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Research Article / Araştırma Makalesi

Evaluation of Antimicrobial Activities of 1,3-Bis Chalcone Derivatives on Cellulose Based Materials

Selüloz Bazlı Malzemeler Üzerindeki 1,3-Bis Kalkon Türevlerinin Antimikrobiyal Aktivitelerinin Değerlendirilmesi

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Abstract

Hospital-acquired infections increase hospital stays and put pressure on national economies. The increasing prevalence of antimicrobial resistance necessitates the development of new antimicrobial agents and the implementation of more effective methods for preventing infections. Antimicrobial textiles are important tools for reducing the spread of hospital-acquired infections and antibiotic resistance genes. In this study, two chalcone compounds (K1 and K2) were synthesized via the Claisen–Schmidt reaction and applied to single jersey fabrics. We evaluated the antimicrobial activity of the fabrics using quantitative and qualitative methods. Scanning electron microscopy and Fourier transform infrared analyses indicated the presence of both chalcone compounds in fabrics treated at concentrations of 1% and above. Quantitative antibacterial analysis showed that cotton fabrics treated with chalcone at concentrations of 1% and 4% inhibited bacterial growth against *Staphylococcus aureus* by 61.00–86.66%.

Keywords: Chalcone, Bioactive compound, Antibacterial activity, Functional textile, Technical textile, Oxa-Michael addition.

Öz

Hastane kaynaklı enfeksiyonlar, hastanede kalış sürelerini artırmakta ve ulusal ekonomiler üzerinde baskı oluşturmaktadır. Antimikrobiyal direncin artması, yeni antimikrobiyal ajanların geliştirilmesini ve enfeksiyonların önlenmesine yönelik daha etkili yöntemlerin uygulanmasını gerektirmektedir. Antimikrobiyal tekstiller, hastane kaynaklı enfeksiyonların ve antibiyotik direnç genlerinin yayılmasını azaltmak için önemli araçlardır. Bu çalışmada, Claisen-Schmidt reaksiyonu yoluyla iki kalkon bileşiği (K1 ve K2) sentezlenmiş ve tek jarse kumaşlara uygulanmıştır. Kumaşların antimikrobiyal aktivitesini nicel ve nitel yöntemler kullanarak değerlendirilmiştir. Taramalı elektron mikroskobu ve Fourier dönüşümlü kızılötesi analizleri, her iki kalkon bileşiğinin de %1 ve üzerindeki konsantrasyonlarda işlem görmüş kumaşlarda bulunduğunu göstermiştir. Kantitatif antibakteriyel analiz, %1 ve %4 konsantrasyonlarında kalkon uygulanan pamuk kumaşların *Staphylococcus aureus*'a karşı %61,00–%86,66 oranında bakteriyel büyümeyi inhibe ettiğini göstermiştir.

Anahtar Kelimeler: Kalkon, Biyoaktif bileşik, Antibakteriyel aktivite, Fonksiyonel tekstil, Teknik tekstil, Oksa-Michael katılması.

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1. Introduction

Hospital-acquired infections are one of the most common problems met during care services. Nosocomial infections affect 5-10% of people cared in hospitals. This situation causes great harm to both human health and national economies. Nosocomial infections cost 10-15 billion Rubles in Russia, €7 billion in Europe, and US$ 6.5 billion in the United States each year (Abramova et al., 2014). In addition to the economic issue, microorganisms in the hospital environment increase antibiotic resistance against effective drugs (Arafa Badr, 2018). Antibiotic resistance causes microorganisms to become resistant to the bacteriostatic or bactericidal effects of antibiotics (Junaid et al., 2022). Although the early years of antibiotics led to a significant reduction in infectious diseases, antibiotic resistance complicates the treatment of many infectious diseases such as pneumoniae, tuberculosis, and gonorrhea. As a result, this increases mortality/morbidity rates, length of hospital stay and medical costs (Sanchez Ramirez et al., 2019). *Staphylococcus aureus (S. aureus)*, *Klebsiella pneumoniae (K. pneumoniae)*, and *Candida albicans (C. albicans)* are among the most frequently encountered human pathogens responsible for a wide range of community- and healthcare-associated infections (Tobin & Zahra, 2025). Therefore, developing textile materials with antimicrobial activity against these pathogens may contribute to reducing the transmission of healthcare-associated infections.

