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Original Article

Comparison of the Effectiveness of Cream Extracts and VEGF Expression on Incision Wounds in Male Wistar Rats Treated with Ethanol Extracts of Papaya Leaf, Fruit Flesh, and Seeds (*Carica Papaya L.*)

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ABSTRACT

Background: Papaya (*Carica papaya Linn*) is a tropical fruit plant known for its medicinal benefits, including wound healing. **Objective:** This study aimed to analyze and compare the potential of papaya leaf, fruit, and seed extracts in accelerating incision wound healing in male Wistar rats. Additionally, we compared the flavonoid content and stability of the extracts in cream formulations. **Materials and Methods:** Creams containing 30% ethanol extracts of papaya leaf, fruit, and seeds were prepared and applied to the backs of male Wistar rats for two weeks. The wound diameter, epithelial thickness, fibroblast count, macrophage count, collagen density, and VEGF expression were measured after two weeks. **Results:** Our findings revealed no significant differences between the effects of the papaya leaf, fruit, and seed ethanol extracts on wound diameter, epithelial thickness, fibroblast count, macrophage count, and collagen density ($p > 0.05$). There were also no significant differences when compared to the negative control group ($p > 0.05$). The highest flavonoid content was found in the ethanol extract of papaya leaves. Application of the ethanol extract creams of leaf, fruit, and seeds had a significant effect on angiogenesis, as evidenced by VEGF expression ($p = 0.002$). **Conclusion:** Papaya leaf, fruit, and seed extracts are promising products for wound healing. Further research is recommended to conduct periodic histopathological examinations during the wound healing process and to explore different extract concentrations for optimal results.
Keywords: Incision wound; Male Wistar rats; Papaya; VEGF.

INTRODUCTION

A wound is a discontinuity, damage, or loss of part or all of the body tissue.[1,2] Wound healing is a dynamic and complex process aimed at restoring tissue integrity and homeostasis. Several studies have shown that the application of papaya leaf extract can accelerate the formation of epithelial tissue in wounds. Previous studies have shown that ointments made from young papaya fruit latex, papaya leaf extract, and papaya fruit extract are effective in accelerating wound healing.[3,4] Data in the review also indicates that the administration of papaya plant parts, including latex, stem, seeds, and leaves, shows good effectiveness in the wound healing process and possesses antibacterial activity against wound infection-causing pathogens.[5] The objective of this study is to compare the effectiveness of ethanol extracts from papaya leaves, papaya fruit, and papaya seeds in the healing of incision wounds.

MATERIALS AND METHODS

Ethics Statement

This study was approved by the Ethics Committee of the Center for Health Research, Universitas Prima Indonesia, with approval number 024/KEPK/UNPRI/IX/2022.

Plant Collection and Identification

The materials used in this study included male Wistar rats, papaya seeds, papaya leaves, papaya fruit, povidone-iodine, ether, decamethonium, lidocaine, 90% ethanol, rat feed, water, aquadest, and rice husks. The research was conducted at the Animal House and Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA), Phytochemistry and Cosmetology Laboratory of the Faculty of Pharmacy, and the Anatomical Pathology Laboratory of the Faculty of Medicine at Universitas Sumatera Utara. Additionally, the plant samples were collected from the Tunas Baru plantation, Jl. Batako Dusun II, Tandam Hilir II, Deli Serdang, North Sumatra.

Preparation of Plant Extracts

Papaya leaf, seed, and fruit extracts were obtained through maceration with 70% ethanol and prepared as creams. Each cream was applied to incised wounds twice daily (morning and evening) in a consistent manner.

Ethanol Extract Preparation

Papaya Leaf Extract

The harvested papaya leaves were washed with running water, dried at room temperature for three

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days, and ground into a fine powder. A total of 800 grams of powdered leaves was macerated with 70% ethanol for 72 hours. The macerate was filtered and evaporated at 40°C using a rotary evaporator to obtain a thick extract.

Papaya Fruit Extract

Papaya fruit was washed, its skin and seeds removed, and the flesh sliced and dried at 50°C. After grinding into powder, 800 grams of the fruit powder was macerated with 70% ethanol for 72 hours, followed by filtration and evaporation at 40°C to yield the extract.

Papaya Seed Extract

Papaya seeds were separated from the flesh, washed, dried, and ground into a fine powder. The 800 grams of seed powder underwent the same maceration, filtration, and evaporation process as the leaf and fruit extracts.

Cream Formulation

To formulate the creams, the oil phase (liquid paraffin, cetyl alcohol, stearic acid, and propylparaben) was heated to 70°C and mixed with the aqueous phase (propylene glycol and methylparaben) at the same temperature. After homogenization, 30% ethanol extracts of papaya leaf, fruit, and seeds were added to the cream base. The 30% ethanol extract was chosen because it is the highest concentration that can be prepared in the local laboratory and has not been studied by previous researchers.

Sample Selection and Grouping

The study included 30 male Wistar rats, 3 months old, weighing 150-250 grams, divided into 5 groups; The sample size was calculated using the Federer formula, resulting in 6 rats per group such as K(-) (negative control with no treatment), K(+) (positive control treated with povidone-iodine), Seed group (treated with papaya seed cream), Leaf group (treated with papaya leaf cream), Fruit group (treated with papaya fruit cream)

Incised Wound Procedure and Treatment Application

Wounds of 1.5 cm in diameter and 0.3 cm in depth were made on the shaved backs of anesthetized rats. Lidocaine was administered as local anesthesia (15-40 mg/kg body weight). Povidone-iodine or papaya extract creams were applied to wounds using cotton bud twice daily.

Histopathological Preparation and VEGF Immunohistochemistry

Skin tissue was fixed, processed, and embedded in paraffin for histological assessment. Sections (4 µm) were stained with Hematoxylin-Eosin and Masson's Trichrome for the evaluation of collagen density, epithelial thickness, and cellular components such as fibroblasts, macrophages, and blood vessels. VEGF expression was assessed using the Allred Scoring System after staining with Rabbit Anti-VEGF Rabbit Polyclonal Antibody IgG, with results calculated based on the proportion and intensity of staining.

