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Electrospun Cellulose Acetate Nanofibers Containing *Clinacanthus nutans* (Phayayo) Crude Extract as Potential Wound Dressings

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Abstract

Extraction of *Clinacanthus nutans* (Burm.f.) lindau (*C. nutans*) or Phayayo (PY) leaves was performed by maceration using ethanol as an extractant. The antioxidant activity was evaluated by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The inhibitory concentration at 50% antioxidant activity (IC_{50}) of PY extract was 15.62 mg/mL. The electrospun cellulose acetate (CA) nanofibers containing PY extract at concentrations of 2.5, 5, and 7.5% w/v and silver (Ag) nanoparticles at 0.1% w/v were fabricated. The addition of PY extract and Ag influenced the viscosities of solutions and therefore affected the morphology and fiber diameters. The electrospun CA fiber mat containing 0.1% Ag and 7.5% PY extract was chosen to investigate its potential for use in wound dressing applications. The degrees of weight loss and water swelling of the electrospun CA/PY7.5/Ag fiber mat after immersion in a phosphate buffer solution (pH 7.4) at 37°C were examined in a range of 2-24 h, and found to increase with immersion time. The antioxidant activity of the fiber mat at the same period of immersion time was also studied, which corresponded with the trends of weight loss and water swelling. The antioxidant activity at 24 h of immersion was 68.3±9.2%. The CA/PY7.5/Ag fiber mat had no antibacterial activity against *Escherichia coli* but slightly inhibited the growth of *Staphylococcus aureus*. The fiber mat also possesses high hydrophilicity, as examined by the contact angle measurement. These results indicate that the CA/PY7.5/Ag fiber mat is a promising material for use as a topical transdermal patch or wound dressing.

Keywords**:** *Cellulose acetate; Electrospinning; Herbal extract; Nanofibers; Phayayo; Wound dressing*

1. Introduction

Wound healing is a process where destroyed tissue is treated or regenerated and then replaced by new tissue. Recently, many interests have focused on the development of wound dressings in a number of aspects, for example, the selection of matrix material, the addition of active agents that help regenerate cells, and the fabrication process. An ideal wound dressing should offer a moist environment to stimulate the proliferation of live cells, absorb wound exudate, prevent infections, and even speed up tissue regeneration. Moreover, it should be permeable to gases (i.e., oxygen and moisture), possess sufficient mechanical properties, be inexpensive, and be biocompatible (Rezvani Ghomi et al., 2019). The selection of polymer types for use as wound dressings is one of the important issues in achieving ideal wound dressings. Cellulose acetate (CA), a synthetic polymer derived from cellulose, was chosen as the carrier for herbal crude extract in this study. According to the non-toxicity or biocompatibility and excellent swelling capability of CA (Wutticharoenmongkol et al., 2019), it is widely used in wound dressing applications.

Not only the selection of polymer matrix but also the addition of bioactive compounds that possess antibacterial, antioxidant, and anti-

inflammatory properties can improve the biological properties of wound dressings. *Clinacanthus nutans* (Burm.f.) Lindau, a plant in the family Acanthaceae, is generally found in tropical Asia, for example, Indonesia, Malaysia, China, and Thailand (Alam et al., 2016). It is also known by the common names *snake grass* (English); *Sabah snake grass, Belalai gajah* (Malaysia); *Dandang Gendis, Ki tajam* (Sunda/ Indonesia); *twist of flowers, alligator flower, e zuihua* (China); and *Phayayo, Phayaplongtong* (Thailand) (Alam et al., 2016). The root and leaves of Phayayo (PY) are used as a traditional medicine to cure a variety of symptoms, for example, diarrhea, diabetes, toothache, ulcers, venom from insect and snake bites, skin rash, incised wounds, burns, and lesions caused by the herpes simplex virus (Alam et al., 2016; Neamsuvan et al., 2015). The major compounds in PY plant are flavonoids, glycosides, glycoglycerolipids, cerebrosides, and monoacyl-monogalatosylglycerol (Alam et al., 2016). Pure compounds and various types of PY extracts revealed several pharmacological properties, such as antiviral (Haetrakul et al., 2018), anti-diabetic (Abdullah, & Kasim, 2017), anti-inflammatory (Kuo et al., 2021; Wanikiat et al., 2008), antioxidant (Alam et al., 2017; Arullappan et al., 2014), and antimicrobial activities against *Bacillus cereus, Escherichia coli* (Arullappan et al., 2014; Sekar & Rashid, 2016), *Salmonella enterica Typhimurium, Candida albicans* (Arullappan et al., 2014), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Sekar, & Rashid, 2016). PY extracts and their pure isolated compounds were also reported to induce wound closure by human gingival fibroblasts (Roeslan et al., 2019).

Moreover, an attempt to enhance the wound healing process can be made by incorporating metal-based nanoparticles into wound dressings. Silver (Ag) nanoparticles, one of the metal-based nanoparticles, are interesting materials for wound dressings due to their excellent antibacterial properties and low toxicity to cells. A number of studies have been conducted on utilizing Ag nanoparticles for wound dressings to control infection of wounds, in which excellent antibacterial activities on a wide range of pathogenic strains were observed (Alven et al., 2021; Eghbalifam et al., 2020).

One of the desired characteristics of an ideal wound dressing is its interconnected porous structure, which allows the permeation of gases, the absorption of exudate, and the growth and migration of regenerated cells. Electrospinning is an interesting technique for creating ultrafine fibers with interconnected pores. According to the remarkable characteristics of the electrospun fibers, which are a high surface area-to-volume or mass ratio and an interconnected porous structure, they have been proposed as the ideal materials in a wide range of applications, especially for biomedical applications, including, tissue engineering (Atila et al., 2022), drug delivery systems (Cui et al., 2018; Liu et al., 2019), and wound healing (Chen et al., 2021; He et al., 2022). The technique of electrospinning is easy, flexible, adjustable, and reasonably priced. High electrical force is used to create ultrafine fibers with sizes ranging from micrometers to nanometers (Doshi, & Reneker, 1995). By applying a high electrostatic field to the polymer solution or polymer melt, the charge species accumulate on the surface of a pendant drop at the nozzle tip. The hemispherical drop destabilizes into a conical shape, which is usually referred to as Taylor's cone, up until the electrostatic field hits the critical value. Above the critical value, the Coulombic repulsion and electrostatic forces overcome the viscoelastic force and surface tension of the polymer liquid, leading to the ejection of a charged polymer jet to the collector (Reneker, & Yarin, 2008). During its flight to the collector, solidification of the polymer jet occurs through either cooling down in the case of the melt or evaporation of solvent in the case of the solution.

