

Correlation of Extract Composition - Azmi A

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Correlation of Extract Composition on Antioxidant Activity of ELECTROSPUN Polyvinylpyrrolidone/*Bassela rubra linn* Leaf Extract Composite

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Abstract. Nanofiber through electrospinning process has been developed as a promising material for wound dressing due to its large porosity and high surface area. This characteristic of nanofiber provides an adequate gas permeability surrounding the wound which prevents the healing failure. The best wound dressing not only maintain a wound to have a good gas permeability but also to have an active agent giving an antibacterial and antiinflammation property. This research aims to combine a synthetic polymer and active agent, polyvinylpyrrolidone (PVP) and *Bassela rubra linn* leaf extract (BRLE), become nanofibrous material. The electrospinning process was conducted in two steps. The first step is to obtain the best voltage of PVP electrospinning by using the voltage of 10, 12, 15, 17 kV. The other step is combining PVP to 2%, 5%, 8% of BRLE. The first step shows the best operation condition is using 12 kV. Hence, it is used to obtain nanofiber with different BRLE percentage. Based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the result indicates that increase the BRLE composition will rise the antioxidant activity because of the smaller diameter size.

Introduction

Nanofiber, fiber in nanometer size below 100 nm have been developed for many applications as filter and membrane, chemical and biological sensor, solar and fuel cell, health care [1]. Electrospinning method can be used to produce nanofiber. The advantages of it are low cost and relatively high production rate compared with other method (e.g. gas-jet technique, melt fibrillation, to nanolithography) which is have limitation in material range, high cost production and low production rate [2]. Nanofiber utilizing for health care which became attention in last few decades is application for wound dressing. Chronic wound is susceptible to healing failure due to microorganism infection and low absorption of oxygen without a good wound dressing. Ideal wound dressing increasing wound healing process, inhibit infection of microorganism, revitalize structure and function of skin. A good wound dressing has criterion including (i) absorbing excess exudate from wound tissue, (ii) maintaining wound temperature, (iii) protecting wound from bacterium infection, (iv) non-toxic and non-allergenic, (v) easy detaching without traumatic effect [3]. Nanofiber is promising a good wound dressing because it has very small fiber diameter dan relatively high porosity, so that may prevent microorganism growing and dehydration, also give a good gas permeability [4]. Many researchers combine nanofiber from synthetic polymer and natural raw material to upgrading wound dressing function. Combination aloe vera with PVA/PVP-I/PEG improve wound healing process based on anti-bacteria and anti-inflammation [5,6]. Binahong plant

1 have been believed by Javanese people (Indonesia) from generation to generation give antiinflammation characteristic for burn wound treatment [9,10]. This plant believed that will heal patient of diabetes mellitus, hypertension, rheumatic, tuberculosis, and asthma because of its phenolic compound. However, it did not documented appropriately [11]. The phenolic compound of this plant shows an antioxidant activity which provides good healing rate. Therefore, this research is aiming to know the effectivity of BRLE concentration in the nanofiber through antioxidant activity analysis using DPPH assay. Scanning Electron Microscopy (SEM) analyzation was used in this experiment to obtain the correlation between antioxidant activity and fiber size distribution.

Material and Method

Material

The *Bassela rubra linn* (Binahong) leaf was purchased from Premium Herb, Madiun city, East Java, Indonesia. Polyvinylpyrrolidone (PVP) with MW 1,300,000 g/mol and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were manufactured from Aldrich Chemistry. Ethanol 99% which being produced by FUJUFILM Wako Pure Chemical Corporation was used as solvent in extraction and electrospinning process.

Method

Preparation. *Bassela rubra linn* leaf was extracted to obtain the phenolic and alkaloid by 200 ml ethanol 99% solvent until the solvent became clear using Soxhlet apparatus. Then the extract was evaporated to concentrate the extract till 10% of the volume left using vacuum evaporator. While, the 10%w PVP was obtained by mix the PVP to ethanol 99% solvent using magnetic stirrer in 30 minutes. The combination precursor of PVP and *Bassela rubra linn* leaf extract (BRLE) was done by adding the BRLE about 2%w, 5%w, and 8%w to the initial solution for 10/2 PVP/BRLE, 10/5 PVP/BRLE, and 10/8 PVP/BRLE respectively.

Synthesis. The nanofiber synthesis was conducted by using electrospinning tool as can be seen at Fig. 1 at which distance between collector and spinneret is 10 cm and precursor rate is 1 ml/hour. The precursor was fed from spinneret to collector by adjusting syringe pump tool (HARVARD APARATUS) at certain flowrate. The spinneret and collector were connected to a high voltage power supply (Matusada) with opposite electron charge. This study was started from synthesis of PVP only in different voltage 10 kV, 12kV, 15kV, 17kV. The first stage was used to obtain the optimal voltage of electrospun PVP so that the next step was the synthesis of PVP/BRLE nanofiber in different composition.

