Amaryllifolius Roxb* In Wistar Rats

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Abstract.

The incision is a condition of tissue discontinuity that is commonly encountered with the risk of prolonged healing time, imperfect wound healing, infection, etc. Assessment of the wound healing activity of the incision is the purpose of this research, which includes an examination of wound contraction, the thickness of collagen fibers, and the number of new blood vessels formation. In total, 25 rats will be divided into 5 test groups, each consisting of 5 rats; the control group (cream base treated), standard group (Povidone Iodine), followed by the treated group with 10%, 20%, and 30% pandan leaf extract ointment. The preliminary screening of phytochemical analysis showed the presence of alkaloids, saponins, flavonoids, tannins, triterpenoids, and steroids and glycosides, with the total content of phenols and flavonoids from the ethanolic extract of Pandan Wangi is 20.95 GAE mg/gram extract, and 23.57 TAE mg/gram extract. Wound contraction examination result showed a significant difference between the treatment groups on day 16 and a sign the pairs of groups after being given the ointment with each concentration ($p < 0.05$), especially in the group with 30% fragrant leaves extract and the standard group. Examination of new blood vessels showed a significant difference ($p < 0.05$), especially with 30% of the leaves extract ointment. There was also a significant difference in collagen density in the group given pandanus ethanol ointment, especially at the 30% pandanus extract content against the group that was only given the ointment base. This research strongly suggests that the fragrant pandan leaf extract ointment is effective in healing incisional wounds and promises the potential for further studies on the pharmacological aspects.

Keywords: Pandanus extract, Ointment, Wound Healing, Angiogenesis, Collagen fiber

I. INTRODUCTION

One of the mechanical damage to the skin is the occurrence of tissue discontinuities caused by cuts, lacerations, friction wounds (abrasions), pulling wounds (avulsions), penetrating wounds (penetration), bites, burns, and surgery. Wounds that occur on the skin will stimulate wound healing that will involve various substrates cells and processes that are physiological processes that include the hemostasis phase characterized by vasoconstriction, primary hemostasis, and secondary hemostasis (Rodrigues et al., 2019). The complex process of wound healing, especially incisions, is still one of the challenges in health, considering the number of cases and treatment methods developed in the current era of globalization. Many studies on wound healing have used natural ingredients such as herbal plants and fruits to be formulated as preparations to help wound healing. A traditional herbal which can be considered is the fragrant pandan leaf (*Pandanus amaryllifolius Roxb*). Fragrant pandan leaves have 2-acetyl-1-pyrroline (ACPY) compounds and oxygenation degradation of yellow carotenoid pigments (Silalahi, 2018). In addition to these compounds, secondary metabolites such as alkaloids, flavonoids, phenols, saponins, and terpenoids in the fragrant pandan leaf make it a traditional diabetes treatment and help the wound healing process. Ningrum et al. (2015) stated that pandan leaves contain carotenoid and xanthophyll compounds. Carotenoids, especially carotene, are precursors of norisoprenoids. Carotenoids have long been known as antioxidant compounds to protect against various degenerative diseases such as cancer, coronary heart disease, or diseases related to aging.

II. METHODS

2.1 Materials

The ingredients in this study were fragrant pandan leaves, aquadest, lanolin, solid paraffin, Cetostearyl alcohol, white vaseline, gauze, oil paper, filter paper, Povidone Iodine, rat pellets, Neutral Buffered Formalin (NBF) 10%, hematoxylin & eosin dye, and aquadest. The tools used are digital scales,

caliper, filter, lumping digital camera, microtome (Leica RM2235), maceration vessel, knife, rotary evaporator, water bath, gel container, stirring rod, object-glass, cover glass, organ storage container.

2.2 Extract Preparation

The fragrant leaves were washed using running water to make pandanus extract and then cut into small pieces and dried for several days at room temperature. The dried leaves were mashed using a blender machine and put into a maceration vessel. The vessel is filled with 70% ethanol in a ratio of 1:6 (Simplified: solvent). Furthermore, the vessel is closed and left for five days in a place protected from the sun and damp, stirring occasionally. When it has been five days, then filtered and re-extracted the dregs, done one time to get more extract. Then the extract was concentrated with a vacuum rotary evaporator at a temperature of 60°C and evaporated through heating until a thick extract was obtained (Aini, 2016; Nagar, 2016; Sugihartini, 2017). After extraction, preliminary phytochemical tests were carried out (Buckley 1966; Richardson and Harborne 1985; Suman et al. 2016).

