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Sustainable antibacterial and wound-healing hydrogels: *Croton confertus*-loaded bacterial cellulose composites

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The development of sustainable, plant-derived antimicrobial polymeric biomaterials is increasingly important for managing infections associated with wound environments, particularly in the context of rising antimicrobial resistance. In this study, low-cost bacterial cellulose (BC) was produced using waste-derived fruit media and subsequently modified with *Croton confertus* leaf extract (CE) through an *ex situ* infusion process to obtain bioactive BC–CE composites. The physicochemical structure of the composites was characterized using FE-SEM and FTIR analyses, which confirmed successful incorporation of phytochemicals into the nanofibrillar cellulose matrix and demonstrated reduced porosity, enhanced hydrogen-bonding interactions, and improved microstructural stability. BC–CE films revealed better moisture-retention capabilities than pure BC, maintaining structural stability for repeated swelling/drying cycles. Antibacterial performance indicated clear inhibition zones (1.28 cm for *Staphylococcus aureus* and 1.11 cm for *Escherichia coli*) and substantial growth containment, with 46% and 36% reductions in bacterial proliferation, respectively. *In vivo* wound-healing experiments further demonstrated accelerated epithelial regeneration and reduced inflammation in BC–CE-treated wounds compared to BC control dressings. Collectively, these findings highlight the synergistic benefits of integrating plant-derived phytochemicals within a sustainable BC platform, providing a cost-effective and biocompatible polymeric biomaterial with promising potential for next-generation wound-care applications with antimicrobial functionality.

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1. Introduction

Microbial infections are a major worldwide health concern, aggravated by the rising threat of multidrug-resistant organisms as a result of the maladministration and overuse of antibiotics.[1–3] According to reports, 4.71 million deaths occurred due to antimicrobial resistance, including 1.14 million deaths attributed to bacterial infections in 204 countries.[4] In order to treat microbial infections and overcome the antibiotic resistance that has been documented for almost all of the antibiotics used in clinical practice, significant efforts have been made to develop novel antimicrobial drugs.[5,6] In addition to the time-consuming process of creating new antibiotics, innovative alternative therapeutic approaches are required to change the dynamics of this struggle.[6,7] Plant extracts have exhibited pronounced effects in this regard due to their various bioactive compounds with antimicrobial qualities. Plant extracts are abundant in secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds.[8] These moieties not only possess broad-spectrum activity but also follow mechanisms to counter traditional bacterial resistance pathways.[9]

Extensive studies have been carried out to develop antimicrobial films enriched with plant extracts including gelatin and chitosan films with cinnamon, guarana and rosemary extracts,[10] gelatin and methyl cellulose with citrus extract,[11] poly(vinyl

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alcohol) (PVA) and poly(vinylpyrrolidone) (PVP) films loaded with *T. indica etc.*,[12] chitosan and gelatin incorporated with grape seed and jabuticaba peel extracts,[13] pectin and starch films with *Gunnera tinctoria* and *Ugni molinae* extracts,[14] agarose and κ-carrageenan-based films with *Cryphaea heteromalla* aqueous extract,[15] gelatin films with *Aloe vera* gel and garlic peel extract,[16] and carboxymethyl cellulose and gelatin films with pomegranate peel extract.[17]

Bacterial cellulose (BC) has gained significant attention over the past few decades as an ideal polymeric platform for fabricating antimicrobial films.[18–20] Produced as an exopolysaccharide by specific bacterial strains, BC shares the fundamental chemical structure of plant-derived cellulose, but lacks accompanying matrix components such as lignin, pectin, and hemicellulose.[21,22] Consequently, BC exhibits exceptionally high crystallinity, remarkable purity, superior liquid-holding capacity, excellent biocompatibility, and greater moldability compared to plant cellulose.[23] Furthermore, it possesses a unique three-dimensional (3D) reticulated network, which provides a larger surface area, good flexibility, high wet tensile strength, excellent permeability and elasticity, making it an excellent substrate for incorporating plant extracts. In addition, the BC matrix contains an ample number of functional groups, which enables chemical interactions between the BC matrix and plant extracts. Moreover, the high surface area and high porosity of BC membranes provide a platform for physical interactions of BC with additives or plant active compounds.[24,25] Numerous studies have documented the addition of plant extracts or active phytomolecules to the BC matrix, which enhances the BC's structural qualities and biocompatibility while also providing therapeutic benefits like antioxidant and antibacterial activities. These studies include the incorporation of neem and sage,[26] *Ageratum conyzoides* L. and *Chromolaena odorata* L.,[27] *Epilobium angustifolium*,[28] *H. rosa*-sinensis,[27] *Boswellia serrata*,[29] *Zingiber officinale* root[30] and pomegranate peel.[31] However, a vast reservoir of medicinally significant plants is available for further investigations to be developed as BC composites for therapeutic purposes.

Croton confertus (*C. confertus*) Baker (Euphorbiaceae) is a tree which grows in desert regions or the dry shrubland biome. It is commonly found in Ethiopia and the Arabian Peninsula. Although no scientific reports are currently available on *Croton confertus*, other Croton species have been studied for their therapeutic effects. *C. linearis* leaves are reported to possess antiprotozoal activity,[32] and essential oils from *C. adipatus*, *C. thurifer*, and *C. collinus* are reported to exhibit antimicrobial activity.[33] In addition, antimicrobial activity from *C. urucurana* Baillon,[34] antimicrobial and modulatory effects from *C. limae*,[35] antimicrobial activity from stem bark extracts of *C. macrostachyus*,[36] antimicrobial and antibiofilm activity from *C. nepetaefolius*[37] and antimicrobial activity of *C. cajucara* on artificial biofilms and planktonic microorganisms[38] have been reported in previous studies. Several compounds, including acetyl aleuritolic acid, β-sitosterol, campesterol, stigmasterol, sonderianin, catechin, gallocatechin germacrene D, *E*-caryophyllene, β-caryophyllene, limonene, 1,8-cineole epi-longipinanol, methyl eugenol, β-phellandrene, myricetrin, and quercetin, are found to be present in Croton spp., which are responsible for its various pharmacological properties.

