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Artırılmış Biyoaktivite için Bazı Flavonoid Karışımlarının Elektroeğirme Tekniği Kullanılarak Enkapsülasyonu ve Kontrollü Salım Çalışmaları

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in vitro ilaç salım,
Naringin,
Rutin hidrat.

ÖZET

Narenciye meyvelerinden elde edilen biyoaktif bileşikler olan rutin hidrat, naringin ve hesperidin kullanılarak polimer bazlı elektroeğirme fiberleri hazırlanmıştır. Bu flavonoidlerin antioksidan, anti-inflamatuar ve anti-kanser gibi çeşitli biyolojik aktiviteleri bulunmaktadır. Elektroeğirme tekniği sayesinde, flavonoidlerin biyolojik kullanılabilirliği artırılarak, ilaç taşıma sistemleri, gıda ambalajları ve kozmetik ürünler gibi farklı alanlarda potansiyel uygulamalar geliştirilmesi amaçlanmıştır. Elde edilen fiberlerin morfolojik ve kimyasal ve karakterizasyonları ile flavonoidlerin fiber matris içerisindeki dağılımı ve salım davranışları belirlenmiştir. Aynı zamanda, nanofiberlerin *Staphylococcus aureus* (*S. aureus*) bakterisine karşı antibakteriyel aktivitesi değerlendirildi. Yapılan çalışmanın sonucunda bağırsak pH'ında (pH 7,4) yapılan dissolüsyon analizinde PLA-MIX fiberi etken maddeleri %20-25 oranında, PCL- MIX fiberi etken maddeleri %21-28 oranında salındı. Antibakteriyel sonuçlar nanofiberlerin %45 oranında PLA karışımında daha fazla bakteriyel inhibisyona neden olduğunu gösterdi. Bu çalışma, narenciye atıklarının değerlendirilmesi ve yeni nesil biyomalzemelerin geliştirilmesi açısından önemli bir katkı sağlamaktadır.

Encapsulation of Some Flavonoid Mixtures Using Electrospinning Technique for Enhanced Bioactivity and Controlled Release Studies

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ABSTRACT

Polymer-based electrospun fibers were prepared using bioactive compounds derived from citrus fruits such as rutin hydrate, naringin and hesperidin. These flavonoids have various biological activities such as antioxidant, anti-inflammatory and anticancer. By increasing the bioavailability of flavonoids through the electrospinning technique, it is aimed to develop potential applications in different areas such as drug delivery systems, food packaging and cosmetic products. The distribution and release behaviors of flavonoids in the fiber matrix were determined by morphological and chemical characterization of the obtained fibers. At the same time, the antibacterial activity of nanofibers against *Staphylococcus aureus (S. aureus)* bacteria was evaluated. As a result of the study, in the dissolution analysis performed at intestinal pH (pH 7.4), the active ingredients of PLA-MIX fiber were released at a rate of 20-25% and PCL-MIX fiber at a rate of 21-28%. Antibacterial results showed that nanofibers caused more bacterial inhibition in a 45% PLA mixture. This study provides an important contribution to the evaluation of citrus wastes and the development of new-generation biomaterials.

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INTRODUCTION

Controlled drug delivery offers numerous advantages as it reduces premature degradation, improves drug uptake, maintains drug concentrations within the therapeutic window, and reduces side effects. Over the last decade, recent advances in genomics and nanotechnologies have made it possible to achieve drug delivery by using specifically designed nanoparticle carriers, particularly in anticancer therapy, vaccines, and theranostic imaging [1].

One popular technique for encapsulating active nutraceutical ingredients into fibers is to combine them with the polymer solution and spin the mixture. The encapsulation of bioactive compounds for ultimate incorporation in food plays a major role in food processing. Capsules constructed of polymers based on lipids, proteins, and carbohydrates coat or encapsulate active components, such as food-bioactive chemicals or living cells, inside micro- or nanoscale-sized particles. Encapsulated bioactive compounds have enhanced stability, bioavailability, and physicochemical properties while being shielded from external stresses. Electrospinning is a feasible method for encapsulating food bioactive substances, as it effectively produces dry, food-grade, nano-scaled materials with good encapsulation efficiencies [2-4].

