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Original Article

Development Of Papaya Leaf Extract/Pva-Based Nanofibers Using Electrospinning Method For Medical Appliaction As A Wound Dressing

Pengembangan Nanofiber Berbasis Ekstrak Daun Pepaya/PVA Menggunakan Metode Elektrospinning untuk Aplikasi Medis sebagai Pembalut Luka

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Abstract

The nanofiber manufacturing process is carried out using the electrospinning method, which produces nanometer-sized fibers. This study used a mice test animal model, where wounds in mice were treated with papaya leaf extract/PVA nanofibers, then compared with a control group using commercial wound dressings. Evaluation was carried out based on wound healing parameters, such as the speed of wound closure and the ability to form an inhibition zone on *E. Coli* bacteria. Based on the results of SEM testing, it was seen that the morphology of papaya leaf extract/PVA nanofibers had been successfully synthesized with the formation of fibers. The results of observations for the inhibition of *E. Coli* bacteria for extract concentrations of 5% and 10% for 7 and 24 hours were 13.33 and 12.43 mm. The application of 10% papaya leaf extract/PVA nanofibers was then tested as a wound dressing on test mice. The results showed that after 9 days, 10% papaya leaf extract/PVA was able to accelerate wound healing in mice. Overall, this research can contribute to the development of nanotechnology-based wound dressing materials that utilize natural resources for medical applications.

Abstrak

Proses pembuatan nanofiber dilakukan menggunakan metode elektrospinning, yang menghasilkan serat berukuran nanometer. Studi ini menggunakan model hewan uji tikus, di mana luka pada tikus diobati dengan nanofiber ekstrak daun pepaya/PVA, kemudian dibandingkan dengan kelompok kontrol yang menggunakan perban luka komersial. Evaluasi dilakukan berdasarkan parameter penyembuhan luka, seperti kecepatan penutupan luka dan kemampuan membentuk zona inhibisi pada bakteri E. Coli. Berdasarkan hasil pengujian SEM, terlihat bahwa morfologi nanofiber ekstrak daun pepaya/PVA telah berhasil disintesis dengan pembentukan serat. Hasil pengamatan untuk inhibisi bakteri E. Coli untuk konsentrasi ekstrak 5% dan 10% selama 7 dan 24 jam adalah 13,33 dan 12,43 mm. Aplikasi nanofiber ekstrak daun pepaya/PVA 10% kemudian diuji sebagai perban luka pada tikus uji. Hasil penelitian menunjukkan bahwa setelah 9 hari, ekstrak daun pepaya 10%/PVA mampu mempercepat penyembuhan luka pada tikus. Secara keseluruhan, penelitian ini dapat berkontribusi pada pengembangan bahan penutup luka berbasis nanoteknologi yang memanfaatkan sumber daya alam untuk aplikasi medis.

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INTRODUCTION

Papaya leaves (Carica papaya) are a traditional herbal plant that has long been used to aid wound healing. Papaya leaves are typically crushed and applied directly to the injured skin. Scientifically, papaya leaves have been shown to have anti-inflammatory effects. Their flavonoid, alkaloid, tannin, and saponin content produces

antimicrobial and antioxidant properties, accelerating wound epithelial formation by stimulating collagen production[1]. Traditional methods such as applying papaya leaves directly to wounds have limitations in terms of hygiene and clinical effectiveness. Conventional bandages only serve to cover wounds without providing antibacterial effects or accelerating tissue regeneration. Therefore, the development of more modern, effective, and safe wound dressing materials is needed to support the wound healing process.

One technology currently being widely developed in the field of biomaterials is electrospinning-based nanofibers. Nanofibers are fascinating materials produced from various polymers and have demonstrated remarkable properties in a variety of biomedical and engineering applications. The high porosity and surface area offered by nanofibers' structural arrangement make them widely used in the medical field[2]. Nanofibers are known to promote tissue formation, enhance blood vessel regeneration, and accelerate wound healing. Compared to conventional wound dressing methods, nanofibers provide better protection against bacterial infection and maintain wound moisture, thus optimizing tissue regeneration.

