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Electrospun of poly(vinyl alcohol) nanofiber as carrier of *Garcinia mangostana* L. pericarp extract

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Abstract. The extract of *Garcinia mangostana* pericarp (GP) contains xanthenes that possesses diverse therapeutic effects. Nevertheless, it has poor solubility in water and poor absorption. Incorporation of GP extracts in nano-delivery system is a promising way to increase GP extracts solubility and absorption. The objective of this study is to prepare poly(vinyl alcohol) (PVA) mats GP nanofiber by electrospinning method. The nanofibers that contained GP extracts were synthesized in a mixture of PVA using single nozzle electrospinning method. The electrospinning parameters used have 13 kV potential with the nozzle-collector distance of 13 cm and flow rate 10 μ l /minute. Scanning electron microscopic (SEM) was evaluated. From the SEM analysis, the average diameter of the fibers was on the nanoscale size. Hence in this study, the PVA nanofiber and PVA/GP nanofiber mats were successfully prepared using the electrospinning method. In addition, applying high voltage in the electrospinning process did not destroy the chemical structure of GP extracts indicated by retained antibacterial activity in vitro. These GP nanofiber mats may provide the good alternative for drug delivery system.

1. Introduction

Electrospinning is an interesting process for fabricating ultrafine fibers with average diameters in sub-micrometer down to nanometer from various type of polymers. The nanofibers produced by electrospinning have unique characteristics such as extremely large surface compared to conventional fibers, small diameter, low density, very tight pore size and high pore volume of nanofiber [1]. The characteristics make them suitable for a variety of applications, such as wound dressing, drug delivery systems, tissue engineering, and filtration [2,3,4,5,6].

Among the polymers used for DDS, poly(vinyl alcohol) (PVA) are often preferred because PVA has been widely used in the biomedical field, its toxicity is very low, biocompatible and biodegradable [6]. Recently, PVA nanofibers have been successfully made from electrospinning pure PVA and mixed PVA with other polymers. For drug and delivery application, blended PVA was used to prepare nanofibers such as PVA/BSA [7] and PVA/curcumin [8]. However, PVA/GP extract nanofibers for drug delivery are little reported.

Garcinia mangostana L is a fruit that grows in Southeast Asia, including Indonesia. Mangosteen is dark purple or reddish, with soft and juicy pulp with a slightly sour taste but sweet and pleasant aroma. the Hull fruit has been used as a traditional medicine to treat diarrhea, abdominal pain, dysentery, pus,



chronic ulcers and infected wounds. The main active substances in the mangosteen are xanthenes (α -mangostin) and their derivatives [9]. They are classified in polyphenol compounds that are commonly found in higher plant families, some of which have been reported α -mangostin to have high antioxidant activity [10,11], antibacterial activity [12] and anti-inflammatory activity [13]. In spite of its benefits, α -mangostin has low solubility in aqueous solution [4] and poorly absorbed [5].

Nanofiber can be used as a drug carrier due to its ability to preserve the compound inside the fiber and its great specific area, hence could improve the absorption of the drug compound [14]. Nanofiber can be produced by using electrospinning efficiently and in relatively shorter time than other methods [15]. However, comprehensive studies that investigate contributing factors affecting in vitro performance of GP nanofiber mats, size, physical characteristics, and efficacy of antibacterial agents for drug delivery system, are still scarce. In this study, GP nanofiber mats (PVA/GP fibers) were used as the drug carrier for GP extract. The effect of electrospinning process parameters on physical characteristics of fiber was described. The electrospun PVA nanofiber mats will be loaded with GP extracts and the efficacy as antibacterial agents for drug delivery system will be investigated. The bioactive chemical compounds in GP extract were determined using thin layer chromatography (TLC). The morphology of the PVA and PVA mats nanofiber analyzed using scanning electron microscopy (SEM). The antibacterial activities of the nanofiber mats were analyzed.

2. Materials and Methods

Poly(vinyl alcohol) with Mw. 89000-98000 was purchased from Sigma Aldrich. The solvent used aqua demineralization from Palembang, Indonesia. *Garcinia mangostana* L. Pericarp (GP) was obtained from the local market in Palembang, Indonesia.

GP were cleaned to remove any residual composite. The hulls of GP were cut into small pieces and dried in a hot air oven at 50°C for 24 h. The dried samples were milled into powder by blender. Dried powder of GP was separately macerate with submerged in ethanol and was macerated for five days. The GP extract was filtered and then evaporated to obtain dry crude extracts.