PLA is FDA-approved for direct contact with biological fluids. One of the key features that makes PLA advantageous for drug carriers is its biodegradability. PLA can easily dissolve in extracellular environments, and its degradation rate can be modulated to achieve the desired effect. For drug carrier systems in particular, the kinetics of this degradation can be extended to maintain the sustained release of medicinal agents. This provides sufficient time for the drug to exert its effect, which is crucial since this therapeutic strategy can potentially be diminished by metabolic processes [5].

Polycaprolactone (PCL) is a highly valued polymer for wound dressing applications due to its exceptional mechanical strength, biocompatibility, and biodegradability. PCL is a commonly utilized polymer in the electrospinning process because of its easy spinnability. Due to its biocompatible and biodegradable properties, PCL is a semi-crystalline polyester that has received FDA approval and has promise for usage in biomedical applications like wound dressing applications and delivery systems. One of the most popular biopolymers for skin regeneration is also easily processed into a variety of forms, including films, mats, membranes, and fibers [6-7].

Rutin is gaining significant attention due to its wide pharmacological potential and non-toxic nature. It also has antioxidant, anti-inflammatory, and anticancer activities thanks to its radical sweeping activity. However, its utility in clinical applications is limited by its poor stability and bioavailability. A bioactive compound's pharmacodynamics and pharmacokinetics may be improved by novel drug delivery methods. For this reason, employing a novel drug distribution mechanism can get around regular restrictions [8,10].

Fruits including oranges, lemons, tomatoes, mandarins, and grapefruit peel contain naringin, which has a low solubility in water and a poor absorption rate in the digestive system. Therefore, encapsulation techniques and release mechanisms can increase its formulation, absorption, and stability. This phytochemical has heart-protective, antimicrobial, anticancer, and antioxidant properties [11].

The most common flavonoid in oranges and lemons is hesperidin, a cheap and abundant byproduct of the citrus family. Its benefits include lowering cholesterol, antioxidant, edema-reducing, hypolipidemic, and vascular protection. Hesperidin has a low solubility in water and limited bioavailability [9].

Studies have demonstrated the high multifunctional bioactivities of flavonoids, including antiviral, antioxidant, antibacterial, anti-inflammatory, and anti-tumor properties. Using the

electrospinning technology, the goal of this work was to encapsulate flavonoid combinations for use in the nutraceutical supplement market as a controlled release mechanism [2-4].

MATERIALS AND METHOD

Materials

Rutin hydrate, naringin, and hesperidin were purchased from Merck, Germany. Polylactic acid (PLA) (Mw~ 60,000), polycaprolactone (PCL) (Mn 80,000), hexafluoro-2-propanol (HFIP), methanol (MeOH), chloroform and hydrochloric acid (HCl) were supplied by Sigma- Aldrich, Germany. Gelatin was bought from SelJel Gelatin, Türkiye. pH 1.2 and pH 7.4 buffer solutions were made in laboratory conditions.

Method

Preparing Electrospun Fibers

Three different flavonoids—rutin, naringin, and hesperidin—along with two different polymers—PCL and PLA—were chosen. The three flavonoids were mixed and the was mixture added to the solution at a rate of 10% PLA after PLA (2.5%) and gelatin (7.5%) were dissolved in 20 mL of HFIP. To prepare the PCL-containing electrospinning solution, 80000 kDa PCL (10%) was dissolved in a 40:60 methanol to chloroform mixture before adding mixed active components. For electrospinning, the resultant solution was put into a 5 mL syringe. With a voltage of 18 kV, a flow rate of 1 mL/h, and a tip-to-collector distance of 15 cm, fibers were created by electrospinning [12-13].

