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Formulation and Evaluation of Papaya Seed Extract Hydrogel (Carica Papaya L.) As Well as its Effectiveness in Repairing Incision Wounds in Mice (Mus Musculus)

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ABSTRACT

Papaya seeds (Carica papaya L.) contain flavonoids, saponins, alkaloids, steroids, and tannins that have been proven effective in accelerating the wound healing process. This research aims to formulate hydrogel preparations from papaya seed extract and test their effectiveness in healing incision wounds in mice. This research is experimental in nature, involving the preparation of a papaya seed extract hydrogel with chitosan as a polymer and PEG 400 as a crosslinker. The papaya seed extract hydrogel was tested on mice (Mus musculus) across five treatment groups. Wound healing data were statistically analyzed using a repeated measures ANOVA test, and the physical evaluation of the hydrogel preparations was conducted descriptively. The results of the study showed that the papaya seed extract hydrogel met the requirements of the hydrogel preparation evaluation tests. The statistical test results indicated a significant effect on wound healing in male mice ($p < 0.05$). Based on the results obtained, the papaya seed extract hydrogel (Carica papaya L.) demonstrated the best incision wound healing effectiveness. In conclusion, papaya seed extract hydrogel, particularly at a 15% concentration, effectively accelerates incision wound healing in mice and meets the physical quality standards for topical hydrogel preparations, making it a promising natural-based wound dressing.

INTRODUCTION

The skin is the largest organ in the human body, covering the entire outer surface and weighing approximately 4.5–5 kg in the average adult, or about 12–15% of total adult body weight (Jiao et al., 2024). In addition to covering the outer surface, the skin plays an important role in protecting the body, including regulating body temperature and responding to stimulation (Cramer et al., 2022). The skin consists of three layers, namely the epidermis, dermis, and subcutaneous tissue (Jiao et al., 2024). The skin is susceptible to different types of trauma, such as burns, scalds, lacerations, and sharp incisions (Zhang et al., 2019). Optimal wound management has encouraged the development of knowledge about wounds, healing, and wound care, as improperly treated wounds may lead to wound healing complications (Nayak et al., 2012). Although human skin has a high regeneration potential, wounds exceeding a certain diameter will not heal spontaneously and require a skin transplant. The wound healing process involves a series of events including inflammation, granulation tissue formation, epithelialization, collagen synthesis, and tissue remodelling (Nayak et al., 2012; Tavakoli &

Klar, 2020).Research on the use of natural ingredients to assist the wound healing process is currently being carried out, one of which involves the use of papaya seeds (Carica papaya L.). Papaya seeds have demonstrated antimicrobial, antioxidant, and anti-inflammatory activity (Kyei-Barffour et al., 2021; Masson-Meyers et al., 2020; Nayak et al., 2012). Papaya seeds are reported to contain tannins, steroids, terpenoids, saponins, phenols, flavonoids, alkaloids, and anthraquinones (Ghaffarilaleh et al., 2019; Masson-Meyers et al., 2020). In addition, papaya seeds also contain potassium, copper, zinc, magnesium, vitamin A, B vitamins, ascorbic acid, and folate (Amin et al., 2019). Other constituents include fatty acids, proteins, fibre, papaya seed oil, carpine, benzyl isothiocyanate, benzyl glucosinolate, glucotropaeoline, cryptoxanthin, oleic acid, benzylthiourea, stearic acid, sitosterol, sericin, and myrosinase enzymes. Papaya seeds also contain protein (28%), crude fibre (32%), and lipids (28%) (Amin et al., 2019; Ávila et al., 2020; Kyei-Barffour et al., 2021; Memudu & Oluwole, 2021).Several studies related to papaya seeds have been conducted. Nayak et al. (2012) demonstrated that ethanol extract of papaya seeds significantly affects the wound healing process, achieving a wound closure value of 89%, attributable to its content of flavonoids, alkaloids, and glycoside compounds. According to Silvy (2021), papaya seed extract at concentrations of 5–15% is able to produce a burn-healing effect with an average effectiveness of 5–7 cm.Another study conducted by Panzarini et al. (2014) showed that papaya seed extract has promising antioxidant activity, as it was proven to protect fibroblast cells from H_2O_2-induced damage. In some developing countries, papaya seed preparations are effectively used in treating ulcers and skin inflammation (Nayak et al., 2012). Excessive production of Reactive Oxygen Species (ROS) in response to the activation of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) in macrophages and neutrophils during the inflammatory phase of wound healing can result in non-healing wounds (Kong et al., 2021). A key factor in wound healing is the degree of inflammation at different stages of the healing process. Inflammation is essential for preventing infection, stimulating angiogenesis, and matrix deposition through the secretion of cytokines and angiogenic factors in the early stages of wound healing; however, excessive or prolonged pathological inflammation leads to delayed wound healing and fibrosis (Kong et al., 2021).One of the delivery systems for wound healing is the hydrogel preparation. Hydrogels have a three-dimensional network of polymer chains and water, which underpins their use in wound repair, as they provide a moist environment conducive to healing, particularly during re-epithelialization. In addition, hydrogels can serve as excellent carriers for topical applications (Seow et al., 2016).One of the natural polymers that can be used in the manufacture of hydrogels is chitosan. Chitosan can be applied in the development of pharmaceutical, biomedical, cosmetic, and food products owing to its non-toxic and biodegradable properties (Zhao et al., 2014). Chitosan structures contain numerous hydroxyl and amine groups that form intermolecular and intramolecular hydrogen bonds, resulting in a strong hydrogen-bonding network. Furthermore, the presence of amino groups in chitosan allows these molecules to be modified to achieve desired properties. Chitosan possesses a hydrophilic group in the form of $–NH_2$, which confers its ability to bind water molecules. By virtue of these physical and chemical properties, chitosan can be applied as a polymer in hydrogel preparations (Liu et al., 2018).Research conducted by Soares et al. (2019) using chitosan polymers produced hydrogels with good physical stability, as indicated by minimal change in preparation pH and the absence of discolouration during the storage period. A further study by Liu et al. (2018) demonstrated that chitosan can serve as an efficient polymer for drug delivery, having been shown to effectively reduce the swelling capacity of hydrogels at a concentration of 2%. Chitosan-based hydrogels can alleviate factors that inhibit wound healing, 6744haracteri wound areas, protect against secondary infections, and produce finer scarring. Chitosan is therefore considered advantageous as a polymer material for wound dressing applications, particularly when modified or combined with other polymers. The combination of chitosan with polyethylene glycol can increase the water solubility of chitosan (Lih et al., 2012). Polyethylene glycol is widely used in pharmaceuticals due to its biocompatibility, non-toxic properties, and good solubility in water and other common solvents (Hasan et al., 2021). According to Chen et al. (2014), the hydrocarbon component of the polyethylene glycol 400 structure helps disrupt hydrogen bonds between water molecules, thereby reducing intermolecular interactions. Hydrogels composed of a combination of chitosan and polyethylene glycol exhibit superior biocompatibility compared to chitosan polymer hydrogels alone.Hydrogel-based skin substitutes have attracted considerable attention due to their unique ability to mimic the microenvironment of natural skin tissue compared to other materials (Tavakoli & Klar, 2020). Clinical studies confirm that moist conditions promote faster wound contraction and can accelerate wound healing by up to 50%. To support the wound healing process more efficiently, hydrogel preparations may incorporate active ingredients from both synthetic medicines and natural sources such as plant extracts (Ali & Ahmed, 2018; Bustamante-Torres et al., 2021; Extracts et al., 2021).This study aims to formulate hydrogel preparations from papaya seed extract (Carica papaya L.) using chitosan as a polymer and PEG 400 as a crosslinker, and to test the effectiveness of the papaya seed extract hydrogel in healing incision wounds in mice (Mus musculus). This research is expected to provide theoretical benefits by enriching knowledge in the pharmaceutical field, particularly in the development of natural ingredient-based hydrogel preparations for wound healing, as well as practical benefits by offering a safe, effective, and economical alternative topical preparation based on papaya seed extract to accelerate the healing of incision wounds.

