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Enhanced Stability and Bioactivity of *Curcuma comosa* Roxb. Extract in Electrospun Gelatin Nanofibers

Chi-Ming Chiu [1,2], Jukkrit Nootem [1], Thanapich Santiwat [1], Choladda Srisuwannaket [1], Kornkanya Pratumyot [1], Wei-Chao Lin [2], Withawat Mingvanish [1] and Nakorn Niamnont [1,*]

[1] Organic Synthesis, Electrochemistry & Natural Product Research Unit, Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok 10140, Thailand

[2] Department of Cosmetic Science, College of Pharmacy and Science, Chia Nan University of Pharmacy and Science, Tainan 71710, Taiwan

* Correspondence: nakorn.nia@kmutt.ac.th

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Abstract: Electrospun fiber can be used as a carrier for releasing active ingredients at the target site to achieve the effects of drug treatment. The objectives of this research work were to study suitable conditions for producing electrospun gelatin fiber loaded with crude *Curcuma comosa* Roxb. extract (CE) and to study antioxidant, anti-tyrosinase and anti-bacterial activities and its freeze–thaw stability as well. To achieve optimal conditions for producing electrospun gelatin fiber, the concentration of gelatin was adjusted to 30% w/v in a co-solvent system of acetic acid/water (9:1 v/v) with a feed rate of 3 mL/h and an applied voltage of 15 kV. The lowest percent loading of 5% (w/v) CE in gelatin nanofiber exhibited the highest DPPH radical scavenging activity of 94% and the highest inhibition of tyrosinase enzyme of 35%. Moreover, the inhibition zones for antibacterial activities against *S. aureus* and *S. epidermidis* were 7.77 ± 0.21 and 7.73 ± 0.12 mm, respectively. The freeze–thaw stability of CE in electrospun gelatin nanofiber was significantly different ($p < 0.05$) after the 4th cycle as compared to CE. Electrospun gelatin nanofiber containing CE also showed the capacity of the release of bioactive ingredients possessing anti-oxidant properties and, therefore, it could potentially be used for face masks.

Keywords: *Curcuma comosa*; electrospinning; gelatin; *S. epidermidis*; *S. aureus*

1. Introduction

Curcuma comosa Roxb. is a species in the genus *Curcuma* of the family Zingiberaceae. It has been used in traditional folk medicine for the treatment of a wide range of diseases in many Asian countries [1,2]. The individual compounds isolated from *C. comosa* have displayed various biological properties such as estrogenic [3], choleretic [4], nematocidal [5], anti-inflammatory [6], antioxidant [7], and antibacterial activities [8]. The active constituents of *C. comosa* extract have been identified as acetophenones, curcuminoids, sesquiterpenes, and diarylheptanoids [9]. Additionally, the major bioactive compounds were diarylheptanoid derivatives at a level of 80–90% in crude extract [10]. Amongst non-phenolic diarylheptanoids, (3R)-1,7-diphenyl-(4E,6E)-4,6-heptadien-3-ol exhibited the highest anti-inflammatory [11], anti-oxidant [12,13] antibacterial activities [14] and anti-tyrosinase. However, its backbone consisting of double bonds is easily oxidized and transformed upon exposure to oxygen, light, and temperature, which decreases its bioactivities [15].

Various encapsulation techniques have been employed to improve the stability of diarylheptanoids using lipid nanoemulsions, beta-cyclodextrin, and calcium alginate [16–18]. These have demonstrated an increase in the stability and bioactivity of diarylheptanoids under various environmental conditions.

However, encapsulation is associated with several limitations such as handling, drug-loading, and upscaling. Therefore, an alternative encapsulation method, known as electrospinning, has been developed to produce continuous fibers in levels of submicron diameters down to nanometer diameters by transforming polymer solutions or polymer melts to nonwoven-fibrous materials using electricity. Furthermore, an electrospun nanofibrous mat exhibited superior properties such as effective drug encapsulation, high drug loading efficacy, wide range of polymers to accommodate compatibility, and the ability to adjust drug releasing level through its high surface to volume ratio [19–21].

Several polymeric materials such as cellulose [22–24], polycaprolactone [25], poly (lactic acid) [26], polyvinyl alcohol [27,28] and gelatin [29,30] have been currently used to encapsulate many drugs or bioactive compounds by electrospinning. Gelatin, a hydrophilic polymer, has commonly been electrospun together with other polymers to carry bioactive compounds due to its biocompatible, nontoxic and biodegradable characteristics [31]. Many research studies have confirmed that bioactive compounds loaded into electrospun gelatin fibers, including silver nanoparticles [32], antibiotic drugs [33], and vitamins A and E [34] showed significant releasing property, increased bioactivity, and highly enhanced stability. Herein, we reported for the first time on the optimal conditions for the fabrication of the electrospun fibers of gelatin loaded with crude *Curcuma comosa* Roxb extract (CE). The bioactivities of the electrospun fibers containing bioactive compounds were also investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, anti-tyrosinase activity assay and antibacterial activity assay. Moreover, its freeze–thaw stability testing was carried out.