Despite the outstanding biocompatibility, mechanical properties, and remarkable crystallinity of BC, its widespread adoption is still restricted due to elevated costs and lack of therapeutic properties. Thus, this study is focused on developing a low-cost BC production strategy utilizing cheaply available local resources as potential media. BC sheets were developed into antimicrobial hydrogels by incorporating an ethanolic extract of *C. confertus*. The rationale for this study stemmed from the simplicity of the *ex situ* methods employed, in contrast to other studies, and the use of a biodegradable BC matrix support, developed from cheap food wastes. Given the well documented medicinal potential of Croton spp. the BC–*Croton confertus* composites are expected to deliver the therapeutic efficacy of the plant extracts. Since, *Croton confertus* has not been previously explored for its therapeutic applications, the present study offers a significant scientific advancement by investigating this plant for the first time in the context of BC-based composite development, distinguishing it from previous reports of BC composites from other plant extracts. The developed composites were subsequently evaluated for their liquid-holding capacity, reswelling behavior, antibacterial activity, and healing potential following their characterization.

2. Materials and methods

2.1. Materials

C. confertus leaves were collected from the Dhofar region, Oman. Fresh coconut water was procured from local markets and used as the primary culture medium. The collected coconut water was thoroughly filtered and sterilized prior to use to ensure a clean, nutrient-rich substrate for bacterial cellulose production. Furthermore, the pH of the juices was adjusted to 5 and sterilized at 70 °C. Sodium hydroxide (≥97.0%, pellets, NaOH), succinic acid (≥99.0%, $C_4H_6O_4$), D-(+)-glucose (≥99.5%, $C_6H_{12}O_6$), acetic acid (glacial ≥99.7%, CH_3CO_2H), peptone and agar ($(C_{12}H_{18}O_9)_n$) were procured from Sigma Aldrich (St. Louis, MO, USA) and used as received without further purification.

2.2. Microbial cultures

MacConkey agar (MA) medium was utilized to culture the BC producing *Gluconacetobacter hansenii* (*G. hansenii*) bacteria. Two representative strains *Escherichia coli* (*E. coli* – Gram-negative) and *Staphylococcus aureus* (*S. aureus* – Gram-positive) were used to assess the antibacterial activity of BC-plant based composites. Luria Bertani (LB) broth was used to culture the strains kept in shaking incubation conditions at 37 °C and 200 rpm for 24 h.[39]

2.3. BC production, harvesting, and purification

Frozen *G. hansenii* were inoculated in synthetic media and incubated at 30 °C and 200 rpm overnight to prepare the pre-culture. 5% pre-culture was further introduced inside the media prepared from leftover fruits and juices and cultured in aerobic conditions at 30 °C for a week. BC sheets appeared at the air and liquid media interface. Sheets were then collected,

cleaned and autoclaved to remove any leftover cell residue. The sheets were stored at 4 °C.

2.4. Soxhlet extraction of *Croton confertus* phytochemicals

The plant leaves were ground into a fine powder after drying under ambient temperature for approximately 14 days. Extraction was done using ethanol and a Soxhlet extractor for 5 h. Excess ethanol was removed on a rotary evaporator delivering a dark greenish mass. The dried extracts were kept at 4 °C until further use.

2.5. Fabrication of BC–bioextract composite films

The BC composite with *Croton confertus* leaf extract (CE) was developed using an *ex situ* composite synthesis strategy. BC sheets were immersed in a beaker containing an aqueous solution of CE (20% w/v) and were kept under stirring for up to 24 h to ensure uniform diffusion of the extract within the BC matrix, allowing absorption of the extracts. The extract concentration (20% w/v) used for BC impregnation was selected based on our previous study, where this concentration enabled efficient incorporation and uniform distribution of plant-derived bioactive compounds within the BC matrix. The developed BC composites with CE were termed as BC–CE.

2.6. Characterization of BC/plant-extract composites

Field emission scanning electron microscopy (FE-SEM, Hitachi S-4800 and EDX-350, Horiba, Tokyo, Japan) was used to determine the morphological characteristics of BC–CE. FTIR was recorded on a PerkinElmer FTIR spectrophotometer (Spectrum GX & Autoimage, USA). The diffraction patterns were recorded using an X-ray diffractometer equipped with Cu Kα radiation (λ = 1.5406 Å) operated at 40 kV and 30 mA. The samples were scanned over a 2θ range of 10°–70° at a scanning rate of 2° min^{-1}.

2.7. Absorption capabilities of the composites

The water absorption capabilities of BC and BC–CE were determined by calculating their water holding capacity. For both water retention time (WRT) and water holding capacity (WHC) analysis, pre-cut (4 cm × 3 cm), freeze dried samples of BC and BC–CE were submerged in water at room temperature in static conditions for the purpose of rewetting. A stabilized wet state is required for the calculation of WRT. BC samples were weighed at different time intervals until they acquired a stable weight or reached a complete dry state. For WHC, the samples were kept in drying chambers to completely remove the water using the formula given below:[40]

$$\text{Water holding capacity} = \frac{\text{mass of water removed during drying (g)}}{\text{dry weight of BC sample (g)}}$$

2.8. Antibacterial activity

The antibacterial activity of BC and BC–CE against the *S. aureus* and *E. coli* strains was determined following the disc diffusion assay and plate count technique.

2.8.1. Disc diffusion assay. BC and BC–CE sheets were cut into round discs (6 mm) and were exposed to UV irradiation for 2 hours for sterilization. These discs were then placed on the surface of LB plates pre-cultured with *S. aureus* and *E. coli*. As the negative control, pure BC discs were taken, while ampicillin discs (10 mg) were utilized as a positive control. The cultured plates were then incubated overnight at 37 °C. Antibacterial activity was determined by measuring the zone of inhibition formed around the discs.

2.8.2. Optical density method. The antibacterial activity of BC and BC–CE films was assessed by the optical density method. Sterilized sample pieces (0.03 g mL^{-1}) were incubated with *E. coli* and *S. aureus* cultures at 37 °C and 150 rpm for 15 h. Growth was monitored by measuring turbidity at 600 nm using a UV-vis spectrophotometer, with blank media serving as a control.

