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Zeynep Cansu Ayturan, Engineering and Natural Sciences Faculty, Konya Technical University,
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Phone : +90 332 2238630

: +90 536 5954591

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E-Mail : iseser@iseser.com
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O 39. EVALUATION OF ANTIBACTERIAL ACTIVITIES OF NYLON 6,6 NANOFIBERS COATED WITH MINT OIL AND MINT EXTRACT

Havva Tutar Kahraman¹, Kezban Buse Altun^{1*}, Merve Musaoğlu¹, Gülsüm Aydın²

¹*Department of Chemical Engineering, Konya Technical University, Konya, Turkey*

²*Department of Biotechnology, Selcuk University, Konya, Turkey*

E-mail: buse906@gmail.com

ABSTRACT: The aim of the study was to fabricate Nylon 6,6 nanofibers coated with essential oil and plant extract to create an antibacterial activity which can be used as a potential food packaging material or biomedical textile. Herein, the target antibacterial material was engineered by coating mint oil and mint extract on Nylon 6,6 nanofibers. Firstly, production of Nylon based nanofibers was performed by electrospinning technique. Then, mint oil and mint extract were decorated onto nanofibers via ultrasonication technique. The nanofibers were characterized using Scanning Electron Microscope (SEM) and Fourier-transform infrared (FT-IR) spectrophotometer. Antibacterial activities of surface coated nanofibers were evaluated using Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*). The inhibition zones were measured and recorded. Nanofibers coated mint oil showed better antibacterial properties as compared to mint extract.

Keywords: Antibacterial, nanofiber, mint oil

1. INTRODUCTION

Tremendous research interest has been directed toward nanofibrous designs generated by electrospinning technique, in the last 10 years. Electrospinning is a versatile method to fabricate nanoscale polymer fibers with diameter in the range of 3–5000 nm (Jiang et al. 2015). Nanofibrous structures obtained via electrospinning method have antimicrobial, anti-inflammatory, and anti-oxidant activity, which are attractive for biomedical applications and food packaging industries (Kohsari et al. 2016). In order to gain these properties to the materials, different types of agents (i.e. antimicrobials, antibiotics, plant extracts and essential oils) can be used (Zhang et al. 2017; Akia et al. 2019). Since ancient times, essential oils are well-known for their antimicrobial properties. The use of essential oils as an antibacterial agents leads to produce novel and eco-friendly nanomaterials. This types of oils can be incorporated into electrospun nanofibers and polymer films to be in a wide range of applications. With the advances in science it is possible to fabricate new materials at nanoscale level. Nanofibers are the member of this type of novel materials. Nanofibers are generally preferable due to having high area and controllable compositions.

The purpose of this study was to develop new nanomaterials having antibacterial activity to be used in different applications. In this respect, nanofibers were obtained using electrospinning technique, then they were coated with mint oil and mint extract. SEM analysis and FT-IR analysis were performed to characterize the structures of nanofibers. Antibacterial activities of materials were evaluated by agar diffusion method.

2. MATERIAL AND METHOD

Nylon 6,6 and formic acid with purity of 99 vol% were obtained from Sigma Aldrich Company. Nanoclay (Cloisite 20A) was purchased from BYK company. Mint extract was prepared using mint plant. Mint oil used in this study, was purchased from local market.

2.1. Preparation of nanofibers

In brief, before fabricating of nanofibers, polymer was dissolved in formic acid. Polymer-clay solution was mixed by magnetic stirrer and ultrasonicator in order to achieve homogeneous solutions. Nanofibers were produced by spinning the polymeric solution with and without nanoclay. Nylon 6,6-formic acid solution was placed in a 10-ml syringe with 19-gage needle tip and electrospun onto a aluminum foil to

produce Nylon nanofibers. Similarly, Nylon 6,6-clay solution was electrospun with different parameters to determine the proper conditions. The distance between collectors and spinneret were maintained at 15 cm. Clay was incorporated into the Nylon 6,6 solution in order to improve the mechanical properties of fibers.

The fabricated nanofibers were coated with mint oil and mint extract by magnetic stirrer and ultrasonicator. The excess solution was removed and the samples were dried at room temperature overnight.