2. Experiments

2.1. Reagents and Apparatus

Gelatin powder from porcine skin with the gel strength of ~300 g Bloom (Type A), 2,2-diphenyl-1-picrylhydrazyl 3,4-dihydroxy-L-phenylalanine (DPPH; ≥98%), phosphate-buffered saline (PBS), 3-(3,4-Dihydroxyphenyl)-L-alanine (L-DOPA), and tyrosinase were purchased from Sigma–Aldrich (St. Louis, MO, USA). All analytical grade reagents of methanol, ethanol, acetone, acetic acid, and ethyl acetate (EA) with 99% purification were obtained from RCI Labscan (Thailand). The other chemicals used were of analytical grade. Powder of *Curcuma comosa* Roxb. rhizome was purchased from a local Thai Herb Trading market in Bangkok, Thailand in May 2016. Muller-Hinton Agar (MHA), Gentamicin, dimethyl sulfoxide (DMSO), *Staphylococcus aureus*, and *Staphylococcus epidermidis* were all supplied by the laboratory at Rangsit University, in Pathum Thani, Thailand.

2.2. Preparation of Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

A total of 500 g of powder of *Curcuma comosa* Roxb. rhizome was macerated with 2 × 500 mL of 95% ethanol at room temperature for 3 days. The ethanol extract solution was then concentrated by using a rotary evaporator to obtain the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE). CE was kept at 4 °C with light protection. The amounts of diarylheptanoid derivatives in CE were measured using high performance liquid chromatography (HPLC, Agilent Technologies, CA, USA) at detector wavelength of 259 nm [35]. The purity of diarylheptanoid derivatives was found to be 89.3% by HPLC technique.

2.3. Identification of Bioactive Compounds of the Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

Preliminary phytochemical testing was used to detect the presence of plant secondary metabolites, i.e., alkaloids, flavonoids, saponins, steroids, tannins, phenols according to Prashanth et al.'s method [36] with little modifications. Furthermore, the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE) powder was characterized by Fourier-transform infrared spectrometer (FTIR, Thermo scientific, CA, USA) in the region of 4000 to 600 cm^{-1} and 400 MHz ^{1}H, and 100 MHz ^{13}C-NMR spectrometer (Avance III HD 400 MHz of NMR, Bruker, Zurich, Switzerland) for determining the presence of diarylheptanoid derivatives in CE. The deuterated solvent for NMR analysis was chloroform-d ($CDCl_3$).

2.4. Electrospinning and Characterization of Gelatin Nanofibrous Mat

The gelatin solutions of known concentrations (20, 25 and 30% (w/v)) were prepared in a mixed solvent system containing acetic acid and H_2O (9:1 (v/v)). Each concentration of the gelatin solution was then loaded into a 5-mL syringe connected with a metal needle of inner diameter of 0.22 mm. The needle was connected to the positive electrode (Gamma high voltage research FL 32174, United States) and the negative electrode was connected to the rotary spinning collector. The distance of needle tip away from the collector was located about 15 cm. Each gelatin solution was injected under the applied voltage of 15 kV at a flow rate of 3 mL/h and nanofibers were collected on the rotary spinning collector at 1200 rpm [24]. In order to study morphology of electrospun nanofibers fabricated under various experimental electrospinning conditions, all nanofiber samples were dried in a vacuum oven at 50 °C for 3 h. After that, each sample was cut into a small piece with a size of 1 × 1 cm, which was coated with gold before examined using a scanning electron microscope (JSM-6610 LV; SEM, JEOL, Tokyo, Japan). The diameters of each electrospun fiber were then determined using ImageJ 1.51 K. software ($n \geq 100$) [29,30].

2.5. Studies of Characteristics of Electrospun Gelatin Nanofibers Loaded with the Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome

Percent loading. To determine the suitable content of the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE) loaded into electrospun gelatin nanofibrous mat, a predetermined weight of CE (1, 5, 10, 15% (w/v)) was dissolved in 2 g of gelatin solution (30% and the solution mixture was electrospun under optimum conditions. Crude diarylheptanoid derivatives extract (1, 5, 10, 15% (w/v)) of a predetermined weight was dissolved in 2 g gelatin solution to generate electrospun fiber under optimum conditions. 10 mg of a gelatin nanofibrous mat loaded with CE was then re-dissolved in 2 mL of methanol. The content of diarylheptanoids in the solution mixture was determined using a UV-Vis spectrometer at 254 nm and 285 nm based on the method of Yingngam et al. [10] and reported as mean value ± SD ($n = 3$).

Anti-oxidant activity. 50 mg of a electrospun gelatin nanofibrous mat containing various concentrations of the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE) (1, 5, 10 and 15% w/v) was re-dissolved in 5 mL of methanol by aid of sonication machine for 5 min and was subjected to examine its DPPH radical scavenging activity in accordance to the method of Devi et al. [37] with some modifications. 100 µL of each sample was mixed with 100 µL of 0.5 mM DPPH solution in methanol. The solution mixture was incubated in the dark at room temperature for 15 min. The absorbance of the solution mixture was measured at 517 nm using Perkin-Elmer multimode plate reader. All tests were done in triplicate.

