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An In Vitro Study of Antibacterial Properties of Electrospun *Hypericum perforatum* Oil-Loaded Poly(lactic Acid) Nonwovens for Potential Biomedical Applications

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Featured Application: The electrospun mats produced may have biomaterial potentials such as therapeutic compound delivery (essential oils) and wound dressings.

Abstract: The growth of population and increase in diseases that cause an enormous demand for biomedical material consumption is a pointer to the pressing need to develop new sustainable biomaterials. Electrospun materials derived from green polymers have gained popularity in recent years for biomedical applications such as tissue engineering, wound dressings, and drug delivery. Among the various bioengineering materials used in the synthesis of a biodegradable polymer, poly(lactic acid) (PLA) has received the most attention from researchers. *Hypericum perforatum* oil (HPO) has antimicrobial activity against a variety of bacteria. This study aimed to investigate the development of an antibacterial sustainable material based on PLA by incorporating HPO via a simple, low-cost electrospinning method. Chemical, morphological, thermal, thickness and, air permeability properties, and in vitro antibacterial activity of the electrospun nonwoven fabric were investigated. Scanning electron microscopy (SEM) was used to examine the morphology of the electrospun nonwoven fabric, which had bead-free morphology ultrafine fibers. Antibacterial tests revealed that the *Hypericum perforatum* oil-loaded poly(lactic acid) nonwoven fabrics obtained had high antibacterial efficiency against *Escherichia coli* and *Staphylococcus aureus*, indicating a strong potential for use in biomedical applications.

Keywords: *Hypericum perforatum* oil; poly(lactic acid); electrospinning; antibacterial; biodegradable

1. Introduction

In recent years, researchers have focused on the development of new materials with a low detrimental impact on the environment, due to the increasing growth of the world's population and pollution. Green polymers are becoming more widely used to achieve the requisite biodegradability. Poly(lactic acid) (PLA) is a biodegradable, recyclable polyester made from renewable feedstock, that is nowadays one of the most promising polymers for commercially replacing poly(styrene) (PS), poly(ethylene terephthalate) (PET), high-density poly(ethylene) (HDPE), and low-density poly(ethylene) (LDPE). Lactic acid is produced as a raw material by fermenting glucose or sucrose and is refined to high purity. PLA has been

used in food packaging, textiles, and, more recently, engineering plastics and currently is a niche product with significant growth potential [1–6]. PLA production represented 10.3% of the global bioplastics production capacity in 2018, reaching almost 220,000 tons/year, with a growth rate of around 60% expected by 2023 [7].

Electrospinning is a promising method for spinning polymer solutions or melts that can be easily scaled up with the use of strong electric fields. The approach is based on the assumption that in a charged polymer solution, strong electrical forces overwhelm weak surface tension forces. A high voltage is used to eject an electrically charged polymer solution jet from the tip (Taylor cone) of a capillary tube and uniformly scatter it over the collecting substrate. Jet then flows in the direction of the external electrical field, elongating as the external and internal electrical fields interact. The jet is sprayed onto a nonwoven mat-like substrate at random. This approach works with synthetic and natural polymers that have been processed to produce fibers with diameters ranging from a few nanometers to several microns [8–13]. Electrospun fibers have sparked a lot of attention due to the ease of fabricating multifunctional materials for usage in a variety of applications such as biomedical, textile, food packaging, batteries, and filters. Especially, electrospun fibers provide a unique opportunity for biomedical applications since they combine biocompatibility with micro-/nanoscale fiber architecture. These days, researchers are more focused on the development of biomaterials that reduce the risk of disease transmission and infection spread. The demand for novel antimicrobial materials is growing [14–20]. Natural bioactive agents have been used in biomedical due to their significant contribution since ancient times. Lately, the use of natural compounds such as plant extracts [21–23], bee products [24–26], and oils [27–29] for developing electrospun fibrous materials in biomedical applications has been studied extensively. *Hypericum perforatum*, a member of the *Hypericaceae* family, has long been regarded as a valuable herbal medicine. Flavonoids, hyperforin, and hypericin are all found in this plant. *Hypericum perforatum* oil (HPO) has long been used topically and orally as a home remedy to treat cuts, burns, depression, hemorrhoids, diabetes, and gastrointestinal ulcers [30–33]. Various articles have been written about the extraordinary properties of electrospun materials containing *Hypericum perforatum*. Poly(ε-caprolactone) (PCL) nanofiber wound dressings containing alcoholic extract of *Hypericum perforatum* have been produced [34]. A nanofiber wound dressing containing *Hypericum perforatum* extract was developed using poly(lactic-co-glycolic acid) (PLGA) [35]. *Hypericum perforatum* oil loaded-poly(ethylene glycol) (PEG) based nanofiber material was produced [36]. The developments of thermoplastic polyurethane (TPU) electrospun mats containing *Hypericum perforatum* extract have been researched and its antibacterial property evaluated [37]. Electrospun PLGA/gelatin (GE) membranes containing *Hypericum capitatum* extract have been developed and their biocompatibility properties investigated [38]. A wound dressing was designed from zein polymer containing *Hypericum perforatum* oil [39]. The cellulose-based fibrous dressing material for the treatment of acute wounds was produced from carboxymethyl cellulose (CMC)/poly (ethylene oxide) (PEO)/*Hypericum perforatum* solutions by needle-free electrospinning method [40]. The production of PEO and chitosan (CS) based fibrous containing *Hypericum perforatum* for wound dressings has been investigated [41]. A new wound dressing was fabricated by electrospinning using *Hypericum perforatum* oil into a mixture of biodegradable PCL/GE blended polymers [42].