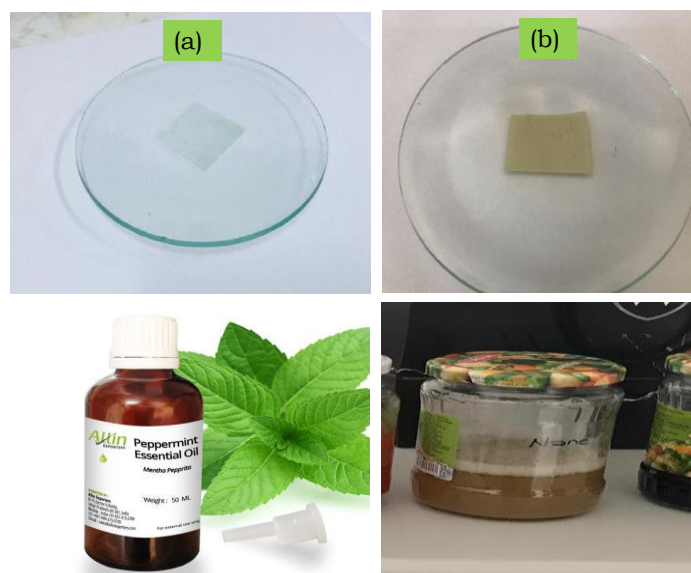


Figure 1. Nanofibers coated with mint oil (a) and mint extract (b)

2.2. Characterization of nanofibers

Fiber morphology was analyzed using Scanning Electron Microscopy (ZEISS evo LS10). FT-IR spectrum was recorded at range 400 to 4000 cm^{-1} at 4 cm^{-1} resolution using a Bruker Vertex FT-IR spectrometer.

2.3. Antibacterial Activity

Antibacterial studies were conducted on the developed Nylon 6,6 nanofiber mats, both including uncoated and mint oil and mint extract coated samples. Antibacterial activities of Nylon 6,6 nanofibers coated with mint oil and mint extract were tested against a Gram-positive (*Staphylococcus aureus*) bacterium and a Gram-negative bacterium (*Escherichia coli*) using agar diffusion method. Fibers cut into equal sizes were placed on nutrient agar plates previously seeded with 0.1 mL of the tested bacteria ($\sim 10^8$ cfu/mL). After 24 hours of incubation at 37°C the diameter of inhibitory zones surrounding the samples were measured. Fibers lacking mint oil and mint extract were used as control.

3. RESEARCH FINDINGS

3.1. SEM analysis

Fig. 2 shows scanning electron micrographs of the fabricated bare Nylon 6,6 fibers. The average diameters were 200-300 nm. As seen from the figure, uniform bead-less fibers were achieved.

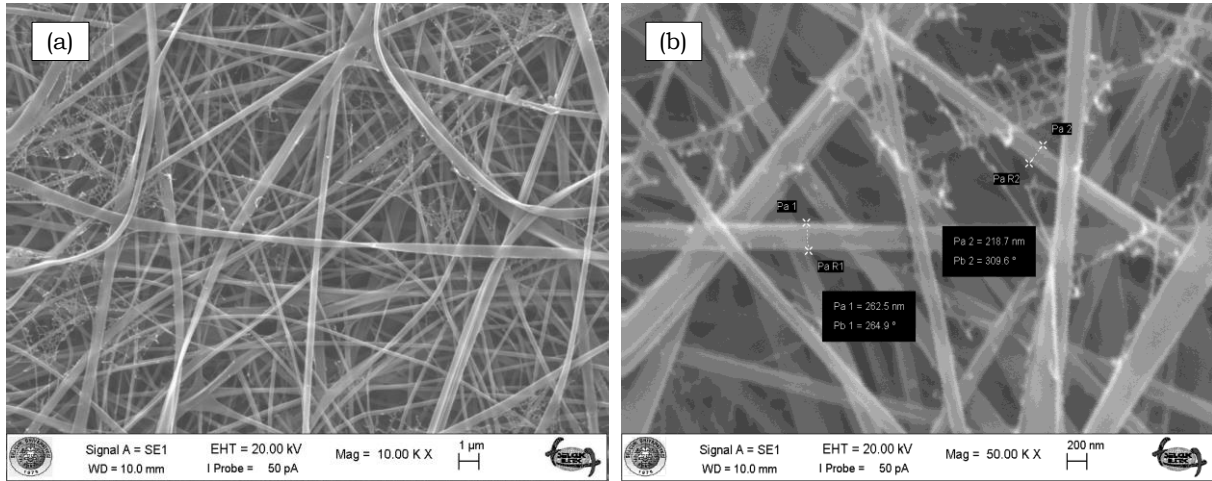


Figure 2. SEM micrographs of bare electrospun Nylon 6,6 nanofibers

Figure 3 and 4, show SEM images of nanofibers coated with mint oil and extract, respectively. As seen from the figure 3, a thin film layer was formed by networking between each fibers. In addition, no deterioration was observed in the structure.