The percentage of DPPH radical scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity } (\%) = \left[\frac{A_0 - (A_1 - A_2)}{A_0}\right] \times 100 \quad (1)$$

where A_0 was the absorbance of the blank solution; A_1 was the absorbance of the nanofiber solution with DPPH; A_2 was the absorbance of the nanofiber solution without DPPH.

Anti-tyrosinase activity. The anti-tyrosinase activity of electrospun gelatin nanofibrous mat loaded with various concentrations of the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE) was carried out based on inhibition of mushroom tyrosinase-catalyzed oxidation of L-DOPA. According to Masamoto et al.'s [38] method with some modifications, 30 µg of each electrospun gelatin nanofibrous mat loaded with various concentrations of CE was dissolved in 3 mL of sodium phosphate buffer pH 6.8 and l.0 mL of a 1.5 mM L-DOPA, 0.1 mL of DMSO with or without a test sample and 1.8 mL of a phosphoric acid (pH 6.8) were mixed well in a test tube. The reaction mixture was preincubated at 25 °C for 10 min, before 0.1 mL of an aqueous tyrosinase solution (100 U/mL, Sigma Chemical Co. (St. Louis, MO, USA)) was added into the reaction mixture and the reaction

was monitored at 475 nm. The control reaction was conducted with DMSO. Each measurement was performed at least in triplicate.

The inhibitory percentage of tyrosinase activity was calculated using the following formula:

$$\text{Tyrosinase inhibitory activity } (\%) = \left[\frac{(A_1 - A_2) - (A_3 - A_4)}{(A_1 - A_2)}\right] \times 100 \quad (2)$$

where A_1 was the absorbance of the blank solution with enzyme; A_2 was the absorbance of the blank solution without enzyme; A_3 was the absorbance of the nanofiber solution with enzyme; A_4 was the absorbance of the nanofiber solution without enzyme.

Anti-bacterial activity. The initial evaluation of anti-bacterial activity of electrospun nanofibrous mat loaded with various concentrations of the crude ethanol extract of *Curcuma comosa* Roxb. rhizome (CE) was carried out using agar diffusion method (Kirby-Bauer test). The anti-bacterial activity testing was done by following the Clinical and Laboratory Standard Institute (CLSI) Guidelines 2017 with some modifications. Two Gram-positive bacteria; *Staphylococcus aureus* and *Staphylococcus epidermidis*, were used for this study. Briefly, electrospun gelatin nanofiber sheets loaded and unloaded 500–1000 µg/mL CE and 0.25 µg/mL gentamicin (positive control) were dissolved in DMSO. Paper discs (6 mm in diameter) were soaked with 5 µL of test samples for 15 min and let them dry in a laminar air flow cabinet for 2 h. After that, they were placed on Mueller-Hinton agar (MHA) plates adjusted to pH 7.2–7.4, which have been inoculated with test bacteria at 37 °C for 18 h. The diameters of inhibition zones (mm) were measured after 18 h. Filter papers containing only DMSO were used a control and no inhibition zones were not observed. Testing was performed in triplicate and repeated three times. The minimum inhibitory concentration (MIC) of electrospun nanofibrous mat loaded with various concentrations of CE was carried out using agar dilution method, which also followed the Clinical & Laboratory Standard Institute (CLSI) Guidelines 2017 with some modifications. The MIC value of the test sample was determined as the lowest concentration of the test sample that completely prevent any turbidity or growth of the test bacteria. All test samples were performed in triplicate.

2.6. Stability of Electrospun Gelatin Nanofibrous Mat Loaded with Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

The stability of electrospun gelatin nanofibrous mat loaded by crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) have been evaluated by using freeze–thaw cycle test via their remaining content of bioactive compounds in CE and remaining DPPH radical scavenging activity [34]. 30 mg of nanofibrous mat mixed with CE dissolved in 3 mL of methanol and an only CE solution in methanol at the same concentration were prepared. All test solutions from gelatin-CE nanofibrous mats and CE solution were freezed in freezer at −5 °C for 16 h and then thawed in a conventional oven at 45 °C for 8 h. After that, all test solutions were determined for the remaining contents of bioactive compounds in CE by comparison of calibration curves of CE using an UV-Vis spectrophotometer at 254 and 285 nm. The remaining DPPH scavenging activity of each test sample at any freeze–thaw cycle were assessed as mentioned before. The freeze/thaw cycle testing were repeated up to 20 times (a duration of around 3 weeks).

2.7. Statistical Analysis

All experiments were carried out in triplicate. The experimental results are reported as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed for the triplicate (n = 3) testing data using SigmaPlot 10.0.