PLA is an aliphatic polyester that is biocompatible and biodegradable. The US Food and Drug Administration has approved PLA for clinical applications. These properties have made PLA-based materials suitable for biomedical applications such as sutures, bone fixation implants, stents, tissue engineering scaffolds, and drug delivery carriers [6,18]. Researchers have been worked about the development antibacterial PLA-based electrospun materials using chitosan and silver [43], thymol [44], propolis [45], silver and vitamin E [46], copaiba (*Copaifera* sp.) oil [47], tetracycline hydrochloride and chitosan [48], cinnamon essential oil [49], thymoquinone [50], tea tree and manuka oil [51], carvacrol [52], clary sage and black pepper [53], terpinen-4-ol [54], epidermal growth factor [55], quercetin [56],

Nigella sativa herbal extract [57], *Eucalyptus* essential oil [58], silver diclofenac complex [59], chitosan, black pepper essential oil and limonene [60], selenium and clarithromycin [61], cefazolin [62], silver nanoparticles [63], zinc oxide [64], thyme essential oil [65], zenian (*Carum copticum)* essential oil [66], birch bark triterpene extract [67], *Capparis spinosa* L. extracts [68], *Plumbago europaea* plant extract [69], curcumin [70], babassu oil [71], and cypress (*Cupressus sempervirens* L.) essential oil [72].

Despite all the efforts mentioned above, the effects of *Hypericum perforatum* oil on the morphology of poly(lactic acid) electrospun mats have not yet been studied. PLA is easily available, biodegradable and renewable [1–6]. *Hypericum perforatum* oil exhibits bioactive properties such as antioxidant, anti-inflammatory, anticarcinogenic, and antifungal [30–33]. Moreover, it is already widely used and accessible in the medical field. It has been proposed that developing an advanced material using a combination of the beneficial properties of *Hypericum perforatum* oil and the advantages of poly(lactic acid) might be an alternative in bioengineering applications. This study contributes to the development of new sustainable materials and opens a new door to other studies.

This research work aims to develop antimicrobial PLA-based nonwoven fabrics containing HPO using a simple, environmentally-friendly method. HPO/PLA nonwoven fabrics were fabricated by electrospinning HPO/PLA aqueous solutions. The morphology, chemical composition, thermal and physical properties of the electrospun nonwovens were investigated. The electrospun HPO/PLA nonwoven fabrics demonstrated good antibacterial activity and have the potential for use in a variety of biomedical applications, particularly wound dressing, due to the combination of the antibacterial activity of the HPO and the favorable biocompatible and biodegradable polymer due to the PLA fibers.

2. Experimental Study

2.1. Materials

HPO (Aromatics of Dreams (Ароматика Мрії), Kiev, Ukraine) was purchased from the local herbal market (Lodz, Poland). Commercial PLA (a thermoplastic fiber-grade resin derived, Ingeo™ 6201D Fiber Grade PLA, the average molecular weight (M_w) 59.1 kg/mol, polydispersity index (M_w/M_n) 1.29, and an L-lactide content of isomer 1.4% D) [2] was obtained from NatureWorks LLC (Minnetonka, USA). Dichloromethane was purchased from POCH Basic (Gliwice, Poland) and acetone was Eurochem Sp. z o.o (Katowice, Poland). All chemicals were used as received, with no further purification. *Escherichia coli* (ATCC 10536) and *Staphylococcus aureus* (ATCC 6538) came from the American Type Culture Collection (ATCC) and are stored at the Department of Environmental Biotechnology, Lodz University of Technology. Cotton gauze was (Matocomp Gaze Comprey, TZMO SA, Torun, Poland) purchased from the local pharmacy (Lodz, Poland).

