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Original Article

SIMPLE ELECTROSPINNING ASSEMBLY FOR THE PREPARATION OF POLYVINYL ALCOHOL NANOFIBERS CONTAINING *PIPER BETLE* (L)

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ABSTRACT

Objective: The aim of this research was to assemble an electrospinning device with some components from used medical devices in hospital, so that it could be utilized to produce nanofibers containing *Piper betle* (L).

Methods: The electrospinning was assembled with the main components were the 20 kV high voltage (hV) power supply (module), the Terumo TE-331 syringe pump and the collector. The resulting device was then evaluated for tool performance. The device was used to produce Polyvinyl Alcohol (PVA)-based nanofibers with *Piper betle* (L) as the active ingredient. The nanofibers produced were then tested for antibacterial activity morphology by Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR).

Results: The polymer solution was pushed by a syringe pump with a flow rate of 15 ml/h into the spinneret. In this electrospinning process, the formed nanofibers appear visually as a thin layer on the collector. With variation in PVA concentration and the same concentration of *Piper betle* l. as the active ingredient, the results showed that the nanofiber carrier did not affect the efficacy provided by *Piper betle* (L). The characterization with SEM revealed that the assembled tool was able to make nanofiber preparations that have fine continuous/fairly regular fibers with an average diameter of 46.479±2.406 nm. Meanwhile, the analysis using FTIR showed the presence of OH stretching groups of phenolic compounds from *Piper betle* (L).

Conclusion: It can be concluded that the electrospinning was successfully assembled from unused medical devices in hospital and proven to produce nanofibers.

Keywords: Electrospinning, Assembly, Nanofiber, Polyvinyl alcohol, Hospital, *Piper betle* (L)

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INTRODUCTION

In recent years, nanotechnology has developed very rapidly and has become an option to overcome problems related to biopharmaceuticals (pharmaceuticals and biomaterials). For this reason, the electrohydrodynamic (EHD) method is the choice among other methods because the process is simple and provides the potential for good physicochemical stability. The working principle of the electrohydrodynamic process is to utilize a high-voltage electrostatic field that is able to spin the polymer solution into nano/nanofibers and particles with fine droplets in the nanometer to micrometer range in one process step. There are two techniques of EHD, electrospinning (electrostatic spinner) and electrospraying (electrostatic sprayer) [1-5]. Electrospinning is a technique used to produce fibers or nanofibers. It can be used as a catalyst, filtration, biosensor, food packaging, cosmetics, and biomedicine. Bioactive components in traditional herbs generally have antibacterial, antiviral, anti-inflammatory, antioxidant, immune regulation, and tissue regeneration effects. The electrospinning technique can be an option because it is simple to work with, easy scalability, material flexibility, and efficiency [6]. Electrospinning is a method for manufacturing nanofibers based on electrostatic phenomena [7]. However, electrospinning devices can be made by utilizing unused medical devices in hospitals.

Materials and medicines that have not been used in the hospital environment may evolve to be disposed of without being used, and this is a problem that must be analyzed more closely [8]. At Ratu Zalecha Regional General Hospital (RSUD), each year, there are around 50 pieces of health and medical equipment that are no longer suitable for use. Based on the Minister of Home Affairs regulation concerning the depreciation of regional and state property, rehabilitation is needed if viewed from the perspective of a useful life of 5 y [9]. Waste Medical equipment at Ratu Zalecha Regional Hospital, such as nebulizers, syringe pumps, baby incubators, and so on are damaged tools that can be repaired or are still functional but not suitable for use. Nevertheless, it is still possible to utilize these materials as a simple tool that can produce nanotechnology products such as fiber material in nanometer sizes. This utilization will improve cost efficiency.

In this research, the electrospinning device was tested for its performance by looking at the success of producing nanofibers using a polyvinyl Alcohol (PVA) base. PVA has many important features, such as being easily available, water-soluble, excellent in film formation and thermostable, and other properties. It can also help to improve fiber spinning by reducing resistance forces in charged polymer solutions [10]. Furthermore, *Piper betle* l. was incorporated as an active ingredient. The phenolic compounds in this material have strong antibacterial and antifungal effects and are effective in inhibiting the growth of a wide range of bacteria [11], so that it has the potential to be developed as a wound dressing with a nanofiber system. The aim of this research was to assemble an electrospinning device with some components from used medical devices so that it could be utilized to produce nanofibers containing *Piper betle* (L).