A large number of investigations on the use of electrospun fibers in wound dressings were revealed. The electrospun CA fibers incorporated with drugs such as amoxicillin (Castillo-Ortega et al., 2011), naproxen (Purnamasari et al., 2022), and herbal extracts such as asiaticoside (Suwantong et al., 2008), curcumin (Suwantong et al., 2007; Tsekova et al., 2017), and Siam weed (Sriyanti et al., 2021) were highlighted as promising wound dressings. Utilizing the ultrafine fibers as the drug carriers in wound dressings provides the outstanding aspect that they allow the drug molecules to diffuse out of the fibers easily due to the high surface area and the porous structure of the ultrafine fiber mats. As a result, sustained drug release can be achieved (Chew et al., 2005; Thairin, & Wutticharoenmongkol, 2022).

In the present work, PY leaf extract was prepared. The electrospun CA fiber mats loaded

with PY extract and Ag nanoparticles were fabricated. The morphology and size of the fibers were examined in relation to the impacts of the concentrations of PY extract loaded. The possibility of using the electrospun fiber mats as wound dressings or topical transdermal patches was investigated. The degrees of water swelling, weight loss, water contact angle, antioxidant, and antibacterial activities of the obtained electrospun fiber mats were examined.

2. Objectives

The objectives of this work were to examine the morphology and size of electrospun fibers in relation to the addition of PY extract and the possibility for using the PY-loaded CA fiber mats in wound dressing applications.

3. Materials and Methods

3.1 Materials

Phayayo (PY) leaves were collected from a region in Pathumthani, Thailand. Cellulose acetate (CA; M_w 100,000 Da; acetyl content 39.8%; hydroxyl content 3.6%) was bought from Acros Organics, (USA). Acetone was purchased from ACI Labscan (Thailand). Ethanol, methanol, *N*,*N*-dimethylacetamide (DMAc), disodium hydrogen phosphate (Na_2HPO_4), and monosodium phosphate monohydrate ($NaH_2PO_4.H_2O$) were purchased from Carlo Erba, (Italy). Silver nanoparticles were purchased from Alfa Aesar (USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (USA). All chemicals were analytical grade and used without further purification.

3.2 Extraction of PY leaves

Fresh PY leaves were gathered, cleaned with water, and let to air dry for 6 h. Small bits of dried PY leaves were divided into 1 g: 10 mL of 95% ethanol before being submerged. The mixture was gradually shaken at room temperature in a closed container for 7 days. Later, solid residue was removed by filtering out the mixture. Solvent was evaporated from the filtrate using a rotary evaporator at 150 rpm and 70°C. The crude extract of PY leaves was obtained as a dark green, sticky solid. Lastly, the PY extract was stored in a desiccator for further use. An equation (1) was used to determine the yield percentage.

$$\text{Extraction yield (\%)} = \frac{\text{weight of the PY extract (g)}}{\text{weight of the initial dried PY leaves (g)}} \times 100 \quad (1)$$

3.3 Electrospinning of PY-loaded CA fiber mats

The CA solution was prepared at 15% w/v in a mixed solvent of 2:1 v/v acetone:DMAc at room temperature. This sample is designated "CA". For the PY-loaded CA solutions, the weighed amounts of PY extract were added to the 15% CA solution at different concentrations of 2.5, 5, and 7.5% w/v. The solutions were magnetically stirred at 60°C until homogeneous solutions were obtained. The PY-loaded CA samples are designated as "CA/PY2.5", "CA/PY5", and "CA/PY7.5", respectively. Next, silver nanoparticles (Ag) were added to the CA/PY2.5, CA/PY5, and CA/PY7.5 solutions to obtain a final concentration of 0.1% w/v. The PY-loaded CA solutions with the addition of Ag are designated as "CA/PY2.5/Ag", "CA/PY5/Ag", and "CA/PY7.5/Ag", respectively. A Cannon-Fenske Routine viscometer was used to measure the kinematic viscosity of each solution at a temperature of 40°C and a constant of 2.351 cSt/s.

Using a Gamma High Voltage Research ES30P-5W power source, each solution was electrospun into a non-woven fiber mat. A stainless steel needle and plastic syringe were used to inject the fluid. The nozzle had a 0.91 mm diameter. The solution at the nozzle tip was exposed to a high voltage of 20 kV. The distance between a tip and a collector was 15 cm. The rotating drum covered with aluminum foil, with a diameter of 7.6 cm and a width of 25 cm, was used to collect the electrospun fibers at a speed of 100 rpm. The solution feed rate was maintained at 1.0 mL/h using a syringe pump. A fiber mat with a thickness of around 60 ± 10 µm was produced after 8 h of electrospinning.

3.4 Morphology of PY-loaded CA fiber mats

The morphology of the electrospun PY-loaded CA fiber mats was investigated using a Hitachi (S-3400N) scanning electron microscope (SEM). The samples were coated with a thin layer of gold using a sputtering coater before being observed under the SEM. The diameters of fiber segments were measured from SEM images using ImageJ software (version 1.52). The average fiber diameters were calculated from 100 measurements. The selected CA/PY/Ag fiber mat was chosen to

examine in the additional study, including water swelling, weight loss, antioxidant activity, antibacterial activity, and water contact angle.