3. Results and Discussion

3.1. Extraction and Characterisation of Diarylheptanoid Derivatives in Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

Crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) was obtained as viscous and dark brown solid with 5.37% yield (*w*/*w*) of dried plant material. The presence of diarylheptanoid derivatives (Figure 1) and bioactive compounds in CE was studied using spectroscopic techniques (Figures S1–S3 in Supplementary Data). Furthermore, the quantity of diarylheptanoid derivatives in CE was estimated to be 89.3% by using HPLC. The ^{1}H-NMR spectrum of CE (Figure S1) showed important peaks at 6.5–7.5 ppm (Ar–H; aromatic protons), 3.3–5.0 ppm (H–Csp^3–OH; protons attached to carbon atoms singly bonded to oxygen), and 2.0–3.0 ppm (HCsp^3-C; aliphatic protons of sp^3 hybridized carbon atoms of diarylheptanoids). In addition, the results of preliminary phytochemical screening testing presented in Table S1 in the Supplementary Data revealed the existence of other secondary metabolites in CE, which may include cardiac glycosides, phenols, flavonoids, tannins, and terpenoids. These results confirm the phytochemical evidences reported previously for CE [35,36].

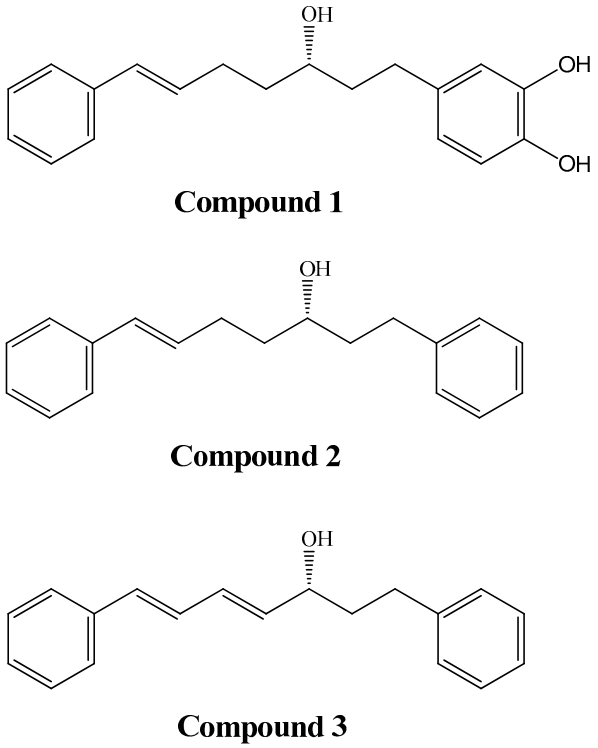

Figure 1. Chemical structures of diarylheptanoids (compound **1**: (3*S*)-1-(3,4-Dihydroxyphenyl)-7-phenyl-(6*E*)-6-hepten-3-ol; compound **2**: (3*R*)-1,7-Diphenyl-(4*E*,6*E*)- 4,6-heptadiene-3-ol; compound **3**: (3*S*)-1,7-Diphenyl-(6*E*)-6-hepten-3-ol) in crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder.

3.2. Effect of Gelatin Concentrations on the Morphology of Electrospun Gelatin Nanofibers

The acetic acid/water co-solvent system at the volumetric ratio of 9:1 was chosen for fabricating gelatin nanofibers by using electrospinning technique [29,30]. The morphology of electrospun gelatin nanofibers fabricated from gelatin solutions in acetic acid/water (9:1 v/v) at various gelatin concentrations was examined using scanning electron microscopy (SEM) From SEM image (Figure 2), the average diameters of electrospun nanofibers obtained from 20, 25, and 30% (w/v) gelatin solutions were 183 ± 29, 367 ± 64 and 547 ± 94 nm, respectively. This may be because polymer molecules and solvents affect the surface tension and the proper evaporation rate of the solution to produce smooth and uniform fibers. Although 20% (w/v) gelatin solution gave electrospun nanofibers the finest average

diameter (mean = 183.4 nm), its nanofibers has relatively low toughness. From SEM micrographs of electrospun nanofibers, gelatin concentrations lower than 25% (w/v) could easily cause the cracking of nanofibers. However, cracking is less likely to be seen in electrospun nanofibers obtained from 30% (w/v) gelatin solution, and its average diameter is relatively in range of 500–600 nm. Therefore, a 30% (w/v) gelatin concentration in acetic acid/water (9:1 v/v) was selected as the optimal condition in this study.

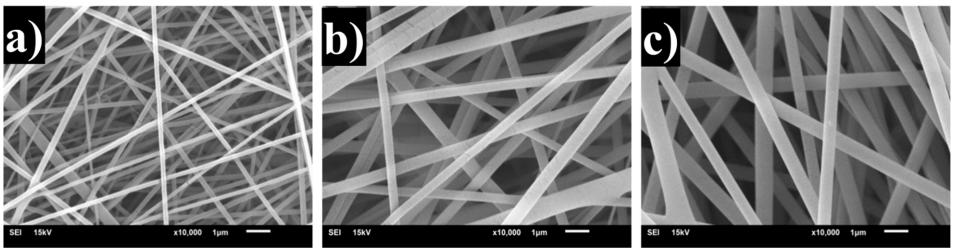

Figure 2. Electrospun gelatin nanofibers fabricated from gelatin solutions in acetic acid/water (9:1 (v/v)) at various gelatin concentrations: (**a**) 20%, (**b**) 25%, and (**c**) 30% (w/v).