MATERIALS AND METHODS

Materials

There were several main materials used; the first was an acrylic glass chamber as a cover of the electrospinning process, which came from the used baby/neonatal incubator equipment (Medixe® IF 5.1

M, Tangerang, Indonesia) that was no longer used in the Hospital Infrastructure and Facilities Installation (IPS-RS) at Ratu Zalecha Regional Hospital. The second material was an electric motor, an iron or metal drum, and used or obsolete syringe pumps (Terumo® TE-331 series, Tokyo, Japan) that were functioning at the IPS-RS workshop at Ratu Zalecha Regional Hospital. Third material was the high-voltage (HV) power supply (module) (Vgate®, Mainland, China). Fourth was the test materials, consisting of *Piper betle* l. leaves obtained from lokgabang village, Banjar regency, South Kalimantan, Indonesia. The plant was determined at Fundamental laboratory, Faculty Mathematics and Natural Sciences, lambung Mangkurat University, number: 041/lB. lABDASAR/II/2024. The other materials were Polyvinyl alcohol (PVA) (Chang Chun Petrochemical, Taipei, Taiwan), Muller Hinton Agar (MHA) (HiMedia®, Mumbai, India), NaCl 0.9% (Otsuka®, lawang, Indonesia), chloramphenicol (Oxoid®, United Kingdom), *Escherichia coli* ATCC252922, *Staphylococcus aureus* ATCC29213.

Tool design

The design of the tool can be seen in fig. 1.

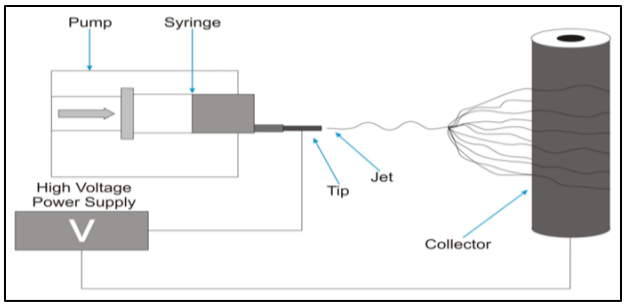


Fig. 1: Electrospinning device design scheme

The design of the electrospinning tool used a high-voltage power supply that formed to a solution from the syringe pump. The solution polymer in a syringe pump (injection pump) out through a needle nozzle (spinneret), which became nano-sized fibers (nanofiber/nanopolymer). The collector drum was made of a conductor material that resulted in electricity flow. A stainless-steel metal pipe was used as a collector for the resulting nanofiber.

Performance test

The performance of tools was performed by producing polyvinyl alcohol nanofiber. PVA powder was taken and weighed on a digital scale until it weighed 15 g to make 15% PVA. Put the PVA in a beaker, and then 100 ml of distilled water was added. A magnetic stirrer was used at a speed of 500–850 rpm until homogeneous. The installed nanofiber tool was prepared with a power supply input cable with a DC adapter (10V to 11.5V), which were connected to the power source and collector and then tested for electrical power and plasma. The red claw cable was connected to the needle nozzle, and the black cable was connected to the collector. 50 ml of 15% PVA solution was inserted into a 50-cc syringe tube attached to the pump, and the distance between the nozzle needle and collector was adjusted to a distance of around 3–5 cm. The syringe pumps were turned on, and the flow was adjusted (10–20 ml/h). The motor in the collector was turned on to rotate the drum 60–90 revolutions per minute. Then, a voltage of 20 kV from the power supply was turned on by pressing the power button of the tool. The Taylor cone was observed at the end of the spinneret and the collector surface. The performance of the tool was categorized as successful if the tool was able to produce filaments or thin layers.