METHOD

Tools

The tools used in this study include water tanks, thermometers, gram scales (O'hauss), sieve number 60, blender (Sachiko), pH meter (HANNA instrument), rotary evaporator, animal scales (Denver), UV-Vis spectrophotometer (Shimadzu), magnetic strirrer (Cimarec), a set of surgical instruments and glassware.

Ingredients

The materials used in this study are papaya seed 6744haracteri, 96% ethanol, chitosan, polyethylene glycol, NaOH, AlCl3, acetic acid, aquades, flannel fabric, gauze, ethanol, anhydrous acetic acid, HCl, H2SO4, FeCl3, anhydrous acetic acid, n-hexane, Woundgel Hydrogel®.

Papaya seed extraction

Preparation of papaya seed 6744haracteri

The ripe papaya fruit is cut, then the seeds are separated from the fruit. The seeds are washed thoroughly using running water, drained, then placed evenly on a baking sheet and then dried by air at room temperature, after drying the papaya seeds are pollinated using a blender and sifted using a 60 mesh sieve so that papaya seed powder is obtained.

2. Fat release of papaya seed extract

Before maceration, fat is first released in papaya seed powder using n-hexane solvent, how: as much as 1000 g of papaya seed 6745haracteri powder is put into a container, 5 liters of n-hexane solvent is added, then let it sit for 24 hours (first), then filtered. Re-soaked with n-hexane solvent at the same volume for 24 hours (second). Furthermore, the pulp obtained is then macerated

3. Papaya seed extraction process

The manufacture of the extract is carried out by the maceration method. Papaya seed pulp that has gone through the fat release process is macerated using 96% ethanol solvent. Simplisia is put in a maserator, then soaked in 5 liters of 95% ethanol solvent for 3x24 hours while stirring occasionally, then filtered using a flannel cloth. The obtained filtrate is evaporated with a rotary evaporator until a thick extract is obtained

Screening phytochemistry

To find out the compounds contained in papaya seed extract, a qualitative analysis was carried out using chemical reagents which included the examination of alkaloid compounds, flavonoids, saponins, tannins, steroids, and glycosides. The flavonoid compound test was carried out by means of 0.5 g of extract, enough magnesium powder was added to oxidize the sample, then 5 drops of concentrated HCl were added. The presence of flavonoids is characterized by the formation of a reddish-black color (Auwal et al., 2014). The saponin compound test was carried out by means of 0.5 g of extract, mixed with 10 ml of distilled water in a test tube, then stirred vigorously for 5 minutes, left for 10 minutes and observed the foam formed. The presence of saponin compounds in the sample is indicated by the formation of foam (Auwal et al., 2014). The alkaloid compound test was carried out by means of 0.5 g of extract being put into the test tube, 5 ml of 10 % NH3 solution was added, then 15 ml of H2SO4 2 N was added, and Mayer reagent was added. The presence of alkaloids in the sample is characterized by the formation of yellowish deposits (Auwal et al., 2014).

