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PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF BACTERIAL CELLULOSE BASED ON *OLEA EUROPAEA* L. LEAF EXTRACTS

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Keywords
Bacterial Cellulose, Olea europaea L., Antioxidant Activity, Bio-composite.

Abstract

This study describes the development of biocomposites of bacterial cellulose (BC) modified with leaf extracts of *Olea europaea* L. by an *ex situ* approach. Olive leaves, an agricultural by-product rich in phenolic compounds, were extracted using an ultrasound-assisted method and incorporated into BC membranes at different concentrations. Structural analyses (FT-IR, SEM) confirmed the successful integration of the extracts as evidenced by hydrogen bonding and morphological changes of the BC nanofibres. Functional analyses showed an improved water holding capacity and swelling ratio at low to medium extract concentrations, while saturation effects occurred at higher concentrations. Antioxidant properties were significantly improved, with DPPH inhibition increasing from 6.8% in pure BC to 65.5% in the fortified samples and total phenolic content reaching over 20,000 µg GAE/g. The originality of this study lies in the *ex situ* modification of BC with olive leaf extracts, which has not been previously reported, and the findings provide a valuable reference for guiding the development of sustainable and bioactive materials for textiles, biomedical applications, and active packaging.

OLEA EUROPAEA L. YAPRAK ÖZÜTLERİNE DAYALI BAKTERİYEL SELÜLOZUN FİZİKOKİMYASAL ÖZELLİKLERİ VE ANTİOKSİDAN AKTİVİTESİ

Anahtar Kelimeler
Bakteriyel Selüloz, Olea europaea L., Antioksidan Aktivite, Biyokompozit,

Öz

Bu çalışma, *Olea europaea* L. yaprak özütleri ile modifiye edilmiş bakteriyel selüloz (BC) biyokompozitlerinin *ex situ* yaklaşımıyla geliştirilmesini açıklamaktadır. Fenolik bileşikler açısından zengin bir tarımsal yan ürün olan zeytin yaprakları, ultrason destekli bir yöntem kullanılarak ekstrakte edilmiş ve farklı konsantrasyonlarda BC membranlarına dahil edilmiştir. Yapısal analizler (FT-IR, SEM), hidrojen bağı ve BC nanoliflerin morfolojik değişiklikleri ile kanıtlandığı üzere, özütlerin başarılı bir şekilde entegre edildiğini doğrulamıştır. Fonksiyonel analizler, düşük ila orta özüt konsantrasyonlarında su tutma kapasitesinde ve şişme oranında iyileşme gösterirken, daha yüksek konsantrasyonlarda doygunluk etkileri meydana gelmiştir. Antioksidan özelliklerin önemli ölçüde iyileştiği, DPPH inhibisyonu saf BC'de %6,8'den güçlendirilmiş numunelerde %65,5'e yükseldiği ve toplam fenolik içerik 20.000 µg GAE/g'nin üzerine çıktığı tespit edilmiştir. Bu çalışmanın özgünlüğü, daha önce bildirilmemiş olan zeytin yaprağı özütleri ile BC'nin *ex situ* modifikasyonunda yatmaktadır ve bulgular, tekstil, biyomedikal uygulamalar ve aktif ambalajlar için sürdürülebilir ve biyoaktif malzemelerin geliştirilmesine rehberlik etmek için değerli bir referans sağlamaktadır.

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Highlights

- *Ex situ* modified BC biocomposites with olive leaf extracts
- Successful integration of phenolics confirmed by FT-IR and SEM
- Enhanced antioxidant activity and total phenolic content in BC
- Sustainable functional materials for future industrial applications

Purpose and Scope

To develop sustainable bacterial cellulose (BC) biocomposites enriched with *Olea europaea* L. leaf extracts, aiming to enhance their physicochemical and antioxidant properties using an agricultural by-product.

Design/methodology/approach

Olive leaves were extracted by ultrasound-assisted methods and incorporated into BC via *ex situ* modification. Structural (FT-IR, SEM) and functional (WHC, swelling, TPC, DPPH) analyses were conducted.

Findings

Olive leaf extracts were successfully integrated into BC, improving water holding, swelling, antioxidant capacity, and phenolic content. DPPH inhibition rose from 6.8% to 65.5%, and TPC exceeded 20,000 µg GAE/g.

Originality

This study is the first to develop BC biocomposites using *Olea europaea* L. leaf extracts obtained by ethanol extraction and incorporated via an *ex situ* modification method. It introduces a novel and eco-friendly approach for producing bioactive composites with potential in textiles, biomedical, and packaging applications.

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1. Introduction

Bacterial cellulose (BC) is a linear polysaccharide consisting of glucopyranose units linked together by β-1,4-glycosidic bonds, synthesized by certain aerobic bacteria such as *Achromobacter, Alcaligenes, Aerobacter, Agrobacterium, Azotobacter*, and *Komagataeibacter* (formerly *Gluconacetobacter*) (Huang et al., 2014). BC production is directly influenced by temperature, pH, nutrient sources, culture medium, and oxygen supply. While media such as HS (Hestrin-Schramm), Yamanaka, Zhou, yeast extract, and CSL fructose are commonly used, kombucha culture also offers a cost-effective and environmentally friendly alternative (Kılınç et al., 2022).

