Clinical Cancer Investigation Journal-ISSN: 2278-0513

Evaluation of Mucoadhesive Films containing Electrospun Gellan Gum Nanofibers for Topical Use of Aphthous Stomatitis

Abstract

Oral inflammation (Stomatitis) is a common disease of the mouth all over the world. The role of oxidative stress in the occurrence of these lesions has been suggested. The plant-derived biological compounds are an important branch of the medical treatment of diseases. The essential oil has antibacterial with antioxidant properties. In the current research, we want to design a system for the treatment of Aphthous Stomatitis using the Mucoadhesive property of polysaccharides in the form of nanofibers along with the loading of essential oil.

Polyvinyl alcohol nanofibers containing Gellan gum and essential oil were prepared by Electrospinning technique. They were cross-linked using glutaraldehyde to increase the stability of the fibers. The average size of nanofibers was measured by Scanning Electron Microscope. The release extract from the fibers was investigated in vitro. Finally, the antibacterial effect of the fibers was determined using the Inhibition zone

The nanofibers had an average size of 641 nm and a relatively uniform structure, and the release of the extract continued for more than 8 hours. In addition, the inhibition zone of Staphylococcus aureus bacteria was measured in this nanofiber of 15 mm. According to the results of this study, these nanofibers will be an effective tool for stomatitis treatment.

Keywords: *Stomatitis, Nanofiber, Polyvinyl alcohol, Gellan gum, Myrtus communis*

Amir norouzi*
Masters Nanobiomimetic, Tehran Azad University of Medical Sciences
Amirnoroozi37@yahoo.com

Sara esnaashari
Medical Nanotechnology P.H.D medical University Tehran

Negar motakef kazemi
P.H.D Nanotechnology - Nanomaterials Tehran Azad University of Medical Sciences

Amirhossein baradaran khaniyan
Associate Accounting Azad University of north Tehran
Amirnoroozi37@yahoo.com

Introduction

Oral inflammation (stomatitis) is a reversible deterioration and is one of the most common oral diseases worldwide. The prevalence of ulcers varies between 5% and 66%. Oral lesions appear more often in the second decade of life and between the ages of 20-30 [1]. The exact cause of these lesions is not known exactly. However recent studies indicate that free radicals may play a role in the etiology of this disease by causing oxidative stress. It seems that oxidative stress is effective in the occurrence of this disease since the role of oxidative stress in the occurrence of inflammatory conditions has been raised and considering the inflammatory nature of recurrent aphthous stomatitis [1]. Now, there is no definitive cure for the rapid treatment of this disease, and the available treatments are mostly Supportive care.

Recently, the use of herbal medicines has received much attention. Because in many myrtles they have less toxicity in addition to lower price [2]. Myrtus communis is a species of flowering plant in the myrtle family Myrtaceae. It is an evergreen shrub native to southern Europe, and North Africa. This plant is found in dry areas of Iran including Kerman, Khorasan, and Kazerun [3]. It is a perennial plant that can grow and be harvested for consecutive years if its leaves and branches are harvested properly. The Myrtus communis has a high sanctity in some regions and is known as the plant around fountains [4]. This plant has evergreen, stable, simple, pointed, leathery, dark green, and very fragrant shrubs. White and fragrant flowers bloom on the plant from May to July. Each flower has five petals and numerous stamens, which eventually develop into fruit after shedding the petals. There are bumps on the stem called "Galls". Like the leaves and fruits of the plant, stem scab has medicinal properties [5].

The Myrtus communis contains various antioxidants and flavonoid compounds such as Myristin, Quercetin, Catechin, Citric and Malic acid, Linalool, Pinene, Tannin, and Vitamin C. This plant. The leaves of this plant have the highest amount of effective medicinal substances of the plant, which have properties such as antibacterial, antifungal, and antiviral properties. Its fruit relieves the pain of the digestive system, tonics the stomach, and gives strength [6]. This plant is used in the treatment of diseases such as diabetes, colds, skin inflammation, superficial wounds, and diarrhea. The plant or essential oil is widely used in Aromatherapy. The essential oil of this plant is used to treat diseases such as bronchitis and asthma. It also cleans the respiratory tract and irritates the nose or throat. Traditional medicine practitioners believe that Myrtus communis helps to reduce white hair in addition to strengthening hair roots [7]. Since the essential oil of this medicinal plant has been shown to have anti-viral and anti-bacterial qualities, numerous medications derived from it have been developed and distributed to treat herpes and acne [8]. Many studies have been conducted to prove its antibacterial

properties on acne biofilms [9] and use it in medicinal and health formulations [10].

In 2010, Babaei et al. [11] investigated the clinical effect of a paste containing myrtle on the treatment of reversible mouth ulcers. In this study, which was conducted as a double-blind controlled clinical trial, 45 patients were randomly treated with a paste containing myrtle or a placebo. Patients were examined after two 6-day treatment periods 4 times a day. It was found that there was a significant decrease in wound size ($p<0.001$), pain intensity ($p<0.05$), and the amount of erythema and exudate ($p<0.001$). These results were obtained while no side effects were recorded in this study.

In another study, the effects of myrtle oil and topical clindamycin on acne vulgaris were compared. This study lasted for 12 weeks with topical medication twice a day. The results showed that inflammation and skin fat in group one had a significant change compared to group two, while no significant change was observed between the side effects of these two groups [12].

In addition, the myrtle oil emulsion was also prepared in a study in 2018. The size of the particles of this emulsion was 30 nm was stable at room temperature and 4 degrees and also had a clear appearance. The antibacterial property of this emulsion was proven by its effect on Staphylococcus aureus, Staphylococcus aureus, and Escherichia coli [13].

In another study (2022), the myrtle essential oil was loaded into combined nanofibers of gelatin and Polycaprolactone for treating vaginal infections. Nanofibers were prepared using Electrospinning and extract in the structure was confirmed by FTIR. Antibacterial and antifungal studies of these fibers showed that this structure was very effective against Trichomonas bacteria and Candida fungus and it seems to be a suitable alternative for treating vaginal infections in the future [14].

At the moment, there is a lot of research being done on the coupling of hydrophilic polymers and polysaccharides to create fibers for coatings and drug delivery structures to the skin[15]. Polyvinyl alcohol has been used in many studies due to its biocompatibility and hydrophilicity [16]. Polyvinyl alcohol is decomposed using biological microorganisms and is very soluble in water due to its greater crystallinity and is used to produce many polymer end products such as surgical sutures, food packaging, etc. It is a very common biopolymer that can be chemically attached to the surface of materials and is easily retained on the surface of water. In addition, other applications of this polymer are in biomedical fields such as contact lenses, heart surgery, drug delivery, and dressing manufacturing [17, 18]. Recently, the antibacterial effect of extracts has been investigated in many studies in modern drug delivery systems [19]. In another study (2020), cellulose acetate nanofibers were made and lemon essential oil was loaded into them. Nanofibers were prepared by electrospinning method and scanning electron microscope images showed that they had a diameter of 440-500 nm. The results of antimicrobial tests showed that these fibers were able to destroy 100% of Escherichia coli and Staphylococcus aureus. It seems that these fibers can be a suitable option for use in wound dressings [19].

In this research, the production of fibers containing myrtle essential oil was developed, considering the special properties that have been proven from the Myrtus communis and its oil.

