International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Optimization of Papaya Leaves and Aloe Vera Extracts as Antibacterial Wound Dressing In PVA/Gelatin Hydrogel

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Abstract

The length of time for wound healing is the main problem in treating infected wounds. Hydrogel is an effective alternative wound dressing that can be prepared from biopolymers. This research aims to optimize and characterize hydrogel film based on *Aloe vera* gel and *Carica papaya* leaves extracts as a wound dressing with antibacterial activity. The hydrogel made in this study was prepared from mixture of polyvinyl alcohol (PVA) and gelatin. The extracts were obtained using maceration method with 96% ethanol as solvent followed by evaporation to obtain concentrated extracts. The extracts were then screened for their secondary metabolites content using phytochemical tests. Aloe vera gel extract contains flavonoids, alkaloids and triterpenoids. Meanwhile, papaya leaves extract contains phenolic, tannins, flavonoids, and steroids. Both extracts showed antibacterial activity against *Staphylococcus aureus* as indicated by the presence of a clear zone in the disc diffusion assay. The average clear zone of aloe vera gel extract and papaya leaves extracts were 5.67 mm and 9.76 mm, respectively. The hydrogel showed good antibacterial activity against *S. aureus* as measured by UV-Vis spectrophotometry and disc diffusion methods. The optimal ratio was obtained at a concentration of 750 ppm aloe vera gel extract and 250 ppm papaya leaves extract. The optimal hydrogel was then characterized using FTIR, swelling ratio, and *in vitro* extract release. FTIR shows the presence of O-H stretching, C-H stretching, C=C stretching aromatic, C-N stretch, N-H group, and C=O stretching. The hydrogel swelled by 19% and release 23% of the extract within 180 minutes. The results proved that PVA/gelatin hydrogel with extracts of aloe vera gel and papaya leaves has antibacterial activity and fulfills the characteristics of wound dressings.

Keywords: aloe vera, gelatin, hydrogel, papaya leaves, wound dressing

Full length article **Corresponding Author*, e-mail: trianing.tyas@unpad.ac.id

1. Introduction

A wound can be defined as a disruption of the integrity of skin, mucous membrane or organ tissue. The wound care management provided influences the length of healing time. Open wounds that are not treated immediately will cause infection. One of the causative agents of infection in wound is bacteria. The most common gram-positive bacterial species found was *Staphylococcus aureus* (79.4%) [1]. In addition, bacteria can cause inflammation, swelling, exudates, and damage to proliferating tissues. These agents also contribute to increased healing time and treatment costs. Currently, the use of alternative drugs other than antibiotics has been widely used by the community to treat infected wounds. One of them is biomaterials that are cheaper and effective in overcoming infection.

Global View Research (2020) reports that the estimated annual growth rate of biomaterials in the global market reaches 15.27%. Biomaterials are natural or synthesized substitutes for the body's biological tissues. Natural-based biomaterials, such as gelatin and collagen are more widely used in skin wound healing [2]. Gelatin is a hydrolyzed form of collagen found in bones or skin. Shaving waste from the tanning process can also be a source of gelatin because it contains collagen and chrome complex. Gelatin has good biocompatibility and is cheaper than other wound dressing. Gelatin can be formulated as films, gels, powders, and hydrogels [3]. Generally, gelatin hydrogel is combined with PVA and various natural active compounds to enhance its benefits [4, 5].

Indonesia has 1.845 species of herbal plants that can be used as medicine and is home to 80% of medicinal plants in the world [6]. Papaya (*C. papaya*) is a plant that has

medicinal properties. Flavonoids, polyphenols, and saponins in papaya leaves have antibacterial properties that can help fight bacterial infections in wounds [7]. However, in some severe cases of infection, the addition of other antibacterials may need to be considered to support optimal healing. Aloe vera is a plant that is widely used by Indonesians and has a designation as a healing plant. Its specialty comes from the gel that is easily absorbed by the skin and its moisturizing properties. Aloe vera can also act as an antibacterial because it contains saponins, flavonoids, tannins, and polyphenols [8]. By combining papaya leaves and aloe vera gel extract, it is expected to utilize the synergy between the active substances and achieve a stronger effect to fight infections. However, there has been no specific research on the gelatin hydrogel mixture of aloe vera gel and papaya leaves combination as antibacterial in healing infectious wounds.

Based on this, further research is needed on the effect of the combination of gelatin hydrogel with papaya leaves and aloe vera gel extract as an antibacterial wound dressing agent in healing infected wounds.

2. Materials and methods

2.1. Research Design

The design of the hydrogel film formulation was carried out using Minitab 17 software, for optimization calculations using 2 level 2 factor factorial design with 2 replications. The results were 8 experiments and are shown in the Table 1.