Kombucha is a slightly sweet and sour drink that is fermented by a symbiotic consortium of bacteria and yeasts. The main substrate for production is tea leaves, and therefore the drink contains numerous biologically active components such as antioxidants, polyphenols, glucuronic acid, and vitamins (Li et al., 2023). During BC production, the symbiotic interaction of bacteria and yeast in the Kombucha culture medium, metabolizes sugar and tea components and forms a three-dimensional, elastic BC layer on the surface within 7–10 days (Kim & Adhikari, 2020; Laavanya vd., 2021).

Unlike plant-derived cellulose, BC is produced in a pure form and does not contain foreign components such as lignin, hemicelluloses or pectin. Thanks to this property, it can be obtained without expensive extraction and chemical purification processes and does not pollute the environment. In addition, BC offers considerable advantages in the production of functional materials due to its high crystallinity, biocompatibility, and biodegradability (Cazón and Vazquez, 2021). Numerous studies in the literature, have shown that plant extracts can be integrated into various materials to improve their functional properties, e.g. antibacterial, antioxidant, UV-protective, or biodegradable. This approach contributes significantly to the development of sustainable, environmentally friendly, and versatile new generation materials using natural components.(Sat et a., 2013; Cuce et al., 2019; Ong et al., 2021; Kilinc et al., 2024).

The use of BC in the form of composites offers significant advantages in terms of improving material and biological properties. BC composites are produced to overcome the limitations of BC and enable a wider range of applications. (Shah et al., 2013). BC has a large surface area due to its three-dimensional, porous nanofibre network structure. The abundant hydroxyl groups in its structure enable the adsorption of molecules through hydrogen bonds and electrostatic interactions. Thanks to these properties, BC can be made suitable for the formation of composite materials by *in situ* (during production) or *ex situ* (after production) modifications (Azeredo et al., 2019; Torres et al., 2019; Cabañas-Romero et al., 2020).

BC composites are considered a potential biomaterial for various areas such as textiles (Cahyaningtyas et al., 2025), biomedical applications (Isopencu et al., 2023), food packaging (Raj et al., 2023) and the development of environmentally friendly materials (Chen et al., 2023).Various approaches to improve the biological and functional properties of BC are reported in the literature. Isopencu et al, (2023) prepared composites with CMC and turmeric extract to overcome the limited biological activity of BC and reported that these composites exhibited high swelling capacity, pronounced antibacterial activity, antioxidant activity, and good cytocompatibility. Fernandes et al, (2020) developed hybrid BC–collagen membranes by enriching BC with phenolic compounds derived from tea and grape pomace and demonstrated that these structures exhibited strong antioxidant properties depending on the phenolic content. Indrianingsih et al, (2020) prepared BC face masks enriched with plant extracts (green tea, hibiscus, roselle) and reported that the highest antioxidant activity was observed in BC enriched with green tea. Ul-Islam et al, (2011) demonstrated that BC-chitosan composites are suitable for biomedical applications by improving their morphological and mechanical properties. Overall, these studies show that BC can be transformed into biocomposites with improved antibacterial, antioxidant, biocompatible, and mechanical properties by enrichment with various components through *ex situ* modifications.

Olive leaf (*Olea europaea*) is a plant material rich in oleuropein, hydroxytyrosol, tyrosol, luteolin, rutin, apigenin glycosides, and various phenolic acids, and has been used for centuries in folk medicine in the Mediterranean region. Olive leaf extract has been reported in the literature to have strong antioxidant and antimicrobial effects; it has been found to be particularly effective against pathogens such as *H. pylori*, *C. jejuni*, and *S. aureus*. Although it is an agricultural waste product, olive leaf is a remarkable natural bioactive source due to its phenolic content (Lee et al., 2010).

This study is based on the ability of BC to retain highly bioactive substances such as phenolic compounds due to its porous structure. The aim of the study is to produce functional bacterial cellulose (BC) biocomposites through ex situ modification using bioactive Olea europaea L. leaf extracts, characterize the structural, morphological, and physicochemical properties of the obtained materials, and evaluate their antioxidant activities. This approach aims to develop sustainable materials that provide a natural and environmentally friendly alternative to traditional synthetic additives, particularly those used in the textile industry. The study also addresses the conversion of agricultural waste olive leaves into high value-added functional and sustainable materials. Phenol-rich olive leaf extracts were successfully integrated into the BC nanofiber network structure using the ex situ method, resulting in innovative BC-based biocomposites with strong antioxidant properties. Thus, a natural, biodegradable, bioactive, and environmentally friendly alternative material for sustainable biotextile applications has been provided. The unique value of this work lies in the production of an innovative and sustainable biocomposite textile material with antioxidant properties by comprehensively integrating waste-based plant extracts with the high-purity nanofiber structure of BC.