3.5 Water swelling and weight loss of PY-loaded CA fiber mats

3.5.1 Preparation of phosphate buffer (pH 7.4)

The simulated physiological circumstances of the wound (i.e., pH 7.4 and 37°C) are used in the proposed application of the electrospun PY-loaded CA fiber mats as wound dressings. The investigations on water swelling, weight loss, and antioxidant activity used the phosphate buffer solution (pH 7.4) as a medium. To prepare 1 L of the phosphate buffer solution (pH 7.4), 20.214 g of $Na_2HPO_4.7H_2O$ and 3.394 g of $NaH_2PO_4.H_2O$ were dissolved in a certain amount of distilled water. The pH of buffer was adjusted to 7.4 with a few drops of concentrated hydrochloric acid or a solution of 1 M sodium hydroxide prior to make up 1 L-volume.

3.5.2 Water swelling and weight loss

After being submerged in a phosphate buffer solution (pH 7.4) at 37°C for a period of 2 to 24 h, the water swelling and weight loss of the electrospun fiber mats were examined. Each fiber mat specimen was cut into a square piece of 2x2 cm^2 and dried in an oven at 40°C for 4 h. The initial dry weight of specimen was noted at M_i. The weight of the submerged specimen was measured and recorded as M at 2, 4, 6, 8, and 24 h. The specimen was then dried for 8 h in a 40°C oven. Lastly, M_d was recorded as the specimen's dry weight. Equations (2) and (3) were used to determine the degrees of water swelling and weight loss, respectively. A triplet of experiments was performed.

$$\text{Water swelling (\%)} = \left(\frac{M-M_i}{M_i}\right) \times 100 \qquad (2)$$

$$\text{Weight loss (\%)} = \left(\frac{M_i-M_d}{M_i}\right) \times 100 \qquad (3)$$

3.6 Antioxidant activity

The antioxidant activities of the PY extract and the electrospun PY-loaded CA fiber mats were studied by the DPPH free radical scavenging assay. A 0.5 mM DPPH solution in methanol was prepared and used as a reagent and a control sample in this study.

For the antioxidant activity of the PY extract, a stock solution of PY extract at a concentration of 50 mg/mL was prepared by using ethanol as a solvent. Subsequently, five different concentrations of PY extract solutions in a range of 14-20 mg/mL were prepared by dilution from the stock solution. After preparing the mixture, which contained 3.0 mL of 0.5 mM DPPH and 1.0 mL of the PY extract solution, it was left in the dark for 30 minutes. Meanwhile, the pristine solution of 0.5 mM DPPH solution was also maintained in a similar condition as those of the tested samples and used as a control sample. Later, the absorbance at 517 nm (λ_{max} of DPPH) of both the control and the tested samples was measured using an I3-Hanon UV-visible spectrophotometer. The reduction in the absorbance of the tested samples from that of the control determined their ability to destroy DPPH free radicals, or, in other words, their antioxidant activity. Equation (4) was used to calculate the antioxidant activity:

$$\text{Antioxidant activity (\%)} = \left(\frac{A_C-A_S}{A_C}\right) \times 100 \qquad (4)$$

where A_C and A_S are the absorbances of the control and the sample, respectively.

The plot between the concentration of PY extract solution and the antioxidant activity was constructed. The concentration of PY extract at 50% antioxidant activity (IC_{50}) was evaluated.

For testing the antioxidant activity of the electrospun PY-loaded CA fiber mats, the tested sample was cut into a 2x2 cm^2 square and submerged in a 40 mL phosphate buffer solution (pH 7.4) at 37°C. 1.0 mL of the immersion medium was taken out at 2, 4, 6, 8, and 24 h after immersion and combined with 3.0 mL of a 0.5 mM DPPH solution. Before measuring the absorbance at 517 nm, the mixture was kept in the dark for 30 minutes. The antioxidant activity was repeatedly determined using equation (4). A triplet of experiments were performed.

3.7 Antibacterial activity

An agar disc diffusion method was used to measure the antibacterial activity of the PY extract and the electrospun PY-loaded CA fiber mats against Gram-positive *Staphylococcus aureus* (*S. aureus*: ATCC 6538) and Gram-negative *Escherichia coli* (*E. coli*: ATCC 8739) bacteria.

In order to test the antibacterial properties of PY extract, a circular filter paper disc was placed in an agar disc containing bacteria and saturated with the PY extract solution at a concentration of 50 mg/mL. The diameter of a paper disc (D_P) was 6

mm. For 24 h, the agar disc was incubated at 37°C. After that, the diameter of the clear zone (D_C), which contained the diameter of the paper disc, was measured. According to equation (5), the diameter of the clear zone was deducted from the diameter of the paper and then divided by two (average from two sides) to get the size of the inhibitory zone. The positive controls for *S. aureus* and *E. coli*, respectively, were vancomycin and gentamicin.

$$\text{Size of inhibition zone (mm)} = \left(\frac{D_C - D_P}{2}\right) \quad (5)$$

For the antibacterial activity of the electrospun PY-loaded CA fiber mats, the fiber mats were cut into a square shape of 2x2 cm^2 and placed in an agar disc. The experiments used a methodology similar to the one just outlined. The size of the inhibition zone was calculated in the same manner as in equation (5).

3.8 Contact angle of CA/PY7.5/Ag fiber mat

The contact angle of the electrospun PY-loaded CA fiber mats was evaluated using an OCA25 Data physics contact angle goniometer at 25°C. The volume of a distilled water droplet was 10.00 µL with a dosing rate of 2.00 µL/s. At least 5 times of experiments were performed.