Phytochemical Analysis (Flavonoids)

Flavonoid content in papaya extracts was quantified using a UV-Vis spectrophotometer at 438 nm, based on a calibration curve of quercetin.

RESULT

Sample Identification

Papaya (*Carica papaya L.*) samples were identified at the FMIPA Laboratory, Universitas Sumatera Utara. This identification aimed to confirm the plant's identity and ensure accurate sample selection for phytochemical analysis. Based on the identification results from the Herbarium Medanense, Universitas Sumatera Utara, the papaya samples were verified as follows:

Table 1. Taxonomic Classification of Papaya (*Carica papaya L.*).

Kingdom	Plantae
Division	*Spermatophyta*
Class	*Dicotyledonae*
Order	*Brassicales*
Family	*Caricales*
Genus	*Carica*
Species	***Carica papaya L.***

Quantitative Phytochemistry (Flavonoids) in Papaya Leaves, Fruit, and Seeds

Phytochemical screening was conducted to identify the secondary metabolites present in the prepared extracts. Previous research has shown that papaya contains flavonoids, alkaloids, tannins, saponins, and triterpenoids. However, this study focused only on the quantitative phytochemical analysis of flavonoids. Observations indicated that the papaya leaf extract had the highest total and average flavonoid content compared to fruit and seed extracts. The quantitative flavonoid analysis using a spectrophotometer is presented in Table 2 below.

Stability Testing of Papaya Leaf, Fruit, and Seed Extract Creams

The stability of the cream formulations was evaluated through organoleptic and homogeneity tests. The organoleptic test focused on changes in the cream's consistency, color, and odor. The homogeneity test was performed by applying a small amount of cream onto a glass surface to check the evenness of the mixture. Ensuring a homogeneous distribution of ingredients is crucial for maximizing the cream's effectiveness.

In the organoleptic test, all the cream formulations exhibited a semi-solid texture and had distinct odors. However, differences in color were noted: papaya leaf extract cream was dark green, papaya fruit extract cream was yellowish-brown, and papaya seed extract cream was white. The homogeneity test confirmed that the extracts were evenly distributed across the formulations, with no visible clumps. This uniformity ensures the even distribution of active ingredients, which is essential for the consistency and efficacy of the cream.

Viscosity Testing

The viscosity of the formulation was determined using a Rheosys Merlin VR viscometer with a 25mm concentric cylinder spindle. The cream was placed into a plate, and the cone was positioned to start the measurement. Measurement parameters were set to be identical for all formulations to ensure uniform treatment, and the process was run on a computer using the Rheosys Micra application.

Viscosity testing was performed to assess the thickness of the cream formulations. A desirable cream viscosity facilitates easy application. An optimal viscosity is indicated by a cream that is neither too thin nor too thick, as viscosity directly affects spreadability and adhesiveness. The results showed variations in viscosity levels among the formulations. Papaya fruit extract cream had the highest viscosity (2100.8 cps) compared to papaya leaf (912.3 cps) and seed extract creams (918.9 cps). These viscosity levels for leaf and seed extract creams were below the Indonesian National Standard (SNI) 1995 recommendation for skin preparations, which is 2000-50,000 cps. Additionally, the amount of active ingredients in a formulation impacts its viscosity; more active ingredients result in lower viscosity.

Table 2. Determination of flavonoid content in papaya leaves, fruit flesh, and seeds.

Sample weight (g)	Sample volume (ml)	FP	Absorbance	Avg. Absorbance	Conc. (µg/ml)	Total Flavonoid Content (mg QE/g extract)	Average Content
Papaya Leaves							
			0.248				
0,0101	10	1	0.248	0.248	18.5714	18.3875	
			0.248				18.4628 (mg QE/g extract)
			0.210				
0,0100	10	1	0.210	0.210	15.3012	15.3012	Or 1.8463 %
			0.210				
			0.302				
0,0107	10	1	0.302	0.302	23.2186	21.6996	
			0.302				
Papaya Fruit							
			0.069				
0,0107	10	1	0.069	0.069	3.1670	2.9598	
			0.069				3.1092 (mg QE/g extract)
			0.074				
0,0108	10	1	0.075	0.074	3.9414	3.6494	Or 0.3109%
			0.074				
			0.066				
0,0107	10	1	0.067	0.066	2.9088	2.7185	
			0.066				
Papaya Seeds							
			0.220				
0,0105	10	1	0.221	0.221	16.2478	15.4741	
			0.221				16.6421 (mg QE/g extract)
			0.261				
0,0110	10	1	0.262	0.261	19.6902	17.9002	Or 1.6642 %
			0.261				
			0.238				
0,0107	10	1	0.239	0.238	17.7108	16.5521	
			0.237				

Table 3. Results of organoleptic and homogeneity testing of papaya leaf, fruit, and seed extract creams.

Formulation	Tests	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Papaya Leaves	**Aroma**	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent
	Colour	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green	Dark Green
	Form	Cream	Cream	Cream	Cream	Cream	Cream
	Homogenity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen
Papaya Fruit	**Aroma**	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent
	Colour	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown	Yellowish-brown
	Form	Cream	Cream	Cream	Cream	Cream	Cream
	Homogenity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen
Papaya Seeds	**Aroma**	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent	Unique scent
	Colour	White	White	White	White	White	White
	Form	Cream	Cream	Cream	Cream	Cream	Cream
	Homogenity	Homogen	Homogen	Homogen	Homogen	Homogen	Homogen

Table 4. Results of the viscosity test for papaya leaf, fruit, and seed extract creams.

