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PRODUCTION AND CHARACTERIZATION OF BACTERIAL CELLULOSE BIOCOMPOSITES BASED ON *THYMUS SIPYLEUS* BOISS. EXTRACT

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Keywords

Bacterial Cellulose,
Ex situ Modification,
Thymus sipyleus Boiss.,
Sustainable Biomaterials,
Natural Antibacterial Agents.

Abstract

The aim of this study was to improve the antibacterial properties of bacterial cellulose (BC) produced by *ex situ* modification in Kombucha culture medium. Bioactive methanol extract from the plant *Thymus sipyleus* Boiss. was added to BC by *ex situ* modification to obtain a bioactive and cost-effective biocomposite. SEM and FTIR analyses confirmed the nanofibers, porous structure and chemical bonding of the extract with the BC nanofibers and revealed that the biocomposites were successfully produced. A significant decrease in water retention and swelling behavior was observed in the biocomposites. The antibacterial activity of the biocomposites was determined according to the AATC100-2004 method. The bactericidal activities of the biocomposites produced were compared with those of the gram-positive bacteria *Staphylococcus aureus* ATCC 25923 and the gram-negative bacteria *Escherichia coli* ATCC 25922. Biocomposite T010 (0.10% extract) showed strong antibacterial activity, reducing *E. coli* by 84.6% and *S. aureus* by 97.54%. The results of this study show that the *T. sipyleus* extract can be used as an effective antibacterial agent at appropriate concentrations and that the BC biocomposite produced by *ex situ* modification has excellent antibacterial properties.

THYMUS SIPYLEUS BOISS. EKSTRAKTI BAZLI BAKTERIYEL SELÜLOZ BIYOKOMPOZITLERININ ÜRETIMI VE KARAKTERIZASYONU

Anahtar Kelimeler

Bakteriyel Selüloz,
Ex situ Modifikasyon,
Thymus sipyleus Boiss.,
Sürdürülebilir Biyomalzemeler,
Doğal Antibakteriyel Ajanlar.

Öz

Bu çalışmanın amacı Kombucha kültür ortamında *ex situ* modifikasyon ile üretilen bakteriyel selülozun (BC) antibakteriyel özelliklerini iyileştirmektir. *Thymus sipyleus* Boiss. bitkisinden elde edilen biyoaktif metanol özütü, BC'ye *ex situ* modifikasyonla eklenerek biyoaktif ve uygun maliyetli bir biyokompozit elde edilmiştir. SEM ve FTIR analizleri, nanofiberleri, gözenekli yapıyı ve özütün BC nanofiberleri ile kimyasal bağlanmasını doğruladı ve biyokompozitlerin başarıyla üretildiği kanıtlanmıştır. Biyokompozitlerde su tutma ve şişme davranışında önemli bir azalma gözlenmiştir. Biyokompozitlerin antibakteriyel aktivitesi AATC100-2004 yöntemine göre belirlenmiştir. Üretilen biyokompozitlerin bakterisidal aktiviteleri, gram pozitif bakteri *Staphylococcus aureus* ATCC 25923 ve gram negatif bakteri *Escherichia coli* ATCC 25922 ile karşılaştırılmıştır. Biyokompozit T010 (%0,10 ekstrakt) güçlü antibakteriyel aktivite göstererek *E. coli*'yi %84,6, *S. aureus*'u ise %97,54 oranında azaltmıştır. Bu çalışmanın sonuçları, *T. sipyleus* ekstraktının uygun konsantrasyonlarda etkili bir antibakteriyel ajan olarak kullanılabileceğini ve *ex situ* modifikasyonla üretilen BC biyokompozitinin mükemmel antibakteriyel özelliklere sahip olduğunu göstermektedir.

Alıntı / Cite

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Highlights (At least 3 and maxium 4 sentences)

- Biocomposites of bacterial cellulose and *T. sipyleus* extract were successfully produced by *ex situ* modification.
- Characterization analyzes confirmed the existence of the biocomposites produced.
- The biocomposite structures showed excellent antibacterial activity at different concentrations.

Purpose and Scope
Preparation of a biocomposite with improved antibacterial properties by the *ex situ* modification technique of bacterial cellulose produced in a static culture medium with methanol extract from the plant *Thymus sipyleus* Boiss.

Design/methodology/approach
In the present study, *ex situ* modification of bacterial cellulose produced under static culture conditions with methanol extract of *T. sipyleus* plant was successfully performed. Antibacterial biocomposite structures were successfully produced and their characterization was investigated.

Findings
The T010 biocomposite produced showed 97,54% antibacterial activity against *S. aureus* bacteria in the antibacterial activity test according to the AATC100-2004 method. Depending on the amount of plant extract, the water holding capacity and swelling ratio decreased.

Originality
In the present study, BC biocomposites with antibacterial properties were prepared using bioactive *T. sipyleus* extract. *T. sipyleus* plant was used for the first time in *ex situ* modification studies with BC.

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1. Introduction

Biomaterials have provided benefits to society for hundreds of years. The development of biomaterials in various fields is essential for sustainability. Owing to the structural uniqueness of biomaterials, their production, processing and functionalization have become simple processes with the latest developments in materials science. Cellulose, a polysaccharide that is insoluble in water, is the most common polymer in nature (Du et al., 2018). While plants are the main source of cellulose, they can also be produced by many bacteria, such as those in the genus Gluconacetobacter, in the form of bacterial cellulose (BC) (Klemm et al., 2006; Gupte et al., 2021).

The BC layer is also referred to in scientific literature as a bacterial cellulose nonwoven surface or biononwoven layer (Ashjaran et al., 2013; Song et al., 2020). While BC has the similar structure to plant cellulose, it can be produced in pure form and does not contain impurities such as lignin, hemicellulose or pectin. This purity of BC offers a significant advantage over plant cellulose, as it does not use environmentally harmful chemicals (Cazón et al., 2021). Several important factors affect bacterial cellulose production. These factors include temperature, pH, carbon sources, culture medium components, oxygen levels and the type of bacteria used. BC production can take place in different culture environments. Some of these include the Hestrin-Schramm (HS) culture medium (Meftahi et al., 2015), the glucose, yeast extract and carbonate culture media, and the Kombucha culture media (Kilinc et al., 2022).

Kombucha is a slightly sweet, slightly sour refreshing drink consumed worldwide. Kombucha is made from the infusion of tea leaves by fermenting a symbiotic culture of bacteria and yeasts that form the 'tea fungus'. A slippery layer of cellulose (bacterial cellulose) and sour broth are the two components of kombucha (İleri et al., 2010). The bacterial cellulose layer was obtained by keeping this mixture at room temperature, preferably in an environment protected from light, for 1 to 3 weeks. Compared with other methods, the production of BC in static culture media is inexpensive and relatively simple and is the most used technique for obtaining BC under laboratory conditions. The resulting BC layer takes the form of a biofilm at the air–liquid interface (Kilinc and Özdemir Kücükçapraz, 2024).