Drug Content, Encapsulation Efficiency and in vitro Drug Release

Nanofibers were cut into small pieces, weighted around 100-120 mg, and transferred into 50 mL falcon tubes. Fibers were dissolved with 1 mL chloroform, 1 ml HCl solution (1% v/v), and 3 ml MetOH mixture, centrifuged at 4000 rpm for 20 min, and sonicated for 5 minutes. Solutions were filtered through a 0.45 µm PTFE filter and analyzed by UV-Vis spectroscopy at the suitable wavelength. The results are shown in Table 1 [14-16].

$$EE\% = \frac{Encapsulated\ API}{Total\ API\ content} \quad (1)\ [16]$$

In vitro drug release was carried out by USP <711>. Two distinct pH ranges will be used in the investigation throughout the dissolution phase. The pH 1.2 and pH 7.4 ranges were determined as these. The right buffer solutions were made, and the dissolving process was induced in various parts of the stomach and intestine. Samples were obtained from the stomach environment every 15 minutes from 0 to 2 hours, and every hour from 2 to 3 hours. Samples were obtained from the intestinal environment every 30 minutes between 0 and 2 hours, and every hour between 2 and 5 hours. The HPLC-PDA was used to analyze the samples. The results are shown in Figures 1-4 [17].

Characterization of Fibers

The ZEISS GeminiSEM 500 scanning electron microscope was used to examine the diameter and morphology of the electrospun fibers. A small piece of the fiber mat was gold-sputter-coated (4.35 nm) and placed on the SEM sample holder. A voltage of 20 kV was used to acquire SEM pictures [18].

Shimadzu IR Tracer-100 Fourier Transform Infrared Spectroscopy was used to examine the fiber's chemical content. Active pharmaceutical ingredients (rutin hydrate, naringin and hesperidin), PLA, gelatin, PCL, and two fibers (PLA-MIX and PCL-MIX) were examined in the mid-IR range (4000-400 cm^{-1}) by 16 scans [18].

Antibacterial Activity Assay

The colony counting method was used to evaluate the antibacterial activity of nanofibers against the *Staphylococcus aureus (S. aureus)* ATCC6538 bacterial strain. Each nanofiber, approximately 1 X 1 cm in diameter, was covered with a sterile film after 100 µL of bacterial solution containing 1.5 × 107 colony-forming units (CFU/mL) was added to its surface. The nanofibers were incubated in the incubator at 37°C for 24 hours. After incubation, the films were washed with 1 mL of PBS solution, and 100 µL was taken and inoculated into blank Nutrient agar media. The number of viable bacterial colonies was determined after a 24-hour incubation period at 37°C. The decrease in the number of bacteria was calculated using the following formula:

$$R(\%) = \frac{(B - A)}{B} * 100 \qquad (2)\ [19]$$

R: %inhibition, **A:** the colonies in the control samples, **B:** the colonies in the nanofiber samples

RESULTS

Encapsulation Efficiency and in vitro Drug Release

Active pharmaceutical ingredient (API) amount determination and encapsulation efficiency were calculated. Encapsulation efficiency is calculated by Equation 1.

Table 1
Encapsulation efficiency of API in PLA and PCL electrospun fibers

API	PLA (%)	PCL (%)
RTN	29.80	15.88
HSP	38.41	25.24
NAR	5.09	1.30

According to the dissolution analysis results, the release percentages in the stomach and intestinal environments for the fiber mat produced using PLA-gelatin were similar. In the fiber mat made with the PCL polymer, the release was greater in the intestinal environment compared to the stomach environment.