Ointment of pandanus extract has been prepared with the following formulation:

Table 1. Ointment Formulation

Composition	Ointment Base	Pandanus Ointment 10%	Pandanus Ointment 20%	Pandanus Ointment 30%
Pandanus extract (ml)	-	1 ml	6	9
Stearic acid (g)	15	15	15	15
Cetyl alcohol (g)	6	6	6	6
Potassium hydroxide (g)	0.7	0.7	0.7	0.7
Methyl Paraben (g)	0.3	0.3	0.3	0.3
Propyl Paraben (g)	0.06	0.06	0.06	0.06
Glycerine (g)	5.0	5.0	5.0	5.0
Propylene glycol (g)	3.0	3.0	3.0	3.0
Aquadest (g)	Add 100	Add 100	Add 100	Add 100

2.3 Animal Experiment

The experimental animal is Wistar rats with a sample size of 25 white male rats, divided into 5 test groups. Each test group consists of 5 white rats calculated by the Federer formula. All rats were placed in animal cages with the same size and pellet food for rats in all treatment groups, with a temperature of 24-27°C, a 12-hour morning cycle, and a 12-hour night cycle. All groups are acclimatized for seven days. On the eighth day, they will be shaved using a razor before wounding; experimental wounds are performed under sterile conditions under anesthesia using ketamine (75 mg/kg i.m) which had previously been fasted. The incision wound was made on the Rat's back tail and pulled up with a length of 6 cm and a depth of 2 mm. Then all groups of rats will be treated in topical application of Povidone Iodine, ointment base, and 10%, 20%, 30% fragrant pandan leaf extract ointment two times a day for 16 days. Then at the end of the study, the rats will be euthanized for taking skin specimens. (Jeong, 2017; Ersel, 2016; Saifudin, 2020; Angkasa, 2019).

Experimental rats were divided into five groups; Control (treated with ointment base), Standard (treated with Povidone-Iodine), PDW 10% (treated with Pandanus extract 10%), PDW 20% (treated with Pandanus extract 20%), PDW 30% (treated with Pandanus extract 30%). The examination of the effectiveness of the incision was carried out on days 0, 4, 8, 12, 16. The evaluated aspects of the incision wound healing activity included wound contraction and histopathological examination (Asrade et al., 2020; Nagar et al., 2016). Wound contractions were measured by measuring the diameter of the wound using a caliper every four days, then Wound contractions were calculated using the following formula (Asrade et al., 2020; Nagar et al., 2016):

$Wound\ contraction\ (\%) = (initial\ wound\ size - wound\ size\ at\ specific\ days) / (wound\ size\ at\ specific\ days) \times 100\%$.

At the end of the study, the test animals will be euthanized using a high dose of ether solution by inhalation. The back area where the skin will be taken will be shaved first from the hair that grows back. Skin samples were taken with a thickness of ± 3 mm to the subcutaneous area along the 2.5 cm. The skin sample was then fixed with Neutral buffered formalin or 10% NBF solution and left for ± 48 hours. This block of skin tissue would be incised again using a microtome thickness of 5 m to prepare histopathological reading preparations. Samples were then stained with Hematoxylin & Eosin (H&E) for the histological examination to observe the formation of new blood vessels and the density of collagen fibers. (Nanda et al, 2017; Nagar et al, 2016). Histological observation criteria for collagen and angiogenesis followed the previous studies (Shakya et al., 2016; Nasiri et al., 2015).

2.4 Statistical Analysis

The statistical analysis used in this study was the One-Way ANOVA test, followed by a post-hoc LSD test. The Shapiro-Wilk test was performed before the ANOVA test to determine the normality. The Levene test was performed to examine the homogeneity of two or more groups of sample data originating from the same population (homogeneous). Then the histopathological changes data were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney-U difference test because there was a significant difference with a significant degree of $p < 0.05$.