The steroid compound test was carried out by means of 0.5 g of extract, put into the test tube, then added 2 ml of 70% ethanol, and 2 ml of chloroform, then added 2 ml of concentrated H2SO4 by dripping slowly from the side of the test tube wall. The formation of red rings indicates the presence of steroids (Auwal et al., 2014). The tannin compound test was carried out by means of 0.5 g of extract, 10 ml of hot water was added, then dripped with FeCl3. The presence of tannins in the sample is characterized by the formation of a blackish-green color (Auwal et al., 2014). The glycoside compound test was carried out by testing with the Libermann Burchard reaction, which was 0.5 g of extract taken, 5 ml of anhydrous acetic acid added, and 10 drops of concentrated sulfuric acid, blue or green color indicating the presence of glycosides (Ministry of Health of the Republic of Indonesia, 1979).

Quantitative analysis of total flavonoid compounds of papaya seed extract

Determination of the maximum wavelength of quercetin

First, a stock solution with a concentration of 1000 bpd is made by means of quercetin weighing as much as 10 mg, then dissolved with distilled water up to 10 ml. then from the stock solution 100 µl is pipetted, then 100 µl of 10 µl AlCl3 is added, homogenized and 100 µl of sodium acetate solution is added, homogenized again and sufficient in volume up to 5 ml with distilled water. Then the absorption is measured in the wavelength range of 400-800 nm and the maximum wavelength is determined.

2. Quecetin standard curve creation

From the stock solution, a series of dilution is made with a concentration of 2, 4, 6, 8, 10 bpj, by spitting the stock solution of 0.2 µl, 0.4 µl, 0.6 µl, 0.8 µl, 10 µl, then 100 µl of 10 µl AlCl3 solution, homogenized, then 100 µl of 1 M acetic acid solution is added, re-homogenized and sufficient volume up to 5 ml with distilled water, Then each concentration was measured for absorption at the maximum wavelength.

3. Determination of total flavonoid levels of papaya seed ethanol extract

The sample was weighed as much as 100 mg and dissolved with distilled water to a volume of 10 ml. The solution was taken 80 µl and put in a 5 ml flask, added 100 µl of 10% AlCl3 solution and 100 µl of 1 M sodium acetate solution, and filled with distilled water. Sample absorption is measured at maximum wavelength.

Formula design

Table 1. Papaya Seed Extract Hydrogel Formula

Ingredients	Concentration (%b/v)			
	F1	F2	F3	F4
Papaya seed extract	5	10	15	-
Kitosan	2	2	2	2
PEG 400	1	1	1	1
Asam asetat	1	1	1	1
NaOH 0,1 M	q.s	q.s	q.s	q.s
Aquades to	100 ml	100 ml	100 ml	100 ml

Source: Research design, 2025

Manufacture of papaya seed extract hydrogel

All ingredients are weighed according to the formula. Chitosan is dissolved in 35 ml of 1% acetic acid solution, then PEG 400 is put into it and stirred using a magnetic stirrer at 300 rpm for 1 hour, after which it is left for 30 minutes. Next, papaya seed extract is added, the pH is set at 4.5-6.5 by adding sodium hydroxide. Next, the volume is sufficient with aquades up to 100 ml, then stirred using a magnetic stirrer until homogeneous. The homogeneous mixture is poured into a petri dish, then put in the refrigerator at -20ºC for 18 hours. It is then stored at room temperature for 4 hours, then heated in the oven at 40ºC for 15 minutes (Lotfipour et al., 2019; Ouyang et al., 2018).

Evaluation of preparations

Organoleptic tests are carried out to determine organoleptic characteristics including color, odor, and texture. By way of visual observation for the color, texture, and smell of the hydrogel preparation. pH Test pH analysis aims to determine the compatibility of the pH of the preparation with the physiological pH of the skin, which is 4.5-6.5. First of all, the pH measuring device to be used is calibrated first using a neutral pH standard solution (pH 6.86) and an acidic pH solution (pH 4.01) until the device shows the pH price, then the electrodes are washed with distilled water, then dried with a tissue. Furthermore, the hydrogel of papaya seed extract is measured for its pH, by means of the electrodes of the pH meter dipped into the hydrogel preparation until the device shows a constant pH price (Malpure et al., 2018).

Swelling index The hydrogel swelling index test is carried out to determine the ability of the hydrogel preparation to expand so that it can absorb water. The swelling index test was carried out by weighing 1 g of samples from each formula, immersed in 5 ml of 5.5 phosphate buffer. Then the sample was reweighted at 5, 10, 20, 30, 45, and 60 minutes. (Waresindo et al., 2021). Gel fraction is carried out by weighing the hydrogel preparation and recorded as (Wo), then the hydrogel is wrapped in gauze and soaked in aquades for 24 hours, then dried again in an oven at 50ºC for 4 hours and weighed as (W1) (Malpure et al., 2018).

Testing the effectiveness of papaya seed extract The experimental animals used in this study were healthy male mice aged 2-3 months with a weight of 30-35 g. 15 mice were prepared, then divided into 5 groups, each group consisted of 3 mice. Before treatment, mice are acclimatized for 7 days. Mice that have been acclimatized for 7 days, then under anesthesia. First of all, the fur on the back of the mouse that will be made to be wounded is shaved first. Then it is cleaned with a cotton swab given 70% alcohol. Next, a cut wound with a length of 1 cm and a depth of 0.2 cm is made using a sterilized scapel (scalpel). The cut wound is made up to the subcutaneous part after which the wound area is cleaned with an alcohol swab.

The treatment is carried out by attaching a hydrogel of papaya seed extract with a weight of 1 g. The administration of papaya seed hydrogel is carried out every day for 14 days. Woundgel is a hydrogel preparation with a high water content and can be used as a wound contact layer. Wound observation was carried out every day visually for 14 days by measuring the length of the cut wound using a caliper.

