Research Article

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Preparation of cypress (*Cupressus sempervirens* L.) essential oil loaded poly(lactic acid) nanofibers

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Abstract: This study investigated the production of poly (lactic acid) (PLA) nanofibers containing cypress (CUP) essential oil (EO) via electrospinning. The nanofibers were produced from polymer solution prepared with different percentages of cypress EO. Cypress EO-containing PLA nanofibers were characterized and some mechanical and thermal properties were examined using thermogravimetric analysis, scanning electron microscopy, Fourier-transform infrared spectroscopy, and dynamic mechanical analysis. The thermal stability of the nanofibers was reduced depending on the percentage of the cypress EO. As the ratio of the cypress EO to polymer matrices was increased, it was observed that the glassy transition temperatures of the nanofibers decreased and their flexibility increased. The T_g value was determined to be 53.74°C for the neat PLA nanofiber, while 51.83°C for the PLA-CUP nanofiber (containing 15% cypress EO). According to the results of releasing trial, the increased amount of cypress EO resulted in less cypress EO releasing from polymer matrices. The nanofibers were observed to exhibit antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The inhibition zone diameter of the nanofibers containing 10% cypress EO was 20 mm for *S. aureus* and 16 mm for *E. coli*, while 10 mm in the presence of Kanamycin.

Keywords: poly(lactic acid), cypress essential oil, electrospinning, nanofibers, controlled release, antibacterial activity

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1 Introduction

Electrospinning produces nanofibers by electrostatic spinning. This method is performed using a jet of polymer solution by means of an electric area to produce synthetic fibers. These fibers typically have different diameters ranging between a few nanometers to a few micrometers [1–4] that have attracted the interest of the scientific community [5]. Among the applications where nanofibers are exploited are nanocatalysis, tissue engineering, protective textile manufacturing, filtration, biomedical, pharmacy, optics, electronics, production of medical equipment, and environmental engineering [6–12]. In recent years, the synthetic additives used in many fields (pharmaceutical, cosmetics, food, agriculture, etc.) have raised much health-related concerns over their side effects [13]. Hence, natural alternatives such as essential oils (EOs), which are classified as "safe" by the United States Food and Drug Administration (FDA) [14], are needed to replace them. EOs have been studied by extensive research to demonstrate their biological properties. Today, different antibacterial compounds such as EOs [15] are introduced in PLA matrices to produce new films with antibacterial

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properties. In addition, the research on the improvement of PLA-based materials with antibacterial properties has experienced a great boom to extend the shelf life and to increase food quality [16]. PLA has attracted great attention due to its production from renewable resources, biocompatibility, biodegradability, moderate mechanical performance, and transparency [17]. The addition of EOs to PLA films has been shown to result in certain physicochemical modifications in pristine polymer matrices [18,19]. Mori et al. [19] report that the incorporation of candeia oil in PLA fibers leads to decreased glassy transition temperatures (T_g) of 30% (15% EO). Souza et al. have observed an 18% reduction in the T_g of PLA fibers in the presence of 20% linalool [18]. The research cited below reports that natural extracts from bioactive plants could potentially be used as plasticizers for PLA, which reflects the mobility of polymer chains and minimizes the rate of interactions between the chains, also observable in the case of PLA-EO films [15]. Cypress (*Cupressus sempervirens* L.) is the only species of Cupressus existing in Tunisia. In fact, this medicinal plant is an ornamental tree from the Cupressaceae family. It grows at high altitudes in different geographies, such as North America, the Mediterranean region, and subtropical Asia. The basic use of this plant is to protect agricultural fields from wind [20].

C. sempervirens EOs have antiseptic, aromatherapeutic, astringent, and anti-inflammatory activities and antispasmodic, astringent, deodorant, and diuretic effects. The fruit of this species is known for its ability to cure diabetes and its antiseptic activity [21–24]. The EO of *C. sempervirens* L. exhibits antimicrobial properties, and so it has potential as a natural antimicrobial agent in human infectious diseases as well as food preservation. In addition, the development of natural antimicrobial agents helps to reduce the negative effects (environmental pollution, resistance) of synthetic chemicals and drugs [25]. Many secondary plant metabolites have been shown to exhibit insecticidal properties. It is known that plants are used to kill or repel insects [26]. The EOs have harmful, anti-agent, and anti-feeding effects on stored product insect pests [27]. The richness of the *C. sempervirens* L. EOs in α-pinene has been confirmed by several authors in different countries, such as Iran (30%) [34], Algeria (44.9%) [35], Morocco (60%) [36], Italy (31%) [37], Egypt (6.9%) [38], and Saudi Arabia (48.6%) [39].

This study aims to produce biopolymer nanofiber matrices with improved thermal, mechanic, and antibacterial properties by introducing natural contents into these matrices. Therefore, cypress EO as an active natural substance and PLA as a biopolymer matrix have been chosen. The different amounts of cypress EO were added into PLA matrices. The prepared nanofibers were characterized by considering some of their properties, such as thermal and mechanical properties, antibacterial properties, and releasing behaviors.

2 Materials and methods

2.1 Materials

Poly(lactic acid) (PLA) was purchased from Natureworks LLC (Nebraska, USA). Dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), and tetrahydrofuran (THF) were used as solvents. The cypress EO extracted by the hydrodistillation method [28] was used as the natural antibacterial agent. The GC-MS analyses [64] revealed the predominant compounds of this EO to be α-pinene (42%), δ-3-carene (21.26%), limonene (5.96%), and α-terpinolene (4.86%).

2.2 Preparation of the EO-containing PLA (PLA-CUP) nanofibers

The formulation was prepared for electrospinning as modified from previous studies [29–31], which shows the capacity of the EO incorporation in solvents for electrospinning. PLA (0.4 g) was dissolved in a mixture of solvents (3 mL of dichloromethane, 1 mL of dimethylformamide, and 1 mL of tetrahydrofuran) with magnetic stirring at room temperature at 2 h. The cypress oils were added to the PLA solution with different weight percentages, i.e., 7.5, 10, and 15% (w/w), and then stirred for 1 h. The prepared polymer solutions were introduced in a 5 mL syringe. The injection of the solution was carried out with a constant flow of 1.5 mL/h. To electrospun the solutions, a voltage of 15 kV and a separation distance of 15 cm was employed.