III. RESULTS

3.1 Phytochemical Analysis

The phytochemical screening of fragrant pandan leaves extracted using ethanol showed the presence of alkaloids, saponins, flavonoids, tannins, triterpenoids, steroids, and glycosides—the details as shown in Table 2.

Table 2. Preliminary Screening of Phytochemistry

Secondary Metabolites	Methods	Interpretation
Alkaloid	Bouchardart	+
	Maeyer	-
	Wagner	-
	Dragendorff	+
Triterpenoid and Steroid	Salkowsky	-
	Lieberman-Burchard	+
Saponin	Aquadest + Alcohol 96%	+
Flavonoid	FeCl ₃ 5%	+
	Mg _(s) + HCl _(p)	+
	NaOH 10% _(p)	-
	H ₂ SO _{4(p)}	+
Tannins	FeCl ₃ 1%	+
Glycoside	H ₂ O + NaOH 10%	+

The analysis was then continued by determining the total content of phenols and flavonoids, as shown in Table 3.

Table 3. Total Phenols and Flavonoids content

Compounds	Total-Content
Phenols (GAE mg/ gram extract)	20.95 \pm 0.39
Flavonoids (QE mg/ gram extract)	23.57 \pm 0.43

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent.

3.2 Physical Characteristics of Ointment

The homogeneity test carried out on all ointment preparations gave homogeneous results for each preparation based on the uniformity of color and the absence of lumps and granules. The pH data showed that the pH values of all ointment preparations ranged from 5.62 to 5.79 and had met the requirements for a safe pH value for the skin, namely pH 5.5 to 6.0.

3.3 Wound Contraction Analysis

Wound contraction as one of the incision wound healing parameters was analyzed according to the normality of the data from each measurement time. The data will be determined for normality using Shapiro-Wilk and Levene's homogeneity test. Then analyzed by One Way ANOVA test at 95% confidence level ($p < 0.05$) with $\alpha = 0.05$. Further analysis was carried out using Post Hoc Tukey HSD (Honestly Significance Different) to determine the level of difference in the influence of each group.

Table 4. Normality Test of Wound Healing Effectivity of Pandanus Ointment

Parameter	<i>p-value</i>	Interpretation
Wound contraction K (-)	0.060	Normal
Wound contraction Povidone Iodine	0.064	Normal
Wound contraction PDW 10%	0.107	Normal
Wound contraction PDW 20%	0.210	Normal
Wound contraction PDW 30%	0.132	Normal

From the data in Table 4, it can be seen that the wound contraction data in the control group, Povidone Iodine, 10%, 20%, and 30% fragrant pandanus, were usually distributed. So that the analysis was continued with the One Way ANOVA test then followed by the Post Hoc Test. The homogeneity test by Levene's method showed that the sig value was $0.070 > 0.05$. It can be concluded that the data was homogeneous. They qualified for the One Way ANOVA test. Based on the ANOVA test results with a Sig value of $0.070 < 0.05$, it can be concluded that there are differences between the means in all treatment groups. To see significant differences in each group, the LSD test was performed, as shown in Table 5.

Table 5. Least Significant Difference (LSD Test of Wound Healing Effectivity

Group	Control (K-)	Standard (PI)	PDW 10	PDW 20	PDW 30
Control (K-)	-	<0.001*	0.407	0.097	<0.001*
Standard (PI)	<0.001*	-	0.072	0.331	0.626
PDW 10	0.407	0.072	-	0.400	<0.001*
PDW 20	0.097	0.331	0.400	-	0.146
PDW 30	<0.001*	0.626	<0.001*	0.146	-

*) significant value ($p > 0.05$)

The results of the Least Significant Difference (LSD) test on wound contraction incisions in rats shown in Table 8 show that there were significant differences between the pairs of groups after being given the ointment with each concentration, namely the difference in wound length in the negative control group (K-) which was only given ointment base with the group that was given Povidone Iodine (PI) and the group that was given pandan fragrance ointment with a concentration of 30% (PDW 30), while there was no significant difference between (K-) and the group that was given pandan fragrance ointment with a concentration of 10% (PDW 10). and 20% (PDW 20). Then there was a significant difference between the group (PDW 10) and the group (PDW 30). In the group (PDW 20), there were no significant differences in all treatment groups. The group (PDW 30) had a significant difference in the (K-) and (PDW 10) groups, while the Povidone Iodine and (PDW 20) groups did not show a significant difference.