2.9. *In vivo* wound healing on mice model

Male BALB/c mice (2–3 weeks old; 25–30 g) were procured from the National Institute of Health (NIH), Islamabad, Pakistan. All animal procedures received approval from the Institutional Review Board (IRB) and adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, US, 2011). Ten mice were randomly assigned into two groups (n = 5 each): one treated with BC and the other with BC–CE. The animals were maintained under pathogen-free conditions with unrestricted access to standard chow. For wound creation, mice were anesthetized by mixing and injecting ketamine (80 mg kg^{-1}) and xylazine (10 mg kg^{-1}), intraperitoneally, respectively. Then, the dorsal area was shaved and cleaned with 70% ethanol. A biopsy punch was used to obtain full-thickness excisions measuring 8 mm in diameter, removing both epidermal and dermal layers. Following excisions, BC scaffolds of uniform dimensions were disinfected using UV light, washed with saline, and then the samples were applied directly to the wounds of the respective groups. We used single applications of BC and BC–CE during the experiment as a non-invasive protocol and to avoid disrupting early-stage cellular migration and re-epithelialization. The wounds were observed on days 0 and 7 to understand the degree of wound healing.

2.10. Statistical analysis

All experiments were performed in triplicate (n = 3), and the results are presented as mean ± standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test to evaluate differences between groups. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Cost-effective BC production and composite fabrication

Fig. 1 provides a schematic outline of the integrated process beginning with the extraction followed by cost effective BC production and culminating in the impregnation of CE into the

BC matrix to obtain bioactive BC–CE composites. As illustrated in Fig. 1A, CE was first prepared from *Croton confertus*, a phytochemically rich medicinal plant containing flavonoids, phenolics, and terpenoids with documented antimicrobial and wound-healing potential.[41,42] The plant material underwent washing, drying, grinding, and ethanolic extraction to yield a concentrated bioactive solution suitable for subsequent incorporation. Following CE preparation, Fig. 1B depicts the formation of BC using coconut water an abundant agro-resource in southern Oman as a low-cost fermentation substrate.[43,44] After filtration and sterilization, coconut water served as the sole culture medium under static conditions, producing thick BC pellicles at the air–liquid interface within 7–10 days. This strategy effectively replaced conventional glucose- and yeast extract-based media, thereby reducing production costs while promoting waste valorisation and environmental sustainability. The resulting BC sheets displayed a homogenous and clear appearance, representing a pure cellulose network with a uniform nanofibrillar structure.

In the final stage, the preformed BC films were impregnated with the previously extracted CE *via ex situ* immersion. The intrinsic porosity and hydrophilicity of the BC matrix facilitated deep penetration and adsorption of phytochemicals through hydrogen bonding and van der Waals interactions.[45,46] The pronounced color transition from off-white to dark brown upon drying provided visual confirmation of successful entrapment. The resulting BC–CE composites retained the inherent flexibility and structural integrity of the bacterial cellulose matrix, which allowed convenient handling during preparation and potential wound-dressing applications.

Overall, two locally available materials, coconut water and *Croton confertus*, were used, which are sustainable and economically viable sources for producing multi-functional biomaterials. This strategy eliminates the use of chemical additives, reduces waste and enables local natural resources to be converted into value-added biomedicals. The resulting BC–CE composites would be supposed to combine the mechanical strength and hydrophilic nature of BC with the inherent antibacterial and antioxidant properties of the natural plant extract. These properties together endow them with excellent prospects for wound dressings, tissue scaffolds and antimicrobial membranes.

3.2. FTIR and XRD analysis of BC, CE and BC–CE composites

The microstructure and molecular interactions of the BC/CE composite membrane were studied by Fourier-transform infrared (FTIR) spectroscopy. FTIR studies help in understanding the constitution of functional groups, hydrogen bonding interactions and certain possible chemical links established by the polymeric hydroxyl moieties of BC with bioactive phytoconstituents of CE

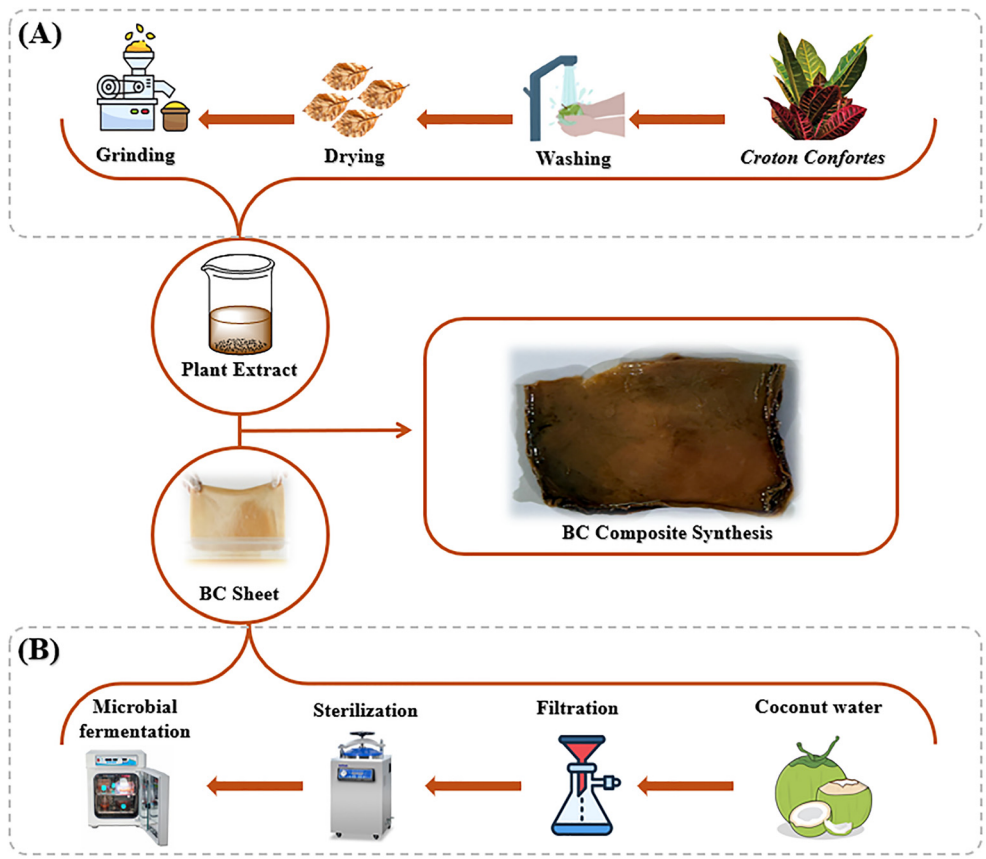

Fig. 1 Schematic demonstration of the formation of BC–CE composite films, showing plant extract preparation (A), BC production (B), and composite formation.

together dictating physical–chemical and biological behavior of the composite.

