Chapter 6

Novel Natural Polymer/Medicinal Plant Extract Electrospun Nanofiber for Cosmeceutical Application

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Additional information is available at the end of the chapter

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Abstract

Zein produced from maize is a hydrophobic protein, which holds great potential for a number of industrial applications, for example, food packaging, pharmaceutical, cosmetic, and biomedical industry. Sorghum, known as important cereal crop worldwide, is a good source of various phytochemicals such as tannins, phenolic acids, anthocyanins, phytosterols, and policosanols, and these phytochemicals have great impact on human health. *Poria cocos*, a well-known traditional East-Asian medicinal plant, is found around the roots of pine trees in Korea and China. As a rapid and efficient process, electrospinning has drawn huge interest among scientists to produce nanostructured polymer materials with excellent properties. In this work, we studied the influence of co-solvent ratio and concentration of zein/medicinal plant extract on the morphologies of nanostructured zein/medicinal plant extract nanomaterials prepared by electrospinning technique from ethanol/water solution. The zein/medicinal extract nanofibers were characterized by field-emission scanning electron microscopy, transmission electron microscopy, thermogravimetric analysis, and differential scanning calorimetric technique. And we were to incorporate medicinal plant resources into the electro-spun zein nanofibers by electrospinning technique to investigate the effect of medicinal extract on the morphologies, antibacterial, antioxidant, and other properties. Zein/medicinal plant extract might have a practical use as a new preservative for cosmeceutical applications.

Keywords: natural polymer, medicinal plant extract, sorghum extract, *Poria cocos* extract, Electrospinning, Nanofiber, Cosmeceutical, zein

1. Introduction

Recently, investigations have been focused on incorporating medicinal plant extracts with natural polymer-based electro-spun nanofibers due to their unique characteristics and applications. Such materials can be prepared by incorporating medicinal plant extract into the nanofibers of natural polymer by electrospinning technique. Electrospinning has generated great interest among scientists due to its very simple, low-cost method to produce nanofiber which has exhibited outstanding properties such as high porosity and a high specific surface area [1–5]. Biomaterials are of huge interest to the scientists due to their inclusive potential applications particularly in tissue engineering and drug delivery [6–8]. Among the various types of biomaterials being developed, electro-spun ultrafine fibers of protein-based biomaterials are preferred for their cosmeceutical and medical applications. Having smaller pores and higher surface area than general fibers, electro-spun fibers have been successfully used in different fields such as tissue engineering scaffolds, biomedical, pharmaceutical, healthcare, biotechnology, and others [9–13]. Medicinal plants act as a rich source for antimicrobial agents and used as drugs and cosmetics due to their medicinal properties [14–18].

The principle of electrospinning is simple; however, it is necessary to control the process as several variables have an influence on the properties of the end product [9, 19–22]. This chapter is concerned with the fabrication of natural polymer/medicinal plant extract electro-spun nanofibers for cosmeceutical application which is one of the most important application areas. Furthermore, the effects of co-solvent ratio and concentration of natural polymer/medicinal plant extract on the morphologies of nanostructured natural polymer/medicinal plant extract electro-spun nanofibers were also investigated. The effectiveness of these bio-nanofibers is demonstrated with a field emission scanning electron microscope (FE-SEM), a transmission electron microscopy (TEM), thermogravimetric analysis (TGA), a differential scanning calorimetry (DSC) and the anti-bacterial performance and antioxidant properties were also discussed.

2. Effect of co-solvent ratios on the morphologies of electro-spun zein/sorghum extract and zein/*Poria cocos* extract nanomaterials

Zein is considered as one of the best understood plant proteins and it is soluble in ethanol/water solution. It presents good cell compatibility and has more hydrophobic characteristics than other proteins as a result of the presence of polar amino acids, proline and glutamine, which are the major constituents of zein [23, 24]. As a biocompatible and biodegradable protein, zein has useful applications in tissue engineering and drug delivery [8, 23–26]. Also, zein has been extensively used in food packaging, cosmetic, pharmaceutical and biomedical industries [24, 27–32]. Another useful plant protein sorghum (*Sorghum bicolor* L. Moench) is an important cereal food crop and in Asia and Africa, it is considered as an important human food resource [33]. It contains various phytochemicals, for example tannins, phenolic acids, anthocyanins, phytosterols, and policosanols and these phytochemicals have great potential to impact human health significantly [34]. According to recent studies, sorghum has antioxidant activity [35,

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36], anti-carcinogenic effects [36, 37], cholesterol-lowering effects [38], antimicrobial effects [37] and it has a potential to reduce the risk of cardiovascular disease [35, 36]. *Poria cocos* is used in traditional East-Asian medicine that is found around the roots of pine trees in Korea and China. As a rapid and efficient process, electrospinning has attracted great interest among scientists to produce nanostructured polymer materials having excellent properties. Though the principle of electrospinning technique is simple; however, it is difficult to control the process and several variables influence the properties of end product [39].