2. Material and Method

2.1. Materials

The leaf samples of *Olea europaea* L. were collected in 2024 in Kırkağaç district of Manisa province (39°09'23.2 "N, 27°39'38.6"E). The collected samples were weighed to calculate their yield rates. In order to preserve the bioactive compounds in the plant tissue during the drying and storage process, the samples were kept away from direct sunlight, in an oxygen-rich environment, and at +4 °C until extraction.

2.2 Extraction methodology of *Olea europaea* L. Leaf

The extraction procedures were carried out using an ultrasonic bath device (Isolab 1.3L, Germany). The leaves of Olea europaea L. , dried at room temperature, were ground to fine particles using a grinder (Fakir Aromatic, Germany). For ultrasonic extraction, 20 g of the ground plant material was placed in a glass volumetric flask and 200 mL of 70% ethanol (C_2H_6O) was added. The extraction process was carried out in an ultrasonic water bath filled with distilled water at a temperature of 34–35 °C for 3 hours. Whatman filter paper No. 2 was used to filter the extracts. Subsequently, the C_2H_6O solvent was completely removed at 35 °C under low pressure using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany). Finally, the extraction yield was calculated from the extracts obtained. (Kılınç et al., 2025a).

2.3. Bacterial cellulose synthesis and purification

A static method was applied in the Kombucha culture for BC production. Tea was brewed by adding 10 g of tea to 1 L of boiled water, followed by the addition of glucose, acetic acid, and Kombucha SCOBY obtained from a probiotic manufacturer in Turkey. The mixture was incubated at room temperature for 14 days, and the resulting BC surfaces were purified (Kılınç and Özdemir Küçükçapraz, 2023). In the purification process, the BC was first soaked in ethanol, then treated by stirring in deionized water at boiling temperature. Subsequently, it was treated in a heated magnetic stirrer with a 3% NaOH solution, and in the final step, neutralization was performed with deionized water adjusted to pH 3 (Kilinc et al 2022).

2.4. *Ex situ* fabrication of biocomposites

Olea europaea L. leaf extracts were prepared in concentrations of 0.05%, 0.15%, and 0.25% in 70% (v/v) ethanol using the *ex situ* preparation method. The solutions were subjected to a 15-minute ultrasonic treatment to ensure homogeneous distribution and reduce particle size. Subsequently, the cleaned BC films were cut into 3x3 cm^2 pieces and immersed in these solutions, and then incubated at room temperature for 24 hours. (Kumar et al., 2023; Kılınç et al., 2025b) Following the synthesis process, the sheets were washed with pure water to remove any free extract residues that might remain on the BC surfaces, ensuring that only the bioactive components integrated into the matrix structure were retained. This approach enabled the effective integration of plant extracts into the BC matrix and represents a novel method to significantly improve the functional properties of the developed biocomposites. Table 1 shows the codes and characteristics of the biocomposites developed in the study and used for the analyses.

Table 1. Codes and characteristics of the biocomposites

Biocomposite codes	Composition
BC	Purified BC layer
BCOE1	Biocomposite incorporated with 0.05% extract
BCOE2	Biocomposite incorporated with 0.15% extract
BCOE3	Biocomposite incorporated with 0.25% extract

Crugeira et al. (2025) study indicated that phenolic compounds in olive pomace (such as hydroxytyrosol, oleuropein, and tyrosol) form molecular interactions (hydrogen bonds) with the hydroxyl (-OH) groups of bacterial cellulose, as shown in the figure 1, and that this interaction alters the properties of the biopolymer. Such an interaction is also thought to occur in this study.