BC has excellent properties, such as a large fibril size, high crystallinity, improved biocompatibility, good mechanics, high permeability, high fluid retention capacity and improved moldability (Brown, 1886; Khattak et al., 2015). Because of its improved properties, BC is considered a promising biopolymer in various fields, such as textiles, medicine, food, electronics and layered construction (Boni et al., 2020; Wei et al., 2020Cazón et al., 2021; Li et al., 2021; Kilinc et al., 2022;). In the textile industry, it is used as temporary artificial skin, as a wound dressing for the treatment of burns and ulcers, and as an adsorbent in other textile products (Lin et al., 2013). Despite the many beneficial properties of BC, its limited antibacterial activity limits its use in many areas (Fatima et al., 2021). The synthesis and modification of hydrogels from BC has recently gained importance for various applications in different fields. Different modification techniques have been used to modify BC and increase its performance, leading to the production of composite structures. *Ex situ* modification is one of these methods (Stumpf et al., 2018). *Ex situ* modification is performed mainly by absorption, which leads to the development of powerful hydrogen bonds between the adsorbed molecules and BC (Lin et al., 2013). Although many studies have investigated the functionalization of BC with antimicrobial polymers and nanomaterials for various applications, research on the functionality of BC with bioactive agents of plant origin is limited (Fatima et al., 20-21). In studies conducted with herbal extracts in the literature, the application of extracts imparts antimicrobial properties to the product (Kilinc et al., 2015; Cüce et al., 2019; Cuce, 2022; Cuce et al., 2022). In the textile industry in particular, plant-based products used in environmentally friendly production are seen as one of the most important textile applications as people become increasingly health-conscious. For this reason, textile products with antibacterial properties are becoming increasingly popular (Korkmaz et al., 2023).

The Lamiaceae family is one of the most widespread flowering plant groups and is known for its biodiversity and medicinal properties. It has high economic value because of its aromatic properties and ease of cultivation (Ndhlala et al., 2024). Each species contributes to overall bioactivity via a complex combination of bioactive compounds. These plants have been used for aromatherapy and antiseptic purposes since ancient times. *Thymus* species have volatile and nonvolatile extracts that are rich in phenolic compounds (Cüce and Basançelebi, 2021; Elbouny et al., 2022). These extracts contain secondary metabolites that have strong antioxidant, anti-inflammatory, antimicrobial, antiviral and anticancer properties (Ustuner et al., 2019, Nilofar et al., 2024; Ndhlala et al., 2024).

Rosmarinic acid has been identified as the main component of the methanol extract of *T. sipyleus*. This compound, an ester of caffeic acid, is widespread in plants of the Lamiaceae family. In addition, *T. sipyleus* contains phenolic compounds such as salvianolic acid, caffeic acid, and ferulic acid and flavonoids such as luteolin, gallocatechin, isorhamnetin and quercetin. Flavonoids and phenolic compounds are secondary metabolites with aromatic rings that are notable for their antibacterial effects (Elansary et al., 2020). In particular, carvacrol has been shown to inhibit the growth of microorganisms such as *E. coli*, *S. aureus* and *P. aeruginosa*. The high contents of carvacrol and thymol explain the high antimicrobial and antioxidant activities of these plants. *Thymus* species are often preferred in industry because they are rich sources of bioactive compounds (Nadeem et al., 2019; Chen et al., 2019; Llorent-Martínez et al., 2022).

Ul-Islam et al. (2021) reported that the addition of *aloe vera* gel to BC by an *ex situ* modification method only filled the gaps between the fibril networks without affecting the morphology of the structure. The BC/*Aloe vera* composite was found to increase mechanical strength and other properties without altering the structural morphology. Asanarong et al. (2020) produced a composite structure of BC by *ex situ* modification by crosslinking papain extract and glutaraldehyde for wound dressings. The results showed that the addition of glutaraldehyde enhanced the binding of papain to BC, the wound dressing exhibited antibacterial properties, and the resulting BC composite structure could be a promising biomaterial for biomedical applications. Barud et al. (2013) produced a biocomposite of BS and Polis extracts by *ex situ* modification. Owing to the caffeic acid and flavonoids contained in the propolis extract, the prepared BS/propolis composite structures presented antioxidant and antibacterial properties.

In this study, BC was modified *ex situ* with a methanol extract from *Thymus sipyleus* Boiss. The aim of this study was to produce biocomposites of bacterial cellulose and *T. sipyleus* (BCT) with low antibacterial activity. The biocomposites were prepared by immersing purified BC in *T. sipyleus* extract via the *ex situ* method. The obtained biocomposites were analyzed in detail with respect to their morphological and physicochemical properties. In addition, the antibacterial activity of the biocomposites against gram-positive *S. aureus* ATCC 25923 and gram-negative *E. coli* ATCC 25922 pathogens was evaluated. Biocomposites prepared from various plant extracts using bacterial cellulose have been prepared previously. However, the preparation of biocomposites using the plant *T. sipyleus* was performed for the first time and will shed light on the literature.

2. Materials and Method

2.1. Materials

The flower and leaf parts of *T. sipyleus* were collected in June and July 2024 in Giresun, Şebinkarahisar. The samples were weighed to calculate the yield rates. To prevent damage to the bioactive components in the plant structure during the drying phase, the samples were protected from direct sunlight in a well-ventilated room and stored until extraction.

2.2. Extraction Process of *T. sipyleus* Boiss.

The extraction process was performed via an ultrasonic bath (Isolab 1.3 L, Germany). The flower and leaf parts of *T. sipyleus* dried at room temperature were ground in a grinder (Fakir Aromatic, Germany). For ultrasonic extraction, 25 g of the ground herbal material was placed on a volumetric glass, and 250 ml of 80% methanol (CH_3OH) was added. The extraction was carried out in an ultrasonic water bath with distilled water at 34–35 °C for 12 h. Whatman No. 2 was used to filter the extraction. The solvent (CH_3OH) used for the extraction was evaporated under low pressure in a rotary evaporator at 35 °C (Heidolph Instruments GmbH & Co. KG, Germany) until no more CH_3OH was present. The efficiency of extraction was then calculated.

2.3. Bacterial Cellulose Production and Purification

Kombucha SCOBY (Bacteria and Yeast Symbiotic Culture) was purchased from a firm producing Kombucha cultures in Türkiye. The nitrogen source required for the production of the Kombucha culture was black tea (7.9 g). The black tea was boiled in 1 L of distilled water for 20 min. After boiling, 100 g of fructose was dissolved in the boiled tea as a carbon source. When the temperature of the prepared solution fell below 24 °C, it was transferred to a glass container sterilized with 70% ethanol solution. One hundred milliliters of 80% acetic acid was added to the container. The prepared mixture was stored for 10 days in a sterile environment at room temperature and protected from direct sunlight. Purification was performed to remove bacteria and other components in the structure of the bacterial cellulose layer obtained after 10 days. For the purification process, the BC layer was first incubated in 96% ethanol for 15 min. The BC was then transferred to a beaker containing 1 L of purified water and processed for 50 min at boiling temperature with constant stirring. The relevant literature reports that the purification of BC with pure water followed by treatment with 3% NaOH does not damage the structure if an effective purification process is desired. Therefore, BC was purified by treatment with 3% NaOH for 95 min at 25 °C and 70 rpm. To neutralize the purified BC, it was treated with acetic acid and distilled water for 30 min at 25 °C and 50 rpm, and the pH was adjusted to 3. The purified BC was stored at +4 °C for later use (Zeng, 2014; Han et al., 2019; Kilinc et al., 2022; Kilinc and Dicle Özdemir, 2024).

2.4. *Ex Situ* Development of BC-*T. sipyleus* Biocomposites

For the preparation of the *T. sipyleus* solution for *ex situ* production, 0.10% *T. sipyleus* extract was dispersed in a beaker with ethanol (70%, v/v). The mixture was sonicated for 20 min to achieve better dissolution and reduce the particle size (Siahaan et al., 2020; Kumar et al., 2023). The same solution was also prepared as 0.15% *T. sipyleus*.