There are many parameters which have great influence on the morphology of the resultant electro-spun materials varying from nanoparticles to nanofibers, having pores on their surface to beaded nanofibers. Electrospinning parameters can be broadly classified into polymer solution parameters and processing conditions that comprise the applied voltage, tip-to-collector distance (TCD), etc. In this work, we have studied the influence of co-solvent ratio and concentration of zein/sorghum extract and zein/*Poria cocos* extract on the morphologies of nanostructured zein/sorghum extract and zein/*Poria cocos* extract nanomaterials prepared by electrospinning technique from ethanol/water solution. The morphological changes were observed in details by FE-SEM and TEM.

2.1. Experimental

2.1.1. Materials

Zein (molecular weight = 35,000) extracted from corn was purchased from Tokyo Chemical Industry Co. Ltd., Japan. Sorghum was collected from National Institute of Crop Science (NICS) (Rural Development Administration, Korea). *Poria cocos* was obtained from cultivation in a plastic bag. Double-distilled water was used with 96% (v/v) ethanol (EtOH) (obtained from Daejung Chemical & Materials Co. Ltd., Korea) as a co-solvent to prepare all solutions.

2.1.2. Preparation of ethanolic extract and water extract of sorghum and Poria cocos

In case of ethanolic extract, at first, the stem and leaves of sorghum (*Sorghum bicolor* L. Moench) and the sliced *Poria cocos* were air dried and chopped. Later, they were extracted three times using 70% ethanol for 14 days at room temperature. The extracts were combined and evaporated for drying under reduced pressure, and temperature was kept bellow 40°C to yield a red gum. In case of water extract, the stem and leaves of sorghum and the air-dried *Poria cocos* were extracted with distilled water by refluxing for 24 hours (three times) on a water bath at 85°C. The extract was filtered through a Buchner funnel using filter paper, concentrated by rotary evaporator, and freeze dried.

2.1.3. Preparation of spinning solution

Zein solutions were obtained by dissolving zein at different volume ratios of ethanol/water [9:1, 8:2, and 7:3 (v/v)] and various zein concentrations were used (10, 15, 20, 25, and 30 wt.%). Zein/sorghum extract blend solutions were prepared by dissolving various amounts of sorghum extracts in 7:3 (v/v) mixture solvent of EtOH/H_2O at room temperature for 10 minutes.

After that, 30 wt.% zein (based on the weight of the solution) was added into the above solution and the mixture was stirred for another 10 minutes. Following this similar method, zein/*Poria cocos* solutions were also prepared for electrospinning.

2.1.4. Electrospinning

Zein solutions were carefully placed into a syringe and a syringe pump was used to deliver the solution through the blunt needle with a controlled solution feeding rate. Electrospinning was carried out under a constant electric field of 10 kV (Chungpa EMT Co., Korea), which was applied to the solution via an alligator clip attached to the syringe needle. An electrically grounded Al foil was used to collect fibers, which was placed at 15 cm vertical distance to the needle tip. Same method as described here was used for electrospinning zein/sorghum extract and zein/*Poria cocos* blend solutions. **Figure 1** shows a schematic diagram of the electrospinning process.

