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Antimicrobial Properties of Biocompatible Poly (Ɛ-Caprolactone) Treated with Plant Extract

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ABSTRACT

Poly (ε-caprolactone) (PCL) was synthesized using ethylene glycol initiator and catalyst accompanied by ring-opening polymerization method. The number average molecular weight (Mn) of the obtained polymer was found to be 4000 gmol-1. In addition, the characteristic signals of PCL were determined with Fourier-transform infrared spectroscopy (FT-IR). Decomposition temperatures were investigated by Thermogravimetric Analysis (TGA) and melting temperatures (Tm) were investigated by Differential Scanning Calorimetry (DSC). Tm of PCL at 57.3 °C was observed. PCL was treated with Rumex patientia L. ethanolic plant extract and its effects on Klebsiella pneumoniae ATCC 700603, Bacillus megaterium DSM32, Staphylococcus aureus ATCC25923, Escherichia coli ATCC25322, and Candida albicans FMC17 microorganisms were examined. It was determined that PCL, which did not show antimicrobial activity, showed antimicrobial activity on some microorganisms after being treated with the plant.

Keywords: Antmicrobial, Crystallinity, FT-IR, Poly ε-caprolactone, *Rumex patientia.*

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Introduction

With the increase in diseases, it is seen that herbal medicines among the drugs used in the treatment have been preferred more in recent years [1]. It is known that these herbal medicines are frequently used to prevent cancer, fight against microorganisms, and eliminate the harmful effects of free radicals [1-3].

Rumex pateintia L. is a medicinal plant belonging to the Polygonaceae family and has more than 200 species [4]. Species of this genus have different pharmacological activities such as anti-inflammatory, antioxidant, cytotoxic, antifertility, antibacterial, laxative, antidiarrheal, antifungal, antipyretic, antiviral activities [5-10]. There are about 25 species of this genus in Turkey and the most common species are *R. patientia L.*, *R. crispus L.*, *R. acetosa L. R. caucasicus RECH.* and *R. alpinus L.* [11]. *Rumex patientia L.* is a perennial herb commonly found and grown in Eastern Europe, especially used in the treatment of different diseases in traditional medicine (Jovin et al., 2011). The root parts of the plant are known to have some preventive effects. The most important of these are antipyretic, anti-inflammatory, wound healing and diuretic effects [12].

As well as, antimicrobial polymers attract attention in the fight against pathogenic microorganisms [13]. Polymers with antimicrobial action or polymers with the ability to be conjugated with other materials with antimicrobial action can be effective in controlling pathogenic microorganisms [14]. For this reason, traditional methods and new generation antimicrobial agents with antimicrobial effect are in high demand.

Poly(ε-caprolactone) PCL, an aliphatic polyester, is of great interest in surgical fields such as drug delivery and tissue engineering [15,16]. PCL has a biocompatible, biopermeable, biodegradable and hydrophobic semi-crystalline structure [17-19]. Bioabsorbable and non-toxic to living organisms, PCL has been developed as a copolymer or blend polymer, providing an even wider field of application [20,21].

PCL with shape memory properties was synthesized using the ring-opening technique. During the synthesis, ethylene glycol was used as initiator and tin(II) octanoate was used as catalyst. The synthesized PCL was characterized by FT-IR, TGA, DSC and XRD. PCL was treated with *Rumex patientia L.* extract in an ultrasonic homogenizer in dimethyl sulfoxide (DMSO) solution. Antimicrobial activities of the blend obtained against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium* and *Candida albicans* microorganisms were investigated.

Materials and Methods

Synthesis of Poly (ε-caprolactone)

The synthesis of Poly (ε-caprolactone) in the presence of ethylene glycol and tin(II) octanoate is schematized in Figure 1 [22]. 5 grams ε-caprolactone was weighed into the reaction flask and then stirred until completely

dissolved in 10 mL of toluene. Then, 0.07 gram ethylene glycol and 2 drops tin (II) octanoate were added and mixed in a magnetic stirrer at room temperature. Ar gas was passed through the reaction flask and the flask was tightly closed. It was taken into an oil bath and the temperature was gradually increased up to 120 °C and stirring was continued for 24 hours. The viscous polymer obtained after 24 hours was precipitated by adding dropwise in cold n-hexane. The resulting poly (ε-caprolactone) was dried in a vacuum oven at 40 °C for 24 hours and then stored in the refrigerator.