RESULTS AND DISCUSSION

Results of papaya seed extraction

The manufacture of papaya seed extract (Carica papaya L.) using the maceration method was chosen for its simplicity and ease, and one of the common methods in the extraction process of natural materials. 96% ethanol was chosen as a solvent because of its ability to attract polar and non-polar active substances, universal, and easy to obtain. The results of the extraction of papaya seeds (Carica papaya L.) using the maceration method with 96% ethanol solvent as much as 5 L produced a thick extract of 13.744% yield.

Table 2. Results of papaya seed extraction

Simplicia weight (g)	Extract weight (g)	Rendemen (%)
1000	137,4485	13,744

Source: Primary data, processed, 2025

The yield value obtained in this study is still eligible for the extract yield value, as written in the Indonesian Herbal Pharmacopoeia (FHI) with the requirement that the yield is not less than 10%.

Phytochemical screening results of papaya seed extract (Carica papaya L.)

The results of the phytochemical screening test have the aim of identifying secondary metabolites that are efficacious in wound healing

Table 3. Result of Screening Phytochemistry

Compounds	Reagents	Results	Remarks
Flavonoid	Magnesium + HCL concentrate	Blackish-red color formation	Positive
Saponins	Distilled water	Foam formed	Positive
Alkaloid	NH3 10% + H2SO4 2 N + Reagen Mayer	Formation of yellowish deposits	Positive
Steroids	Ethanol 70% + Chloroform + H2SO4 pointed	Formed red rings	Positive
Tannins	FeCl3	Formed a blackish-green color	Positive
Glikosida	Liberman Burchard	No blue or green color formed	Negatives

Source: Laboratory test results, 2025

The flavonoid test obtained a positive result by being marked with a blackish-red color, in the flavonoid test the metals Mg and HCl were added. The purpose of adding Mg and HCl metals is to reduce the benzopiron nucleus present in the flavonoid structure so that red or orange 6748haracter salts are formed. Mg and HCl powders react to form H2 gas bubbles (Auwal et al., 2014). The saponin test was positive with a marked presence of foam. According to the literature, if positive saponins are identified if foam is more than 1 cm. Froth indicates the presence of glycosides that have the ability to form foam in hydrolyzed water (Auwal et al., 2014)

Alkaloid test Positive results are obtained by marking the presence of cloudiness and deposits, if there are yellow deposits, it indicates positive for the presence of alkaloids. The addition of mayer reagents will cause the nitrogen in the alkaloids to react by precipitation (Auwal et al., 2014). Tannin test Identification of tannin compounds reacted with FeCl3 obtained blackish-green color results, solution color change due to a reduction reaction, tannins are a class of polyphenol compounds that are able to reduce iron (III) to iron (II) (Auwal et al., 2014). The glycoside test was performed with the Liberman burchard reagent, but no blue or green color was formed.

Quantitative analysis of papaya seed extract compounds

8. Determination of Maximum Absorption Wavelength

The determination of the maximum wavelength has the purpose of determining the wavelength of the measurement where the complex between quercetin and $AlCl_3$ provides optimal absorbance. Measurements at the maximum wavelength will provide the most absorbance change for each unit of rate, so that if remeasurements and replication are to be carried out, it will minimize the occurrence of measurement errors. Based on the observations that have been made, the maximum wavelength is at 424 nm which is in accordance with the literature, because the quercetin wavelength has a maximum wavelength ranging from 400-450 nm.

b. Quercetin Standard Solution Absorbance Measurement

The choice of quercetin as a standard solution is because quercetin is the most widely distributed compound found in plants. Quercetin and its glycosides are in the amount of about 60-70% of the flavonoids. And also because it is a flavonoid compound that can react with AlCl3 to form a complex. The addition of AlCl3 aims to form a complex, so that there is a shift in wavelength towards visible (visible) characterized by the solution producing a more yellow color. Meanwhile, the addition of potassium acetate to maintain the wavelength in the visible area. Absorption measurements of each of the standard quercetin solution concentrations were performed at a maximum wavelength of 424 nm. The measurement of the absorbance of the standard quercetin solution is made in five concentrations, namely the concentration of 0, 2, 4, 6, 8 ppm which is measured in absorption at the maximum wavelength.

Concentration (ppm)	Absorbansi
0	0
2	0,170
4	0,334
6	0.443
8	0,619

Based on the results of the measurement of the absorbance of the quercetin solution, the absorbance value is obtained, it can be seen that the greater the concentration of quercetin solution used, the greater the absorbance of the quercetin solution. The raw result of quercetin is plotted between its content and absorbance, so that the linear regrease equation is y = 0.0738x + 0.0241, with an R value of 0.9962.