2.2. Papaya leaves and aloe vera gel extraction

Extraction carried out by maceration with 96% ethanol as solvent with 1:2 ratio crude material to solvent. The maceration was performed for 24 hours followed by filtering the extract and evaporation using rotary vacuum evaporator at 60°C [8, 9].

2.3. Phytochemical Screening Test

Phytochemical screening testing of papaya leaves extract and aloe vera gel extract is carried out to identify the presence of active compounds contained in the extract. Phytochemical screening testing consists of phenolic, tannin, flavonoid, alkaloid, saponin, steroid and triterpenoid tests [10].

2.4. Antibacterial Assay using Disc Diffusion Method

The disc diffusion method was carried out using disc paper (Whatman No.42, ⌀ 6 mm) soaked into the test material to absorb the active substances in it. Furthermore, the disc paper that has contained the test material is placed on the surface of agar media that has been inoculated with *S. aureus* ATCC 25923 bacterial cultures. The media was then incubated for 24 hours at 37°C. After 24 hours, the diameter of clear zone was recorded [11]. For antibacterial assay of hydrogel, the paper disc was substituted by hydrogel disc (⌀ 6 mm).

2.5. Preparation of Hydrogel Film

The PVA/gelatin hydrogel were prepared by heating 20 g of PVA in 200 mL distilled water with 400 rpm stirring until a clear solution was obtained. Afterward, 5 g of gelatin and 0.01 mL of 37% HCl was added to the solution. The mixture was then stirred (400 rpm) at 75°C for 30 minutes. The solution was divided to 10 equal volumes, eight of which was added by the combination of extracts as presented on Table 1. One was added by gentamycin to give 1000 ppm final concentration, while the last one was leave blank without any addition as negative control. The mixture was stirred manually, followed by sonication for 20 minutes and casting on petri dish. The samples were then dried in an oven at 40°C for 48 hours [5].

2.6. Antibacterial Assay using Spectrophotometry Method

Antibacterial activity of hydrogel was assayed using disc diffusion method, as previously describe, and UV-Vis spectrophotometry. The assay using spectrophotometry is described below. The *S. aureus* ATCC 25923 culture that has been left overnight was adjusted to a standard bacterial suspension with an OD value of 0.1. The test solution consisting of sterilized 20 mL MHB media, 100 µL *S. aureus* bacterial culture, and hydrogel (⌀ 38 mm). The mixture was then shaken at room temperature with 180 rpm shaking speed. The OD value at 600 nm was recorded every two hours for eight hours. The results obtained was plotted to get slope value, which indicate the bacterial growth rate (µ) [12, 13].

2.7. Fourier Transform Infrared (FTIR) Analysis

Hydrogel films were prepared, grounded, and mixed at a ratio of 1.0% with potassium bromide (KBr) powder. They were then dried for 24 hours at 120°C. With a scan speed of 2 mm/sec and a resolution of 4 cm^{-1}, FTIR spectra were obtained in the range of 4000-400 cm^{-1} [5].

2.8. Swelling ratio test

The hydrogel's capacity to swell was examined using the swelling ratio test. The hydrogel film (⌀ 38 mm) was immersed in 8 mL distilled water. Then the diameter was measured after 5, 15, 25, 35, 45, 55, and 60 minutes of immersion [14]. Equation (1) was used to calculate the swelling ratio [15].

$$\text{Swelling ratio } \% = \frac{d_s - d_d}{d_d} \times 100\ \% \quad \text{... (Eq.1)}$$

Where:
d_s = diameter of distilled water-soaked hydrogel film
d_d = initial diameter of hydrogel film

2.9. In Vitro Extract Release Test

The extract release test was used to see the ability of the hydrogel to release the extract. The speed of extract release can be assessed from the percentage calculation of the mass amount of drug released into the liquid. The hydrogel film was put into 20 mL of distilled water. Samples of the solution (5 mL) were pipetted every 30 minutes for three hours. The concentration of drug released was determined by UV-Vis spectrophotometry [16]. The percentage of active substance released was determined by Equation (2).

$$\text{Drug release } (\%) = \frac{r}{t} \times 100\% \quad \text{... (Eq.2)}$$

Where:
r = active substance release at time t
t = total active substance loaded into the film

Table 1. Ratio of aloe vera and papaya leaves extract on hydrogel film formulation

Run number	Aloe vera extract concentration (ppm)	Papaya leaves extract concentration (ppm)
1.	750	750
2.	250	250
3.	250	750
4.	750	250
5.	750	750
6.	250	250
7.	250	750
8.	750	250

Table 2. Phytochemical test results of extracts

Metabolites	Papaya leaves extract		Aloe vera extract	
	This study	Reference [18]	This study	Reference [19]
Phenolics	+	+	-	-
Tannin	+	+	-	+
Flavonoid	+	+	+	+
Alkaloid	-	+	+	+
Saponin	-	+	-	-
Steroid	+	+	-	-
Triterpenoid	-	-	+	+

