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Encapsulation of flaxseed oil and eucalyptus essential oil using electrospinning

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ABSTRACT

The use of medicinal plants in food has a long history. Flaxseed oil and eucalyptus essential oil are highly regarded due to their biological properties. In this research, the aim is to investigate the antimicrobial properties of zein nanofibers containing flaxseed oil and eucalyptus essential oil to control *staphylococcus aureus* and *escherichia coli* bacteria in a laboratory environment. For this purpose, flaxseed oil mixed with eucalyptus essential oil in zein solution. Then, it was converted into nanofibers using an electrospinning machine. In order to study the characteristics and antimicrobial properties of the nanofibers, tests including scanning electron microscopy (SEM), fiber diameter determination with Image J software, atomic force microscopy (AFM), X-ray diffraction (XRD), thermal gravimetric analysis (TGA) were performed. Investigation of antimicrobial properties of produced nanofibers by disk diffusion method were performed. The SEM results showed that the morphology of the electrospun fibers was uniform and free of beads. AFM images represented three-dimensional and tubular images of fibers obtained from electrospinning zein/flaxseed oil/eucalyptus essential oil. The X-ray diffraction pattern showed an increase in the crystallinity intensity of the treatments compared to the control sample. Based on thermal analysis results, eucalyptus and flaxseed oil increased the thermal stability of zein nanofibers. The results showed that the addition of flaxseed oil to eucalyptus essential oil strengthened the antimicrobial properties of nanofibers. According to the results of the present research, the zein/flaxseed oil/eucalyptus essential oil fibers can be used as natural antimicrobials.

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1- Introduction

Considering that the food industry today seeks to use less chemicals, there is a growing acceptance for the use of natural food additives with antimicrobial and antioxidant properties that do not have any harmful effects on the body [1]. Essential oils (EOs) extracted from spices have antibacterial and antioxidant properties that have made them a favorite additive in the food industry. In addition, most of them are generally recognized as safe (GRAS) [1].

Flax is a dicotyledonous plant with the scientific name *Linum Usitatissimum*, belonging to the category of flowering plants, Dicotyledons, Malpigiaceae order, flax family. The seed of the plant is rich in fiber, protein and oil. Humans have been consuming flax seeds since the beginning of early civilizations. In ancient Egypt and Greece, it was used for medical purposes, mainly to relieve abdominal pain and as a source of energy [2].

Flaxseed oil is a rich source of essential fatty acids, especially alpha-linolenic acid (ALA), which is an omega-3 fatty acid. In addition to the oral uses of this oil, it is known as an antioxidant, anti-inflammatory and pain reliever, anti-fibrosis drug, anti-diabetes, stabilizer of blood sugar level, antiviral and bactericidal [2].

Research has shown that flaxseed oil exhibits broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria. For example, a study by Sing et al reported that flaxseed oil showed inhibitory effects against common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [3].

Eucalyptus, with the scientific name *Eucalyptus globulus*, is one of the medicinal trees that has been considered for a long time due to its antimicrobial effects and other properties. This plant is rich in polyphenols, and the important composition of its leaf essential oil can be eucalyptus or cineol, which varies depending on the type and habitat conditions [4]. The essential oil of this plant is colorless or reddish-yellow and has a very strong, cool, burning and very volatile smell, which is extracted by various methods, including distillation. Spain, Australia and Portugal are the most important producers of eucalyptus essential oil [5]. The therapeutic properties of eucalyptus include expectorant, general disinfectant, antispasmodic, wound healing, and antiparasitic. In China and Japan, eucalyptus leaf essential oil is used as an antibiotic and anthelmintic. This substance is used as a stimulant and softener of the chest and in cases of asthma and chronic bronchitis [6]. In addition, eucalyptus essential oil has shown antimicrobial activity on a wide range of Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Shigella dysentery*, *Salmonella paratyphi*, *Escherichia coli*, *Bacillus cereus*, and *Candida albicans* fungus [7]. However, their use in the food industry has been limited due to economic limitations, being expensive, having a spicy taste, and affecting the taste of food. In addition, these compounds in food are sensitive to oxygen, high heat, light, etc., and are insoluble in water. For some uses, it is necessary to control their release. Therefore, the encapsulation of active components (liquid, solid or gas state) in a layer of different polymers (protein, polysaccharide or lipid) is used to release the active component under controlled conditions and desired time in the food matrix to prevents bad taste due to excessive consumption of food [8]. It also has a long-term antioxidant and antibacterial effect for food preservation. However, a general limitation for all encapsulation methods is the absence of a suitable contact surface area for the capsules, which might not allow the maximum delivery of nutrients to the body. It has been reported that the dissolution rate of the drugs increases with increasing contact surface area. Increasing the contact surface area can lead to better absorption and drug delivery to the body tissues. These researchers reported that nanofibers due to having a wide contact surface area are able to deliver drugs better compared to particles such as microcapsules that have a spherical cross-section. Therefore, the use of nanofibers as an alternative and possibly superior method of food fortification has received much attention.

