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Comparative Analysis of Clove and Eucalyptus Essential Oils-Based PVP/Gelatin Nanofibers

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ABSTRACT

This study aimed to produce polyvinylpyrrolidone/gelatin (PVP/GEL) nanofibers based on clove essential oil (CLEO) and eucalyptus essential oil (EEO) through emulsion electrospinning. Firstly, solution properties such as Gas Chromatography–Mass Spectrometer (GC-MS) profile, viscosity, conductivity, and surface tension were investigated. Then, nanofibers were produced under optimum process parameters and characterized using Scanning Electron Microscope (SEM), Fourier Transform Infrared Spectroscopy (FT-IR), and Ultraviolet-visible (UV–vis) spectroscopy. Lastly, antibacterial activity was determined via the disc diffusion method against Staphylococcus aureus *(S. aureus), Escherichia coli (E. coli), Candida albicans (C. albicans), Pseudomonas aeruginosa (P. aeruginosa), and Enterococcus faecalis (E. faecalis)*. The major components of CLEO and EEO were determined to be eugenol and 1,8-cineole, respectively. Conductivity and surface tension decreased and viscosity increased with increasing concentration of either essential oil. Generally, addition of CLEO to the polymer solution yielded quite fine nanofibers and eliminated beads. Moreover, CLEO nanofibers displayed larger inhibition zones than did EEO nanofibers.

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KEYWORDS

Polyvinylpyrrolidone, gelatin, clove essential oil, eucalyptus essential oil, emulsion electrospinning, antibacterial nanofibers

1. INTRODUCTION

Spices and their essential oils have played important roles in traditional medicine in many countries [1]. Essential oils are obtained from non-woody part of plants such as the flowers, leaves, roots, and branches [2]. In recent years, natural antibacterial additives have become more attractive than synthetic products, and essential oils are promising alternatives for synthetic antimicrobials, being both natural and cheap. In particular, essential oils can be utilized instead of medicine in the soap, perfume, and toiletries industries in light of their natural antibacterial, antifungal, and fragrance properties. Nevertheless, essential oils have drawbacks such as their volatility, oxidation, and easy degradation with exposure to light and temperature [3; 4]. These limitations can be reduced by protecting the essential oils with different methods such as microencapsulation [5], coaxial electrospinning [6], beta-cyclodextrin [7], nanoparticles [8], and emulsion electrospinning [9; 10].

For this research, clove essential oil (CLEO) and eucalyptus essential oil (EEO) were chosen as natural antibacterial agents to be encapsulated with biocompatible PVP/GEL nanofibers. Eucalyptus oil has been placed in the category Generally Regarded as Safe by the U.S. Food and Drug Administration, and is classified as non-toxic [11]. Generally, EEO can be used in the food, perfumery, and pharmaceutical industries [12]. At low concentrations, it is also used extensively in soaps, detergents, and perfumes [13]. Moreover, it can be used in pharmaceuticals to treat pharyngitis, bronchitis, and sinusitis [14]. In addition, EEO has some biological activities, such as: anti-microbial, fungicidal, insecticidal/insect repellent, herbicidal, acaricidal, and nematicidal activity [2]. Among the various

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components of eucalyptus oil, 1,8-cineole is the most important, being largely responsible for its pesticidal properties; in fact, this is a characteristic compound of the genus *Eucalyptus*. EEO that is rich in 1,8-cineole (more than 70%) can be used commercially [15; 16].

Eugenol is the main component of CLEO, which is extracted from the dried flower buds of the clove, and is approved as a food additive by the U.S. Food and Drug Administration. [10; 17]. It has a number of biological activities, including: antibacterial, fungicidal, anticarcinogenic [18], antimutagenic (potential) [19], antitumor, insecticidal activity [20], and anaesthetic [21].

Nanofibers have unique properties that increase effectiveness for drug delivery and controlled release, such as: small fiber diameter (nm), high porosity, small and open pore structure, large specific surface area (m^2/g), and high loading capacity [22-24]. Nanofibers can gain functionality through being loaded with proteins, bioactive molecules, drugs, and essential oils. Emulsion electrospinning is a popular alternative to co-axial electrospinning that can be used to produce core-sheath nanofibers [25]. Specifically, this approach enables the incorporation of hydrophobic agents such as essential oils into the structure of water-based nanofibers. More importantly, extra apparatus and processes are not required to produce emulsion electrospun nanofibers [9; 26]. In addition, this approach can be used to produce green nanofibers, i.e. without using harmful and toxic solvents. Emulsion electrospinning can be enabled to produce green nanofibers. Green treatments and eco-friendly products gain importance in recent years to prevent environmental pollution, air pollution, water pollution and protect human health and reduce using synthetic plastic materials. Green electrospinning is a type of new approach for clean and safe production [27]. The main goal of this study is the encapsulation of essential oils in the nanofiber structure without using any toxic solvents or extra apparatus.

The critical step in emulsion electrospinning is accurate preparation of the emulsion polymer solution. In this study, polyvinylpyrrolidone (PVP) and gelatin (GEL) were used as polymers to produce antibacterial emulsion electrospun nanofibers. These polymers were chosen because of several advantages such as biocompatible, good cell adhesion, non-toxic, water-soluble which are important for the intended end-use [28-32].

Limited studies exist in the literature concerning nanofibers based on essential oils and essential oil components, such as: polyvinylalcohol/eugenol [33], polylactic acid/candeia essential oil [34], chitosan/polyethylene oxide/ cinnamaldehyde [35], and PVP/cinnamon essential oil [9]. This study contributes new information about the emulsion electrospinning of PVP with CLEO and EEO with application to the fields of biomedicine. The results of this research will add to information green electrospinning, which has become a very important approach in recent years. Moreover, a deep analysis of the literature revealed there is no prior study to have performed a systematic comparison of two essential oils spanning from solution properties to fiber morphology and antibacterial activity. Therefore, this research adds useful new information for future studies.