The cleaned BC layer (2x2 cm^2) was immersed in the prepared solution. It was removed from the solution after being kept in an environment away from light for 30 h. Excess plant extract on the produced biocomposite was removed by washing with distilled water. Table 1 shows the codes and properties of the biocomposites produced in the study and used in the analyses.

Table 1. Sample Codes and Properties

Sample code	Properties
T010	Biocomposite produced using 0.10% extract
T015	Biocomposite produced using 0.15% extract
BC	Purified BC surface

2.5. Characterization

2.5.1. SEM and FTIR Analysis

SEM analyses were performed with a Fei Quanta 250 Feg instrument. The surface morphology of the samples was visualized by scanning electron microscopy (SEM). The chemical interactions between BC and the BC-*T. sipyleus* biocomposite (BCT) were investigated via Fourier transform infrared (FTIR) spectroscopy. To understand the chemical structure of the produced biocomposites, FTIR spectra were measured between 4000 and 400 cm^{-1} via the attenuated total reflection (ATR) technique.

2.5.2. Water Holding Capacity

The BC was dried at room temperature, and the T010 and T015 biocomposites at different concentrations were cut into 2 × 2 cm2 pieces. The dry samples were kept in water for 1 h under static conditions at room temperature and weighed. The samples were then dried at room temperature for 24 h and weighed again. The WHC was then calculated via equation (1) (Ul-Islam et al., 2023).

$$\text{WHC [\%]} = \frac{(\text{Ww-Wd})}{\text{Wd}} \times 100 \quad (1)$$

Ww = Wet weight of the sample
Wd = Dry weight of the sample

2.5.4. Swelling Ratio

To test the swelling rate, BC dried at room temperature was soaked in deionized water for 48 h and weighed after wiping off dripping water. The swelling rate is calculated using the following equation. (2) (Gao et al., 2023).

$$\text{Swelling Ratio [\%]} = \frac{\text{Wr}-\text{Wd}}{\text{Wd}} \times 100 \quad (2)$$

(Wr refers to the wet weight of the dried sample after rehydration, Wd refers to the weight of the dried sample)

2.6. Antibacterial Activity

For antibacterial activity, gram-positive *Staphylococcus aureus* ATCC 25923 and gram-negative *Escherichia coli* ATCC 25922 strains were applied to plant extracts prepared with CH_3OH in an ultrasonic bath. The antibacterial properties of the BCT biocomposite and the BC produced by *ex situ* modification were quantitatively determined according to AATCC 100-2004 (Cuce, 2022). The bacterial colonies formed in Muller–Hinton agar (MHA) medium were counted. The decrease in bacterial count was calculated according to equation (3).

$$R\ [\%] = \frac{B-A}{B} \times 100 \quad (3)$$

R represents the percentage decrease, B represents the number of bacterial colonies at the beginning of the test (0 h), and A represents the number of colonies after 24 h of contact with the composite structures obtained via the *ex situ* modification method.

3. Results and Discussion

3.1. Development of BC-*T. sipyleus* Biocomposites

In this study, BC-based biocomposites will be obtained thanks to the positive properties of plant extracts. As a result of the *ex situ* modification process, biocomposite (T010) containing 0.10% *T. sipyleus* extract, biocomposite (T015) containing 0.15% *T. sipyleus* extract were obtained.

The surface chemistry of BC enables chemical interactions with different materials, and its unique network structure facilitates the impregnation of substances. Therefore, BC can serve as an ideal substrate for the production of bioactive materials with plant extracts (Fatima et al., 2021). Plant extracts contain many medically important compounds, such as flavonoids, phenols, alkaloids, chalcones, and amines. The aim of this study was therefore to develop an antibacterial biocomposite based on BC with plant extracts. Ultrasonic extraction is preferred because of its compliance with the concept of sustainable "green chemistry", as it allows extracts to be obtained in a short time, with high quality and purity, and requires the use of a minimal amount of solvent (Shirsath et al., 2021). The extract of *T. sipyleus* was obtained via ultrasonic extraction, with a yield of 18.42%.

The efficiency of the extraction process varies depending on the type of solvent and the method used (Cuce et al., 2022). The literature indicates that the methanol extract of T. sipyleus prepared via the Soxhlet method has an efficiency of 18.79%. In view of these results, the efficiency of the methanol extract prepared in the ultrasonic bath confirms the data in the literature (Polat et al., 2007).

In this study, BC-based biocomposites were obtained because of the positive properties of the plant extracts. In the *ex situ* modification process, a biocomposite (T010) containing 0.10% *T. sipyleus* extract and a biocomposite (T015) containing 0.15% *T. sipyleus* extract were obtained.

3.2. Characterization of Biocomposites

BC is known to have a porous structure in the form of a net-like fibril. This was confirmed by SEM images. As shown in the SEM images of the BC biocomposite structures prepared by the *ex situ* modification method with T. sipyleus, the *T. sipyleus* particles were successfully impregnated into the BC, and no deterioration of the network structure was observed (Kumar et al., 2023). The porous structure increases the penetration rate of solutions or adds plant extracts into the BC matrix (Kamal et al., 2022). In the structure of the T010 and T015 biocomposites, unlike the SEM image of BC, the number of oil molecules bound to the plant extract increased in a concentration-dependent manner. Furthermore, the biocomposites T010 and T015 exhibited a comparatively reduced porosity and a more compact, net-like structural configuration. This finding is interpreted as an indication that the plant extracts were bound to and penetrated into the BC fibers (Ul Islam et al., 2023). This result is also supported by the water holding capacity and swelling ratio results. The plant extract is expected to penetrate the BC surface and impart antibacterial properties to the biocomposite through further interaction (Hungund et al., 2016; Fernandes et al., 2020). Cross-sectional and surface SEM images of the T010 and T015 biocomposites and BC are shown in Figure 1.