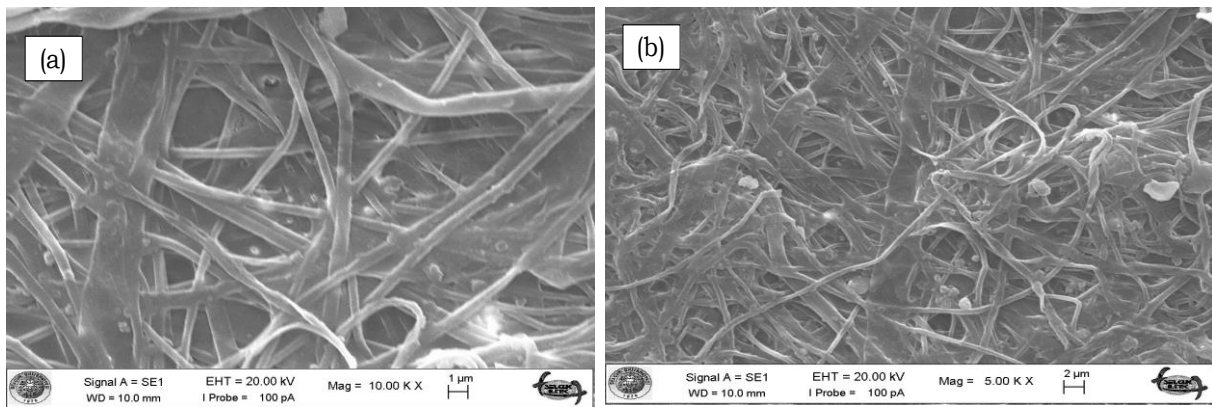


Figure 3. SEM micrographs of electrospun Nylon 6,6 nanofibers coated with mint oil

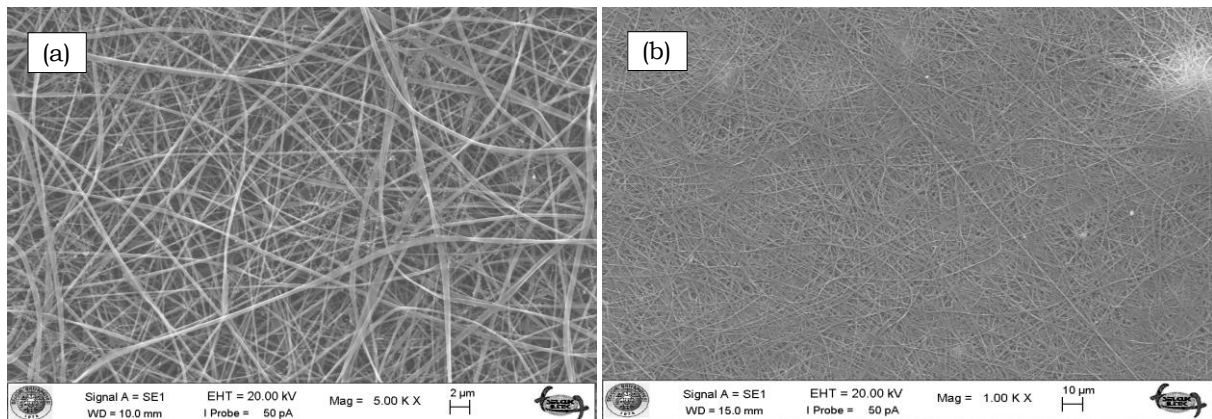


Figure 4. SEM micrographs of electrospun Nylon 6,6 nanofibers coated with mint extract

3.2. FT-IR analysis

Figure 5 shows the FT-IR spectra of obtained Nylon 6,6 and coated nanofibrous mats. Nylon 6,6 has some characteristic peaks at 3300 cm^{-1} : (O-H deformation); $2940\text{ ve } 2860\text{ cm}^{-1}$: (C-H stretching and O-H groups); 1640 cm^{-1} : (C=O deformation); 1420 cm^{-1} : (C-O-H deformation); 1256 cm^{-1} : (C-O

stretching). Oil immobilized nanofiber has some characteristic peaks at 3060, 3030, 1490, 1450, 1370 ve 1030, 758 ve 698 cm^{-1} . However, many peaks of sample coincide with the main peaks of Nylon 6,6 fibers. The shift in the absorption values of the main peaks of the mint oil loaded fiber, indicates the presence of volatile oil on the fiber surface. New peak formation is not observed for fibers coated with mint extract. However, the intensities of peaks mainly decreased.