Methods and materials

Preparation of solutions needed for electrospinning

First, the combination of polyvinyl alcohol 10% by weight by volume and gellan gum in concentrations of 1%, 1.5%, and 2% by weight by volume in ratios of 3:1 was investigated to prepare nanofibers. Since gellan gum is a very viscous substance, its 2% solution did not come out of the nozzle due to its high viscosity, and this problem was not solved even by increasing the temperature to 40 °C. In another example, the concentration of 1% of gelatin in combination with polyvinyl alcohol with the same ratio as before was studied, but the obtained fibers did not have a proper morphology, and droplets and grains were abundantly found among the fibers. But the concentration of 1.5% by weight and volume of gellan was the best alternative for fiber production and electrospinning was performed as follows:

Polymer solutions of polyvinyl alcohol 10% by weight and volume and gellan gum 1.5% by weight and volume were prepared in deionized water. Erlenmeyer flask was used in the required volume to prepare both solutions. First, a proper amount of deionized water was poured into the Erlenmeyer flask, then polyvinyl alcohol and gellan gum polymers were slowly added to the Erlenmeyer flask and placed on the stirrer at 500 rpm. The volume of the solutions reached the required amount after complete dissolution by adding deionized water. To prepare the fibers containing Myrtle extract, an aqueous solution of this extract with a concentration of 200 mg/ml was prepared. Polyvinyl alcohol and gellan gum solutions were mixed at a ratio of 3:1 to perform electrospinning of nanofibers. It is necessary to place the gellan gum solution under a stirrer at a temperature of 40 °C because it becomes gelatinous and solid at ambient temperature. Then the final solution was entered in a 2 ml syringe without a washer and then placed in the electrospinning machine and the nanofiber manufacturing process was performed in the following conditions. To make nanofibers containing extract, all the steps are the same as the solution of nanofibers without extract, except that 200 mg of extract is added to the solution and stirred for 30 minutes at 40°C and 300 rpm until a uniform solution is obtained.

Electrospinning process

It is necessary to optimize the fiber manufacturing process. Gellan gum alone cannot form fibers and is thrown from the nozzle as a droplet. Therefore, we used polyvinyl alcohol polymer as the main substrate for the formation of fibers to obtain nanofibers with a small and uniform diameter. To obtain fibers, 2 ml of the precursor solution was electrospinning at a rate of 0.5 ml/min for 3-4 hours.

For this purpose, the percentages and parameters researched and proven in previous studies were used. The electrospinning conditions of nanofibers with and without myrtle extract that led to the formation of nanofibers with suitable diameters and without droplets are summarized (Table 1).

Table 1: Factors affecting electrospinning

Parameters	Nanofibers with extract	Nanofibers without extract
Distance	18 cm	18 cm
Temperature	30-40 °C	30-40 °C
Voltage	22 kV	22 kV
The exit acceleration of the solution	0.7 mL/hr	0.5 mL/hr
The rotation speed of the collecting plate	150 rpm	150 rpm

Fiber cross-linking

One of the important factors in using nanofibers is their strength. For this purpose, it is necessary to stabilize the nanofibers and form network links and transverse links between them. In this study, polyvinyl alcohol polymer, which is the main structure of these fibers, is a completely hydrophilic polymer that dissolves quickly and its structure is destroyed when exposed to a wet environment. Therefore, considering that the purpose of using these fibers is inside the body, it is necessary to increase their stability in the wet environment, and this is possible by forming cross-links from various materials such as glutaraldehyde, formaldehyde, and acetaldehyde for cross-linking nanofibrous structures.

Sterilization of nanofibers

Samples of gridded felts were prepared in different plots. Then the prepared samples are placed in a sterile plate without a lid and placed in the sterilization machine for 30 minutes under UV light to disinfection.

Bacterial culture

Escherichia coli (PTTC:1338, ATTC:10536) and Staphylococcus aureus bacteria (PTTC:1431, ATTC:25923) respectively an indicator of Gram-negative and Gram-positive pathogenic bacteria from Iran's Fungi and Bacteria Collection Center (Iranian Research Organization for Science and Technology) was prepared as active cultivation. We get active from bacteria.

by transferring 2 to 4 colonies of E. coli to Nutrient Broth nutrient medium and 2 to 4 colonies of Staphylococcus aureus bacteria to Tryptic Soy Broth nutrient medium and placing these closed containers in a shaker incubator for 24 hours at 37 °C in the broth environment.

Investigating the antibacterial effect of nanofibers on agar

In total, 10 microliters of bacterial suspension were used on agar culture medium (for Staphylococcus aureus from Tryptic Soy Agar culture medium and for E. coli from Nutrient Agar culture medium) to investigate the antibacterial effect of nanofibers.

Findings

Preparation of Nanofibers

First, a homogeneous solution of polyvinyl alcohol, gellan, and myrtle essential oil was prepared with the proportions stated in the previous chapter. Electrospinning of polyvinyl alcohol polymer solution and gellan gum with and without myrtle extract was performed under the following conditions. The distance between the tip of the needle and the plate of the collector is 18 cm, the voltage is 22 kV, the temperature is 30-40 °C, the rotation speed of the collector is 150 rpm, and the exit speed of the solution from the nozzle is 0.5-0.7 ml per hour.

In this study, 1. The optimal conditions include several myrtles. The polymer should be completely dissolved in its solvent and a uniform solution should be obtained. 2. A droplet of polymer (solution) should appear at the end of the needle. 3. Create a Taylor cone. 4. The polymer jet should appear towards the collector. 5. A white substance will appear on the collector. 6. Electron microscope photographs show fibers without nodes. First, the various parameters of the electrospinning machine were assessed. The results showed that due to the high viscosity of the solution, regular fibers are not formed if the distance between the nozzle and the collector is less than 18 cm, and in many parts of the fibers, grains, and drops of the solution can be seen.

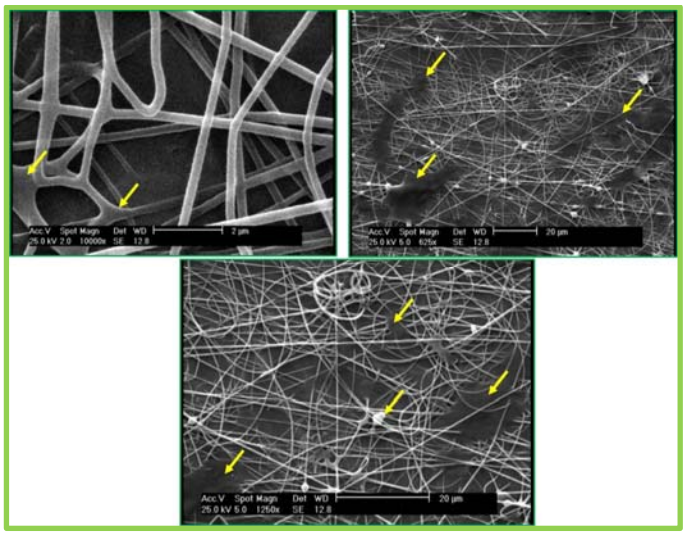
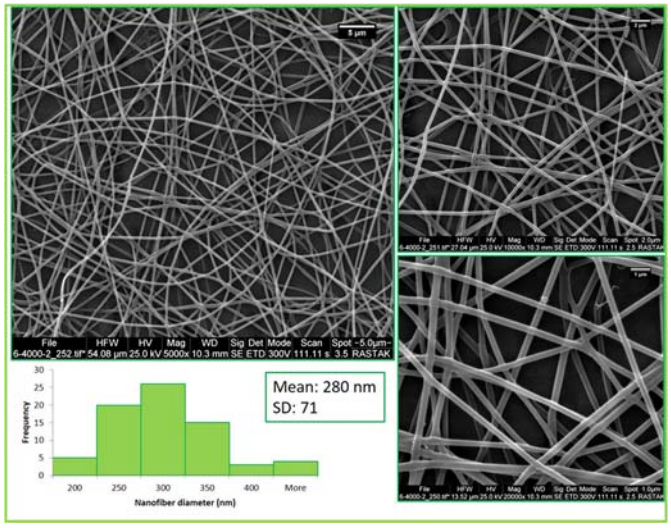
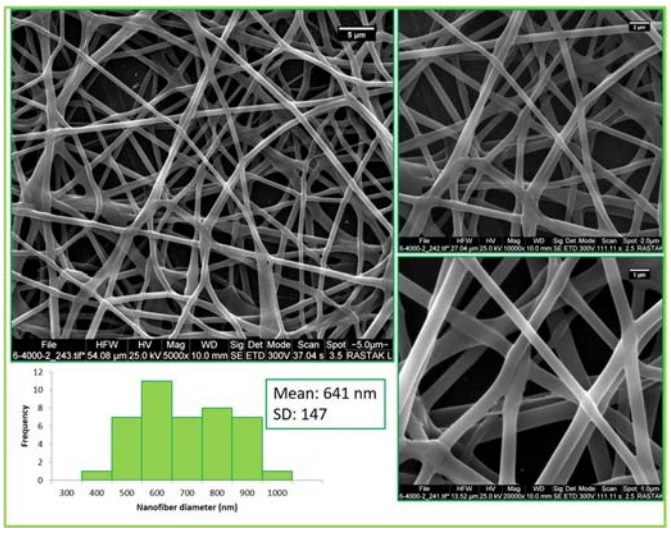


Figure 1: Scanning electron microscope image of fibers at a distance of less than 18 cm

In addition, the voltage cannot overcome the surface tension of the solution if the injection speed of the solution is high and the solution is thrown in droplet form in many parts of the felt. Ultimately, by adjusting various parameters, we were able to produce fibers with the right size and structure. After preparing the necessary fibers, the final felt from each group was subjected to Transmission Electron Microscope imaging. Then, at least 20 fibers from each group were measured to determine the average fiber diameter. Figures 3 and 4 show the electron microscope image of nanofibers without essential oil and with essential oil, respectively. Nanofibers without essential oil and with essential oil had an average size of 280 nm and 641 nm and a size distribution of 500-200 nm and 1000-400 nm, respectively (Figure 2).