3.3. Effect of Various Concentrations of Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powderloaded on the Morphology of Electrospun Gelatin Nanofibers

The optimal conditions for electrospinning were chosen to fabricate gelatin nanofibrous mat containing crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) with various concentrations (1, 5, 10 and 15% (w/v)). Figure 3 showed that the gelatin nanofibers loaded with 5% (w/v) of CE were relatively with an average diameter of 1089 ± 611.38 nm. However, 10 and 15% (w/v) of CE made gelatin solutions more viscous and resulted in an increase in the average diameters of gelatin nanofibers, which might cause to the effect of the high CE extract concentration with the increase viscosity, and reduced conductivity to main affect in an increase the average diameters of the electrospun gelatin nanofibrous mat. Thus, the addition of 5% (w/v) of CE into 30% w/v of gelatin solution is the most suitable condition in this study.

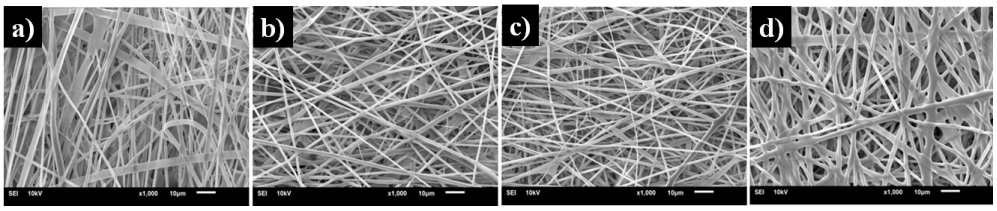

Figure 3. Electrospun gelatin nanofibers with various concentrations of crude ethanol extract of *Curcuma comosa* Roxb.rhizome powder: (**a**) 1%, (**b**) 5%, (**c**) 10%, and (**d**) 15% (w/v) in acetic acid/H_2O (9:1 (v/v)).

3.4. Effect of Percent Loading on Anti-Oxidant Activity of Electrospun Gelatin Nanofibrous Mat Loaded with Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

The effect of percent loading of crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) on anti-oxidant activity of electrospun gelatin nanofibers, which were fabricated from 30% (w/v) gelatin solution in acetic acid/H_2O (9:1 (v/v)) was explored by DPPH radical scavenging assay. Figure 4 presents the plot of percent loading of CE against DPPH radical scavenging rate. The percent loadings at 1, 5, 10, and 15% w/v yielded DPPH radical scavenging rates of 35%, 94%, 94%, and 94.5%, respectively. However, the DPPH radical scavenging rates at the percent loadings of 5, 10 and 15% w/w, they were not statistically significant ($p < 0.05$). Therefore, to achieve significant free radical scavenging activity, the lowest percent loading with the highest DPPH radical scavenging rate of 5% w/v was preferred. This showed that CE was a good source of natural antioxidants.

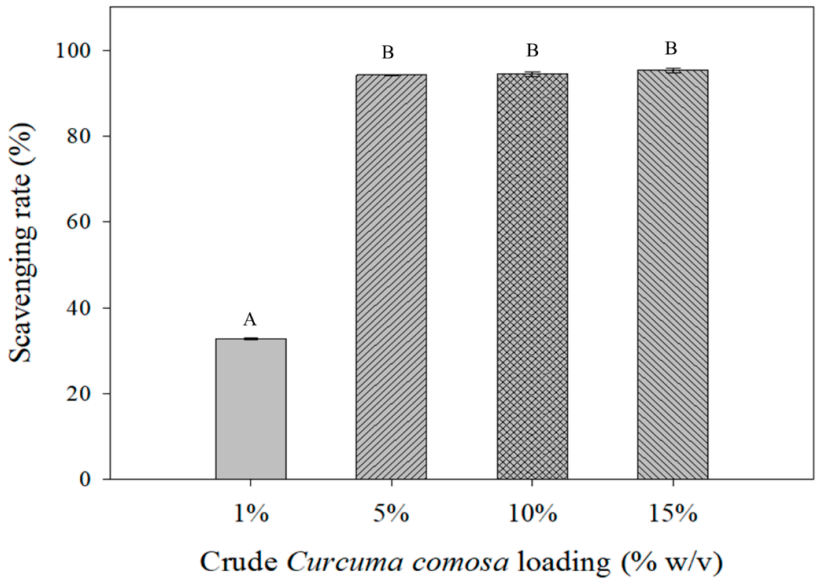

Figure 4. Effect of percent loading of crude ethanol extract of *Curcuma comosa* Roxb. powder in electrospun gelatin nanofibers on DPPH radical-scavenging activity. ^{A,B} Means with different capital letter superscripts are significantly different ($p < 0.05$).