The characteristic absorption peaks of cellulose fibers were observed in the FTIR spectrum of pristine BC (Fig. 2a): a broad band at 3340–3360 cm^{-1}, which is due to stretching vibrations of intermolecular O–H groups, confirming the presence of an extensive hydrogen bonding network (cellulose fibrils) as well as a peak at about 2890 cm^{-1} that relates to the aliphatic C–H vibration bond. In addition, the bands in the range between 1640 and 1650 cm^{-1} are from bending vibrations due to adsorbed water.[47,48] Similarly, the well resolved peaks at 1050–1150 cm^{-1} result from C–O–C stretching of β-(1 → 4)-glycosidic linkages and imply that there was no significant perturbation of the cellulose backbone.[49] The CE spectrum revealed strong absorptions around 3300–3400 cm^{-1} (O–H stretching of phenolic compounds), 2920 cm^{-1} (C–H stretching of alkane groups), and a pronounced peak at 1720–1730 cm^{-1} corresponding to C═O stretching vibrations of esters, flavonoids, and terpenoids. Additional bands between 1500 and 1600 cm^{-1} indicate the presence of aromatic C═C stretching, while the signals at 1020–1080 cm^{-1} correspond to C–O stretching in alcohols and ethers.[50–52] These characteristic peaks validated the existence of polyphenolic, flavonoid and terpenoid groups (important active compounds of CE), as a bioactive component having an effect on antioxidant and antimicrobial properties. The FTIR spectrum of the BC–CE composite demonstrated an obvious broadening and slight shift of the O–H stretching band from 3340 cm^{-1} to approximately 3320 cm^{-1}, signifying stronger hydrogen bonding interactions between the hydroxyl groups of BC and phenolic compounds of CE. The C═O stretching band observed at 1725 cm^{-1} in pure CE decreased in intensity in BC–CE, suggesting possible intermolecular interactions between CE's carbonyl groups and BC hydroxyls. In addition, the C–O–C and C–O bands in the region of 1050–1150 cm^{-1} were more evident, which suggested that new hydrogen-bonded networks are formed and thus the CE components dispersed uniformly within the BC matrix.[53]

These spectral changes altogether represent the physical adsorption and chemical interactions between BC and CE, without disrupting the cellulose structure. Such interactions are expected to improve the antimicrobial, antioxidant, and hydrophobic properties of the BC–CE films, making them suitable for use in future biomedical applications like wound dressings or aseptic packaging materials.[26,54]

The XRD patterns of BC and BC–CE are presented in Fig. 2b. As evident, the pure BC showed peaks at $2\theta \approx 14–15^{\circ}$, $16–17^{\circ}$, and $22–23^{\circ}$, corresponding to the cellulose I crystalline planes. These peaks are usually ascribed to the (1−10), (110), and (200) crystallographic planes, confirming the bacterial cellulose nanofibrils' highly organized structure.

After adding *Croton confertus* plant extract to the BC matrix, the composite hydrogel retained the primary diffraction peaks associated with cellulose I, showing that bacterial cellulose's core crystalline structure was conserved. Compared to pristine BC, the composite sample had a slightly lower peak intensity and broadening. This shows a partial decrease in crystallinity due to phytochemical components interacting with the cellulose fibrils' hydrogen-bonding network. Plant extracted molecules in the BC nanofibrillar network may disrupt intermolecular hydrogen bonds, resulting in a somewhat more amorphous form while maintaining the cellulose crystalline framework. Importantly, no further crystalline peaks belonging to different crystalline phases of the plant extract were observed, indicating that the extract is well diffused in the BC matrix, likely amorphous or molecularly distributed. The XRD results show that the *Croton confertus* extract does not significantly change the intrinsic crystalline structure of bacterial cellulose, but modest peak intensity changes imply good extract-BC network interaction. This confirms the hydrogel system's structural integrity and complements SEM and FTIR data.

3.3. Morphology and absorbing capabilities of the produced BC composites

SEM images of the pristine BC and BC–CE composites are shown in Fig. 3A. The pure BC showed a well-defined, 3D nanofibrillar network with a highly porous and uniform structure.[55,56] The random network of nanofibers was entangled to create an interconnected web of well-defined porous structures similar to the cellulose synthesized by Acetobacter species under static culture.[25,57] Such homogeneous fibrillar geometry facilitates

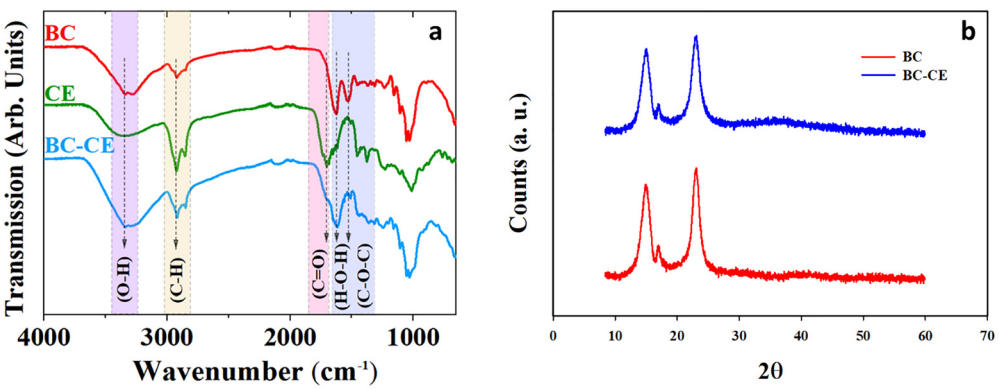

Fig. 2 (a) FT-IR spectra of BC, CE, and the BC–CE composite highlighting the characteristic functional-group regions. (b) XRD spectra of the BC and BC–CE composites.