4. Results and Discussion

4.1 Yield, Antioxidant, and antibacterial activity of PY extract

PY extract was prepared by maceration extraction using 99% ethanol as a solvent. The process was performed at room temperature, as a cold process, to preserve the active phytochemical compounds from heat degradation. The crude extract of PY leaves was obtained as a dark green sticky solid with an average yield percentage of 3.59 ± 0.57%. The antioxidant activity of PY extract at concentrations in the range of 14-20 mg/mL, based on the DPPH free radical scavenging assay, was evaluated. Figure 1 shows the plot between the concentration of PY extract and antioxidant activity, which was in a range of 43.1 – 72.7 %. PY extract has a half-maximal inhibitory concentration (IC_{50}) of 15.62 mg/mL, which is the level at which 50% of DPPH free radicals are scavenged. Interestingly, the IC_{50} of PY weed extract was lower than those of some plant extracts revealed in the literature, such as *Vernonia canescens*, *Clibadium funzike*, *Calea angosturana*, and *Pentacalia Americana* (IC_{50} ranged from 245-848 mg/L or µg/mL) (Mosquera et al., 2007), which determines the excellent antioxidant activity of PY extract. An agar disc diffusion method was used to test PY extract's antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria. Table 1 displays photographs of the bacteria-cultured plates as well as the dimensions of the inhibition zones. It was found that PY extract exhibited slight antibacterial activity against both bacteria.

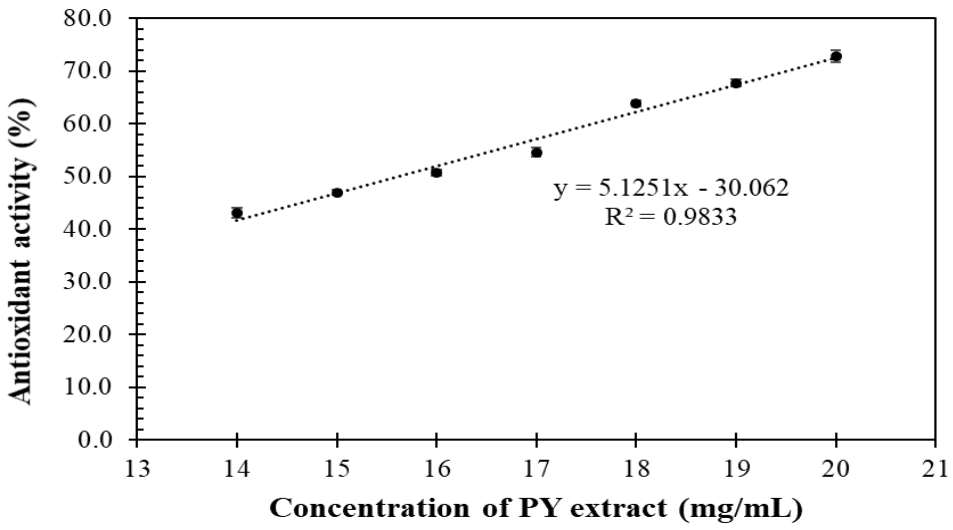


Figure 1 Antioxidant activity of PY extract as determined by DPPH assay.

Table 1 Antibacterial activity of PY extract against *S. aureus* and *E. coli* as determined by an agar disc diffusion method

Type of bacteria	***S. aureus***	***E. coli***
Photographs of agar plates		
Size of inhibition zone (mm)	0.35 ± 0.08	0.22 ± 0.02

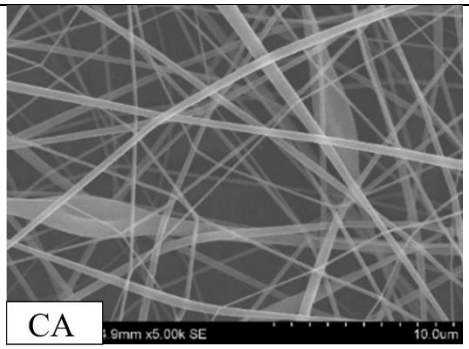
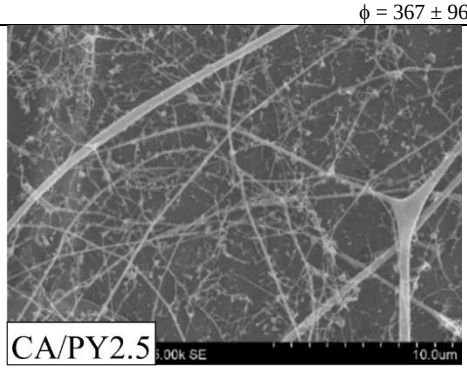
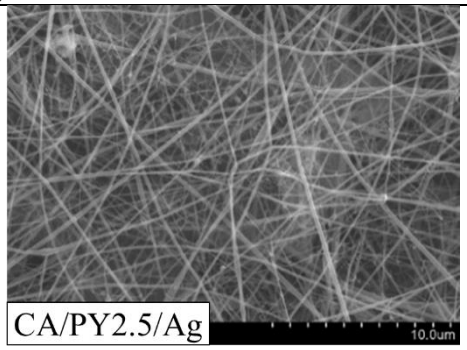
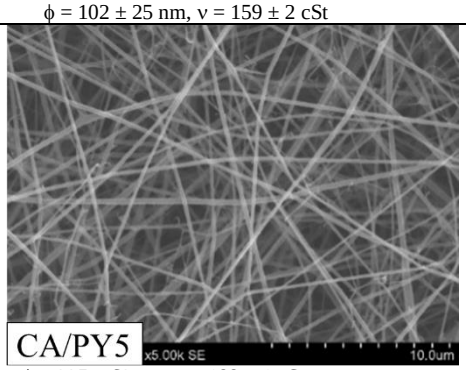
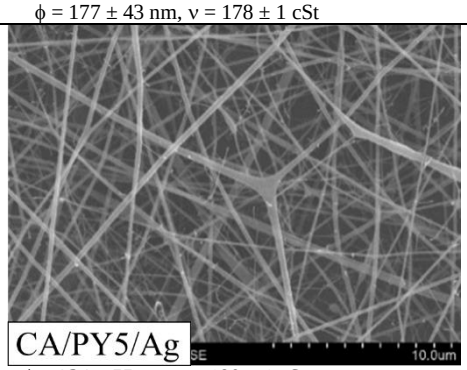
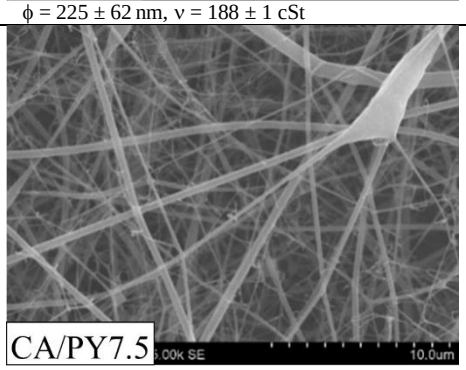
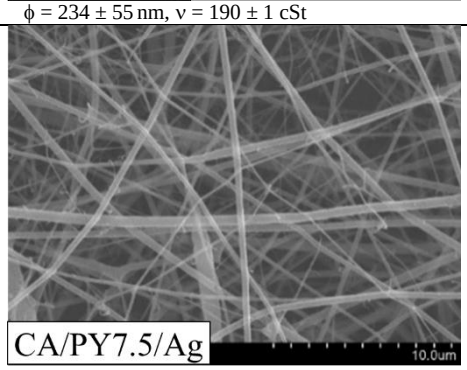
Based on these results, PY extract has both antioxidant and antibacterial properties, indicating its potential application as an active ingredient in wound dressings. Therefore, the electrospun CA fibers loaded with PY extract were further fabricated and investigated. In addition, according to the slight antibacterial activity of PY extract, Ag nanoparticles were also loaded into the electrospun fiber mats in order to enhance the antibacterial activity. The degrees of water swelling, weight loss, water contact angle, antioxidant, and antibacterial properties of the electrospun PY-loaded CA fiber mats were evaluated.