3.5. Effect of Percent Loading on Anti-Tyrosinase Activity of Electrospun Gelatin Nanofibrous Mat Loaded with Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

Figure 5 is the plot of anti-tyrosinase activity of electrospun gelatin nanofibrous mat against percent loading of crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE). The results showed that, at the percent loading of 1% (w/v) of CE, the inhibition rate of tyrosinase enzyme was 30%. As the percent loading of CE increased from 5% to 15% (w/v), the inhibition rate of tyrosinase enzyme slightly increased from 35% to 37%. The lowest percent CE loading with the highest anti-tyrosinase activity of 5% w/v was therefore selected due to its good performance in inhibiting melanin production.

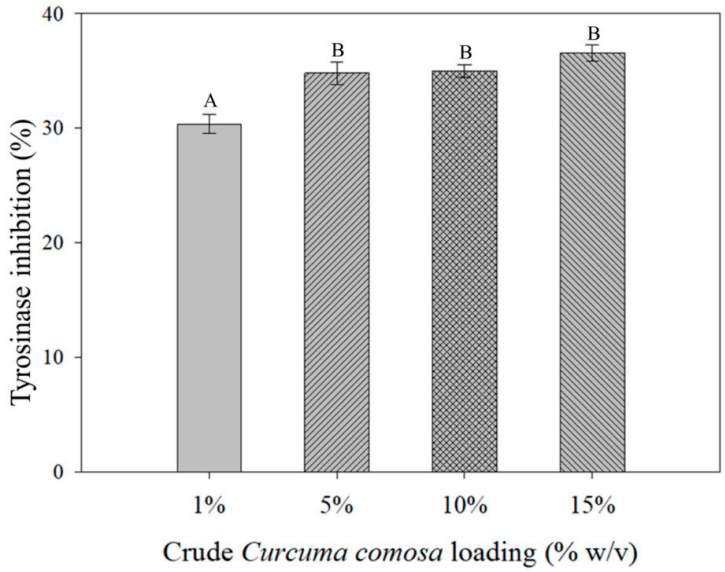

Figure 5. Effect of percent loading of crude ethanol extract of *Curcuma comosa* Roxb. powder in electrospun gelatin nanofibers on anti-tyrosinase activity. ^{A,B} Means with different capital letter superscripts are significantly different ($p < 0.05$).

3.6. Effect of Percent Loading on Antibacterial Activity of Electrospun Gelatin Nanofibrous Mat Loaded with Crude Ethanol Extract of Curcuma comosa Roxb. Rhizome Powder

Electrospun gelatin nanofiber sheets loaded with various concentrations of crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) were tested for their antibacterial activity by the disc diffusion method and by the broth dilution method. Gentamicin was used for this study as a positive control. It gave inhibitory activity against *S. aureus* with the inhibition zone of 24.30 ± 0.00 mm and MIC of 0.25 µg/mL and against *S. epidermidis* with the inhibition zone of 30.50 ± 0.17 mm and MIC of 0.25 µg/mL. The results of the antibacterial studies with regard to the zone of inhibition are shown in Table 1. The electrospun gelatin nanofibrous mat without CE loading (blank nanofiber) showed no inhibitory activity against *S. aureus* and *S. epidermidis*. CE solution showed inhibitory activity against *S. aureus* with the inhibition zone of 9.80 ± 0.17 mm and MIC of 1000 µg/mL and against *S. epidermidis* with the inhibition zone of 9.93 ± 0.06 mm and MIC of 500.0 µg/mL (Figure 6). The electrospun gelatin nanofibrous mat with CE loading of 5% (w/v) (CE nanofiber) showed inhibitory activity against *S. aureus* with the inhibition zone of 7.77 ± 0.21 mm and against *S. epidermidis* with the inhibition zone of 7.73 ± 0.12 mm (Table 1). This means that CE nanofiber could inhibit *S. epidermidis* and *S. aureus* in median level of inhibition. This may be due to the good adhesion of the bioactive compounds in electrospun gelatin nanofibrous mat. Although, the limit of maximum loading of CE concentration in nanofibrous mat of the work was low concentration level of bioactive compounds. Additionally, CE nanofiber could be the efficacy of the antimicrobial treatment for atopic dermatitis (AD, eczema) as a common inflammatory skin.