Formulation	Viscosity Value Before Cycling Test (CPS)	Viscosity Value After Cycling Test (CPS)
Papaya Leaves	912.3	744.2
Papaya Fruit	2100.8	1513.6
Papaya Seeds	918.9	771.1

Cps, centipoise.

pH Measurement Test of Formulations

The pH of topical formulations is a critical parameter, affecting stability, effectiveness, and user comfort. The ideal pH for skin products is between 4.5 and 6.5 to prevent skin irritation. The pH of the cream formulations was measured using a pH meter. Based on the pH evaluation in Tables 5 all formulations fell within the safe pH range for the skin.

Table 5. pH measurement results of papaya leaves, fruit dan seed extract cream.

Storage Time	pH Measurement Results			
	Papaya Component			Mean ± SD
Papaya Leaves				
Cycle 1	6.47	6.48	6.47	6.47 ± 0.03
Cycle 2	6.44	6.43	6.43	6.43 ± 0.03
Cycle 3	6.39	6.40	6.42	6.40 ± 0.00
Cycle 4	6.36	6.36	6.35	6.35 ± 0.06
Cycle 5	6.32	6.32	6.33	6.32 ± 0.06
Cycle 6	6.17	6.18	6.19	6.18 ± 0.00
Papaya Fruit				
Cycle 1	6.41	6.40	6.41	6.40 ± 0.06
Cycle 2	6.38	6.39	6.38	6.38 ± 0.03
Cycle 3	6.37	6.38	6.37	6.37 ± 0.03
Cycle 4	6.35	6.34	6.33	6.34 ± 0.00
Cycle 5	6.34	6.34	6.34	6.34 ± 0.00
Cycle 6	6.32	6.32	6.31	6.32 ± 0.06
Papaya Seed				
Cycle 1	6.46	6.47	6.46	6.46 ± 0.03
Cycle 2	6.44	6.45	6.44	6.44 ± 0.03
Cycle 3	6.41	6.42	6.42	6.41 ± 0.06
Cycle 4	6.38	6.38	6.39	6.38 ± 0.03
Cycle 5	6.36	6.37	6.37	6.36 ± 0.06
Cycle 6	6.33	6.33	6.33	6.33 ± 0.00

Table 6. Comparison of average incisional wound lengths between groups.

Groups	D-0 (cm)	D-3 (cm)	p	D-7 (cm)	p	D-14 (cm)	p
K (-)	1.5 ± 0	1.32 ± 0.13		1.28 ± 0.11		0.11 ± 0.26	
K (+)	1.5 ± 0	1.26 ± 0.26		1.205 ± 0.21		0.105 ± 0.26	
Fruit	1.5 ± 0	1.34 ± 0.17	0.959[a]	1.27 ± 0.15	0.92[a]	0.07 ± 0.16	0.88[b]
Seeds	1.5 ± 0	1.28 ± 0.19		1.25 ± 0.2		0.14 ± 0.35	
Leaves	1.5 ± 0	1.28 ± 0.14		1.24 ± 0.09		0 ± 0	

[a]One-way ANOVA, Kruskal-Wallis test. Data are presented as mean ± standard deviation.

Table 7. Comparison of average epithelial thickness among groups.

Group	Epithelial Tissue Thickness (µm)	p-value
K+	105.84 ± 20.85	
K-	21.84 ± 0	
Seeds	95.99 ± 78.82	0.519*
Fruit	103.02 ± 93.03	
Leaves	73.45 ± 15.8	

*Kruskal-Wallis test. Data are presented as mean ± standard deviation.

Table 8. Comparison of fibroblast cell count among groups.

Group	Fibroblast Cell Count (n)	p-value
K+	42.67 ± 20.92	
K-	31.17 ± 7.9	
Seeds	32.33 ± 10.19	0.541*
Fruit	38.67 ± 13.69	
Leaves	39.5 ± 11.17	

*One-way ANOVA test. Data are presented as mean ± standard deviation.

Table 9. Comparison of macrophage cell count among groups.

Group	Macrophage cell count (n)	p-value
K+	43.67 ± 20.52	
K-	48.67 ± 34.76	
Seeds	46.5 ± 26.59	0.771*
Fruit	66.67 ± 42.04	
Leaves	52 ± 33.06	

*Kruskal-Wallis test. Data are presented as mean ± standard deviation.

Clinical Description of Incisional Wound Healing

On the 7th day post-acclimatization, incisions (1.5 cm in diameter and 0.3 cm deep) were made on the backs of male Wistar rats. The rats were divided into five groups: a negative control (natural healing), a positive control (treated with povidone iodine), and three treatment groups (treated with creams containing papaya leaf, fruit, or seed extracts). Wound measurements were taken on days 3, 7, and 14 to track the healing process. On day 3, all groups, including the negative control, showed a reduction in wound size. The greatest reduction was observed in the positive control group, but there were no statistically significant differences between the groups on day 3 (p=0.959), day 7 (p=0.92), or day 14 (p=0.88).

Based on the results in Table 6 above, it was found that at the initial measurement (H-0), the incisional wounds on the backs of the rats were of the same size. To statistically assess the effects of the application of seed, fruit, and leaf extract creams, the average wound length data were analyzed. After performing normality testing using the Shapiro-Wilk method and homogeneity testing using Levene's test, the results showed that the data were normally distributed and homogeneous (p>0.05). Thus, one-way ANOVA was conducted to determine the differences in the effects of the treatments.

On day 3 of observation, a reduction in wound size was noted in all treatment groups, including the negative control group. The largest average reduction in wound size was observed in the positive control group (1.26 ± 0.26 cm), while the smallest average reduction was found in the group treated with papaya fruit extract cream (1.34 ± 0.17 cm). One-way ANOVA showed no statistically significant differences in the average wound size among the five groups on day 3 (p=0.959). Since no significant differences were found, further testing with post hoc Tukey HSD (Honest Significant Difference) was not conducted.