2. EXPERIMENTAL

2.1 Materials

PVP (360.000 g/mol) was used as a polymer, gelatin from porcine skin (gel strength 300, Type A) was used as a co-polymer, ultra-pure water (UPW) was used as a solvent, surfactant (Cremophor RH 40) was used as an emulsifier, and clove essential oil (CLEO) and eucalyptus essential oil (EEO) were used as natural antibacterial agents. PVP and gelatin polymers were purchased from Sigma Aldrich Corporation (St. Louis, MO, USA), surfactant was supplied by Ersa Chemistry (İzmir, Turkey), the essential oils were bought from Botalife (Isparta, Turkey), and UPW was obtained from a Millipore Milli-Q System with conductivity of 18.0 MΩ.cm. Cremophor RH 40 was used as a surfactant. This surfactant can be used medical and cosmetic applications. Moreover, it does not demonstrate any cytotoxic effect [36;37]. The PVP and GEL polymer concentrations were 12 wt % and 6 wt %, respectively. The surfactant concentration was kept constant at 3 wt % for all solutions. CLEO and EEO were used at concentrations of 1/3/5 wt % and 1/3/5/7 wt %, respectively (Table 1). All solutions were prepared under the same conditions, with such as stirring time, stirring speed (rpm), and temperature held constant.

Table 1. Composition of PVP/UPW and GEL/UPW polymer solutions with essential oils (CLEO & EEO)

Sample Codes	PVP/UPW polymer concentration (wt %)	GEL/UPW polymer concentration (wt %)	Surfactant concentration (wt %)	CLEO concentration (wt %)	EEO concentration (wt %)
PVP/GEL	12	6	3	-	-
CLEO1	12	6	3	1	-
CLEO3	12	6	3	3	-
CLEO5	12	6	3	5	-
CLEO7	12	6	3	7	-
EEO1	12	6	3	-	1
EEO3	12	6	3	-	3
EEO5	12	6	3	-	5
EEO7	12	6	3	-	7

2.2 Methods

Firstly, PVP/GEL emulsion solutions with various concentrations of CLEO and EEO were prepared under the same conditions using a magnetic stirrer. Next, polymer solution properties were determined, such as viscosity (Lamy Rheology, B-One Touch Screen) under shear rate 5 s^{-1}, conductivity (Selecta CD 2005), and surface tension (Biolin Scintific Sigma 702) by the Wilhelmy plate method.

GC-MS analysis was carried out to determine essential oil components with details and this analyze was performed under the conditions given in Table 2.

Then, nanofibers were produced via conventional laboratory scale electrospinning (Figure 1). The greatest advantage of emulsion electrospinning is that it does not require extra apparatus to produce nanofibers encapsulating essential oils.

During the spinning process, all nanofibers were produced under the same experimental parameters (Table 3). The power supply came from Matsusada Precision Inc. (Kusatsu, Japan) and the solution feed pump from New Era Pump Systems (Farmingdale, NY, USA). All nanofibers were produced for an hour and collected on alumina foil.

Table 2. GC-MS analysis conditions

Device	Shimadzu (Japan) GC- 2010 Plus Shimadzu GCMS-QP2010 SE (Detector)
Detector Temperature	250 °C
Flow rate (mL/min)	1.0
Detector	70 eV
Ionising Type	EI
Carrier Gas	Helium
Capillary Column	Restek Rx-5Sil MS 30 m x 0.25 mm, 0.25 µm film thickness, catalog no: Restek 13623
Oven Temperature Program	60 °C raised to 250 °C at a rate of 5 °C/min, then held at 250 °C for 20 min
Reference Library	Wiley, Nist, Tutor, FFNSC
Sample Preparation	30 µl essential oil added into 970 µl hexane, of which 1 µl solution was injected from vial
Split ratio	1:10

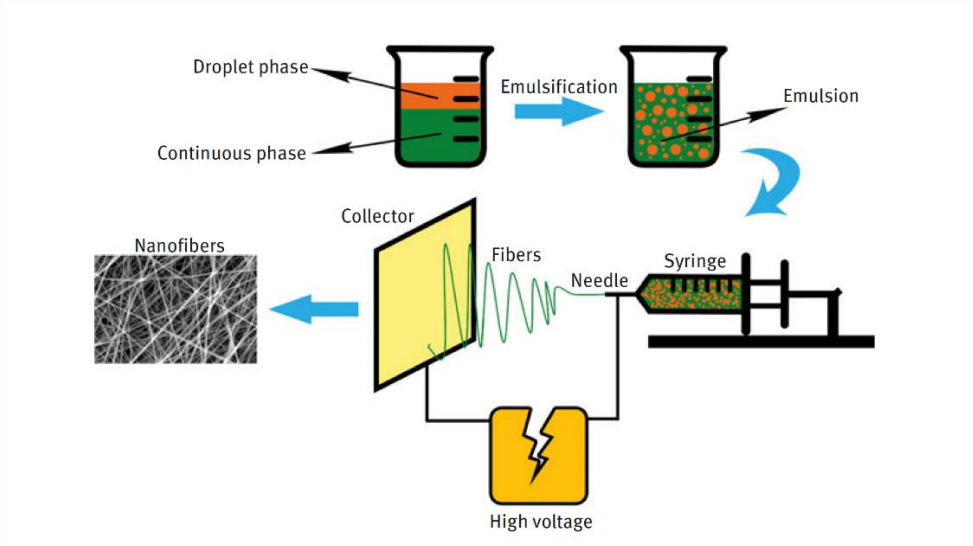


Figure 1. Schematic representation of emulsion electrospinning [38]

Table 3. Electrospinning process parameters

Voltage (kV)	Distance between electrodes (cm)	Feed rate (mL/h)	Humidity (%)	Temperature (°C)	Needle Diameter (mm)	Spinning Duration (min)
24.6	17.0	0.5	30±1	22.5±1	0.8	60

The morphology of emulsion electrospun nanofibers was analyzed with by scanning electron microscopy (SEM) with a FEI Quanta 250 FEG instrument. Fiber diameters were measured using ImageJ software on 100 fibers obtained from different parts of the electrospun web. Then, the fiber diameter uniformity coefficient was calculated with a method that uses the same principle as for molar mass distribution in chemistry. First, the number average and weight average values were calculated using formulas (1) and (2) given below.

$$A_n = \frac{\sum n_i d_i}{\sum n_i} \quad \text{(number average)} \qquad (1)$$

$$A_w = \frac{\sum n_i d_i^2}{\sum n_i d_i} \quad \text{(weight average)} \qquad (2)$$

d_i : fiber diameter

n_i : fiber number

Then, the fiber uniformity coefficient was determined as the ratio of Aw/An. An optimal value is close to 1, which represents uniform fibers [39]. Fiber diameter histogram curves were obtained using statistical analysis software.