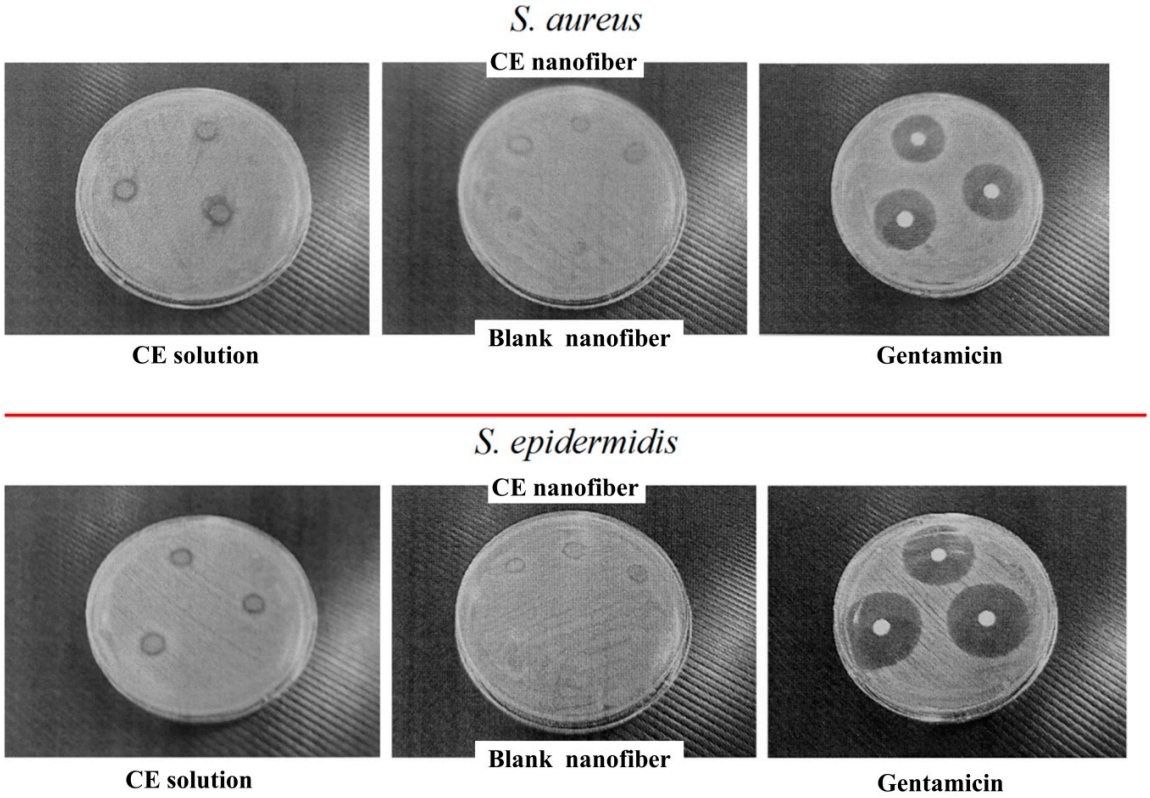

Figure 6. Effect of 5% w/v crude extract of electrospun fiber on antibacterial activity inhibition. Antibacterial activity of crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) in electrospun gelatin nanofibrous mat; 5% (w/v) CE solution, blank nanofiber, 5% (w/v) CE nanofiber, and gentamicin positive control (5% (w/v)). Test disc diameter is 6 mm.

Table 1. Inhibition zone and MIC value of 5% (w/v) crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder crude extract on electrospun gelatin nanofiber sheets.

Sample	*S. aureus*		*S. epidermidis*	
	Inhibition Zone [a] (mm)	MIC (μg/mL)	Inhibition Zone [a] (mm)	MIC (μg/mL)
CE solution	9.80 ± 0.17	1000	9.93 ± 0.06	500
Blank nanofiber	NA *	-	NA *	-
CE nanofiber	7.77 ± 0.21	-	7.73 ± 0.12	-
Gentamicin	24.30 ± 0.00	0.25	30.50 ± 0.17	0.25

* NA = no activity, [a] include disc diameter (6.0 mm).

3.7. Stability of Crude Extract-Loaded Gelatin Nanofibrous Mat

The stability of crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) loaded into electrospun gelatin nanofibrous mat was investigated using a freeze–thaw cycle test. The concentrations of bioactive compounds released from electrospun gelatin nanofibrous mats were determined by following the method's Li [33]. At the 20th cycle, the released concentration of bioactive compounds from tested electrospun gelatin nanofibrous mat was 620 mg/L, which was significantly higher than the remaining concentration of CE in the tested solution (540 mg/L) (Figure 7). This confirmed that the bioactive compounds loaded into the gelatin nanofiber showed increasing stability. Moreover, Figure 8 showed that the DPPH radical scavenging activity of both free CE and CE loaded into electrospun gelatin nanofibrous mat decreased rapidly with progressing freeze–thaw cycles. However, free CE exhibited decreasing activity faster than CE loaded into electrospun gelatin nanofibrous mats. At the 20th cycle, the electrospun gelatin nanofibrous mats loaded with CE had the DPPH radical scavenging rate of 93% whereas the free CE had the DPPH radical scavenging rate of 89%. The results clearly indicate that electrospun gelatin nanofibrous mat can be used as a drug carrier for CE.