On the 7th day of observation, the wound healing process was ongoing, as indicated by a reduction in the size of the incisional wound. The largest average reduction in wound size was observed in the positive control group (1.205 ± 0.21 cm). Statistical testing on day 7 also revealed no significant difference in the average wound size among the five groups (p=0.92). By the end of the 14th day of observation, the best wound healing was noted in the group treated with papaya leaf extract cream. However, no significant difference in average wound size was found among the five treatment groups (p=0.88).

Comparison of Histopathology in the Wound Healing Process

Histopathological evaluation was performed by assessing epithelial thickness, fibroblast and macrophage cell counts, blood vessel count, and collagen density. On day 14, epithelial thickness was measured, and while the positive control group showed the greatest epithelial thickness, no significant differences were found among the groups (p=0.519).

Comparison of Epithelial Thickness

On the 14th day, the epithelial tissue thickness of the test animals was observed in all five groups to assess the wound healing process. To determine whether there were differences in epithelial tissue thickness among the five groups, the collected data were first tested for normality using the Shapiro-Wilk method and for homogeneity using Levene's test. Since some data were not normally distributed (p<0.05), the Kruskal-Wallis test, an alternative to one-way ANOVA, was conducted to determine the effect of the different treatments.

The results of this study show that the largest average epithelial tissue thickness was observed in the positive control group (105.84 ± 20.85 µm), while the smallest epithelial tissue thickness was found in the

Table 10. Comparison of Collagen Density Among Groups.

Group	Collagen Density (%)	p-value
K+	31.64 ± 6.02	
K-	27.37 ± 8.59	
Seeds	37.94 ± 5.24	0.130*
Fruit	37.77 ± 10.03	
Leaves	37.08 ± 9.85	

*One-way ANOVA test. Data are presented as mean ± standard deviation.

Table 11. Comparison of VEGF expression among groups.

Group	Proportion (%)	p-value	Intensity (score)	p-value	Allred score	p-value
K+	28.00 ± 6.45		2.33 ± 0.517		5.50 ± 0.84	
K-	17.56 ± 4.66		1.17 ± 0.408		4.17 ± 0.41	
Seeds	28.61 ± 4.15	0.001*	2.17 ± 0.408	0.002*	5.33 ± 0.82	0.002*
Fruit	17.81 ± 2.37		1.17 ± 0.408		4.17 ± 0.41	
Leaves	19.98 ± 2.49		1.5 ± 0.547		4.50 ± 0.55	

*Kruskal-Wallis test. Data are presented as mean ± standard deviation.

Table 12. Mann-Whitney Test results for VEGF expression.

Variables	p-value	Interpretation
K- and K+	0.016	There is a significant difference
Seeds and K+	0.522	There is no significant difference
Fruit and K+	0.004	There is a significant difference
Leafs and K+	0.006	There is a significant difference
Seeds and Fruit	0.004	There is a significant difference
Seeds and Leaves	0.004	There is a significant difference
Fruit and Leaves	0.262	There is no significant difference

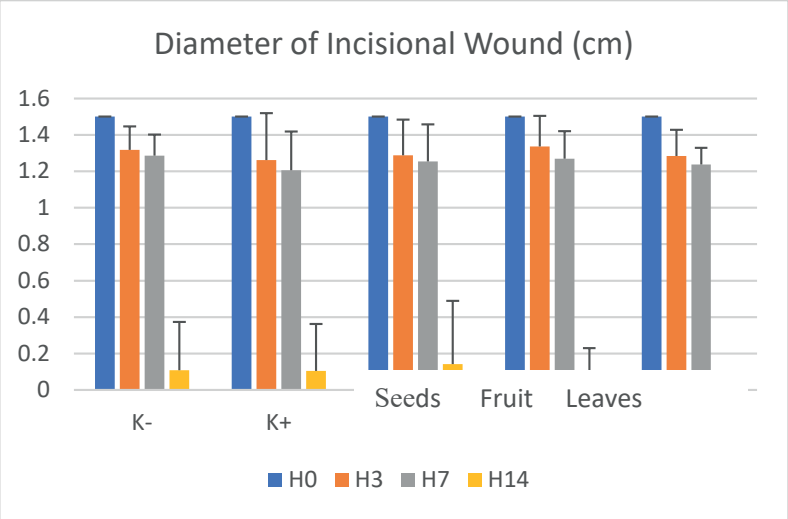

Figure 1. Diagram comparing the average diameter of incisional wounds in test animals on days 0, 3, 7, and 14.

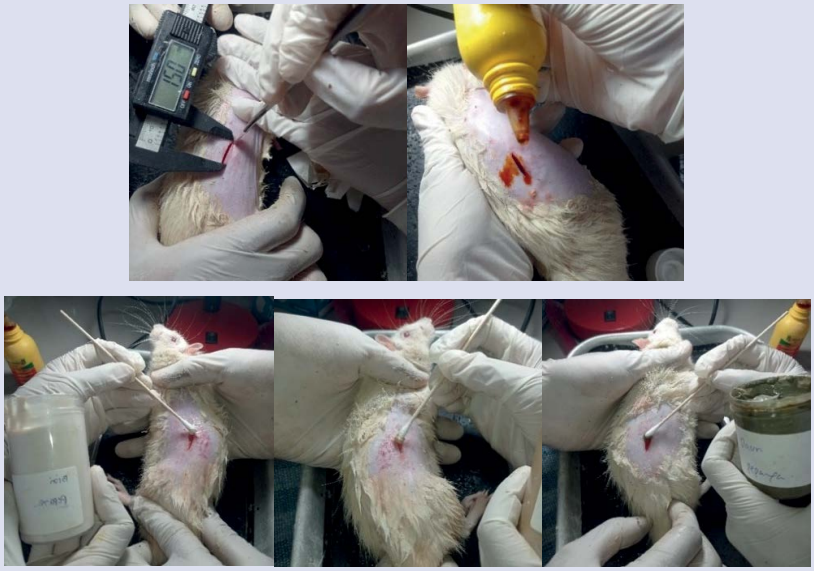

Figure 2. A. Incisional wound treatment with a diameter of 1.5 cm; B. Application of povidone iodine to incisional wounds (positive control); C. Application of papaya seed extract cream; D. Application of papaya fruit extract cream; E. Application of papaya leaf extract cream.