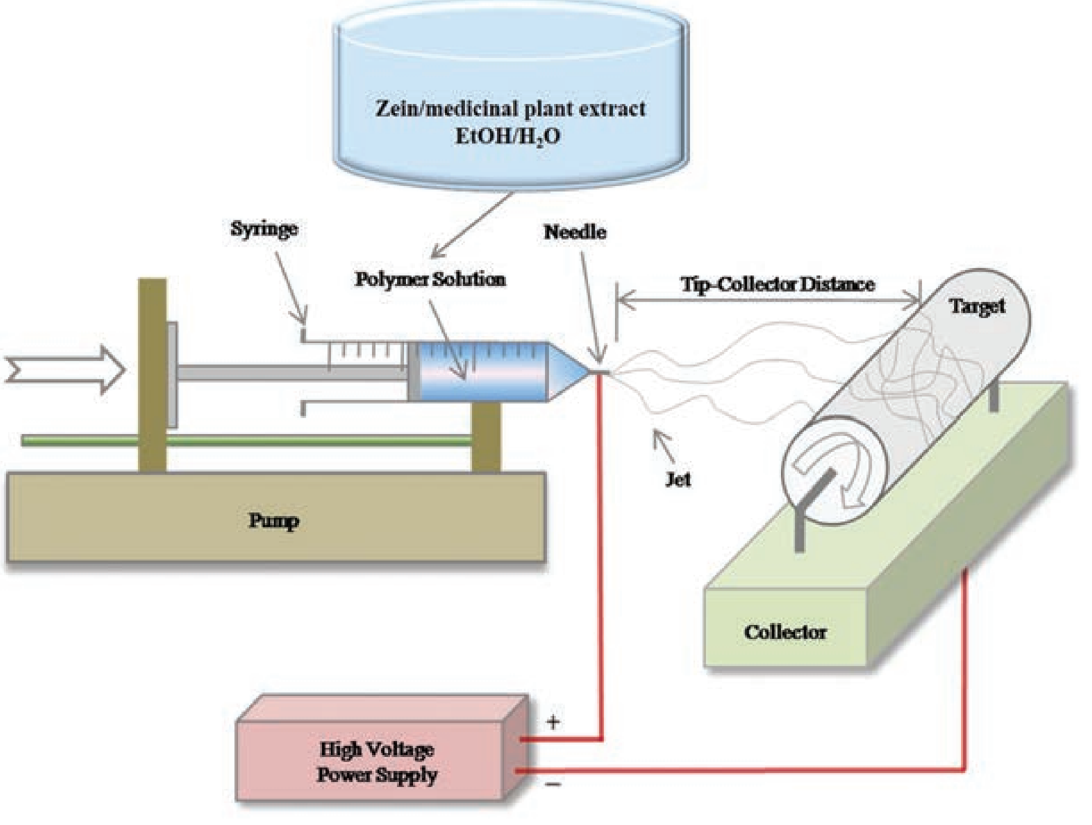

Figure 1. Schematic representation of the electrospinning process.

2.1.5. Characterization

FE-SEM images of the electro-spun nanofibers of zein/*Poria cocos* extract were captured using microscope (JSM-6380, JEOL Ltd., Japan) after platinum coating. The TEM (H-7600, Hitachi Ltd., Japan) analysis was conducted with an accelerating voltage of 100 kV.

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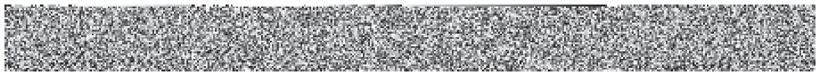

Figure 2. FE-SEM images of electro-spun zein nanofibers prepared by different zein concentrations of (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%, and (e) 30 wt.% [EtOH/H_2O ratio of 70:30 (v/v), TCD = 15 cm and applied voltage = 10 kV].

Figure 2 shows the variation in the FE-SEM morphologies of the 10, 15, 20, 25, and 30 wt.% of zein electro-spun nanofibers from the 7:3 (v/v) of ethanol/water co-solvents. At a fixed applied voltage (10 kV) and TCD (15 cm), the effects of the polymer solution concentration on the morphology of zein nanofiber are illustrated. At low concentration of polymer solution (10 wt.% and 15 wt.%), mostly beads were produced as shown in **Figures 2a** and **2b**. However, when the zein concentration was increased, fibrous products started getting formed and beads density decreased gradually (**Figures 2c** and **2d**). Finally, uniform zein nanofiber mats having no bead defects were observed at 30% zein concentration (**Figure 2e**). Ultrafine zein electro-spun nanofiber mats in a nanometer range (300–500 nm) prepared in the aqueous solutions are presented in **Figure 2e**. The key factors that control the formation of the beaded fibers are charge density carried by the jet, the viscoelasticity, and surface tension of the solution [21]. The effect of the different volume ratios of ethanol/water in the polymer solution was also evaluated by changing the ethanol content from 70% to 90% (v/v) and the results are presented in **Figures 3** and **4**. However, the most regular-diameter ultrafine nanofibers were found at EtOH/water ratio of 70:30 and 30 wt.% zein concentration (**Figure 2e**).