Table 3. Extract inhibition zone diameter results

Concentration (ppm)	Aloe vera inhibition zone diameter (mm)	Papaya leaves inhibition zone diameter (mm)
Control (+)	18	27,55
Control (-)	-	-
100 ppm	-	-
500 ppm	-	11,5
1000 ppm	-	6,6
5000 ppm	-	11,2

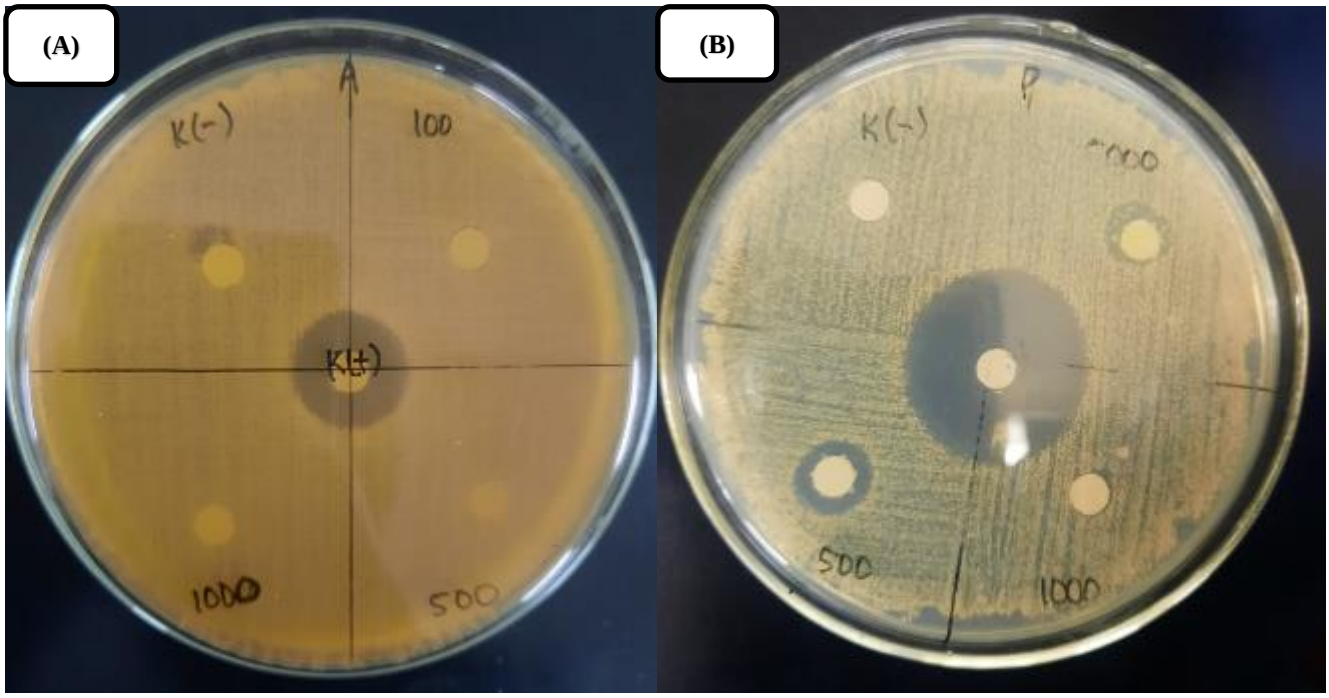

Figure 1. Antibacterial assay results of (A). Aloe vera extracts, (B). Papaya leaves extracts using disc diffusion method

Table 4. Antibacterial assay results of PVA/gelatin hydrogel using spectrophotometry method

Hydrogel Sample	Aloe vera extract concentration (ppm)	Papaya leaves extract concentration (ppm)	μ (AU/hour)
P1	750	750	0.019
	250	250	0.030
	750	250	0.010
	250	750	0.015
P2	750	750	0.025
	250	250	0.017
	750	250	0.018
	250	750	0.031
Control (+)			-0.006

Note:
P1 = treatment 1
P2 = treatment 2

Table 5. ANOVA table of aloe vera and papaya leaves extract effect on growth rate of *S. aureus*

Factorial Regression: Slope 2 versus Blocks, Aloe, Papaya leaves

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	4	0.000156	0.000039	0.52	0.735
Blocks	1	0.000036	0.000036	0.48	0.539
Linear	2	0.000083	0.000042	0.55	0.625
Aloe	1	0.000055	0.000055	0.73	0.456
Papaya leaves	1	0.000028	0.000028	0.37	0.585
2-Way Interactions	1	0.000036	0.000036	0.48	0.539
Aloe*Papaya leaves	1	0.000036	0.000036	0.48	0.539
Error	3	0.000226	0.000075		
Total	7	0.000382			