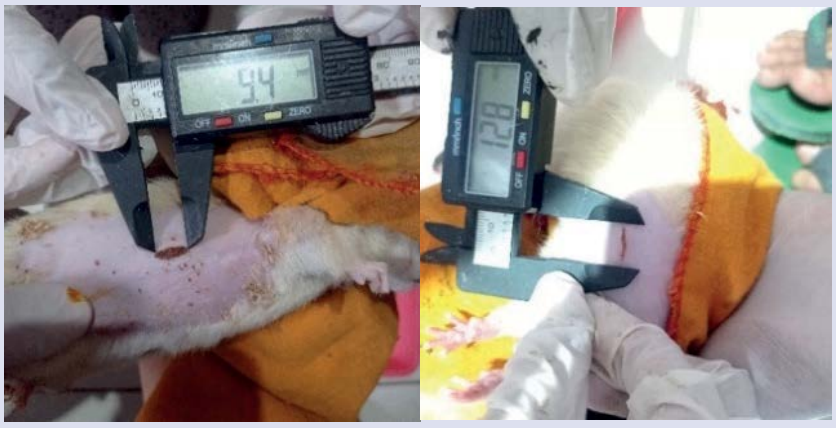

Figure 3. A. Wound diameter on day 3 for povidone iodine treatment; B. Wound diameter on day 3 for papaya fruit cream treatment.

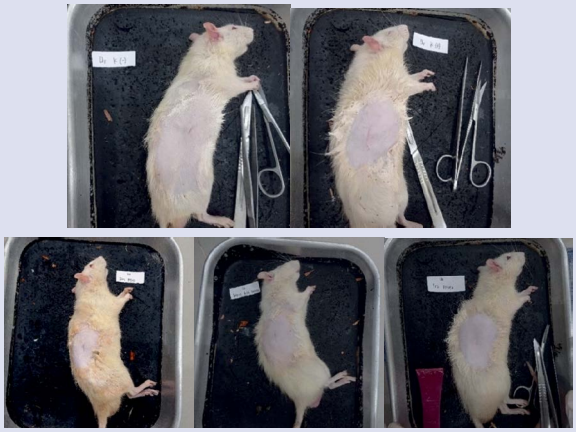

Figure 4. A. Wound diameter on day 14 for negative control; B. Wound diameter on day 14 for positive control; C. Wound diameter on day 14 for papaya leaf treatment; D. Wound diameter on day 14 for papaya fruit cream Treatment; E. Wound diameter on day 14 for papaya seed treatment.

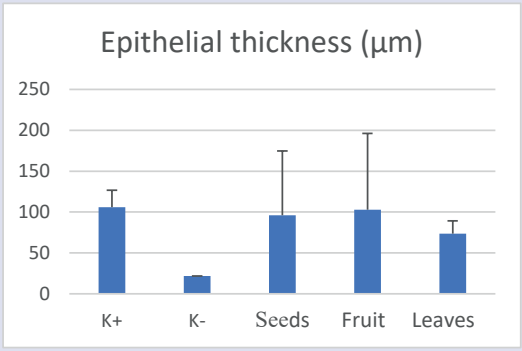

Figure 5. Diagram of epithelial thickness comparison among groups.

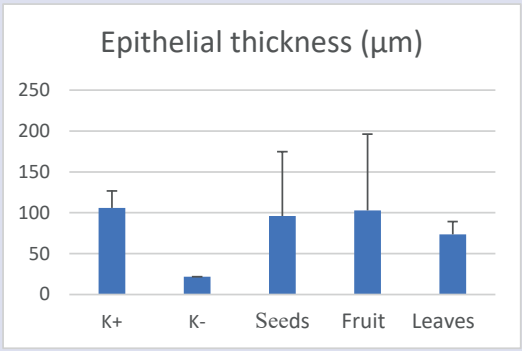

Figure 6. Diagram of fibroblast cell count comparison among groups.

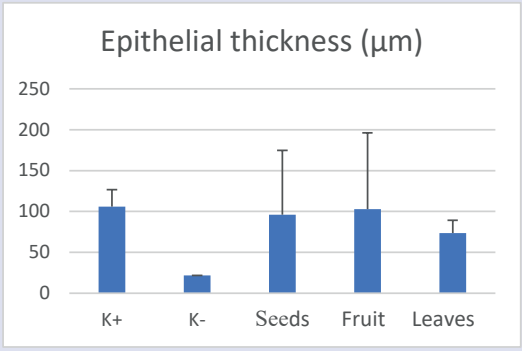

Figure 7. Diagram of macrophage cell count comparison among groups.

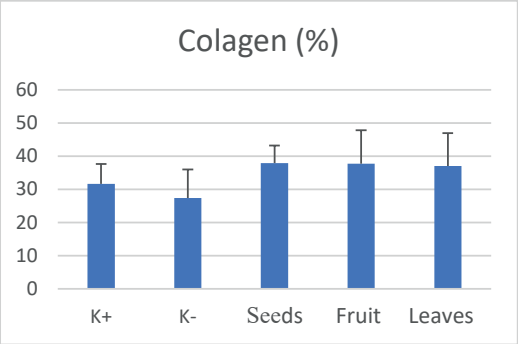

Figure 8. Diagram of collagen density comparison among groups.