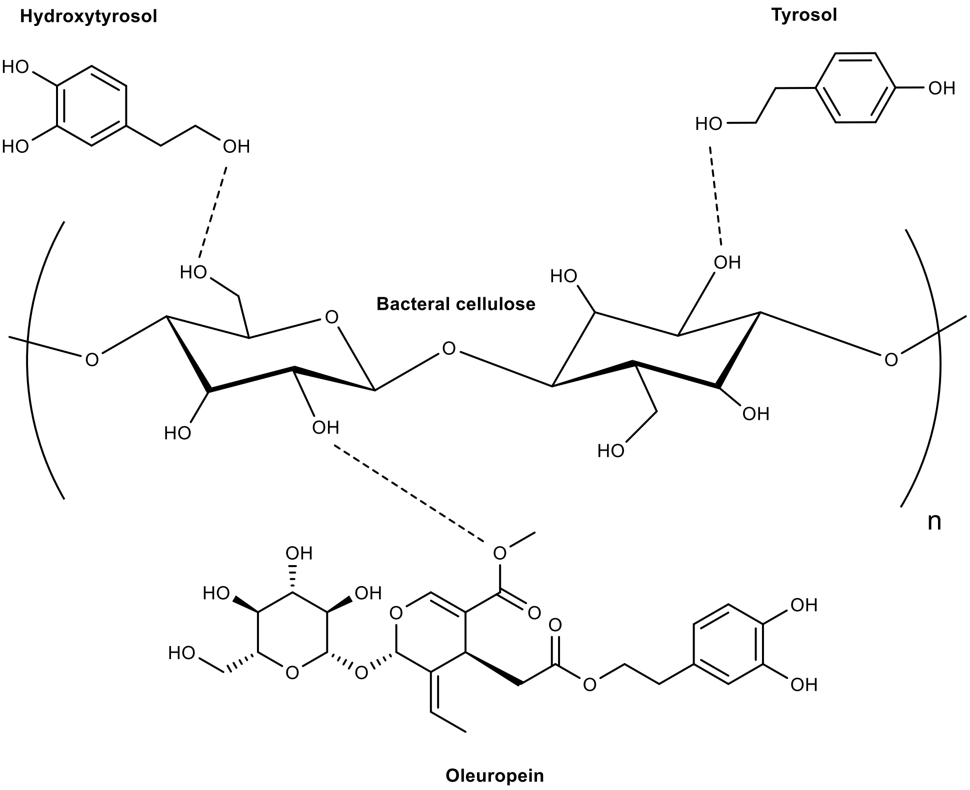

Figure 1. The possible binding mechanism of bacterial cellulose with *Olea europaea* L. Leaf extract

2.5. Structural and physicochemical characterization

2.5.1. FTIR and SEM analysis

The chemical structure of the biocomposites produced was analyzed using Fourier transform infrared spectroscopy (*FT-IR*). The spectra were recorded in the 4000–400 cm^{-1} range using the Attenuated Total Reflection (ATR) technique. *FT-IR* analysis was performed to determine the chemical interactions of the extract additive with the cellulose matrix and the changes in functional groups. The surface morphology of the samples was examined using SEM using a Fei Quanta 250 Feg instrument. SEM analysis was performed to determine the fibre distribution, pore structure, and surface properties of the biocomposites.

2.5.2. Swelling ratio

The dried BC samples were immersed in deionised water and allowed to stand for 48 hours, then weighed after the excess water was removed from the surface. The swelling ratio was calculated according to Equation 1 (Gao et al., 2023). This method was used to evaluate the swelling behaviour of the biocomposites after contact with water.

$$\text{Swelling Ratio } [\%] = \frac{\text{Wr}-\text{Wd}}{\text{Wd}} \times 100 \qquad (1)$$

W_r is the weight of the wet sample obtained after immersion in water; W_d is the weight of the sample dried at the start.

2.5.3. Water Holding Capacity

The BC samples dried at room temperature and the biocomposites prepared at different concentrations were cut into 3×3 cm^2 pieces. The dry samples were soaked in pure water for 1 hour under static conditions at room temperature and then weighed. The samples were then dried again for 24 hours at room temperature and weighed again (Ogrizek et al., 2021). Based on this data, the water holding capacity (WHC) was calculated according to equation (2).

$$\text{WHC } [\%] = \frac{(\text{Ww-Wd})}{\text{Wd}} \times 100 \qquad (2)$$

W_w = Moist (rehydrated) weight of the sample;

W_d = Dry (anhydrous) weight of the sample

2.6. Antioxidant and bioactivity evaluation

2.6.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1- picrylhydrazyl) radical scavenging method was used to determine the ability of the samples to neutralize free radicals and thus assess their antioxidant potential. 0.3 g of each sample was weighed and transferred to Falcon tubes, which were then filled to 10 ml with distilled water. The samples were incubated in a shaker at 250 rpm for 24 hours. Then 0.0015 ml of the sample was removed from each tube and transferred to new Falcon tubes. To these samples, 0.8 ml DPPH solution and 1.3 ml ethanol were added. The prepared mixtures were incubated in the dark for 30 minutes, and absorbance measurements were performed at a wavelength of 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1601). The DPPH radical scavenging activities were determined using equation (3), and the results were calculated as Trolox equivalents (TE) using the equation obtained from the Trolox standard curve (Gülçin et al., 2004; Sukhtezari et al., 2017).

$$\%\text{DPPH radical scavenging act.} = \frac{C-S}{C} \times 100 \quad (3)$$

C= Control absorbance value

S= Sample absorbance value

2.6.2. Total phenolic content (TPC)

The determination of the total phenolic content was carried out to determine the amount of phenolic compounds present in the samples. As phenolic compounds are known for their strong antioxidant properties, this analysis allowed the evaluation of their contribution to the antioxidant capacity and provided information on the bioactive potential of the samples. 0.3 grammes of each sample were weighed. The samples were placed in Falcon tubes and made up to 10 ml with distilled water. The samples were shaken at 250 rpm for 24 hours. After shaking, 0.1 ml of the sample was removed from each Falcon tube. To these samples, were added successively 0.20 ml Folin-Ciocalteu phenol reagent, 0.2 ml 20% sodium carbonate, and 4.5 ml water. The absorbance of the liquid phase was measured at 760 nm using a spectrophotometer (Shimadzu UV-1601). The total phenol content of the sample was calculated in µg GAE/g substance using the equation from the gallic acid calibration curve (Demirbaş and Şat, 2023).

2.7. Statistical analysis

Duncan multiple comparison test with confidence interval at $p < 0.05$ significance level was applied to determine the differences between the groups.