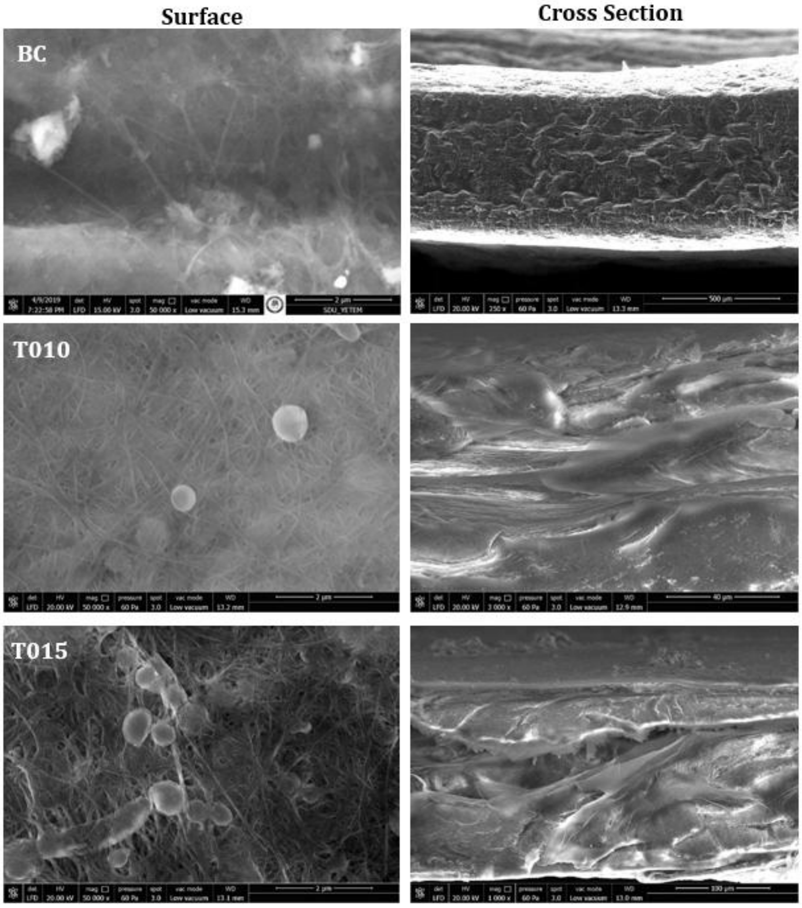


Figure 1. SEM Images of BC, T010 and T015

To investigate the presence of active biomolecules in the *T. sipyleus* extract of the biocomposites T010 and T015, the FTIR spectrum was analyzed. The C-O-C vibration peak can be identified in the fingerprint region about 1058 cm^{-1} (Ul Islam et al., 2023). The vibrations at 1050- 1500 cm^{-1} in samples T015 and T010 are thought to be due to the presence of thymol and the C-N stretching vibrations of the amines. The vibrations between 1350 and 1450 cm^{-1} are indicative of the presence of –OH bonds, which are characteristic of phenolic compounds. The observation of characteristic C-OH stretching vibrations of C=O ester groups in the range of 1550- 1560 cm^{-1} provides evidence that these compounds have been incorporated into the polymer matrix formed by BC (Razali et al., 2019; Nasr et al.,2022; Rigueto et al., 2024). The different FTIR peaks of the prepared samples are listed in Table 2 (Ul Islam et al., 2023; Bodea et al., 2022; Razali et al., 2019; Jiji et al., 2019; Sukhtezari et al., 2017).

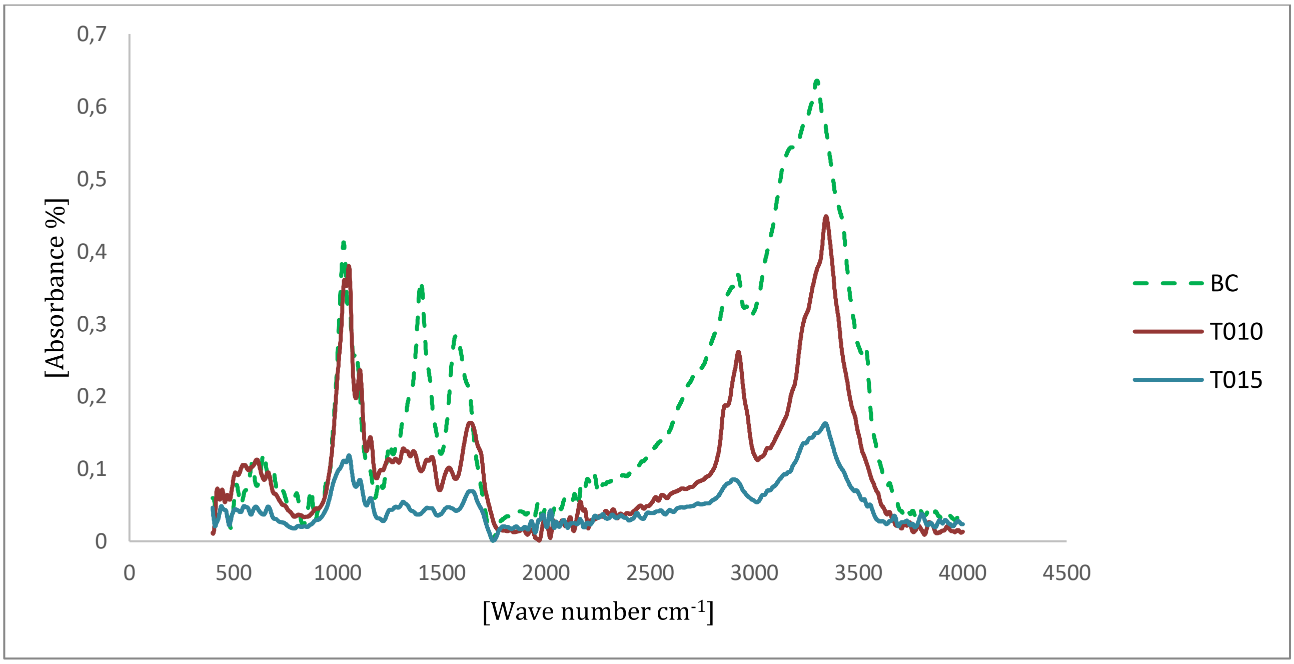


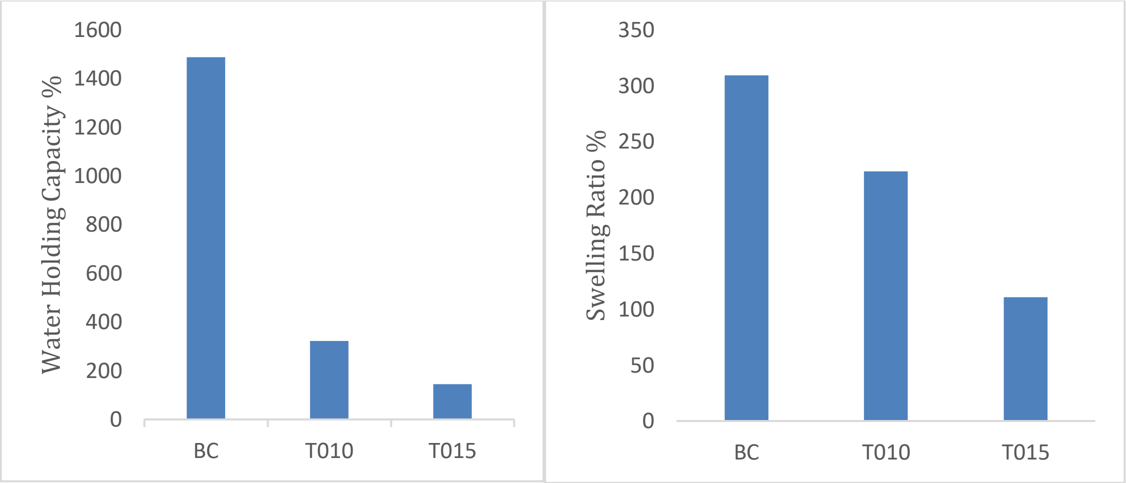
Figure 2. FTIR Spectrum of BC, T010 and T015