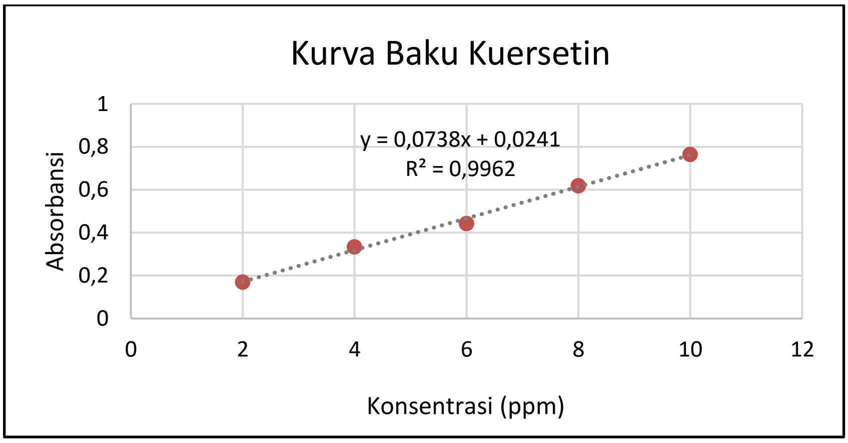

Figure 1. Quercetin Standard Curve for Determination of Total Flavonoid Levels

Source: Primary data, processed, 2025

After the absorbance of the quercetin solution was obtained, the flavonoid content of papaya seed ethanol extract was determined, carried out by making a test solution, then tested 3 times and measured at a wavelength of 395 nm, with the result of consecutive sample measurements of 0.000; 0,446; 0,447; 0,440.

Testing	Wavelength	Absorbansi
Replication I	395 nm	0,446
Replication II	395 nm	0.447
Replication III	395 nm	0,440

After the sample absorbance was obtained, a calculation was carried out to determine the flavonoid level of papaya seed ethanol extract, the absorption produced from papaya seed ethanol extract at three replications provided a value that was not much different. Furthermore, the concentration of each absorption was calculated using a linear regression equation, namely y = 0.0738x + 0.0241, with an R value of 0.9962. The results of the calculation of test extract 1 were 8.2105 ppm, test 2 was 8.2043 ppm, and test 3 was 8.2353 ppm. The results are converted into mg/ml units and then calculated as a percentage of the level. The results of the calculation of the percentage of flavonoid content in the ethanol extract of papaya seeds in test 1 were 0.48079%, test 2 was 0.48051% and test 3 was 0.48191%.

Table 4. Kadar flavonoid ekstrak biji papaya

No	**Absorbansi**	**Concentration**		**flavonoid**	
		mμ/ml	mg/ml	%	mg QE/100 g
1	0,446	8,2105	0,008211	0,48079	480.79
2	0,447	8,2043	0,008204	0,48051	480.51
3	0,440	8,2353	0,008235	0,48191	481.91
Average				0,48107	481,07
Standard deviation				±0,0094	±0,94068

Source: Primary data, processed, 2025

Ethanol extract of positive papaya seed extract contains flavonoid compounds. The levels in ethanol extract of papaya seed extract have flavonoid levels with an average level of 481.07 mgQE/100g.

Uji organoleptis

The results of organoleptic tests during 12-day storage (6 cycles) can be seen in cycles 0, F1, F2, F3, and F4 of the gel preparation do not experience changes in texture, color, and aroma until the 6th cycle. The 6750haracterize test of hydrogels was carried out by visually observing the color, odor, and texture of the preparation. Organoleptic tests on the four formulas with different extract levels obtained F1 results with a concentration of 5% light brown extract and a typical smell of papaya seed extract, while F2 has a more intense brown color than F1. F3 formula has a very intense hydrogel brown color, with a distinctive extract smell, and with a texture that is easy to apply to the skin.

Table 4. Organoleptic Test Results

Formula	**Parameter**	**Before storage**	**After storage**
F1	Color	Coklat	Coklat
	Construction	Khas EBP	Khas EBP
	Tekstur	Semi-compact	Semi-compact
F2	Color	Coklat	Coklat
	Construction	Khas EBP	Khas EBP
	Tekstur	Semi-compact	Semi-compact
F3	Color	Coklat	Coklat
	Construction	Khas EBP	Khas EBP
	tekstur	Semi-compact	Semi-compact
F4	Color	Clear	Clear
	Construction	Odorless	Odorless
	Tekstur	Semi-compact	Semi-compact

Source: Primary data, processed, 2025

pH Test

The pH test results on papaya seed extract hydrogel preparations during 12-day storage (6 cycles) F1, F2, F3, F4 ranged from 6.78-4.65, which showed that the preparation decreased after storage, but was still within the pH range of the preparation acceptable to the skin, so it can be ensured that the resulting hydrogel is stable and has a pH range (4.5-8). Based on statistical tests using Reapeted Measures Anova, a significance result of 0.000 (<0.05) was obtained, which means that there is a significant difference between the formulations in the pH test.

Table 5. pH Test Results

Siklus	K-	5%	10%	15%
0	6,78 ± 0,08	6,30 ± 0,34	6,30 ± 0,21	5,52 ± 0,34
1	6,15 ± 0,13	5,63 ± 0,33	5,36 ± 0,04	5,16 ± 0,05
2	6,13 ± 0,16	5,58 ± 0,37	5,23 ± 0,11	4,97 ± 0,06
3	6,08 ± 0,11	5,14 ± 0,12	5,33 ± 0,11	5,06 ± 0,06
4	5,88 ± 0,10	4,67 ± 0,21	4,81 ± 0,04	4,65 ± 0,04
5	5,81 ± 0,07	4,65 ± 0,11	4,82 ± 0,16	4,63 ± 0,29
6	5,80 ± 0,13	4,76 ± 0,16	4,77 ± 0,13	4,88 ± 0,09
Say.		0,000		

Source: Primary data, processed, 2025

Remarks: The pH test data listed is the mean value of SD ±, Sig. >0.05 there is no significant difference, Sig. <0.05 there is a significant difference

Uji swelling index

The swelling index data showed a consistent increase from the 5th minute to the 60th minute, illustrating the hydrogel's ability to absorb water gradually and steadily. In the 5th minute, the average swelling value was 1.98, then increased to 3.00 in the 10th minute, 3.66 in the 20th minute, to reach 5.52% in the 60th minute. This ascending pattern indicates that the hydrogel structure has good expandability, which is important in retaining moisture in the wound area as well as aiding in the absorption of exudate. The relatively small standard value of deviation indicates that the characteristics of hydrogel development are quite consistent between replications. Based on the statistical test using Reapeted Measures Anova, a significance result was obtained which was 0.000 (<0.05), which means that there is a significant difference between the formulations in the swelling index test.