Electrospinning is one of the new methods of encapsulation that is capable of producing a polymer layer with a nanofiber structure. Nanofibers produced by electrospinning are used in the preparation of local drug delivery systems, drug encapsulation, vitamins, etc. [9]. Electrospinning is a method that produces

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nanofibers with electrostatic driving force. Nanofibers are made from a liquid or molten polymer solution that is transferred from a capillary tube to an area with a high electric field. When the electrostatic forces dominate the surface tension of the liquid, a Taylor cone is formed and a narrow jet is rapidly accelerated towards the target (collector) which is connected to the ground or has an opposite charge. Instability in this jet causes intense movement of the jet, which in turn increases and narrows the jet and causes the evaporation of the solvent or the cooling of the melt, and the formation of nanofibers on the target surface [10]. Nanofibers are used as active coatings to transfer bioactive compounds to food products.

Zein is the main protein of corn, constituting approximately 45-50% of the corn protein. Zein is insoluble in water. The reason for the lack of solubility in water is the presence of large amounts of non-polar amino acids such as leucine, proline and alanine. At present, due to having exceptional features such as film formation, high heat resistance, and high resistance to oxygen, zein has found many applications in the food and pharmaceutical industries [11]. The aim of the current research was the encapsulation of flaxseed oil and eucalyptus essential oil using the electrospinning method, and investigating the characteristics and antimicrobial properties of the resulting fiber membrane.

2- Materials and Methods

2-1- Materials

Zein powder with catalog number Z3625 from Sigma Aldrich Company, Acetic acid with 99.7% purity from Merck (Germany), Eucalyptus essential oil from Barij Essence Company (Iran), Flaxseed oil from Exir Company (Iran), 96% ethanol from Iran Hamoon-Teb Company and double-distilled deionized water were obtained from Samen Serum Company (Iran).

2-2- Preparation of electrospinning solution

The electrospinning solution was prepared by adding flaxseed oil (5%) and eucalyptus essential oil (0, 2.5, 5 and 10%) to the zein solution. To prepare zein solution (27%), the required amount of zein powder was dissolved in a flask with glacial acetic acid and made up to volume. The resulting solution was kept at room temperature for 24 hours until the existing bubbles are removed and intermolecular bonds are formed in the zein and a homogeneous solution is obtained [12].

2-3-Electrospinning process

A single-axis electrospinning machine equipped with a high-voltage power supply in the range of 0 to 35 kV was used to prepare nanofibers containing eucalyptus essential oil and flaxseed oil. A stainless steel needle with a diameter of 0.9 mm (G18) was used as a thread maker. The polymer solution was inserted into 5 ml syringes and transferred to the needle through a narrow tube. The syringe was placed in a horizontal position on a digitally controlled pump, so that the needle was located vertically and facing the collecting drum. The needle was connected to an electrode with positive polarity and a high voltage energy source. After the formation of the Taylor cone, a jet of positively charged polymer solution was formed, which, after traveling the distance between the needle tip and the collector, settled on the collector in the form of non-woven fibers. The collector was previously covered with aluminum foil. Electrospinning was performed at room temperature for 30 minutes [13].

2-4- Scanning electron microscope (SEM)

The morphology of the fibers obtained from electrospinning was examined using a scanning electron microscope at four magnifications of 1000, 2000, 5000 and 10000 times. For this purpose, the samples were cut in dimensions of 0.5 x 0.5 cm^2 on special bases and before taking pictures, the samples were covered by gold-palladium alloy. Since the polymer nanofibers must be covered by a conductive material and because gold easily vaporizes, and bombarding it with high-energy electrons gives a favorable result for the detection of secondary electrons, therefore gold is used as a coating. SEM was performed at a working distance of 13 mm and a voltage of 5 kV. Using the Image J engineering software, the diameter of 50 fibers of each production series, which were randomly selected, was measured and the average of these data was considered as the average diameter of the fibers of that image [14].

2-5- Atomic force microscope (AFM)

Atomic force microscope (AFM) was used to better describe the surface topography of electrospun fibers [11]. Images were scanned in non-contact mode in air using commercial

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Si consoles (Ara Research Company, Iran) with a resonance frequency of 180 kHz.