A



B

Figure 2: A)Scanning electron microscope image of nanofibers without extract and fiber size distribution
B) Scanning electron microscope image of nanofibers with extract and fiber size distribution

Nanofiber cross-linking

Glutaraldehyde was used as a cross-linking agent to improve and enhance the mechanical properties of nanofibers. The average diameter and morphology of nanofibers of both groups with and without extracts were investigated after 24 hours of exposure to glutaraldehyde vapor. The scanning electron microscope image of nanofibers without reticulated extract showed that the nanofibers had an increase in diameter their average diameter reached 415 nm, and their size distribution was also 300-700 nm. The average size of nanofibers with extract also increased and reached 805 nm and the size distribution was 1400-500 nm by adding glutaraldehyde.

Definition of the standard curve of the extract

The extract prepared in a deionized water medium was used to prepare the standard curve of the extract concentration concerning the absorbance of different concentrations and their absorbance was measured with a UV-Vis spectroscopic device at a wavelength of 264 nm. Then the equation of absorption to concentration ratio was obtained and the R^2 value of the standard curve was calculated. Let's that that $R^2 = 0.9883$, it indicates sufficient accuracy and repeatability of the standard curve.

Determine of extract loading percentage in nanofibers

A certain amount of fibers was dispersed in deionized water and completely dissolved by a stirrer. Then its absorbance was read using UV-VIS spectroscopy at 264 nm wavelength. The loading percentage was calculated according to the formula stated in the previous chapter. The results of this test on 3

nanofiber felts at different times of synthesis showed that the average loading percentage of the extract in these fibers was 25±8%.

Determination of extract release curve from nanofibers

A 100 ml bottle was used to check the release of the extract and felt fibers were completely placed in the bottle. Figure (3) shows that the release of the extract from the nanofibers was 2-step. The orange curve shows the release of the extract from non-networked fibers and the blue curve shows the release of the extract from the networked fibers. This figure shows that the release in non-reticulated fibers continued up to 6 hours and reached 100% in the sixth hour. In networked fibers, the release was slower and in the eighth hour, 70% of the extract was released.

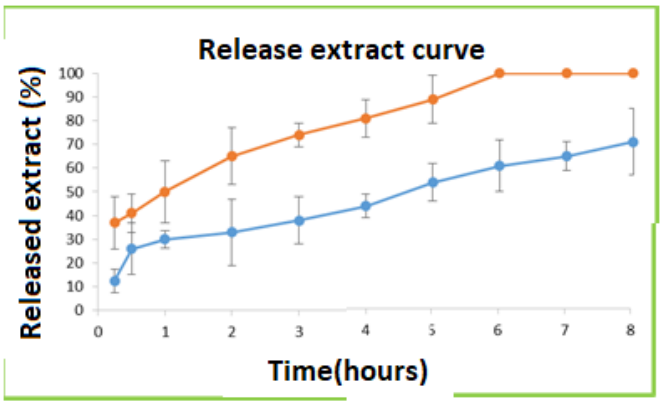


Figure 3: Release extract curve in vitro

Investigating chemical bonds established in nanofibers using ATR FTIR

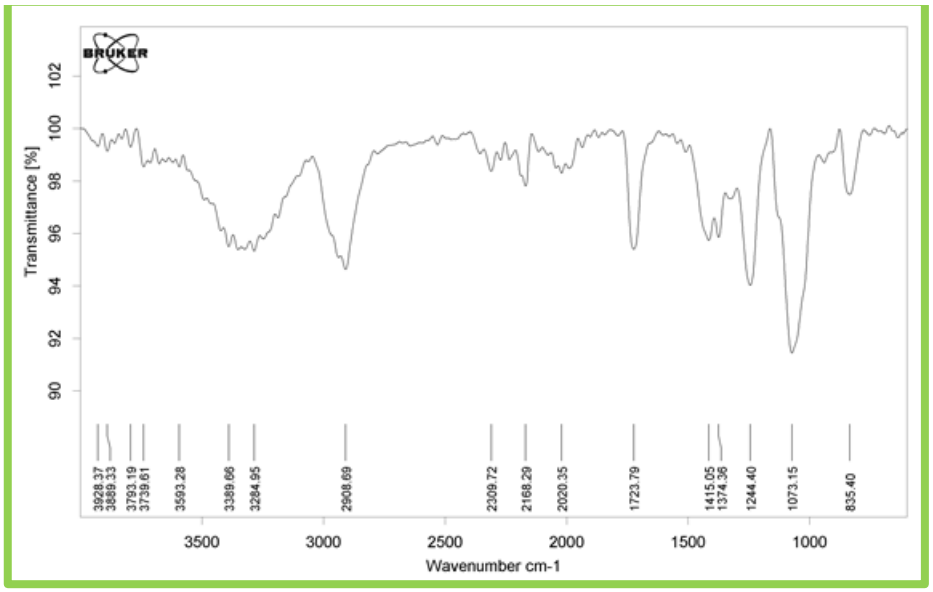
In the structure of polyvinyl alcohol, there are C-O, C=O, CH, OH, and C-C bonds. A sharp peak at the wavelength of 2908, which represents the OH group, is observed in the spectrum of polyvinyl alcohol. In the spectrum of polyvinyl alcohol at about 1073 cm-1 and 11723 cm-1, it has been proved that they are related to C-O and C=O stretching, respectively. Polysaccharides have a significant number of hydroxyl groups that show a broad absorption band in the region above 3000 cm-1[20]. In the spectrum of gellan, a wide and rounded band can be seen around 3281 cm-1, which indicates that this polymer is a polysaccharide. In addition, a weak peak can be seen at 2887 cm-1, which indicates the stretching vibration of the C-H bond. The 1600 cm-1 peak also indicates the O-H bending state related to the connected water molecules. Weak peaks around the 1244 to 1415 region indicate variable C-H bond angles [21]. The weak peaks observed around 1374 cm-1 can confirm this.

The region cm^{-1} 910 to 1300 is called the Fingerprint region, and this region is the result of the combination of vibration interaction that causes a specific fingerprint for each specific combination. The good compatibility of the spectra of the two compounds in the range of all frequencies, especially in the fingerprint region, strongly indicates the same molecular structure [22]. Each specific polysaccharide also has a specific band in the region of 1000-1200 cm-1, which is related to the stretching state of carbohydrate rings and side groups -C-O-C, C-OH, and C-H. Therefore, the sharp peak at 1073 cm^{-1} indicates the saccharide structure of gellan.