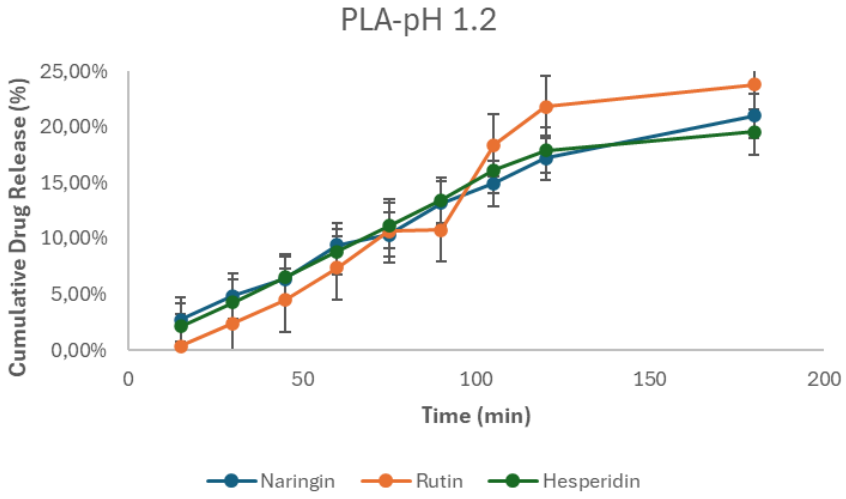

Figure 1
Cumulative drug release of API's from PLA fiber at pH 1.2

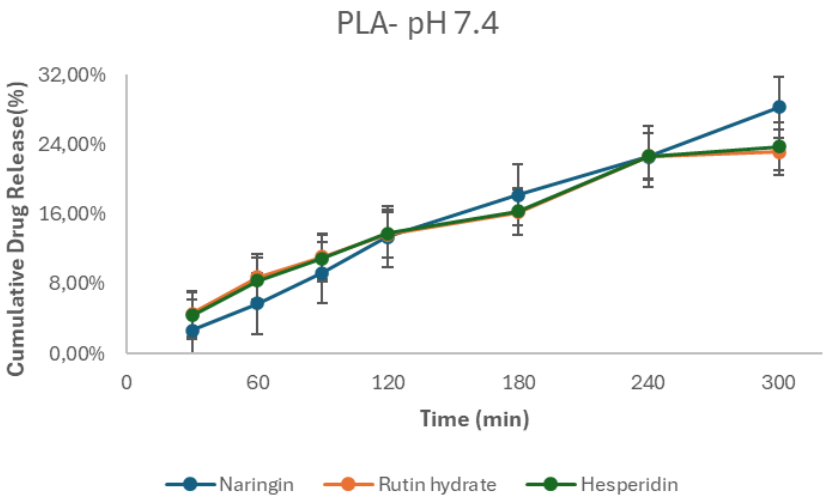

Figure 2
Cumulative drug release of API's from PLA fiber at pH 7.4

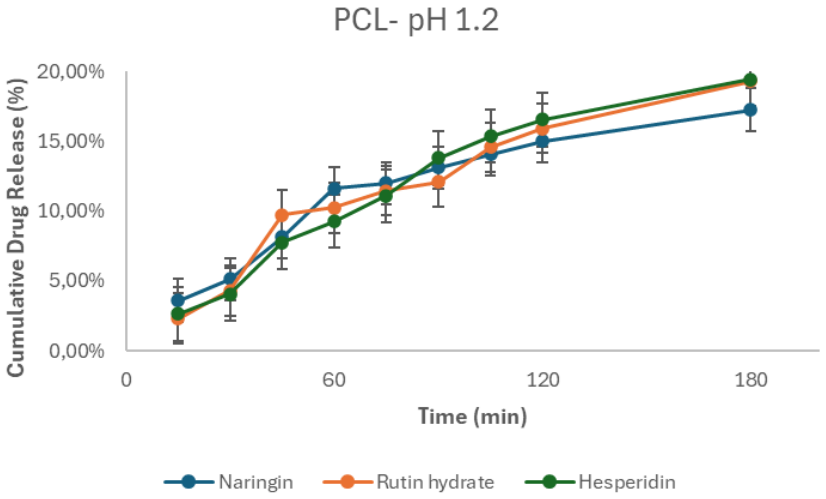

Figure 3
Cumulative drug release of API's from PCL fiber at pH 1.2

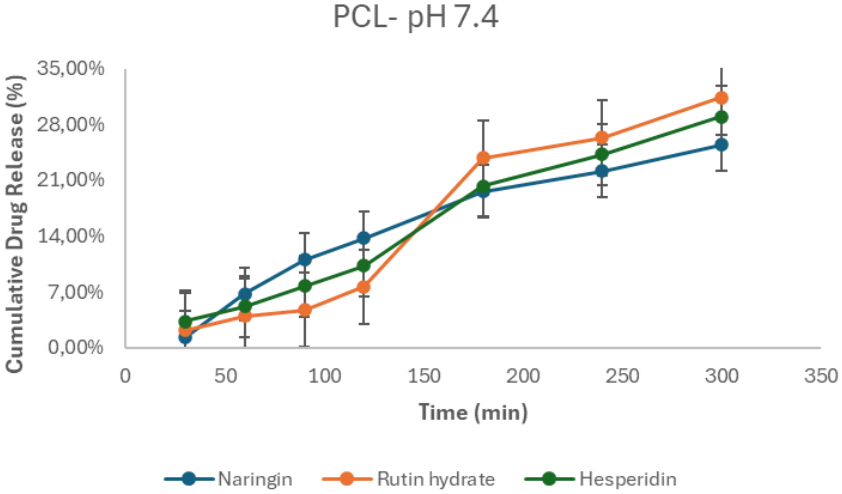

Figure 4
Cumulative drug release of API's from PCL fiber at pH 7.4

FESEM Imaging

In the FE-SEM images of the PCL-MIX mat (Fig. 5), it was seen that the fibers were smooth, and no bead formation was observed. The fiber diameter average thickness is over 800 nm. In the FE-SEM images of the PLA-MIX mat (Fig. 6), it was seen that the fibers were smooth, and no bead formation was observed. The fiber diameter average thickness is over 400 nm.