Extraction of *Piper betle* l. folium

Fresh *Piper betle* l. folium was extracted using the inundation method using aquadest with a concentration of 25% [12]. *Piper betle* l. folium was washed and cleaned with running water, chopped, and then weighed at 250g. The *Piper betle* l. folium that had been weighed were put into a stainless-steel pan, and 1000 ml (1 L) of sterile distilled water was added. After infundation process, the mixture was filtered using filter paper. Liquid extract used for preparation of PVA nanofibers.

Preparation of PVA-*Piper betle* l. nanofibers

The process of making PVA-*Piper betle* l. nanofibers was carried out using assembled electrospinning system equipment. This formulation used three variations of PVA concentration, namely 5%, 10% and 15%. The initial stage of the process was 50 ml of each mixture of PVA and liquid extract of *Piper betle* l. folium put into a volume syringe, which was then flowed into the syringe spinneret or needle. When the polymer solution flows from the needle to the tip (spinneret), it must be ensured that there were no air bubbles trapped inside. The metal end of the spinneret was connected to the positive pole of the high-voltage power source by tightening the thread on the spinneret support. Observations were made on the electrospinning process that influence the formation of nanofibers in the collector by the polymer solution concentration, the distance of the spinneret to the collector, and the electrical voltage used. The electric voltage used in the experiment was 20 kV and the distance between the spinneret and the collector was 3–5 cm. The electrospinning process was carried out at room temperature and lasted for 8–10 h until a nanofiber layer was formed on the collector. The manufacturing process was the same as the performance test.

Antibacterial test activity

The test bacteria were first diluted with a NaCl solution according to a turbidity standard of 0.5 McFarland. Holes were made in MHA (Mueller Hinton Agar) media, and the test bacteria were inoculated. A thin layer of PVA-*Piper betle* l. nanofibers was cut into circles of 5 mm, and placed into the holes in the MHA medium and incubated at 37 °C for 24 h. As a result, a clear zone formed around the hole [11, 13]. The zone of inhibition of the sample was calculated by subtracting the diameter obtained from the diameter of the sample (5 mm). The PVA-*Piper betle* l. nanofibers samples with antibacterial capabilities were selected for further morphological examination. The test result data obtained was then analyzed statistically using SPSS 27.

Characterization nanofibers

Nanofibers were examined for surface morphology by Scanning Electron Microscope (SEM) (Thermo Fischer®) and functional group analysis by Fourier transform infrared spectroscopy (FTIR) in KBr pellets (Perkin-Elmer®). The samples were first frozen using the freeze-dry method for 23 h until they became powder to measure particle diameter estimates using software [14, 15].

RESULTS AND DISCUSSION

Test tool performance and preparation of PVA-*Piper betle* l. nanofibers

The design and manufacture of the electrospinning tool in the research has been assembled and tested, and the tool's function has been successfully carried out. The electrospinning tool installed

used a horizontal system where the collector roll/drum was in front of the spinneret (syringe no. 18G). The collector with spinneret was 3-5 cm away, so the polymer solution jet was moved towards the collector drum (diameter 10 cm; l 21 cm), which was rotated by a DC motor. The collector drum rotation was set at 120 revolutions per minute. The system circuit in fig. 2 consists of a high-voltage (hV) module (power supply) of 20 kV, a syringe pump (injection pump) and a roll/drum collector with an electric power source. The equipment and materials needed to make the tool design were shown at table 1.

Table 1: Materials and tools used in making electrospinning tools

No.	Item	Description
1	Material	Stomach tube hose (Fr 14 (4,7 mm); l = 35 cm, acrylic, stainless steel pipe), Polyvinyl alcohol powder (PVA).
2	Power Supply	Output Voltage: 20 KV
3	Syringe Pump	Automatic syringe/syringe flow control Maximum syringe capacity 50 cc
4	Collector type	Pipe/stainless steel drum (d 10 cm; l 21 cm)
5	Spinneret	Material: Stainless steel and Teflon. Hole: 1 obtained from syringe 50 cc Syringe. The nozzle was replaced with syringe needle/syringe no 18G