The absorption band observed in samples T015 and T010 in the range of 2940–2980 cm^{-1} is indicative of the C–H stretching of CH_2 groups, CH_3 and CH_2 in aliphatic compounds. This is consistent with the antisymmetric or symmetric stretching of CH and the bonding of CH_3 with oxygen (O) or nitrogen (N). This finding also suggests the presence of chlorophyll groups (Beldjilali et al., 2020). Figure 2 shows the FTIR spectra of BC, T010 and T015. The FTIR spectrum of BC shows the 1058 cm^{-1} peak, which corresponds C-O-C stretching; the 1403 cm^{-1} peak, which corresponds to C-H stretching; the 1458 cm^{-1} peak, which corresponds to C-C stretching; the 2898 cm^{-1} peak, which corresponds to C-H stretching; and the 3307 cm^{-1} peak, which corresponds to strong H-bonding. These peaks are indicative of the presence of BC and its characteristic functional groups. The peak at 1403 cm^{-1}, which is attributed to C–H stretching, is a notable feature of the FTIR spectrum of BC. However, this peak disappeared when T. sipyleus extract was added (Fatima et al., 2021; Sukhtezari et al. 2017; Ashori et al. 2012). Furthermore, an additional peak corresponding to the aromatic COOH group appeared at 1647 cm^{-1} and 1651 cm-1 in T010 and T015, respectively, as the *T. sipyleus* extract contains various chemical components, such as flavonoids and phenolic compounds (Fatima et al., 2022). The peak observed at 3350 cm^{-1} can also be interpreted as the OH stretching of the phenolic/carboxylic group in the extract (Beldjilali et al., 2020). The adsorption band between 3200 and 3500 cm^{-1} present in all the spectra is attributable to the stretching vibrations within and between the O–H bonds of cellulose (alcohols, phenols and carboxylic acids) are also indicative of the strong hydrogen bonding of the hydroxyl groups of T. sipyleus with BC (Panggabean et al., 2024; Bodea et al., 2022; Jiji et al.,2019).

Table 2. FT-IR Spectra of BC, T010 and T015 Biocomposite

	O-H	C-H	C=O	COOH	C-C	C-N	C-O-C
BC	3307	2898	-	-	1458	-	1058
T010	3350	2944	1556	1647	1512	1068	1056
T015	3350	2980	1551	1651	1508	1095	1060

BC has strong pores and a hydrophilic surface structure, which results in a high water holding capacity. Fluid-based materials, particularly hydrophilic polymers, have the ability to absorb more than 100 times their own dry weight of water (Ul Islam et al., 2012). BC is known to form a network structure by interconnecting fibrils. Owing to its network structure, BC has excellent mechanical and crystalline properties. Moreover, this network structure is the reason for the high porosity of BC (Ul Islam et al., 2013). The WHC and degree of swelling decreased in the T010 and T15 biocomposite structures. The WHC and degree of swelling directly decreased in response to increasing *T. sipyleus* concentration. It is hypothesized that the plant extract present in the prepared biocomposites adhered to the BC matrix, thereby closing the pores on the surface. With increasing extract concentration, the number of closed pores also increased, resulting in a significant decrease in the WHC and swelling ratio of T015. Figure 3a shows the WHC of BC, T010 and T015. Figure 3b shows the swelling ratios of BC, T010 and T015.



a b

Figure 3. a) Water Holding Capacity of BC, T010 and T015, **b)** Swelling Ratio of BC, T010 and T015

3.3. Antibacterial Activity of BC and Biocomposites

Owing to the various phenolic compounds they contain, plant extracts play an important role because of their antibacterial properties. The literature reports that BC has no antibacterial activity and that phenolic compounds such as luteolin, caffeic acid, gallic acid, vanillic acid, kaempferol and p-hydroxybenzoic acid found in *Thymus* species have powerful antioxidant and antimicrobial activities. (Li et al., 2019; Ul Islam et al., 2023). In one study, *ex situ* modified with Bc and pomegranate (Punica granatum L.) peel extracts and antibacterial activity tests for *E. coli* and *S. aureus* revealed %R values of 52% and 100%, respectively. The combination of Bc and pomegranate (Punica granatum L.) peel showed excellent antibacterial activity against *S. aureus* (Ul Islam et al., 2023). The results of the AATC100-2004 test for the antibacterial activity of BC confirm the data in the literature; the value for %R reduction is 0%. According to the AATC100-2004 antibacterial activity test, which was based on the percentage reduction in bacterial count, the percentage reduction rate of the *ex situ* modified T010 biocomposite was 84.6% for *E. coli* and 97.54% for *S. aureus*. The %R value of the T015 biocomposite was found to be 82.18% for *E. coli* and 84.45% for *S. aureus*. The results revealed that the E010 biocomposite had strong antibacterial activity against both *E. coli* and *S. aureus* strains. These findings indicate that the phenolic compounds in the *T. sipyleus* extract are more effective against *S. aureus* bacteria. In this study, increasing the concentration of *T. sipyleus* extract above 0.1% did not affect the antibacterial properties of the extract. Gram-negative bacteria are known to have an outer phospholipid membrane that is impermeable to hydrophobic (like phenolic) molecules. The antibacterial activity of the produced composites may have resulted in lower antibacterial activity against *E. coli* bacteria because *T. sipyleus* has developed resistance to plant extracts because of the outer phospholipid membranes of the bacteria (Ustuner et al., 2019). In this study, a 10% concentration was found to be the optimum value for a *T. sipyleus*-based biocomposite via *ex situ* modification with BC. The fact that the antibacterial effect did not increase at concentrations above 0.1% is probably due to saturation of the BC with the plant extract. Figure 4 shows the %R results from the antibacterial activity tests of the BC, T010 and T015 samples.

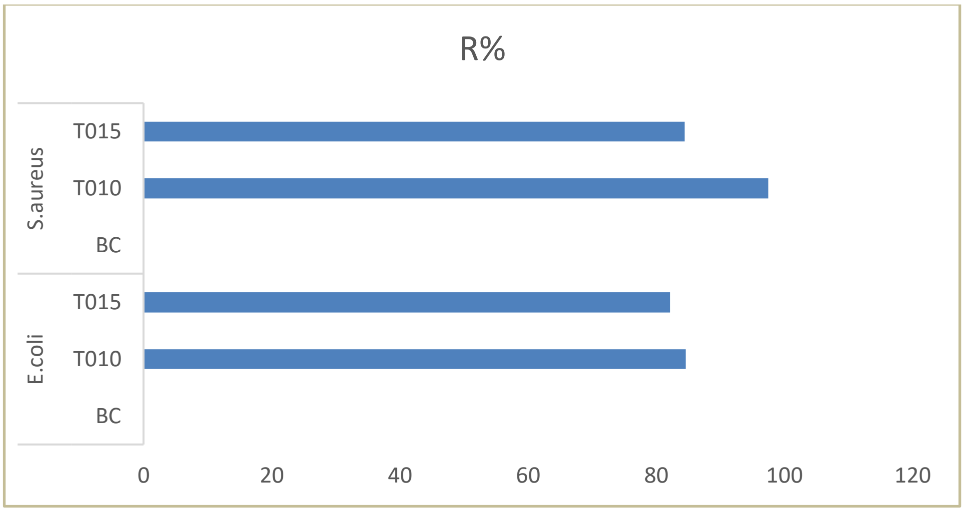


Figure 4. %R Results in AATC100-2004 Antibacterial Activity Test

The abundant porous structure of BC likely contributes positively to the development of antibacterial properties by trapping the plant extract. Figure 5 shows the AATC 100-2004 analysis of the BC and T010, T015 biocomposite structure against *E. coli*, whereas Figure 6 shows the AATC 100-2004 analysis against *S. aureus*.