FT-IR was used for chemical characterization. Specifically, spectroscopic analyses of the nanofibers were carried out on a KBr disc using a Perkin Elmer Spectrum BX instrument. IR spectra were recorded in the 400–4000 cm^{-1} range with a resolution of 4 cm^{-1}. This analysis was carried out to determine the presence of essential oils (CLEO and EEO) and accuracy of the polymers (PVP and GEL) in the nanofiber structure.

UV–vis spectroscopy was performed using a Perkin Elmer Lambda 20 model, with measurement taken over the wavelength range of 190–500 nm. The purpose of this UV–vis measurement is to determine the increasement tendency of CLEO and EEO into the nanoweb structure.

Lastly, the disc diffusion method was used to analyze the antibacterial activity of emulsion electrospun nanofibers. For disc diffusion, all nanofibers were cut into 9 × 9 mm pieces. Petri dishes were spread with 100 µl 0.5 McFarland turbidity suspensions of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *C. albicans* (ATCC 10231), *P. aeruginosa* (ATCC 27853) and *E. faecalis* (ATCC 29212) and incubated aerobically for 24 h at 35 ± 2 °C. After incubation, the inhibition zone diameters were measured with a ruler. Greater diameter of the zone around the disc demonstrated the greater antimicrobial effects of a tested substance [40; 41].

3. RESULTS & DISCUSSION

3.1 Results of Solution Properties

GC-MS analysis was carried out to determine the constituents of CLEO and EEO. In all, 25 components were identified, of which eugenol (72.83 %) was identified as the major component of CLEO and 1-8 cineole (86.23 %) as the main constituent of EEO (Table 4). It is well known that essential oil composition may vary with regard to climatic, seasonal, and geographic conditions; harvest period; and distillation technique [42]. Some studies reported that different percentage of eugenol and 1-8 cineole were main component of CLEO and EEO, respectively [43-46].

Table 4. Constituents of CLEO and EEO by GC-MS analysis

Component Name	CLEO (Area %)	EEO (Area %)
α-Pinene	0.02	3.89
β-Pinene	0.01	0.26
β-Myrcene	-	0.26
Phellandrene	-	0.21
Cyclohexene	-	0.04
O-Cymene	-	2.98
Isodurene	-	0.44
p-Cymene	-	2.59
1,8-Cineole	**-**	**86.23**
trans-β-Ocimene	-	0.03
γ-Terpinen	-	2.10
α- Terpinolene	-	0.09
Linalool	1.20	0.09
Butanoic acid	-	0.02
trans-Pinocarveol	-	0.12
4-Terpineol	-	0.24
β-Fenchyl alcohol	-	0.43
Eugenol	**72.83**	-
β-Caryophyllene	15.75	-
α–humulene	5.22	-
Calamenene	0.12	-
Calacorene	0.10	-
Eugenol acetate	4.42	-
Caryophyllene oxide	0.06	

Based on solution properties assays, conductivity values decreased with increasing essential oil concentration for both CLEO and EEO. The reductions of conductivity at 1, 5, and 7 wt % were similar for both essential oils. However, at 3 wt %, conductivity decreased sharply for EEO but only slightly for CLEO (Figure 2). Conductivity is related to the number of ions in the polymer solution. As essential oils are not soluble in water, increasing essential oil concentration reduces the number of ions, and conductivity values decrease, too [47].

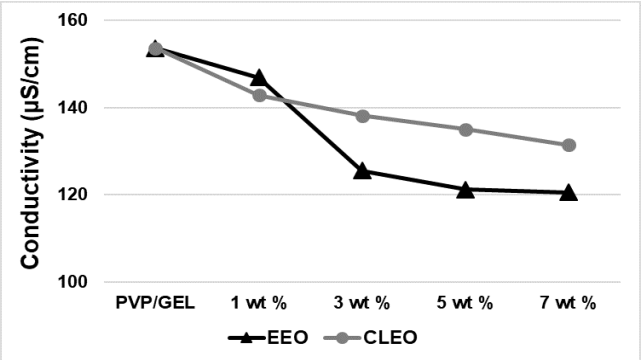


Figure 2. Conductivity of PVP/GEL nanofibers with various CLEO and EEO concentrations

Generally, viscosity values increased with EEO and CLEO concentration (Figure 3). A critical point of dramatic increase was between 1 and 3 wt % for EEO and between 5 and 7 wt % for CLEO. At other concentrations, the viscosity increased only slightly. As it has been seen from GC-MS analyzes, major components of eugenol and 1,8 cineole play an important role for solution properties because polymers, solvent and surfactant concentrations are the same in the polymer solution for both essential oils. For this reason, molecular weight of eugenol is 164,2 g/mol [48] and includes 72.83 % into the CLEO and molecular weight of 1,8 cineole is 154.25 g/mol [49] and includes 86.23 % into the EEO. It is possible to say, eugenol and 1,8 cineole molecular weight very close to each other but amount of these major components in the essential oil quite different (CLEO<EEO). It is thought that, viscosity increases dramatically between 1 and 3 wt % for EEO and between 5 and 7 wt % for CLEO because of this reason. In a previous study, [9] reported that viscosity of a polymer solution containing cinnamon essential oil increased with added essential oil.