3. Result and Discussion

3.1 Fabrication of BC- *Olea europaea* L. Leaf Based Biocomposites

The utilization of agricultural waste is an important approach for the production of sustainable and environmentally friendly materials (Korkmaz et al., 2023). In this study, functionalized biocomposites were successfully produced using Olea europaea L. leaf extract, which is rich in bioactive compounds. The ultrasonic extraction method is compatible with the concept of "green chemistry" and provides an environmentally friendly and efficient method by reducing solvent consumption, shortening processing time, and causing less degradation of phenolic compounds (Alifakı et al., 2018).

The extraction efficiency was determined to be 14.83%. In the literature, various studies conducted with the leaf extract of *Olea europaea* L. reported that the yield varies between 14.5% and 17.6%, depending on variables such as the type of solvent used, solvent concentration, solid-liquid ratio, and processing time (Salık et al., 2023). These differences show the effects of extraction conditions on the solubility of phenolic compounds and the amount of extract obtained. The fact that the yield value obtained in this study is consistent with the above-mentioned range therefore speaks in favour of the effectiveness of the method and conditions used (Shu et al., 2003; Spietelun et al., 2013). The extract obtained was integrated into bacterial cellulose using the *ex situ* modification method, resulting in the development of sustainable, bioactive biocomposites.

3.2. Structural and physicochemical characterization

In FT-IR analysis, the broad bands between 3480–3520 cm^{-1} are associated with the O–H and N–H stretching vibrations and are formed by the action of hydrogen bonds. These bands reflect both the hydroxyl groups of the cellulose and the phenolic OH groups of the plant extracts, which supports the successful integration of the extract into the structure (Costa et al., 2017; Gorgieva and Trček, 2019; Beldjilali et al., 2020). Changes in the OH band in the range 3250–3300 cm^{-1} also confirm the differentiation of hydrogen bonds and the integration of the extract into the BC structure (Jafarizad et al., 2015; Kamal et al., 2022). In pure cellulose, the 1400-1450 cm^{-1} band is associated with CH_2 bending vibrations and is indicative of the crystalline structure, but with the addition of the extract, this peak is significantly reduced; this suggests that phenolic compounds disrupt the crystalline order and increase the amorphous region by forming hydrogen bonds with the hydroxyl groups of cellulose (Oh et al., 2005; Ciolacu et al., 2011). The FT-IR spectra of BC and BC/Olea europaea *ex situ* modified biocomposites are also shown in Figure 2. The 1100–1150 cm^{-1} band reflects the characteristic signals of C–O–C stretching vibrations associated with β-1,4-glycosidic bonds in the cellulose structure and alcoholic C–O bonds. The changes observed in this region indicate that BC fibres interact with phenolic components in the plant extract to form structural bonds and that the modification process is successful (Klemm et al., 2005; Ashori et al., 2012). The band at 1031 cm^{-1} is associated with the characteristic C–O–C stretching vibration of cellulose and confirms the presence of cellulose-based material (Fatima et al., 2022). Table 2 provides the bond names in the FTIR analysis of the biocomposites.

Table 2. FT-IR analysis bonds

Wave Number (cm^{-1})	Bonding
3480–3520	O–H (Hydroxyl) and N–H (Amin) stretching band
3250–3300	O–H (Hydroxyl) band
1400–1450	CH_2 bending vibrations
1100–1150	C–O–C tension (β-1,4-glycosidic bonds) and C–O bonds
1031	C–O–C