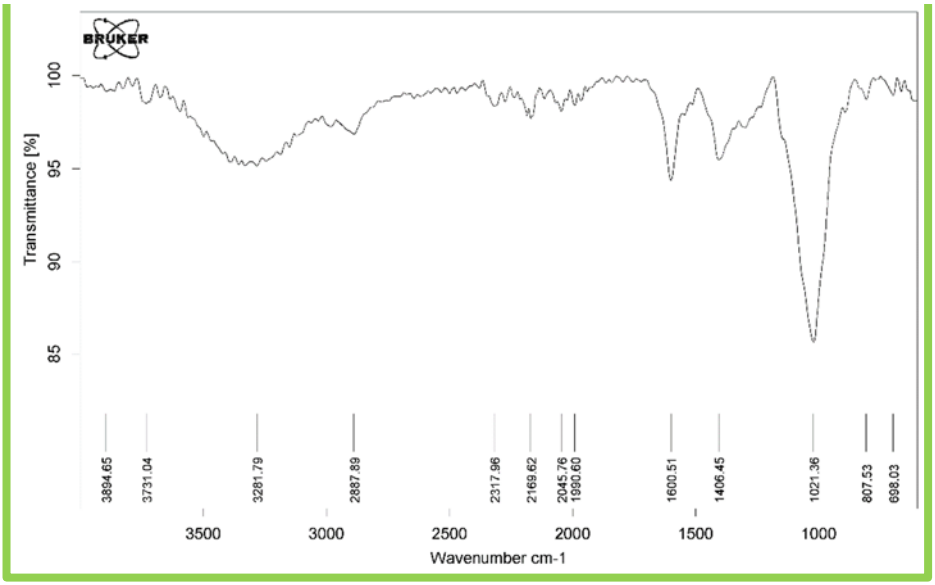
Peaks 1032, 1199, 1339, 1603, 3284, and 2913 cm-1 are related to the structure of the extract. One of the main peaks of the myrtle extract is the peak related to the O-H stretching bond in the range of 3000-3700 cm-1, which in our study had a broad peak at 3284 cm^{-1}.

In addition, there was a sharp peak at 2913 cm^{-1}, which is due to the vibration of the C-H group bond. In our study, a peak was seen in the range of 1339, which is due to the bending of methyl bonds. In our study, polyvinyl alcohol and gellan gum showed peaks in the range of 2400-3300, which is due to a hydroxyl group in the structure. In addition, a small peak of 1500 was observed in the combination of polyvinyl alcohol and gellan gum. There are peaks at 3200 to 3400 due to the hydroxyl groups that were confirmed in the structure of polyvinyl alcohol, extract, gellan gum, and nanofibers [23].

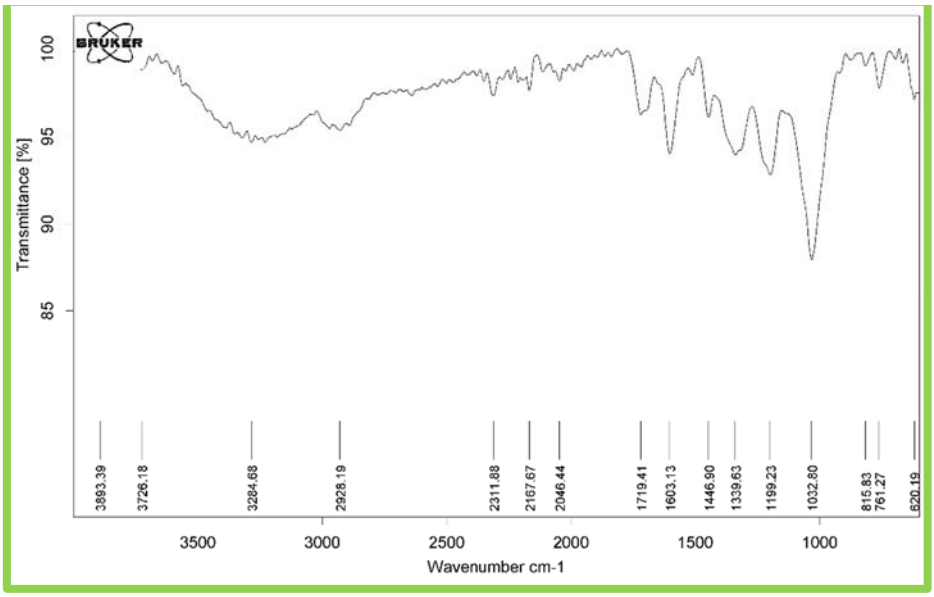
The spectrum of nanofibers loaded with the extract shows all three characteristic peaks of polyvinyl alcohol, gellan gum, and the characteristic bands related to the presence of the extract. (Figure 4)


BRUKER
Transmittance [%]
102
100
98
96
94
92
90
3928.37
3889.33
3793.19
3739.61
3593.28
3369.66
3284.95
2908.69
2309.72
2168.29
2020.35
1723.79
1415.05
1374.36
1244.40
1073.15
835.40
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

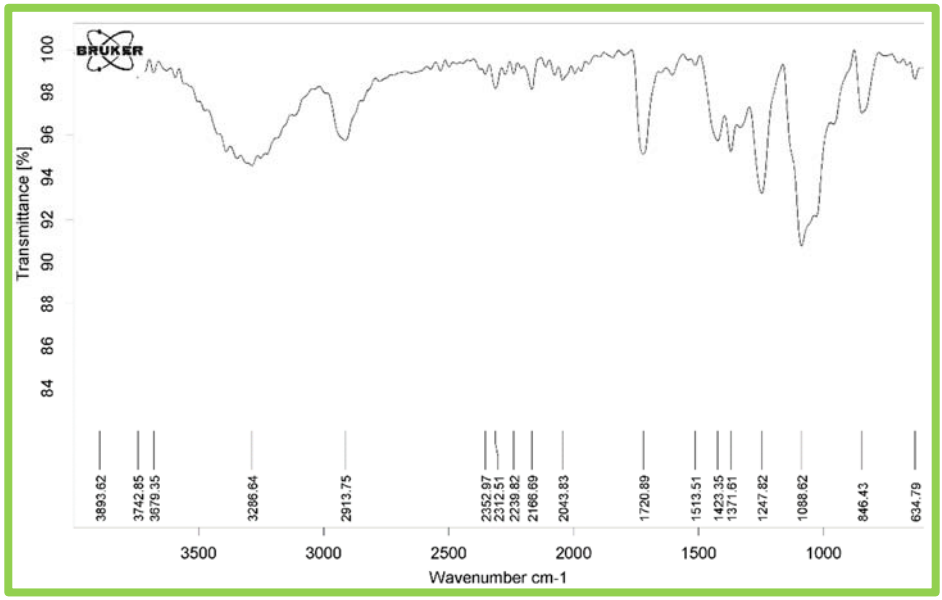
A


BRUKER
Transmittance [%]
100
95
90
85
3894.65
3731.04
3281.79
2887.89
2317.96
2169.62
2045.76
1990.60
1600.51
1406.45
1021.36
807.53
698.03
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

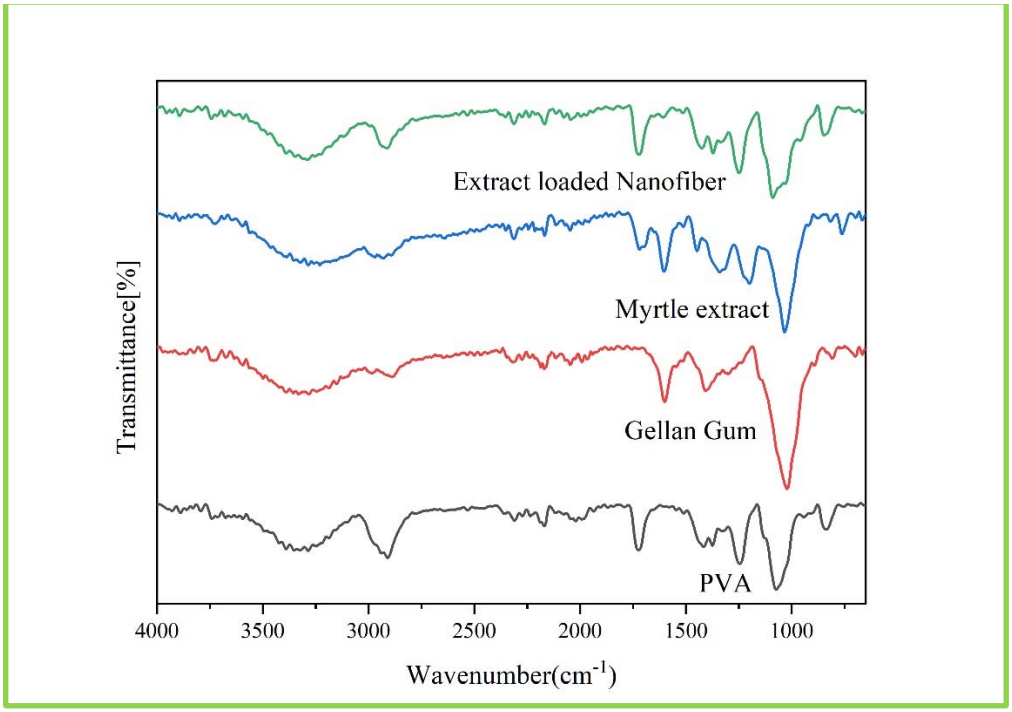
B


BRUKER
Transmittance [%]
100
95
90
85
3893.39
3726.18
3284.68
2928.19
2311.88
2167.67
2046.44
1719.41
1603.13
1446.90
1339.63
1199.23
1032.80
815.83
761.27
620.19
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