4.2 Electrospinning of PY-loaded CA fiber mats

The kinematic viscosities of the neat and PY-loaded CA solutions as-prepared were tested before electrospinning. During the electrospinning procedure, 20 kV of applied voltage and a fixed tip-to-needle distance of 15 cm were used. The selected SEM images and the average diameters (ϕ) of the electrospun CA fibers with various PY extract contents and either with or without Ag, along with the kinematic viscosities (ν) of the as-prepared solutions, are illustrated in Table 2. The electrospinning of the neat CA fibers was achieved using a solvent of 2:1 acetone:DMAc (Liu, & Hsieh, 2002; Wutticharoenmongkol et al., 2019). The electrospun CA fibers that were produced had a rounded cross-sectional form and smooth surfaces. A few beaded fibers were observed. The average fiber diameter was 367 ± 96 nm, which was comparable to the electrospun CA fibers presented in one of our colleagues' research (i.e., the average fiber diameter of about 335 ± 75 to 342 ± 116 nm, which were electrospun at 18-21 kV) (Wutticharoenmongkol et al., 2019).

Investigations were done into how PY contents affected the size and morphology of the electrospun fibers. At 2.5% PY loading, the average fiber diameter of the obtained electrospun CA/PY2.5 fibers was substantially reduced (i.e., 102 ± 25 nm), and the surface of the fibers was not smooth (see Table 2). The smaller fiber diameter was due to the drastically decreasing viscosity of the as-prepared solution. It is widely known that the viscosity of the solution is one of the key parameters to designate the size of the electrospun fibers. The less viscosity of the solution, which indicates a smaller viscoelastic force, referred to the less ability of the charged jet to withstand the stretching from the Coulombic repulsion and the electrostatic forces, resulting in a smaller fiber diameter (Okutan et al., 2014).

Table 2 Selected SEM images and average diameters (ϕ) of the electrospun CA fibers with various PY extract content, either with or without Ag, along with the kinematic viscosities (ν) of the as-prepared solutions

PY content (%)	SEM images of electrospun fiber	
0	 ϕ = 367 ± 96 nm, ν = 205 ± 1 cSt	
2.5	 ϕ = 102 ± 25 nm, ν = 159 ± 2 cSt	 ϕ = 177 ± 43 nm, ν = 178 ± 1 cSt
5	 ϕ = 225 ± 62 nm, ν = 188 ± 1 cSt	 ϕ = 234 ± 55 nm, ν = 190 ± 1 cSt
7.5	 ϕ = 239 ± 78 nm, ν = 190 ± 1 cSt	 ϕ = 294 ± 81 nm, ν = 195 ± 1 cSt

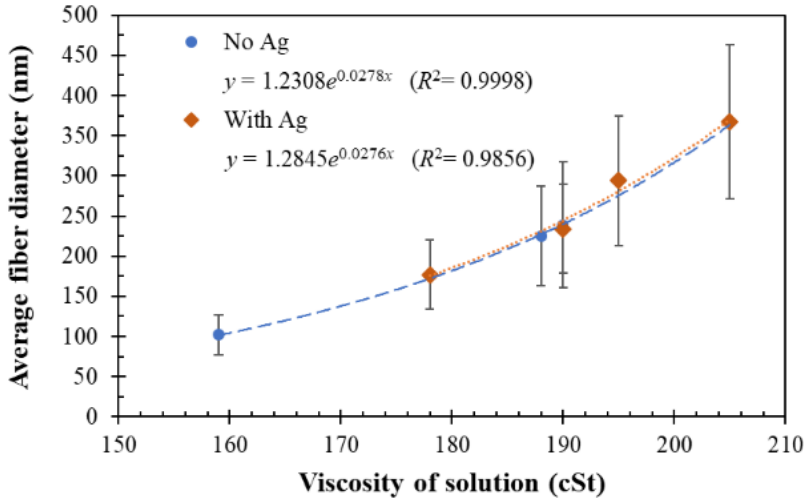


Figure 2 Average fiber diameters of the electrospun CA/PY fibers, either with or without Ag as a function of the viscosity of solution.