3.4 Histopathology Analysis

In addition to wound contraction, other parameters that are also evaluated in assessing wound healing are the formation of new blood vessels and the density of collagen fibers through histopathological examination with Hematoxylin & Eosin (H&E) staining. The formation of new blood vessels based on angiogenesis is shown in Figure 1.

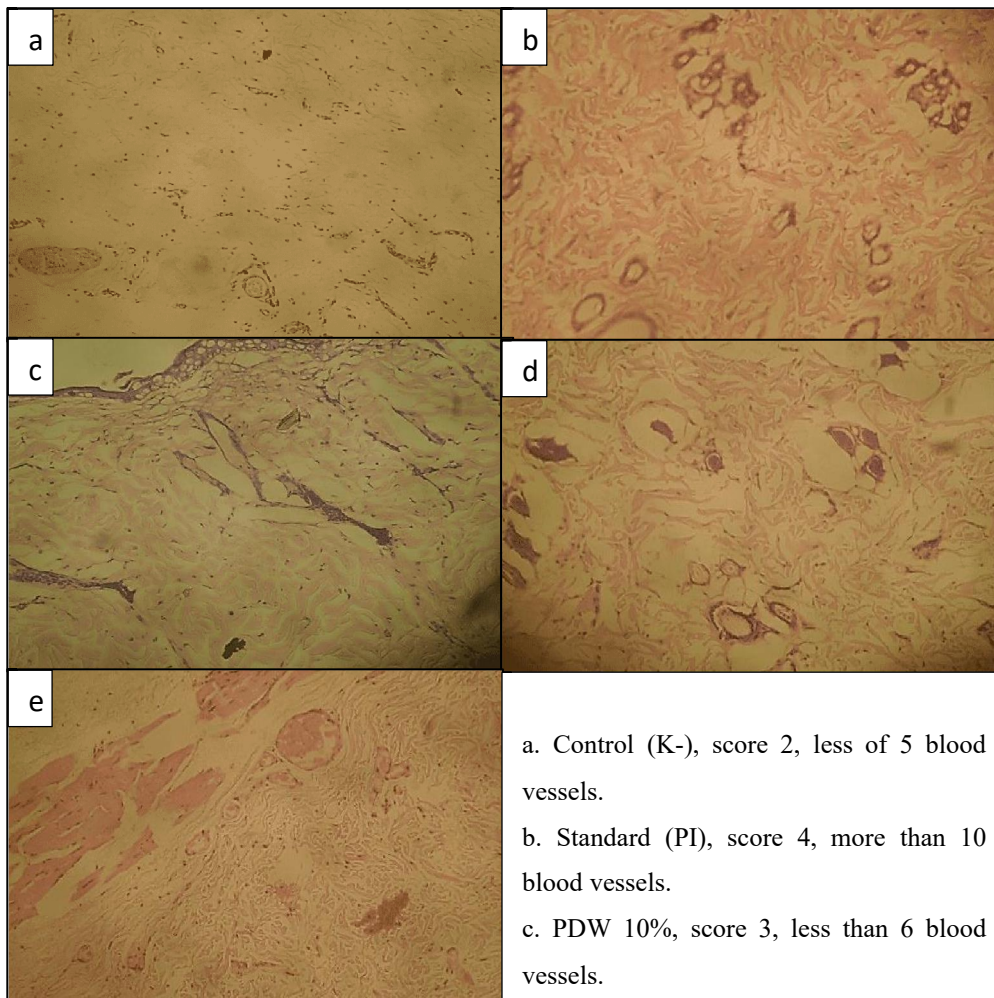


Fig 1. Formation of new blood vessels of Control (a), Standard (b), PDW 10% (c), PDW 20% (d), and PDW 30% (e),

Furthermore, the statistical analysis using Mann Whitney-U on the new blood vessels formation is shown in Table 6.