C


BRUKER
Transmittance [%]
100
98
96
94
92
90
88
86
84
3893.62
3742.85
3679.35
3286.64
2913.75
2352.97
2312.51
2238.82
2166.69
2043.83
1720.89
1513.51
1423.35
1371.61
1247.82
1088.62
846.43
634.79
3500
3000
2500
2000
1500
1000
Wavenumber cm-1

D

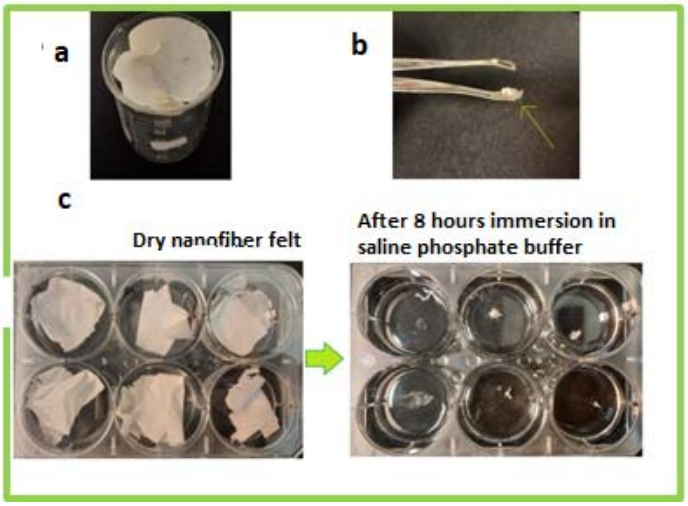


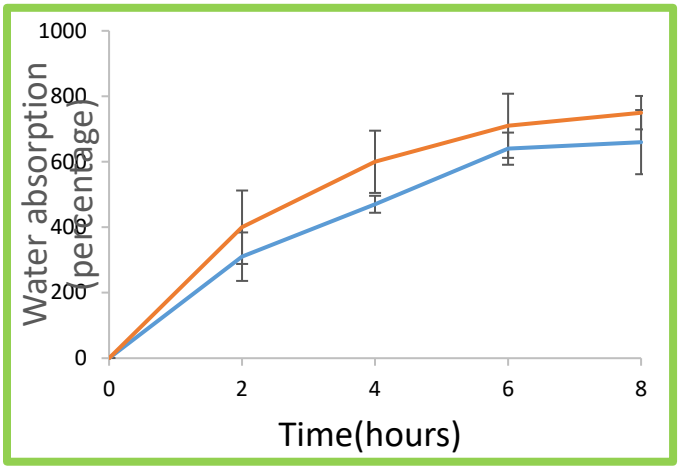
E

Figure 4:
A) FTIR spectrum of polyvinyl alcohol polymer
B) FTIR spectrum of gellan gum
C)FTIR spectrum of myrtle extract
D)ATR spectrum of polyvinyl alcohol nanofibers and gellan gum-containing extract
E)Cumulative chart of ATR

Investigating the moisture absorption capacity of nanofibers

Water absorption was measured during 2, 4, 6, and 8 hours after placing the samples in the saline phosphate buffer. Considering that the non-cross-linked samples were not very stable in the water environment and dissolved quickly, only the cross-linked samples were used for this test. Filter paper was used to weigh and dehydrate the nanofibers (Figure 5a). Figure (5b, c) shows the water absorption of nanofibers after 2 hours and 8 hours. Figure 5e shows that the nanofibers began to absorb water quickly due to the hydrophilicity of polyvinyl alcohol, and the percentage of water absorption in nanofibers without extract was 310±74, 470±26, 640±49, and 660±98% after 2, 4, 6 and 8 hours respectively. Water absorption in fibers with extract was slightly faster, which was 400±112, 600±95, 710±49 and 750±98% after 2, 4, 6 and 8 hours, respectively.





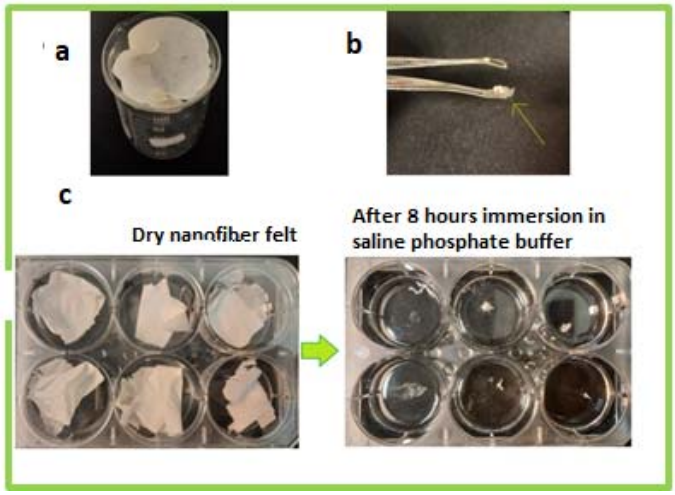
e

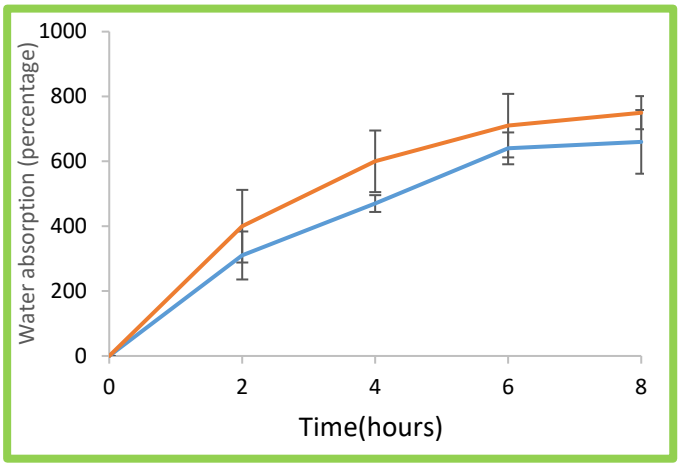
Figure 5. a) use of filter paper to dehydrate fiber felt, b) gelation of nanofibers after 2 hours of being in a wet environment, and c) structure change and dissolution of nanofibers after 8 hours

e) Water absorption percentage of nanofibers (orange: nanofibers with extract, blue: nanofibers without extract)

Investigating the moisture absorption capacity of nanofibers

Water absorption was measured during 2, 4, 6, and 8 hours after placing the samples in the saline phosphate buffer. Considering that the non-cross-linked samples were not very stable in the water environment and dissolved quickly, only the cross-linked samples were used for this test. Filter paper was used to weigh and dehydrate the nanofibers (Figure 6a). Figure (6b, c) shows the water absorption of nanofibers after 2 hours and 8 hours. Figure 6e shows that the nanofibers began to absorb water quickly due to the hydrophilicity of polyvinyl alcohol, and the percentage of water absorption in nanofibers without extract was 310±74, 470±26, 640±49, and 660±98% after 2, 4, 6 and 8 hours respectively. Water absorption in fibers with extract was slightly faster, which was 400±112, 600±95, 710±49 and 750±98% after 2, 4, 6 and 8 hours, respectively.





e

Figure 6: a) use of filter paper to dehydrate fiber felt, b) gelation of nanofibers after 2 hours of being in a wet environment, and c) structure change and dissolution of nanofibers after 8 hours

e: Water absorption percentage of nanofibers (orange: nanofibers with extract, blue: nanofibers without extract)

Determine the contact angle

The contact angle of the droplet and the samples was measured on all 4 samples at the ZMOT moment (Figure 7). The contact angle in samples 1, 2, 3, and 4 was 41.5, 78.6, 41.9, and 42.7 degrees, respectively, which indicates the relative hydrophilicity of all samples. It was expected to confirm the hydrophilicity of the surface of the samples due to the presence of polyvinyl alcohol in the samples (Table 2).