2.2. Methods

2.2.1. Preparation of Solutions for Electrospinning

Initially, a PLA 9% solution was prepared by dissolving in mixed solvents of dichloromethane and acetone 50:50 (*v/v*) at room temperature (RT) and stirring for 8 h. Subsequently, the 3HPO/PLA and 5HPO/PLA solutions (*v/v*) were prepared at RT temperature by dissolving in 3:97 (*v/v*) and 5:95 (*v/v*) HPO/PLA mixtures, respectively. HPO/PLA electrospun were blended and mixed to get homogenous solutions. A magnetic stirrer (Magnetic motor stirrer MS 11, WIGO, Warsaw, Poland) was used to prepare the solutions. Three solutions were obtained for electrospinning: PLA, 3HPO/PLA, and 5HPO/PLA.

2.2.2. Preparation of Electrospun Nonwoven Fabrics

Electrospun PLA and HPO/PLA nonwoven fabrics were fabricated by a homemade electrospinning device (Lodz University of Technology, Lodz, Poland). A needle with a flattened tip (inner diameter = 1.2 mm) was used for electrospinning. The solution was fed at a rate of 5 mL/h at a voltage of 22 kV. The electrospun fibers were collected on aluminum foil at a distance of 15 cm between the needle tip and the collector. Nonwoven

fabrics were gathered on a cylindrical drum rotating (circumference = 1 m) at 35 rpm. All electrospinning trials were done at room conditions (temperature = 25 ± 2 °C; relative humidity = 50 ± 5%). For each electrospun nonwoven sample, 50 mL of polymer solutions were used.

2.2.3. Measurements and Characterizations

The surface structure and morphology of electrospun nonwovens fabrics were observed via scanning electron microscopy (Nova™ NanoSEM 230, FEI Company, Hillsboro, OR, USA). The diameters of the fibers were measured using imaging software (ImageJ, National Institutes of Health, Madison, WI, USA). The average fiber diameter and distribution for each experiment were estimated using micrographs of fiber morphology and 100 random measurements.

The chemical structure of the HPO, PLA, and HPO/PLA electrospun samples was analyzed by Fourier transform infrared spectroscopy (FTIR; Nicolet 6700, Thermo Electron Corp., Madison, WI, USA) with an attenuated total reflectance (ATR) accessory. All spectra were recorded in the wavelength range from 4000 to 600 cm^{-1}. The investigation was carried out using the SpectraGryph program (Dr. Friedrich Menges, Oberstdorf, Germany).

The thermogravimetric analysis of all electrospun mats was carried out with a Perkin Elmer TGA 7 thermal analyser in a platinum measuring cell and using the Pyris program for data handling. The measurements were performed in artificial air with a 20 °C/min heating rate. Electrospun mats were heated up to 600 °C, starting from room temperature. Electrospun mats were acclimatized for at least one day in dry conditions (humidity below 5%) before measurement. The thermogravimetric analyzer is controlling sample weight before and during measurement.

The thickness of the electrospun nonwoven fabrics was measured using a digital thickness gauge for nonwovens (Checkline J-40-V, Electromatic Equipment Co., New York, NY, USA) at ten points. It was reported values were the average and standard deviations of obtained results.

The weight of the electrospun nonwoven fabrics was measured using a digital balance (Radwag WPS 510/C, Radom, Poland) at five different samples (5 × 5 cm^2). It was reported values were the average and standard deviations of obtained results. The weight of the PLA, 3HPO/PLA, and 5HPO/PLA electrospun nonwoven fabrics were measured as 2.86 ± 0.37 g/m^2, 3.04 ± 0.29 g/m^2, and 3.13 ± 0.88 g/m^2, respectively.

The air permeability of the electrospun nonwoven fabrics was measured using a Textest FX 3300 air permeability tester (Textest AG, Schwerzenbach, Switzerland) by fixing the airflow rate at 100 Pa with a test area of 20 cm^2 at ten points. It was reported values were the average values and standard deviations of obtained results.

2.2.4. In Vitro Antibacterial Activity

The antibacterial efficiencies of the electrospun nonwoven fabrics were quantitatively evaluated according to the AATCC 100–1999 method "Antibacterial Finishes on Textile Materials". Before starting the test, the microorganisms were activated in TSB medium (Tryptic Soy Broth, Merck, Darmstadt, Germany) at 37 ± °C for 24–48 h. The samples with a mass of 0.1000 ± 0.001 g were placed in sterile plastic containers with a volume of 100 mL. Then, 1 mL of inoculum was applied to the samples with the following densities: $3.5 \times 10^6 \pm 1.0 \times 10^6$ (*Escherichia coli)* and $1.5 \times 10^6 \pm 1.1 \times 10^6$ (*Staphylococcus aureus*). The inoculum density was determined by the culture method. The samples were then incubated for 24 h at a temperature of 37 ± 2 °C. The microorganisms were washed out of the samples immediately after inoculation (t = 0) and after 24 h of incubation (t = 24 h). Then it shook for 5 min in 50 mL of 0.85% sterile NaCl (Merck, Darmstadt, Germany) solution. The number of bacteria was determined by the culture method using TSA medium (Tryptic Soy Agar, Merck, Darmstadt, Germany) for 24 h at a temperature of 37 ± 2 °C. The colonies were counted after incubation, and the result was provided in a colony-forming unit (cfu)/g of

samples. The tests were carried out in two separate repeats. The reduction in the number of bacteria was calculated using the following Equation:

$$\text{Reduction rate (\%)} = [(A - B)/A] \times 100 \quad (1)$$

where A is the number of bacteria from the flask containing the treated substrate after the 0 contact time and B is the number of bacteria for the addition of treated substrate at 24 h contact time.