Table 6. Mann Whitney-U Test of the New Blood Formation

Group	Control (K-)	Standard (PI)	PDW 10	PDW 20	PDW 30
Control (K-)	-	<0.05*	<0.05*	<0.05*	<0.05*
Standard (PI)	<0.05*	-	<0.05*	0.824	0.276
PDW 10	<0.05*	<0.05*	-	<0.05*	<0.05*
PDW 20	<0.05*	0.824	<0.05*	-	<0.05*
PDW 30	<0.05*	0.276	<0.05*	<0.05*	-

*) significant value ($p < 0.05$)

In the group of control (K-) given the ointment base to the group given Povidone-iodine (PI), the group given the pandan fragrance extract ointment 10% (PDW10), 20% (PDW20), and 30% (PDW30) showed a significant difference ($p = 0.05$). This shows that there are differences in blood vessel formation between the K(-) group and the Povidone (PDW10), (PDW20), and (PDW30) groups. The (K-) and (PDW10) groups showed a significant difference in the Povidone group. It can be interpreted that there are

differences in the formation of blood vessels. Meanwhile, there was no significant difference in the Povidone group (PDW20) and (PDW30). In the group (PDW10) against Povidone, (K-), (PDW20), and (PDW30)) showed a significant difference. In the (PDW20) and (PDW30) against (K-) groups, (PDW10) showed a significant difference. In contrast, the groups (PDW20) and (PDW30) against Povidone showed no significant difference. It can be interpreted that there is no difference in the formation of blood vessels.

Based on the criteria for observing the histological picture of collagen density, the results are shown in Figure 2.