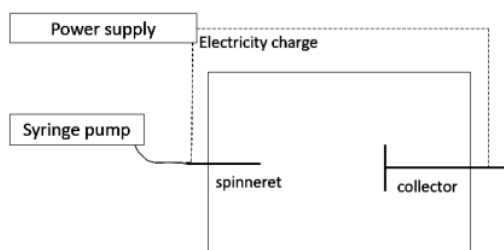


Fig. 1. Electrospinning system schematic illustration

Analysis. The phenolic compound of the extract composition of BRLE was analyzed by high performance liquid chromatography (HPLC) with a Jasco MD-2010 Plus under the following condition: column Inertsil ODS-3; flow rate 1 mL/min; eluent CH₃CN/H₂O = 20/80; detector UV 280 nm; temperature 313 K. The morphology of the nanofibers was characterized by Scanning Electron Microscopy (SEM), JEOL, JSM-6390LV, operating at 10 kV and 10 μ A.

Antioxidant activity. The radical scavenging ability of PVP/BRLE nanofiber was analyzed by antioxidant activity using DPPH assay. This method needs measuring the absorbance of mixture of 50 ppm of DPPH solution and 50 ppm of the nanofiber in ethanol with ratio 1:1 (*Asample*) and the

absorbance of 25 ppm of DPPH solution in ethanol ($A_{control}$) by UV-Visible Spectrophotometer [12]. Then, the antioxidant activity (AA) was calculated by following equation

$$\%AA = (A_{control} - A_{sample})/A_{control} * 100 \quad (1)$$

Both the sample and the control solution was incubated in 2 hours in the dark place and the absorbance of the solution was determined at the peak level of DPPH solution which found at 515 nm wavelength.

Result and Discussion

The composition of BRLE which was determined by HPLC method shows the major of phenolic compound type in the extract. Based on this analyzation, BRLE has a major phenolic substance type which is determined by means of standard of phenolic compound comparison. Fig. 2 shows that a peak of gallic acid, at retention time about 2.68, rise dramatically compared by those of others [13].

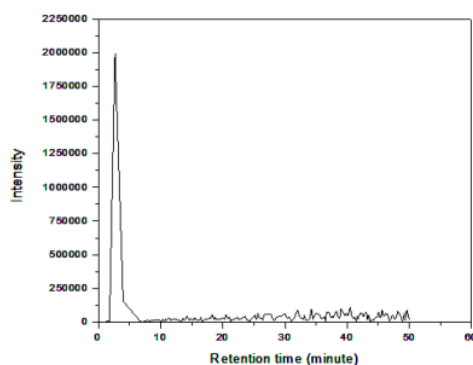


Fig. 2. High Pressure Liquid Chromatography of Bassella rubra linn leaf extract

The morphology of PVP nanofiber was examined by SEM method with 5000 times of magnification. The Fig 3 indicates that the nanofiber diameter is range from 41 to 400 nm. This weight percentage allows the nanofiber produced without a bead. From these images, then the diameter size distribution of nanofibers was determined by software analysis of ImageJ as well as the mean diameter and the deviation standard.

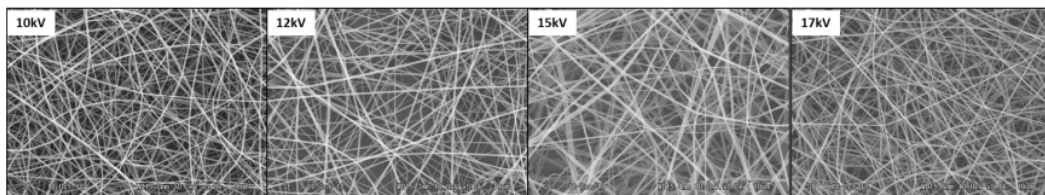


Fig. 3. SEM image of PVP nanofiber of 10%w in ethanol with different voltage

Fig 4 describes the nanofiber diameter size distribution of the PVP nanofiber. The figure indicates that operating condition of electrospinning process at the voltage of 12 kV gives the best result in the diameter size and size distribution which is indicated by the smallest particle size and standard deviation of 246.3 nm and 99.2 respectively. Operation under this optimum voltage results increasing of the nanofiber diameter because the charge is not enough to pull the material [14]. While, the voltage above 12 kV makes the diameter of nanofiber product thicker at 15kV because at this voltage raise the time consumption of the precursor released from spinneret to collector [15]. Increasing the voltage at 17 kV provides a multi-jet performance in the tip of spinneret so that the mean diameter falls from those of 15 kV as well as improvement of the nanofiber diameter size distribution.