Nanofiber manufacturing methods have been developed using various techniques, such as electrospinning, template synthesis, centrifugal spinning, blow spinning, self-assembly, and microfluidic spinning. However, compared to other methods, electrospinning is considered the simplest, lowest-cost, and most practical technique for producing micro- to nano-sized fibers[3–5]. In the electrospinning process, a polymer solution is subjected to a high electrical voltage to form nanofibers that are collected in a collector. Some synthetic polymers commonly used in nanofiber production include polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), gelatin, and chitosan. Among these polymers, PVA is widely used because it is biocompatible, non-toxic, easily soluble in water, and able to form nanofibers with good stability for medical applications[6].

Previous research has shown that the combination of natural extracts with synthetic polymers can increase the effectiveness of nanofiber-based wound dressings. Ahlawat, et al[7] used papaya leaves that had previously been treated by drying them into powder, then added with PVA/gelatin to make nanofibers through the electrospinning method to be applied as wound dressings. The results showed that the formed nanofibers had antibacterial properties and for testing this nanofiber wound dressing also showed good potential for use. In addition Huang Z, et al[8], it was reported that the combination of PVA polymer with sodium alginate produced a wound dressing with good antibacterial properties.

Based on this, this research will focus on the utilization of papaya leaf extract, which has not been optimally utilized and is often discarded as waste. Therefore, this papaya leaf juice will be further utilized by adding PVA polymer using the electrospinning method. The resulting nanofibers will then be tested for their morphological characteristics, antibacterial activity against Escherichia coli, and their ability as a wound dressing in test animals. The development of nanofibers based on papaya leaf extract/PVA is expected to be an alternative modern wound dressing that is more hygienic, effective, and environmentally friendly compared to traditional methods.

METHODS

Tools and Materials

Tools. sieves, baking pans, knives, cutting boards, Erlenmeyer flasks, glass beakers, sieves, filter paper, aluminum foil, cling wrap, cotton wool, aluminum foil, electrospinning equipment, Erlenmeyer flasks, Petri dishes, hot plates, magnetic stirrers, incubator shakers, incubators, spirtus, and autoclaves.

Materials. papaya leaves, distilled water, PVA, 95% alcohol, NA and NB media, dan amoxilin.

Research Procedure

Research Stages

This research was conducted by initially preparing the main ingredients: papaya leaf extract and PVA solution. These two ingredients were then mixed to create 5% and 10% electrospinning solutions, which were used to fabricate nanofibers. The resulting nanofibers were then tested for antibacterial activity and wound dressing properties on experimental animals.

Research Procedure

Papaya Leaf Extraction

Papaya leaves were finely chopped, then distilled water was added and blended. The mixture was then collected in a glass beaker and left to stand for 24 hours. The liquid and solids were then separated, and the resulting solids were then dried. The dried product was called papaya leaf extract.

Electrospinning Solution Preparation

In this step, a 10% PVA solution was prepared by weighing 10 grams of PVA powder and adding 100 mL of distilled water while stirring for 1 hour at 85°C. After the PVA was completely dissolved, papaya leaf extract was slowly added to the prepared PVA solution and stirred for 30 minutes at 85°C. The papaya leaf extract added varied between 5 and 10% wt of the PVA weight.

Nanofiber Fabrication

The PVA/Electrospinning solution was cooled to room temperature, then transferred to a 10 mL plastic syringe with a flow rate of 1 mL/h. Electrospinning used a high-voltage DC supply with a voltage of 15 kV. The collector measured 8 x 8 cm, with a distance of 10 cm from the collector to the needle tip. Alligator clips from the high-voltage supply, with the positive (+) current lead, were clamped to the end of the spinneret, and the other, the negative (-) lead, was clamped to the collector. The electrospinned nanofibers were collected on aluminum foil and stored.