2-6- Thermal gravimetric analysis (TGA)

This experiment was conducted to investigate the behavior of pure zein nanofibers and nanofibers loaded with eucalyptus and flaxseed oil against heat. Analysis was done on polymer solution and different concentrations of zein-eucalyptus-flaxseed oil solution. TGA analyzes were performed on a SDT-Q600 (TA Instruments, USA) with a scanning speed of 20 °C/min. While the samples (4.8 mg) received heat, their temperature was analyzed from 25 to 700 °C with a temperature increase rate of 10 °C/min [15].

2-7- X-ray diffraction (XRD)

X-ray diffraction test was used to investigate the mixing and dispersion of different compounds and to determine the physical condition of eucalyptus in electrospun zein fibers. The test was performed on four samples, which are samples loaded at levels of 0, 2.5, 5 and 10% eucalyptus essential oil and 5% flaxseed oil. In this test, the samples were scanned using an X-ray diffractometer, with a CuKα beam with a wavelength of λ=1.5418 Angstroms and an incident angle of 2θ from 10 to 40 degrees, at ambient temperature, and their X-ray diffraction spectrum was compared [16].

2-8- Antimicrobial test of nanofiber membrane

The disk diffusion method was used to measure the antimicrobial properties of electrospun fiber mats against model microbial species, including *S. aureus* (ATCC 1112) and *E. coli* (ATCC 1330) according to the method described by Hosseini et al. (2021). The nanofibrous membranes made of 10 mm disks were separated. 100 microliters of broth culture with 10^5-10^6 CFU/mL of bacteria was cultured on Mueller Hinton agar plate at 37°C for 24 hours. When the incubation was finished, the diameter of the inhibition zone was measured [9].

2-9- Encapsulation efficiency

In this study, the amount of active substances on the surface of nanofibers was measured by immersing the electrospun membrane in hexane solvent [11]. Zein does not dissolve in hexane, but flaxseed oil and eucalyptus essential oil do easily. The amount of absorption and the amount of dissolved oil/essence was determined at a wavelength of 275 nm using a UV/vis spectrophotometer. The amount of active substances on the surface of the zein electrospun fibers was subtracted from the total of the active substances loaded in the electrospun fibers. Therefore, the encapsulation efficiency was obtained according to the following formula:

$$\text{Encapsulation efficiency (\%)} = \frac{Ot-Od}{Ot} \times 100$$

Where the O_t showed the total amount of essential oil (in theory) and O_d showed the amount of essential oil dissolved in hexane.

2-10- Statistical design

The experiment was carried out in the form of a completely randomized design. SAS software version 1.9 was used to compare the mean of the data using Duncan's test.

3- Results and discussion

3-1- Morphology and fiber diameter

SEM images of electrospun fibers in samples containing essential oil and flaxseed oil can be seen in Figure 1. These images were analyzed by Image J software, the average fiber diameter was calculated and the calculation results are listed in Table 1. According to the images, all the fibers have a proper morphology, free bead, and are uniform. As can be seen in Table 1, with the increase in the amount of the essential oil, the average diameter of the fibers increases and also the electrical conductivity decreases. In general, with the increase in the electric conductivity of the solution, the charge density increases, so the increase in the charges resulting from the solution increases the elasticity of the solution, and this leads to the production of fibers with a small diameter. The results are in accordance with the results of Hosseini et al. (2021) and Ghasemi et al. (2022) [11, 9]. In addition, concentration is another factor that can increase the fiber diameter. It has been reported that the most important parameter in controlling the morphology and diameter of electrospun fibers in the electrospinning process is the concentration of the solution. As the concentration increases, the diameter of the fiber also increases. This is probably due to the greater resistance of high concentration solutions that are drawn to the collector [11]. Afshar et al. (2017) reported in a study that by adding peppermint essential oil to chitosan-bovine protein solution, electrical conductivity decreased, which is due to the non-ionic structure of peppermint essential oil. In addition, increasing the electrical conductivity