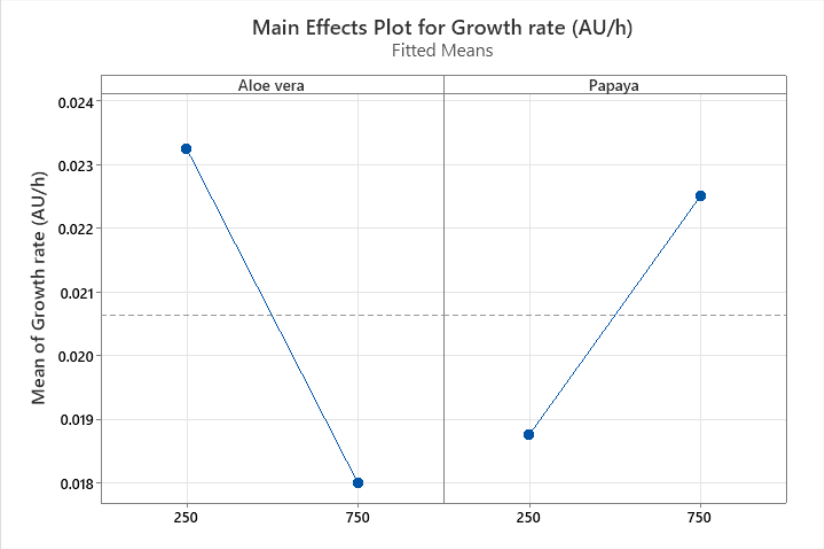

Figure 2. Main effects plot of aloe vera and papaya leaves extracts on *S. aureus* growth rate (µ)

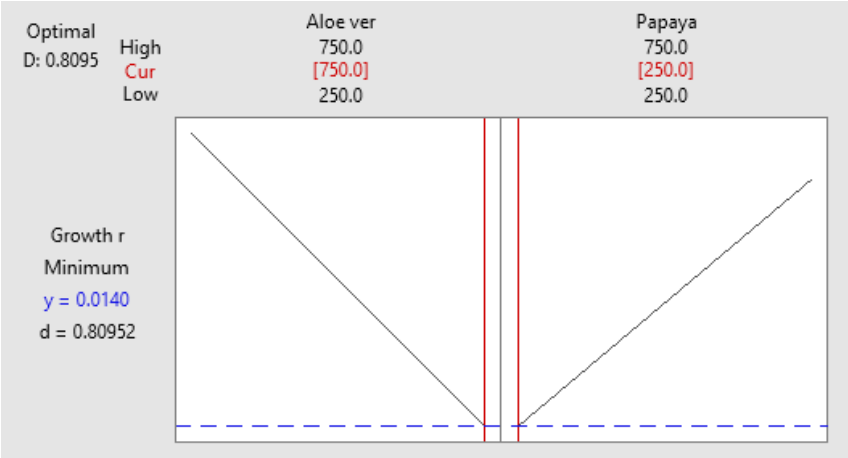

Figure 3. Response optimization of aloe vera and papaya leaves extracts on antibacterial activity of PVA/gelatin hydrogel with the lowest *S. aureus* growth rate (µ)

Table 6. Antibacterial assay results of PVA/gelatin hydrogel using disc diffusion method

Aloe vera extract concentration (ppm)	Papaya leaves extract concentration (ppm)	Hydrogel inhibition zone diameter (mm)
750	750	11
250	250	9.1
250	750	10.1
750	250	11.4
Control (+)		30.5
Control (-)		-