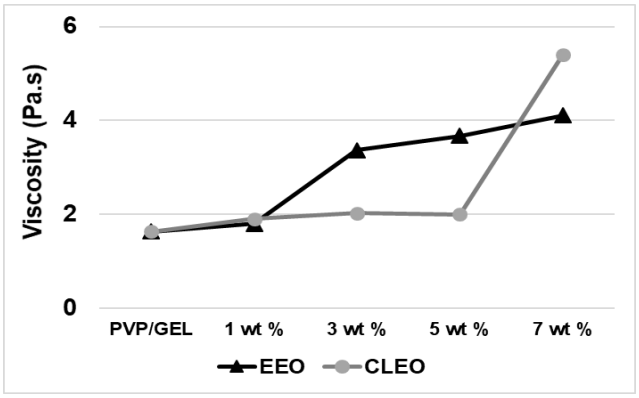


Figure 3. Viscosity of PVP/GEL nanofibers with various CLEO and EEO concentrations

Surface tension values of the PVP/GEL polymer solutions containing CLEO decreased considerably with all CLEO concentrations except 7 wt %, while those with EEO were not affected by the addition of more oil (Figure 4). As it is known from the literature, there is a relationship between surface tension and cohesion force of polymer solution. Cohesion force is the meaning of the same type of molecular attraction [50]. For this reason, surface tension can increase above critical concentration value of essential oil in the PVP/GEL polymer solution. Generally, the surface tension of PVP/GEL with EEO is significantly higher than that of the corresponding solution with CLEO. It is well known from the literature [24] that there is a strong relation between surface tension and spinning performance; this is reflected in the observation that PVP/GEL with 7 wt % CLEO was non-spinnable.

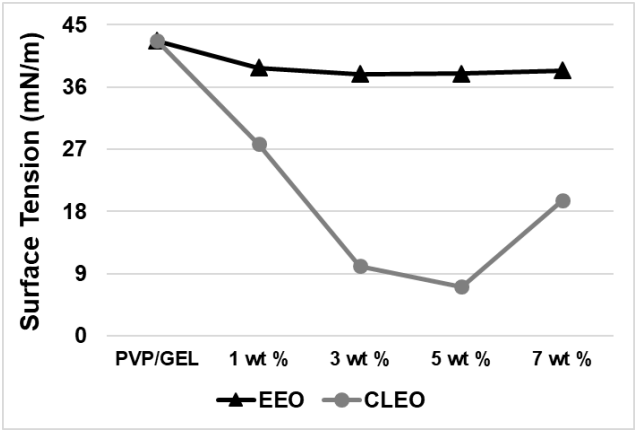


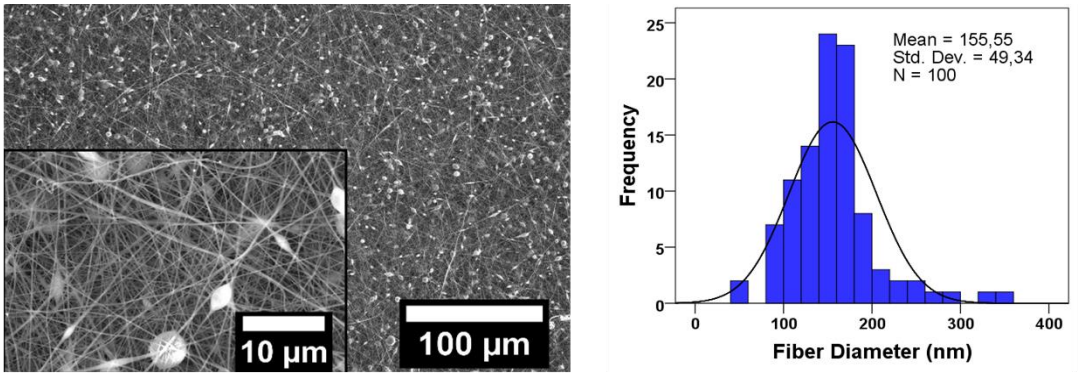
Figure 4. Surface tension of PVP/GEL solutions with various CLEO and EEO concentrations

3.2 Fiber Morphology Results

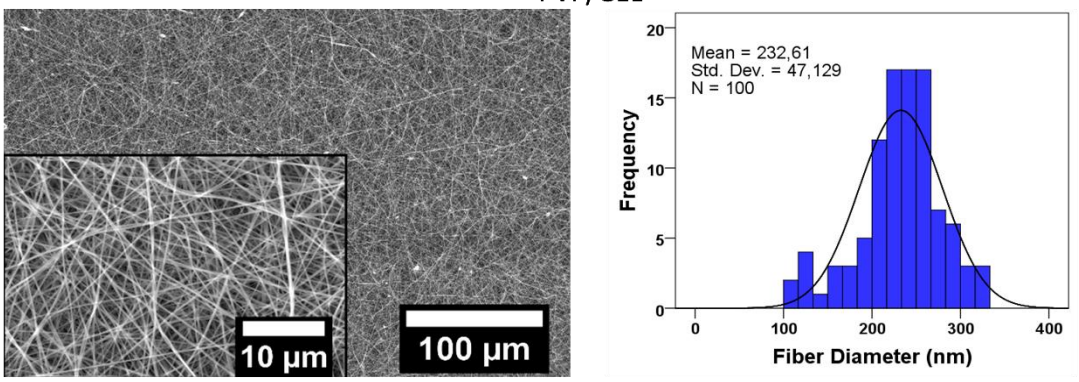
SEM images and fiber diameter histograms of PVP/GEL nanofibers alone and with various concentrations of CLEO are given in Figure 5.

Many beads were observed in the structure of PVP/GEL nanofibers without CLEO, and the average fiber diameter was unimodal and very low (155.55 nm). The addition of 1 and 3 wt % of CLEO to the PVP/GEL solution enhanced fiber morphology and eliminated beads from the nanofiber structure. Meanwhile, average fiber diameter increased with CLEO concentration, and diameter distributions were unimodal for PVP/GEL/CLEO1 and PVP/GEL/CLEO3. However, a sticky and membranous structure occurred at 5 wt % CLEO; therefore, it was not possible to measure the diameter of those nanofibers. In addition, the spinning performance of the solution with 5 wt % CLEO was very low, and the solution with 7 wt % CLEO was non-spinnable. Overall, these fiber morphology results are compatible with the solution properties results for PVP/GEL/CLEO nanofibers. According to the histogram diagrams; it is possible to say, fiber diameter curves were unimodal for sample of PVP/GEL, PVP/GEL/CLEO1 and PVP/GEL/CLEO3.