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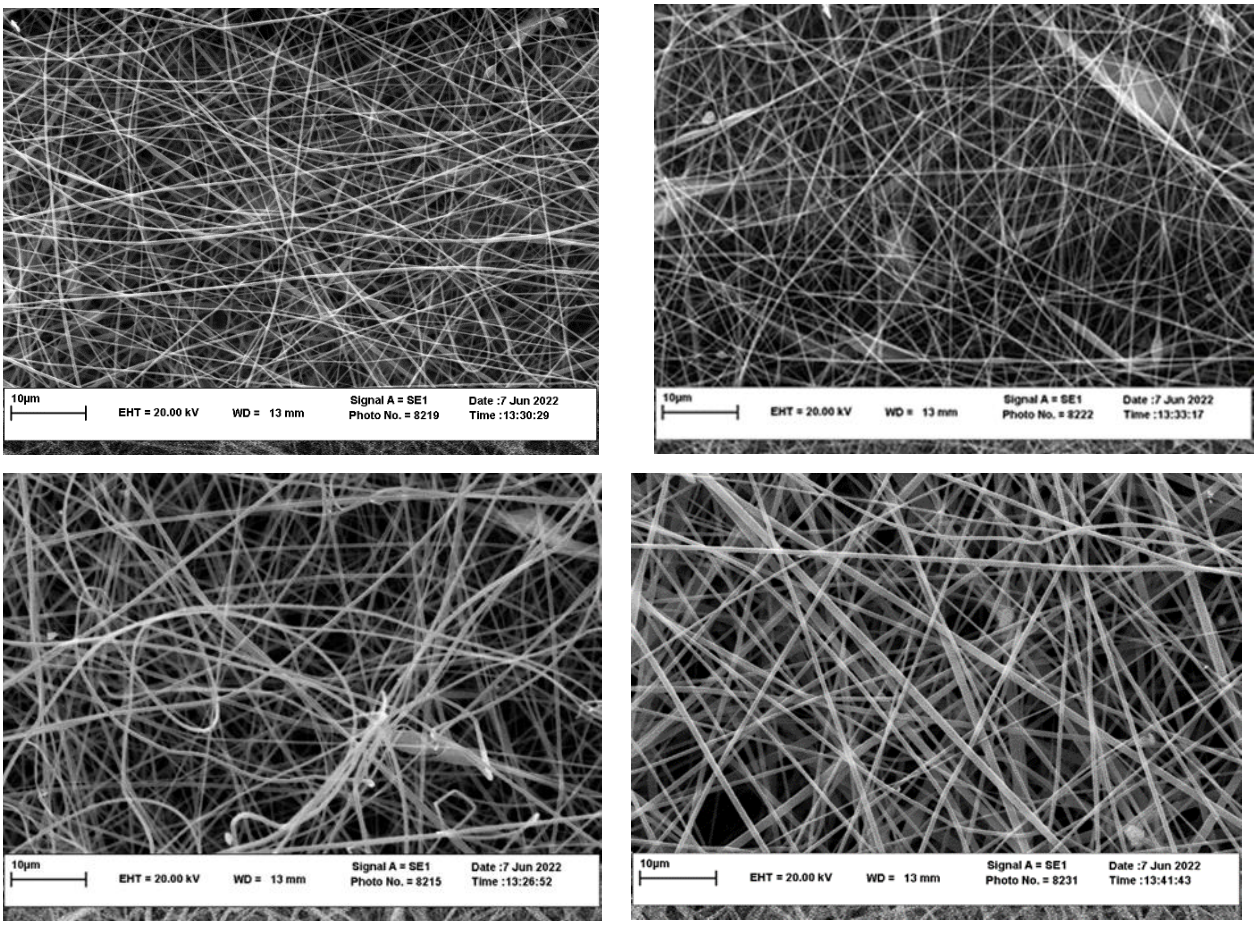
of the solution causes a significant decrease in the diameter of nanofibers [20].

Atomic force microscope (AFM) was used to better describe the surface topography of electrospun fibers. Figure 2 shows the three-dimensional AFM images of fibers obtained from electrospinning containing flaxseed oil and eucalyptus essential oil. AFM images show that all the electrospun zein nanofibers are tubular and no other structure is formed, which confirms the SEM images. The result is similar to the findings of Miri et al. (2016) [13]. AFM images show the tubular shape of electrospun fibers better than SEM images. It seems that tubular fibers are formed when a highly volatile solvent system such as acetic acid is used for electrospinning [13]. It can be concluded that the encapsulation of eucalyptus essential oil and flaxseed oil did not change the morphology of electrospun zein fibers, but the diameter of electrospun fibers increased with increasing the concentration of essential oil. The results are consistent with Tilaghi et al. [17].



(c) (d)

Figure 1 SEM images of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers: (a) 0%, (b) 2.5%, (c) 5%, and (d) 10%.

Table 1 Morpholgy, average diameter and electrical conductivity of flaxseed oil/eucalyptus essential oil/zein electrospun fibers *

Concentration (%)	Morphology	SEM	EC

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0	Free bead		24.1
2.5	Free bead	360	20.3
5	Free bead	480	18.96
10	Free bead	600	15.6