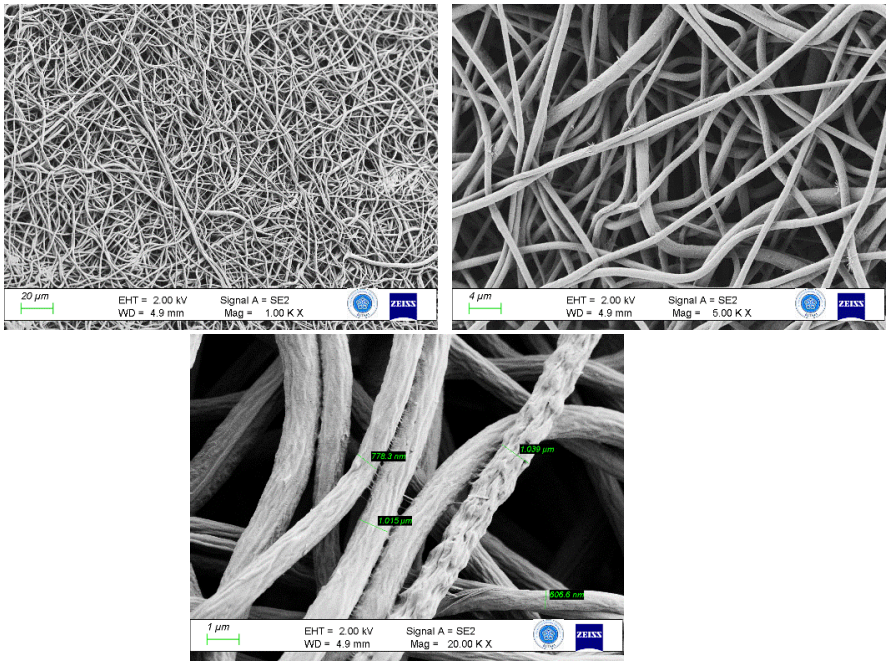

Figure 5
PCL-MIX FESEM images

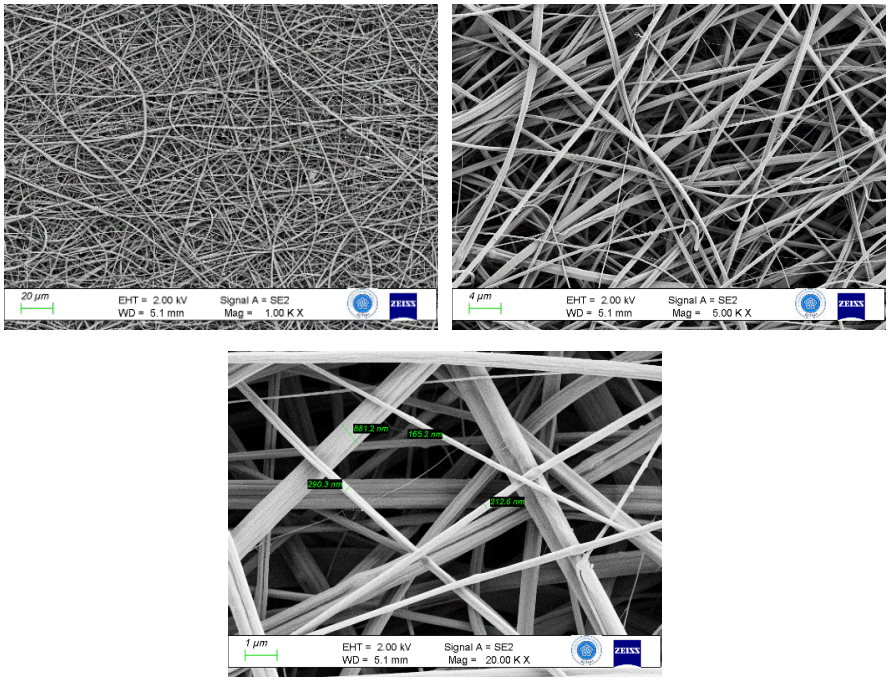

Figure 6
PLA-MIX FESEM images

FT-IR Spectroscopy

In Figure 7, the spectrum of electrospun fibers prepared with PCL polymer, the peak observed at 3500-3300 cm^{-1} was the hydroxyl (-OH) peak. The peak belonging to the alkene group (C=C) was observed at 3000-2900 cm^{-1}. Alkyl (C-H) was observed at 2900-2800 cm^{-1}. The C=O bond was observed at 1760 cm^{-1}. It was called the aromatic ring around 1700-1650 cm^{-1}. There was a carbonyl group and O-C-O structure at 1200-1150 cm^{-1} [20-23].