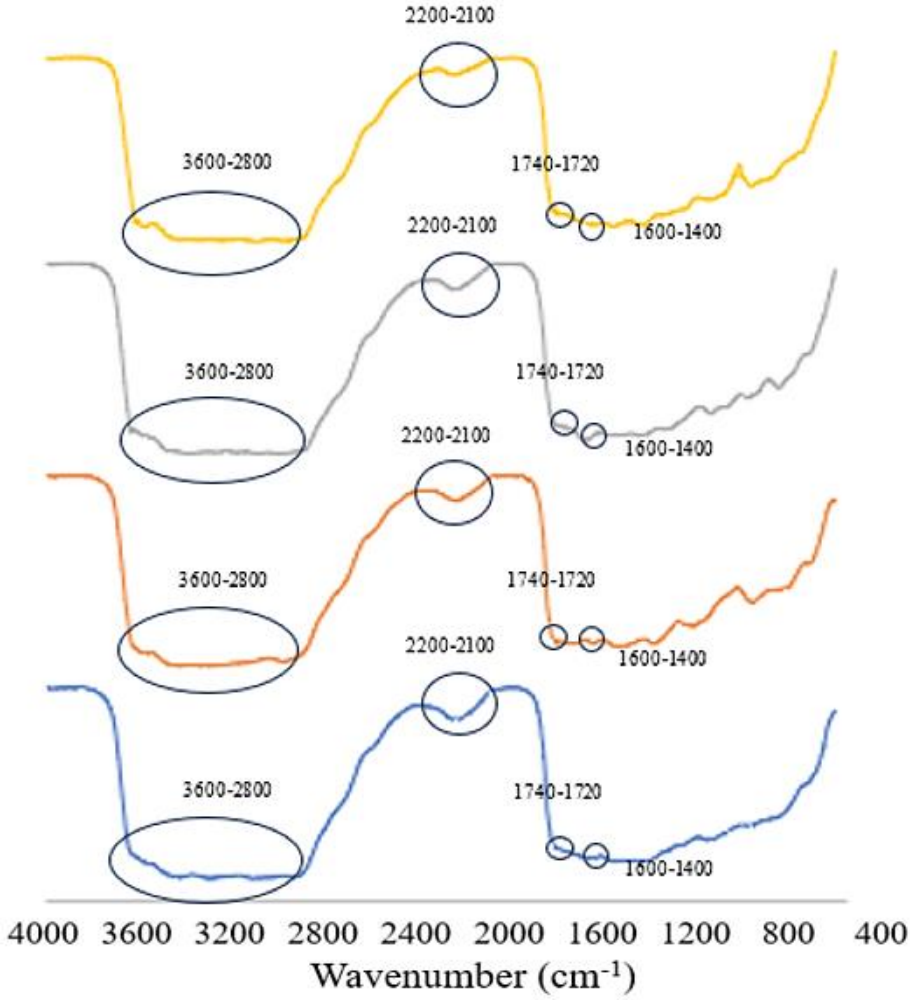

Figure 4. FTIR spectrum of PVA/gelatin hydrogel (blue), PVA/gelatin hydrogel impregnated with aloe vera (orange), papaya leaves (grey), and the optimum hydrogel (yellow)

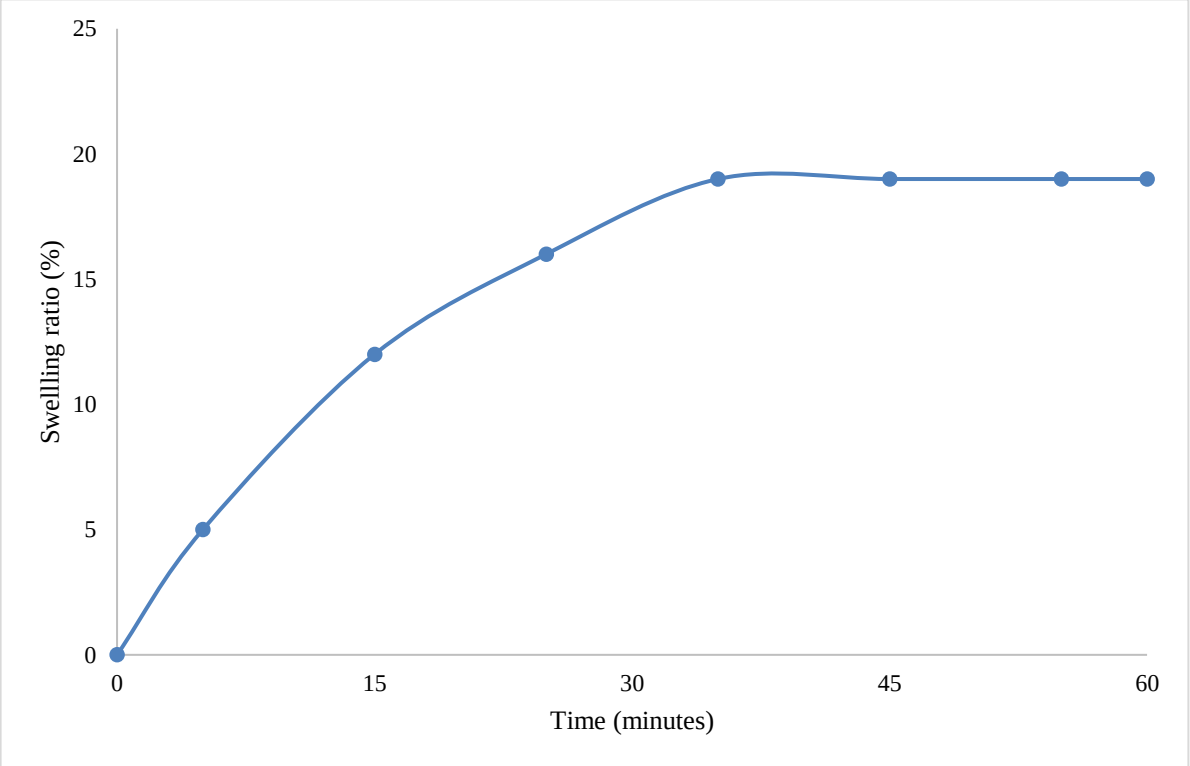

Figure 5. Swelling ratio of the PVA/gelatin hydrogel impregnated with 750 ppm aloe vera and 250 ppm papaya leaves extracts