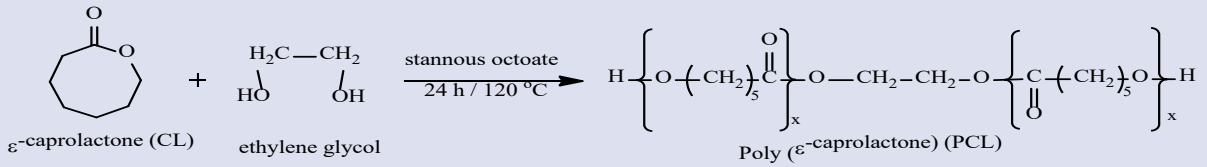

Figure 1. Polymerization of ε-caprolactone

Preparation of the Rumex patientia extract

The *Rumex Patientia* plant collected from Elazığ, Turkey, was dried in a cool, sun-free environment. Then, 20 grams the plant, which was pulverized with a blender, was taken and dissolved in 200 mL ethanol. The plant solution, which was dispersed in an ultrasonic homogenizer for 30 minutes, was shaken in a shaker incubator at 100 rpm for 24 hours at room temperature. The filtrate obtained by filtration of the solution was removed with ethanol at 35 °C with the evaporator [23]. *R. patientia* extract, the solvent of which was removed, was stored at -20 °C to interact with the polymer.

Treatment of PCL with Rumex patientia

R.patientia plant, the extract of which was prepared, was dispersed with PCL in Dimethyl sulfoxide solvent at a ratio of 1:1 for 1 hour in an ultrasonic homogenizer. After mixing for about 30 minutes on a magnetic stirrer, it was prepared for antimicrobial measurements [24].

Test Microorganisms

Bacillus megaterium DSM32, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC25322 and *Candida albicans* FMC17 microorganisms obtained from the culture collection of Fırat University Faculty of Science Department of Biology Microbiology Laboratory were used.

Antimicrobial Assay of Poly (ε-caprolactone) Treated with Rumex Patientia

The antimicrobial activity of PCL treated with *R.patientia* were determined according to the disk diffusion method [25]. Bacterial strains were inoculated into Nutrient Broth (Difco) for 24 hours at 35 ± 1°C, and the yeast strain was incubated for a longer time (48 hours) in Malt Extract Broth (Difco) at 25 ± 1°C. While the prepared bacterial culture was inoculated on Müeller Hinton Agar as 10^6 bacteria ml^{-1}, the yeast broth was inoculated on Sabouraud Dextrose Agar as 10^4 yeast ml^{-1} (Both inoculations were made at 1% rate). Then, after the shaking process was finished, 25 mL was taken and placed in sterile petri dishes with a diameter of 9 cm. In this way, homogeneous distribution of the medium was achieved. Antimicrobial discs with a diameter of 6 mm were impregnated with 100 µl of the prepared extracts at a concentration of 10 ppm and carefully placed on solid agar medium. Prepared petri dishes were incubated at 4 °C for 1.5-2 hours. Yeast inoculated plates were incubated at 25 ± 0.1°C for 72 hours, while bacteria inoculated plates were incubated at 37 ± 0.1°C for 24 hours. Standard discs of streptomycin sulfate 10 µg $disk^{-1}$ and Ceftrioxan 30 µg $disk^{-1}$ were used as controls. Dimethyl sulfoxide (DMSO) was used as negative control and inhibition zones were measured in mm.

Results and Discussion

FTIR Results

In Figure 1, the FTIR spectrum of poly (ε-caprolactone) is given. The characteristic signal of PCL at 1713 cm^{-1} in the FTIR spectrum belongs to the –C=O stretching vibration. The binary signal at 2943-2860 cm^{-1} belongs to aliphatic -CH, -CH_2 asymmetric and symmetric stretching vibrations, respectively [26]. Signals in the range of 1468 cm^{-1} – 1165 cm^{-1} are attributed to the -CH_2 deformation signals of PCL [27]. In addition, the signals of asymmetric and symmetric stretching vibrations of the C-O-C bond in the polymer structure were observed at 1240 cm^{-1} and 1165 cm^{-1}, respectively [28].The signal at 725 cm^{-1} is the shaking vibration of the methylene group in PCL [29, 30].