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Figure 3. FE-SEM images of electro-spun zein nanofibers prepared by different zein concentrations of (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%, and (e) 30 wt.% [EtOH/H_2O ratio of 80:20 (v/v), TCD = 15 cm and applied voltage = 10 kV].

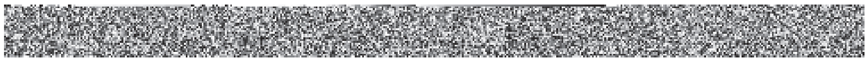

Figure 4. FE-SEM images of electro-spun zein nanofibers prepared by different zein concentrations of (a) 10 wt.%, (b) 15 wt.%, (c) 20 wt.%, (d) 25 wt.%, and (e) 30 wt.% [EtOH/H_2O ratio of 90:10 (v/v), TCD = 15 cm and applied voltage = 10 kV].

FE-SEM morphologies of electro-spun zein nanofibers containing various quantities of sorghum (0, 5, 10, and 20 wt.%) and *Poria cocos* extract (0, 5, 10, and 15 wt.%) are presented in **Figure 5** and **6**, respectively. At a fixed applied voltage (10 kV) and TCD (15 cm), the typical morphology of only zein nanofibers is shown in **Figure 5a**. The morphologies of the nanofibers were gradually changed when the amount of sorghum extract was increased from 0 to 20 wt. %. A slight reduction in the fiber diameters was observed with the increase of sorghum extract content. In **Figure 5b**, it can be clearly seen that nanofibers were splattered as 5 wt.% sorghum extract was added to zein solution. With the increase of the amount of sorghum extract, splashing of the nanofibers was increased and they started forming branched nanofibers that were connected with others (**Figures 5c** and **5d**).

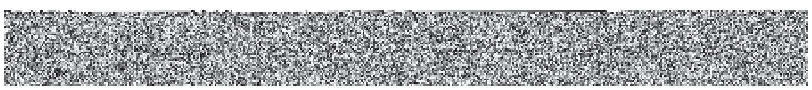

Figure 5. FE-SEM images of electro-spun zein nanofibers containing different sorghum extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.%, and (d) 20 wt.% [zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

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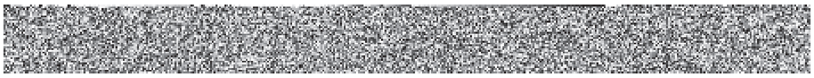

Figure 6. FE-SEM images of electro-spun zein nanofibers containing different *Poria cocos* extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.%, and (d) 15 wt.% [zein concentration = 30 wt.%, EtOH/H_2O ratio of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

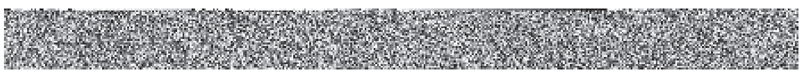

Figure 7. TEM images of electro-spun zein nanofibers containing different sorghum extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.%, and (d) 20 wt.% [zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

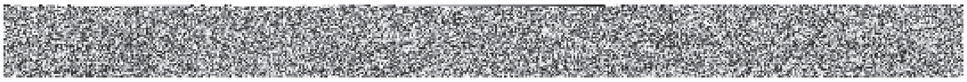

Figure 8. TEM images of electro-spun zein nanofibers containing different *Poria cocos* extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.%, and (d) 15 wt.% [zein concentration = 30 wt.%, EtOH/H_2O ratio of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

The modified physical properties that developed in zein/sorghum extract solutions are responsible for these morphological changes [32]. An opposite trend was observed in case of zein/*Poria cocos* nanofibers as the fiber diameters expanded slightly with the increase of the amount of *Poria cocos* extract. Also, nanofibers showed the tendency of formation of rough surfaces when the *Poria cocos* extract was added; however, nanofibers containing 15 wt.% *Poria cocos* showed the maximum surface smoothness in comparison to those with 5 and 10 wt.% solutions (**Figures 6b**, **6c**, and **6d**). For preparing both types of electro-spun nanofibers (zein/sorghum extract and zein/ *Poria cocos* extract), a 7:3 (v/v) mixture solvent of EtOH/H_2O was used. The morphological changes of zein/sorghum extract and zein/ *Poria cocos* extract were also observed from their TEM images listed in **Figures 7** and **8**, respectively. The TEM images clearly show that both zein/sorghum extract and zein/ *Poria cocos* extract nanofibers were crinkled with the increase of sorghum extract and *Poria cocos* extract, respectively. However, folding is slight for *Poria cocos* extract in comparison to sorghum extract (**Figures 7b**, **7c**, **7d**, **8b**, **8c**, and **8d**). From FE-SEM and TEM images, it can be clearly observed that sorghum extract and *Poria cocos* extract could alter the zein morphology very effectively.