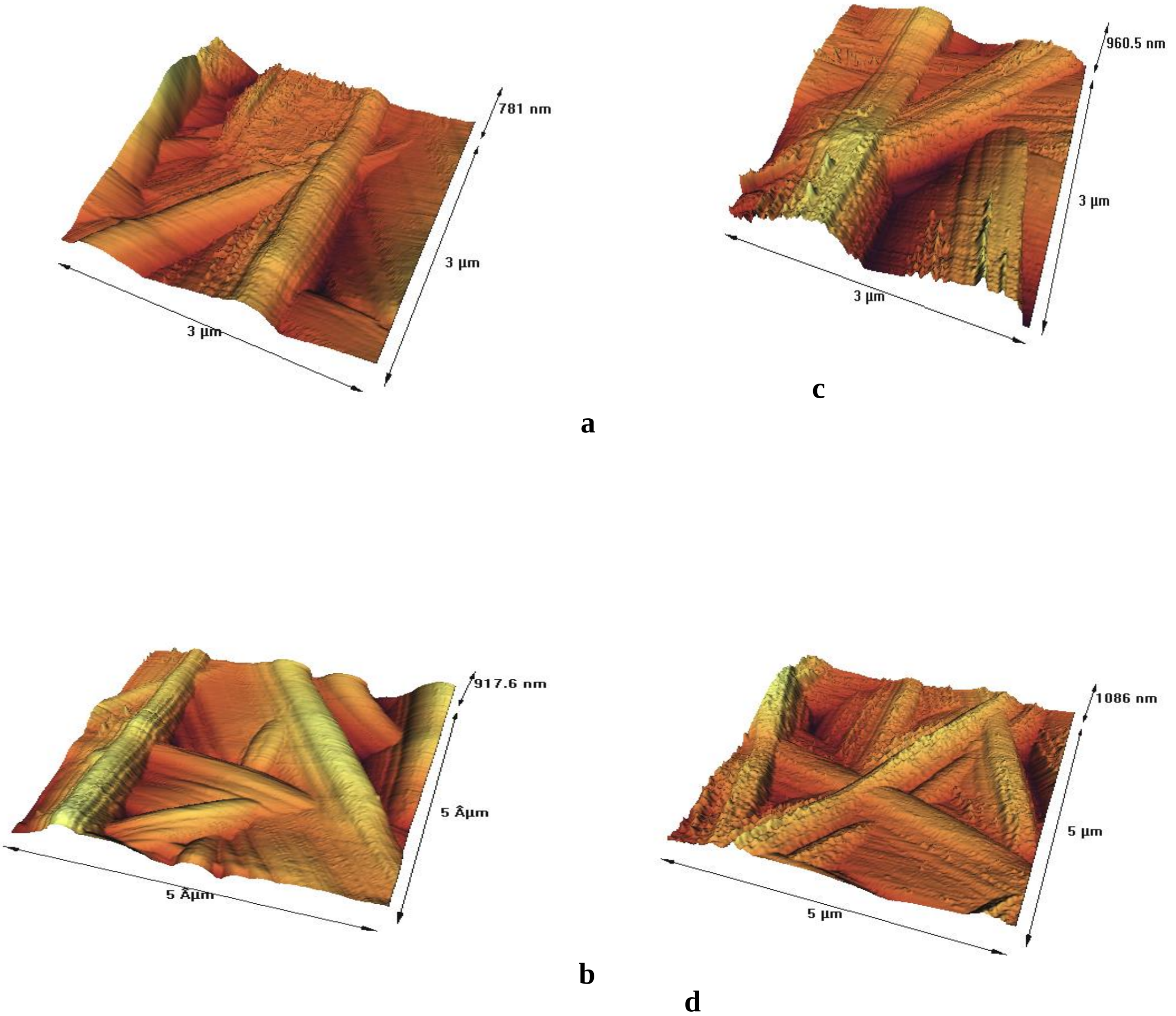

Figure 2 AFM images of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers: (a) 0%, (b) 2.5%, (c) 5%, and (d) 10%.

3-2- X-ray diffraction test (XRD)

As a result of the collision X-rays with the atoms of a substance, electrons move and oscillate, causing the incident X-rays to be

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scattered within the atom. If the phases are the same, scattered X-rays reinforce each other. In amorphous materials, X-rays weaken each other and the scattering phenomenon does not occur. The XRD patterns of zein nanofibers containing 5% flaxseed oil and 0% eucalyptus essential oil show two peaks at angles θ=2.98◦ and θ=2.19◦, respectively, which are related to the distance between the spiral plates. The neighbors in the protein structure (Å, d210) and the distance between the planes of the alpha helix structures (Å, d1 4.5) in the zein configuration. The peaks obtained for zein nanofibers are comparable to the results of Huang et al. [18]. X-ray diffraction patterns of the loaded samples show that the intensity of the peaks has increased compared to the control sample, which indicates the effect of eucalyptus essential oil on d2 and d1. This means that the increase of essential oil causes an increase in molecular aggregations and the content of alpha helix structure, which is probably due to the hydrophobic reaction between flaxseed oil, essential oil and zein. As the X-ray diffraction patterns show, the intensity of the peaks has increased compared to the control sample, which indicates an increase in the intensity of crystallinity compared to the control sample, which could be due to the increase in molecular aggregation [9]. This increase in the intensity of zein peaks by adding amorphous phases to it has also been observed in other studies [9, 19]. In a research, Afshar et al stated that the percentage of crystallization decreases with the formation of protein-polymer complex (chitosan). In fact, with the formation of this complex at each stage, the peak becomes wider and its intensity decreases. In other words, the crystalline structure moves towards the amorphous structure [20].