2.3 Characterization of the PLA-CUP nanofibers

Poly(lactic acid) nanofibers can be characterized by considering some mechanical and thermal properties using thermogravimetric analysis (TGA), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and dynamic mechanical analysis (DMA). In this

study, the characterization methods listed below were used for the characterization of the PLA-CUP nanofibers.

2.3.1 Scanning electron microscopy

The morphology of the nanofibers containing neat PLA and PLA-CUP nanofibers was characterized using an SEM (JEOL JSM-7100-F) with 15 kV working voltage as modified from Cesur et al. [61] and Yang et al. [63].

2.3.2 Fourier-transform infrared spectral analysis

FTIR is a qualitative and quantitative analytical technique for identifying functional groups that appear as an absorption band. It is used to subject a molecule to IR radiation. The IR spectra of the neat PLA and PLA-CUP nanofibers, ranging from 600 to 4,000 cm^{-1}, were recorded using the PerkinElmer's FTIR "spectrum one" model, controlled by a computer equipped with a processing software program featuring a resolution of 2 cm^{-1} [62].

2.3.3 Thermogravimetric analysis

TGA is a technique of thermal analysis adopted to measure the change in the mass of a sample as a function of time at a temperature or at a specified temperature. The analysis was performed with PerkinElmer TGA 8000. The thermal stabilities of the PLA and PLA-CUP nanofibers were analyzed according to the modified version of the procedure in Yang et al. [63]. A 3 mg sample test was conducted in a nitrogen atmosphere at a heating rate of 10°C/min at 30–550°C.

2.3.4 Dynamic mechanical analysis

DMA of the PLA and PLA-CUP nanofibers containing EO was performed on a PerkinElmer DMA 8000 by employing the modified process available in Yang et al. [63]. The samples were heated from 30 to 100°C at 1 Hz at a heating rate of 3°C/min.

2.4 *In-vitro* release studies

The cypress EO content of the various electrospun samples was calculated according to a calibration curve. This calibration curve was prepared by dissolving the EOs in ethanol, covering a broad range of their concentrations (2.5–20 ppm), and then applying a linear regression (R^2 = 0.9994). Next, the solution absorbance was examined at 203.8 nm for the cypress EOs. In a microtube, 20 mg of the nanofibers at different obtained concentrations was dissolved in 5 mL of 60% phosphate-buffered saline (PBS) (pH = 7.4) + 40% ethanol [32] and incubated at room temperature. At well-determined time intervals, the supernatant of each sample was measured by a UV-visible spectrophotometer.

The ratio of the PBS and ethanol was 60:40. About 600 mL of PBS and 400 mL of absolute ethanol were mixed in a volumetric flask of 1 L. The PBS was prepared by dissolving 8 g of NaCl, 2.0 g of KCl, 14.4 g of Na_2HPO_4, and 2.4 g of KH_2PO_4 in 800 mL of distilled water. After the solution was mixed thoroughly, the pH was regulated to 7.4.

The cumulative amount of the cypress EO released from the PLA-CUP nanofibers was determined by measuring the cypress EO concentrations (ppm) in the release medium at specific time intervals using the UV-visible spectrophotometer at 208.3 nm and converted to the released amount (μg) considering the volume of the release medium (mL). The cumulative percentage of the released cypress EO was represented by the formula (1):

$$\text{Cumulative release percentage} = \sum_{t=0}^{t} \frac{M_t}{M_0} \times 100 \quad (1)$$

M_t: Cumulative amount of the cypress EO released at each sampling point. M_0: Initial weight of the cypress EO loaded in the sample.

2.5 Antibacterial activities of the nanofibers

The antibacterial activity of the nanofibers was qualitatively tested by the disc diffusion method against the Gram (+) strains *S. aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212 and the Gram (−) strains *Pseudomonas aeruginosa* PA01 and *E. coli* ATCC 25922. Initially, 100 μL of the bacterial suspension (0.5 McFarland turbidity) was spread on the Luria Bertani Agar (LBA) plate. Then, the nanofibers (diameter = 15 mm) were placed on the LBA plate and incubated at 37°C for 24 h. Finally, the diameters of the inhibition zones around the nanofibers were measured [33].

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 SEM analysis

The structure of the PLA-CUP nanofibers (10%) was analyzed by SEM. Using the SEM observations, an examination of the modifications in the morphology of the PLA nanofibers was performed after introducing the cypress EO. Usually, the surface morphology of a neat PLA is smooth and homogeneous and contains no pores and cracks [40]. According to the SEM images of the neat PLA and PLA-CUP nanofibers in Figure 1, the diameters of the nanofibers