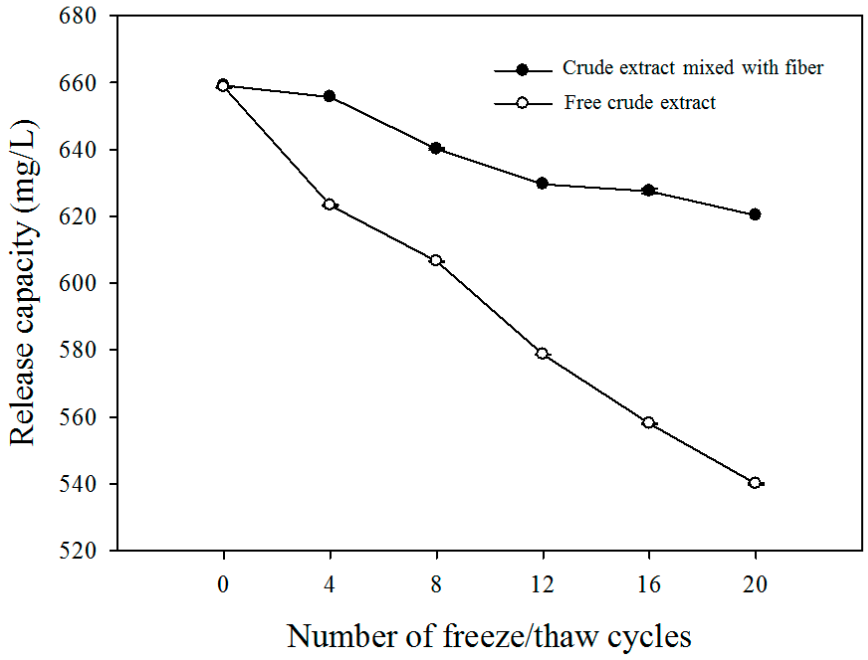

Figure 7. Change in concentrations of bioactive compounds in crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) released from electrospun gelatin nanofibrous mats during freeze–thaw cycle testing; (•): electrospun gelatin nanofibrous mats loaded with 5% (w/v) of CE and (o): 5% (w/v) of only CE solution.

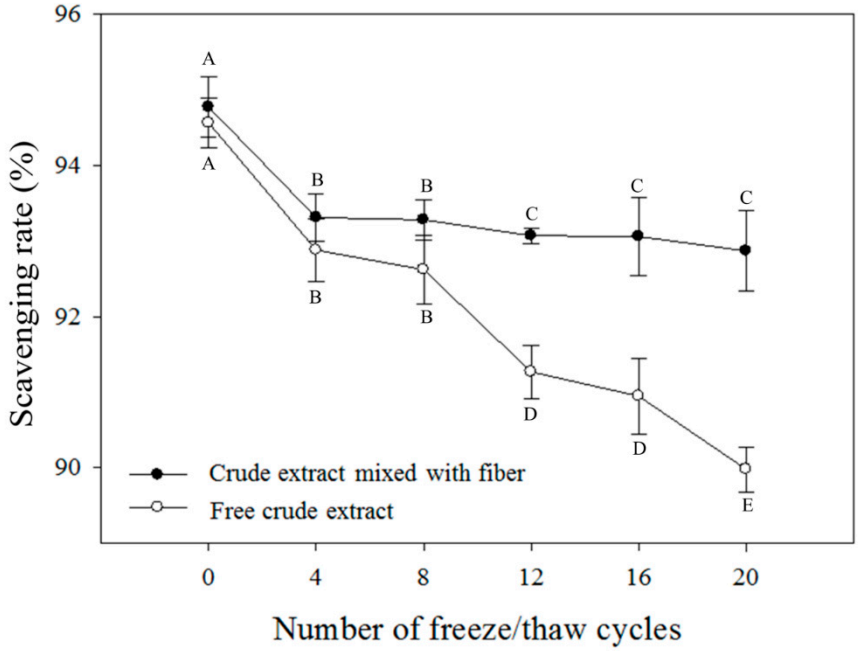

Figure 8. Change in percent of DPPH radical scavenging activity of bioactive compounds in crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) released from electrospun gelatin nanofibrous mat during freeze–thaw cycle testing; (•): electrospun gelatin nanofiber sheets loaded with 5% (w/v) of CE and (o): 5% (w/v) of CE solution. ^{A,B,C,D,E} Means with different capital letter superscripts are significantly different ($p < 0.05$).

4. Conclusions

The suitable conditions for fabricating electrospun gelatin nanofibrous mat loaded with crude ethanol extract of *Curcuma comosa* Roxb. rhizome powder (CE) were 30% (w/v) gelatin solution in acetic acid/H_2O (9:1 (v/v)) mixed with 5% (w/v) of CE. The electrospun gelatin nanofibrous mat loaded with 5% (w/v) of CE showed significant antioxidant activity, and anti-tyrosinase activity. In addition, it exhibited antibacterial activity against *S. aureus* and *S. epidermidis* as compared to the positive control gentamicin. Notably, the electrospun gelatin nanofibrous mat was proved to stabilize the bioactive compunds in CE under an accelerated storage condition. The results showed that drugs loaded into electrospun gelatin nanofibers were appropriate for facial mask applications.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-6439/7/9/76/s1, Figure S1: 400 MHz ^{1}H-NMR spectrum of crude *Curcuma comosa* Roxb. extract., Table S1: Results of premilinary phytochemical screening of the crude ethanol extract of *Curcuma comosa* Roxb.

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