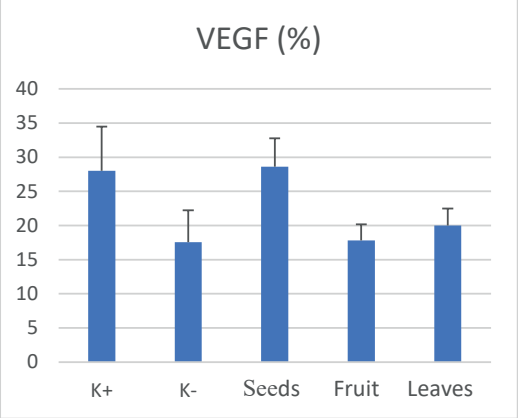

Figure 9. Diagram of VEGF expression proportion comparison among groups.

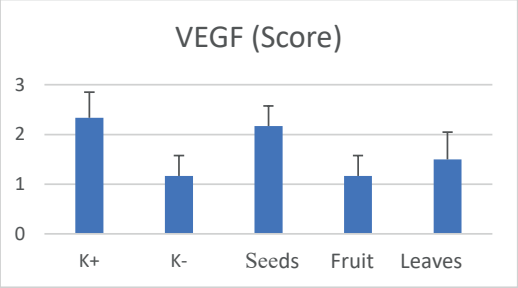

Figure 10. Diagram of VEGF expression intensity comparison among groups.

negative control group (21.84 ± 0 µm). Among the treatment groups receiving extract creams, the largest average epithelial tissue thickness was observed in the group treated with papaya fruit extract cream (103.02 ± 93.03 µm). However, statistical testing revealed no significant difference in epithelial tissue thickness among the five different treatment groups (p=0.519).

Comparison of Fibroblast Cell Count

The results of the normality test using the Shapiro-Wilk method and the homogeneity test using Levene's test indicated that the fibroblast cell count data on the 14th day of observation were normally distributed and homogeneous (p>0.05). Therefore, a one-way ANOVA test was conducted to determine the differences among the groups.

The results of this study show that the highest number of fibroblast cells was observed in the positive control group (42.67 ± 20.92), while the lowest was found in the negative control group (31.17 ± 7.90). Among the treatment groups receiving extract creams, the highest and closest result to the positive control group was observed in the group treated with papaya leaf extract cream (39.50 ± 11.17). However, statistical testing revealed no significant differences in fibroblast cell count among the five different treatment groups (p=0.541). Since no significant differences were found, further testing with post hoc Tukey HSD (Honest Significant Difference) was not conducted.

Comparison of Macrophage Cell Count

The results of the normality test using the Shapiro-Wilk method indicated that the macrophage cell count data on the 14th day of observation were not normally distributed (p<0.05). Therefore, the Kruskal-Wallis test was used as an alternative to one-way ANOVA to determine the differences among the groups.

The results of this study show that the highest number of macrophage cells was observed in the group treated with papaya fruit extract cream (66.67 ± 42.04), while the lowest was found in the group treated with povidone iodine (43.67 ± 20.52). However, statistical testing using the

Kruskal-Wallis test revealed no significant difference in the number of macrophage cells among the five different treatment groups (p=0.771).

Comparison of Collagen Density

The results of the normality test using the Shapiro-Wilk method and the homogeneity test using Levene's test indicated that the collagen density data on the 14th day of observation were normally distributed and homogeneous (p>0.05). Therefore, a one-way ANOVA test was conducted to determine the differences among the groups.

The results of this study show that collagen density in the treatment group receiving the extract cream was higher compared to the control group. Among the treatment groups receiving the extract cream, the highest collagen density was observed in the group treated with papaya seed extract cream (37.94 ± 5.24). However, statistical testing using one-way ANOVA revealed no significant differences in collagen density among the five different treatment groups (p=0.130). Since no significant differences were found, further testing with post hoc Tukey HSD (Honest Significant Difference) was not conducted.

Comparison of VEGF Expression Using Immunohistochemistry

The comparison of blood vessel formation was observed by evaluating VEGF expression on the 14th day using immunohistochemistry. VEGF expression was assessed by evaluating both the proportion and intensity, which were summed to obtain the results.

On the 14th day, observations were made on VEGF expression in the test animals across the five groups to assess the wound healing process. To evaluate whether there were differences in epithelial tissue thickness among the five groups, the collected data were first tested for normality using the Shapiro-Wilk method and for homogeneity using Levene's test. Since some data were not normally distributed (p<0.05), the Kruskal-Wallis test, an alternative to one-way ANOVA, was conducted to determine the effect of different treatments. The results of this study show that the highest average proportion and intensity of VEGF expression were observed in the group treated with papaya seed extract cream, with values of 28.61 ± 4.15 and 2.17 ± 0.408, respectively. Statistical testing revealed significant differences in both proportion (p=0.001) and intensity (p=0.002) of VEGF expression among the five different treatment groups. In the Allred score calculation, all groups exhibited moderate positive strength for VEGF expression. The group treated with papaya seed extract cream had the highest average score among the groups (5.33 ± 0.82). Statistical testing indicated a significant difference in Allred scores among the five different treatment groups (p=0.002).

Based on the table above, it can be concluded that the effectiveness of applying cream in the rat incision wound model was best achieved with the papaya seed extract cream, as it demonstrated effectiveness similar to the group treated with povidone iodine in the angiogenesis process, as represented by VEGF expression (p>0.05)

DISCUSSION

Effects of Papaya Extract on Wound Size

According to the 2010 National Workshop on Medicinal Plants data, out of a total of 40,000 plant species worldwide, Indonesia has 30,000 plant species, including 940 species with medicinal properties.[6,7] Similar to papaya fruit, topical application of papaya extract (*Carica papaya L.)* on incision wound models has been extensively studied to observe its effects on wound healing. Papaya extract contains secondary metabolites like terpenoids, alkaloids, flavonoids, and saponins, which can aid in the wound healing process[25]. Among these metabolites, flavonoids have been widely reported in previous studies to possess wound-healing properties due to their anti-inflammatory, angiogenic, re-epithelialization, and antioxidant effects.[8,9,10] Flavonoids have antimicrobial effects by inhibiting bacterial cell wall permeability due to their hydroxyl groups, which can alter organic compounds and nutrient transport, resulting in highly toxic effects on bacteria.[4] Flavonoids play a crucial role in antioxidant and anti-inflammatory activities during the wound healing process. The ability of flavonoids in the wound healing process is to inhibit uncontrolled inflammation. Additionally, flavonoids play a role in enhancing wound contraction speed, increasing collagen deposition, forming granulation tissue, and accelerating epithelialization.[11] Therefore, papaya ethanol extract can be used as a herbal source for potential drug formulation.