3. Results and Discussion

3.1. Morphology of the Electrospun Nonwoven Fabrics

In this research, various solutions containing different concentrations of HPO were prepared and used in the development of fibers to investigate the effect of electrospinning solution composition on the structure of the nonwoven fabrics and the electrospinning process. SEM was used to evaluate the morphologies and fiber diameter distributions of the PLA and HPO/PLA electrospun nonwoven fabrics, as shown in Figure 1. PLA electrospun fibers were estimated to have a diameter of 1.71 ± 1.02 µm. This result is in agreement with the data previously reported by Štular et al., who founded a similar average diameter for electrospun PLA fibers (~1.4 µm) [73]. Smooth cylindrical and continuous fibers were obtained from the PLA electrospinning solution. The average diameters of 3HPO/PLA and 5HPO/PLA electrospun fibers were 1.68 ± 0.58 µm, and 1.51 ± 0.64 µm, respectively. The diameter of the fibers decreased slightly after adding HPO. In addition, the fiber diameter distribution of HPO/PLA fibers was narrower than that of PLA fibers, as measured by the lower standard deviation in the graphs presented in Figure 1. This is possible since HPO is a natural surfactant, and its addition induces a reduction in surface tension of the resulting solution [74]. Lower surface tension reduces the resistance to the applied electric force, which causes the jet to stretch more, resulting in a thinner fiber diameter [75]. The fiber diameter of the HPO/PLA electrospun mats containing various amounts of HPO revealed that the fibers were randomly aligned and had thin diameters. Akşit et al. observed that increasing the amount of *Hypericum capitatum* extract within the PLGA/GE nanofibrous mats caused the fiber diameter to drop as the viscosity of the polymer solution decreased [38]. In addition, HPO loaded-PLA electrospun nonwoven fabrics appear to have favorable fiber diameter (1.5–1.7 µm) for cell adhesion and attachment, which is an advantage in biomedical applications, such as wound dressings and scaffolding. PLGA fibers with diameters ranging from 0.4 to 1.4 µm are suitable for cell attachment, according to researchers who have investigated the importance of the fiber diameter on the attachment of fibroblast cells [76]. To sum up, SEM images show that both PLA and HPO/PLA electrospun mats displayed a smooth and uniform morphology.