Nanofiber Antibacterial Test

Escherichia coli ATCC 6538 bacteria were subcultured on Nutrient (NA) media using the continuous streak method, then incubated in an incubator for 24 hours at 37°C. The test bacteria were inoculated into 30 ml of Nutrient Broth (NB) media, then incubated in a shaker incubator at 37°C at 150 rpm overnight. The bacterial cell count was calculated using a McFarland 0.5 standard (1.5 x 108). Sterile Nutrient Agar (NA) media was poured into sterile Petri dishes (15-20 ml) and allowed to solidify. A 100 µL suspension of the test bacteria was taken and spread aseptically on the surface of the NA media. Sterile paper discs were immersed for 30 minutes in each nanofiber solution, a positive control of liquid amoxicillin, and sterile distilled water as a negative control. The soaked paper discs were taken using sterile tweezers and placed on the surface of the NA media. One petri dish consisted of 4 discs, namely discs for 10% nanofiber, 5% nanofiber, positive control, and negative control. The agar media was incubated in an incubator at 37^{o}C for 12 to 24 hours. Observe and calculate the diameter of the inhibition zone formed around the paper disc after inoculation. The inhibition zone indicates the ability of nanofibers to inhibit the growth of *E. coli* ATCC 6538 bacteria.

Characterization

The 5% nanofiber characterization process was conducted using SEM to determine the morphology and success of nanofiber formation.

Nanofiber Testing on Test Animals

Preparation of Test Animals

The test animals used in this study were male mice (Mus musculus) aged 2-3 months, weighing 20 grams.

Wound Creation

Before wounding the mice, they were anesthetized by inhaling 10% ether. Then, the fur around the mice's backs was shaved to a diameter of 3 cm and cleaned with 70% alcohol. An incision 1 cm long and 2 mm deep was made on the mice's backs.

Nanofiber Attachment to Test Animals

Treatment was administered immediately after wounding by attaching the nanofibers to the wound area. Wound healing activity was measured by measuring the diameter of the wound surface 24 hours after nanofiber application.

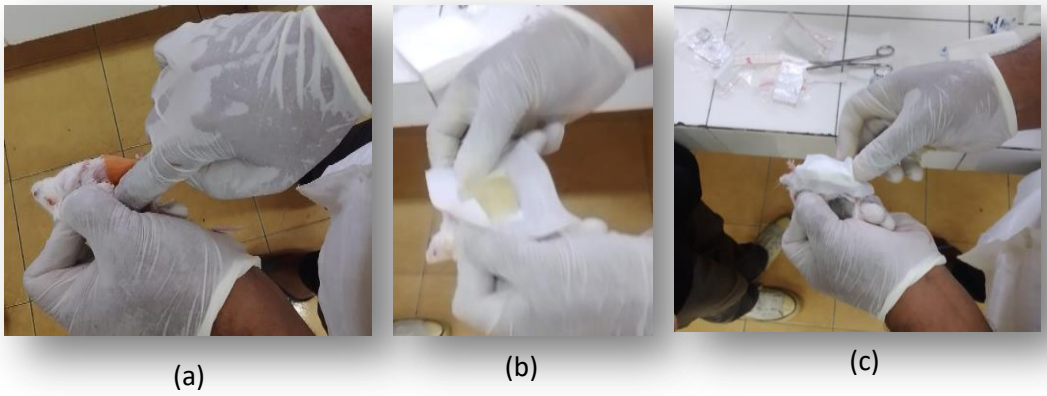


Figure 1. Wound Dressing Process (a) Store-Based Plaster (Hansaplast), (b) 10% Papaya Leaf Extract/PVA Mixture, and

RESULT AND DISCUSSION

Results and Discussion

Research Results

Papaya Leaf Extract/PVA Nanofiber Fabrication

Papaya leaf extract/PVA nanofibers were successfully fabricated using the electrospinning method at papaya leaf extract concentrations of 5% and 10%. The electrospinning process was carried out at a voltage of 15 kV, a flow rate of 1 mL/h, and a collector distance of 10 cm for 5 hours, resulting in papaya extract/PVA nanofibers (Figure 2).

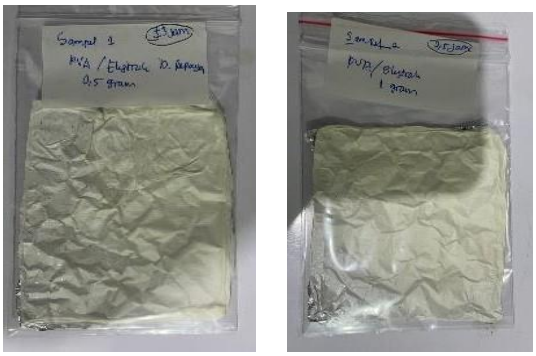


Figure 2. Results of the Electrospinning Process

Visually, it shows that nanofibers are formed continuously and relatively evenly on the collector surface. The resulting nanofibers were then analyzed using Scanning Electron Microscopy (SEM) to determine the morphology of the formed fibers. The SEM results on 5% papaya leaf extract/PVA nanofibers show the formation of nanofibers with a relatively

homogeneous and interconnected distribution. In addition, no significant amount of beads or beads were found on the fiber surface. The morphology of the SEM nanofibers can be seen in Figure 3.