Number of nanofiber samples:

1) Without extract and not cross-liked: 41.5
2) Without extract and cross-linked: 78.6
3) Without extract and not cross-liked: 41.9
4) With extract and cross-liked: 42.7

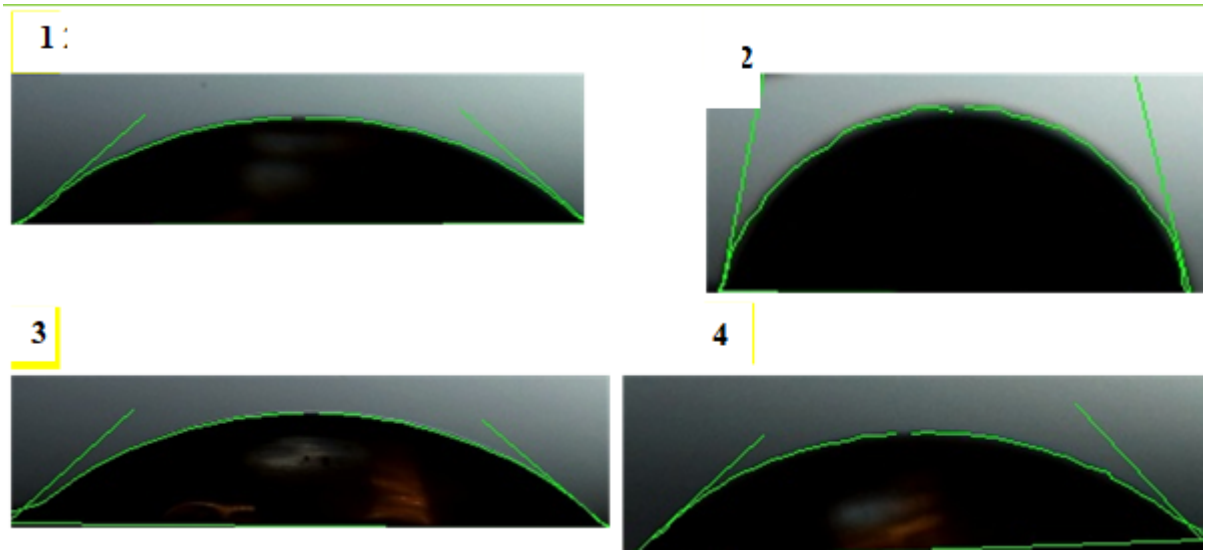


Figure 7: Angles of nanofibers 1) without extract and not cross-liked, 2) without extract and cross-liked, 3) without extract and cross-liked, and 4) with extract and cross-liked

Bacterial culture

In this experiment, it was first necessary to sterilize the nanofibers. For this purpose, the fibers were cut into appropriate dimensions and exposed to UV radiation for 30 minutes.Then, the stages of bacterial cultivation and determination of the aura of non-growth were carried out as follows:

Escherichia coli bacteria (PTTC:1338, ATTC:10536) and Staphylococcus aureus bacteria (PTTC:1431, ATTC:25923) respectively as an indicator of gram-negative and gram-positive pathogenic bacteria from Iran's Fungi and Bacteria Collection Center (Iranian Research Organization for Science and Technology) was prepared as active cultivation. We get active from bacteria by transferring 2 to 4 colonies of E. coli to Nutrient Broth nutrient medium and 2 to 4 colonies of Staphylococcus aureus bacteria to Tryptic Soy Broth nutrient medium and placing these closed containers in a shaker incubator for 24 hours at 37 °C in the broth environment.

Determination of antibacterial properties of nanofibers

The 10 microliters of bacterial suspension were poured on the agar culture medium (tryptic soy agar was used for Staphylococcus aureus and Nutrient Agar was used for E. coli) by a micropipette with a volume of 100 µml to check the antibacterial effect of nanofibers. Then, it was spread by the L-shaped glass rod under the hood. The sterilized nanofiber samples were placed under UV light by sterile forceps on the agar medium containing bacterial culture. In total, 2 plates were cultured as a positive control, one containing E. coli and the other with Staphylococcus bacteria. Nanofiber samples without extract were placed on agar without bacterial culture in addition to the plates containing the sample and as a negative control. All the plates were incubated in a 37 °C incubator for 24 hours. The penicillin disc was also used as a reference. Figure 22-4-a shows that in each petri dish containing bacteria, two cross-linked felts with and without extract were used, and part of the fibers in the environment dissolved and transformed after 2 hours. The results of this test show that in the plate containing E. coli bacteria, the growth of the bacteria is completely observed, and the nanofibers had no effect on this bacterium after 24 hours. But, In the plate containing Staphylococcus aureus bacteria, the non-cross-linked aura of bacteria in nanofibers with extract is quite clear. Figure 4-22 b. The diameter of the halo of non-growth was measured using a caliper and its amount was 19 mm in penicillin and 15 mm in nanofiber felt with extract, which indicates the antibacterial effect of these fibers against Staphylococcus aureus bacteria. In addition, fibers without extract had no effect against this bacterium.

Discussion

In this study, the possibility of spinning gellan polysaccharide as a microbial polysaccharide with polyvinyl alcohol polymer was discussed. It was found that the aqueous solution of this polysaccharide at a weight-to-volume percent (% w/v) in combination with polyvinyl alcohol solution at 10% w/v can be spun. It can understand the concentration in which the warping and complexity of the chains of this polymer have reached such a level that during the exit of the aqueous solution from the nozzle and the evaporation of the aqueous solvent, the polymer chains can form a jet, and be drawn towards the collector, and create uniform fibers.

This property was investigated in other concentrations and ratios in the present study, but no suitable fibers were obtained. The combined polymer and polysaccharide solution of this study were spun at the temperature of 30-40 °C. This is due to the high viscosity of polyvinyl alcohol polymer and the gelling

property of gellan gum, which is also observed in other polysaccharides. One of the influencing factors on the molecular scale structure is the homogeneity of the polymer solution. Many charged polysaccharides are easily hydrated but difficult to completely dissolve. The solution acquires the structural property of a weak gel, which prevents the development of the tensile force necessary for the formation of a stable jet and a Taylor cone [24]. In the present study, a stable jet and a Taylor cone were well formed, which indicated sufficient tensile force in the solution.

In this study, the fibers were not formed by reducing the voltage and they left the nozzle in droplet form. Voltage change shows a double effect on the diameter of the nanofibers. The applied voltage may affect various factors such as the volume of polymer removed from the tip of the needle, the amount of jet stretching by electric force, the morphology of the jet (one or more jets), and so on. The balance between these factors determines the final diameter of electrospun fibers [25]. Therefore, if the applied voltage is not enough, it cannot overcome the surface tension force of the droplet, and the fibers are not formed, which seems to be the reason for the non-formation of fibers in this study is also the high surface tension of the solution and the lack of overpowering voltage of less than 22 kV on it. Liu et al showed a non-linear increase in the diameter of the nanofibers on Poly Lactic-co-Glycolic Acid (PLGA) electrospun nanofibers with increasing voltage [26]. On the other hand, in our study, the diameter size distribution of nanofibers at the highest concentration (10% w/w) was not symmetrical, which could be due to the formation of multiple jets at high voltages [27]. Polymer concentration or solution viscosity is one of the parameters that play an important role in determining the diameter of nanofibers [28]. The viscosity of the polymer solution also increases when the concentration of the polymer increases, which is caused by the increase in chain entanglements and therefore the higher resistance of the polymer solution against stretching by the charges on the jet. As a result, thicker fibers are formed. Another effect of increasing viscosity is reducing the instability of the jet, which results in shortening the jet path from the nozzle to the collector. This shortening of the jet path shows that less elongation is applied on the polymer jet, which causes the creation of fibers with a larger diameter. Polymer solutions also became more viscous with increasing concentration, and the diameter of nanofibers also increased with this increase.