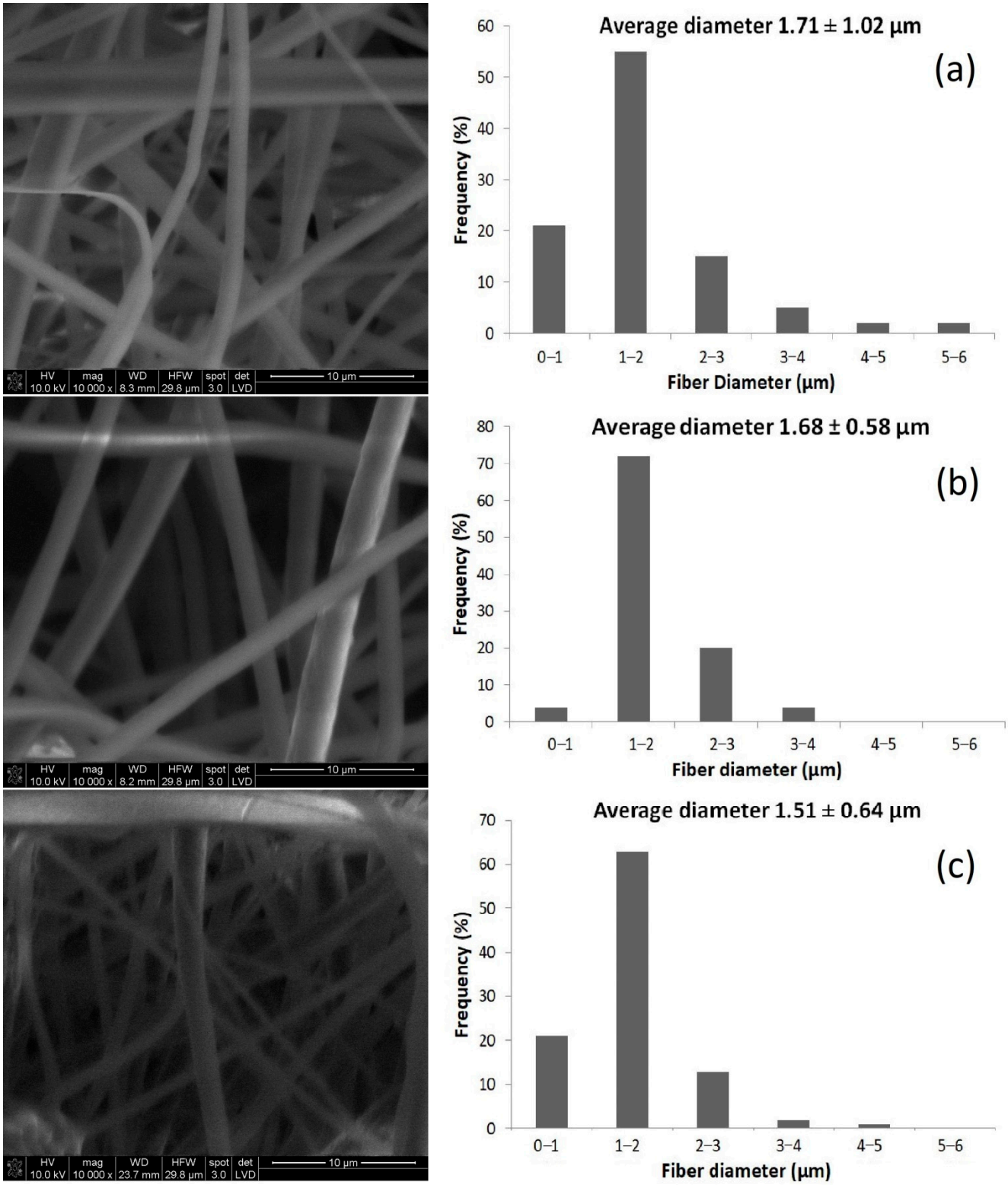

Figure 1. SEM images and fiber diameter distributions of the electrospun nonwoven fabrics obtained from (**a**) PLA, (**b**) 3HPO/PLA and (**c**) 5HPO/PLA (×10,000) (average ± standard deviation).

3.2. ATR-FTIR Analysis of the Electrospun Nonwoven Fabrics

The functional groups of HPO, PLA, and HPO/PLA electrospun nonwoven fabrics were determined by ATR-FTIR spectroscopy (Figure 2).

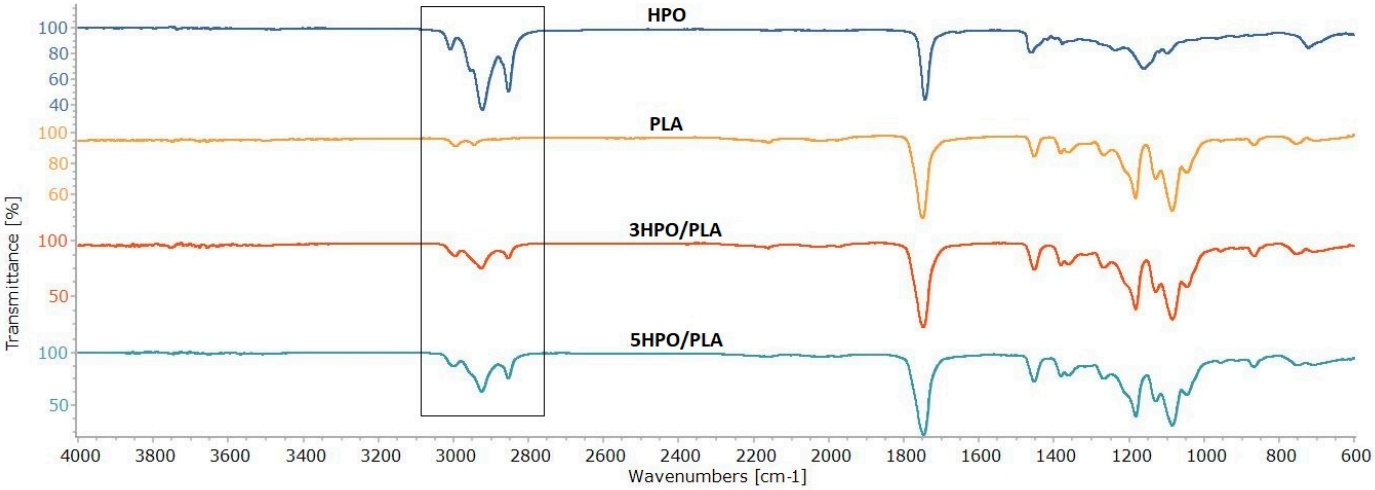

Figure 2. ATR-FTIR spectra of the HPO, the PLA, and the HPO/PLA electrospun nonwoven fabrics.