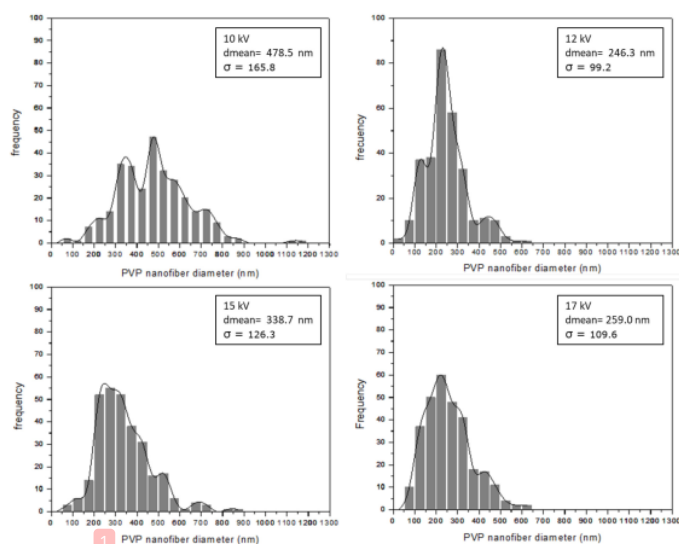


Fig. 4. PVP nanofiber diameter size distribution

Since the minimum of the diameter and the size distribution were obtained by using operating condition at 12 kV, the 10/2; 10/5; 10/8 of PVP/BRLE synthesis were conducted by this condition. The morphology and diameter size distribution of the PVP/BRLE nanofibers were attached in Fig 5 which represent reduction of nanofiber diameter instead of those of 10/5 PVP/BRLE. The increasing of mean diameter from 10/2 PVP/BRLE to 10/5 PVP/BRLE is caused by the addition of extract which increases the viscosity of the solution, while the reduction of the mean diameter of 10/8 PVP/BRLE is caused by the higher viscous solution which was clogged at the spinneret tip. Thus, it makes the spinneret produce a multi-jet of solution to the collector.

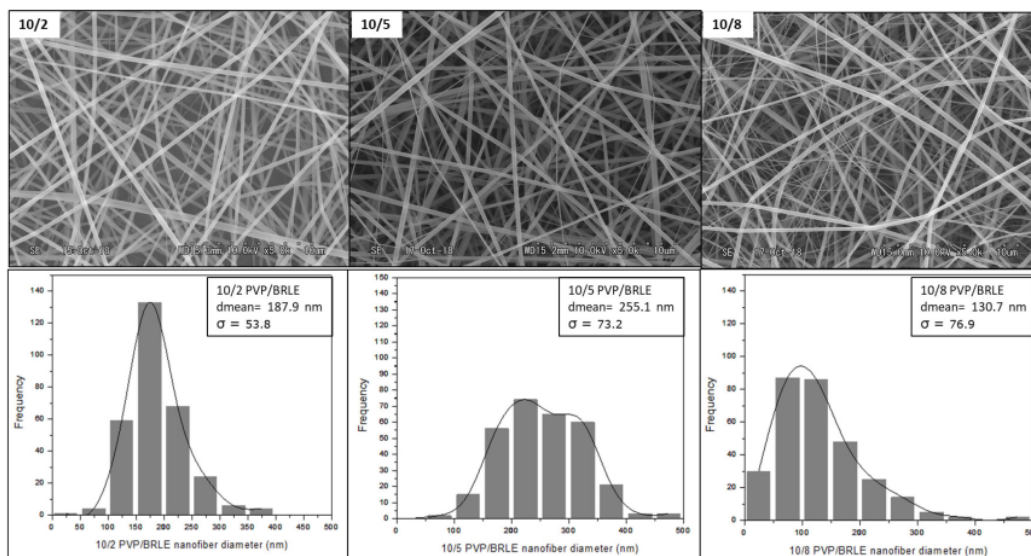


Fig. 5. SEM image and nanofiber diameter of PVP/BRLE at various composition

The antioxidant activity was studied here due to wound healing activity relation that the presence of antioxidant will increase collagen formation in healing process. Based on Table 1, the antioxidant activity can be connected to nanofiber diameter size and distribution. The best antioxidant activity can be obtained from the composition of 10% PVP and 8% BRLE in ethanol

solution because it has the smallest mean diameter size in comparison with other compositions. The number of dominant diameter size of nanofiber at this composition, under 150 nm, may indicate the optimum product. The smaller nanofiber diameter, the higher antioxidant content [16]. It is caused by the increase of polar substance from BRLE elevates the dielectric properties of spinning solution and produces a good spinnability. Therefore, the 10/5 PVP/BRLE and 10/2 PVP/BRLE which have higher diameter size ranging from 180 to 260 nm have smaller antioxidant activity.

Table 1. Antioxidant activity of nanofiber composite of PVP and BRLE

Composition	nanofiber diameter size (nm)	Antioxidant activity (%)
10% PVP and 2% BRLE in etOH solution	187.9 ± 53.8	21.4
10% PVP and 5% BRLE in etOH solution	255.1 ± 73.2	19.5
10% PVP and 8% BRLE in etOH solution	130.7 ± 76.9	34.9

Conclusion

The nanofiber composite form combination of PVP and *Bassella rubra* linn extract can be synthesized by determining the optimum operating condition of electrospinning of PVP nanofiber. The optimum voltage is obtained from 12 kV in the fixed distance from spinneret to collector and the flowrate of precursor at 10 cm and 1 ml/hour in turn. Based on this voltage, the best oxidant scavenging activity is found from the composition of 10% PVP and 8% BRLE. The concentration of BRLE affects to the diameter size of nanofiber. The higher mean diameter of nanofiber, the smaller antioxidant activity.

Acknowledgment

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