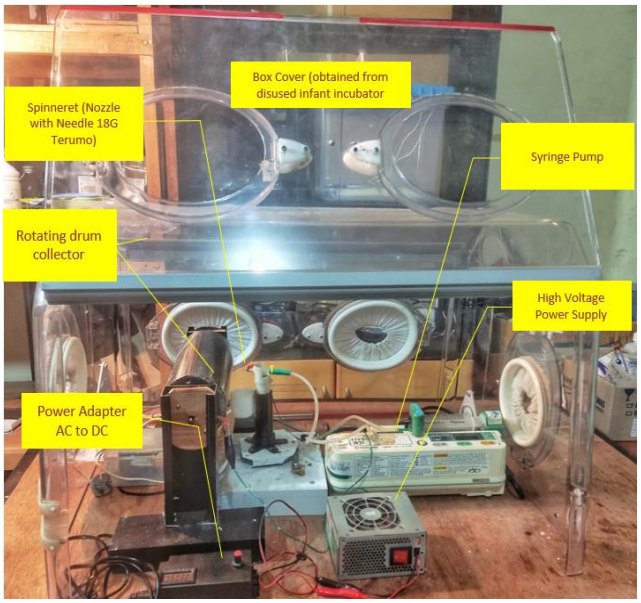


Fig. 2: Assembled electrospinning tool used during nanofiber production

The viscous polymer solution was pushed by a syringe pump with a flowrate of 15 ml/h. At this stage, the electrospinning process used a fairly thick solution, which was pushed by a syringe pump into the spinneret. The viscosity of the polymer solution of nanofibers could simply be seen from the formation of a Taylor cone at the end of the spinneret (fig. 3.) at the electrospinning process. The process was operated at a voltage of 20 kV with a spinneret distance of 12 cm from the collector surface and took approximately 6 h. The syringe pump used was an unused tool that was usually utilized as an aid in intravenous therapy for patients in inpatient rooms at the Ratu Zalecha Regional Hospital.

(a) **(b)**

Fig. 3: The electrospinning process, (a) Illustration of a taylor cone at the end of a spinneret (adapted from Adrienne H, 2011) [16], (b) Taylor cone was captured during the electrospinning process

Fibers are produced in electrospinning by static electrical forces acting on a polymer solution as it passes through an electric field. The process begins when an electric field is created by a high-voltage power supply between a conductive capillary (needle or spinneret) containing a polymer solution and a grounded collector plate. The polymer solution is held in the form of droplets by surface tension, and when the electric field moves from a high-voltage source to the needle and then to the solution, a charge is induced on the droplet surface. At this time, a different charge (repulsion force) also forms and pulls the droplet towards the electric field. With the increasing electric field, the charge of the drop*let al.* so increases, forming a conical shape for the sphere droplet. This is called Taylor cone (fig. 3). If the force of the electric field affects the repulsion force until it exceeds the surface tension, the loaded solution is pulled into the electric field to the ground plate. When the solution is pulled through the electric field, the liquid filament, as the liquid jet accelerates towards the grounded collector, internal and external charges cause it to whip around the field. This action causes the polymer chains in the solution to stretch and slide past each other, simultaneously evaporating the solvent in the solution. This whipping movement makes the fibers on the grounded collector small enough to be classified as nanofibers [16].

In the electrospinning process, the nanofibers that were formed visually look like a thin layer on the collector. It indicated the assembled device was capable of forming a layer of nanofibers, as seen in fig. 4. The morphology and size of the fibers were further clarified in the SEM results.

The formation of nanofibers by the electrospinning process was strongly influenced by the viscosity of the polymer solution used. Therefore, nanofibers were made with different variations (5%, 10%, 15%) of PVA, and incorporated with liquid extract of *Piper betle* l. folium as the active ingredient. The result shows the assembled tool also proved to be able to produce nanofibers with the three formulas. The PVA-*Piper betle* l. nanofibers were then continued to the antibacterial activity test.