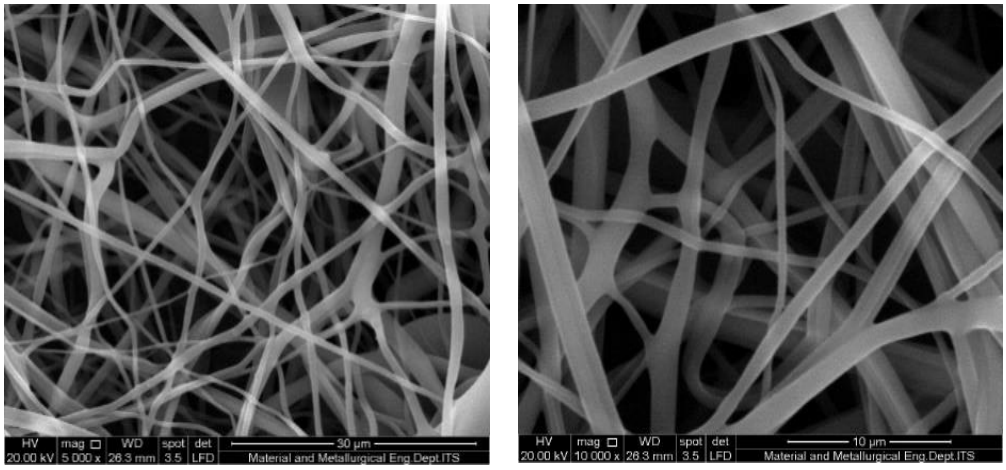


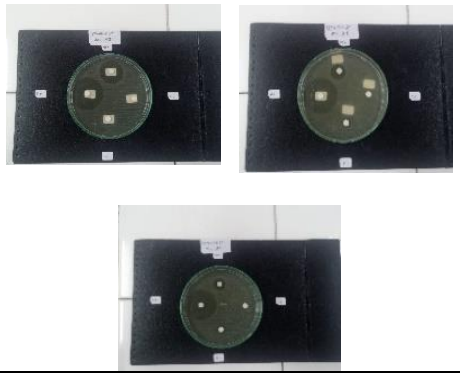
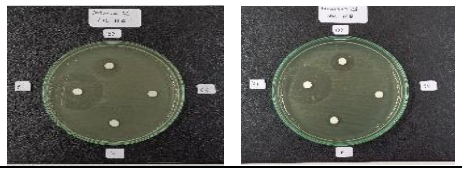
Figure 3. SEM Test Results for 5% Nanofiber (a) 5000x magnification and (b) 10000x magnification

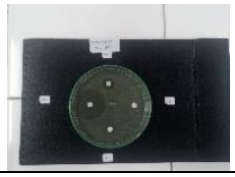
The homogeneous fiber distribution indicates that the electrospinning parameters used have been able to produce a stable polymer jet during the spinning process. According to Li dan Xia[9], the intrinsic properties of the solution such as polymer type, viscosity, electrical conductivity, and operating conditions such as electric field, distance between spinneret and collector, and the surrounding environment have an important role in determining the morphology and diameter of the resulting nanofiber[8].

Antibacterial Testing

Antibacterial testing was conducted using the disc diffusion method against *Escherichia coli* ATCC 6538. Testing was performed on 5% and 10% papaya leaf extract/PVA nanofibers, with amoxicillin as a positive control (K^+) and sterile distilled water as a negative control (K^-). The results are presented in Table 1.