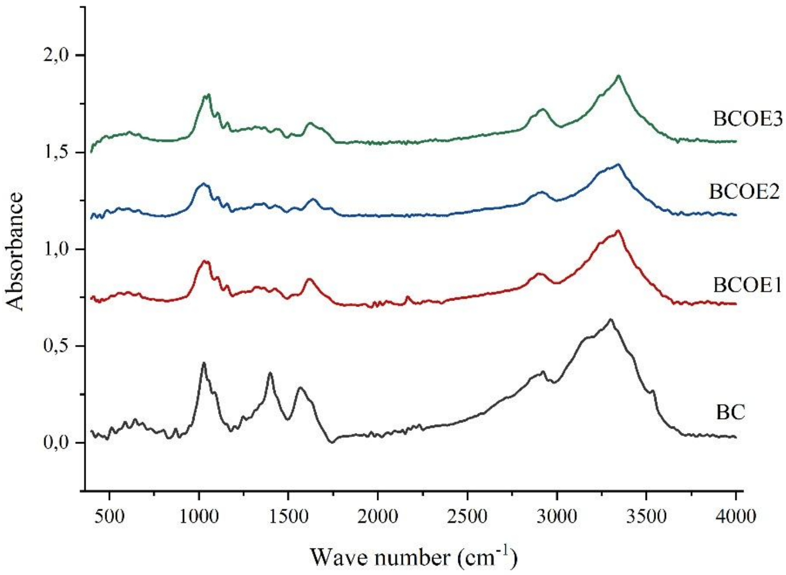

Figure 2. FTIR graph of the produced BC, BCOE1, BCOE2, BCOE3

The surface and internal morphology of pure BC and BCOE biocomposites was analysed by SEM. Pure BC exhibited a multilayered network structure consisting of interlocking nanofibres and containing both large and small pores (Adan et al., 2025). Figure 3 shows SEM images of BC and BCOE biocomposites. As can be seen in the images, this structure, consisting of ribbon-like microfibrils, contributes significantly to the high mechanical strength and water retention capacity of BC (Sozcu et al., 2024). Furthermore, the absence of any bacterial residues in pure BC confirms the effectiveness of the applied cleaning process. With increasing OE concentration, clear differences in the morphology of the biocomposites were observed. The irregular distribution of nanofibres indicates the formation of porous areas in the biocomposites, which is consistent with previous studies in the literature (Mocanu et al., 2019). SEM images of BC biocomposites prepared with OE addition show that the extract forms an irregular coating on the cellulose nanofibres and is integrated into the structure (Amorim et al., 2022). This coating effect is believed to contribute to the improvement of the antioxidant properties of the biocomposites, together with the transfer of the bioactive components of the extract to the nanofibres. The results show that OE-containing BC biocomposites can be successfully produced and provided with functional properties.