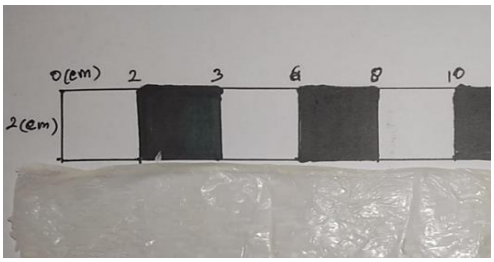


Fig. 4: PVA nanofiber layer (film) resulting from the electrospinning process from the assembled tool

Antibacterial activity test of PVA-*Piper betle* l. nanofibers

As for the nanofibers that were made, it was continued by determining the bacterial inhibitory activity PVA-*Piper betle* l. nanofibers. This activity was characterized by the appearance of an inhibitory zone in the form of a clear zone around the hole in the media. The antibacterial activity test was carried out using the agar diffusion method against the bacteria *S. aureus* and *E. coli*, with positive control of chloramphenicol.

The role of PVA is as a base material to produce electrospun scaffolds. PVA has many important features, such as being easily available, water-soluble, excellent in film formation and thermostable, and other properties. It can also help to improve fiber spinning by reducing resistance forces in charged polymer solutions [10].

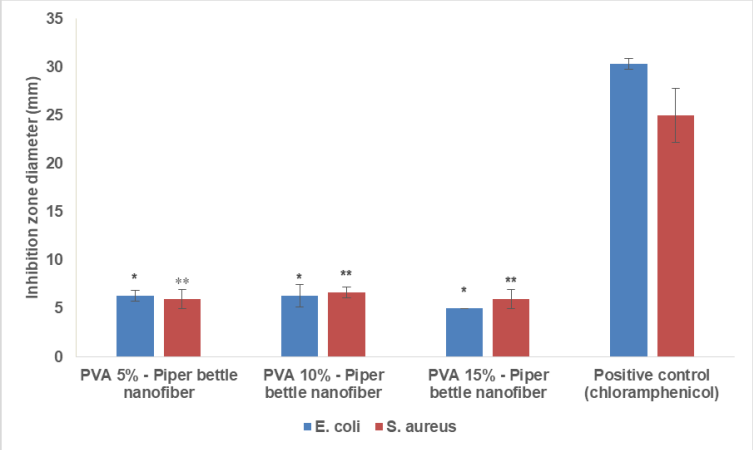


Fig. 5: Antibacterial activities of PVA-*Piper betle* l. nanofibers. Error bars indicate SD values, (n=3), *Kruskal-Wallis obtained no difference (P>0.05) between the inhibitory zone values for all PVA-Piper betle l. nanofibers, **One Way Anova obtained no difference (P>0.05) between the inhibitory zone values for all PVA-Piper betle l. nanofibers

With variation in PVA concentration and the same concentration of *Piper betle* l. as the active ingredient, the results showed that the nanofiber carrier did not affect the efficacy provided by *Piper betle l.* It indicated that it has the potential to be developed as a wound dressing with a nanofiber system. In general, *Piper betle l.* contained various bioactive compounds such as alkaloids, glycosides, reducing sugars, saponins, total phenols (phenols and polyphenols), flavonoids, essential oils, carbohydrates, amino acids, and even steroid compounds. Some of these various contents were bioactive, responsible for being able to kill bacteria (bactericide) and inhibit microbial growth (bacteriostatic). The content of these compounds was very much determined by the origin of the plant, for example, the height and solvent during the extraction process. The main bioactive compounds from polyphenols, such as allylpyrocatechol, cavicol, and cavibetol can damage the formation of cell walls and membranes. If cell wall formation was incomplete, the bacteria were damaged by the environment. Cells whose membranes were damaged and affected cell contents and disrupted energy metabolism and growth were also included. The mechanism of the antibacterial ability of flavonoids was disrupting the potassium concentration of gram-positive bacteria, which caused dysfunction of their cytoplasmic membranes. The inhibitory effect of tannins is caused by tannic acid because it can make changes in potassium concentration, inhibit enzyme production, and inhibit enzymatic reactions [17–20].