Textile materials provide a very favorable environment for the reproduction of microorganisms, (Shariatinia et al., 2017) and this helps the spread of resistant microorganisms. Microorganisms also cause problems such as decrease in color quality (Arafa Badr, 2018) and performance values (El-Khatib et al., 2020), cause unpleasant odors (Elsayed et al., 2015) and decrease in the life cycle of the product in textiles. Especially in the COVID-19 pandemic, the interest in antimicrobial agents and antimicrobial textile products has increased. Antimicrobial agents of natural or synthetic origin have an inhibitory effect on microbial growth (bacteriostatic) or a lethal effect on microorganisms (bactericidal) (Singh et al., 2012). Many antimicrobial agents have adverse effects on the consumer and the environment. For this reason, it is necessary to use non-toxic and environmentally friendly antimicrobials in textile products (Elsayed et al., 2015).

Textile manufacturers and researchers try to provide antimicrobial textiles by bonding bacteriostatic and bactericidal structures to the textile surface (Abo El-Ola et al., 2019). Some substances are attached to fiber surfaces to produce antimicrobial textiles: metal ions such as zinc oxide (Subash et al., 2011), copper oxide, silver (Elsayed et al., 2015; Singh et al., 2012); natural substances such as chitosan (Shanmugam et al., 2016); some synthetic biopolymers such as poly(butylene succinate) (Muñoz-Bonilla et al., 2019). In addition, surface modification processes such as plasma technology are also used in antimicrobial textile studies (Ražić et al., 2017).

Chalcones are precursors of flavonoids (Pastor-Villaescusa et al., 2018) and can be obtained synthetically or isolated from plants (Cazarolli et al., 2013). Flavonoid compounds exist in many types of fruit and vegetable (Xiao, 2022). Many studies have shown that flavonoids have antibacterial (Ali & Dixit, 2012), antiallergic (Sato & Tamura, 2015) and antioxidant (Tajammal et al., 2022) activity. Chalcones, first synthesized in the 1880s (Sulpizio et al., 2018), have a wide spectrum of biological activity, including antimicrobial, anticancer, antioxidant, anti-inflammatory and antiproliferative effects (Chu et al., 2018; Go et al., 2012; Gürdere et al., 2020). Although the most popular method is the Claisen-Schmidt reaction to synthesize chalcones (Sharma & Saraswat, 2021; Gürdere et al., 2016), some different methods are also used such as coupling reactions (Heck, Suzuki, Julia-Kocienski), Friedel-Crafts reaction, Photo-Fries rearrangement (Rammohan et al., 2020). Furthermore, chalcone derivatives have been widely used as key intermediates in the synthesis of biologically active heterocyclic compounds, further highlighting their importance in medicinal chemistry (Gezegen et al., 2021; Korkmaz et al., 2021).

In this study, we aimed to synthesize 1,3-bis chalcone compounds whose activities are given in the literature and apply them to cotton fabrics in order to obtain antimicrobial properties (Tutar et al., 2019). The chalcone derivatives were synthesized by the Claisen-Schmidt reaction, and their structures were confirmed by Nuclear magnetic resonance (NMR) and Fourier-transform infrared (FTIR) spectroscopy. Cotton fabrics treated with chalcones in dimethyl sulfoxide (DMSO) at pH 8-9. The surface morphology of the fabric samples was examined using scanning electron microscopy (SEM), and the functional groups were examined using FTIR. The antimicrobial properties were evaluated using the agar diffusion method against gram-positive, gram-negative, and a fungal strain. Additionally, the antibacterial activity of the fabrics was quantitatively evaluated using a method similar to ASTM E2149.

2. Materials and Methods

2.1. Materials

In this study, 100% cotton and 100% polyester single jersey fabrics were used (ready to dye, 115 g/m^2). Synthesis of chalcone compounds and antimicrobial studies were carried out in Sivas Cumhuriyet University Faculty of Health Science. In addition, the application of compounds to fabrics was carried out in antimicrobial laboratory at Bursa Uludağ University, Textile Engineering Department.