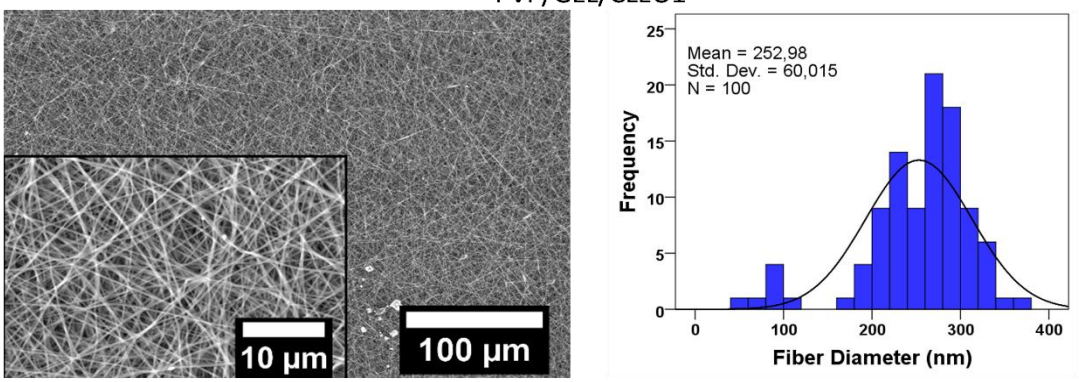
SEM images and fiber diameter histograms of PVP/GEL nanofibers alone and with various concentrations of EEO are given in Figure 6.



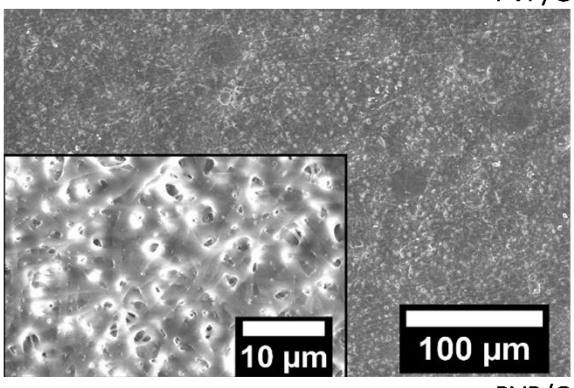
PVP/GEL



PVP/GEL/CLEO1



PVP/GEL/CLEO3



PVP/GEL/CLEO5

Figure 5. SEM images (1.000x-10.000x) and histograms of PVP/GEL nanofiber samples with various concentrations of CLEO

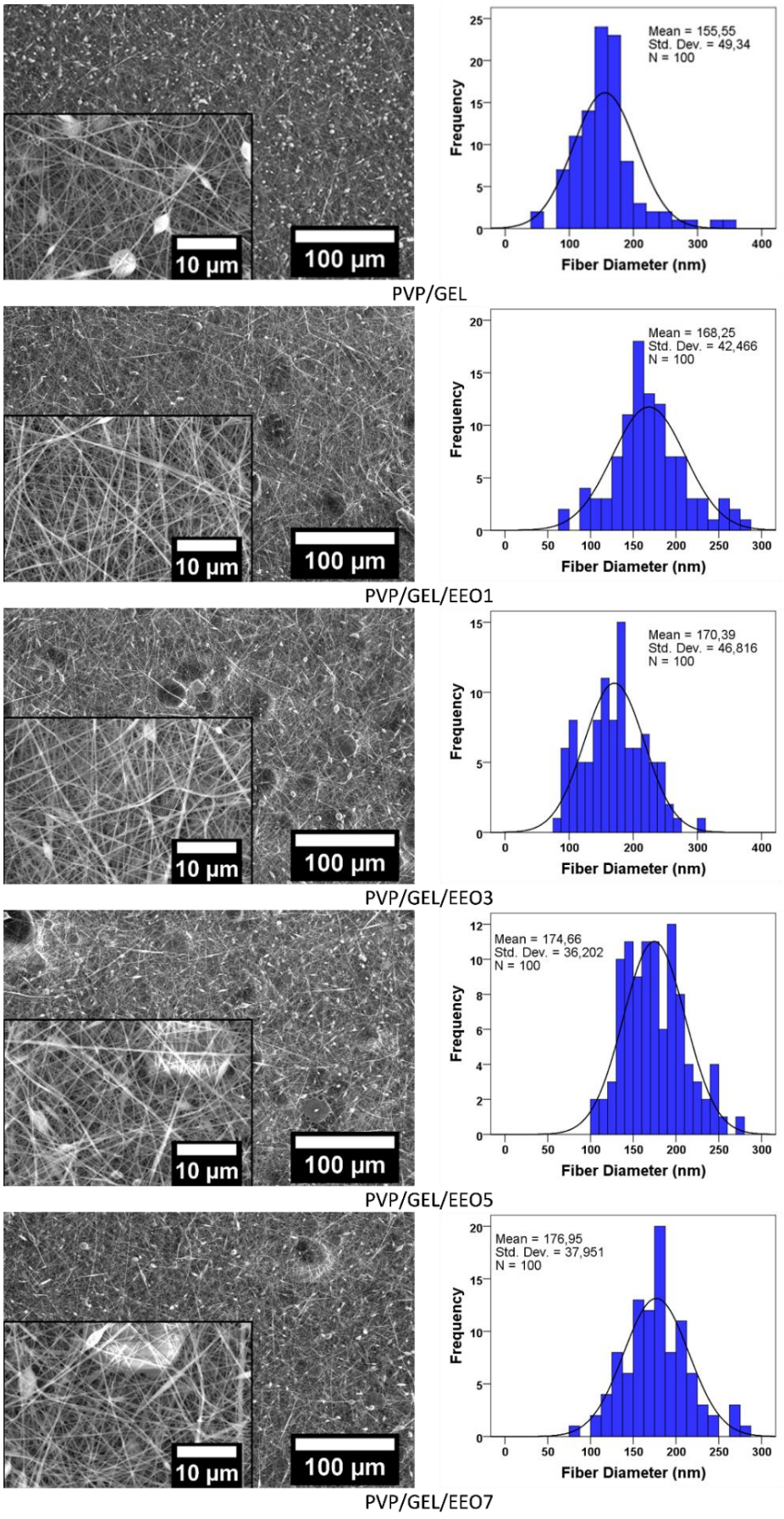


Figure 6. SEM images (1.000x-10.000x) and histograms of PVP/GEL nanofiber samples with various concentrations of EEO