excellent water holding capacity, permeability, and mechanical flexibility making BC a potential material toward biomedical and tissue-engineering applications.[58,59] A continuous and uniform pore distribution was observed, which may correspond to the natural organization of cellulose microfibrils into a hydrogen bonded and crystallized structure during biosynthesis. The composite samples exhibited a striking morphological change after the CE was introduced (Fig. 3A, lower panel). The nanofibrils became thicker and denser, and some were aggregated because of the plant extract component deposition within and on the BC network. The surface also showed low porosity and packed open voids. This lower pore size could be caused by phenolic and terpenoid compounds of the extract, which are adsorbed on cellulose hydroxyl groups through hydrogen bonding and π–π stacking.[46,60] Such interactions result in the compaction of the BC, which is consistent with visual dark coloration and increased density observed macroscopically for the composite film. The presence of bio-extract molecules in the nanocellulose matrix not only improves the antibacterial/antioxidant properties, but also increases secondary interfacial cross-linking.[24,61]

After CE addition, a clear morphological change was visibly observed in the composite (Fig. 3A, lower panel). The nanofibrils seemed to be thicker, denser and partly aggregated, according to the fact that phytochemical components of the plant extract are successfully deposited both into and onto the BC network. Such open voids were also partially blocked on the surface, leading to decreased porosity and closed-cell region formation. This decrease in pore size can be attributed to the adsorption of phenolic and terpenoid compounds from the extract, that interact with hydroxyl groups on the surface of cellulose through hydrogen bonding.[46,60] These interactions lead to densification of the BC structure, consistent with the darker color and higher density observed macroscopically in the composite films. The incorporation of bio-extract molecules within the nanocellulose matrix not only enhances antibacterial and antioxidant properties but also improves structural integrity by forming secondary interfacial cross-links.[24,61]

The quantified pore-size distribution from image analysis (see Fig. 3(B)) is consistent with the morphological findings. A broader distribution of pore size (0.05–0.25 μm) with a maximum frequency peak, centered at 0.10 μm, was observed in the raw BC sample and thus demonstrated an open cellular porous network. In contrast, the BC–CE composites established a far narrower pore-size distribution with most pores centered under 0.05 μm (Fig. 3). The shift towards smaller pore sizes and new "closed-cell" peaks (highlighted in red) suggests partial filling of the voids therein by extract molecules and some sealing-off of these by extract molecules. This behavior is in accordance with previous results reported for low-porosity BC-based composites loaded with bioactive agents, including plant polyphenols, essential oils or nanoparticles.[62,63]

The general microstructural development demonstrates that CE strongly interacts with the BC nanofibers, leading to a dense and less porous matrix. The observed reduction in pore size after incorporation of the *Croton confertus* extract likely reflects partial filling of nanoscale voids within the bacterial cellulose network rather than complete pore closure. SEM observations confirm that the three-dimensional nanofibrillar architecture of BC remains preserved, with the extract distributed along the fibrillar matrix. This structural configuration may contribute to improved retention of phytochemicals within the composite while maintaining the inherent porous structure of bacterial cellulose. Importantly, bacterial cellulose membranes are widely recognized for their high porosity, excellent moisture retention, and favourable oxygen permeability, properties that make them highly suitable for wound dressing applications. The interconnected nanofibrillar network of BC typically provides continuous pathways for gas diffusion and water vapor

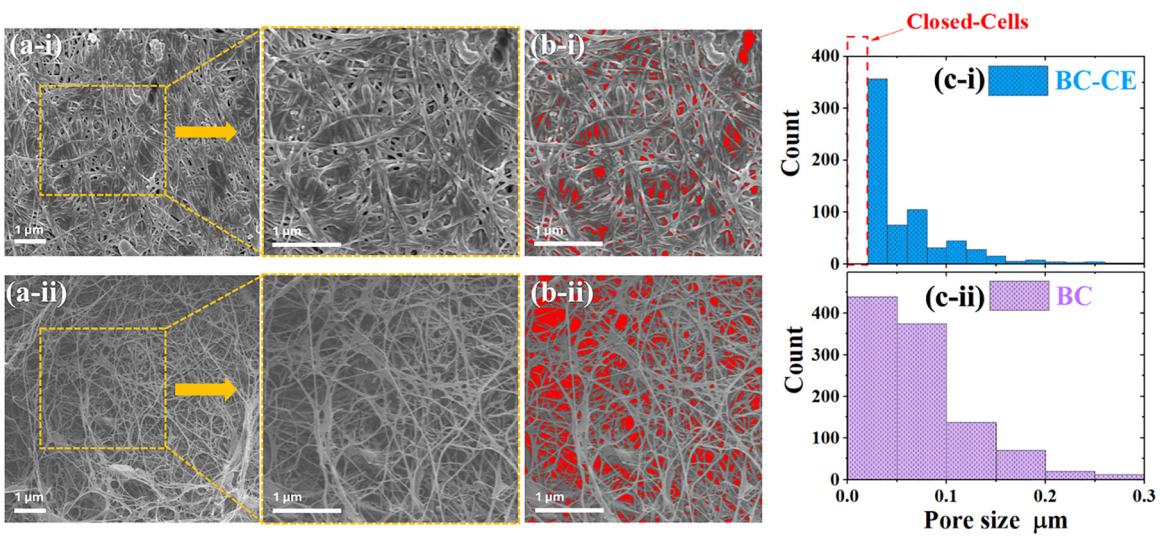

Fig. 3 FESEM images and pore size distribution of BC and the BC–CE composites. (a) and (b) Surface morphology of BC–CE (a-i, b-i) and pure BC (a-ii) and (b-ii) showing denser fibre packing and reduced porosity in the composite. (c-i) and (c-ii) Pore size distribution histograms indicating smaller and more closed pores in BC–CE compared to pure BC.