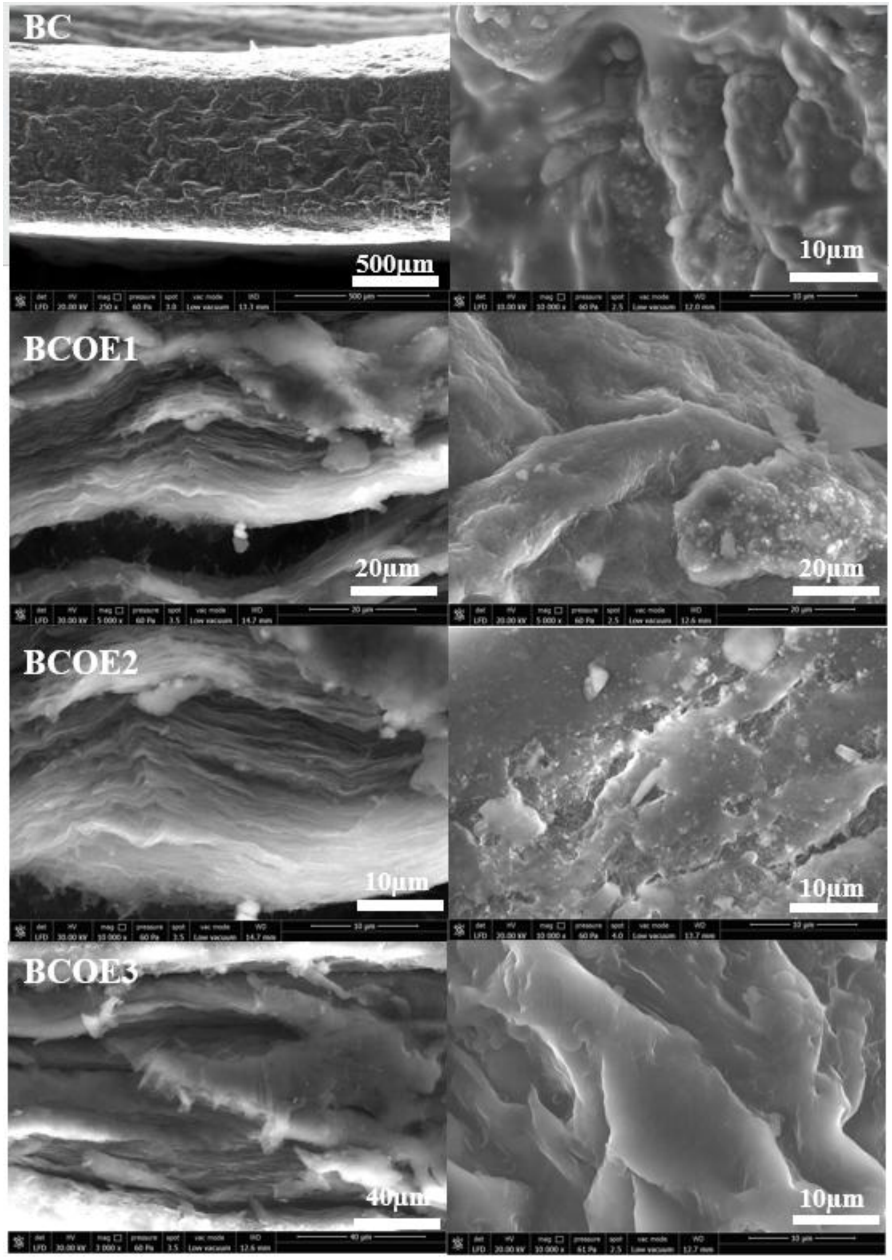

Figure 3. SEM images of BC, BCOE1, BCOE2 and BCOE3

When plant extract is added to bacterial cellulose (BC) through ex situ modification, the change in water holding capacity (WHC) can have a bidirectional effect depending on the concentration. At low concentrations, the hydrophilic (-OH) groups of the extract can bind to the BC network at the molecular level, creating new hydrogen bonding sites for water and thus increasing WHC (Kotcharat et al.,2022). Any reinforcing material added to BC impairs its natural mesh structure and consequently its absorption capacity. Figure 4 shows the water holding capacity and degree of swelling of BC and the biocomposites produced. In a study examining composite structures with Aloe Vera (AV) added at different concentrations via ex situ modification, it was reported that at low concentrations, the water holding capacity (WHC) and swelling ratio increased compared to pure BC. However, a significant decrease in these properties was observed at higher concentrations. This decrease was attributed to the aggregation of AV particles at high concentrations, which physically block the pores of the BC network (Ul-Islam et al., 2021). It is hypothesised that the coating effect observed in the SEM images causes the extract components to bind to the surface of the fibrils, and create new hydrophilic areas. This increases the distance between the nanofibrils and helps water to diffuse and be retained more easily thanks to the hydrophilic groups created by the structure. Therefore, it can be observed that the water retention and swelling ratio values of BCOE and BCOE2 biocomposites are slightly higher than those of pure BC. It is hypothesised that the lower water retention and swelling ratio of the BCOE3 sample compared to other concentrations is related to the decrease in porosity, masking of the hydrophilic groups, and stiffening of the network structure on the BC biocomposite due to the addition of the extract at high concentrations (Nasresfahani et al., 2025).

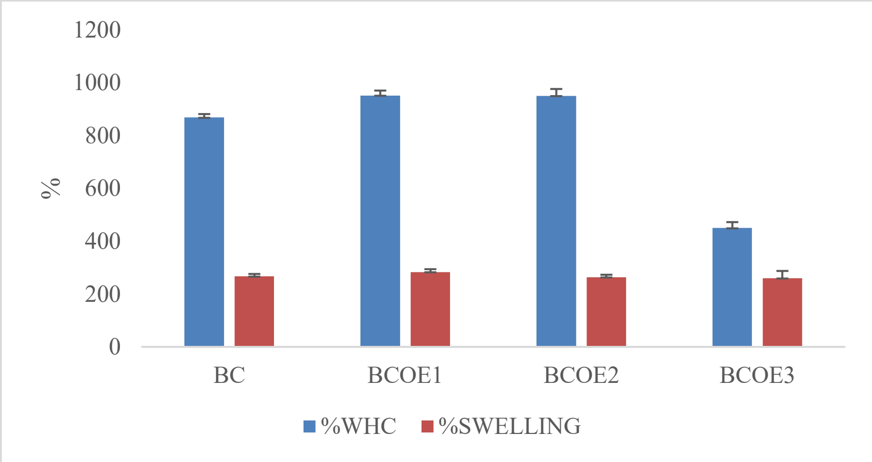

Figure 4. WHC and Swelling ratio of BC and biocomposites

Duncan's multiple comparison test was applied to compare the mean values between samples and determine statistical differences ($p<0.05$). The water holding capacity and Duncan test results for the samples are presented in Table 3. Based on these data, the samples were classified into three different groups. In this classification, no significant difference was observed between BCOE1 and BCOE2, while a significant difference was observed between these samples and BC and BCOE3. The swelling (%) and Duncan test results for the samples are presented in Table4. According to Duncan's analysis, all groups were placed in the same statistical subgroup, and it was determined that the addition of extract had no significant effect on swelling capacity ($p < 0.05$).

Table 3. Duncan test results for water holding capacity

Sample	N	Duncan Grouping	WHC(%) (Mean ± SD)	Increase (%)
BC	3	B	868.15 ± 13.25	0
BCOE1	3	C	951.66 ± 18.69	+9.62
BCOE2	3	C	950.33 ± 25.90	+9.47
BCOE3	3	A	449.70 ± 22.66	-48.20
There is no statistically significant difference between values indicated by the same letter in the same column (Duncan, p < 0.05).				

Table 4. Duncan test results for swelling ratio

Sample	N	Duncan Grouping	Swelling(%) Mean ± SD	Increase (%)
BC	3	A	266.77 ± 9.29	0
BCOE1	3	A	283.33 ± 10.83	+6.21
BCOE2	3	A	263.79 ± 9.26	-1.12
BCOE3	3	A	259.76 ± 27.70	-2.63
There is no statistically significant difference between values indicated by the same letter in the same column (Duncan, p < 0.05).				

3.3. Antioxidant and bioactivity evaluation

BC has a relatively low antioxidant capacity; this is to be expected as cellulose itself has only limited functional groups with radical scavenging effects (Sukhtezari et al., 2017; El-wakil., 2019). A gradual and significant increase in DPPH inhibition levels is observed with the addition of OE extract. This increase can be explained by the presence of phytochemicals with strong antioxidant properties, such as phenolic compounds and flavonoids, in the plant extracts (Nowak et al., 2021). Table 5 shows the DPPH data for BC and the produced biocomposites BCOE1, BCOE2 and BCOE3. As the amount of extract in the biocomposites produced increased, the radical scavenging activity also increased. This indicates a positive correlation between the amount of extract and the antioxidant capacity. However, the increase is not linear, but shows a trend approaching saturation. At high extract levels, the increase could be lower as the phenolic groups that can bind to the matrix are limited or the extract reaches saturation in the BC structure. The addition of OE extract significantly improved the antioxidant properties of BC. In particular, the integration of phenolic compounds into the matrix increased the free radical scavenging activity, demonstrating that a controlled increase in the amount of extract is an effective strategy to improve the functional properties of biocomposites. The relevant results are confirmed by the Duncan test (p<0.05) performed on µg TE/g values. According to the comparison test, the samples were grouped into three different groups. While no significant difference was found between the BCOE2 and BCOE3 samples, a significant difference was observed between these samples and the BCOE1 and BC samples (Table6).