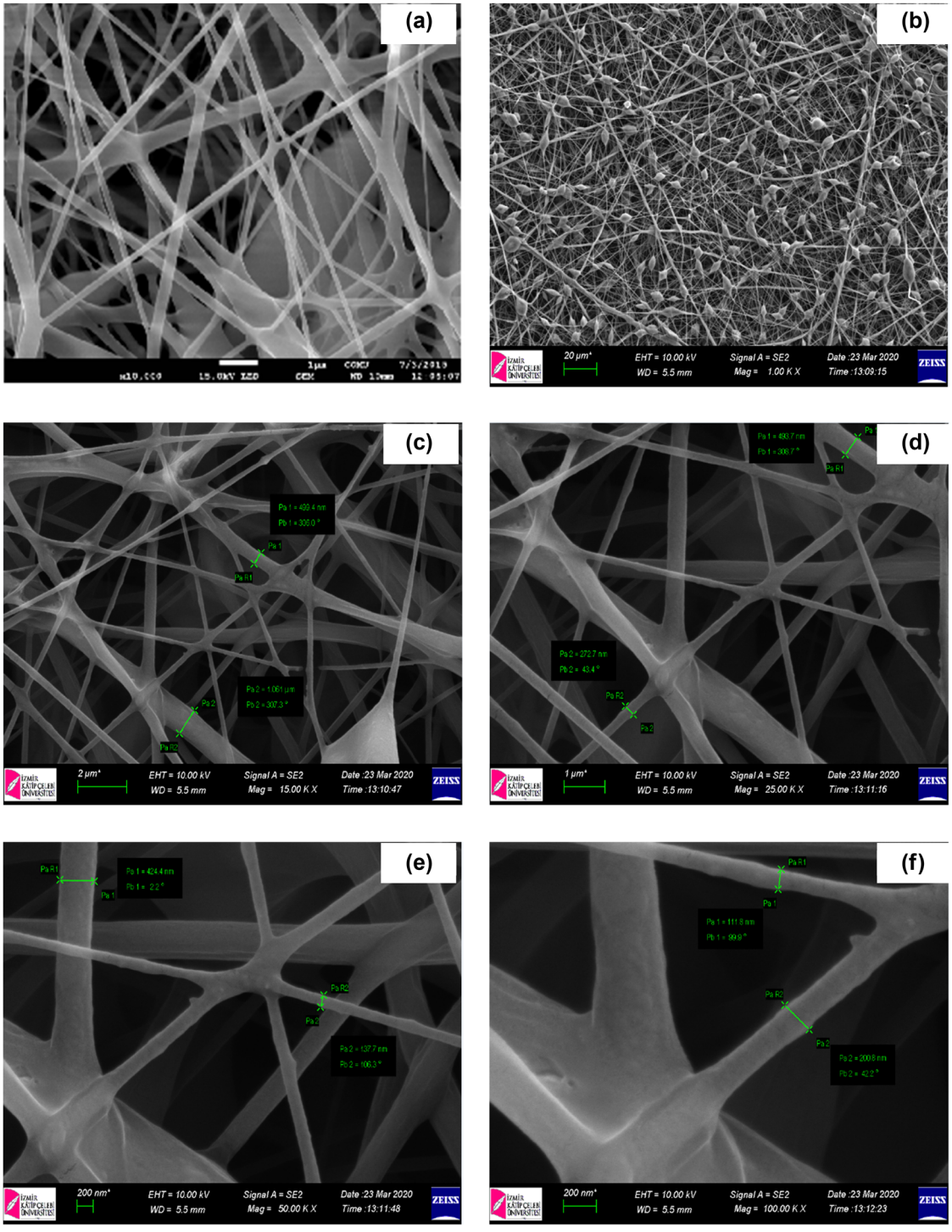

Figure 1: SEM images of the nanofibers (a) neat PLA, (b–f) PLA-CUP nanofibers.

ranged between 111 and 1,061 nm. The average diameter of the neat PLA nanofibers was 607 ± 197 nm and the PLA-CUP was 400 ± 288 nm. As these results indicated, the diameters of the neat PLA nanofibers were larger than those of EO-loaded PLA nanofibers [40].

3.2 FTIR analysis

According to the FTIR spectra of the neat PLA nanofibers in Figure 2, the peaks at 2.990 and 2.940 cm^{-1} were attributed to –CH– stretching, the peak at 1.760 cm^{-1} to C═O carbonyl group, the peaks at 1.180 and 1.080 cm^{-1} to –C–O– stretching, and the peak at 860 cm^{-1} to –C–COO stretching [42]. The FTIR spectra of PLA-CUP showed the presence of the functional groups' characteristics of this type of nanofiber. The band of high-intensity fine at around 3.350 cm^{-1}, which we could attribute to the free OH vibrators, was observed in the FTIR graph. The existence of absorption bands attributed to the two modes of vibration of symmetrical elongation at around 2.980 cm^{-1} of the CH_2 and asymmetrical at around 2.920 cm^{-1} of CH_3 groups. The two bands of the group –CH at around 1.470 and 1.380 cm^{-1} were also observed in the FTIR graph [43]. The band at 765 cm^{-1} represents the alkene bond of C═C.

3.3 TGA of the neat PLA and PLA-CUP (15%) nanofibers

According to Figure 3, the neat PLA nanofiber showed a single-step process decomposition [44]. The incorporation of EO into the polymer matrix resulted in additional degradation maximums. These additional peaks, which

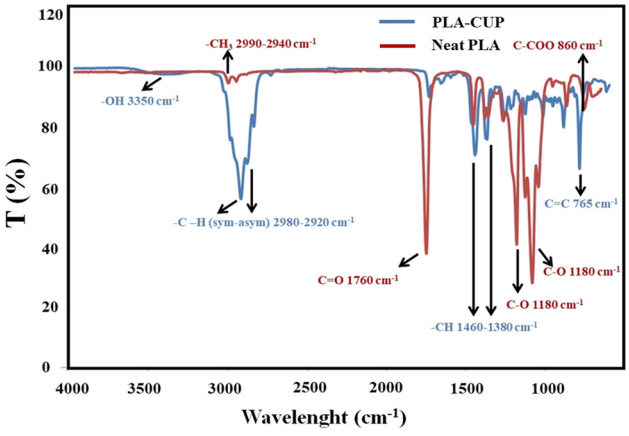

Figure 2: FT-IR spectrum of neat PLA and PLA-CUP nanofibers.