Flavonoids have potential as wound treatments due to their antioxidant, immunostimulatory, and antibacterial properties. They directly influence cellular signaling pathways involved in healing, including Wnt/β-catenin, TGF-β, JNK, Nrf2/ARE, and NF-κB pathways.[12]

The results of this study indicate that there was no statistically significant difference in wound size among the groups treated with ethanol extracts from papaya seeds, fruit flesh, and leaves compared to the control group. Although observationally, the best wound healing was seen in the group treated with ethanol extract cream from papaya leaves, the application of ethanol extracts from seeds, fruit flesh, and leaves did not statistically accelerate wound healing. This finding is consistent with the study by Nurhidayah et al., which also reported that the application of 10%, 20%, and 30% papaya leaf extract gel did not have statistically significant differences compared to the positive control group using povidone iodine and the negative control with 0.9% NaCl (p=0.770).[13] In contrast, the findings in this study are contrary to the study conducted by Hakim et al., which reported that topical application of 75% papaya fruit extract could accelerate the healing process of skin incisions in rats.[2] In line with the research by Hakim et al., Darin and Ajisman also found that papaya latex extract has an effect in accelerating the healing time of skin incisions. The average time to reach the proliferation phase of wound healing in the four groups in that study was as follows: approximately 14 days (negative control), approximately 12 days (50% concentration), approximately 10 days (75% concentration), and approximately 8 days (100% concentration)[4]. It is important to note that the majority of extract concentrations used in the studies previously mentioned were higher compared to the concentration used in this research. A study conducted by Wan et al. on rats found that therapeutic activity was directly related to higher doses of flavonoids.[14] Therefore, the researchers suggest the need for further studies to understand the differences in papaya extract concentrations in topical preparations and their effects on wound healing processes. A wound is an occurrence of discontinuity, damage, or loss of part or all of body tissue. This event is generally caused by changes in temperature, trauma from sharp or blunt objects, explosions, chemicals, animal bites, or electric shocks.[5] In this study, no statistically significant difference was found in wound size between the K(+) and K(-) groups. Similar results were also reported by several previous studies.[11,13] In the negative control group, although no therapeutic agents were provided for wound healing, the incision wounds in the rats were able to close on their own due to the natural protective and restorative capabilities of body tissues. When a wound closes quickly, the risk of infection can also decrease. During the observation period in this study, no infections were observed in the incision wound models in rats, as indicated by the absence of pus formation.

Phytochemical analysis by Nayak et al. revealed that ethanolic papaya seed extract contains glycosides, including glucotropaeolin, which has antimicrobial properties due to its benzyl-isothiocyanate content.[15] Myrosin, a protein in papaya seeds, hydrolyzes glucotropaeolin into rhamnose, sulfate ions, and benzyl-isothiocyanate. Additionally, alkaloids in the extract disrupt bacterial cell wall formation by targeting the peptidoglycan component, leading to bacterial cell death.[16]

Effects of Papaya Extract on Epithelial Thickness

The wound healing process can be microscopically observed by examining histopathological changes such as cellular infiltration, collagen production, neovascularization, and epithelial thickness. Once granulation tissue has formed, the epithelialization process begins. During the proliferative phase, wound contraction occurs, which involves the centripetal movement from the edges of the wound toward its center. The wound contracts at an average rate of 0.6 to 0.75 mm per day, gradually narrowing until it eventually closes. Myofibroblasts, which are involved in wound contraction, contain actin and myosin similar to the contraction system in smooth muscle, allowing them to contract and stretch. The contraction of the wound in the later stages of healing is observed through changes in the wound's shape, a reduction in the open wound area, and resulting smaller wound area.[17]

Although a noticeable difference in epithelial tissue thickness was observed compared to the negative control group, the results of this study indicate that the application of 30% ethanol extract creams from papaya seeds, fruit pulp, and leaves did not show a significant difference compared to the positive and negative control groups in terms of re-epithelialization, as measured by epithelial tissue thickness in incision wounds ($p>0.05$). The lack of significant differences between the treatment and positive control groups in this study suggests that the effectiveness of ethanol extracts from papaya seeds, fruit pulp, and leaves is comparable to that of povidone-iodine in the re-epithelialization process. This finding contrasts with the study by Nasution and Batubara, which reported that 100% papaya leaf extract was 200% more effective in wound healing compared to 0.1% gentamicin as the positive control. This was evident from the epithelial thickness in incision wounds, which reached 355.18 µm with papaya leaf extract, compared to 265.12 µm with 0.1% gentamicin.[18]

In the study, the researchers observed that statistically significant differences in epithelialization were only evident on day 3. This result may be attributed to the fact that epithelialization in incision wounds begins within the first 48 hours after the injury. Based on these findings, papaya leaf extract is effective in wound healing up to the proliferative phase, with a healing time of 3-14 days.

Several studies have shown that the application of papaya leaf extract is an effective alternative for accelerating epithelialization in wound healing. This is because papaya leaves contain saponins, compounds that stimulate collagen formation in the wound healing process. Additionally, papaya leaves contain vitamins C and E, as well as beta-carotene, which function as antioxidants to neutralize free radicals produced by neutrophil phagocytosis of debris and bacteria during wound healing. Furthermore, papaya leaves contain the enzyme papain, which accelerates macrophage activity by increasing interleukin production, which is crucial for wound healing and preventing widespread infection.[19]

In addition to the differences in papaya extract concentrations used in previous studies, the researchers recommend that epithelial thickness be monitored regularly. This would help determine whether the application of ethanol papaya extract cream can accelerate re-epithelialization in the wound healing process.