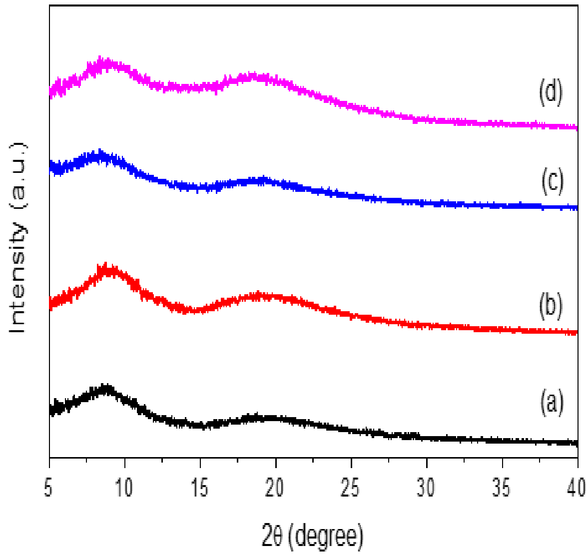

Figure 3 X-ray diffraction patterns of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers: (a) 0%, (b) 2.5%, (c) 5%, and (d) 10%.

3-3- Differential Scanning calorimetry (DSC) Thermal Analysis

The thermal properties of zein nanofibers were investigated by DSC technique. As shown in Figure 4, the DSC of nanofibers showed a peak at a temperature of 80 to 90 °C, which is due to the evaporation of the volatile components of the molecules, which is due to the presence of volatile components in the encapsulated materials. This temperature is lower than the 0% sample. In the range of 250 to 300 °C, peaks are observed which related to the folding of the protein chain. With increasing the concentration of essential oil and the presence of flaxseed oil, the folding temperature of the protein chain of the nanofibers increased, which can be due to the reaction between the carrier and the loaded active compounds. DSC test results show that eucalyptus essential oil and flaxseed oil are successfully encapsulated in zein electrospun fibers.

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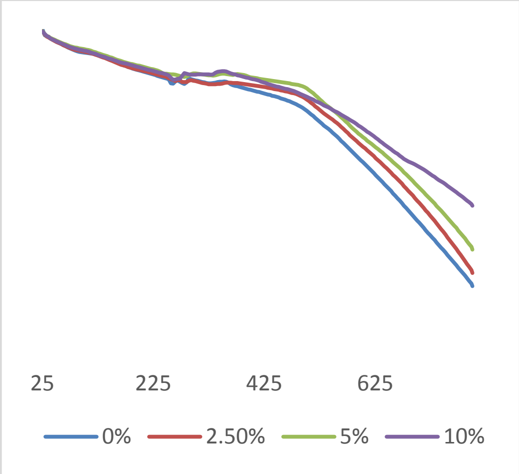
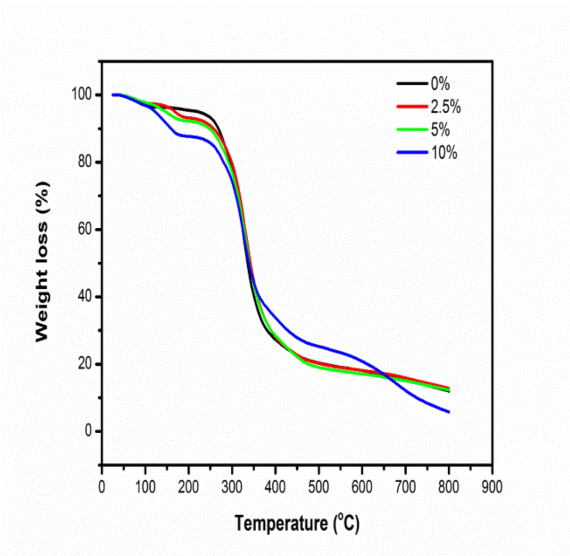


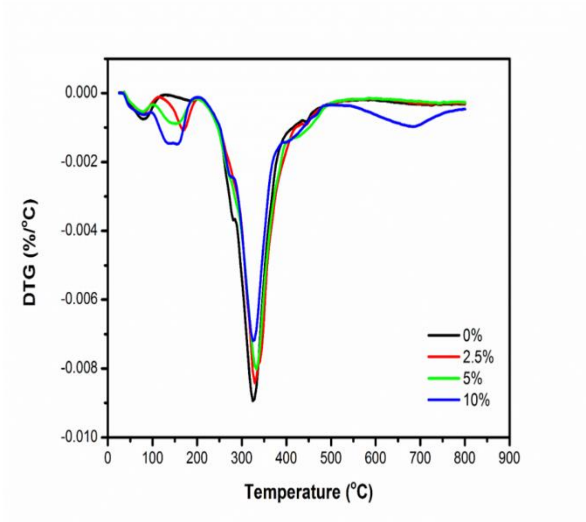
Figure 4 DSC thermograms of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers.