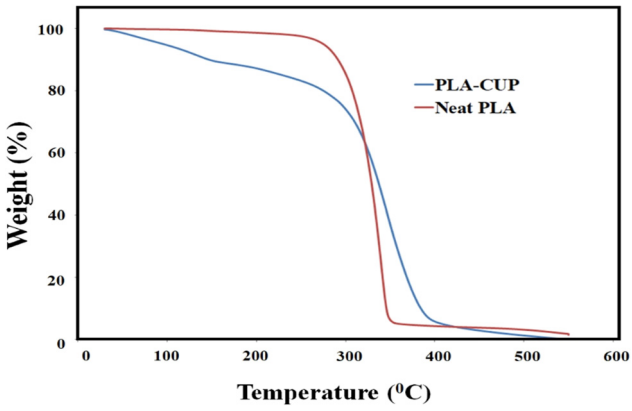

Figure 3: TGA curves of neat PLA and PLA-CUP (% 15) nanofibers.

were observed at around 150 and 230°C, belonged to the EO in the matrix, and these peaks corresponded to the degradation and evaporation of EO. The weight loss at around 70°C in the derivative TGA graph suggests the evaporation of the adsorbed water. The maximum degradation rate of the PLA nanofiber is 337.90°C. This peak shifted to 345.06°C because of the incorporation of the EO. The thermal stability of the PLA nanofiber was improved with the added EO [44]. Previous works have shown that the incorporation of EO has resulted in decreased thermal stability of polymer matrices [58–60].

3.4 Dynamic mechanical analysis

As clear from Figure 4, the T_g value was 53.74°C for the neat PLA nanofiber, while the T_g value was 51.83°C for the PLA-CUP nanofiber (containing 15% EO). The PLA-CUP nanofibers exhibited a lower T_g than the neat polymer for both samples. The insertion of EO into the PLA matrix leads to decreased T_g and increased elasticity [15]. The change range of T_g for PLA mats has been reported between 17 and 35°C for low molecular weight molecules, such as citrates, terpenes, and oil [45–47]. The introduction of fillers potentially acting as plasticizers increase polymer chain mobility and polymer-free volume and decreases T_g of polymer mats [45,48,49]. It can also be observed that the elasticity of the PLA nanofiber mats has been increased by adding EOs. The EOs behave as plasticizers, which leads to increased free volume and decreased interactions between polymer chains. These changes lead to improved chain mobility and flexibility [49]. Zhang et al. [47] reported that the EOs work as plasticizers for PLA, lowering the glassy transition temperature of EO-containing composite fibers by up to 60% and increasing elongation-at-break and tensile strength up to 12 times.

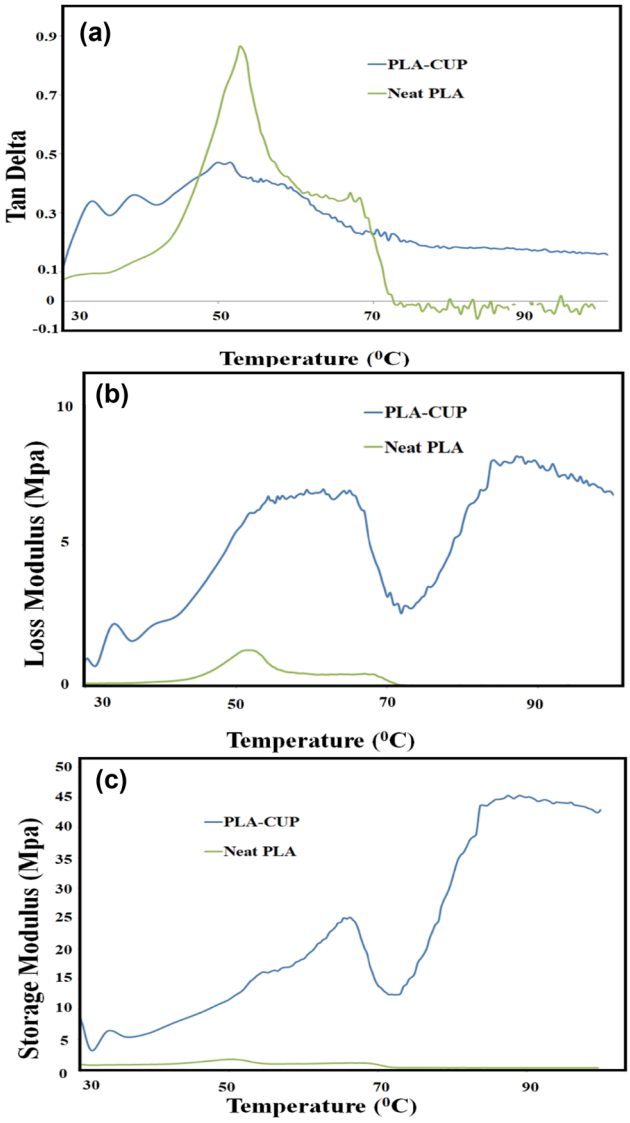

Figure 4: Thermal properties (a) Tan Delta, (b) Loss Modukus, (c) Storage Modulus of the neat PLA and the 15% EO added PLA-CUP nanofibers.

3.5 *In-vitro* release studies

The releasing behavior of the PLA-CUP nanofibers is shown in Figure 5. The PLA-CUP nanofibers were released in about 70 h. In 7 h, burst releases were observed for the PLA-CUP nanofibers. According to the results, the lowest percentage of EO (7.5%) in the nanofibers exhibited the best releasing behavior and the amount of releasing decreased as the EO percentage increased. This ratio corresponded to approximately 60% for the nanofibers containing 10% EO and to around 50% for the nanofibers containing 15% EO. About 10% EO-containing PLA nanofibers have been reported to release approximately 50% more EO than 15% EO-containing PLA nanofibers [50].