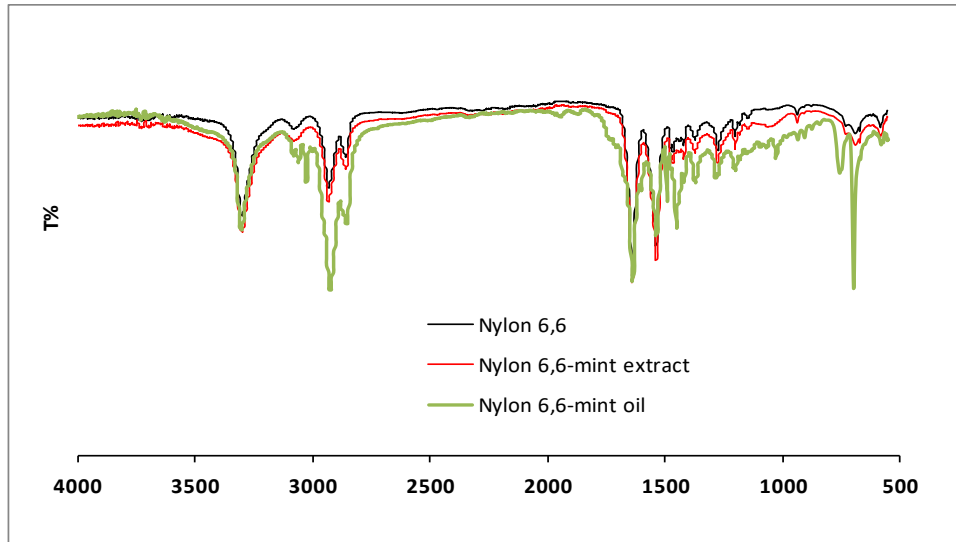


Figure 5. FT-IR spectrum of electrospun nanofibers

3.3. Assay of Antibacterial Activity

The results of antibacterial activity test were illustrated in Figure 6 and 7. The control samples exhibited no inhibitory effect on *E. coli* and *S. aureus*. Fibers coated with mint extract also showed no antibacterial activity against both of the test bacteria. Inhibitory zones around the fibers coated with mint oil was observed verifying the antibacterial effect of the fibers against both *E. coli* and *S. aureus*. The degree of inhibition was similar against both the Gram-positive and the Gram-negative bacterium. Addition of clay besides the mint oil or the mint extract resulted in disappearance of antibacterial activity of the fibers.