The three formulas made had the same antibacterial effect against *E. coli* and *S. aureus*; thus one of the formulas, PVA 10%-*Piper betle* l nanofiber, was selected to be characterized in the form of FTIR analysis and morphology using Scanning Electron Microscopy.

FTIR analysis

The FTIR examination resulted from the PVA 10%-*Piper betle* l. nanofiber showed a spectrum with 16 peaks, which appear prominently in fig. 6. In general, it consisted of the compound types such as alcohol, alkane (alkyl), ketone, and alcohol (primary) in table 3. The main peaks were absorption bands 3428, 2943, 2923, 1733, 1715, 1434, and 1095 (cm-1). In FTIR examination, the frequency of functional groups was in the wave range of 4000–400 cm^{-1}, and the wave range of 1400–400 cm-1 was the fingerprint area. The peak band area of 400–1400 cm^{-1} was a fingerprint area where infrared vibrations overlapped and were formed by many absorption bands, so not all bands could be analyzed [21].

The absorption at wavenumber 3428 cm^{-1} was due to the presence of OH stretching groups in phenolic compounds with strong and wide peaks. The strong, sharp absorption peaks at 2943 and 2923 cm^{-1} were at C-H stretching vibrations, and the 1434 cm^{-1} C-H bending peak of alkane. The strong and sharp peak/wave band 1733–1715 cm^{-1} was the C=O stretching vibration as a ketone. For the peak band of 1095 cm^{-1}, the sharp width was probably related to the C-O stretching of alcohol [22-25].

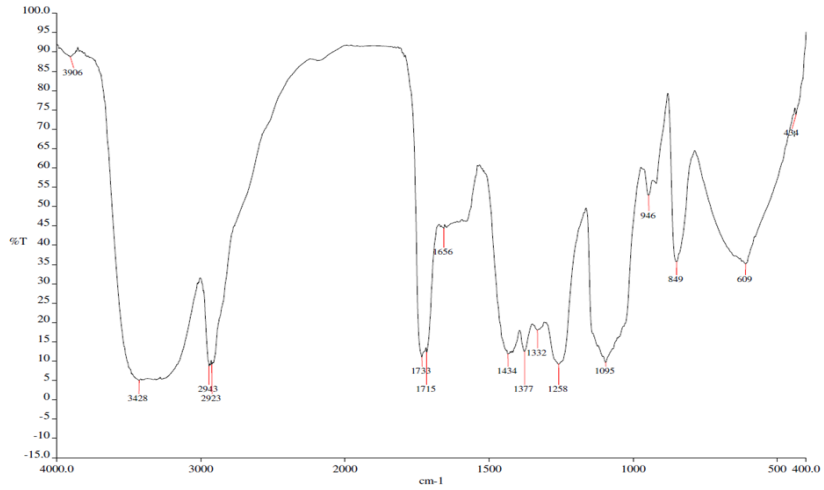


Fig. 6: FTIR spectra of PVA 10%-*Piper betle* l. nanofiber, X= Wavenumber cm-1; Y= % Transmitant

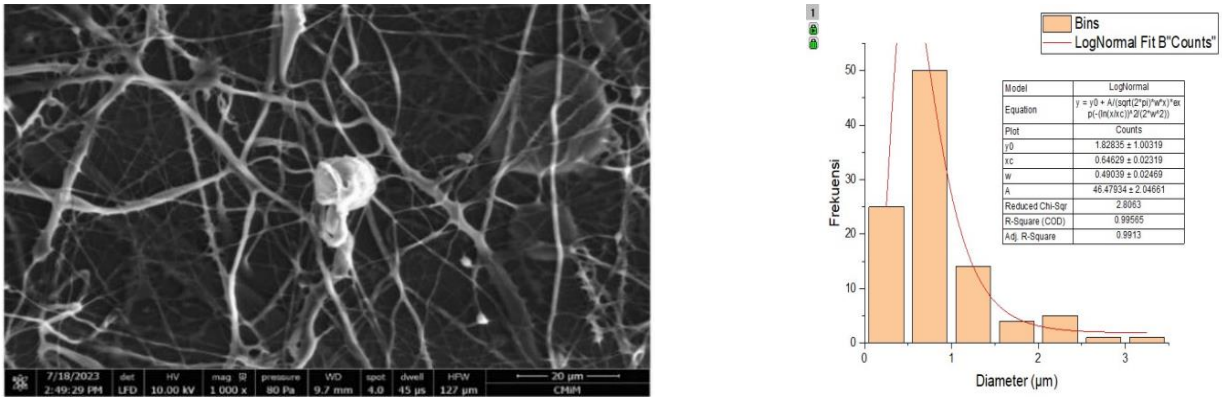
Table 2: Peak wavelength absorption of PVA 10%-*Piper betle* l nanofiber