At 5 and 7.5% PY loadings, the viscosities of the as-prepared solutions increased with increasing PY contents, but they were still lower than that of the neat CA solution. The average fiber diameters of the electrospun CA/PY5 and CA/PY7.5 fibers corresponded with the viscosities of the solutions, which were 225 ± 62 and 239 ± 78 nm, respectively. Both CA/PY5 and CA/PY7.5 fibers had round cross-sectional forms and smooth surfaces.

In this study, Ag nanoparticles were also loaded into the as-prepared solutions at 0.1% w/v and fabricated into the electrospun fibers in order to enhance the antibacterial activity of the fiber mats. The morphological appearance and size of the fibers were examined in relation to the impacts of the addition of Ag. For each PY loading content (i.e., 2.5, 5, and 7.5%), the viscosities of the solutions increased with the addition of Ag nanoparticles, especially the CA/PY2.5/Ag sample. Repeatedly, the average fiber diameters of the electrospun fibers corresponded well with the viscosities of the solutions. The average fiber diameters of the CA/PY2.5/Ag, CA/PY5/Ag, and CA/PY7.5/Ag were 177 ± 43, 234 ± 55, and 294 ± 81 nm, respectively. Mit-uppatham et al. (2004) demonstrated the relationship between the average fiber diameters and the viscosities of polyamide-6 solutions in an exponential equation. In this study, the exponential growth equations were also observed with a good correlation between the average fiber diameters and the viscosities of CA/PY solutions either with or without Ag addition, as presented in Figure 2.

For the proposed use as wound dressings, the electrospun CA/PY7.5/Ag fiber mat, with the highest concentration of PY in this study, was chosen to be investigated for further experiments, including the degrees of water swelling, weight loss, water contact angle, antioxidant, and antibacterial activities. It should be noted that the attempt to increase more than 7.5% PY in the CA solutions resulted in a non-homogeneous mixture.

4.3 Water swelling and weight loss of CA/PY7.5/Ag fiber mat

Two important characteristics of the materials used for wound dressings are their levels of water swelling and weight loss. These parameters notably correspond to the release behaviors of the drug or active molecules from the carriers (Lamoudi et al., 2016). The degree of water swelling indicates the capability of the matrix to hold water both in bulk and in the inter-porous space of the non-woven structure of the electrospun fibers and therefore relates to the ability to allow drug or active molecules to diffuse out (Cortes et al., 2020; Sutananta et al., 1995). Moreover, one of the release mechanisms can be explained with regards to the degree of weight loss of the matrix in which the drug molecules can be released due to erosion of materials (Sutananta et al., 1995).

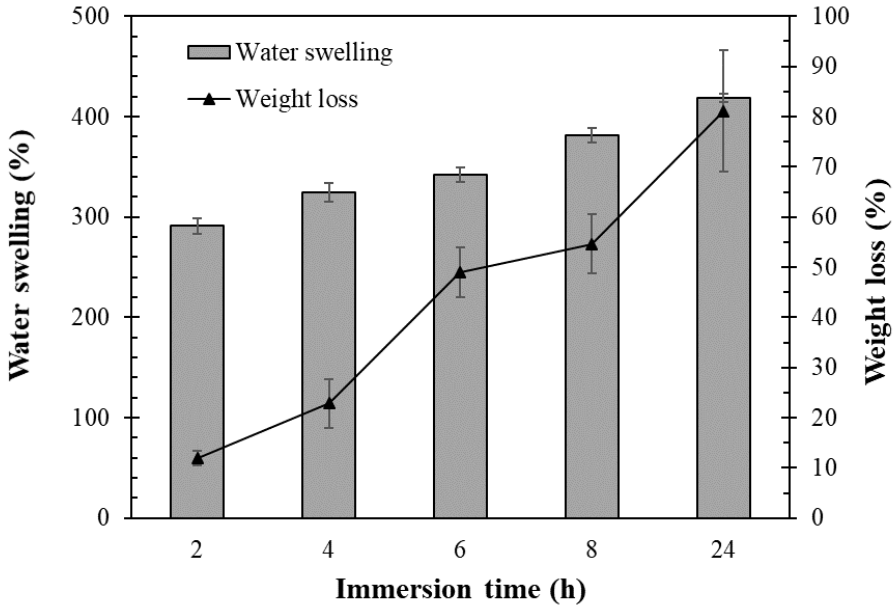


Figure 3 Water swelling and weight loss of CA/PY7.5/Ag fiber mat after immersion in a phosphate buffer solution (pH 7.4) at different time points.

Figure 3 shows the percentages of water swelling and weight loss of the electrospun CA/PY7.5/Ag fiber mats after immersion in a phosphate buffer solution (pH7.4) at 37°C at different time points ranging from 2-24 h. The degrees of water swelling increased with immersion time, which were about 291 ± 8, 324 ± 10, 342 ± 7, 382 ± 7, and 418 ± 4% at 2, 4, 6, 8, and 24 h, respectively. The values of water swelling were comparable and even higher than the electrospun CA fibers containing 40% gallic acid reported in the literature review (i.e., 352% in acetate buffer (pH 5.5) and 379% in normal saline (pH 7.0) at 48 h of immersion) (Wutticharoenmongkol et al., 2019). The excellent water swelling indicates the potential for use as wound healing materials.

The amounts of weight loss also showed a similar trend. The percentages of weight loss were 12.0 ± 1.4, 22.8 ± 4.9, 49.0 ± 5.0, 54.7 ± 5.9, and 81.1 ± 12.1%, at 2, 4, 6, 8, and 24 h, respectively. The amounts of the water swelling and weight loss of the electrospun CA/PY7.5/Ag were remarkably high according to the nature of CA, which is a hydrogel that has a high ability to absorb water (Wutticharoenmongkol et al., 2019). Also, the nano-fibrous structure that provides a high surface area-to-volume ratio and the interconnected pores could allow more surfaces to contact and hold the medium (Suwantong et al., 2007). Interestingly, it seemed that the water swelling of the electrospun fiber mats was initially dominant for the first 2 h. However, the water swelling did not change much after that. On the other hand, the weight loss of the fiber mats seemed to dominate in a late period of time, in which the values drastically increased from about 12% to 55% when immersion time increased from 2 to 8 h. After that, the degrees of weight loss were increased to about 81% for the next 16 h (at 24 h of immersion). In the next section, antioxidant activity is further discussed in relation to the effects of water swelling and weight loss.