2.2. Effect of extraction conditions on yield

Extract yield of sorghum and *Poria cocos* using water and 70% EtOH is summarized in **Table 1**. For indicating the effects of the extraction conditions, extraction yield was used. In case of sorghum, 70% EtOH extract yield rate (1.414%) was maximum followed by water. For *Poria cocos*, the trend was similar as observed in the case of sorghum.

Medicinal plant extracts		Total (g)	Yield rate (%)
Water extract	Sorghum	0.9468	1.1482
	Poria cocos	0.8259	0.9177
70 % EtOH extract	Sorghum	1.5846	1.7414
	Poria cocos	1.3820	1.5356

Table 1. Yield rate of the extracts of sorghum and *Poria cocos* using water and ethanol.

3. Novel zein/medicinal plant extract electro-spun nanofibers for cosmeceutical application

Due to biocompatible nature, bio-nanofibers have superiority over their synthetic counterparts and have wide range of applications. Among the various types of bio-nanofibers being developed, extensive efforts are currently being made for the development of protein-based bio-nanofibers. Nanofibers have a large surface-to-mass ratio that makes them promising candidates for advanced material devices [40]. A number of methods have been developed to spin nanofibers, among them electrospinning is one of the methods using electrical charge to draw nanoscale fibers from polymer liquid solutions [20, 41]. Natural nanofibers are preferred products over synthetic nanofibers as synthetic nanofibers are environmentally toxic [40].

The major purpose of this section is the incorporation of medicinal plant resources (sorghum extract and *Poria cocos* extract) into the electro-spun zein nanofibers by electrospinning technique to investigate the effects of medicinal extract on the morphologies, antibacterial and antioxidant properties of zein nanofibers. The prepared zein/medicinal extract nanofibers were characterized by FE-SEM, TGA, and DSC techniques. Photoshop 5 was used to measure the fiber diameter. Using 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) assay and superoxide dismutase (SOD) assay, antioxidant properties of Zein/*Poria cocos* plant extract were investigated and improved antioxidant capacities were found. Zein nanofibers containing sorghum extract were found to be effective to control the growth of bacteria. The significant anti-bacterial activity of zein/sorghum extract nanofibers and improved antioxidant capacities of zein/*Poria cocos* extract nanofibers suggested that they might have a practical use of new preservative for cosmeceutical and medical applications.

3.1. Experimental

3.1.1. Materials

Zein (molecular weight = 35,000) extracted from corn was purchased from Tokyo Chemical Industry Co. Ltd., Japan. Sorghum was collected from National Institute of Crop Science (NICS) (Rural Development Administration, Korea). *Poria cocos* was obtained from plastic bag cultivation method. Double-distilled water was used with 96% (v/v) EtOH (Daejung Chemical & Materials Co. Ltd, Korea) as a co-solvent to prepare electrospinning solution.

3.1.2. Electrospinning

Zein/medicinal plant resources (sorghum extract and *Poria cocos* extract) blend solution was carefully loaded into a syringe and the solution was delivered through blunt needle tip with a controlled flow rate by means of a single syringe pump. During electrospinning, the voltage was kept at 10 kV and applied to the solution via an alligator clip adhered to the syringe needle. Fibers were deposited on an electrically grounded Al foil and the distance between the needle and collector was maintained at 15 cm [42, 43].

3.1.3. Determination of antioxidant capacity

3.1.3.1. Free radical scavenging by the use of DPPH radical

The free radical scavenging activity was measured using DPPH radical following the protocols of Brand Williams modified by Miliauskas [44, 45]. Maximum absorption of DPPH radicals is at 515 nm and by an antioxidant compound, DPPH disappears with reduction. The DPPH solution in methanol (6×10^{-5} M) was required to prepare daily and 3 ml of this solution was mixed with 100 μl of methanolic solutions of *Poria cocos* extracts. In a water bath, the samples were incubated for 20 minutes at 37°C, and then the decrease in absorbance was measured at 515 nm (β). The measurement was carried out in triplicate and based on the following formula, DPPH activity was calculated.

$$\text{Inhibition}\,\% = [(\alpha - \beta)] \times 100$$

where, α= absorbance of the blank sample and β = absorbance of the *Poria cocos* extract.