Table 5. BC and biocomposite of DPPH data

Sample	DPPH Inhibition(%)	microgram TE/g
BC	6.870229008	370.3703704
BCOE1	20.48346056	1427.160494
BCOE2	60.55979644	4538.271605
BCOE3	65.5216285	4923.45679

Table 6. Duncan test results for DPPH data

Sample	N	Duncan Grouping	DPPH (µg TE/g) Mean ± SD	Increase (%)
BC(Control)	3	A	370.37 ± 185.04	0
BCOE1	3	B	1427.16 ± 152.05	+285.33%
BCOE2	3	C	4538.27 ± 377.51	+1125.33%
BCOE3	3	C	4923.46 ± 197.28	+1229.33%
There is no statistically significant difference between values indicated by the same letter in the same column (Duncan, p < 0.05).				

The total phenolic content of BC was found to be quite low (1350 µg GAE/g). This can be explained by the fact that the BC structure does not contain naturally occurring phenolic compounds and the measured value is probably due to traces of residues present in the environment or surroundings. The addition of the plant extract significantly increased the phenolic content, reaching 5526 µg GAE/g in the low extract sample. This increase shows that the extract was successfully integrated into the BC structure. In the medium extract sample, the phenolic content increased to 20876 µg GAE/g, indicating that BC can absorb a large amount of phenolic compounds due to its large surface area and porous structure. However, the measured value in the high extract sample (20785 µg GAE/g) remained almost unchanged, indicating that the BC matrix reaches saturation with phenolic compounds at a certain level (Nowak et al., 2021; Cazón et al., 2024). The Duncan test results also confirm this situation. The test results grouped the samples into three different classes. As in the DPPH analysis, the BCOE2(C) and BCOE3(C) samples were in the same class, while the BC(A) and BC0E1(B) samples were in different groups. Table 7 contains duncan data , data on the total content of phenolic compounds in BC and the produced biocomposites BCOE1, BCOE2 and BCOE3.

Table 7. BC and biocomposite of total phenolic compounds data and Duncan Test results

Sample	N	Duncan Grouping	TPC (µg GAE/g) (Mean ± SD)	Increase (%)
BC(Control)	3	A	1350.57 ± 137.06	0
BCOE1	3	B	5526.82 ± 241.42	+309.2%
BC0E2	3	C	20876.44 ± 1349.89	+1445.3%
BCOE3	3	C	20785.44 ± 598.35	+1439.8%
There is no statistically significant difference between values indicated by the same letter in the same column (Duncan, p < 0.05).				

When the results were evaluated collectively, the BCOE2 sample (0.15% extract) achieved maximum antioxidant activity (60.5% DPPH) while maintaining the highest water retention capacity. It was observed that the additional extract used in BCOE3 (0.25%) did not contribute significantly to performance. This finding indicates that the BCOE2 concentration should be preferred in industrial-scale production in terms of cost-effectiveness.

4. Conclusion

In this study, an extract obtained from agricultural waste *Olea europaea* L. (olive) leaves was incorporated into bacterial cellulose using an ex situ modification method to produce a sustainable and environmentally friendly biocomposite. FTIR and SEM analyses confirmed that the plant extract components were effectively integrated into the BC matrix via hydrogen bonds and that the biocomposites were successfully produced. A noteworthy finding of the study is the effect of extract concentration on water holding capacity (WHC) and swelling ratio. At low and medium extract concentrations (BCOE1 and BCOE2), an increase in water holding capacity was observed due to interactions between the extract's hydrophilic groups and the BC network. However, at high concentrations (BCOE3), a decrease in water holding capacity was observed due to the saturation of extract particles, which physically blocked the pores and stiffened the network structure. This clearly demonstrates the importance of optimising concentration for successful biocomposite production via ex situ modification. Functionally, the developed biocomposites exhibited superior antioxidant properties. The DPPH radical scavenging activity, which was 6.8% in pure BC, increased to 65.5% after modification with high-concentration extract (BCOE3), and the total phenolic content exceeded 20,000 µg GAE/g. Consequently, this study serves as an important example of both converting agricultural waste into value-added products and producing sustainable materials in accordance with green chemistry principles. The resulting BC/Olea europaea biocomposites have promising potential for biomedical applications such as wound dressings, active food packaging, and sustainable textiles due to their high antioxidant capacity and biocompatible structures.

In future studies, the effects of different extract concentrations on the swelling behaviour, water retention, and mechanical properties of BC biocomposites can be evaluated more comprehensively; additionally, the controlled release behaviour of phenolic compounds can be studied in detail regarding their applicability in wound dressings and food packaging.

Conflict of Interest

No conflict of interest was declared by the authors.

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