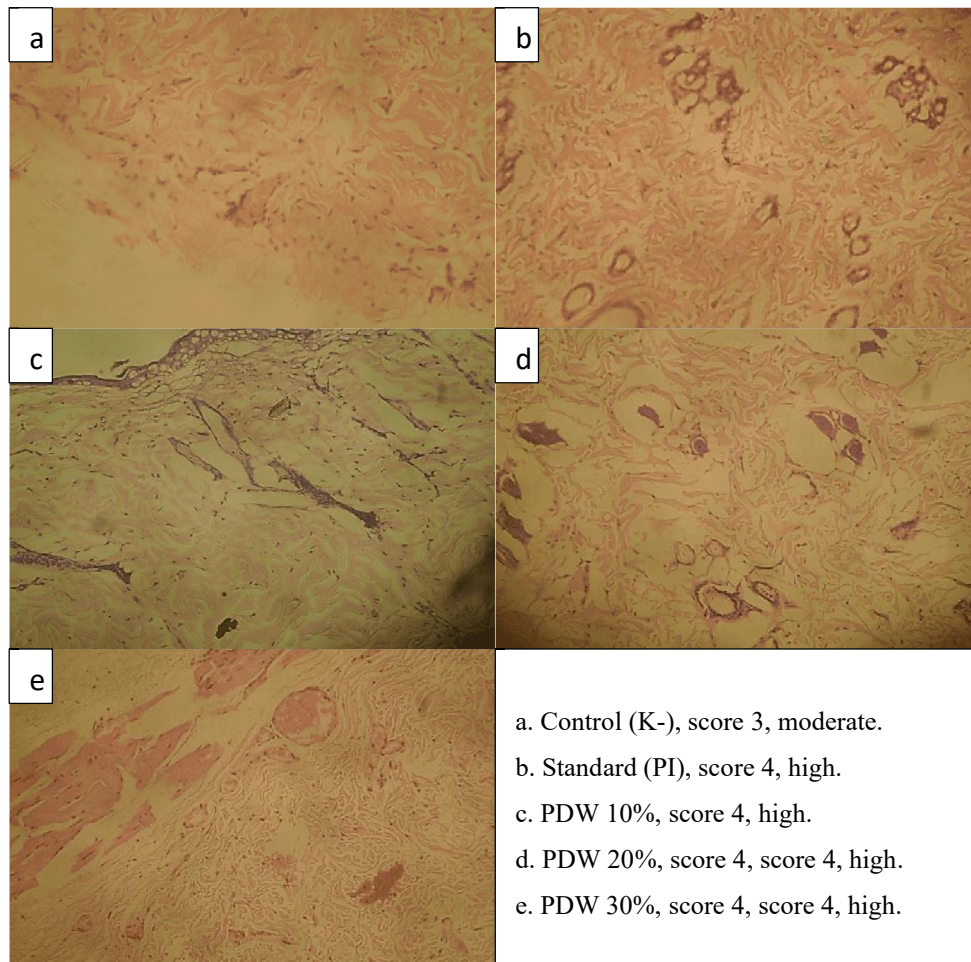


Fig 2. Collagen Density of Control (a), Standard (b), PDW 10% (c), PDW 20% (d), and PDW 30% (e)

In the group (K-) given the ointment base to the group given Povidone-iodine (PI), the group given the pandan fragrance extract ointment 10% (PDW10), 20% (PDW20), and 30% (PDW30) showed a significant difference ($p = 0.05$). This indicates that there is a difference in collagen density between the K(-) group and the Povidone (PDW10), (PDW20), and (PDW30) groups. Meanwhile, in the Povidone group (PDW10), (PDW20), and (PDW30), there was no significant difference. It can be interpreted that there is no difference in collagen density between the Povidone (PDW10), (PDW20), and (PDW30) groups.

Table 7. Mann Whitney-U Test of the Collagen Density

Group	Control (K-)	Standard (PI)	PDW 10	PDW 20	PDW 30
Control (K-)	-	<0.05*	<0.05*	<0.05*	<0.05*
Standard (PI)	<0.05*	-	0.108	0.190	0.200
PDW 10	<0.05*	0.091	-	0.091	0.071
PDW 20	<0.05*	0.190	0.091	-	0.140
PDW 30	<0.05*	0.200	0.071	0.140	-

*) significant value ($p < 0.05$)

IV. DISCUSSION

The number of compounds in fragrant pandan leaves (*Pandanus amaryllifolius* Roxb) plays a role in wound healing. Some theories state that flavonoids are known to play a role in capturing free radicals or can function as natural antioxidants. The antioxidant activity allows flavonoids to capture or neutralize free radicals to repair damaged tissue. (Lolok, et al 2020; Dewanti, et al 2017). In this study, it was found that there was a reasonably good flavonoid content from the extraction method that had been carried out so that it was able to affect the healing of cuts in rats which can be seen in the results of the research parameters that have been described, this is also reinforced by research conducted by Dewi et al. (2019) in pandan leaf plants which from the results of his research showed the optimum flavonoid content with a long extraction time of 40 minutes which was 7.1%, which means that an increase in temperature and extraction time will affect the compounds in solution, the extraction temperature is too high and the extraction time is too high. The length of time and the optimum time limit can cause the loss of compounds in solution due to evaporation; if the extraction temperature is too low, it will cause not all active compounds to be extracted from the material and result in low active compounds obtained (Dewi et al., 2019). Wound healing on fragrant pandan leaves, which contain flavonoids in powder mask preparations, also shows the ability to inhibit skin damage, maintain normal skin regeneration, and play an essential role in absorbing and neutralizing free radicals.

On the other hand, the saponins contained in the fragrant Padan leaves were found with the formation of foam on phytochemical examination, also contributing as antioxidants in the formation of skin collagen tissue which is needed in the formation of new tissue in wound healing, maintaining the balance of oil and skin moisture. The ability of flavonoids in *Pandanus amaryllifolius* Roxb to scavenge free radicals was also found in the reduced histological changes in the liver induced by carbon tetrachloride (CCl₄) compared to the group that did not receive treatment with *Pandanus amaryllifolius*. Shameenii A. et al. (2021) explained that the antioxidant and free radical scavenging properties of *Pandanus amaryllifolius* could protect the liver from extreme damage caused by carbon tetrachloride (CCl₄). At the same time, research related to fragrant pandan leaf extract can inhibit the cytotoxic effect of the Head And Neck Squamous Cell Carcinoma Cell Line (HNSCC cell line; HN31) (Suwannakul, 2018). This may be due to the fragrant pandan leaf extract, which shows a high content of phenolics, flavonoids, carotenoid compounds, and xanthophylls. Carotenoids, especially carotene, are precursors of norisoprenoids.