3.1.3.2. Free radical scavenging by the superoxide dismutase (SOD) assay

The superoxide anion scavenging activity of *Poria cocos* extract was determined by the WST (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)2H-tetrazolium, monosodium salt) reduction method, using SOD assay kit-WST. In this method, O^2 reduced WST-1 and produced yellow formazan which is spectrophotometrically measured at 450 nm. Antioxidants are capable to inhibit yellow WST formation. All experiments were done in triplicate. The percentage of inhibition of superoxide radicals was measured using the formula discussed earlier.

3.1.4. Determination of anti-microbial efficacy

For investigating anti-bacterial performance of zein/medicinal plant extract, *Klebsiella pneumonia* (ATCC 4352) was used. To prepare samples, nanofibers were dispersed into a viscous aqueous solution containing 0.05 wt.% of polyoxyethylenesorbitan monooleate (TWEEN 80, Sigma Aldrich). After 18 hours of incubation at 37°C, a mixed culture of microorganisms was obtained on tryptone soya broth. After that, 0.4 g of sample was inoculated with 0.2 g of the microorganism suspension to adjust the initial concentration of bacteria to 1.3×10^5 ea/ml. Finally, the inoculants were mixed homogeneously with the samples and stored at 37°C.

3.1.5. Characterization

Average fiber diameters of the zein/*Poria cocos* extract and zein/sorghum extract nanofiber were determined using Photoshop 5. The thermal properties of electro-spun zein/sorghum and zein/*Poria cocos* extract were studied with TGA (model Q-50) and DSC (Q-10) from TA instruments under the nitrogen gas atmosphere. Using DPPH radical scavenging assay and SOD assay, antioxidant properties of zein/*Poria cocos* plant extract were investigated. The anti-bacterial performance test of zein/sorghum extract was carried out on *Klebsiella pneumonia*.

3.2. Results and discussion

3.2.1. Morphology

Figure 9 presents the dependence of the average diameters of the zein/sorghum and zein/*Poria cocos* extract nanofibers on the contents of sorghum and *Poria cocos* extracts, respectively. The result indicates a dramatic decrease in fiber diameter when 5 wt.% sorghum extract was added to zein nanofiber. However, nanofibers retained their previous shape (average diameter around 600 nm) when 10 wt.% sorghum extract was added. Moreover, there is no huge change in fiber diameter, when the sorghum extract contents increased from 10 to 20 wt.%. On the other hand, when the *Poria cocos* extract contents increased from 5 to 15 wt.%, a slight increase in fiber diameter was observed.

Figure 9. Average diameter of zein/sorghum extract and zein/*Poria cocos* extract nanofibers containing various sorghum extracts and *Poria cocos* extracts, respectively [zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

3.2.2. Thermal properties

Temperature		50-150°C	150-350°C	350-450°C	Above 500°C
Concentration		Dehydration and decomposition of the solvent	Decomposition of the sorghum extract	Decomposition of thezein	Residue
Zein	Sorghum extract				
30 wt.%	-	92.12%	34.17%	20.25%	18.51%
30 wt.%	5 wt.%	92.94%	34.62%	21.81%	19.35%
30 wt.%	10 wt.%	93.49%	38.15%	26.48%	24.64%
30 wt.%	20 wt.%	94.87%	43.65%	30.03%	27.72%

Table 2. TGA data of electro-spun zein nanofibers containing different sorghum extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.%, and (d) 20 wt.% [zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

To measure thermal properties of zein/sorghum extract and zein/*Poria cocos* extract nanofibers, TGA and DSC were used in nitrogen atmosphere. **Table 2** presents the TGA data of decomposition temperature obtained from zein nanofibers containing 0, 5, 10, and 20 wt.% of sorghum extract. From **Table 2**, it can be seen that, zein/sorghum extract nanofiber showed three typical weight loss regions. The first region (50–150°C) was due to dehydration and decomposition of the solvent, the second region (150–350°C) was due to the decomposition of the sorghum extract and the third region (350–450°C) was due to thermal degradation of zein. After thermal decomposition, the lowest amount of residue was obtained from pure zein nanofibers (18.51%) and highest amount of residue was obtained from zein nanofiber containing 20 wt.% of sorghum extract. It can be concluded that higher thermal properties could be obtained at higher weight percentage of sorghum extract. Similar effect was observed in case of zein/*Poria cocos* nanofibers as shown in **Table 3**.