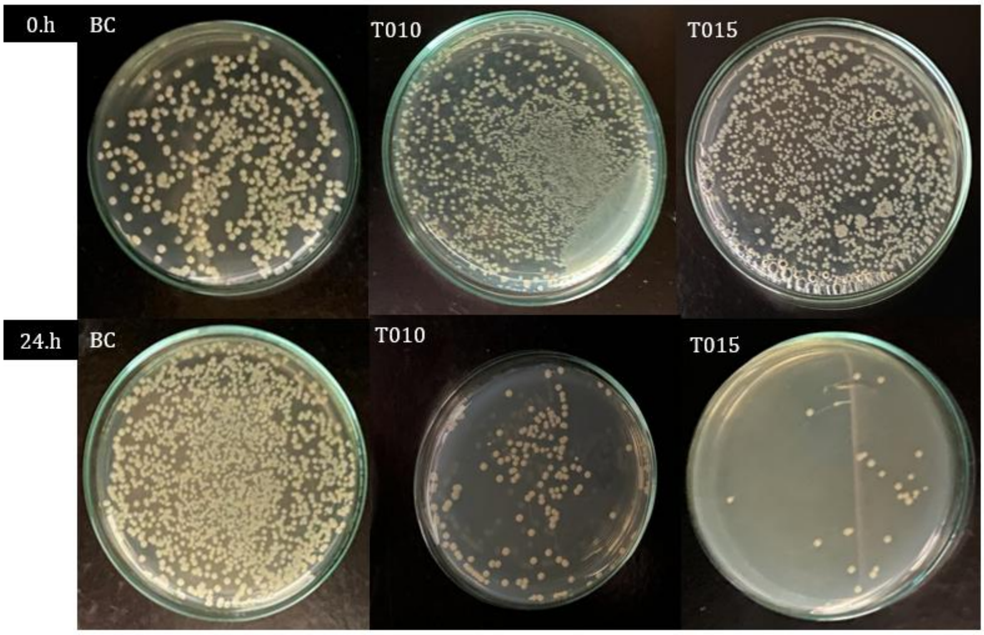


Figure 5. AATC 100 Analysis of BC, T010 and T015 Against *E. coli* at 0. h and 24. h

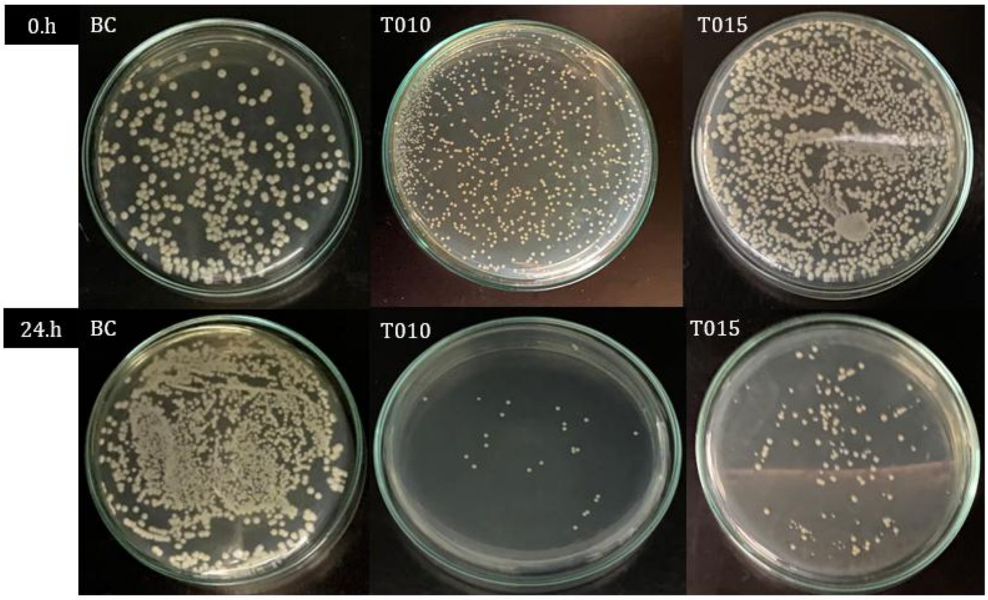


Figure 6. AATC 100 Analysis of BC, T010 and T015 Against *S. Aureus* At 0. H and 24. H

4. Conclusion

Owing to their easy availability, low price and medicinal benefits, plant extracts are known to be excellent supplements. This study was conducted to impart antibacterial properties to BC via the CH_3OH extract of *T. sipyleus*, as no similar study has been conducted with *T. sipyleus*. The BC-based antibacterial biocomposite enriched with *T. sipyleus* extract was prepared via an *ex situ* modification method. The results of the water holding and swelling ratio tests revealed that the ratio decreased with increasing extract concentration due to the closure of the pores in the BC with the *T. sipyleus* plant extract. The results of the FTIR and SEM analyses also proved that the extract was successfully incorporated into the BC matrix. The T010 obtained from the prepared biocomposites showed excellent antibacterial activity against both *S. aureus* (97.54%) and *E. coli* (84.6%). The high WHC of BC significantly increased the uptake of plant extracts, thus resulting in high antibacterial activity. The fact that the *T. sipyleus* plant extract used at a concentration greater than 0.1% did not positively increase the antibacterial activity suggests that greater antibacterial effects can be achieved with lower amounts of plant extract in future studies. On the basis of these effects, it is assumed that the biocomposites obtained can be used in the textile industry, especially in medical textiles, in products where an antibacterial effect is required and that they can lead to the development of new sustainable biocomposites.

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Conflict of Interest

No conflict of interest was declared by the authors.

References

Asanarong, O., Quan, V. M., Boonrungsiman, S., Sukyai, P., 2020. Bioactive Wound Dressing Using Bacterial Cellulose Loaded with Papain Composite. Morphology, Loading/Release And Antibacterial Properties. European Polymer Journal, 143, 110224.
Ashjaran, A., Yazdanshenas, M., Rashidi, A., Khajavi, R., Rezaee, A., 2013. Overview of Bio Nanofabric from Bacterial Cellulose. Journal of the Textile Institute, 104, 121–31.

Ashori, A., Sheykhnazari, S., Tabarsa, T., Shakeri, A., Golalipour, M., 2012. Bacterial Cellulose/Silica Nanocomposites: Preparation and Characterization. Carbohydrate Polymers, 90 (1), 413-418.

Barud, H. D. S., de Araújo Júnior, A. M., Saska, S., Mestieri, L. B., Campos, J. A. D. B., De Freitas, R. M., Berretta, A. A., 2013. Antimicrobial Brazilian Propolis (EPP-AF) Containing Biocellulose Membranes as Promising Biomaterial for Skin Wound Healing. Evidence-Based Complementary and Alternative Medicine, (1), 703024.

Beldjilali, M., Mekhissi, K., Khane, Y., Chaibi, W., Belarbi, L., Bousalem, S., 2020. Antibacterial and Antifungal Efficacy of Silver Nanoparticles Biosynthesized Using Leaf Extract of *SİTU algeriensis*. Journal of Inorganic and Organometallic Polymers and Materials, 30 (6), 2126-2133.

Bodea, I. M., Cătunescu, G. M., Pop, C. R., Fiț, N. I., David, A. P., Dudescu, M. C., Beteg, F. I., 2022. Antimicrobial Properties of Bacterial Cellulose Films Enriched With Bioactive Herbal Extracts Obtained by Microwave-Assisted Extraction. Polymers, 14 (7), 1435.

Boni, B. O. O., Lamboni, L., Bakadia, B. M., Hussein, S. A., Yang, G., 2020. Combining Silk Sericin and Surface Micropatterns in Bacterial Cellulose Dressings to Control Fibrosis and Enhance Wound Healing. Engineered Science, 10, 68-77.

Brown, A.J., 1886. XLIII. On an Acetic Ferment which forms Cellulose. Journal of the Chemical Society, 49, 432–439.