Table 6. Swelling index test results

Time	5%(Mean ± SD)	10%(Mean ± SD)	15%(Mean ± SD)	K- (Mean ± SD)
5 minutes	1,72 ± 0,10	1,79 ± 0,13	1,98 ± 0,06	1,72 ± 0,13
10 minutes	2,43 ± 0,20	2,36 ± 0,17	3,00 ± 0,20	2,40 ± 0,27
20 minutes	2,93 ± 0,05	2,88 ± 0,02	3,66 ± 0,26	2,92 ± 0,06
30 minutes	3,33 ± 0,11	3,33 ± 0,10	4,01 ± 0,23	3,28 ± 0,06
40 minutes	3,92 ± 0,06	3,91 ± 0,04	4,61 ± 0,31	3,92 ± 0,03
60 minutes	4,57 ± 0,20	4,70 ± 0,07	5,52 ± 0,25	4,63 ± 0,12
Sign.		0,000		

Source: Primary data, processed, 2025

Remarks: The swelling index test data listed is the mean value of SD ±, Sig. >0.05 there is no significant difference, Sig. <0.05 there is a significant difference

Gel fraction test

Table 7. Gel fraction test results

Parameter	F1 (Mean ± SD)	F2 (Mean ± SD)	F3 (Mean ± SD)	F4 (Mean ± SD)
Starting weight (g)	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00
Dry weight (g)	0,47 ± 0,07	0,53 ± 0,03	0,85 ± 0,03	0,35 ± 0,03

Source: Primary data, processed, 2025

Based on the gel fraction test, the results of the gel fraction were obtained consecutively in formulas 1, 2, 3, and 4, namely 47%, 53%, 86%, and 34%. This value illustrates that most hydrogels are able to maintain their mesh structure even through the drying process. The high value of the gel fraction indicates a good crosslinking rate, so the hydrogel is not easily destroyed or decomposed. The highest gel fraction value is found in the formula with a papaya seed extract content of 15%. While the gel fraction is lowest in the negative control formula without extract.

Results of the effectiveness test of papaya seed extract hydrogel

This test used 15 male mice divided into 5 groups. Group I was given a hydrogel of papaya seed extract with a concentration of 5%, group II was given a hydrogel of papaya seed extract with a concentration of 10%, group III was given a hydrogel of papaya seed extract with a concentration of 15%, group IV was given hydrogel without extract as a negative control, group 5 was given a hydrogel® woundgel as a positive control. The test animals were adapted for 7 days before the test animals were given treatments aimed at avoiding stress in mice.

Table 8. Results of Wound Healing Effectiveness Test in Mice

Day	K− (Mean ± SD)	K+ (Mean ± SD)	(5%) (Mean ± SD)	(10%) (Mean ± SD)	(15%) (Mean ± SD)
0	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00
2	1,00 ± 0,00	0,95 ± 0,02	1,00 ± 0,00	1,00 ± 0,00	1,00 ± 0,00
4	1,00 ± 0,00	0,86 ± 0,02	1,00 ± 0,00	1,00 ± 0,00	0,96 ± 0,02
6	1,00 ± 0,00	0,74 ± 0,03	0,99 ± 0,01	0,95 ± 0,02	0,85 ± 0,02
8	0,96 ± 0,02	0,60 ± 0,01	0,96 ± 0,01	0,91 ± 0,01	0,76 ± 0,02
10	0,89 ± 0,01	0,44 ± 0,05	0,83 ± 0,01	0,87 ± 0,01	0,41 ± 0,01
12	0,86 ± 0,02	0,21 ± 0,01	0,79 ± 0,01	0,77 ± 0,02	0,31 ± 0,02
14	0,78 ± 0,02	0,05 ± 0,04	0,73 ± 0,01	0,66 ± 0,04	0,23 ± 0,02
Sign.			0,000		

Source: Primary data, processed, 2025

In the mouse wound data, in all treatment groups, namely negative control (K−), positive control (K+), and treatment group with hydrogel application (F1, F2, and F3), there was a significant change in the size of the cut wound from day 0 to day 14. A p-value much smaller than 0.05 indicates that there was a significant difference in wound size between observation times in each group, so it can be concluded that the entire group experienced a marked change in wound size during the observation period.

The change in the Mean Rank value in each group showed a consistent pattern of decreasing wound size over time. In the early days of observation (day 0 to day 5), the Mean Rank value was still relatively high, reflecting the size of the cut wound that was still large in mm. Furthermore, from day 6 to day 14, there was a gradual and significant decrease in the

Mean Rank, indicating that the size of the wound was getting smaller and the healing process took place progressively in all treatment groups.

When compared between groups, the F3 group showed a faster decline in Mean Rank since the early days than the other groups, followed by the F2 and F1 groups. The positive control group (K+) also showed a steady decline in Mean Rank, while the negative control group (K−) experienced a relatively slower decline. This pattern indicates that the application of hydrogels, especially in F3 formulations, has a more optimal effect in accelerating the reduction of the cut size (mm) compared to the negative control, as well as approaching the effectiveness of positive control.