transmission, which are essential for maintaining an aerobic environment that supports tissue regeneration and wound healing. Therefore, although nanoscale pore dimensions may be reduced after extract incorporation, the composite structure is expected to retain sufficient permeability for oxygen exchange while enabling localized availability of bioactive phytochemicals. This level of microstructural compaction may be beneficial for biomedical applications where moderate permeability and retention of bioactive molecules within the BC network are desirable. The lower pore connectivity further improves the water holding performance of the composite, which is important for long-term wound healing activity.[64,65]

3.4. Liquid absorption and retention behavior

The dynamic water absorption and release behavior of pure BC and BC–CE films was studied to assess their potential as moisture-regulating biomaterials. Efficient moisture management is of particular importance for biomedical applications such as wound dressings, where a moist and non-macerating condition expedites tissue repair and prevents cellular damage by dehydration.[66] The high initial hydration of both samples is shown in Fig. 4(A), and this decreased slowly during the test because of natural evaporation. The water content in untreated BC declined sharply and got almost completely evaporated after 50 h. In sharp contrast, the BC–CE composite demonstrated a significantly higher level of residual moisture content up to 60 h monitoring time. This increased moisture retention potential could be related to the accumulation of phytoconstituents in Croton, which contain polyphenolic compounds, terpenoids and hydrophilic polysaccharides. These components become incorporated within the BC nanofibrillar framework, which encourage a hydrogen-bonding process with water molecules.[67,68] Such molecular interactions not only lead to a higher density of hydrophilic sites but also generate a tortuous diffusion path that delays the evaporation of water, thereby enhancing long-term moisture stability.

The reswelling ability of both materials was also evaluated through 5 consecutive swelling–drying cycles (Fig. 4(B)). As anticipated, the WHC in both BC and BC–CE decreased gradually as a function of cycles owing to limited pore collapse or microstructural fatigue and gradual loss of fiber flexibility due to repeated dehydration phenomena. Nevertheless, throughout the cycles, BC–CE always showed higher WHC than that of pristine BC and was proven to be more stable on rehydration. This feature indicates that the CE components work as reinforcing and crosslinking agents, stabilizing cellulose microfibrils, and appear to mitigate excessive pore compaction and help maintain the structural integrity of the BC network during repeated swelling–drying cycles. The preservation of WHC in BC–CE might also be associated with the interfacial interactions of extract molecules and cellulose hydroxyl groups, which further alloys the microstructural reordering of the BC matrix during drying. These observations are in line with previous studies on other plant-based additives, including *Aloe vera*, neem and sage, where bioactive ingredients were found to improve the hydrogel elasticity and reduce structural collapse upon repetitive reswelling.[24,45] Additionally, the antioxidant and antimicrobial nature of Croton metabolites could help to maintain the structure of cellulose through preventing oxidative breakage and microbial deterioration during prolonged hydration exposure.[69]

Taken together, the results indicated that Croton modification enhanced the short-term water retention and long-term structural robustness of BC matrices. The BC–CE hydrogel possesses controlled maintenance of moisture balance, dimensional stability and mechanical rigidity, which are excellent properties for biomaterial dressings and environmentally responsive hydrogel applications. The potential of BC/CE biocomposites as hydro-stable carriers for the phytochemical's delivery can be attributed to their unique three-dimensional nanofiber architecture and the multifunctional properties of CE, thus providing a sustainable choice of naturally functionalized biocomposites with improved hydro-stability and therapeutic benefit.

3.5. Antibacterial assessment, bacteriostatic and bactericidal activity of plant extract-modified BC films

Phytochemical studies have revealed that medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids, tannins, terpenes and phenolics, which was reported for

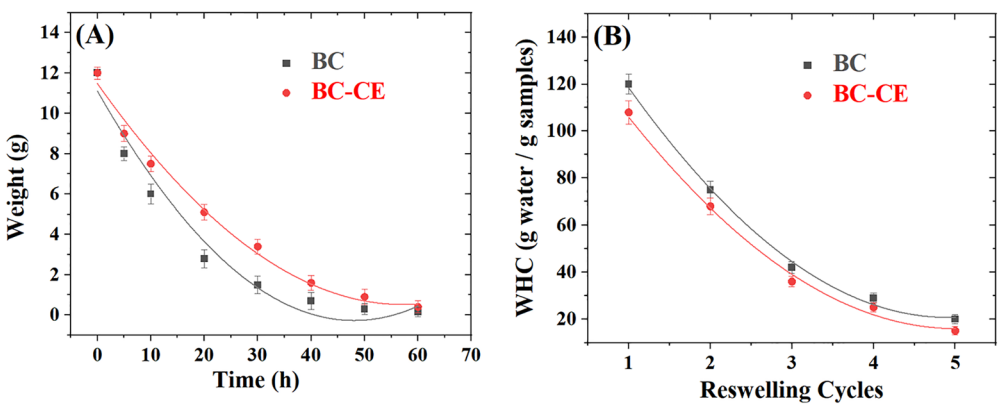

Fig. 4 (A) Time-dependent water retention behavior of BC and BC–CE films over 60 h and (B) water–WHC of BC and BC–CE during five consecutive reswelling drying cycles. Data are presented as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's *post hoc* test ($p < 0.05$).

their synergistic antimicrobial activity.[70,71] These phytochemicals interfere with the bacterial membrane, essential enzymes and quorum sensing and efflux systems.[72] The antibacterial activity of the fabricated BC–CE films was assessed against *E. coli* and *S. aureus* through disc diffusion analysis (DDA) and optical density (OD_{600}) growth inhibition assays (Fig. 5A and B). In the DDA (Fig. 5A), the BC–CE composite indicated that it had obvious inhibition zones of 1.28 cm against *S. aureus* and 1.11 cm against *E. coli*, while pure BC showed no inhibition, proving it to be non-bactericidal in nature. The positive control, ampicillin, displayed inhibition zones of 3.85 cm for *S. aureus* and 3.42 cm for *E. coli*. The greater susceptibility of *S. aureus* compared to *E. coli* can be attributed to the simpler cell wall architecture of Gram-positive bacteria, which allows entry of hydrophobic phytochemicals. These findings agree with previous studies on Croton species, where methanolic extracts of *C. urucurana* and *C. menyharthii* exhibited MIC values of 1.5–5 mg mL^{-1} against the same bacterial strains. As compared to former reports where plant extracts were combined with BC, BC–CE composites showed slightly higher activity. For example, in a recent study, *S. baicalensis* extracts were used to make BC–SBAC composites, which exhibited a 1.28 cm zone against *S. aureus* and a 1 cm zone against *E. coli*. In previous reports, *Ageratum conyzoides* L. leaf extract (BC–AC) exhibited a 0.98 cm zone of inhibition against *S. typhimurium* and 0.84 cm zone against *E. coli* in disc diffusion, and the *Chromolaena odorata* L. leaf extract (BC–CO) showed 0.97 cm and 0.68 cm zone of inhibition against *S. typhimurium* and *E. coli*, respectively. Here, the BC–CE composites showed almost similar inhibition zones of 1.28 cm against *S. aureus* and a higher zone of 1.11 cm against *E. coli*.