Cazón, P., Vázquez, M., 2021. Improving Bacterial Cellulose Films by *Ex-Situ* and *İn-Situ* Modifications. A review. Food Hydrocolloids, 113, 106514.

Chen, J., Chen, C., Liang, G., Xu, X., Hao, Q., Sun, D., 2019. *In Situ* Preparation of Bacterial Cellulose with Antimicrobial Properties from Bioconversion of Mulberry Leaves. Carbohydrate Polymers, 220, 170–175.

Cuce, M., 2022. Investigation of color, fastness, and antimicrobial properties of wool fabrics dyed with *Rosa canina* leaf extract. Journal of Natural Fibers, 19, 823-834.

Cuce, M., Kılınc, M., Kılınc, N., 2019. Investıgation of Color and Antimicrobial Properties of Wool Fabrics Dyed with Polygonum Cognatum Natural Dye Extracts. 'In International 19 May Innovative Scientific Approaches Congress, Samsun, Turkey', 1, 1211-24.

Cuce, M., Kilinc, M., Kilinc, N., Tekin, A., Kut, D., 2022. Color, Fastness, and Antimicrobial Properties of Wool Fabrics Dyed with *Helichrysum arenarium* Subsp. *aucheri* Extract. Journal of Natural Fibers, 19 (3), 797-809.

Cüce, M., Basançelebi, O., 2021. Comparison of Volatile Constituents, Antioxidant and Antimicrobial Activities of *Thymus leucotrichus* (Lamiaceae) Stem and Leaves Essential Oils from Both Natural Resources and *In Vitro* Derived Shoots. Journal of Essential Oil Bearing Plants, 24 (5), 1097-1112.

Du, R., Zhao, F., Peng Q., Zhou, Z., Han, Y., 2018. Production and Characterization of Bacterial Cellulose Produced by *Gluconacetobacter xylinus* İsolated from Chinese Persimmon Vinegar. Carbohydrate Polymers, 194, 200-207.

Elansary, H. O., Szopa, A., Kubica, P., Ekiert, H., Klimek-Szczykutowicz, M., El-Ansary, D. O., Mahmoud, E. A., 2020. Polyphenol Profile and Antimicrobial and Cytotoxic Activities of Natural *Mentha× piperita* And *Mentha longifolia* Populations İn Northern Saudi Arabia. Processes, 8, 1-17.

Elbouny, H., Ouahzizi, B., Bouhlali, E.D.T, Sellam, K., Alem, C., 2022. Pharmacological, Biological and Phytochemical aspects Of *Thymus munbyanus* Boiss. & Reut. A Review. Plant Science Today. 9, 399–404.

Fatima, A., Yasir, S., Khan M. S., Manan S., Ullah M. W., Ul-Islam M., 2021. Plant Extract-Loaded Bacterial Cellulose Composite Membrane for Potential Biomedical Applications. Journal of Bioresources and Bioproducts, 6, 26-32.

Fatima, A., Yasir, S., Ul-Islam, M., Kamal, T., Ahmad, M. W., Abbas, Y., Yang, G., 2022. Ex Situ Development and Characterization of Green Antibacterial Bacterial Cellulose-Based Composites for Potential Biomedical Applications. Advanced Composites and Hybrid Materials, 1-15.

Fernandes, I. D. A. A., Maciel, G. M., de Oliveira, A. L. M. S., Miorim, A. J. F., Fontana, J. D., Ribeiro, V. R., Haminiuk, C. W. I., 2020. Hybrid Bacterial Cellulose-Collagen Membranes Production in Culture Media Enriched with Antioxidant Compounds from Plant Extracts. Polymer Engineering & Science, 60, 2814-2826.

Gao, G., Niu, S., Li, T., Zhang, Y., Zha, X., Shi, Z., Ma, T., 2023. Fabrication of Bacterial Cellulose Composites with Antimicrobial Properties by *İn Situ* Modification Utilizing the Specific Function-Suspension Containing Water-İnsoluble Magnolol. International Journal of Biological Macromolecules, 239, 124329.

Gupte, Y., Kulkarni, A., Raut, B., Sarkar, P., Choudhury, R., Chawande, A., Dasgupta, S., 2021. Characterization of Nanocellulose Production by Strains of *Komagataeibacter* sp. İsolated from Organic Waste and Kombucha. Carbohydrate polymers, 266, 118176.

Han, J., Shim, E., Kim, H. R., 2019. Effects of Cultivation, Washing, and Bleaching Conditions on Bacterial Cellulose Fabric Production. Textile Research Journal, 89, 1094-1104.

Hungund, B, Siddhan, P, Basavaraj, H., 2016. Biosynthesis of Bacterial Cellulose and İmparting Antibacterial Property Through Novel Bio-Agents Biosynthesis of Bacterial Cellulose İmparting Antibacterial Property Through Novel Bio-Agents. Research Journal of Biotechnology, 11, 86–93.

İleri, T., Taşçı, F., Şahindokuyucu, F., 2010. Kombucha ve Sağlık Üzerine Etkileri. Uludag University Journal of the Faculty of Veterinary Medicine, 29, 69-77.

Jiji, S., Udhayakumar, S., Rose, C., Muralidharan, C., Kadirvelu, K., 2019. Thymol Enriched Bacterial Cellulose Hydrogel As Effective Material for Third Degree Burn Wound Repair. International journal of biological macromolecules, 122, 452-460.

Kamal, T., Ul-Islam, M., Khan, S. B., Bakhsh, E. M., & Chani, M. T. S., 2022. Development of plant extract impregnated bacterial cellulose as a green antimicrobial composite for potential biomedical applications. Industrial Crops and Products, 187, 115337.

Khattak, W.A., Khan, T., Ul-Islam, M., 2015. Production, Characterization and Physico-Mechanical Properties of Bacterial Cellulose from İndustrial Wastes. Journal of Polymers and the Environment, 23, 45–53.

Kilinc, M., Ay, E., Kut, D., 2022. Thermal, Chemical and Mechanical Properties of Regenerated Bacterial Cellulose Coated Cotton Fabric. Journal of Natural Fibers, 19, 7834-7851.

Kilinc, M., Canbolat, S., Merdan, N., Dayioglu, H., Akin, F., 2015. Investigation of The Color, Fastness and Antimicrobial Properties of Wool Fabrics Dyed with the Natural Dye Extracted from The Cone of *Chamaecyparis lawsoniana*. Procedia-Social and Behavioral Sciences, 195, 2152-2159.

Kilinc, N., Özdemir, Küçükçapraz, D., 2024. Production of Bacterial Cellulose Based on Bio Nonwoven / Nonwoven Composites for Medical Textile Applications. Textile and Apparel, 33, 357-365.

Klemm, D., Schumann, D., Kramer, F., Heßler, N., Hornung, M., Schmauder, H. P., Marsch, S., 2006. Nanocelluloses as İnnovative Polymers in Research and Application. İn 'Polysaccharides İi', (Ed(S).: Klemm D.), Springer, Germany, Vol. 1, 49-96.

Korkmaz, G., Kılınç, M., Kılınç, N., Kut, Y. D. 2023. The Role of Surface Modification Methods for Sustainable Textiles. In Roadmap to Sustainable Textiles. IntechOpen.

Kumar, M., Kumar, V., Saran, S., 2023. Efficient Production of Bacterial Cellulose Based Composites Using Zein Protein Extracted from Corn Gluten Meal. Journal of Food Science and Technology, 60, 1026-1035.