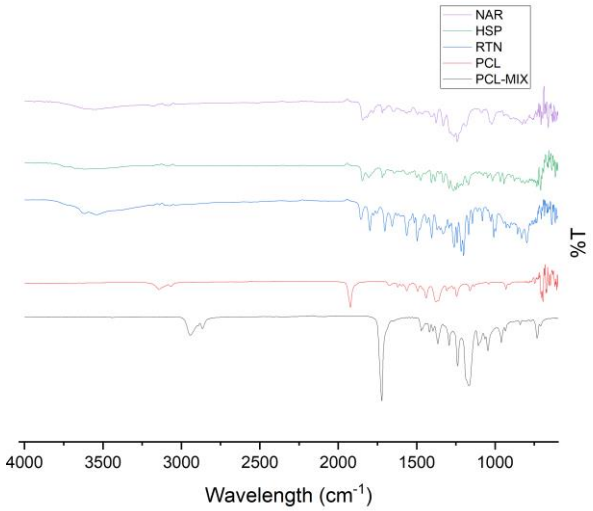

Figure 7
FT-IR spectra of citrus flavonoids, PCL and PCL-MIX fiber

In Figure 8, spectrum of electrospun fibers prepared with PLA-Gelatin polymer, the peak observed at 3500-3300 cm^{-1} is the hydroxyl (-OH) peak. The peak observed at 3000 cm^{-1} indicates the alkene group (C=C). The peak observed at 2900-2800 cm^{-1} belongs to the alkyl (C-H) group. The peaks observed at 1750 and 1700 cm^{-1} are due to carboxyl and ester bonds, respectively. The peak observed at 1650 cm^{-1} is the amide (C=O) group. The peak observed at 1550 cm^{-1} is the stretching vibration of C-N bonds. The peak observed at 1260 cm^{-1} is due to C-O-C bonds. [20-23].

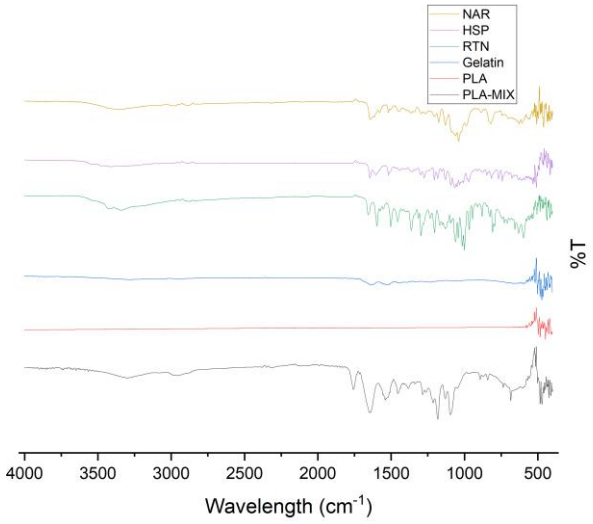

Figure 8
FT-IR spectra of citrus flavonoids, PLA, Gelatin and PLA-MIX fiber

Antibacterial Assay

It has been demonstrated that rutin hydrate has significant antibacterial properties. Rutin's strong antibacterial action was demonstrated by a study that found that at 1.87 mg/mL and 25 °C, it could suppress the growth of Staphylococcus aureus by up to 100%. Moreover, rutin has been shown to amplify the antibacterial properties of other substances, indicating a synergistic potential that may be helpful in fighting resistant bacterial strains. This is especially important when it comes to methicillin-resistant *Staphylococcus aureus* (MRSA), since rutin's capacity to stop bacterial growth may help develop more potent treatment plans [24-25].

Another flavonoid that is mostly present in citrus fruits, naringin, has likewise shown strong antibacterial qualities. Studies have demonstrated that naringin is an efficient means of inhibiting the growth of Staphylococcus aureus, especially in diseases that mirror infections, including osteomyelitis. Naringin works by perhaps rupturing the membranes surrounding bacterial cells or by blocking particular metabolic processes, which stops the growth of bacteria. Furthermore, naringin's involvement in improving the efficacy of other antimicrobial drugs emphasizes its potential as a valuable component in medicinal formulations against bacterial infections [26-27].

Like naringin, hesperidin is a flavonoid that is present in citrus peels and has been linked to several health advantages, including antibacterial action. Studies have revealed that hesperidin can suppress biofilm formation by Staphylococcus aureus, which plays a significant role in the persistence and resistance of bacterial infections. Hesperidin's capacity to prevent the formation of biofilms implies that it may be used with other antibiotics to increase their efficacy in treating infections linked to biofilms. Furthermore, as oxidative stress is known to play a role in bacterial survival and virulence, hesperidin's antioxidant qualities may also contribute to its antibacterial actions [28-29].

In the antibacterial studies, no inhibition zone was observed, but bacterial inhibition percentages were measured as 30.04±3.15% and 45.94±4.6% for PCL and PLA, respectively.