To provide additional insights into these observations, bacterial growth inhibition was measured through OD_{600}

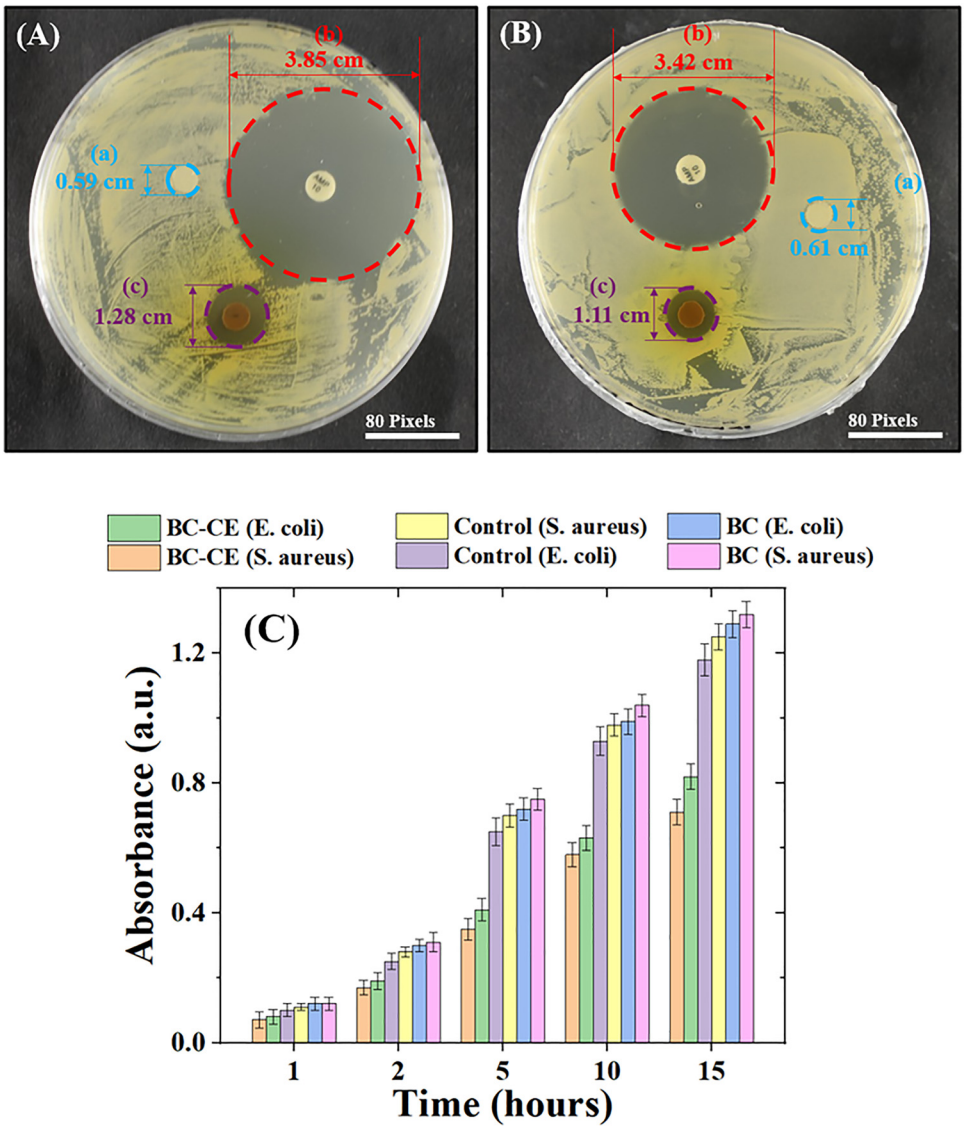

Fig. 5 Antibacterial activity of (a) pristine BC disc, (b) ampicillin drug and (c) the BC–CE composite against *S. aureus* (A) and *E. coli* (B) evaluated using a disc diffusion assay, and by optical density measurements at OD_{600} (C). Data are presented as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's *post hoc* test ($p < 0.05$).

measurements over a 15-hour incubation period (Fig. 5B). The pure BC treated samples showed normal bacterial growth curves, reaching final absorbance values of 1.32 ± 0.04 a.u. for *S. aureus* and 1.29 ± 0.04 a.u. for *E. coli*. In contrast, the growth of cultures treated with BC–CE films was remarkably reduced amounting to 0.71 ± 0.04 a.u. for *S. aureus* and 0.82 ± 0.04 a.u. for *E. coli*, representing an approximately 46% and 36% reduction in bacterial proliferation relative to the control, respectively. The increased lag phase and lower final OD values confirm the bacteriostatic effect of the BC–CE films and may be associated with the presence of bioactive phytochemicals retained within the BC nanofibrillar matrix.

Overall, the superior antibacterial ability of the BC–CE composites is attributed to the combined activity between bioactive phytochemicals uniformly deposited in cellulose, following a mechanism for controlled release and sustained efficacy. *S. aureus* was more sensitive to metabolites from CE, whereas *E. coli* showed a moderate resistance owing to the presence of the outer membrane. The addition of CE greatly improved the antibacterial properties of BC, demonstrating its promise as a natural-based environmentally friendly antimicrobial biomaterial to be used in wound dressing, scaffolds and active packaging.