Table 1. Antibacterial Test Results and Inhibition Zone Calculation

Time	Result	Inhibition Zone			
		K+	K-	5%	10%
7 Jam		28 mm	-	-	13,33 mm
24 Jam		29,56 mm	-	-	12,43 mm



The inhibition zone formed indicates that 10% papaya leaf extract/PVA nanofibers have antibacterial activity against E. coli. This antibacterial activity is influenced by the content of bioactive compounds in papaya leaves distributed in the nanofiber matrix. According to Kiran Peter et al[10], papaya leaf extract has been proven effective against Gram-negative and Gram-positive bacteria tested, showing a broad spectrum of activity and analysis of phytochemical content capable of inhibiting bacterial growth.

Nanofiber Testing on Animals

Nanofiber Testing on Animals

Wound dressing testing was conducted on mice with a 1 cm incision. The treatments used consisted of conventional plaster (Hansaplast), a 10% papaya leaf extract/PVA solution, and 10% papaya leaf extract/PVA nanofibers (Figure 4).



Figure 4. Wound Closure Process (a) Store Plaster (Hansaplast), (b) 10% Papaya Leaf Extract/PVA Solution, and (c) 10% Nanofiber

Observations over 9 days showed that the papaya leaf extract nanofiber/10% PVA provided the fastest wound closure compared to the other treatments. On day 9, the wound treated with the nanofiber had completely closed, with a wound length of 0 cm. The results of the wound closure observations can be seen in Table 2.

Table 2. Wound Closure Observation Results

Variabel	Panjang		
	Awal	Hari ke-1	Hari ke-9
Plester Hansaplast	1 cm	0,9 cm	0,2 cm
Larutan Ekstrak Daun Pepaya/PVA 10%	1 cm	0,7 cm	0,1 cm
Nanofiber 10%	1 cm	0,6 cm	0,0 cm

Discussion

Morphology of Papaya Leaf Extract/PVA Nanofibers

The successful formation of nanofibers in this study demonstrates that the combination of papaya leaf extract and PVA can be successfully fabricated using the electrospinning method. PVA is a polymer widely used in biomaterial applications due to its biocompatibility, non-toxicity, and ability to form nanofibers with good stability[6,11].

During the electrospinning process, a high-voltage power supply (15 kV) is applied to the papaya leaf extract/PVA, creating a repulsion between charges on the drop surface at the end of the capillary that competes with the surface tension, which stabilizes the drop. When a critical condition is reached, the surface charge repulsion will dominate, the hemispherical surface of the liquid at the end elongates to form a cone shape known as a Taylor cone. The polymer jet will experience disruption, resulting in bending and continued spiral formation until the nanofibers are finally collected in the collector in solid form. Electrospinning parameters such as electric voltage, flow rate, and solution viscosity greatly influence the morphology of the resulting nanofibers[12].

SEM results show that the nanofibers have a relatively homogeneous fiber distribution and do not exhibit significant bead formation. The absence of bead formation indicates that the electrospinning parameters used are optimal. According to Bhardwaj and Kundu[13], electrospinning is an effective method for producing nanofibers with a continuous structure and high surface area.

SEM results show that nanofibers have a relatively homogeneous fiber distribution and do not show significant bead formation. The absence of bead formation indicates that electrospinning parameters such as voltage, viscosity, and flow rate are appropriate. Huang et al[8] explained that too low a solution viscosity can cause bead formation on nanofibers. In addition, Zargham et al[5] stated that the stability of the flow rate greatly affects the fiber morphology and distribution of nanofiber deposition. According to Kenry and Lim[14], the ECM-like structure of nanofibers can increase cell adhesion and proliferation, thereby accelerating skin tissue regeneration. According to Wang Q et al[15] also reported that electrospun nanofibers have high porosity and good exudate absorption capabilities, making them very potential for use in biomedical applications.

In addition, the small pore size of nanofibers is able to inhibit the penetration of microorganisms from the external environment but still allows good oxygen exchange[16]. Xue et al[17], explained that the high surface area of nanofibers allows the gradual release of active substances, thereby increasing the effectiveness of biomaterials in medical applications.

The results of this study are consistent with those of Ahlawat et al[7], who reported that papaya leaf extract/PVA-gelatin-based nanofibers successfully demonstrated homogeneous fiber morphology and had the potential to be used as wound dressings. Research by Augustine et al[18] also demonstrated that electrospun nanofibers have a high ability to support tissue regeneration because they are able to maintain an optimal wound environment.