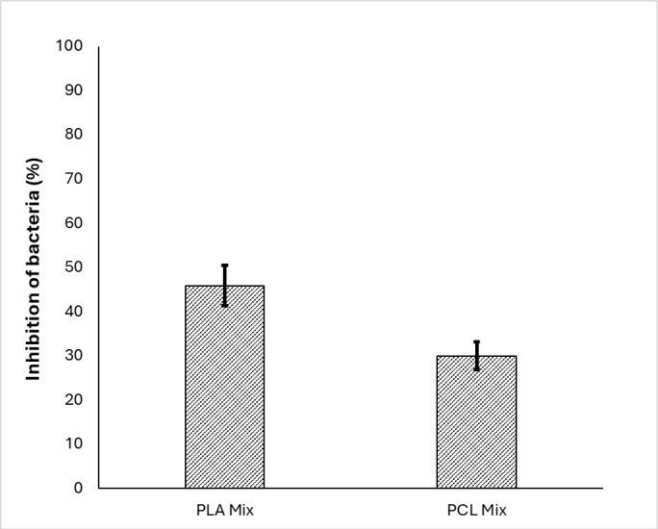

Figure 9
Inhibition of Staphylococcus aureus bacteria in PLA-MIX and PCL-MIX

DISCUSSION AND CONCLUSION

In the study, PLA and PCL electrospun fibers loaded with a flavonoid mixture were analyzed. No bead formation was observed in both produced fibers, and the fiber mats were produced smoothly. Dissolution analysis was performed at stomach pH for 3 hours and at intestinal pH for 5 hours, and it

was observed that the cumulative drug release was above 20% in both environments. In the FE-SEM images of the PCL-MIX fiber mat, it was observed that the fibers were dispersed, and no bead formation was observed. The fiber diameters were 800 nm on average. In the FESEM images of the PLA-MIX fiber mat, it was observed that the fibers were more uniformly placed, and no bead formation was observed. The fiber diameters were 200 nm on average. In the FT-IR spectra of the PCL-MIX fiber, PCL, and flavonoids compared, it was observed that the flavonoids peaked in the PCL-MIX fiber mat. Likewise, in the FT-IR spectra comparing PLA-MIX fiber, PLA, and flavonoids, it was observed that flavonoids peaked in the PLA-MIX fiber mat. *Staphylococcus aureus* is a Gram-positive, aerobic, highly pathogenic bacteria that produces toxins. It is one of the primary microbial food contaminants that cause a variety of ailments in both humans and animals. The bacterial inhibition percentage in the PCL-MIX fiber mat was measured as 30.04±3.15%. While the bacterial inhibition percentage in the PLA-MIX fiber mat was measured as 45.94±4.6%. These results showed that rutin hydrate- hesperidin-naringin-loaded PLA fiber mats can be used to gather antibacterial surfaces for different applications. While cumulative release of substances from PCL nanofiber mats is higher than the release of substances from PLA nanofiber mats, the antibacterial activities of PLA-MIX can cause by nanofiber morphologies and diameters [30-31].

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Ethical Statement

This article is the revised and developed version of the unpublished conference presentation entitled "Polymer-Based Encapsulation of Flavonoids via Electrospinning for Nutraceutical Applications", presented as a poster at the 6th International Congress on Biosensors.

Author Contributions

Research Design (CRediT 1) D.T.A. (%70) - E.K. (%30)
Data Collection (CRediT 2) D.T.A. (%90) - E.K. (%10)
Research - Data Analysis - Validation (CRediT 3-4-6-11) D.T.A. (%80) - E.K. (%20)
Writing the Article (CRediT 12-13) Author 1 D.T.A. (%80) - E.K. (%20)
Revision and Improvement of the Text (CRediT 14) D.T.A. (%50) - E.K. (%50)

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Conflict of interest

The authors declare no conflict of interest for the present study.

Sustainable Development Goals (SDG)

Sustainable Development Goals: 3 Good Health and Well-Being

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