Chemicals and suppliers: Muller Hinton Broth (MHB) and Muller Hinton Agar (MHA) from Merck and Conda, DMSO from Carlo Erba, 2-Acetylthiophene and 3',4'-Dimethoxyacetophenone from Acros Organic, isophthalaldehyde from Tokyo Chemical Industry, ethanol absolute from Isolab, sodium hydroxide (NaOH) from Chemsolute.

2.2. General Information

Infrared (IR) spectra were recorded using a Bruker Tensor II (OPUS, Karlsruhe, Germany) and a Jasco FT/IR-430 spectrometer (JASCO, Tokyo, Japan). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a 600 MHz Agilent Premium COMPACT NMR spectrometer (Agilent Technologies, Santa Clara, CA, USA). SEM (Tescan Mira3XMU, Czechia) was employed for better understanding the surface features of the cotton fabrics. SEM images were taken at 10 kV accelerating voltage at a 10 mm analytical working distance with secondary electron imaging detector. The samples were gold coated for 1 min as 5 nm for preventing the electron charging on surface (Quorum RS150T, Birmingham, UK).

2.3. Synthesis of 1,3-Bis Chalcone Compounds

Compounds K1 and K2 were synthesized according to the literature method with minor modifications (Tutar et al., 2019). For the synthesis of compound K1, 30 mmol isophthalaldehyde (A) and 60 mmol 3',4'-dimethoxyacetophenone (B) were added to a reaction flask containing 180 mL of ethanol in a 1:2 mole ratio. It was stirred (500 rpm) on a magnetic stirrer for five minutes until a clear solution was obtained. After adding 2.5 M 5 mL of NaOH solution to the mixture, it was stirred at room temperature (25 °C) for 4 hours to complete the reaction. After, the mixture was transferred to a beaker containing 300 mL of distilled water and neutralized with dilute HCl (10%). The obtained yellow precipitate (K1 compound) was filtered from whatman paper and dried at room temperature (Parveen et al., 2014). Compound K2 was synthesized by reacting isophthalaldehyde (37 mmol) and 2-acetylthiophene (74.5 mmol) in ethanol in the presence of 5 mL of 2.5 M NaOH (Figure 1a), following the same reaction conditions described for K1 (Tutar et al., 2019).

2.4. Application of 1,3-bis chalcone compounds to fabrics

Cotton and polyester fabrics (10 × 10 cm^2) were treated simultaneously in the same treatment bath containing 100 mL of DMSO in which the chalcone compounds were dissolved. The treatment was carried out at 25 °C under stirring at 250 rpm for 30 min (pH 8–9). Both fabric types were treated under identical conditions; however, only the cotton fabrics were further characterized and evaluated for antimicrobial activity in this study. Therefore, only the results obtained for cotton fabrics are presented and discussed. Based on the literature (Hasan et al., 2021), the binding of chalcone compounds to cellulose was proposed to occur through an Oxa-Michael addition between the hydroxyl groups of cellulose and the α,β-unsaturated carbonyl groups of chalcone (Figure 1b). Cotton fabrics treated with K1 at concentrations of 0.25%, 0.5%, 1%, and 4% were labeled A1–A4, respectively, whereas those treated with K2 at the same concentrations were labeled A5–A8.