4.4 Antioxidant activity of CA/PY7.5/Ag fiber mat

The DPPH free radical scavenging experiment was used to determine the antioxidant activity of the electrospun CA/PY7.5/Ag fiber mat. The fiber mats were submerged in a phosphate buffer solution (pH 7.4) at 37°C, and the releasing media was then gathered and tested for antioxidant activity. The experiments were carried out at various time points ranging from 2-24 h. The reduction in the absorbance at 517 nm (λ_{max} of DPPH) compared to that of the control referred to the ability to scavenge the hydrogen radicals of DPPH.

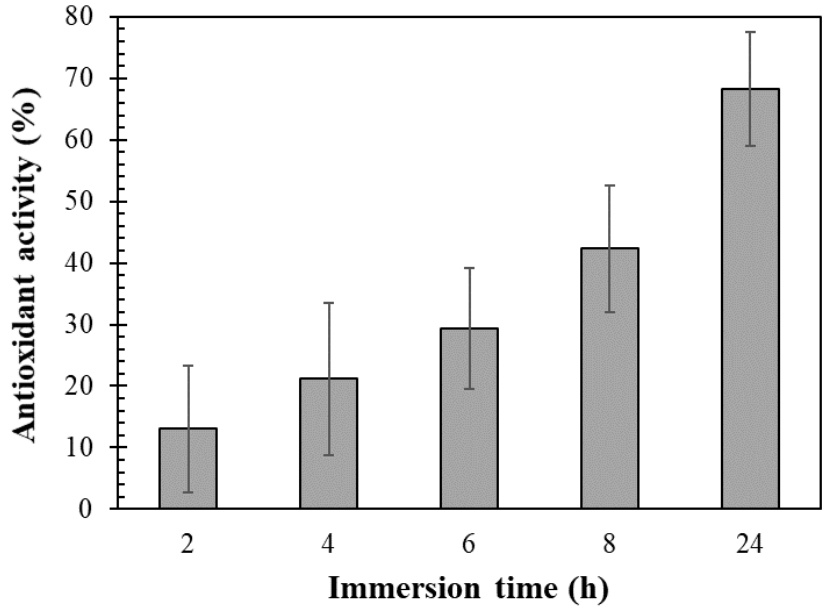


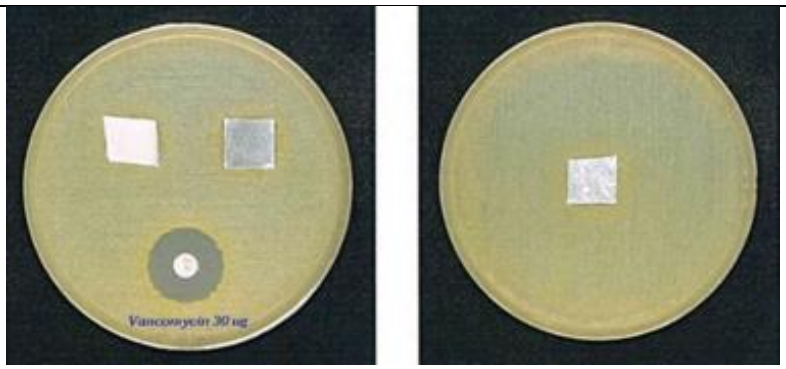
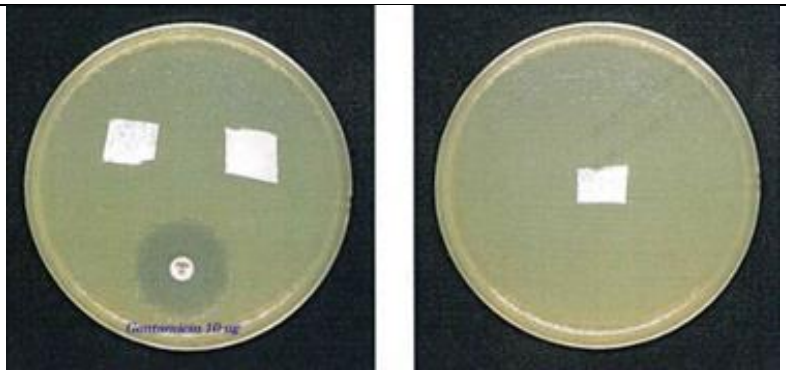
Figure 4 Antioxidant activity of CA/PY7.5/Ag fiber mat after being submerged in a phosphate buffer solution (pH 7.4) at different time points.

Figure 4 illustrates the antioxidant activity of the electrospun CA/PY7.5/Ag fiber mats after being submerged in a phosphate buffer solution (pH 7.4) at different time points. The antioxidant activities at 2, 4, 6, 8, and 24 h of immersion were 13.1 ± 10.2, 21.2 ± 12.4, 29.3 ± 9.8, 42.3 ± 10.3, and 68.3 ± 9.2%, respectively. It was noticed that the antioxidant activities increased with increasing immersion time, which could be due to the greater release of PY extract from the electrospun fiber mats as time increased. The values of antioxidant activities corresponded to the degrees of water swelling and weight loss, which increased with immersion time. As mentioned earlier in the previous section, the drug release behaviors may be explained in terms of both the weight loss that enables drug molecules to be released due to matrix erosion and the water swelling that permits drug molecules to diffuse out from the matrix. The excellent antioxidant activity of the electrospun CA/PY7.5/Ag fiber mat has drawn attention to its further investigation for use as wound dressings that can suppress the oxidation stress of the wounds.