Thin layer chromatography was performed to determine the bioactive chemical compounds in GP extract such as xanthone, alkaloid, flavonoid, saponin, and fenol by separated with different polarities in TLC plates. TLC plate was made of Aluminum plate and coated by silica as adsorbent material. The liquid GP extracts spotted on the TLC plates. The TLC plates were dipped in several eluents as mobile phase and to separate the chemical compounds. The eluents used were n-hexane:ethyl acetate (7:3), butanol:acetic acid:water (4:1:5), methanol:chloroform:ethyl acetate (1:95:4). Dragendorff and Lieberman Burchard were used as spray reagent. Sitroborat was used as vapor reagent and FeCl₃ for fenol test. The TLC plates with samples spotted on it were identified under UV lamp with wavelength 254 and 366 nm.

Poly(vinyl)alcohol/PVA were dissolved in aqua demineralization at a solution concentration of 12 wt%. The solution was stirred at the rotational speed of 500 rpm for at 80°C, followed by stirring for 8 hour. GP extract was dried, powdered, and extracted through room temperature extraction (maceration) using ethanol. Then, the crude extract of *Garcinia mangostana* pericarp was dissolved in ethanol at a solution concentration of 15 wt%. GP extract solution was mixed with PVA solution at various mass ratio of PVA:GP were 8:0 (GP0), 8:2 (GP1), 8:4 (GP2) and 8:8 (GP3). GP0 solution mass was constant in GP1, GP2, and GP3. Each precursor solution (GP0, GP1, GP2, and GP3) was homogenized by using stirrer, then transferred into a 10 mL syringe (Terumo®) with the inner diameter of 15 mm. The syringe was connected to a 38 mm-length nozzle with diameter of 0.8 mm. The electrospinning of GP0, GP1, GP2, and GP3 solution was run at fixed parameters, comprised of the voltage of 13 kV, solution flow rate of 10 μ L/minute, nozzle-collector distance of 12 cm, relative humidity of 40-50% and the chamber temperature of 30-33°C.

The morphology of GP0, GP1, GP2 and GP3 fibers was analyzed by using scanning electron microscopy (SEM) (JEOL-JSM-6650LA). Samples were coated with electrically conductive carbon. The image was observed at a 10 kV and magnified 1000 times. The fiber size distribution was analyzed by using Origin Pro v7. The antibacterial activity of the GP nanofiber mats were tested with

bacteria *Staphylococcus aureus* ATCC 25921, by the disc diffusion method. Gram-positive *Staphylococcus aureus* used in this study were obtained from Farmasi Laboratory, Universitas Sriwijaya (Unsri-Indonesia). Bacterial suspensions were prepared fresh by growing a single colony in a nutrient broth. After that, each of the GP nanofiber mats specimens (0.7 mm in diameter) was placed on top of the smeared and then the plate was incubated at 37°C for 24 h. The zone of inhibition (ZOI) was determined as the total diameter (nm) of GP nanofiber mats-filter paper.

3. Results and Discussion

3.1 Thin layer chromatography

In column chromatography, the samples are determined in the mobile phase to limit the amount of liquid used. The liquid from GP extract chromatography is dominated by reversed-phase separations. The mobile phase used by methanol in aqueous buffer was 90%. In TLC, the mobile phase is removed before detection, so it cannot interfere with measurements. Figure 1 shows the TLC plate under the UV illumination lamp with wavelengths 255 and 366 nm.

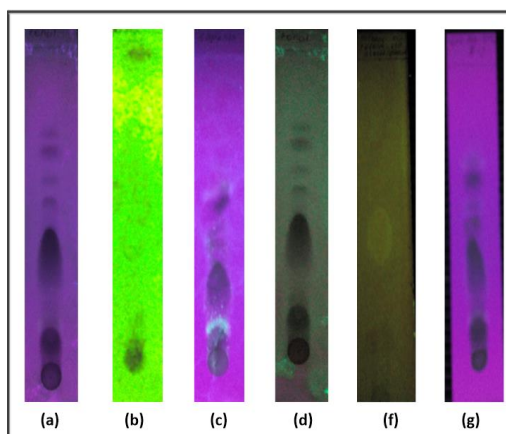


Figure 1. TLC plates under UV illumination lamp with wavelengths 254 and 366 nm (a) Xanthone, (b) Fenol, (c) Flavonoid, (c) Saponin, (d) Fenol, (e) Alkaloid, (f) Terpenoid.