A higher entry rate leads to a larger amount of polymer to be released in time, although the input rate has an optimal interval. Entry velocity should be adjusted so that a balanced Taylor cone is formed. Increasing the penetration rate up to a certain value until the Taylor cone is stable causes thicker fibers to form. However, fibers are obtained in which solvent evaporation does not occur sufficiently, with an excessive increase in the entry rate, and flat and network-like objects are obtained instead of fibers. There should always be a minimum of solution at the nozzle tip. Because the solvent evaporates at the tip of the needle and the electrospinning process stops below this threshold. In the present study, fibers were not formed by increasing the entry rate, and droplet ejection mode was developed.

In this study, the electrospinning process was not carried out at temperatures below 30 °C due to the solution gelatinization and syringe closure. In addition, fibers were not formed at higher temperatures due to the rapid evaporation of the solvent. The prepared nanofibers lost the least amount of water in their nanofiber structure in contact due to the solubility of polyvinyl alcohol in water. Although the solubility of this biocompatible and biodegradable polysaccharide in water is an advantage for using this polymer in biological works, on the other hand, the low physical resistance of this polymer is a challenge for its use. One of the solutions is to strengthen the cross-link of the nanofiber structure with cross-linker materials in cases where water-soluble polymers are used. Collagen and gelatin have been used for other polymers as well. The nanofibrous meshes were cross-linked by glutaraldehyde vapor after production. It was found that the average diameter of fibers with extract increased by 200 nm after cross-linking by comparing SEM images before and after exposure to glutaraldehyde. This result has also been observed in other studies in increasing the diameter of nanofibers [29].

In the present study, polyvinyl alcohol and gum were used in a ratio of 3:1. While in a study, polyvinyl alcohol nanofibers and gellan gum were made in combination with polycaprolactone to transfer pentoxifylline for wound treatment. In this study, a 1:1 combination of polyvinyl alcohol and gellan gum was used to make fibers by electrospinning. The average diameter of these fibers (110-86 nm) was measured by a scanning electron microscope. While the diameter of the nanofibers in our study was greater, it seems that the presence of polycaprolactone in the structure and the increase in the concentration of gum caused the decrease in the diameter of the fibers [30]. In other studies, the size of polyvinyl alcohol nanofibers is 150-250 nm, which increases with the addition of drugs in the structure or network [31].

IR spectrum can be used to check the purity and structure of nanofiber samples with and without extract after nanofiber fabrication. In addition, ATR-FTIR spectroscopy is a valuable tool for monitoring the structural changes of biopolymers. In this study, the IR spectrum of polyvinyl alcohol, extract, gellan gum, and nanofibers of the combination of polyvinyl alcohol and gellan gum with extract was investigated through the ATR-FTIR method to investigate the change in the structure of polyvinyl alcohol polymer during the electrospinning process.

In the structure of polyvinyl alcohol, there are C-O, C=O, CH, OH, and C-C bonds. A long peak at the wavelength of 2908, which represents the OH group, is observed in the spectrum of polyvinyl alcohol. It has been shown in the spectrum of polyvinyl alcohol at about 1073 cm-1 and 11723 cm-1 that are related to C-O and C=O stretching, respectively.

In other studies, polyvinyl alcohol and gellan gum have peaks in the range of 2400-3300, which is due to the hydroxyl group in the structure. In addition, a small peak of 1500 was observed in the combination of polyvinyl alcohol and gellan gum, which is present in the nanofibers of our study and is due to the carbonyl groups [32]. Peaks at 3200-3400 are also due to hydroxyl groups, which were also confirmed in our studied structures.

Peaks 1032, 1199, 1339, 1603, 3284, and 2913 cm^{-1} are related to the structure of the extract. In other studies, peaks similar to the present study have been obtained. One of the main peaks related to the myrtle extract is the peak related to the O-H stretching bond in the range of 3000-3700 cm-1, which in our study had a broad peak at 3284 cm^{-1}. There was also a sharp peak at 2913 cm^{-1}, which is due to the vibration of the C-H group bond. It has also been observed at 2900 cm^{-1}in other studies. In addition, a large number of small peaks in the range of 1300-1400 cm^{-1} have been reported in studies [33], which is due to the bending of methyl bonds. In our study, a peak was also seen in the range of 1339. Therefore, the peaks obtained from this study are consistent with other studies [34].

Polyvinyl alcohol and gellan gum were used to characterize the manufactured nanofibers after confirming the structure of the extract. The contact angle of nanofibers indicates their hydrophilicity which is one of the important points for their use in the medical industry. In this study, the contact angle of nanofibers in 3 samples was 30-40 degrees. Similar studies that used the combination of polyvinyl alcohol and gellan gum to make nanofibers also reported the same amount of hydrophilicity. Polyvinyl alcohol alone is a very hydrophilic polymer and has a contact angle of about 60 degrees [35].

The percentage of water absorption in the present study was very similar to other studies based on polyvinyl alcohol and after 8 hours 600 to 800 times has been reported [36]. In a study, the combination of polyvinyl alcohol and chitosan in a ratio of 1 to 1 led to the formation of fibers with 50 times moisture absorption, which decreased by 20 times with the increase in the percentage of chitosan. It shows that polyvinyl alcohol was the main reason for moisture absorption in this study [38]. In another study, the water absorption percentage of polyvinyl alcohol hydrogels reached 910% after 30 min [39].

In addition, the investigation of the degradability of nanofibers shows time-dependent degradability. In nanofibers without Cross-linked extract, 4±2, 25±5, 32±4, and 48±9 percent of the fibers were destroyed after 2, 4, 6, and 8 hours, respectively. In another study, the degradation percentage of polyvinyl alcohol and gellan gum nanofibers was 25% after 72 hours. This value was much lower than the fiber degradation results in our study, which could be due to the change in the ratio of 3:7 resin to polymer. In addition, in this study, nanofibers were stable for up to 14 days in a humid environment, but in our study, they became relatively unstable after 6-8 hours [23].

In the study of the antibacterial effect of this extract alone and the Niosome structure at low concentrations, it was ineffective on E. coli, but with the increase in the concentration, a growth halo was formed and it has even reached 12 mm by increasing the concentration to 8 mg/ml. But like the present study, the extract was more effective on Staphylococcus aureus bacteria. About 14 mm of non-growth aura was given in concentrations of 0.5 mg/ml both in the extract structure and in the noisome structure, which with increasing the concentration up to 8 mg/ml, the lack of growth aura in the free extract and niosome structure reached 29 and 35 mm [40].

Conclusion

Gellan gum nanofiber felts with polyvinyl alcohol containing myrtle extract as a biodegradable, biocompatible, and usable polymer were successfully electrospuned for the first time in different ratios.

Conflict of interest:

None.

Financial support:

None.

Ethics statement:

None.