3-4- Thermogravimetric analysis (TGA)

In order to check the thermal properties of the examined samples, TGA test was used and TGA and DTG graphs are shown in Figure 5 and weight loss values related to each stage are given in Table 2.



(a)



(b)

Figure 5 (a) TGA and (b) DTG of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers.

Table 2 Weight loss values related to each step for the examined samples

Sample	First step (%)	Second step (%)	Third step (%)	Remained ash (%)
	25-200 °C	200-500 °C	500-800 °C	800 °C
0%	4.55	83.45		12
2.5%	6.83	80.33		12.84

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5%	7.77	79.85		12.38
10%	12.31	62.49	19.39	5.81

According to Figure 5, the first stage of weight loss in the temperature range of 25 to 200 °C for all samples is a visible endothermic peak, which is related to the evaporation of water absorbed on the surface, structural water and other solvents in the samples [21, 22]. According to Table 2, the highest surface water absorption was related to the 10%, then followed by the 5%. The lowest amount of water in the structure belongs to the 0%. In fact, it seems that with increasing the concentration of essential oil in the structure, higher water absorption has occurred.

The next stage of weight loss in the range of 200 to 800 °C is the thermal decomposition of amino acids with low thermal stability and primary protein structure of zein [23]. In addition, the compounds in flaxseed oil and eucalyptus essential oil are also destroyed in this temperature range [24].

According to Figure 5 and Table 2, it is clear that at this stage, the increasing the concentration caused less weight loss, which can be related to the more crystallininty structure of the samples containing more concentration of essential oil. In fact, the alignment of most of the chains by increasing the percentage of essential oil due to the van der Waals interactions between essential oil compounds, flaxseed oil and zein, has caused that in addition to increasing the crystallinity of the structure, its thermal stability has also increased.

In addition, in the 10%, there is an endothermic peak in the temperature range of 550 to 800 °C, which is not visible in other samples. It is related to the thermal decomposition of complexes of amino acids with flaxseed oil and eucalyptus essential oil [23]. In addition, according to Table 2, the amount of remaining ash in the 2.5% sample was about 0.84% more than the 0% sample, which could be due to the presence of essential oil compounds in the ash. However, it is interesting to note that with a further increase in the concentration, the amount of remaining ash has decreased and in the 10%, the amount of remaining ash has decreased drastically and reached about 5.8%. This could mean that the essential oil and flaxseed oil compounds have been able to intensify the thermal decomposition of the zein structure by creating an interaction with the zein structure, like a catalyst.

3-5- Encapsulation efficiency

In encapsulation, efficiency is an important determining factor that determines how effectively a capsule can preserve active ingredients [9]. The results of encapsulation efficiency were 68 ± 1.5%, 72 ± 2% and 78 ± 5% for electrospun fibers. The results were similar to the results of the studies of Hosseini et al. (2021) and Ghasemi et al. (2022) [9, 11].

3-6- Antimicrobial activity results of fibers containing flaxseed oil and eucalyptus essential oil

Antibacterial activity of nanofiber membrane plays an important role in its food applications. Therefore, the antibacterial activity of zein nanofiber membrane containing flaxseed oil and eucalyptus essential oil was evaluated against *Staphylococcus aureus* and *Escherichia coli*. The results of the antimicrobial activity of nanofibers against bacteria can be seen in Table 5. The nanofiber membrane containing flaxseed oil showed strong antibacterial activity against *Staphylococcus aureus* (17 mm) and *Escherichia coli* (16 mm). Many reports reported the strong antimicrobial ability of flaxseed oil [2, 25]. The antimicrobial activity of flaxseed oil has been attributed to its bioactive components, especially the presence of omega-3 fatty acids, alpha-linolenic acid (ALA) and lignans in flaxseed oil, such as secoisolariciresinol diglucoside [3, 25, 26]. The results are similar to the results of Mohimeni et al. [27]. Nanofiber membranes containing flaxseed oil and eucalyptus essential oil showed strong antibacterial activity with inhibition zone diameters of 19, 19.5, 21.4 mm against *Staphylococcus aureus* and 18, 19, 21 mm against *Escherichia coli*. As the results show, adding flaxseed oil to eucalyptus essential oil enhances its antimicrobial properties. The presence of some compounds such as alpha-pinene in eucalyptus essential oil or all compounds can contribute to the antimicrobial activity of the essential oil. In general, essential oils can have

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antimicrobial activity against microorganisms by destroying the cell wall, reacting with membrane proteins, increasing the permeability of the cell wall, and leaking the cell contents [11].