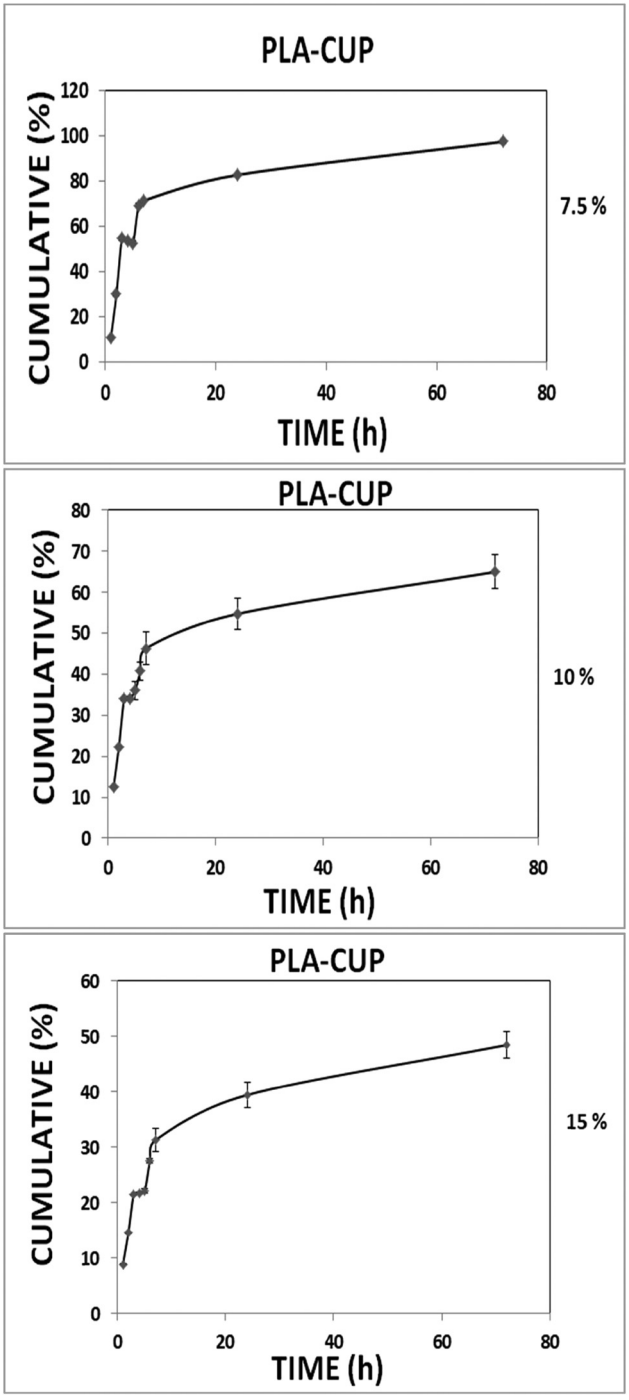

Figure 5: Releasing behavior of the different amount (7.5, 10, and 15%) of cypress EO loaded.

3.6 Antibacterial activities of the PLA-CUP nanofibers

To the authors' best knowledge, no previous research has been reported on the antibacterial activities of PLA-CUP nanofibers. The antibacterial activities of the PLA-CUP

Table 1: The antibacterial activity of the cypress EO-loaded nanofibers

	Inhibition zone diameters (mm)			
(Nanofibers 1 cm^2)	*S. aureus*	*E. faecalis*	*E. coli*	*P. aeruginosa*
Cypress EO (7.5%)	*	*	*	*
Cypress EO (10%)	20 ± 1.1	*	16 ± 1.2	*
Cypress EO (15%)	22 ± 2.1	*	25 ± 2.2	*
Kanamycin (5 µg)	10 ± 1.1	9 ± 0.7	11 ± 1.1	9 ± 0.7

*: No inhibition.

nanofibers at different concentrations (7.5, 10, and 15%) against four bacterial strains were tested by the agar diffusion method. The diameters of the inhibition zones were found to have varied depending on the tested bacterial strain (Table 1).

The study of the neat PLA nanofibers resulted in no antimicrobial property against the researched strains. These results were confirmed by other studies [51–55], which have shown no antimicrobial property for the neat PLA film [51–55]. It may be thought that the hydrophobic nature of pure PLA has limited its antimicrobial property [56]. The results of the current work showed that the PLA-CUP nanofibers exhibited a moderate inhibitory activity at 10% EO concentration against the two tested strains (*S. aureus* 20 ± 1.1 mm and *E. coli* 16 ± 1.2 mm). At this concentration, *E. coli* is a Gram (−) bacterium, which was sensitive to the PLA-CUP nanofibers. At 15% EO concentration, the PLA-CUP nanofibers demonstrated a moderate inhibitory activity against the tested strains (*S. aureus* 22 ± 2.1 mm and *E. coli* 25 ± 2.2 mm). *S. aureus* is a Gram (+) bacterium, which was sensitive to the PLA-CUP nanofibers. At 7.5% EO concentration, the PLA-CUP nanofibers exert no inhibitory effect on all the four tested strains. *C. sempervirens* L. EO mainly contains monoterpene hydrocarbons (91.98%), incorporating α-pinene as the main constituent (42%) and δ-3-carene (21.26%) as the second most prominent constituent. In fact, cypress EO has been reported to show antibacterial activity due to the α-pinene therein. This oil has antioxidant and antiseptic properties that may account for its antibacterial activity [57].

4 Conclusion

The chemical analyses [64] revealed that cypress EO contains 22 components. The components with the highest ratios were α-pinene (42%), δ-3-carene (21.26%), limonene (5.96%), and α-terpinolene (4.86%). The antibacterial effects of cypress EO may have originated from these components. The thermal stability of the mats was determined to have decreased with the added cypress EO. In the cypress EO-containing, PLA nanofiber mats were observed to undergo a two-step decomposition process. The T_g value decreased as the elasticity of the final mats was increased by adding cypress EO to the polymer matrices. The cypress EO-loaded nanofibers showed antibacterial activity against Gram (+) (*S. aureus*) and Gram (−) bacterium (*E. coli*). The inhibition zone diameter of the nanofibers contained 10% cypress oil was 20 mm for *S. aureus* and 16 mm for *E. coli*, while it was 10 mm for Kanamycin. The "good" releasing behavior of the 7.5% EO-containing nanofibers suggests that the active ingredient can make a significant contribution to drug design by reducing manufacturing costs. Besides, the reduced release with an increase in the amount of EO additives may offer benefits for long-term controlled drug release. As a result of the present study, it was understood that cypress EO can act as a plasticizer when inserted into a PLA matrix. All in all, cypress EO can be regarded as a natural antibacterial agent besides its capacity to increase the flexibility of PLA nanofibers.