The characteristic peaks of the HPO observed at 3308^{-1}, 2853^{-1}, 1743 cm^{-1}, and 844 cm^{-1} were assigned to aromatic C-H stretching, aliphatic C-H stretching, C=O stretching, and aromatic rings, respectively [34,41]. The signal at 1237 cm^{-1} typically overlaps, most notably C–O ester, which is found in oils [77]. The characteristic peaks of the PLA electrospun fabrics observed at 2994 cm^{-1}, 2944 cm^{-1},1745 cm^{-1}, 1182 cm^{-1}, 1085 cm^{-1}, and 1045 cm^{-1} were assigned to C=O groups, asymmetric and symmetric stretching vibrations of the C-O-C group, and C-CH_3 stretching, respectively [78,79]. The characteristic peaks of the 3HPO/PLA electrospun fabrics observed at 2995 cm^{-1}, 2926 cm^{-1}, and 2854 cm^{-1}, assigned to –CH_3 asymmetric, –CH_3 symmetric, C-H stretching. The characteristic peaks of the 5HPO/PLA electrospun fabrics observed at 2997 cm^{-1}, 2925 cm^{-1}, and 2854 cm^{-1}, were assigned to –CH_3 asymmetric, –CH_3 symmetric, C-H stretching. A small shift from 2994 cm^{-1} (PLA) to 2995–2997 cm^{-1} (3HPO/PLA-5HPO/PLA) was observed. This shift in the absorption peak shows the miscibility and interaction of HPO and PLA. With the addition of HPO to PLA fibers, the spectra of PLA electrospun nonwoven fabrics changed and the adsorption bands in the frequency range 3000–2800 cm^{-1} were broadened due to CH stretching vibrations of different groups in HPO. The peak intensity of the stretching of CH bands, which corresponds to the varying concentration of the HPO compound, shows that HPO was successfully incorporated into the PLA fibers.

3.3. Thermal Properties of the Electrospun Nonwoven Fabrics

Weight loss as a function of temperature due to degradation is measured by thermogravimetric analysis (TGA). In the TGA curves, the onset temperature, where the deterioration process began, and the end temperature, where the combustion process ended, were both evaluated. The TGA profiles of PLA and HPO/PLA electrospun nonwoven fabrics are shown in Figure 3. Thermal degradation of PLA occurs in a single weight loss phase, as evidenced by the TGA curves. PLA has higher onset degradation temperatures than PLA/HPO blends. The profile for PLA degradation peak was at ~368 °C [79] and it was completely decomposed at ~444 °C, associated with the loss of ester groups by unzipping depolymerization [80]. On the other hand, the HPO/PLA samples of both the 3HPO/PLA and the 5HPO/PLA electrospun nonwoven fabrics showed nearly two steps of degradation, as shown in Figure 4. These degradations occurred at temperatures ranging from ~360–354 °C to ~500–550 °C, respectively.

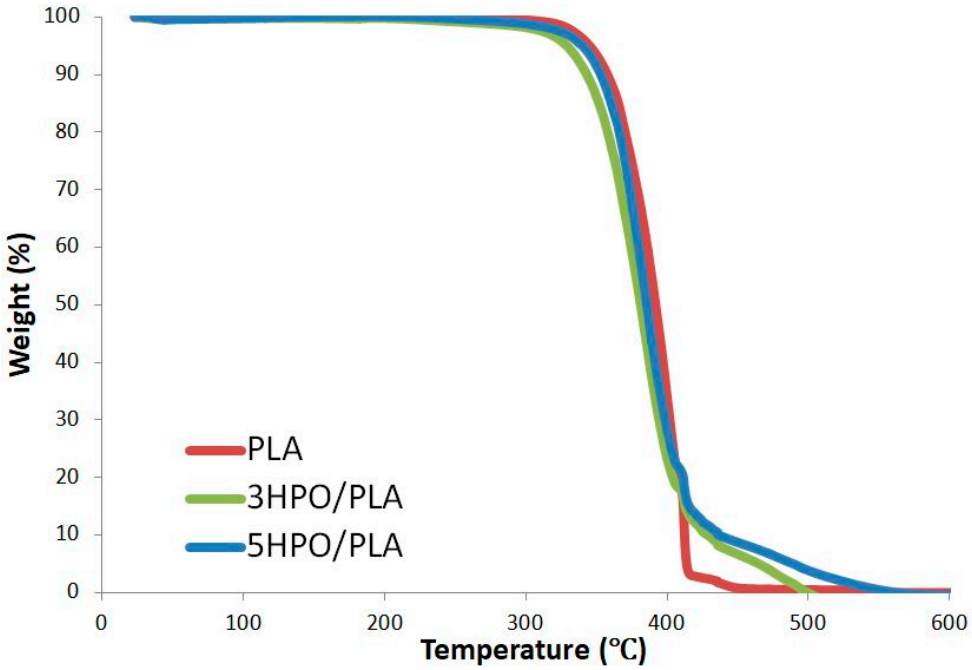

Figure 3. TGA thermograms of the PLA, and HPO-loaded PLA electrospun nonwoven fabrics.