References

1. Al-Muluk, N.S., Investigation of the effect of purslane plant extract in the treatment of recurrent oral thrush
2. Çağlayan, F., et al., *Oxidative stress and myeloperoxidase levels in saliva of patients with recurrent aphthous stomatitis.* Oral diseases, 2008. **14**(8): p. 700-704.
3. Dip, E.C., N.A. Pereira, and P.D. Fernandes, *Ability of eugenol to reduce tongue edema induced by Dieffenbachia picta Schott in mice.* Toxicon, 2004. **43**(6): p. 729-735.
4. Mahmoodi Bardzardi, M., S .Ghazanfari, and S. Sharifi, *Growth performance, carcass characteristics, antibody titer and blood parameters in broiler chickens fed dietary myrtle (Myrtus communis) essential oil as an alternative to antibiotic*

growth promoter. Poultry Science Journal, 2 .٠١٤ :(١)٢p. 37-49.
5. Amensour, M., et al., *Total phenolic content and antioxidant activity of myrtle (Myrtus communis) extracts.* Natural Product Communications, 2009. **4**(6): p. 1934578X0900400616.
6. Serce, S., et al., *Antioxidant activities and fatty acid composition of wild grown myrtle (Myrtus communis L.) fruits.* Pharmacognosy magazine, 2010. **6**(21): p. 9.
7. Bejeshk, M., et al ,.*Anti-inflammatory and anti-remodeling effects of myrtenol in the lungs of asthmatic rats: Histopathological and biochemical findings.* Allergologia et Immunopathologia, 2019. **47**(2): p. 185-193.
8. Bajalan, I. and A.G. Pirbalouti, *Variation in antibacterial activity and chemical compositions of essential oil from different populations of myrtle.* Industrial Crops and Products, 2014. **61**: p. 303-307.
9. Kılıç, S., et al., *Efficacy of two plant extracts against acne vulgaris: initial results of microbiological tests and cell culture studies.* Journal of cosmetic dermatology, 2019. **18**(4): p. 1061-1065.
10. Pereira, P., et al., *Potential of supercritical fluid myrtle extracts as an active ingredient and co-preservative for cosmetic and topical pharmaceutical applications.* Sustainable Chemistry and Pharmacy, 2022. **28**: p. 100739.
11. Babaee, N., et al., *The efficacy of a paste containing Myrtus communis (Myrtle) in the management of recurrent aphthous stomatitis: a randomized controlled trial.* Clinical oral investigations, 2010. **14**(1): p. 65-70.
12. Salmanian, M., et al., *The effects of myrtle (myrtus communis) and clindamycin topical solution in the treatment of mild to moderate acne vulgaris: A comparative split-face study.* Journal of Pharmacopuncture, 2020. **23**(4): p. 220.
13. Nirmal, N.P., et al., *Formulation, characterisation and antibacterial activity of lemon myrtle and anise myrtle essential oil in water nanoemulsion.* Food chemistry, 2018. **254**: p. 1-7.
14. Bellu, E., et al., *Myrtle-Functionalized Nanofibers Modulate Vaginal Cell Population Behavior While Counteracting Microbial Proliferation.* Plants, 2022. **11** :(١٢)p. 1577.
15. Zhu, L.-F., et al., *Engineering of Ganoderma lucidum polysaccharide loaded polyvinyl alcohol nanofibers for biopharmaceutical delivery.* Journal of Drug Delivery Science and Technology, 2019. **50**: p. 208-216.
16. Jiffrin, R ,.et al., *Electrospun nanofiber composites for drug delivery: A review on current progresses.* Polymers, 2022. **14**(18): p. 3725.
17. Mardare, D. and K. Matyjaszewski, *" Living" radical polymerization of vinyl acetate.* Macromolecules, 1994. **27**(3): p. 645-64.٩
18. Debiagi, F., et al., *Biodegradable active packaging based on cassava bagasse, polyvinyl alcohol and essential oils.* Industrial Crops and Products, 2014. **52**: p. 664-670.
19. Beikzadeh, S., et al., *Cellulose acetate electrospun nanofibers encapsulating Lemon Myrtle essential oil as active agent with potent and sustainable antimicrobial activity.* Reactive and Functional Polymers, 2020. **157**: p. 104769.
20. YanpingWang, Z.A., Wu Feng, Chao Li, Shiying Song, *Physicochemical properties of exopolysaccharide produced by Lactobacillus kefiranofaciens ZW3 isolated from Tibet kefir.* International Journal of Biological Macromolecules, 2008. **43**: p. 283–288.
21. Mehran Ghasemlou , F.K., Kambiz Jahanbin , Seyed Mohammad Taghi Gharibzahedi,Salman Taheri, *Structural investigation and response surface optimisation for improvement of kefiran production yield from a low-cost culture medium.* Food Chemistry, 2012. **133**: p. 383–389.
22. *Handbook of Instrumental Techniques for Analytical Chemistry*.
23. Aadil, K.R., et al., *Investigation of poly (vinyl) alcohol-gellan gum based nanofiber as scaffolds for tissue engineering applications.* Journal of Drug Delivery Science and Technology, 2019. **54**: p. 101276.
24. Aadil, K.R., et al., *Investigation of poly (vinyl) alcohol-gellan gum based nanofiber as scaffolds for tissue engineering applications.* Journal of Drug Delivery Science and Technology, 2019. **54**: p. 101276.
25. Saowakon Wongsasulak *, M.P., Jochen Weiss , Pitt Supaphol,Tipaporn Yoovidhya, *Electrospinning of food-grade nanofibers from cellulose acetate and egg albumen blends.* Journal of Food Engineering, 2010. **98**: p. 370–376.
26. Tan, S., et al., *Systematic parameter study for ultra-fine fiber fabrication via electrospinning process.* Polymer, 2005. **46**(16): p. 6128-6134.
27. Liu, F., et al., *Effect of Processing Variables on the Morphology of Electrospun Poly [(lactic acid)-co-(glycolic acid)] Nanofibers.* Macromolecular

Materials and Engineering, 2009. **294**(10): p. 666-672.
28. Sencadas, V., et al., *Determination of the parameters affecting electrospun chitosan fiber size distribution and morphology.* Carbohydrate Polymers, 2012. **87**(2): p. 1295-1301.
29. Jia, Y.-T., et al ,.*Fabrication and characterization of poly (vinyl alcohol)/chitosan blend nanofibers produced by electrospinning method.* Carbohydrate Polymers, 2007. **67**(3): p. 403-409.
30. Wu, S.-C., et al., *Cell adhesion and proliferation enhancement by gelatin nanofiber scaffolds.* Journal of Bioactive and Compatible Polymers, 2011. **26**(6): p. 565-577.
31. Shahravi, Z., et al., *Multifunctional electrospun polyvinyl alcohol/gellan gum/polycaprolactone nanofibrous membrane containing pentoxifylline to accelerate wound healing.* Polymer Bulletin, 2022: p. 1-21.
32. Aadil, K.R., et al., *Fabrication of biocompatible alginate-poly (vinyl alcohol) nanofibers scaffolds for tissue engineering applications.* Materials Technology, 2018. **33**(8): p. 507-512.
33. Wang, F ,.Y. Wen, and T. Bai, *The composite hydrogels of polyvinyl alcohol–gellan gum-Ca2+ with improved network structure and mechanical property.* Materials Science and Engineering: C, 2016. **69**: p. 268-275.
34. Cheikh, D., et al., *Alginate bionanocomposite films containing sepiolite modified with polyphenols from myrtle berries extract.* International Journal of Biological Macromolecules, 2020. **165**: p. 2079-2088.
35. Agatonovic-Kustrin, S., et al., *High-performance thin-layer chromatography linked with (bio) assays and FTIR-ATR spectroscopy as a method for discovery and quantification of bioactive components in native Australian plants.* Journal of pharmaceutical and biomedical analysis, 2020. **184**: p. 1.١٣٢٠٨
36. Vashisth, P. and V. Pruthi, *Synthesis and characterization of crosslinked gellan/PVA nanofibers for tissue engineering application.* Materials Science and Engineering: C, 2016. **67**: p. 304-312.
37. Suzuki, A. and S. Sasaki, *Swelling and mechanical properties of physically crosslinked poly (vinyl alcohol) hydrogels.* Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine, 2015. **229**(12): p. 828-844.
38. Zhou, Y., D. Yang, and J. Nie, *Effect of PVA content on morphology, swelling and mechanical property of crosslinked chitosan/PVA nanofibre.* Plastics, rubber and composites, 2007. **36**(6): p. 254-258.
39. Xu, Z., et al., *Morphological and swelling behavior of cellulose nanofiber (CNF)/poly (vinyl alcohol) (PVA) hydrogels: poly (ethylene glycol)(PEG) as porogen.* RSC advances, 2016. **6**(49): p. 43626-.٤٣٦٣٣
40. Raeiszadeh, M., et al., *Phytoniosome: a novel drug delivery for myrtle extract.* Iranian journal of pharmaceutical research: IJPR, 2018. **17**(3): p. 804.