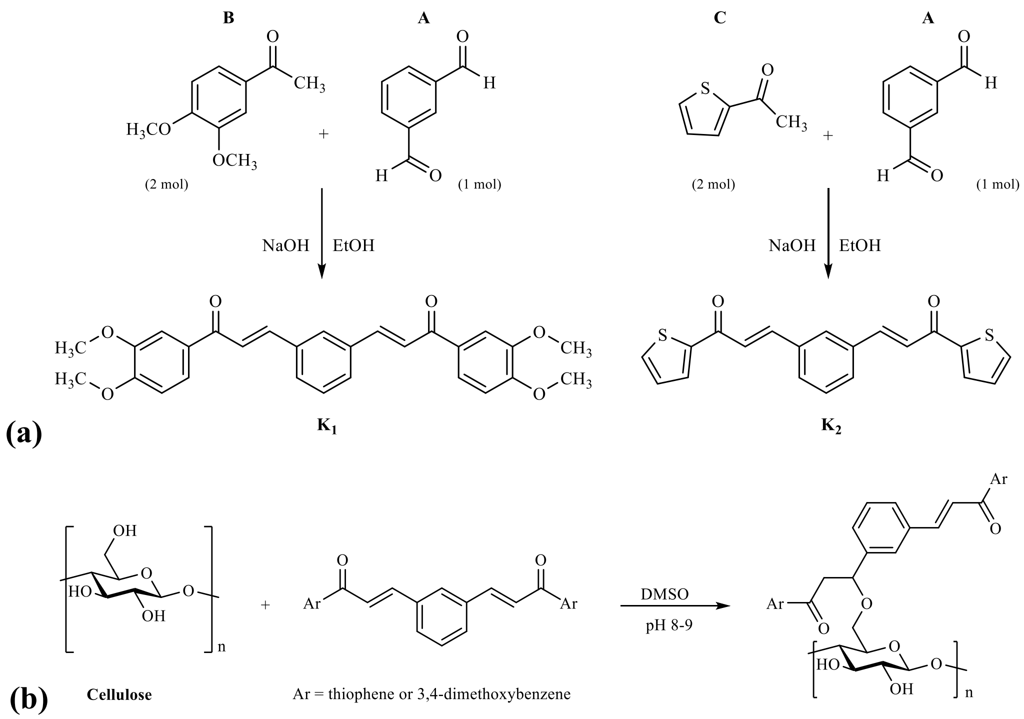

Figure 1. Synthesis of chalcones (a), reaction of chalcone-cellulose binding by Oxa-Michael Addition (b)

2.5. Antimicrobial Activity

We performed a qualitative antimicrobial evaluation using the agar diffusion method (Shateri-Khalilabad et al., 2017) and a quantitative antibacterial evaluation using a method similar to ASTM E2149 (Guzińska et al., 2018), according to the literature, with some modifications.

2.5.1. Qualitative evaluation

Antimicrobial activity of the fabrics was evaluated against *S. aureus*, *K. pneumoniae* and *C. albicans* microorganism strains using the agar diffusion method, similar to the literature. Microorganism strains fixed at a 0.5 McFarland standard were inoculated onto agar media using the spread plate method with swabs. The fabrics were cut in 15 x 15 mm^2 and placed on the inoculated plates as 4 fabrics on one plate. The plates were then incubated at 37 °C for 24 h. The samples' zone formation was examined as a result of the incubation period. Antibiotic discs, untreated fabrics, and DMSO treated fabrics were used as controls. Fluconazole (FCA 25) and nystatin (NS 100) antibiotic discs were used against *C. albicans* fungal strain, and chloramphenicol (C30) antibiotic discs were used against *S. aureus, K. pneumoniae* bacterial strains.

2.5.2. Quantitative evaluation

0.85% NaCl solution (saline) was prepared in distilled water. Ratio of 20/1 saline/MHB mixture was prepared in 20 mL volumes and sterilized. The treated fabrics (approximately 0.1 g) were sterilized in an autoclave under appropriate conditions. We evaluated antibacterial activity of the fabrics treated with chalcones at 1% and 4 % concentrations.

A colony of *S. aureus* bacteria strain was inoculated with 10 mL of MHB with a swab stick and incubated at 37 °C 120 rpm for 18 hours. After incubation, the initial microorganism concentration was adjusted to approximately 1–5x10^5 colony-forming units (CFU)/mL in flasks. The treated fabrics were added to the relevant flasks. A flask without fabric was used as a growth control. All flasks were incubated at 37 °C, 120 rpm for 24 hours in a shaker incubator.

After incubation, dilutions (10^{-1} to 10^{-6}) were prepared by making serial dilutions of the inoculum. 100 µL of dilutions were spread with a drigalski spatula on MHA plates. The colonies were counted after 24 h of incubation at 37 °C. The inhibition rate (%R) of bacterial growth for the treated fabrics was calculated according to Equation (2.1). The tests were performed in duplicate for each sample.

$$\%R = \frac{A-B}{A} \times 100 \quad (2.1)$$

A: The quantity of colonies formed in the growth control,

B: The quantity of colonies formed subsequent to contact with the treated fabric.