Table 3 Inhibition zone diameter of flaxseed oil/ eucalyptus essential oil/zein electrospun fibers against *S. aureus* and *E. coli* *

	S. aureus	*E. coli*
0	0^{c}	0^{c}
2.5	19 ± 0.8^{b}	18 ± 1.3^{b}
5	19.5 ± 1^{b}	19 ± 0.9^{b}
10	21.4 ± 1.3^{a}	21 ± 1.4^{a}

* Different letters (a, b, c, d) within the same column represent significant statistically differences ($p < 0.05$) between 0, 2.5, 5, and 10% concentrations. (n=3).

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4- Conclusions

According to the results obtained from the SEM images, all the zein electrospun fibers are uniform and free of beads. With increasing the content of the essential oil, the diameter of the fibers also increased, that can be due to the decreasing of electrical conductivity. AFM images showed that all the electrospun fibers are tubular. The XRD patterns obtained from the electrospun fibers had two diffraction halos and indicated that the intensity of the peaks of the treated samples increased compared to the control sample. The results obtained from thermal analysis showed that the thermal stability of zein nanofibers carrying essential oil and flaxseed oil increased. According to the research conducted on two bacteria, *Escherichia coli* and *Staphylococcus aureus*, it was concluded that the electrospun fibers of zein containing flaxseed oil and eucalyptus essential oil had antimicrobial properties. The results showed that adding flaxseed oil to eucalyptus essential oil strengthened the antimicrobial properties of nanofibers. It is resulted that flaxseed oil/eucalyptus essential oil/zein nanofibers can be used to control the microbial agents.

5- Acknowledgement

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مقاله علمی_پژوهشی

انکپسولاسیون اسانس روغن دانه کتان و اکالیپتوس با استفاده از الکتروریسی

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چکیده

استفاده از گیاهان دارویی در مواد غذایی دارای سابقه طولانی است. روغن دانه کتان و اسانس اکالیپتوس به¬علت دارا بودن خواص زیستی بسیار مورد توجه قرار دارد. در این تحقیق، هدف بررسی خاصیت ضد میکروبی نانوالیاف زئین حاوی روغن دانه کتان و اسانس اکالیپتوس برای کنترل باکتری‌های *استافیلوکوکوس اورئوس* و *اشرشیا کلی* در محیط آزمایشگاهی می‌باشد. بدین منظور روغن دانه کتان و اسانس اکالیپتوس در محلول زئین مخلوط گردید. سپس به‌وسیله‌ی دستگاه الکتروریسی به نانوالیاف تبدیل شد. جهت بررسی ویژگی‌ها و خاصیت ضد میکروبی نانوالیاف حاصل، آزمایشاتی شامل میکروسکوپ الکترونی روبشی (SEM)، تعیین قطر الیاف با نرم‌افزار Image J، میکروسکوپ نیرو اتمی (AFM)، پراش اشعه ایکس (XRD)، آنالیز حرارتی گرماسنجی وزنی (TGA)، راندمان انکپسولاسیون به روش اسپکتروفتومتری و بررسی خاصیت ضد میکروبی نانوالیاف تولیدی به روش دیسک دیفیوژن انجام شد. نتایج SEM نشان داد که مورفولوژی الیاف الکتروریسی شده، یکنواخت و فاقد گویچه بودند. تصاویر AFM بیانگر تصاویر سه بعدی و لوله‌ای شکل الیاف حاصل از الکتروریسی زئین/روغن دانه کتان/اسانس اکالیپتوس می‌باشد. الگوی پراش اشعه ایکس نشان دهنده افزایش شدت بلورینگی تیمارها نسبت به نمونه شاهد است. بر اساس نتایج آنالیز حرارتی، روغن دانه کتان و اکالیپتوس و باعث افزایش پایداری حرارتی نانوالیاف زئین شده است. نتایج نشان داد که افزودن روغن دانه کتان به اسانس اکالیپتوس باعث تقویت خاصیت ضدمیکروبی نانوالیاف شد. با توجه به نتایج تحقیق حاضر، می‌توان الیاف زئین/روغن دانه کتان/اسانس اکالیپتوس را به عنوان ضد میکروب‌های طبیعی استفاده نمود.

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