Temperature		50-150°C	150-350°C	350-450°C	Above 500°C
Concentration		Dehydration and decomposition of the solvent	Decomposition of the *Poria cocos* extract	Decomposition of the zein	Residue
Zein	*Poria cocos* extract				
30 wt.%	-	92.24%	35.26%	19.16%	17.15%
30 wt.%	5 wt.%	92.81%	35.71%	20.84%	18.45%
30 wt.%	10 wt.%	93.24%	37.66%	23.14%	22.16%
30 wt.%	20 wt.%	94.11%	39.72%	29.18%	25.72%

Table 3. TGA data of electro-spun zein nanofibers containing with different *Poria cocos* extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.% and (d) 20 wt.% (zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV).

Zein/sorghum extract concentration	T_g (°C)
(a) 30 wt.%	155.60
(b) 30 wt.%/5 wt.%	159.32
(c) 30 wt.%/10 wt.%	160.47
(d) 30 wt.%/20 wt.%	166.77

Table 4. T_g values of zein and zein/sorghum extract fibers containing different sorghum extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.% and (d) 20 wt.% [zein concentration = 30 wt.%, EtOH/H_2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

The DSC data of electro-spun zein nanofibers containing different sorghum extracts are shown in **Table 4**. The lowest glass transition temperature (155.60°C) and melting temperature were obtained from only zein nanofiber. Both glass transition temperature and melting temperature of zein/sorghum extract nanofibers were shifted towards higher temperature values with the higher weight percentage of sorghum extract. Similar effect was also observed when the *Poria cocos* extract was added to zein nanofiber (**Table 5**). These results suggest that sorghum extract and *Poria cocos* extract have an influence on the thermal properties of zein nanofibers.

Zein/*Poria cocos* extract concentration	Tg (°C)
(a) 30 wt.%	155.60
(b) 30 wt.%/5 wt.%	158.12
(c) 30 wt.%/10 wt.%	159.48
(d) 30 wt.%/15 wt.%	161.37

Table 5. T_g values of zein and zein/*Poria cocos* extract fibers containing different *Poria cocos* extracts: (a) 0 wt.%, (b) 5 wt.%, (c) 10 wt.% and (d) 15 wt.% [zein concentration = 30 wt.%, EtOH/H2O of 7:3 (v/v), TCD = 15 cm and applied voltage = 10 kV].

3.2.3. Antioxidant activities

Radical scavenging capacities of *Poria cocos* extract were determined using DPPH and SOD assay and results are presented in **Tables 6** and **7**. 15 wt.% *Poria cocos* extract showed the highest antioxidant capacity (98.69 ± 1.49%) of DPPH inhibition, followed by 10 wt.% *Poria cocos* extract (97.75 ± 1.42%), and 5 wt.% *Poria cocos* extract (96.88 ± 0.91%) which is the lowest antioxidant capacity in this assay.

Sample	Concentration (%)	Scavenging activity (%)
Poria cocos	5	96.88 ± 0.91
	10	97.75 ± 1.42
	15	98.69 ± 1.49

Table 6. DPPH radical scavenging activities of ethanol extract from *Poria cocos*.

Sample	Concentration (%)	SOD activity (%)
Poria cocos	5	66.51 ± 6.45
	10	77.57 ± 4.34
	15	83.49 ± 5.73

Table 7. SOD activities of ethanol extract from *Poria cocos*.

Results obtained using SOD assay differed from those observed for DPPH assays. 15 wt.% *Poria cocos* showed the highest antioxidant capacities (83.49 ± 5.73%) followed by 10 wt.% *Poria cocos* extract (77.57 ± 4.34%). In this assay, 5 wt.% *Poria cocos* showed the lowest antioxidant potential (66.51 ± 6.45%). Therefore, both DPPH and SOD assay results suggest that antioxidant capacity increased with the increased weight percentage of *Poria cocos* extract.