4.5 Antibacterial activity of CA/PY7.5/Ag fiber mat

An agar disc diffusion method was used to assess the antibacterial activity of the electrospun CA/PY7.5/Ag fiber mat against *S. aureus* and *E. coli*. Table 3 displays images of the bacteria-cultured plates as well as the dimensions of the inhibition zones. Since no inhibition zone was visible, it was determined that the CA/PY7.5/Ag fiber mat lacked antibacterial action against *E. coli*. However, the fiber mat slightly inhibited the growth of *S. aureus*, and an inhibition zone of about 0.50 mm was discovered. The antibacterial activity against *S. aureus* could be due to the presence of both PY extract and Ag nanoparticles. In spite of that, the antibacterial activity is still low, which could be due to the insufficient amounts of PY and Ag. Therefore, higher amounts of Ag nanoparticles are suggested for the investigation in future work. Even though the electrospun CA/PY7.5/Ag fiber mat exhibited a little antibacterial property, it revealed excellent antioxidant activity, making it a potentially useful material for topical transdermal patches and wound dressings.

Table 3 Photographs of the bacteria-cultured plates and the sizes of inhibition zones of CA/PY7.5/Ag fiber mat as evaluated by an agar disc diffusion method

Type of bacteria	Photographs of agar plates	Size of inhibition zone (mm)
S. aureus		0.50 ± 0.00
E. coli		No clear zone observed

4.6 Water contact angle of CA/PY7.5/Ag fiber mat

An ideal wound dressing should meet the desired characteristics including the good permeation of gases (i.e., oxygen and moisture vapor) to pass through the wound; having sufficient mechanical properties; preventing contaminants from entering the wound; easily to be removed, acceptable cost; biocompatible or non-toxic; and the hydrophilicity that enable it to absorb wound exudate and maintain the moisture around the wound (Rezvani Ghomi et al., 2019). In this study, the hydrophilicity of the electrospun CA/PY7.5/Ag fiber mat was evaluated by measuring its water contact angle. The CA, polymer matrix in this work, is insoluble in water but only swells due to its moderate hydrophilicity regarding the hydroxyl and ester functional groups in the polymer chains. CA is frequently used in wound dressing applications as a drug release carrier. It was found that the contact angle of the electrospun CA/PY7.5/Ag fiber mat cannot be measured due to the rapid disappearance of water droplets into the fiber mats. This observation expresses the excellent hydrophilicity of the material. It could be due to both the hydrophilic nature of CA and PY themselves and the porous structure of the electrospun fibers, which leads to the penetration of liquid by capillary force.

In order to clarify this observation, the cast film of CA/PY7.5/Ag with the smooth surface was fabricated by a solvent-casting technique and tested for the contact angle. It was found that the contact angle of the CA/PY7.5/Ag cast film was 57.07 ± 10.93° (see Figure 5) which indicates the high hydrophilicity of the components. Therefore, it can be restated that the excellent hydrophilicity of the electrospun CA/PY7.5/Ag fiber mat was due to both the hydrophilic nature of the components and the interconnected porous structure of the non-woven fibers.

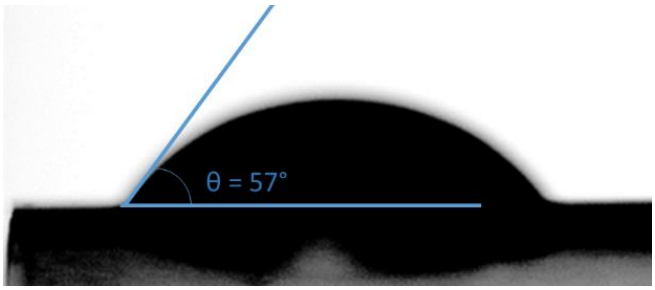


Figure 5 A water droplet on the surface of CA/PY7.5/Ag cast film from a contact angle measurement.

According to the overall findings, the electrospun CA/PY7.5/Ag fiber mat had a high level of antioxidant activity (68.3 ± 9.2% at 24 h of immersion), which was consistent with the trends of water swelling and weight loss. In addition, the fiber mat possesses slight antibacterial activity against *S. aureus* and high hydrophilicity. The electrospun CA/PY7.5/Ag fiber mat revealed its potential as a promising material for use as a topical transdermal patch or wound dressing.

5. Conclusion

In this work, Phayayo (PY) leaf extract, which is well known for its anti-inflammatory, antioxidant, and antibacterial characteristics and used as local medicine for treatment of various symptoms such as diarrhea, diabetes, ulcers, venom from insect bites, skin rash, incised wounds, and burns, was encapsulated in the electrospun CA fiber mats. The antioxidant and antibacterial activities of PY extract were evaluated. The half-maximal inhibitory concentration (IC_{50}) as determined by the DPPH assay of PY extract was 15.62 mg/mL. The addition of PY extract and Ag into CA solutions affected the viscosities of the solutions and therefore affected the morphology and fiber diameters. The possibility of using the chosen electrospun fiber mat (CA/PY7.5/Ag) as patches for healing wounds was examined. The degrees of weight loss and water swelling of the electrospun CA/PY7.5/Ag fiber mat after being submerged in a phosphate buffer solution (pH 7.4) at 37°C were measured in a range of 2-24 h, which increased with immersion time. The antioxidant activities of the releasing medium after immersion of the fiber mats in the phosphate buffer solution were also examined. The antioxidant activities were found to increase with immersion time, which reached 68.3 ± 9.2% at 24 h of immersion. The trend of antioxidant activity corresponded with those of water swelling and weight loss. The high values of water swelling, weight loss, and antioxidant activity of the electrospun fiber mats could be due to their high surface area-to-volume ratio and highly porous structure. The CA/PY7.5/Ag fiber mat had no antibacterial activity against *E. coli* but moderately inhibited the growth of *S. aureus*. The fiber mat also exhibited high hydrophilicity, as examined by the contact angle measurement. Based on the overall results, the CA/PY7.5/Ag fiber mat showed potential for applications as a topical transdermal patch or wound dressing.

6. Acknowledgments

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7. Conflict of interest

The authors declare that they have no conflict of interest.

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