The nanofibers produced from PVP/GEL/EEO solutions were very fine and had unimodal distribution curves. However, poor nanoweb quality was observed for all samples, with beads consistently present in the nanofiber structures. Increasing EEO concentration did not affect the prevalence of beads in the nanoweb structure. The literature supports that increasing polymer concentration can eliminate beads from the fibers [24]. Figure 7 shows, average fiber diameter increased significant with addition of CLEO. However, altering EEO concentration did not affect the average fiber diameter. Taking all these results as a whole, it is possible to say that overall, average fiber diameter increased with viscosity, and spinning performance increased with increased conductivity and decreased surface tension.

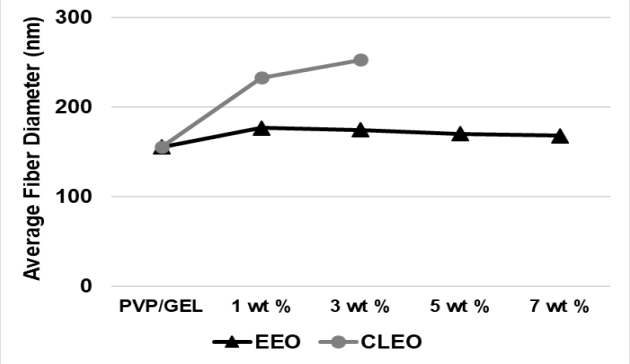


Figure 7. Average fiber diameter of PVP/GEL solutions with various CLEO and EEO concentrations

Based on the fiber diameter uniformity coefficient determination, the most uniform nanofibers were obtained from 1 wt % CLEO and EEO (Figure 8). Generally, the uniformity of fiber diameter was not affected by CLEO and EEO concentration.

All solution properties and fiber morphology results are given in Table 5.

FT-IR spectroscopy confirmed the presence of PVP, GEL, CLEO, and EEO in the chemical structures of nanofibers (Figure 9 and Figure 10).

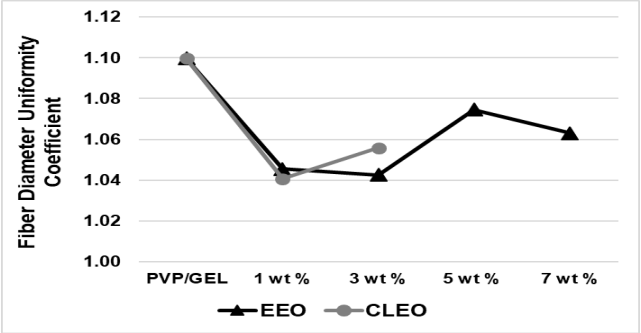


Figure 8. Fiber diameter uniformity coefficients of PVP/GEL solutions with various CLEO and EEO concentrations

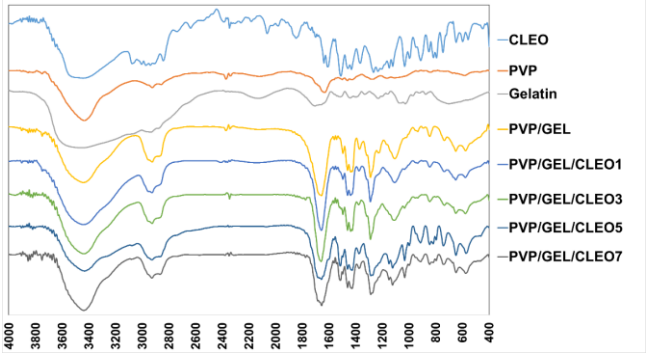


Figure 9. FT-IR spectra of PVP/GEL/CLEO nanofibers

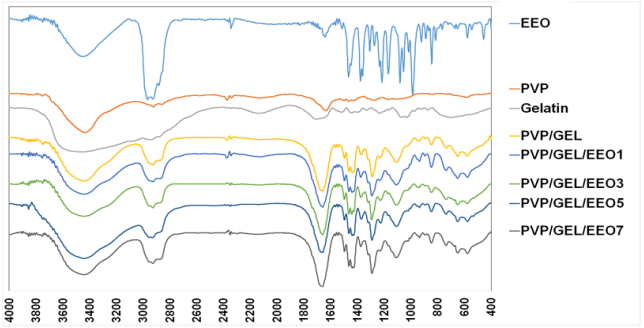


Figure 10. FT-IR spectra of PVP/GEL/EEO nanofibers

Table 5. Solution properties and fiber morphology for all samples

Sample Codes	Conductivity (µS/cm)	Surface Tension (mN/m)	Viscosity (Pa.s) (shear rate 5^{-1})	Weight Average Diameter (A_w)(nm)	Number Average Diameter (A_n)(nm)	Fiber Diameter Uniformity Coefficient (A_w/A_n)	Nanoweb Morphology
PVP/GEL	153.6	42.63	1.63	171.046	155.55±49.3	1.0996	Beaded
PVP/GEL/CLEO1	142.8	27.76	1.90	242.092	232.62±47.1	1.0407	Smoothest
PVP/GEL/CLEO3	138.1	10.08	2.02	267.079	252.98±60.0	1.0557	Smoothest
PVP/GEL/CLEO5	135.0	7.13	1.99	-	-	-	Sticky
PVP/GEL/CLEO7	131.4	19.65	5.39	-	-	-	Non–spinnable
PVP/GEL/EEO1	146.9	38.80	1.80	184.982	176.93±37.9	1.0455	Beaded
PVP/GEL/EEO3	125.5	37.87	3.36	182.028	174.60±36.1	1.0425	Beaded
PVP/GEL/EEO5	121.2	37.90	3.67	183.177	170.45±46.7	1.0746	Beaded
PVP/GEL/EEO7	120.5	38.38	4.10	178.811	168.19±42.4	1.0630	Beaded