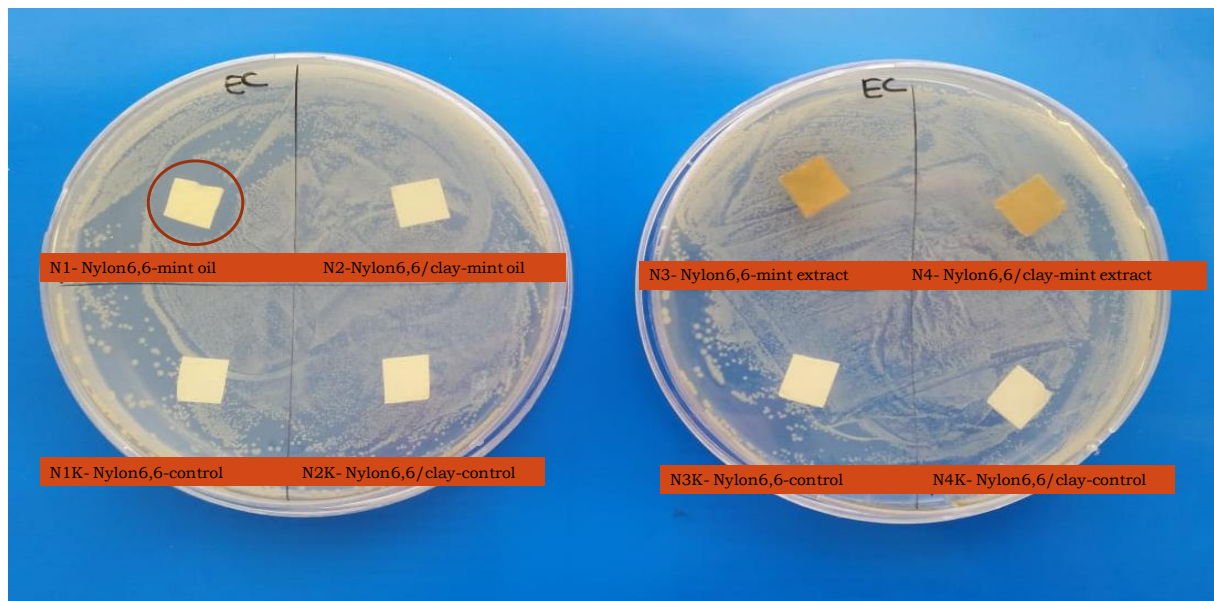


Figure 6. Inhibition zones for nanofibers against bacteria *Escherichia coli*

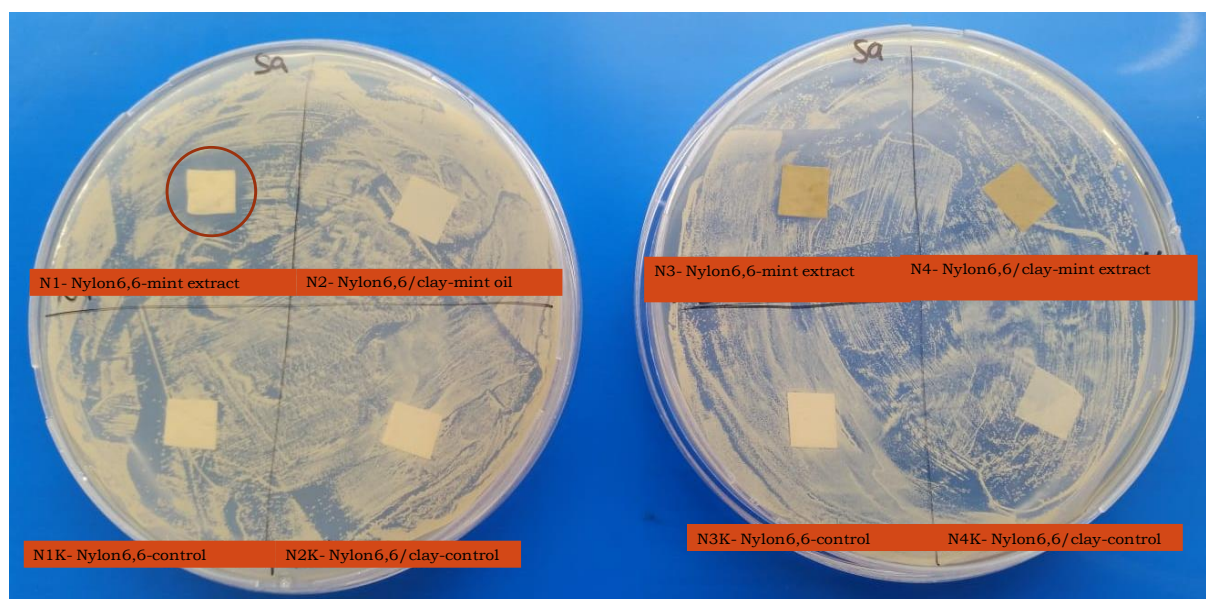


Figure 7. Inhibition zones for nanofibers against bacteria *Staphylococcus aureus*

4. CONCLUSIONS

In this work, an environmentally benign material and process for antibacterial application was proposed. In the first step of the process, Nylon 6,6 nanofibers were developed utilizing a spinning process. Fibers with an average diameter of 200-300 nm were collected as nonwoven mats. The antibacterial activity against two pathogenic bacteria species, *E. coli* and *S. aureus*, was evaluated for the developed nanofiber mats. According to the results, mint oil provides an antibacterial activity to the fibers. However, this effect was not observed in nanofibers coated with mint extract. Furthermore, nanofibers including clay had no antibacterial activity. Consequently, this product would be used for various applications including self-sterilizing textiles and food packages.

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