3.6. Wound healing evaluation

Fig. 6 reveals the *in vivo* wound-healing efficiency of the pure BC membrane and BC–CE composite. At day 0 (no treatment), the wound sites in both groups exhibited fresh epithelial disruption and redness (circled areas). After immediate dressing with BC and BC–CE (day 0 after treatment), both materials adhered well to the moist wound bed, giving rise to a flexible and permeable membrane. Noticeably, the BC–CE film possessed better conformability, benefitting from its dense but slightly more flexible texture (as listed in the morphological analysis), by which it could accurately fit the wound site and enhance water retention.

On day 7, wound contraction and epithelial closure were significantly different between groups. The wound treated with BC maintained partial redness and incomplete closure; however, the wound treated with BC–CE showed a markedly reduced lesion size, a decreased level of inflammation and evidence of new epithelium formation (Fig. 6, lower panel). The augmented wound-healing activity of BC–CE can be attributed to the collaborative effect of bioactive components present in the plant extract, such as flavonoids, phenolics and terpenoids, which are well-known for their potential antibacterial, anti-inflammatory and antioxidant activities.[73–75] These compounds might have inhibited local microbial growth, reduced oxidative stress and promoted fibroblast proliferation and collagen deposition.[75,76] Moreover, the structural features of BC are essential in this healing process. Its high water-holding capacity and gas permeability maintain a moist environment conducive to tissue regeneration.[77,78] The addition of CE further facilitated the environment for wound healing through controlling the exudate levels and enhancing local oxygen exchange. Phenolic molecules from the extract may have established hydrogen-bond, leading to secondary interfacial cross-links that boosted mechanical stability and sustained the gradual release of phytochemicals.[79,80] This controlled release would have allowed extended antiseptic action on the wound surface, contributing to healing acceleration by forming granulation tissue and epithelialization.

Similar outcomes have been reported for BC composites loaded with other plant extracts such as *Aloe vera*, *Centella asiatica*, *Piper betle*, and *Lonicera japonica*, where phytochemical

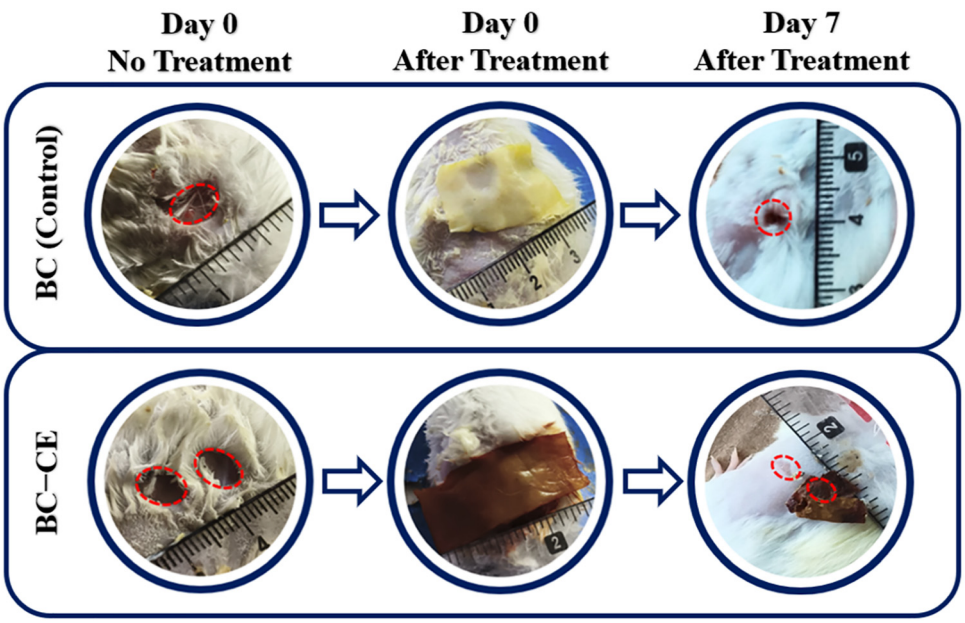

Fig. 6 *In vivo* wound-healing evaluation of BC (control) and BC–CE composite dressings. Images show wound appearance on day 0 before treatment, immediately after applying the respective dressings, and on day 7 post-treatment. BC–CE-treated wounds exhibit markedly enhanced contraction and epithelial regeneration compared to the BC control.

incorporation pointedly augmented wound closure and reduced the inflammatory response compared to pure BC.[81,82] Thus, the observed healing progression in BC–CE-treated wounds displayed the successful biofunctionalization of BC *via* a green, *ex situ* approach, yielding a cost-effective and sustainable wound-care material. Although the BC matrix is expected to facilitate diffusion of phytochemicals due to its porous nanofibrillar architecture, quantitative release kinetics were not investigated in the present study and will be explored in future work.

4. Conclusions

This study presents a sustainable, high-performance antibacterial composite produced by infusing *Croton confertus* leaf extract into a low-cost, waste-derived bacterial cellulose composite. The *ex situ* incorporation approach enabled uniform loading of phytochemicals without compromising the cellulose network. FTIR and FE-SEM analyses confirmed strong interfacial interactions and microstructural densification, resulting in improved water-retention capacity and enhanced hydrogel stability. The BC–CE composites exhibited strong antibacterial activity against both Gram-positive and Gram-negative strains, likely associated with the presence of CE-derived bioactive metabolites incorporated within the BC matrix. *In vivo* evaluation further demonstrated markedly faster wound contraction and improved epithelial regeneration compared to pure BC, underscoring the synergistic therapeutic effects of the plant extract and BC scaffold. Overall, BC–CE emerges as an eco-friendly, scalable biomaterial suitable for antimicrobial dressings and wound-healing applications.[83] The strategy utilizes locally available resources, lowers production costs, and offers a green pathway for developing functional biopolymer composites. Future work may focus on optimizing extract loading, understanding release mechanisms, and conducting broader preclinical validation to support clinical translation. Furthermore, the use of readily available substrates and a simple fabrication strategy suggests promising scalability and translational potential of the BC–CE composite for practical biomedical applications, particularly in sustainable wound-care materials.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data supporting the findings of this study are included within the article in the form of figures and tables. No external datasets or software code were generated or used during this study.

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