3. Findings and Discussion

Chalcone compounds were characterized by NMR and FTIR. The compounds were applied to %100 cotton fabrics directly in room conditions. Fabrics were characterized by FTIR and SEM analysis and evaluated for antimicrobial activity.

3.1. NMR Analysis

The ^{1}H-NMR and ^{13}C-NMR results of the K1 and K2 chalcone compounds confirmed successful synthesis and showed good agreement with literature (Korkmaz, 2019; Parveen et al., 2014; Tutar et al., 2019).

3.2. FTIR Spectra

We used ATR-FTIR spectroscopy to investigate the chemical structures of the fabrics and chalcone compounds in the wavenumber range of 4000–400 cm^{-1} (Figure 2). Figure 2a shows the FTIR spectra of untreated cotton fabric and compounds K1 and K2. Figure 2b and 2c show the FTIR spectra of fabrics treated with K1 and K2 at concentrations of 0.25%, 0.5%, 1%, and 4%, respectively.

We observed new bands in the treated fabrics at approximately 1650, 1600–1580, and 1515 cm^{-1} (Figure 2b, 2c), which correspond to C=O stretching and C=C double bond vibrations associated with chalcones (Parveen et al., 2014; Tutar et al., 2019). These signals indicate the presence of chalcone compounds on the fabric surface. Overall, the FTIR results support the presence of chalcone compounds on the fabric surface and show a consistent increase in the intensity of these bands with increasing chalcone concentration.