Effects of Papaya Extract on Fibroblast Count and Collagen Density

The results of this study showed that the highest number of fibroblasts was found in the positive control group (42.67 ± 20.92), while the lowest was observed in the negative control group (31.17 ± 7.9) on day 14. The application of ethanol extract creams from papaya seeds, fruit pulp, and leaves did not show statistically significant differences in either fibroblast count or collagen density compared to the control groups. These results contrast with a study by Sim et al., which reported significant differences in fibroblast count ($p<0.05$). In that study, Sim et al. compared ethanol extracts of papaya leaves at concentrations of 25%, 50%, 75%, and 100%, and found that the 100% concentration was the most effective in increasing fibroblast count, while the 25% concentration was least effective.[20]

Fibroblasts play a crucial role during proliferative phase of wound healing in producing proteins necessary for wound healing, including collagen. Research by Zulkefli et al. supports the role of flavonoids in increasing fibroblast numbers, concluding that the increase in fibroblasts is attributed to flavonoid compounds.[12] Papaya seeds, fruit pulp, and leaves contain saponins and flavonoids. Flavonoids treat wounds, acting as astringents, antimicrobials, and promoting collagen growth through increased fibroblast activity. Saponins stimulate collagen formation, cleanse wounds, and act as antibacterial agents by disrupting bacterial cell membranes.[5]

Fibroblast proliferation in wound healing is stimulated by interleukin-1β (IL-1β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF). Increased collagen production, critical for tissue repair, occurs as fibroblasts secrete the extracellular matrix (ECM), with collagen being a major component. Collagen scar tissue reorganizes and strengthens over several months, and the goal of the maturation phase is to refine this tissue into strong, high-quality healing tissue. Previous research shows that fibroblast proliferation and collagen density begin between days 7-21, marking the entry into the proliferative phase.

Effects of Papaya Extract on Macrophage Count

The application of ethanol extract creams from papaya seeds, fruit pulp, and leaves in this study did not show statistically significant differences in macrophage count compared to the control group ($p>0.05$). These results contrast with findings from a study by Parampasi and Soemarno, which reported that 70% ethanol extract of papaya leaves could increase macrophage count and collagen formation in incision wound healing[21]. This outcome was attributed to the papain enzyme present in papaya leaf extract, which has anti-inflammatory and analgesic effects through two different mechanisms.

Papain neutralizes inflammatory mediators like kinins and prostaglandins, inhibiting pain receptors and preventing vascular spasms, leading to increased blood flow and vasodilation through its antihistamine action. It also enhances plasma protein and immune complex activity, reducing edema and pain from fluid pressure. Additionally, papain accelerates macrophage activity by boosting interleukin production, essential for wound healing and infection prevention.

As a facial cream and for wound debridement, papain's antiedema and anti-inflammatory effects work alongside vitamins A, C, and E to inhibit inflammation. Carica papaya prevents necrosis, wound surface fibrosis, infection, and skin keratosis. Both papain and chymopapain exhibit antimicrobial and proteolytic enzyme activities.

Saponins in papaya leaf extract enhance monocyte proliferation, increasing macrophage numbers and boosting fibroblast activity in wound tissue. These steroid or triterpenoid glycosides stimulate Vascular Endothelial Growth Factor (VEGF), promoting macrophage migration to the wound area. Saponins also contribute to collagen formation, possess antiseptic properties, and have antioxidant effects, supporting wound healing.

Effects of Papaya Extract on Angiogenesis

The results of this study indicate that the application of creams containing extracts from papaya seeds, fruit pulp, and leaves has an impact on the angiogenesis process, as evidenced by VEGF expression

($p<0.05$). Compared to other formulations, the cream made from papaya seed extract showed the best results in the incision wound model in rats, matching the effectiveness of the povidone-iodine group in promoting angiogenesis, as represented by VEGF expression. These findings are consistent with a study by Nurhidayah et al., which reported a significant effect of papaya leaf extract gel on angiogenesis. In that study, the researchers found that the effect of papaya leaf extract gel on angiogenesis was most effective at a concentration of 20%, compared to 10% and 30% concentrations.[13]

Angiogenesis, the formation of new blood vessels, is crucial for wound healing. The faster new vessels form, the quicker tissue repair occurs, leading to faster wound healing. In this study, VEGF expression was observed only on day 14, so early angiogenesis stages could not be assessed. Previous studies, including Nurhidayah et al., reported angiogenesis beginning on day three, with maximal angiogenesis observed between days three and seven. During this phase, new blood vessels form from pre-existing vessels, invading the wound clot and integrating into the granulation tissue network.[13]

CONCLUSION

No significant differences were found between the administration of ethanol extract creams from papaya leaves, fruits, and seeds in terms of wound diameter, epithelial thickness, fibroblast count, macrophages, or collagen density ($p>0.05$). There were also no significant differences when compared to the negative control group ($p>0.05$). The highest flavonoid content was found in the ethanol extract of papaya leaves. The application of ethanol extract creams from papaya leaves, fruits, and seeds had a significant impact on the angiogenesis process, as evidenced by VEGF expression ($p=0.002$). Given these findings, it is recommended that future clinical trials focus on optimizing the concentrations of papaya extracts and investigate their potential in combination with other wound healing agents. Additionally, further studies on the long-term effects and safety of papaya extracts in wound care could provide valuable insights for their clinical use. Extracts from papaya leaves, fruits, and seeds show promise as natural adjuncts to enhance wound healing in clinical settings.

CONFLICTS OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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