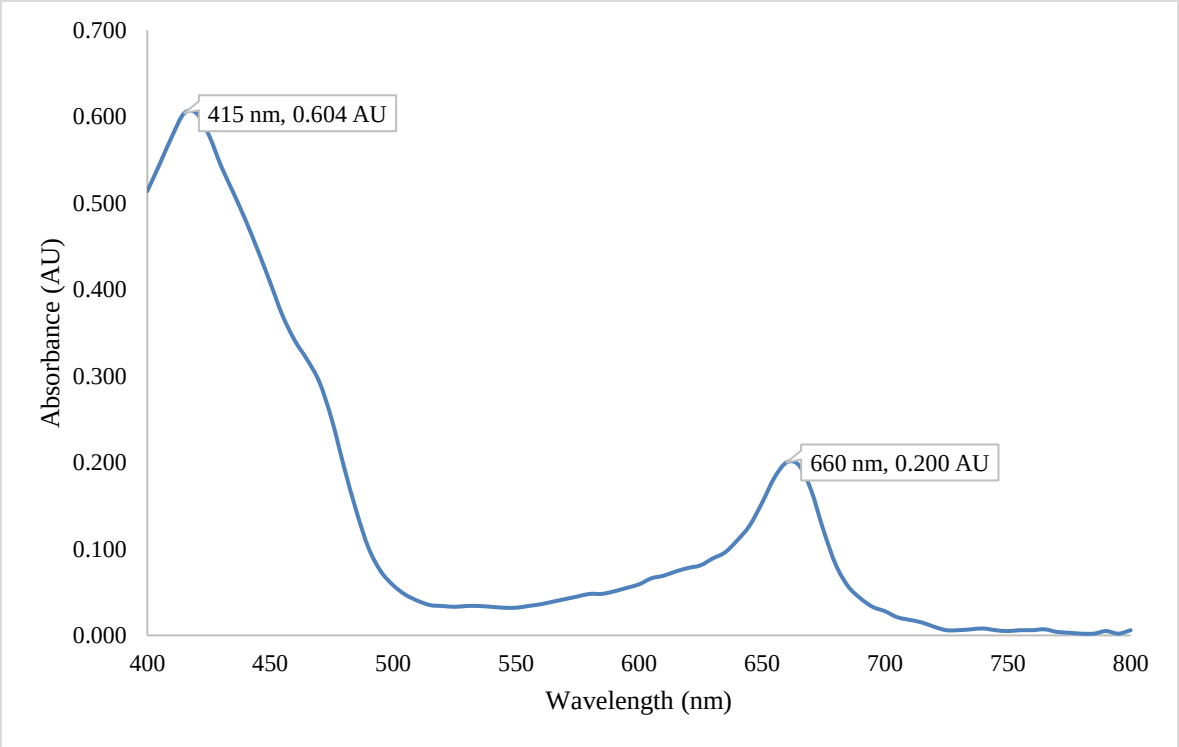

Figure 6. Visible spectrum of 50 ppm papaya leaves extract

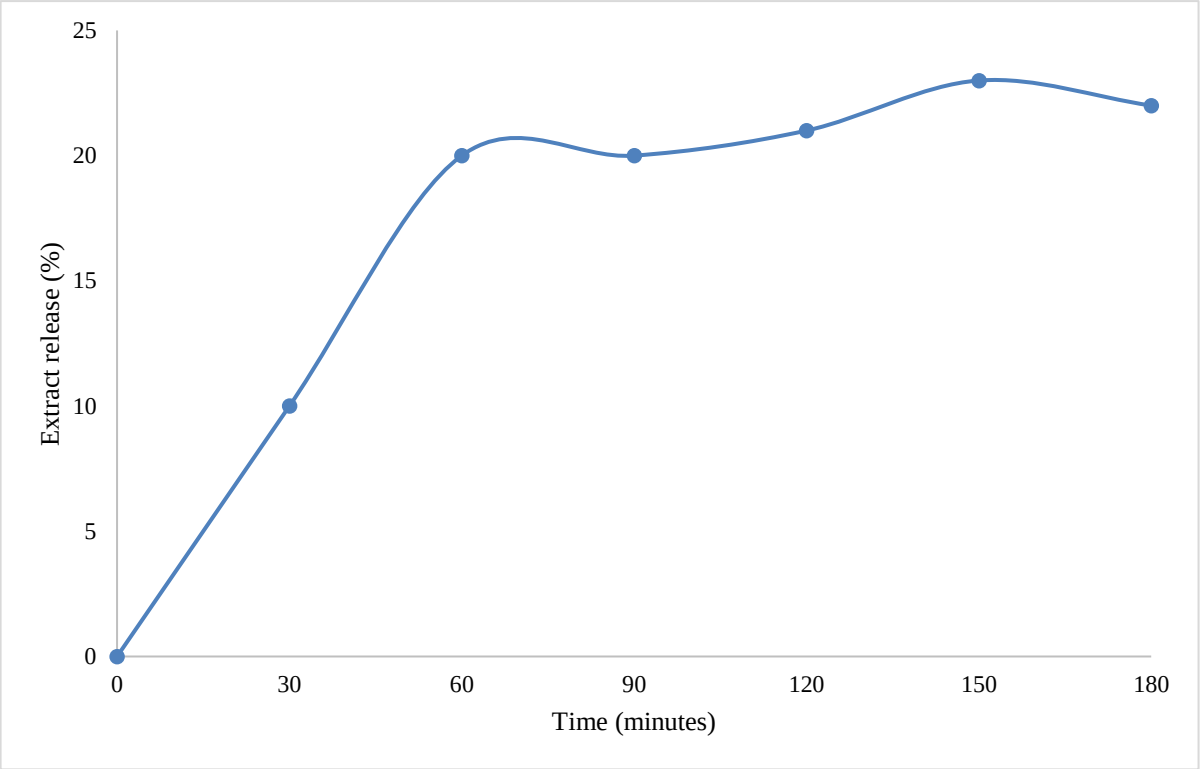

Figure 7. *In vitro* extract release at 660 nm from PVA/gelatin hydrogel

3. Results and Discussion

3.1. Extraction

The yield of papaya leaves extract was 5.77%. Meanwhile, the total yield of aloe vera extract was much greater than papaya leaves, which is 16.26%. This can be caused by the ratio between the solvent and the extract used. These extraction results are also supported by the statement of Rifai *et al.* [17], which suggests that the higher the amount of solvent, the more optimal the target compounds are released. Therefore, the extraction yield will increase.

3.2. Phytochemical test

Based on Table 2, not all tested compounds were identified in each extract. After testing, papaya leaves extract showed positive results for phenolic, tannins, flavonoids, and steroids. Meanwhile, aloe vera extract showed positive results for flavonoids, alkaloids, and triterpenoid. Papaya leaves and aloe vera have diverse secondary metabolites. The present study did not detect alkaloid on the papaya leaves extract. This result is in agreement with result described by Kusumo *et al*. [20], the absence of alkaloid might be due to extract concentration process is not perfect so that alkaloids are difficult to identify. Identification of saponin in both extracts yielded negative results. Shaking of the solution was done after heating and produced a medium intensity froth. However, after standing for 10 minutes, the foam decreased and did not last long. Based on the statement of Rivai [21], there are several factors that affect the saponin content in plants, such as environmental factors, types, and parts of plants used and places to grow. The negative results might be due to unsupported environmental factors that produces the saponin.

3.3. Extract Antibacterial Assay

Based on data in Table 3, aloe vera extract did not show any antibacterial activity. While, papaya leaves extract showed relatively high clear zone, which indicate that papaya leaves extract has a good antibacterial activity. This result is in agreement with some studies described by Syah *et al.* [7].

3.4. Hydrogel Antibacterial Assay

The antibacterial activity of hydrogels is presented on Table 4 and the ANOVA table resulted from the analysis of the data is presented on Table 5. The ANOVA result shows that both of the extracts did not have any significant effect on antibacterial activity ($P>0.05$). The main effect plot (Figure 2) indicates that higher concentration aloe vera lead to lower μ; while higher papaya leaves, extract shows higher μ. When combined, the highest antibacterial activity was detected when the highest aloe vera extract and the lowest papaya leaves extract were prepared (Figure 3). This result shows that each of the extracts have their own antibacterial activity and does not interact with each other. The results of the hydrogel antibacterial test using UV-Vis spectrophotometry were also supported by disc diffusion antibacterial assay as presented on Table 6. Based on the data in Table 6, the data shows that the best extract combination to give the highest antibacterial activity was 750 ppm aloe vera extract and 250 ppm papaya leaves extract, which is in agreement with the spectrophotometry assay.