3.2.4. Anti-bacterial ability

The anti-microbial performance of zein and zein/sorghum extract nanocomposite against *Klebsiella pneumonia* (ATCC 4352) was evaluated and results are shown in **Figure 11**. The anti-bacterial potency of only zein and zein/sorghum extract nanocomposite was assessed by counting the number of remaining bacteria with the storage time at 25°C. As shown in **Figure 11**, only zein nanocomposite has the anti-bacterial ability; however, in the test sample, a number of bacteria remained constant for a long time.

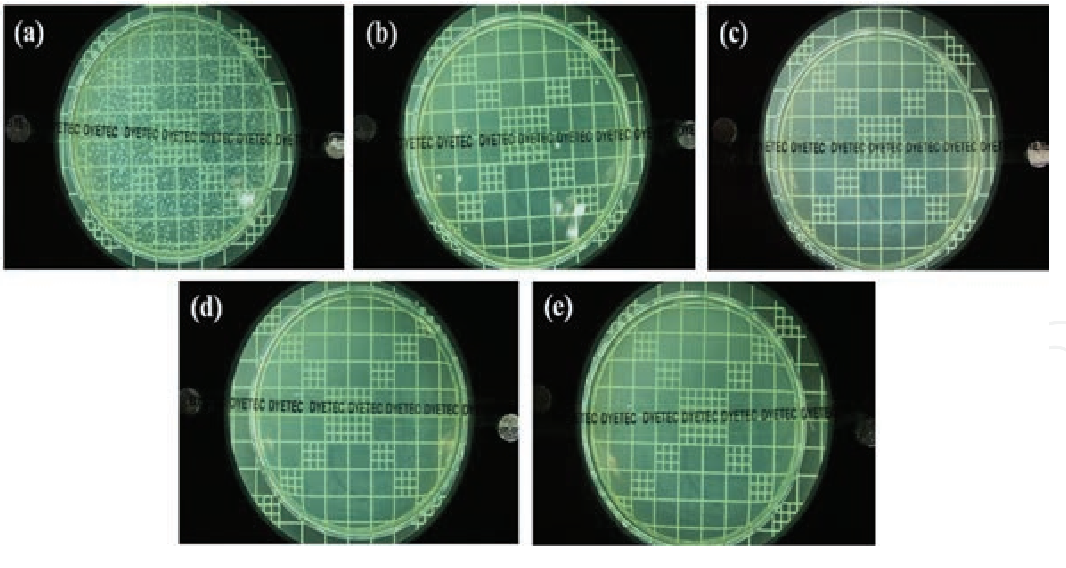

Figure 10. Antibacterial ability test with *Klebsiella pneumonia* (a) blank and electro-spun zein nanofibers containing different sorghum extracts: (b) 0 wt.%, (c) 5 wt.%, (d) 10 wt.% and (e) 20 wt.% (after 4 days).

On the other hand, zein/sorghum extract nanocomposites presented a remarkable decrease in the number of bacteria (**Figure 11c**, **11d**, and **11e**). These results recommended that only a small proportion of sorghum extract can make zein more competent against bacteria. The increase in the concentration of the sorghum extract stimulates decrease in the number of bacteria.

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4. Conclusion

Different volume ratios (v/v) of ethanol/water solutions and zein concentration can affect the morphology of the electro-spun zein nanofibers. Uniform zein nanofiber mats having average diameter around 600 nm could be obtained from the 7:3 (v/v) of ethanol with zein concentration of 30 wt.%. Different amounts of sorghum and *Poria cocos* extracts have been successfully incorporated into zein nanofibers by electrospinning technique from 7:3 (v/v) EtOH/H_2O solutions. Both sorghum extract and *Poria cocos* extract have shown remarkable effect on the structures and morphology of zein/sorghum extract zein/*Poria cocos* extract nanofibers and caused splattering, crinkling, and decreasing the diameter of the zein nanofibers due to decreased viscosity. Moreover, zein/sorghum extract and zein/*Poria cocos* extract nanofibers showed improved thermal property. Antioxidant properties of different wt.% of *Poria cocos* extracts were investigated using DPPH assay and SOD assay and found that 15 wt.% *Poria cocos* extract possessed highest capacities in both methods used, and hence could be a possible rich source of natural antioxidants. An improved performance was achieved from zein/sorghum extract nanofibers against bacteria compared to only zein nanofibers. The significant anti-bacterial activity of zein/sorghum extract nanofibers and improved antioxidant capacities of zein/*Poria cocos* extract nanofibers recommended that they might have a practical application as a new preservative for cosmeceutical and medical applications.

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