TLPC plates showed that there was xanthone compound, was known by yellow [16], There was Green and yellow stain on the TLC plate as an indicator if GP extract has flavonoid compounds. The GP extract has saponin compound (shown by the greenish blue stain on the TLC plate), fenol (shown by blue-black stain), alkaloid (shown by yellow stain), and terpenoid (shown by blue-black stain). From the TLC result, the GP extract has several antioxidant compounds such as xanthone, flavonoid, fenol, and saponin. Besides antioxidant compounds, GP extract also has several antibacterial compounds such as alkaloid and terpenoid.

3.2 Morphology and diameter of nanofiber

The SEM images of GP0 nanofiber and GP1, GP2, GP3 nanofiber mats were shown in Figure 2. It is shown that all precursor solutions of PVA/GP extract concentrations (GP0, GP1, GP2, and GP3) were successfully transformed into beads-free nanofibers mats. Since the beads-free nanofibers membranes can be obtained from a stable jet [5], the process parameters were set at voltage 13 kV, flow rate 0.5 $\mu\text{L}/\text{minute}$ and the distance between needle and collector 12 cm of since they are suitable to produce stable jet during the electrospinning process.

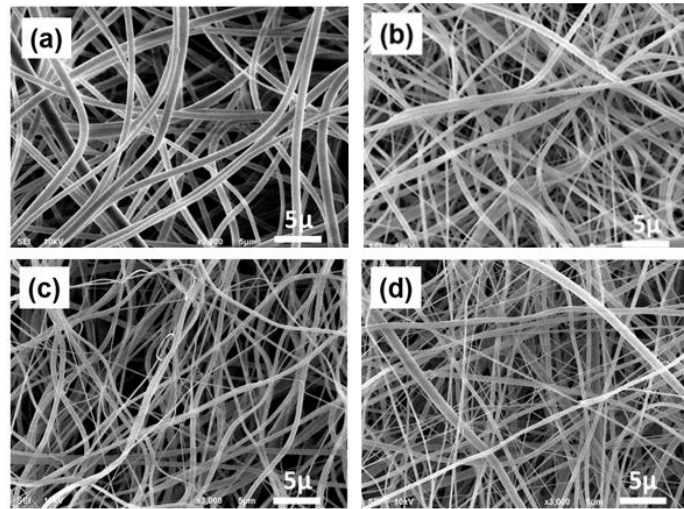


Figure 2. Scanning electron microscopy (3000x) of (a) GP0 nanofiber and (b) GP1, (c) GP2, (d) GP3 nanofiber mats

The distribution diameter of the nanofibers are shown in Figure 3. The average fibers diameters of GP0 fiber and GP1, GP2, GP3 nanofiber mats were found to be 836, 691, 519 and 387 nm, respectively. It was observed that the solution with less GP extract solution created a higher average diameter of the fiber. The decreasing diameter by increasing the mass of GP extract solution can be related to the number of the polymer chain in the precursor. The addition of GP extract solution decreased the mass concentration of the polymer to the total volume solution that would also decrease the diameter of the fibers chain [15,17]. Therefore, the diameter of nanofibers will decrease with increasing mass of GP extract solution.

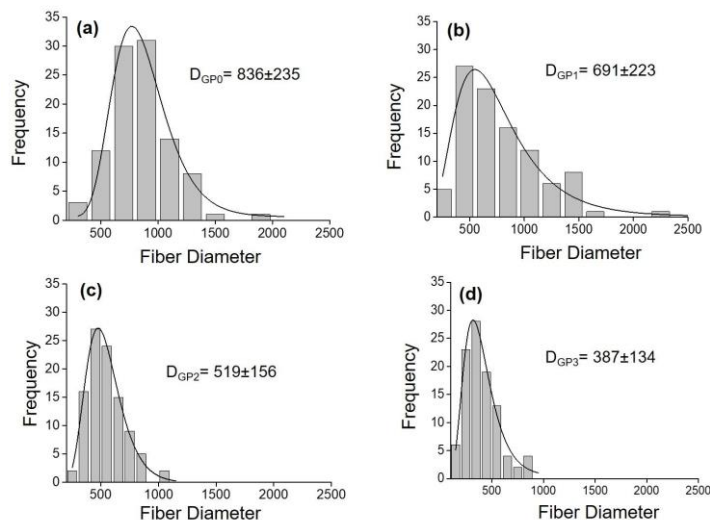


Figure 3. Distribution diameter of (a) GP0 nanofiber and (b) GP1, (c) GP2, (d) GP3 nanofiber mats