3.5. FTIR Analysis

Based on Figure 4, six functional groups can be identified in the hydrogel. In the highest peak (3600-3200 cm^{-1}), O-H stretching and C-H stretching groups were identified. The length of this number range is because it includes two functional groups. These groups come from hydroxyl in PVA, glycerol, gelatin, and phenolic compounds from aloe vera and papaya leaves extract. Furthermore, the peak at 3200-2800 cm^{-1} indicates the presence of C-H stretching. C-H come from organic compounds and in PVA, glycerol, gelatin, and phenolic compounds from aloe vera and papaya leaves extract. At the peak of 2400-2000 cm^{-1} there was a C=C aromatic stretching group. Aromatic C=C stretching is detected by the presence of peak at 2200-2100 cm^{-1} which attributed to aromatic compounds in extract of aloe vera and papaya leaves extract [22]. The type of flavonoid identified is thought to be flavonol due to the presence of a hydroxyl group [23]. Furthermore, peak at 2000-1600 cm^{-1} there is a C=O stretching group. This group comes from acetyl group, especially in gelatin. The peak wave number at 1600-1400 cm^{-1} indicates the presence of C-N stretch groups and N-H groups that include amide II in gelatin. Then the C=O stretching group binds to COO, indicating the amide I group of gelatin [24].

3.6. Swelling Ratio

Based on Figure 5, hydrogel initial diameter of 3.8 cm expands and reaches the highest percentage in the 35th minute to the 60th minute, which amounted to 19%. The graph shows the longer the time the hydrogel is immersed, the value of the swelling ratio will increase and finally consistent at the 35th minute until the end of the test. The ability of hydrogels to expand is supported by several factors, such as the density of polymer network, crosslinking, polymer hydrophilicity, and concentration. In this study, gelatin combined with papaya leaves extract and aloe vera were added to the hydrogel formulation to bind the hydrogel structure. The percentage of the result has also exceeded the swelling ratio specification standard, where the swelling ratio value must reach 12.5% within one hour [25].

The occurrence of an increase in the ability to swell is explained in the research of Elgadir & Adam [26], which states that the addition of papaya leaves extract concentration will increase the thickness of the hydrogel. This phenomenon is due to the competition of two polymers (gelatin and extract) in absorbing water, thus causing an increase in swelling. Then, the addition of aloe vera extract also has an impact on the hydrogel structure. Hydrogels with a high concentration of aloe vera have a low crosslinking ability. The retraction force between polymer chains is reduced and allows the absorption of a larger amount of water. According to Periera [27[, the decrease in calcium content in hydrogels is in line with the increase in aloe vera concentration because aloe vera does not crosslink with calcium, so the swelling of the film will increase. In this study, the concentration of aloe vera is greater than the concentration of papaya leaves, so the effect of the swelling ratio is greatly influenced by the role of aloe vera extract.

3.7. In Vitro Extract Release

Prior to *in vitro* extract release testing, it is necessary to determine the wavelength that can detect extract that were impregnated in hydrogel. The visible spectrum of papaya leaves extract is presented in Figure 6. There are two detected peaks in this test, which are at 415 nm and 660 nm. For the *in vitro* extract release the 660 nm wavelength was selected because the moderate absorbance value at low concentration (50 ppm). The maximum wavelength then used for the release of extract. The result of *in vitro* extract release is presented on Figure 7. Based on the data presented in Figure 7, the release of the active substance starts at the 30th minute, which is 10%. The graph shows an increase from the 30th minute to the 180th minute. The drug was released gradually, which started at 10% at 30th minute and then slowly began to increase until it reached 22% in the 180th minute. This result indicate that the extract released in controlled manner and suitable for the application as wound dressing [28].

Hydrogel extract release is related to swelling behavior, which is important in the hydrogel structure. The soaked hydrogel will increase in size and the drug release occurs by diffusion. The hydrophilicity of the hydrogel helps the drug to enter the aqueous solution and then diffuse into the system. The decrease that occurred at the last check could be due to the limited movement of PVA and gelatin chains influenced by the high concentration of cross linkers. In addition, it will also inhibit the absorption of water into the cross linker network which affects the extract release activity of the hydrogel.

4. Conclusions

Based on the result of present study, the highest antibacterial activity was found at a concentration of 750 ppm aloe vera extract and 250 ppm papaya leaves extract in PVA/gelatin hydrogel. Furthermore, the optimum hydrogels have wound dressing characteristics after checking FTIR analysis, swelling ratio test, and *in vitro* extract release test. The FTIR analysis confirm the presence of O-H stretching, C-H stretching, C=C, C=O stretching and peptide bond. The highest swelling ratio test was 19% which exceed the standard specification of the swelling ratio of 12.5%, and the *in vitro* extract release result was 22% release in 180 minutes. In conclusion, the data collected confirm that PVA/gelatin hydrogel impregnated with aloe vera extract and papaya leaves extracts is suitable of wound dressing.

Conflict of Interest

There is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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