Li, X., He, T.; Wang, X., Shen, M., Yan, X., Fan, S., Wang, L., Wang, X., Xu, X., Sui, H., 2019. Traditional Uses, Chemical Constituents and Biological Activities of Plants from the Genus *Thymus*. Chemistry Biodiversity, 16, e1900254.

Li, Y., Guo, J., Li, M., Tang, Y., Murugadoss, V., Seok, I., Luo, Y., 2021. Recent Application of Cellulose Gel in Flexible Sensing a Review. ES Food Agroforestry, 4, 9-27.

Lin, W. C., Lien, C. C.,- Yeh, H. J., Yu, C.M., Hsu, S., 2013. Bacterial Cellulose and Bacterial Cellulose–Chitosan Membranes for Wound Dressing Applications. Carbohydrate Polymers, 94, 603–611.

Llorent-Martínez, E. J., Ruiz-Medina, A., Zengin, G., Ak, G., Jugreet, S., Mahomoodally, M. F., Chiavaroli, A. 2022. New Biological and Chemical Evidences of Two Lamiaceae Species (*Thymbra capitata* and *Thymus sipyleus* Subsp. *rosulans*): *in Vitro*, in Silico and *ex Vivo* Approaches. Molecules, 27(24), 9029.

Meftahi, A., Khajavi, R., Rashidi, A., Rahimi, M. K., Bahador, A., 2015. Effect of Purification on Nano Microbial Cellulose Pellicle Properties. Procedia Materials Science, 11, 206-211.

Nadeem, M., Imran, M., Aslam Gondal, T., Imran, A., Shahbaz, M., Muhammad Amir, R., Wasim Sajid, M., Batool Qaisrani, T., Atif, M., Hussain, G., 2019. Therapeutic Potential of Rosmarinic Acid: A Comprehensive Review. Applied Science., 9, 3139.

Nasr, AM., Mortagi, YI., Elwahab, NHA., Alfaifi, MY., Shati, AA., Elbehairi, SEI., Elshaarawy, RFM., Kamal, I., 2022. Upgrading the Transdermal Biomedical Capabilities of Thyme Essential Oil Nanoemulsions Using Amphiphilic Oligochitosan Vehicles. Pharmaceutics 14 (7), 1350.

Ndhlala, A. R., Işık, M., Kavaz Yüksel, A., Dikici, E., 2024. Phenolic Content Analysis of Two Species Belonging to the Lamiaceae Family: Antioxidant, Anticholinergic, and Antibacterial Activities. Molecules, 29 (2), 480.

Nilofar, N., Zengin, G., Acar, M., Bouyayha, A., Youssra, A., Eldahshan, O., Fahmy, N., 2024. Assessing the Chemical Composition, Antioxidant and Enzyme İnhibitory Effects of *Pentapleura subulifera* and *Cyclotrichium glabrescens* Extracts. Chemistry, Biodiversity, 2 (2), e202301651.

Panggabean, H., Ahmad, S. T., Sukardi, S., Yahfizham, Y., Thahir, M. T., 2024. Bacterial Cellulose Powder as a Filler in a Matrix Composite from Oil Palm Trunk. Journal of Fibers and Polymer Composites, 3 (1), 33-40.

Polat, Z. A., Tepe, B., Vural, A. 2007. In Vitro Effectiveness of *Thymus sipyleus* subsp. *sipyleus* sar. sipyleus on Acanthamoeba castellanii and İts Cytotoxic Potential on Corneal Cells. Parasitology research, 101, 1551-1555.

Razali, NA, Conte, M, McGregor, J., 2019. The Role of İmpurities in the La_2O_3 Catalysed Carboxylation of Crude Glycerol. Catalysis Letters, 149:1403–1414.

Rigueto, C. V. T., de Vasconcelos, G., Rosseto, M., Krein, D. D. C., Oliveira, F., Freitas, C. P., Pizzutti, I. R., 2024. Composite Films from Steam-Exploded Gelatin and Thyme Essential Oil: Production, Characterization and Application as Coatings. Journal of Polymers and the Environment, 1-13.

Shirsath, S. R., Sonawane, S. H., Gogate, P. R., 2021. Intensification of Extraction of Natural Products Using Ultrasonic İrradiations a Review of Current Status. Chemical Engineering and Processing: Process Intensification, 53, 10-23.

Siahaan, R. C., Sitorus, M., Mulia, R., Gea, S., 2020. *In vitro* İnvestigation of Bacterial Cellulose/Turmeric Extract (Bc-Te) Nanocomposite for Burn Wound Dressing. '1st International Conference on Chemical Science and Technology Innovation. Indonesia', 1, 297-300.

Song, J. E., Cavaco-Paulo, A., Silva C., Kim, H. R., 2020. Improvement of Bacterial Cellulose Nonwoven Fabrics by Physical Entrapment of Lauryl Gallate Oligomers. Textile Research Journal, 90,166-178.

Stumpf, T.R., Yang, X., Zhang, J., Cao, X., 2018. In Situ and Ex Situ Modifications of Bacterial Cellulose for Applications in Tissue Engineering. Materials Science and Engineering, C 82, 372–383.

Sukhtezari, S., Almasi, H., Pirsa, S., Zandi, M., Pirouzifard, M., 2017. Development of Bacterial Cellulose Based Slow-Release Active Films by İncorporation of *Scrophularia striata* Boiss. Extract. Carbohydrate Polymers, 156, 340-350.

Ul-Islam, M, Ha, JH, Khan, T, Park, JK., 2013. Efects of Glucuronic Acid Oligomers on the Production, Structure and Properties of Bacterial Cellulose. Carbohydrate Polymers, 92:360–366.

Ul-Islam, M., Ahmad, F., Fatima, A., Shah, N., Yasir, S., Ahmad, M. W., Ullah, M. W., 2021. Ex Situ Synthesis and Characterization of High Strength Multipurpose Bacterial Cellulose-*Aloe vera* Hydrogels. Frontiers in Bioengineering and Biotechnology, 9, 601988.

Ul-Islam, M., Alhajaim, W., Fatima, A., Yasir, S., Kamal, T., Abbas, Y., Yang, G., 2023. Development of Low-Cost Bacterial Cellulose-Pomegranate Peel Extract-Based Antibacterial Composite for Potential Biomedical Applications. International Journal of Biological Macromolecules, 231, 123269.

Ul-Islam, M., Khan, T., Park, J. K., 2012. Water Holding and Release Properties of Bacterial Cellulose Obtained by *In Situ* and *Ex Situ* Modification. Carbohydrate Polymers, 88, 596-603.

Ustuner, O., Anlas, C.,Bakirel, T., Ustun-Alkan, F., Diren Sigirci, B., Ak, S., Akpulat, H.A., Donmez, C., Koca-Caliskan, U., 2019. *In Vitro* Evaluation of Antioxidant, Anti-İnflammatory, Antimicrobial and Wound Healing Potential of *Thymus sipyleus* Boiss. subsp. *rosulans* (Borbas) Jalas. Molecules, 24, 3353.

Wei, J, Wang, B, Li, Z., 2020. A 3D-Printable TEMPO-Oxidized Bacterial Cellulose/Alginate Hydrogel with Enhanced Stability Via Nanoclay İncorporation. Carbohydrate Polymers, 238, 116207.

Zeng, M., 2014. Bacterial Cellulose: Fabrication, Characterization and Biocompability Studies. Universitat Autònoma de Barcelona, Departament de química, Facultat de Ciències, Doctoral Dissertion, Barselona.