The presence of these secondary metabolites indicates that *Pandanus amaryllifolius* has high antioxidant activity, so it contributes to its hepatoprotective activity by inhibiting oxidative stress that can cause inflammation, cardiovascular disease, degenerative diseases diabetes, cancer development, aging. Research conducted by Suryani et al. on pandan leaf ethanol extract (*Pandanus amaryllifolius*) and the fractionation process showed that the ethyl acetate fraction of pandan leaf ethanol extract had a higher reducing ability than ethanol extract. However, its DPPH radical scavenging power was higher. low with the calculation of EC₅₀ between fractions of hexane, ethyl acetate, ethanol extract, and commercial vitamin E respectively 0.90; 8.66; 4.51; and 11.76 mg/mL, which indicates the ethyl acetate fraction of pandan leaf ethanol extract has the potential as a source of natural antioxidants that help wound healing. (Suryani, 2017; Ismail et al., 2017; Rahmi, 2021; Shameenii, A. et al., 2021). This study is also in line with research conducted by Safitri et al. (2020), showing that in wound healing, flavonoids also have an anti-inflammatory effect by inhibiting signals in microglia cells, resulting in impaired inducible nitric oxide synthase (iNOS) and decreased nitric oxide (NO) levels.). This results in analgesic and neuroprotective effects so that pain in the skin affected by wound trauma is reduced. Besides that, flavonoids can also stimulate vascular endothelial cell growth factor (VEGF) in the angiogenesis process, which is very important in the healing process by stimulating growth factors. such as PDGF, EGF, TGF- β , and FGF.

The difference in this study is the type of plant used, namely pandanus leaf plants were used, while the study of Safitri et al. (2021) used red spinach leaves. In addition, fragrant pandan leaves also have antimicrobial activity (MICs range 30 - 125 g/mL), especially against *Porphyromonas gingivalis*, *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus salivarius*, which is strongly influenced by the total content of flavonoids and phenols with a total value of phenol 57.25 \pm 0.02 mg/g and IC₅₀ 110 \pm 36.42 were examined by the DPPH method. The anti-inflammatory, antioxidant and antimicrobial

content in 50% pandan leaves extract also showed accelerated healing in post-gingivectomy wound models in Wistar rats. It can increase the number of blood vessels with p -value = 0.000 ($p < 0.05$) through examination of histological preparations with staining. Hematoxylin-eosin compared with 2% CMC-Na and Ivoclar gel. The increase in the number of new blood vessels is strongly influenced by growth factors such as PDGF, VEGF, and bFGF, as well as endothelial progenitor cells (Nofikasari, 2016). In line with this study, rats that received routine treatment with pandan leaf extract ointment with one of the wound healing parameters, namely collagen thickness, and angiogenesis, showed significant differences compared to the untreated group.

The use of pandan leaves in solid preparations (ointments, creams, gels) in other studies has also begun to be carried out; this aims to maintain its effectiveness and facilitate its application to various types of wounds with various dosage forms in the medical world, one of which is in research that observes the role of the dosage form. Fragrant pandan leaf gel with 70% ethanol extract on burn healing, the formula with 12.5% fragrant pandan leaves resulted in the most effective percentage of burn healing, 99.33%, with a healing time of 16 days. The preparation of pandan leaf ethanol extract gel based on Hydroxy Propyl Methyl Cellulose showed the results of physical stability tests, including organoleptic, pH, and dispersibility tests. This shows that the manufacture of solid preparations of pandan leaf extract gives good results. (Dewanti et al., 2020). However, another study tested the effectiveness by using an ointment base preparation on fragrant pandan leaves on male white mice with a concentration of 5%, 7.5%, 10%. It shows better results at 13 days with a healing percentage of up to 100% at a 10% ointment concentration. In research, Rini et al. (2018) also found the content of saponins, flavonoids, alkaloids, tannins, and polyphenols. The flavonoid content of pandan leaves can also accelerate the wound healing process by inhibiting the inflammatory process.