Antibacterial Activity of Nanofibers

The antibacterial test used culture media (NA and NB) as a growth medium for *E. coli* bacteria, which served to assess the antibacterial activity of nanofibers as a wound dressing preparation using papaya leaf extract/PVA. The disc diffusion method was used in this antibacterial test, with positive (K^+) and negative (K^-) controls added at concentrations of 5 and 10%. The positive control used was amoxicillin for comparison, and the negative control used

was distilled water because distilled water does not contain toxins or substances that can inhibit or kill bacterial growth[19].

Nanofiber papaya leaf extract/10% PVA showed antibacterial activity against Escherichia coli with the formation of inhibition zones of 13.33 mm and 12.43 mm. This antibacterial activity is influenced by the content of flavonoids, alkaloids, tannins, and saponins in papaya leaves which are able to damage bacterial cell membranes[1]. Flavonoids are known to be able to inhibit nucleic acid synthesis and damage bacterial membrane permeability, while tannins can cause protein denaturation in bacterial cell walls[20]. In addition, as a Gram-negative bacterium, Escherichia coli has an outer membrane of lipopolysaccharide (LPS) which functions as a permeability barrier to hydrophobic compounds including alkaloids [6]. The relatively thin peptidoglycan layer (5-10% of the cell wall), makes it less susceptible to direct damage by alkaloids with the mechanism of action of alkaloids, namely inhibition of peptidoglycan synthesis through interference with the transpeptidase enzyme. So it can be seen that by increasing the concentration from 5% to 10%, it shows that high doses can overcome the permeability barrier of E. Coli[21].

The inhibition zone of nanofibers was still lower than the positive control amoxicillin because synthetic antibiotics have a more specific mechanism of action against E. coli bacteria. Nevertheless, papaya leaf extract nanofibers still show potential as a natural antibacterial for wound dressing applications. Ahmed et al[22], explained that the antibacterial effectiveness of nanofibers is influenced by the concentration of the active ingredient and the ability to release bioactive substances from the polymer matrix. The gradual release of active substances (controlled release) is one of the main advantages of nanofibers compared to conventional wound dressings[23].

The results of this study support the research of Unnithan et al[24], which reported that biomaterial-based electrospun nanofibers have good antibacterial activity when combined with natural active ingredients. Research by Sonia and Sharma[25] also showed that polymer-based nanofibers have a more effective ability to deliver active substances than conventional membranes.

Nanofiber's Ability as a Wound Dressing

The results of animal tests showed that nanofibers containing papaya leaf extract/10% PVA provided the fastest wound healing process compared to other treatments. By day 9, the wounds treated with nanofibers had completely closed, with a healing rate reaching 100%.

The ability of nanofibers to accelerate wound healing is influenced by the combination of the nanofiber's physical structure and the bioactive content of papaya leaf extract. The nanofiber's structure, which resembles the ECM, supports fibroblast migration and the formation of new epithelial tissue. Furthermore, the flavonoids and papain enzyme in papaya leaves help reduce inflammation and accelerate skin tissue regeneration[1].

Moura et al[26], explained that modern wound dressings must be able to create a moist environment in the wound for optimal tissue regeneration. Nanofibers have the ability to maintain wound moisture while absorbing excess exudate, resulting in faster healing compared to conventional wound dressings.

Abrigo et al[27], stated that electrospun nanofibers can increase cell proliferation and accelerate wound healing because their fiber structure supports natural cell growth. The results of this study also showed that nanofibers provide better physical protection against microbial contamination than conventional plasters. Jiang Z et al[28], stated that nanofibers with high porosity can inhibit microbial growth while maintaining air exchange on the wound surface.

Overall, the results of this study indicate that papaya leaf extract/PVA nanofibers have good potential as a modern wound dressing because they can provide antibacterial activity, maintain wound moisture, and accelerate the wound healing process compared to conventional methods.

CONCLUSION

The conclusion drawn from this study is that the fabrication of papaya leaf extract/PVA nanofibers with concentrations of 5% and 10% has been successfully synthesized using the electrospinning method. Testing of nanofibers against E. coli bacteria showed an inhibition zone of 13.33 and 12.43 mm for nanofibers with a concentration of 10%.

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