In the FT-IR spectrum of PVP, an OH stretching peak was observed at 3434 cm^{-1}. This sharp peak was attributed to the presence of water, and also showed at 3439 cm^{-1} in PVP/GEL, 3444 cm^{-1} in PVP/GEL/CLEO1, 3434 cm^{-1} in PVP/GEL/CLEO3, 3432 cm^{-1} in PVP/GEL/CLEO5, 3435 cm^{-1} PVP/GEL/CLEO7, 3447 cm^{-1} in PVP/GEL/EEO1, 3446 cm^{-1} in PVP/GEL/EEO3, 3445 cm^{-1} in PVP/GEL/EEO5, and 3447 cm^{-1} in PVP/GEL/EEO7. Also, in the PVP polymer spectrum was another peak at 2924 cm^{-1}. This peak appears at 2925 cm^{-1}, 2927 cm^{-1}, 2926 cm^{-1}, 2926 cm^{-1}, 2927 cm^{-1}, 2927 cm^{-1}, 2926 cm^{-1}, 2927 cm^{-1}, 2926 cm^{-1} in the spectra of PVP/GEL, PVP/GEL/CLEO1, PVP/GEL/CLEO3, PVP/GEL/CLEO5, PVP/GEL/CLEO7, PVP/GEL/EEO1, PVP/GEL/EEO3, PVP/GEL/EEO5, and PVP/GEL/EEO7, respectively. A C=O peak occurred at 1635 cm^{-1} in the spectrum of PVP and at 1650 cm^{-1}, 1655 cm^{-1}, 1657 cm^{-1}, 1656 cm^{-1}, and 1658 cm^{-1} in the spectra of all nanofiber samples [31; 51; 52]. The spectra of gelatin and nanofiber samples showed different absorption bands related to amide I (C=O stretch), amide II (N-H bend and C-H stretch), and amide III (C-N stretch plus N-H in phase bending); these peaks appeared around 1705 cm^{-1}, 1516 cm^{-1}, and 1232 cm^{-1}, respectively, in the spectra of gelatin [53].

In the spectrum of CLEO, peaks characteristic of eugenol, which is the major component of CLEO, were clearly seen. These peaks occurred at 3522 cm^{-1} (O-H stretching), 1231 cm^{-1} (C-O bending), and at 1609 cm^{-1}, 1512 cm^{-1}, and 1430 cm^{-1} (C-C stretching vibrations in the phenyl ring). These peaks also appeared in the spectrum of the PVP/GEL/CLEO nanofiber sample [1]. In the spectrum of EEO, there was a CH_3 symmetrical deformation peak at 1375 cm^{-1}. This peak also appeared at 1373 cm^{-1}, 1373 cm^{-1}, 1369 cm^{-1}, and 1373 cm^{-1} in the spectra of PVP/GEL/EEO1, PVP/GEL/EEO3, PVP/GEL/EEO5, and PVP/GEL/EEO7, respectively. Another characteristic C-O-C asymmetrical peak at 1215 cm^{-1} was attributed to 1,8-cineole, which is the main component of EEO. This peak occurred at 1231 cm^{-1} in PVP/GEL/EEO1, 1229 cm^{-1} in PVP/GEL/EEO3, 1224 cm^{-1} in PVP/GEL/EEO5, and 1223 cm^{-1} in PVP/GEL/EEO7 [54]. Consequently, the results from GC-MS and FT-IR analyses are compatible with one another in terms of the major components of each solution.

Figure 11 shows the comparative analysis of UV-vis spectra and absorbance values for PVP/GEL nanofibers scanned between 250 and 300 nm. The emulsion electrospun nanofibers were scanned at 280 nm and 274 nm for CLEO and EEO, respectively [55; 56]. The purpose of this UV-vis measurement is to determine the increasement tendency of essential oil into the nanofiber structure. Generally, the results were consistent with expected CLEO and EEO concentrations. In other words, the absorbance values increased with essential oil concentrations. Absorbance values of PVP/GEL/CLEO1, PVP/GEL/CLEO3 and PVP/GEL/CLEO5 were obtained at 0.244, 0.694 and 0.750, respectively at 280 nm. Similarly, absorbance values were determined at 0.047 for PVP/GEL/EEO1, at 0.096 for PVP/GEL/EEO3, at 0.143 for PVP/GEL/EEO5 and at 0.319 for PVP/GEL/EEO7. Moreover, indicated that no undesirable reactions occurred between polymer solution and additives.

3.3 Antibacterial Activity Results

Lastly, antibacterial activity of PVP/GEL nanofibers with CLEO and EEO was determined by the disc diffusion method in culture plates of *S. aureus*, *E. coli*, *P. aeruginosa*, *E. faecalis*, and *C. albicans* (Figure 12). According to the Figure 12, CLEO and PVP/GEL/CLEO nanofibers clearly showed better antibacterial activity than EEO and PVP/GEL/EEO nanofibers. It was observed that there is no zone formation for PS (polymer solution with surfactant). Moreover, pure CLEO and EEO displayed antibacterial activity proportional with their nanofiber samples, and inhibition zone sizes increased with increasing EEO and CLEO concentrations. These results support that the emulsions were prepared correctly and the essential oils successfully encapsulated into the nanofiber structure (Figure 12).

The inhibition zone sizes from disc diffusion assays are plotted in Figure 13. For all microbes tested, the largest zone diameters obtained for nanofiber samples were from PVP/GEL/CLEO5.