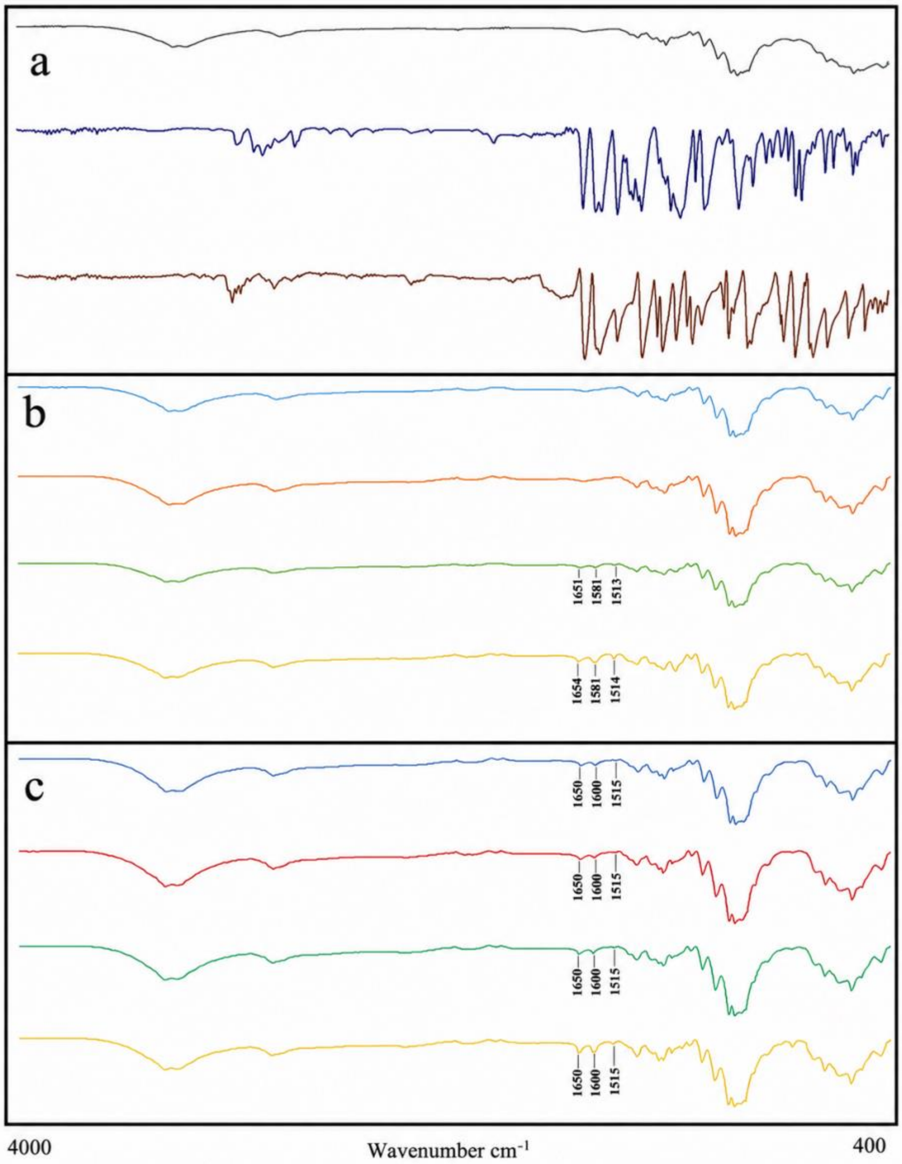

Figure 2. FTIR spectra of untreated cotton fabric, K1, K2 (a); A1, A2, A3, and A4 (b); A5, A6, A7, and A8 cotton fabrics (c) respectively from top to bottom

3.3. Antimicrobial Evaluation

3.3.1. Qualitative evaluation

Positive controls exhibited very limited effect against the *C. albicans* fungal strain (6,5-7 mm). Control C30 formed 13,75 and 35,5 mm inhibition zone for *S. aureus* and *K. pneumoniae* respectively. The negative controls were exhibited no inhibition zones.

Although FTIR spectra show that the two chalcone compounds are present in all fabrics treated above 1% concentration, we observed no inhibition zone of the treated fabrics. Chalcones are insoluble in water, and their limited diffusion in the aqueous agar medium may have prevented the formation of an inhibition zone. We observed a similar situation in another study (Ibrahim et al., 2021). In that study, although no antimicrobial activity was observed in the agar, a 97-99% reduction in bacteria was detected using the quantitative method (AATCC 100).

3.3.2. Quantitative evaluation

The initial bacterial concentration was determined to be 3.6×10^5 CFU/mL. Figure 3 shows the colony counts obtained from the growth control and treated fabric samples at the end of the incubation period. The antibacterial activity of the fabrics was calculated by considering the difference between the growth control group and the chalcone-treated fabric samples, as specified in Eq. 2.1. Cotton fabrics A3 and A7 treated with 1% chalcone exhibited over 60% inhibition of bacterial growth. The 4% chalcone-treated fabrics exhibited an inhibition rate ranging from 83.33% to 86.66% (Table 1).

No distinct characteristic peaks were observed in the FTIR analysis of fabrics treated with 0.5% or 0.25% chalcone (Figure 2). Therefore, quantitative antibacterial analysis was performed only on fabric samples treated with 1% and 4% concentrations. The results suggest that increasing the chalcone concentration enhances its presence on the fabric surface and consequently improves the antibacterial activity of the treated fabrics.

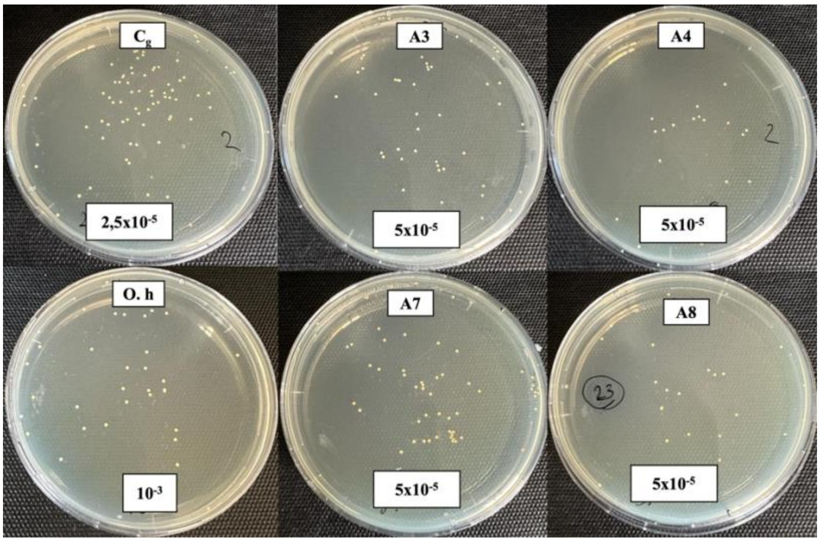

Figure 3. Colonies from the inoculation sample at the 0-hour and 24-hour contact times