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Conflict of interest: The authors declare no conflict of interest.

Data availability statement: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

[1] Greiner A, Wendorff JH. Cover picture: electrospinning: a fascinating method for the preparation of ultrathin fibers. Angew Chem Int Ed Engl. 2007;46(30):5633–703. doi: 10.1002/anie.200604646.

[2] Dzenis Y. Spinning continuous fibers for nanotechnology. Science. 2004;304(5679):1917–9. doi: 10.1126/science.1099074.

[3] Li D, McCann JT, Xia Y, Marquez M. Electrospinning: a simple and versatile technique for producing ceramic nanofibers and nanotubes. J Am Ceram Soc. 2006;89(6):1861–9. doi: 10.1111/j.1551-2916.2006.00989.x.

[4] Frenot A, Chronakis IS. Polymer nanofibers assembled by electrospinning. Curr Opin Colloid Interface Sci. 2003;8(1):64–75. doi: 10.1016/S1359-0294(03)00004-9.

[5] Ahn YC, Park SK, Kim GT, Hwang YJ, Lee CG, Shin HS, et al. Development of high efficiency nanofilters made of nanofibers. Curr Appl Phys. 2006;6(6):1030–5. doi: 10.1016/j.cap.2005.07.013.

[6] Luu Y, Kim K, Hsiao B, Chu B, Hadjiargyrou M. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA–PEG block copolymers. J Control Rel. 2003;89(2):341–53. doi: 10.1016/S0168-3659(03)00097-X.

[7] Subbiah T, Bhat GS, Tock RW, Parameswaran S, Ramkumar SS. Electrospinning of nanofibers. J Appl Polym Sci. 2005;96(2):557–69. doi: 10.1002/app.21481.

[8] Ramakrishna S, Fujihara K, Teo WE, Yong T, Ma Z, Ramaseshan R. Electrospun nanofibers: solving global issues. Mater Today. 2006;9(3):40–50. doi: 10.1016/S1369-7021(06)71389-X.

[9] Taepaiboon P, Rungsardthong U, Supaphol P. Drug-loaded electrospun mats of poly(vinyl alcohol) fibres and their release characteristics of four model drugs. Nanotechnology. 2006;17(9):2317–29.

[10] Lee KY, Jeong L, Kang YO, Lee SJ, Park WH. Electrospinning of polysaccharides for regenerative medicine. Adv Drug Deliv Rev. 2009;61(12):1020–32. doi: 10.1016/j.addr.2009.07.006.

[11] Barnes CP, Pemble CW, Brand DD, Simpson DG, Bowlin GL. Cross-linking electrospun type II collagen tissue engineering scaffolds with carbodiimide in ethanol. Tissue Eng. 2007;13(7):1593–605. doi: 10.1089/ten.2006.0292.

[12] Welle A, Kröger M, Döring M, Niederer K, Pindel E, Chronakis IS. Electrospun aliphatic polycarbonates as tailored tissue scaffold materials. Biomaterials. 2007;28(13):2211–9. doi: 10.1016/j.biomaterials.2007.01.024.

[13] Reyes-Jurado F, Franco-Vega A, Ramírez-Corona N, Palou E, López-Malo A. Essential oils: antimicrobial activities, extraction methods, and their modeling. Food Eng Rev. 2015;7(3):275–97. doi: 10.1007/s12393-014-9099-2.

[14] López P, Sánchez C, Batlle R, Nerín C. Development of flexible antimicrobial films using essential oils as active agents. J Agric Food Chem. 2007;55(21):8814–24. doi: 10.1021/jf071737b.

[15] Qin Y, Li W, Liu D, Yuan M, Li L. Development of active packaging film made from poly(lactic acid) incorporated essential oil. Prog Org Coat. 2017;103:76–82. doi: 10.3390/molecules22071170.

[16] Wang H, Liu H, Chu C, She Y, Jiang S, Zhai L, et al. Diffusion and antibacterial properties of nisin-loaded chitosan/poly(L-lactic acid) towards development of active food packaging film. Food Bioproc Tech. 2015;8(8):1657–67. doi: 10.1007/s11947-015-1522-z.

[17] Auras LLT, Selke SEM, Tsuji H, editors. Poly(lactic acid): synthesis, structures, properties, processing, and applications. 1st ed. New York: Wiley; 2010.

[18] Souza MA, Oliveira JE, Medeiros ES, Glenn GM, Mattoso LH. Controlled release of linalool using nanofibrous membranes of poly(lactic acid) obtained by electrospinning and solution blow spinning: a comparative study. J Nanosci Nanotechnol. 2015;15(8):5628–36. doi: 10.1166/jnn.2015.9692.

[19] Mori CLSO, dos Passos NA, Oliveira JE, Altoé TF, Mori FA, Mattoso LHC, et al. Nanostructured polylactic acid/candeia essential oil mats obtained by electrospinning. J Nanomater. 2015;2015:1–9. doi: 10.1155/2015/439253.

[20] Castells AA, Danti R, Llusia J, Rocca GD, Barberini S, Penuelas J. Strong induction of minor terpenes in Italian cypress, Cupressus sempervirens, in response to infection by the fungus Seiridium cardinale. J Chem Ecol. 2015;41(3):224–43. doi: 10.1007/s10886-015-0554-1.

[21] Bruni A, Ballero M, Poli F. Quantitative ethnopharmacological study of the Campidano valley and Urzulei district, Sardinia, Italy. J Ethnopharmacol. 1997;57(2):97–124. doi: 10.1016/S0378-8741(97)00055-X.