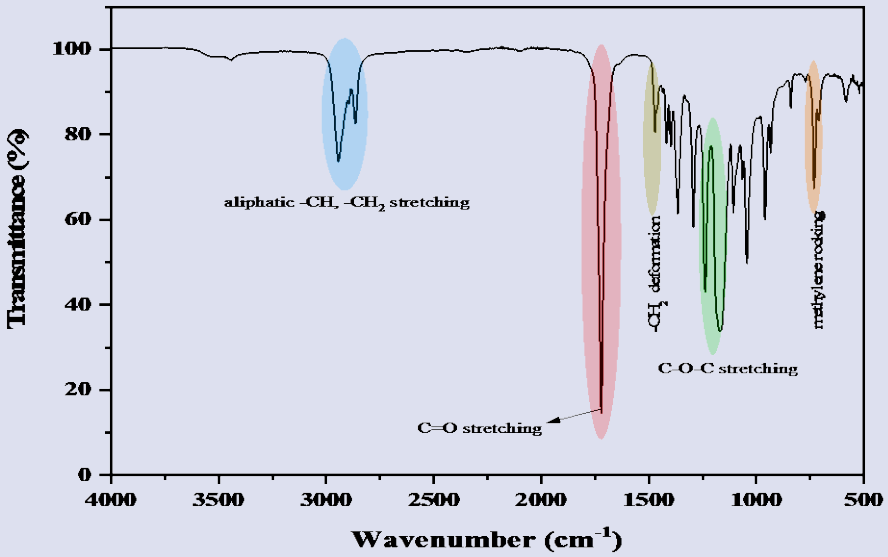

Figure 2. FTIR spectra of PCL

Thermal Results

The thermal properties of PCL synthesized by ring-opening polymerization were investigated. While the TGA curve of PCL is given in Figure 3, the DSC curve that gives the melting temperature of PCL is seen in Figure 4. In the TGA curve, it was observed that PCL decomposed in a single step. It was determined that the initial decomposition temperature (T_i) was 249.5 °C and the amount of residue at 500 °C was 5.9%. The peak of melting temperature (T_m) of PCL is around 57.5 °C, glass transition (T_g) was not observed in our experimental group.

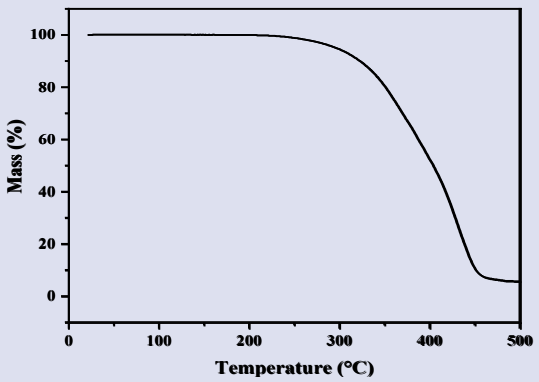

Figure 3. TGA curve of PCL

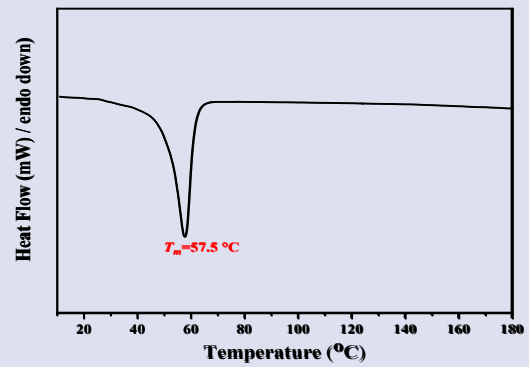

Figure 4. DSC curve of PCL

XRD Results

Figure 4 shows the XRD pattern of PCL at room temperature. PCL, which has a semi-crystalline orthorhombic structure, has 21.3 degrees (110) plane and 23.6 degrees (200) plane signals in the XRD spectrum. In addition, using the Debye-Scherer equation, the crystallinity of PCL was calculated as 53.6% from the XRD spectrum.

$$D = K\lambda/(\beta cos\theta) \quad (1)$$

β: The full width of the maximum half-peak (FWHM)
λ: The wavelength of the x-ray
θ: The angle of the maximum peak

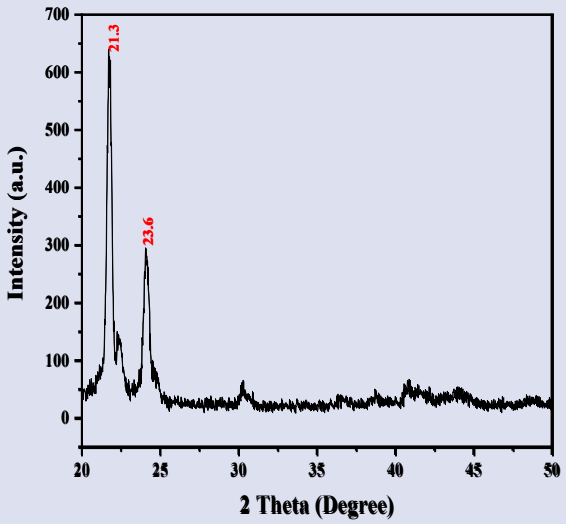

Figure 5. XRD spectra of PCL

Antimicrobial Activity Results

The antimicrobial activities of the ethanolic extract of *R. Patientia*, PCL, and PCL samples treated with *R. Patientia* against *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus megaterium* and *Candida albicans* microorganisms are given in Figure 5, and the zone diameters are given in Table 1.