Compound type	Group	Area frequency (cm-1)	Intensity	Literature supporter
Alcohol	O-H (stretching)	3100–3500	Strong and wide	[21, 23]
Alkana (alkyl)	C-H (stretching)	2900–3000	Strong and sharp	[21, 23]
	C-H (bending)	1415–1440	Medium	[21]
Keton	C=O (stretching)	1730–1740	Strong and sharp	[21, 23, 24]
Alcohol (primer)	C-O (stretching)	1130–1080	Wide and sharp	[21, 25]

The FTIR spectrum of *Piper betle l*. generally showed a wide band in the range of 3500–3100 cm^{-1} (around 3400 cm^{-1}), which was the O-H group of phenolic compounds. Apart from that, there was a peak band at 1600 cm^{-1} which could be attributed to the C-O vibration of ketones, aldehydes, and carboxylic acids or deformation of the amine group [26, 27]. Meanwhile, in PVA, the peak absorption band was around 3500–3200 cm-1 which was the O–H group. For the absorption band 3000-2850 cm^{-1} was the C-H group, the C=C absorption band was around 2800-2700 cm^{-1} and the C-O group is in the absorption band, 1300-1000 cm-1. In FTIR for PVA-Betel composite, all main absorption peaks occur wider and sharper with a slight shift. This was related to the occurrence of bonds between the PVA and Betel functional groups, especially in O-H [27].

SEM analysis

The morphology of PVA 10%-*Piper betle* l nanofiber was measured using SEM analysis (fig. 7).



(a) **(b)**

Fig. 7: The morphology of PVA 10%-*Piper betle* l nanofiber; (a) SEM results at 1000x magnification; (b) Results of nanofiber size calculations with image J

The SEM results observed were in the form of pores with a fiber structure that looked continuous or quite regular, with few beads, branching, less straight, and smooth. The diameter was measured at 46.479±2.406 nm using the ImageJ application. For lumps, it was possible that the PVA-10%-Betel solution that was from the spinneret hole would flow too quickly into the collector drum. Due to the influence of the spinneret to the collector distance, a closer distance would lead to the PVA 10%-*Piper betle* l nanofiber solution to be drawn by an electric field to the collector before nanofiber formation.

CONCLUSION

Electrospinning is a method for manufacturing nanofibers based on electrostatic phenomena. The advantages of this method are simple to work with, easy scalability, material flexibility, and high efficiency. However, the production of nanofibers by electrospinning often requires high technology. Electrospinning devices can be made by utilizing unused medical devices in hospitals. The assembling of an electrospinning device with some components from used medical devices had been conducted in Ratu Zalecha hospital, so that it could be utilized to produce nanofibers containing *Piper betle* (L). This research concluded that the assembled equipment was proven to produce nanofibers. The characterization with SEM revealed that the assembled tool was able to make nanofiber preparations that have fine continuous/fairly regular fibers with an average diameter of 46.479±2.406 nm. Meanwhile, the analysis using FTIR showed the presence of OH stretching groups of phenolic compounds from *Piper betle* (L). The electrospinning was successfully assembled from disused medical devices in hospitals and proven to produce nanofibers. This utilization will improve the cost efficiency in Ratu Zalecha hospital.

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AUTHORS CONTRIBUTIONS

All authors contributed equally to the conception and design of the study, data collection, analysis and interpretation of the results. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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