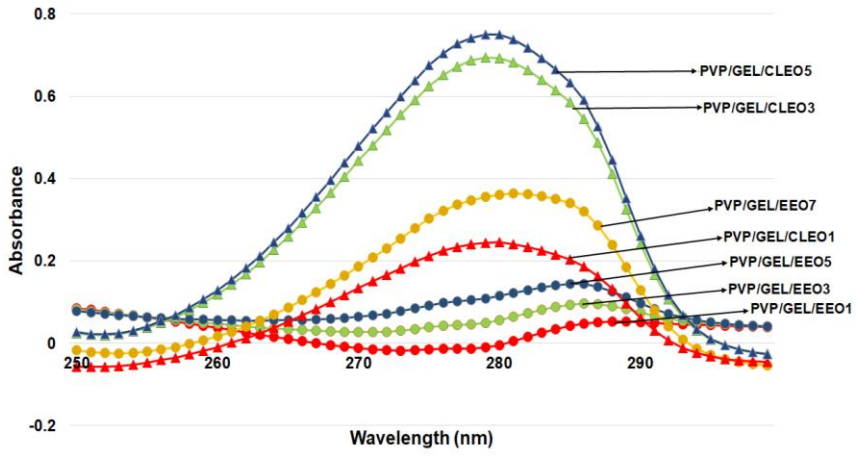


Figure 11. UV-vis spectra of CLEO and EEO in PVP/GEL nanofibers

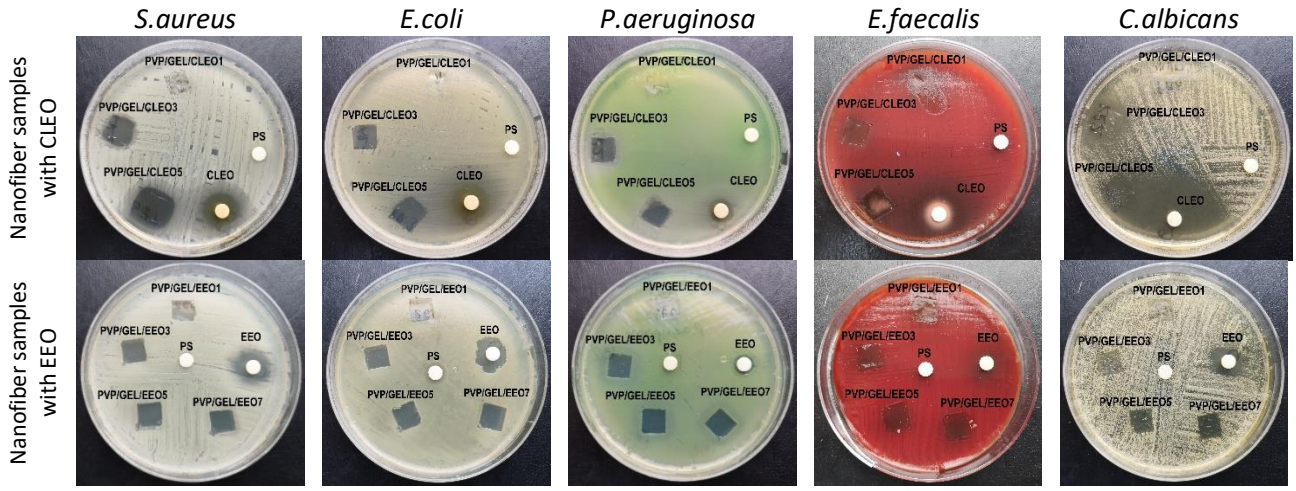


Figure 12. Images of disc diffusion assays for CLEO, EEO, PS and all nanofiber samples

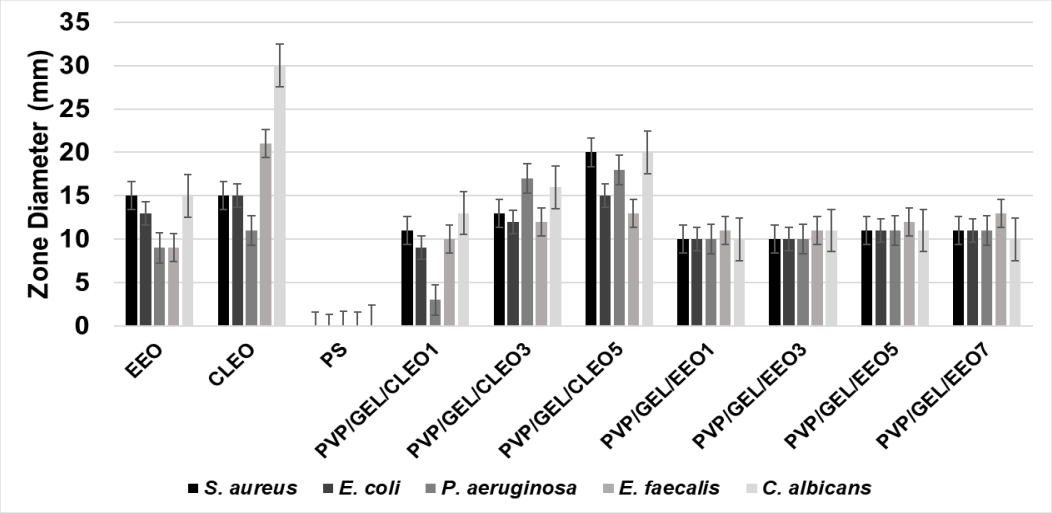


Figure 13. Comparative plot of antibacterial activity for PVP/GEL nanofibers with various concentrations of CLEO and EEO

4. CONCLUSIONS

In this study, antibacterial PVP/GEL nanofibers incorporating various concentrations of CLEO and EEO were produced by emulsion electrospinning. According to the results; eugenol and 1,8-cineole were identified as the major components of CLEO and EEO, respectively. The determination of solution properties revealed that conductivity and surface tension values decreased and viscosity increased with essential oil concentration. Fine and bead-free nanowebs were obtained with CLEO-based nanofibers, while ultra-fine and beady nanofibrous surfaces were obtained with EEO. The most uniform nanofibers were obtained with 1 wt % concentration for both essential oils. FT-IR analyses confirmed that the polymers (PVP and GEL) and essential oils (CLEO and EEO) were present as expected in the nanofiber chemical structures. Finally, in the assay of antibacterial activity, CLEO and CLEO-based nanofibers demonstrated larger inhibition zones than did EEO and EEO-based nanofibers. Of tested species, *C. albicans* was the most impacted by CLEO-based nanofibers, while *E. faecalis* was the most affected by EEO-based nanofibers. Generally, essential oils and nanofibers displayed antibacterial activities proportional with each other. The authors thought that this study will provide a bridge between essential oils and nanostructures for use in a suitable green application area for biomedical applications.

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