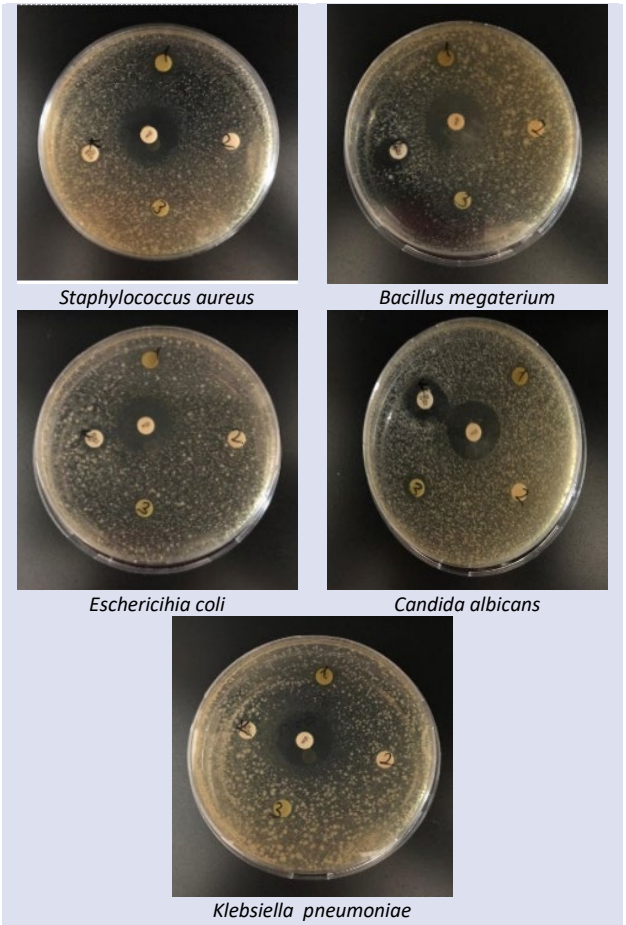

Figure 6. The antimicrobial assay of *Rumex patientia* treated with Poly ε-caprolactone against the bacterial pathogens

While R. patientia extract did not have an antimicrobial effect against E. coli, B. megaterium, K. pneumoniae and C. albicans, it showed an antimicrobial effect (10 mm) against S. aureus. PCL did not form an inhibition zone against the microorganisms used. It was determined that PCL treated with R. Patientia had antimicrobial activity against gram-positive bacteria (B.megaterium, S.aures) and yeast strain (C.albicans), but did not show antimicrobial activity against gram-negative bacteria (E.coli, K.pneumoniae). Antimicrobial polymers have often designed by mimic antimicrobial peptides [31]. Although the polymer used in the study does not have the potential to be an antimicrobial polymer, it is very important in terms of being an antimicrobial peptide in combination with the plant. PCL treated with R. patientia appears to have little antimicrobial effect compared to controls. The reason for this varies according to the solvent used, the amount of concentration, polymer and plant species.

Table 1. Inhibitory zone diameters (mm) of *Rumex patientia L.* treated with PCL

Microorganism	*R. patientia*	PCL	*R. patientia* Interacted with PCL	Streptomycin	Ceftriaxone
E. coli	-	-	-	20	8
B. megaterium	-	-	8	20	11
K. peumoniae	-	-	-	19	9
S. aureus	8	-	7	20	8
C. albicans	-	-	9	21	16

Conclusion

The number average molecular weight (M_n) of PCL synthesized by ring opening was determined to be 4000 $gmol^{-1}$. The characteristic signals of the synthesized PCL (-C=O stretching vibration at 1713 cm^{-1}) were determined by the FT-IR spectrum. In addition, it was calculated from the XRD spectrum that the crystallinity of PCL was 53%. It was determined that *R. Patientia* plant extract had antimicrobial effect only against *S. aureus,* while *R. Patientia* plant, which interacted with PCL, showed antimicrobial effect against *B. Megaterium*, *S. Aureus* and *C. Albicans* microorganisms. As a result, it was observed that the polymer, which did not show any antimicrobial effect, increased its antimicrobial properties in the plant after being treated with the plant extract.

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Conflicts of interest

The authors state that did not have conflict of interest.

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