The nanofiber mats with higher GP extract content are less homogeneous, as indicated by the coefficient of variation (CP). As reported by Matulevicius et al. (2016), the standard ratio of geometric deviation to the average fiber diameter (variance coefficient) is a useful way of evaluating the homogeneity of the mixture in electrospun fibers. Non-homogeneous fibers are generally indicated by the variance coefficient of 0.3 or higher. In construction, the coefficient of variance below 0.3 indicates a homogeneous fiber [18]. The GP1, GP2, and GP3 nanofiber mats with the CVs of 0.32,

0.31, and 0.35, respectively. Therefore, it can be concluded that the addition of GP extract decreases the homogeneity of fiber. The lower fiber homogeneity may also be caused by current fluctuations during the electrospinning process [5] as the PID control action to maintain a constant current is not applied to the current electrospinning system. The last factor that might contribute to reducing homogeneity is the use of high voltage (13 kV).

3.3 Antibacterial activity of GP extracts nanofiber mats

The antibacterial activity of the GP extracts nanofiber mats against *S. Aureus* bacteria was evaluated by disc diffusion method. Photographic images showing the antibacterial activity of the PVA nanofiber mats loaded with GP extracts against *S. aureus* is Figure 4.

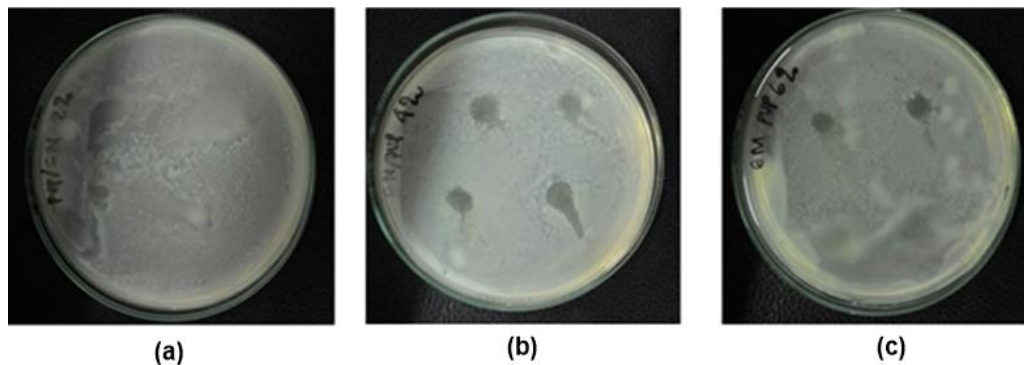


Figure 4. The test of antibacterial activities of (a) GP1, (b) GP2 and (c) GP3 Nanofiber mats

Table 1. The results of the average of inhibition zone diameter of the growth of *S. aureus* GP extract and GP nanofiber mats

Sample	Inhibition Zone Diameter (cm)
GP extract	0.8
GP1 nanofiber mats	-
GP2 nanofiber mats	0.4
GP3 nanofiber mats	0.7

From Table 1 the results of the test bacteria show that GP extract has high potential for antibacterial activity. The antibacterial activity for the GP2 nanofiber mats which showed low antibacterial activity with the inhibition zone of 0.4 cm and for GP3 nanofiber mats which showed moderate potential for antibacterial activity with the inhibition zone of 0.7 cm. Zone of inhibitions (ZOIs) for Gram-positive *S. aureus* increased from 0.4 to 0.7 cm with decrease diameter and increasing the mass of GP in nanofiber mats. The increase of GP extract concentrations will enhance their inhibition zone diameter (antibacterial activities) caused by the increase of bioactive contents of the extracted compound [3]. Various xanthenes found in the stomach of the mangosteen, including α -mangostin, β -mangostin, and γ -mangostin, have biological activity. Among other things, α -mangostin has the most antibacterial activity [19], and the nature of nanofibers, where the surface area at a given volume is very high, played the significant role for the increase inhibition zone diameter (antibacterial activities) of GP nanofiber mats. In addition, applying a high voltage in the electrospinning process not damaging the chemical structure of GP extract indicated by retained antibacterial activity in vitro.

4. Conclusion

Poly(vinyl alcohol) (PVA) nanofiber mats loaded with GP extracts were successfully prepared by the electrospinning method. Variation of the initial contents of GP extract had no effect on the morphology of fiber. All nanofibers (GP0, GP1, GP2, and GP3) produced have the morphology of beads-free. The average diameters of both the bare and the PVA fibers loaded with GP extracts ranged

between 836 to 387 nm. The addition of GM extract decreases the homogeneity of fiber and the lower homogeneity of fibers might also be caused by the fluctuation of current during the electrospinning process. These nanofiber mats loaded with GP extracts may provide a good alternative for drug delivery system.

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