Overall, the test results showed that the entire treatment had a significant effect on the day-to-day change in the size of the cut. Nevertheless, the hydrogel treatment group (F1–F3), especially F3, showed a faster and more consistent pattern of wound healing, thus supporting the effectiveness of hydrogel as an alternative therapy in accelerating the healing of slices based on a decrease in wound size.

CONCLUSION

This study demonstrated that papaya seed extract (Carica papaya L.), containing bioactive compounds such as flavonoids, saponins, alkaloids, tannins, and steroids, was successfully formulated into hydrogel preparations based on chitosan and polyethylene glycol (PEG 400). The resulting hydrogel preparations exhibited good physical characteristics and met the requirements of topical preparation evaluation, encompassing organoleptic tests, pH, swelling index, and gel fraction. All formulas demonstrated adequate stability during storage and a pH corresponding to the physiological pH of the skin, rendering them safe for topical use. The results of the swelling index and gel fraction tests showed that increasing the concentration of papaya seed extract had an effect on the hydrogel's ability to absorb water and maintain its polymer network structure.

Formulas with an extract concentration of 15% yielded the highest swelling index and gel fraction values, indicating a superior crosslinking rate as well as an optimal ability to retain moisture in the wound area — an important factor in the wound healing process.An examination of incision wound healing effectiveness in male mice (Mus musculus) showed that all treatment groups experienced a significant reduction in wound length during the observation period ($p < 0.05$). The papaya seed extract hydrogel provided a better healing effect than the negative control. Formulas with an extract concentration of 15% showed the most optimal acceleration of wound closure, 6753haracterized by faster and more consistent wound size reduction compared to formulas with concentrations of 5% and 10%, and approaching the effectiveness of the positive control. Overall, the results of this study demonstrate that papaya seed extract hydrogel has the potential to be developed as a natural ingredient-based topical preparation for accelerating the healing of incision wounds. The 15% extract concentration represents the most effective formula and may serve as a basis for the development of wound-healing hydrogel preparations in further research.

REFERENCES

Ali, A., & Ahmed, S. (2018). Recent Advances in Edible Polymer Based Hydrogels as a Sustainable Alternative to Conventional Polymers [Review-article]. Journal of

Agricultural and Food Chemistry, 66(27), 6940–6967. https://doi.org/10.1021/acs.jafc.8b01052

Amin, A. H., Bughdadi, F. A., Abo-Zaid, M. A., Ismail, A. H., El-Agamy, S. A., Alqahtani, A., El-Sayyad, H. I. H., Rezk, B. M., & Ramadan, M. F. (2019). Immunomodulatory effect of papaya (Carica papaya) pulp and seed extracts as a potential natural treatment for bacterial stress. Journal of Food Biochemistry, 43(12), 1–11. https://doi.org/10.1111/jfbc.13050

Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Veterinary Research Forum : An International Quarterly Journal, 5(2), 95–100. http://www.ncbi.nlm.nih.gov/pubmed/25568701%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4279630

Ávila, S., Kugo, M., Silveira Hornung, P., Apea-Bah, F. B., Songok, E. M., & Beta, T. (2020). Carica papaya seed enhances phytochemicals and functional properties in cornmeal porridges. Food Chemistry, 323(April), 126808. https://doi.org/10.1016/j.foodchem.2020.126808

Bustamante-Torres, M., Romero-Fierro, D., Arcentales-Vera, B., Palomino, K., Magaña, H., & Bucio, E. (2021). Hydrogels classification according to the physical or chemical interactions and as stimuli-sensitive materials. Gels, 7(4), 1–25. https://doi.org/10.3390/gels7040182

Cramer, M. N., Gagnon, D., Laitano, O., & Crandall, C. G. (2022). Human temperature regulation under heat stress in health, disease, and injury. *Physiological Reviews*.

Chen, Z., Zhao, M., Liu, K., Wan, Y., Li, X., & Feng, G. (2014). Novel chitosan hydrogel formed by ethylene glycol chitosan, 1,6-diisocyanatohexan and polyethylene glycol-400 for tissue engineering scaffold: In vitro and in vivo evaluation. Journal of Materials Science: Materials in Medicine, 25(8), 1903–1913. https://doi.org/10.1007/s10856-014-5223-3

Extracts, T., Fritea, L., Andreea, I., Mintas, A., Antonescu, A., & Groza, F. M. (2021). Evaluation of Wound Healing Potential of Novel Hydrogel.

Ghaffarilaleh, V., Fisher, D., & Henkel, R. (2019). Carica papaya seed extract slows human sperm. Journal of Ethnopharmacology, 241(May). https://doi.org/10.1016/j.jep.2019.111972

Hasan, M. M., Uddin, M. F., Zabin, N., Shakil, M. S., Alam, M., Achal, F. J., Ara Begum, M. H., Hossen, M. S., Hasan, M. A., & Morshed, M. M. (2021). Fabrication and Characterization of Chitosan-Polyethylene Glycol (Ch-Peg) Based Hydrogels and Evaluation of Their Potency in Rat Skin Wound Model. International Journal of Biomaterials, 2021, 1–11. https://doi.org/10.1155/2021/4877344

Jiao, Q., Zhi, L., You, B., Wang, G., Wu, N., & Jia, Y. (2024). Skin homeostasis: Mechanism and influencing factors. Journal of Cosmetic Dermatology, 23(5), 1518–1526. https://doi.org/10.1111/jocd.16155

Kong, Y. R., Jong, Y. X., Balakrishnan, M., Bok, Z. K., Weng, J. K. K., Tay, K. C., Goh, B. H., Ong, Y. S., Chan, K. G., Lee, L. H., & Khaw, K. Y. (2021). Beneficial role of carica papaya extracts and phytochemicals on oxidative stress and related diseases: A mini review. Biology, 10(4), 1–20. https://doi.org/10.3390/biology10040287