Table 1. Antibacterial activity test results of the cotton fabrics

Sample	Treated Compound	Final Concentration	% R
C_g	-	3.00 x 10^7	-
A_3	K1	1.06 x 10^7	64.66
A_4	K1	5.00 x 10^6	83.33
A_7	K2	1.17 x 10^7	61.00
A_8	K2	4.00 x 10^6	86.66

C_g: Growth control
%R: Bacterial growth inhibition rate

3.4. SEM Analysis

SEM images clearly reveal the presence of chalcone compounds on the surface of the cotton fibers (Figure 4). Increasing the chalcone concentration in the bath resulted in a greater amount of chalcone on the fiber surface. This increase is particularly evident when comparing images A7 and A8. In addition to the expected binding mechanism, physical accumulation of the compound can also be observed in image A8. FTIR spectra also showed stronger characteristic bands at the 4% concentration. The quantitative antibacterial results showed that fabrics treated with higher chalcone concentrations exhibited greater antibacterial activity. Overall, the SEM, FTIR, and quantitative antibacterial results showed a consistent trend with increasing chalcone concentration.

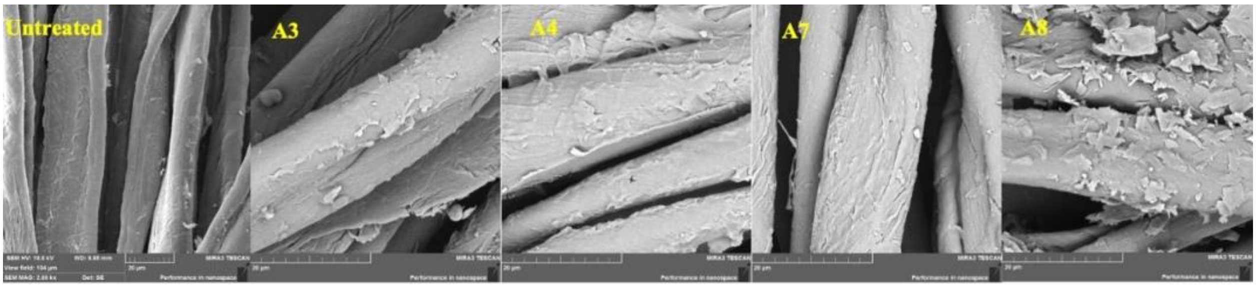

Figure 4. SEM images of untreated and chalcone-treated cotton fabrics

4. Conclusions and Recommendations

This study is a preliminary investigation aimed at revealing the potential applicability of 1,3-bis-chalcones as antimicrobial compounds in cotton fabrics. In this study, we synthesized two 1,3-bis-chalcone derivatives as described in the literature (Tutar et al., 2019). These compounds were previously reported to exhibit antibacterial activity using the microdilution method (MIC: 100-400 μg/mL). We treated cotton fabrics with the chalcone compounds and evaluated the antibacterial activity of these fabrics. FTIR and SEM analyses confirmed the presence of chalcone compounds on the fabrics. Quantitative antibacterial evaluation showed that the fabrics exhibited 61.00–86.66% inhibition of bacterial growth against *S. aureus*. Future studies are planned to apply different chalcone derivatives to fabrics and investigate their antimicrobial properties, adhesion mechanisms, toxicity, and washing durability.

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Authors’ Contributions

Gürsel Korkmaz performed the experiments and prepared the manuscript. Hayreddin Gezegen contributed to the synthesis studies and verification of the synthesis reactions. Uğur Tutar contributed to the antimicrobial studies, including the quantitative antibacterial evaluation, and verification of the antimicrobial tests. Y. Dilek Kut contributed to the fabric application studies and manuscript preparation. All authors read and approved the final version of the paper.

Statement of Conflicts of Interest

The authors declare no conflict of interest.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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