Flavonoids inhibit wound inflammation in several ways, namely by inhibiting capillary permeability, inhibiting arachidonic acid metabolism so that prostaglandin production is reduced, inhibiting the secretion of enzymes in lysosomes, which are inflammatory mediators inhibiting mediators of inflammatory cell proliferation in wounds. So in this study, the ointment formulation was the choice compared to other topical formulations. In line with this study, the effectiveness of burn healing can also be assessed from the acceleration of angiogenesis regeneration, collagen fiber thickness, and wound contraction. (Rini et al, 2018; Rokhmah et al, 2021). Analysis of pandan leaves with ethanol-water extract of leaves by immersion, or microwave-assisted extraction (MAE) method showed the presence of phenolic and flavonoid compounds and have antioxidant activity. Pandan leaf extract contains free amino acids such as aspartic acid, serine, lactic acid glutamate, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, lysine, isoleucine, leucine and phenylalanine, glucose, and fructose which may be precursors of ACPY (2-acetyl-1-pyrroline) and may play an essential role. In another study, pandan extract showed good potential for bioactive compounds such as Pandan leaves containing several bioactive compounds of carotenoids, flavonoids such as catechins, naringin, kaempferol, rutin, epicatechin, myricetin, luteolin, quercetin and phenolic acids including gallic acid, cinnamic acid, ferulic acid, caffeic acid. Leaf extract (*Pandanus amaryllifolius*) showed antioxidant activity and which is higher than the extract in the root of *Pandanus amaryllifolius*, thus pandan leaf extract has the potential as a suitable natural antioxidant, which can serve as an alternative to synthetic antioxidants used in topical emulsions of plant extracts.

Pandanus amaryllifolius to obtain more optimal antioxidant activity to accelerate and affect wound healing which includes wound contraction, formation of new blood vessels, and density of collagen fibers formulated in topical preparations in the form of ointment formulations. (Silalahi, 2018; Bhuyan & Sonowal, 2021). Tannins contained in *Pandanus amaryllifolius* are very complex organic compounds that are amorphous, have a phenolic hydroxyl group, and can form stable cross-links with proteins and biopolymers. A study conducted by Dewi et al. (2019) showed that the optimal tannin content in pandan (*Pandanus amaryllifolius*) leaves obtained using distilled water was 60 minutes. Phytochemical screening of *P. amaryllifolius* extract showed the presence of tannins, alkaloids, saponins, terpenoids, and flavonoids except for pholabanin and cardiac glycosides. The total phenolic content (TPC) was 35.99 ± 0.04 mg GAE/g and the total flavonoid content (TFC) was 59.96 ± 0.013 mg CAE/g extract. The tannin content in the ethanolic extract of fragrant pandan leaves is useful as an astringent that causes shrinkage of skin pores, stops exudate

and light bleeding so that it can cover wounds, and prevent bleeding in wounds that inhibit the inflammatory process (Ambreen & Mirza, 2020; Shameenii, 2004). A., et al, 2021; Rokhmah et al, 2021; Dewi et al, 2019).

V. CONCLUSION

Phytochemical test of Pandan Wangi Ethanol extract, which is made, contains chemical compounds in alkaloids, saponins, flavonoids, tannins, triterpenoids and steroids, and glycosides. The administration of Pandan Wangi Ethanol extract ointment is effective in healing incisional wounds. This can be seen from the significant difference in wound contraction on the 16th day, where the P-value = <0.05. This indicates a significant difference in the healing of the rat incision wound. On the density of collagen, There was a significant difference in the group given the 10%, 20%, and 30% ethanol extract of pandan fragrance ointment against the group that was only given the ointment base. This can be seen from the value of $p = <0.05$, which means that there is an ointment of fragrant pandan extract that is effective in forming collagen in rat incision wounds. There was also a significant difference in the formation of new blood vessels in the group given the 10%, 20%, and 30% ethanol extract of pandan fragrance ointment against the group that was only given the ointment base. This can be seen from the value of $p = <0.05$, which means that there is an effective pandan fragrance extract ointment in forming new blood vessels in the rat incision wound.

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