Kyei-Barffour, I., Kwarkoh, R. K. B., Acheampong, D. O., Brah, A. S., Akwetey, S. A., & Aboagye, B. (2021). Alkaloidal extract from Carica papaya seeds ameliorates CCl4-induced hepatocellular carcinoma in rats. Heliyon, 7(8), e07849. https://doi.org/10.1016/j.heliyon.2021.e07849

Lih, E., Lee, J. S., Park, K. M., & Park, K. D. (2012). Rapidly curable chitosan-PEG hydrogels as tissue adhesives for hemostasis and wound healing. Acta Biomaterialia, 8(9), 3261–3269. https://doi.org/10.1016/j.actbio.2012.05.001

Liu, H., Wang, C., Li, C., Qin, Y., Wang, Z., Yang, F., Li, Z., & Wang, J. (2018). A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. RSC Advances, 8(14), 7533–7549. https://doi.org/10.1039/c7ra13510f

Lotfipour, F., Alami-Milani, M., Salatin, S., Hadavi, A., & Jelvehgari, M. (2019). Freeze-thaw-induced cross-linked PVA/chitosan for oxytetracycline-loaded wound dressing: The experimental design and optimization. Research in Pharmaceutical Sciences, 14(2), 175–189. https://doi.org/10.4103/1735-5362.253365

Malpure, P. S., Patil, S. S., More, Y. M., & Nikam, P. P. (2018). A Review On- Hydrogel. 8(May).

Masson-Meyers, D. S., Andrade, T. A. M., Caetano, G. F., Guimaraes, F. R., Leite, M. N., Leite, S. N., & Frade, M. A. C. (2020). Experimental models and methods for cutaneous wound healing assessment. International Journal of Experimental Pathology, 101(1–2), 21–37. https://doi.org/10.1111/iep.12346

Memudu, A. E., & Oluwole, T. J. (2021). The contraceptive potential of carica papaya seed on oestrus cycle, progesterone, and histomorphology of the utero-ovarian tissue of adult wistar rats. Jornal Brasileiro de Reproducao Assistida, 25(1), 34–43. https://doi.org/10.5935/1518-0557.20200023

Nayak, B. S., Ramdeen, R., Adogwa, A., Ramsubhag, A., & Marshall, J. R. (2012). Wound-healing potential of an ethanol extract of Carica papaya (Caricaceae) seeds. International Wound Journal, 9(6), 650–655. https://doi.org/10.1111/j.1742-481X.2011.00933.x

Ouyang, Q. Q., Hu, Z., Lin, Z. P., Quan, W. Y., Deng, Y. F., Li, S. D., Li, P. W., & Chen, Y. (2018). Chitosan hydrogel in combination with marine peptides from tilapia for burns healing. International Journal of Biological Macromolecules, 112, 1191–1198. https://doi.org/10.1016/j.ijbiomac.2018.01.217

Panzarini, E., Dwikat, M., Mariano, S., Vergallo, C., & Dini, L. (2014). Administration dependent antioxidant effect of carica papaya seeds water extract. Evidence-Based Complementary and Alternative Medicine, 2014. https://doi.org/10.1155/2014/281508

Seow, W. Y., Salgado, G., Lane, E. B., & Hauser, C. A. E. (2016). Transparent crosslinked ultrashort peptide hydrogel dressing with high shape-fidelity accelerates healing of full-thickness excision wounds. Scientific Reports, 6(August), 1–12. https://doi.org/10.1038/srep32670

Silvy, N. (2021). Formulasi dan Uji Efektivitas Gel Ekstrak Biji Pepaya (Carica Papaya L.) Terhadap Penyembuhan Luka Bakar Pada Kelinci. Karya Tulis Ilmiah, 7(2), 107–115.

Soares, L., Leite Miliao, G., Tonole, B., Souza, G., Soares, N., Teixeira, A., Coimbra, J., & De Oliveira, E. B. (2019). Chitosan dispersed in aqueous solutions of acetic, glycolic, propionic or lactic acid as a thickener/stabilizer agent of O/W emulsions produced by ultrasonic homogenization. Ultrasonics Sonochemistry, 59, 104754. https://doi.org/10.1016/j.ultsonch.2019.104754

Tavakoli, S., & Klar, A. S. (2020). Advanced hydrogels as wound dressings. Biomolecules, 10(8), 1–20. https://doi.org/10.3390/biom10081169

Waresindo, W. X., Luthfianti, H. R., Edikresnha, D., Suciati, T., Noor, F. A., & Khairurrijal, K. (2021). A freeze-thaw PVA hydrogel loaded with guava leaf extract: physical and antibacterial properties. RSC Advances, 11(48), 30156–30171. https://doi.org/10.1039/d1ra04092h

Zhang, Y., Jiang, M., Zhang, Y., Cao, Q., Wang, X., Han, Y., Sun, G., Li, Y., & Zhou, J. (2019). Novel lignin–chitosan–PVA composite hydrogel for wound dressing. Materials Science and Engineering C, 104(October 2018), 110002. https://doi.org/10.1016/j.msec.2019.110002

Zhao, P., Liu, H., Deng, H., Xiao, L., Qin, C., Du, Y., & Shi, X. (2014). A study of chitosan hydrogel with embedded mesoporous silica nanoparticles loaded by ibuprofen as a dual stimuli-responsive drug release system for surface coating of titanium implants. Colloids and Surfaces B: Biointerfaces, 123, 657–663. https://doi.org/10.1016/j.colsurfb.2014.10.013