[22] Duke JA, Godwin MB, duCellier J, Duke PAK. Handbook of medicinal herbs. 2nd ed. Washington: CRC press; 2002.

[23] Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. J Ethnopharmacol. 2002;83(3):251–65. doi: 10.1016/S0378-8741(02)00253-2.

[24] Manganelli REU, Camangi F, Tomei PE. Curing animals with plants: traditional usage in Tuscany (Italy). J Ethnopharmacol. 2001;78(2–3):171–91. doi: 10.1016/S0378-8741(01)00341-5.

[25] Mazari K, Bendimerad N, Bekhechi C, Fernandez X. Chemical composition and antimicrobial activity of essential oils isolated from Algerian Juniperus phoenicea L. and Cupressus sempervirens L. J Med Plants Res. 2010;4:959–64. doi: 10.5897/JMPR10.169.

[26] Broussalis AM, Ferraro GE, Martino VS, Pinzón R, Coussio JD, Alvarez JC. Argentine plants as potential source of insecticidal compounds. J Ethnopharmacol. 1999;67(2):219–23. doi: 10.1016/S0378-8741(98)00216-5.

[27] Tapondjou A, Adler C, Fontem D, Bouda H, Reichmuth C. Bioactivities of cymol and essential oils of Cupressus sempervirens and Eucalyptus saligna against Sitophilus zeamais Motschulsky and Tribolium confusum du Val. J Stored Prod Res. 2005;41(1):91–102. doi: 10.1016/j.jspr.2004.01.004.

[28] Argui H, Suner SC, Periz ÇD, Ulusoy S, Ben-Attia M, Coşkun Y, et al. Fabrication, characterization, in vitro release, and some

biological activities of eucalyptus essential oil loaded poly (lactic acid) nanofibers. Acta Sci Microbiol. 2021;4(2):54–63. doi: 10.31080/ASMI.2020.04.0768.

[29] Angeles M, Cheng HL, Velankar SS. Emulsion electrospinning: composite fibers from drop breakup during electrospinning. Polym Adv Technol. 2008;19(7):728–33. doi: 10.1002/pat.1031.

[30] Rieger KA, Schiffman JD. Electrospinning an essential oil: Cinnamaldehyde enhances the antimicrobial efficacy of chitosan/poly(ethylene oxide) nanofibers. Carbohydr Polym. 2014;113:561–8. doi: 10.1016/j.carbpol.2014.06.075.

[31] de Oliveira Mori CLS, dos Passos NA, Oliveira JE, Mattoso LHC, Mori FA, Carvalho AG, et al. Electrospinning of zein/tannin bionanofibers. Ind Crop Prod. 2014;52:298–304. doi: 10.1016/j.indcrop.2013.10.047.

[32] Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F. Two-step method for encapsulation of oregano essential oil in chito.san nanoparticles: preparation, characterization and in vitro release study. Carbohydr Polym. 2013;95(1):50–6. doi: 10.1016/j.carbpol.2013.02.031.

[33] Hufford C, Funderburk M, Morgan J, Robertson L. Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera* L. J Pharm Sci. 1975;64(5):789–92. doi: 10.1002/jps.2600640512.

[34] Khayyat MH, Emami SA, Rahimizadeh M, Bazzaz BSF, Assili J. Chemical constituents of Cupressus sempervirens L. cv. Cereiformis Rehd. essential oils. Iran J Pharm Sci. 2005;1(1):39–42.

[35] Chanegriha N, Baâliouamer A, Meklati BY, Bonvin JF, Alamercery S. Chemical composition of Algerian cypress essential oil. J Essent Oil Res. 1993;5(6):671–4. doi: 10.1080/10412905.1993.9698304.

[36] Rebbas K, Bounar R, Gharzouli R, Ramdani M, Djellouli Y, Alatou D. Plantes d'intérêt médicinale et écologique dans la région d'Ouanougha (M'sila, Algérie). Phytothérapie. 2012;10:131–42. doi: 10.1007/s10298-012-0701-6.

[37] Romeo FV, De Luca S, Piscopo A, Poiana M. Antimicrobial effect of some essential oils. J Essent Oil Res. 2008;20(4):373–9. doi: 10.1080/10412905.2008.9700034.

[38] Elansary HO, Salem MZ, Ashmawy NA, Yacout MM. Chemical composition, antibacterial and antioxidant activities of leaves essential oils from *Syzygium cumini* L., *Cupressus sempervirens* L. and *Lantana camara* L. from Egypt. J Agric Sci. 2012;4(10):144–52. doi: 10.5539/jas.v4n10p144.

[39] Selim SA, Adam ME, Hassan SM, Albalawi AR. Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (Cupressus sempervirens L.). BMC Compl Alternat Med. 2014;14(179):1–8. doi: 10.1186/1472-6882-14-179.

[40] Javidi Z, Hosseini SF, Rezaei M. Development of flexible bactericidal films based on poly(lactic acid) and essential oil and its effectiveness to reduce microbial growth of refrigerated rainbow trout. LWT-Food Sci Technol. 2016;72:251–60. doi: 10.1016/j.lwt.2016.04.052.

[41] Pankongadisak P, Sangklin S, Chuysinuan P, Suwantong O, Supaphol P. The use of electrospun curcumin-loaded poly(L-lactic acid) fiber mats as wound dressing materials. J Drug Deliv Sci Technol. 2019;53:101–21. doi: 10.1016/j.jddst.2019.06.018.

[42] Kister G, Cassanas G, Vert M. Effects of morphology, conformation and configuration on the IR and Raman spectra of various poly(lactic acid)s. Polymer. 1998;39(2):267–73. doi: 10.1016/S0032-3861(97)00229-2.

[43] Pradhitaa M, Masruri M, Rahmana MF. Study catalytic oxidation of α-pinene using hydrogen peroxide-iron(III) chloride. Proceedings of the Conference of ICPESS. Indonesia; 2015 May 20–22.