The thermal properties of the two HPO/PLA electrospun nonwoven fabrics showed almost identical trends. The addition of HPO resulted in a slight decrease in onset temperature (~10–15 °C), indicating that blended samples have low thermal stability, accounted for by the presence of HPO dispersed in the polymer. When the HPO content is increased from 3% to 5%, the temperature of decomposition increased remarkably, while the temperature of onset thermal degradation decreased insignificantly. The decomposition temperature of the 5HPO/PLA blend is ~50 °C higher than that of the 3HPO/PLA due to the presence of HPO in the matrix. The large difference in decomposition temperatures between the two starting materials may contribute to the degradation mechanism. This reassembly produces a physical barrier on the material's surface, reducing the permeability of volatile degradation products out of the blend and, as a result, delaying the blend's breakdown [77,81]. This result is similar to results in the literature, which reported that the thermal behavior of epoxidized soybean oil (a simultaneous compatibilizer and plasticizer) modified PLA natural rubber-based compound [81]. The thermal properties indicate that the HPO/PLA electrospun fabrics have higher decomposition temperatures than PLA.

3.4. Thickness and Air Permeability of the Electrospun Nonwoven Fabrics

Figure 4 shows the thickness and air permeability test results of PLA and HPO/PLA electrospun nonwoven fabrics. Thickness is an essential characteristic of textile materials since it has a direct influence on performance attributes such as permeability. The thicknesses of the PLA, 3HPO/PLA, and 5HPO/PLA electrospun nonwoven fabrics were measured as 215 ± 12 µm, 221 ± 8 µm, and 224 ± 6 µm, respectively. The volume of the electrospinning solutions was used to regulate the thickness. Although all electrospun nonwoven fabrics were made in the same way, the slightly higher thickness value of the HPO/PLA blended fabrics can be attributed to the existence of lower surface tension that reduces resistance to applied electric force, causing fibers to accumulate in a narrower area on the collector. Thus, the presence of HPO into PLA electrospinning solution increased nonwoven mat thickness.

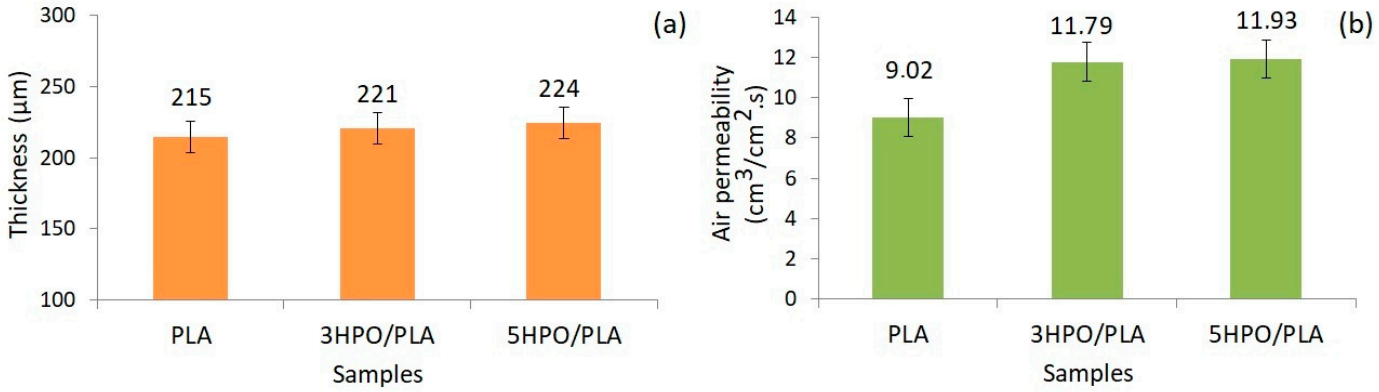

Figure 4. The thickness (**a**) and, air permeability (**b**) test results of PLA and HPO/PLA electrospun nonwoven fabrics.

Air permeability is important for wound dressing, to allow oxygen in which plays a vital role in wound healing [82]. The role of oxygen in wound healing can be summarized as follows; the oxidative killing of bacteria, re-epithelialization, angiogenesis, and collagen synthesis [83]. Parameters that may affect the air permeability of materials include fiber diameter, thickness, and porosity [84]. The air permeability of the PLA, 3HPO/PLA, and 5HPO/PLA electrospun nonwoven fabrics were measured as 9.02 ± 0.55 cm^3/cm^2.s, 11.79 ± 1.23 cm^3/cm^2.s and, 11.93 ± 0.77 cm^3/cm^2.s, respectively. Only a minor difference in air permeability was observed after the addition of HPO in PLA electrospun nonwoven fabric. The air permeability values indicated that the addition of HPO to the PLA electrospun fabrics caused an increase. Air permeability is still a complicated phenomenon for electrospun mats because airflow may affect the geometry of nanofibers due to the mats' lightness and thinness [85]. This increase in air permeability can be explained by the fact that the fine fibers are more affected by the air flow since the intermolecular interaction changes with the addition of HPO to the structure. Avci and Gergeroglu reported that the air permeability values of herbal extract loaded-thermoplastic polyurethane electrospun mats were increased after the addition of extract into a polymer [37]. The measurement results showed that the presence of HPO in the PLA electrospun nonwoven fabric had a minor influence on the thickness and air permeability of samples. The fact that all samples are breathable is promising for the development of PLA-based electrospun biomedical materials such as wound dressings.