[44] Tawakkal IS, Cran MJ, Miltz J, Bigger SW. A review of poly(lactic acid)-based materials for antimicrobial packaging. J Food Sci. 2014;79:1477–90. doi: 10.1111/1750-3841.12534.

[45] Mekonnen T, Mussone P, Khalil H, Bressler D. Progress in bio-based plastics and plasticizing modifications. J Mater Chem A. 2013;1(43):13379–98. doi: 10.1039/C3TA12555F.

[46] Arrieta MP, López J, Ferrándiz S, Peltzer MA. Characterization of PLA-limonene blends for food packaging applications. Polym Test. 2013;32(4):760–8. doi: 10.1016/j.polymertesting.2013.03.016.

[47] Zhang W, Huang C, Kusmartseva O, Thomas NL, Mele E. Electrospinning of polylactic acid fibres containing tea tree and manuka oil. React Funct Polym. 2017;117:106–11. doi: 10.1016/j.reactfunctpolym.2017.06.013.

[48] Nagarajan V, Mohanty AK, Misra M. Perspective on polylactic acid (PLA) based sustainable materials for durable applications: Focus on toughness and heat resistance. ACS Sustain Chem Eng. 2016;4(6):2899–916. doi: 10.1021/acssuschemeng.6b00321.

[49] Vieira MGA, da Silva MA, dos Santos LO, Beppu MM. Natural-based plasticizers and biopolymer films: a review. Eur Polym J. 2011;47(3):254–63. doi: 10.1016/j.eurpolymj.2010.12.011.

[50] Liu Y, Liang X, Zhang R, Lan W, Qin W. Fabrication of electrospun polylactic acid/cinnamaldehyde/β-cyclodextrin fibers as an antimicrobial wound dressing. Polymers. 2017;9(10):464–78. doi: 10.3390/polym9100464.

[51] Erdohan ZÖ, Çam B, Turhan KN. Characterization of antimicrobial polylactic acid based films. J Food Eng. 2013;119(2):308–15. doi: 10.1016/j.jfoodeng.2013.05.043.

[52] Jin T, Zhang H. Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging. J Food Sci. 2008;73(3):M127–34. doi: 10.1111/j.1750-3841.2008.00681.x.

[53] Jin T. Inactivation of Listeria monocytogenes in skim milk and liquid egg white by antimicrobial bottle coating with polylactic acid and nisin. J Food Sci. 2010;75(2):M83–8. doi: 10.1111/j.1750-3841.2009.01480.x.

[54] Liu L, Finkenstadt V, Liu CK, Jin T, Fishman M, Hicks K. Preparation of poly(lactic acid) and pectin composite films intended for applications in antimicrobial packaging. J Appl Polym Sci. 2007;106(2):801–10. doi: 10.1002/app.26590.

[55] Llana-Ruiz-Cabello M, Pichardo S, Bermúdez JM, Baños A, Núñez C, Guillamón E, et al. Development of PLA films containing oregano essential oil (Origanum vulgare L. virens) intended for use in food packaging. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2016;33(8):1374–86. doi: 10.1080/19440049.2016.1204666.

[56] Rhim JW, Hong SI, Ha CS. Tensile water vapor barrier and antimicrobial properties of PLA/nanoclay composite films. LWT-Food Sci Technol. 2009;42(2):612–7. doi: 10.1016/j.lwt.2008.02.015.

[57] Bouaoun D, Hilan C, Garabeth F, Sfeir R. Étude de l'activité antimicrobienne de l'huile essentielle d'une plante sauvage Prangos asperula Boiss. Phytothérapie. 2007;5(3):129–34. doi: 10.1007/s10298-007-0238-2.
[58] Akgün M, Başaran İ, Suner SC, Oral A. Geraniol and cinnamaldehyde as natural antibacterial additives for poly(lactic acid) and their plasticizing effects. J Polym Eng. 2020;40:38–48. doi: 10.1515/polyeng-2019-0198.
[59] Yahyaoui M, Gordobil O, Herrera DR, Abderrabba M, Labidi J. Development of novel antimicrobial films based on poly(lactic acid) and essential oils. React Funct Polym. 2016;109:1–8. doi: 10.1016/j.reactfunctpolym.2016.09.001.
[60] Qin Y, Yang J, Xue J. Characterization of antimicrobial poly(lactic acid)/poly(trimethylene carbonate) films with cinnamaldehyde. J Mater Sci. 2015;50:1150–8. doi: 10.1007/s10853-014-8671-8.
[61] Cesur S, Oktar FN, Ekren N, Kilic O, Alkaya DB, Seyhan SA, et al. Preparation and characterization of electrospun polylactic acid/sodium alginate/orange oyster shell composite nanofiber for biomedical application. J Aust Ceram Soc. 2020;56:533–43. doi: 10.1007/s41779-019-00363-1.
[62] Khan SA, Khan SB, Khan LU, Farooq A, Akhtar K, Asiri AM. Fourier transform infrared spectroscopy: Fundamentals and application in functional groups and nanomaterials characterization. In: Sharma S, editors. Handbook of materials characterization. Cham: Springer; 2018. doi: 10.1007/978-3-319-92955-2_9.
[63] Yang Z, Li X, Si J, Cui Z, Peng K. Morphological, mechanical and thermal properties of poly(lactic acid) (PLA)/cellulose nanofibrils (CNF) composites nanofiber for tissue engineering. J Wuhan Univ Technol-Mat Sci Ed. 2019;34:207–15. doi: 10.1007/s11595-019-2037-7.
[64] Argui H, Ben Youchret-Zalleza O, Suner SC, Periz ÇD, Türker G, Ulusoy S, et al. Isolation, chemical composition, physicochemical properties, and antibacterial activity of *Cupressus sempervirens* L. JEOBP. 2021. doi: 10.1080/0972060X.2021.1924083.