3.5. In Vitro Antibacterial Activity of the Electrospun Nonwoven Fabrics

The antibacterial efficiency of the cotton gauze, PLA, and 5HPO/PLA electrospun nonwoven fabrics against *Staphylococcus aureus* and *Escherichia coli* were tested. The results of the test are given in Table 1. The images of the petri dishes demonstrating antibacterial activity of samples have been given as Supplementary Materials (Figure S1).

Table 1. Antibacterial activity of the electrospun nonwoven fabrics.

Samples	Reduction Rate (%)	
	Staphylococcus aureus	***Escherichia coli***
Cotton gauze	No Reduction	No Reduction
PLA	No Reduction	No Reduction
5HPO/PLA	>99.99	>99.99

The cotton gauze and PLA electrospun nonwoven did not exhibit a growth inhibitory effect against *Staphylococcus aureus* and *Escherichia coli.* However, the HPO/PLA electrospun nonwoven fabric exhibited a good growth inhibitory effect against *Staphylococcus aureus* (>99.99) and *Escherichia coli* (>99.99). The outcome is consistent with the literature that reported that the antimicrobial activity of the *Hypericum perforatum* oil-loaded electro-

spun PEG-PCL membranes has good antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* This result can be explained by the intrinsic antimicrobial properties of HPO. HPO contains naphthodianthrones, hypericin, hyperforin, and flavonoids, which are responsible for their antibacterial properties [36,38,40,86]. HPO is a lipophilic compound that, has been demonstrated to disrupt cellular membranes in bacteria and fungi, hence inhibiting cellular respiration and ionic transport [87]. These findings support the contention that HPO/PLA electrospun nonwoven fabrics can be used as an antibacterial material in potential biomedical fields.

Hypericum perforatum oil has long been used as a traditional treatment method for wound treatment [30–33,86,87]. *Staphylococcus aureus* and *Escherichia coli* are predominant pathogens in wounds, and the source of most pathogenic contamination has been reported to be associated with exogenous contamination from contaminated wound dressing devices from the hospital setting or with the patient's normal flora [88–90]. At this point, the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* of the HPO-loaded PLA electrospun nonwoven may be beneficial in the healing process. For example, in comparison with conventional dressings (cotton gauze), PLA electrospun nonwoven fabrics loaded with HPO eliminate the disadvantages of the lack of antibacterial properties. The HPO-loaded PLA electrospun nonwoven fabric is simple and low-cost to produce, using commercially available technologies. In our effort to develop a biomaterial for biomedical engineering, we prepared a green fibrous material based on PLA loaded with HPO, which was assessed for the first time for bactericidal properties. This study shows that more studies should be done to emphasize the positive effects of HPO and PLA on human health and the environment and to address their possible impact.

Preliminary measurements of *Hypericum perforatum oil*-load poly(lactic acid) electrospun mat as an antibacterial material was performed in vitro analysis and this study investigated some performance properties required for its use as a textile material. Further research should include characterization of the *Hypericum perforatum oil*-load poly(lactic acid) electrospun in a clinical report to establish its practical application, and assessment of fiber-cell interaction and wound healing.

4. Conclusions

In this study, the HPO-loaded PLA electrospun nonwoven fabric as a potential antibacterial medium was successfully fabricated by a green strategy. The incorporation of HPO onto the PLA fibers showed good antibacterial behavior against *Staphylococcus aureus* and *Escherichia coli* bacteria. The diameters of the electrospun nonwoven fibers were determined to be in the micrometer range. ATR-FTIR results confirmed the presence of HPO incorporated within ultrafine PLA fibers. In the interaction between HPO and PLA, the incorporation of HPO slightly influenced the nonwoven fabric's thermal stability. However, little difference in air permeability and thickness characteristics was observed between PLA and HPO/PLA samples. Overall, our findings indicated that the successful preparation of such HPO loaded-PLA nonwoven fabrics with good antibacterial properties could serve as a model for designing and developing innovative antibacterial materials for various biomedical fields such as drug delivery and wound dressing.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/app11178219/s1, Figure S1: Images of the petri dishes showing the antibacterial activity of samples.

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