BACTERIAL CELLULOSE MEMBRANES ENRICHED WITH BIOACTIVE COMPOUNDS FROM AVOCADO SEEDS

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Lately, biologically active materials are an extensive topic of research and the employment of bacterial cellulose membranes (BCMs) as carriers has proven to be one of the more interesting applications, especially in the biomedical, cosmetic, food, and pharmaceutical fields. However, bacterial cellulose itself is not antibacterially effective, which is one of the most desired property. To increase its applicability, BCMs can be modified or enriched with biologically active compounds. While humanity is striving for an extensive strategy for the transition to circular economy, the food wastes present a sustainable and renewable source that can be processed into value-added products. The production of BCMs by cultivation of *Komagataeibacter hansenii* was carried out. BCMs were further enriched with bioactive compounds obtained from avocado seeds using ethanol as a solvent. The developed BCMs have shown great potential with their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* for further applications in biomedicine, cosmetic, food, and pharmaceutical industries.

Keywords:
bacterial cellulose,
nanocellulose,
avocado seed,
antibacterial activity,
membrane

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1 Introduction

The claim for sustainable biologically active materials has remarkably increased in recent times, due to their characteristics that make them appropriate as an extensive potential for applications in biomedical, cosmetic, food, and pharmaceutical fields.

Komagataeibacter hansenii is one of the acetic acid bacteria which produce bacterial cellulose (BC). Under the static conditions, at the air-liquid interface, BC membranes (BCMs) are formed, exhibiting three dimensional open fibrous network structure (Fatima et al. 2023). By means of its hydrophilic nature, nontoxicity, flexibility, biocompatibility, good barrier and mechanical properties, BC have been extensively researched and used (Bodea et al. 2020). The porosity and high specific surface area ensures high liquid absorption capacity (Zheng et al. 2020). It was already applied as a base for artificial blood vessels, implants, stents, part of the drug delivery systems, and mostly as a wound dressing material (Swingler et al. 2021; Rajwade et al. 2015). Most frequently, BC dressings are based on wet form, as it provides suitable moisture degree, which is advantageous for healing of the wounds (Nuutila and Eriksson 2021).

However, one of the most sought-after characteristics of advanced biologically active materials is antimicrobial activity, which BC itself lacks. Therefore, BC is commonly modified with divergent antimicrobial agents, such as metal/metal oxide nanoparticles, antibiotics, and organic compounds (e.g., amino acids, curcumin, chitosan,…) (Zielińska et al. 2022; Krasowski et al. 2021; Moritz et al. 2014).

As the occurrence of drug-resistant bacteria is increasing rapidly (Xuan et al. 2023), there is a constant search for antimicrobials. Plants produce a wide range of phytochemicals, being a rich source of different active compounds (Bergonzi et al. 2022). On the other hand, huge waste of food commodities and by-products occurred due to improper food handling at every step of post-harvest. Plant extracts, obtained from different food wastes are hence a promising alternative, as they prove to be a renewable resource that can be processed into products with added value.

Avocado seeds (AS) are one of the underutilized inedible parts of fruit, which are discarded and present enormous quantity of waste biomass. AS are rich in biologically active substances such as polyphenols, acetogenins, triterpenoids, and others (Jimenez et al. 2021). In our recent study (Kupnik et al. 2023), AS extracts exhibited significant antimicrobial activity against 13 out of 15 tested microorganisms, which often pose a problem in antimicrobial resistance, are transmitted by food or are colonized during food packaging. High concentrations of antimicrobial compounds, i.e., hesperidin, 2,3-dihydroxybenzoic acid, and vanillin, were found in AS extracts, which can synergistically contribute to high antimicrobial properties of AS extracts.

Therefore, the objective of presented study was to produce BCMs enriched with AS extract in order to prepare effective biocomposites for potential antibacterial applications in divergent fields and industries.

2 Methods

2.1 Production of BCMs

First, for the growth and production of BCMs, Hestrin and Schramm medium was used. The HS medium was prepared as followed: 2.0% (w/v) glucose, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract, 0.27% (w/v) Na_2HPO_4, and 0.15% (w/v) citric acid with the pH of 6.0. The inoculum was prepared by transferring *K. hansenii* in 50 mL of HS medium. The suspension was then shaken with 150 revolutions per minute at 27 °C for 48 hours. The suspension was used to inoculate prepared flasks with 100 mL of production media, followed by static cultivation at 27 °C until membranes were formed. Obtained BCMs were gathered, washed with dH_20 and submerged in 1.0 M NaOH solution for 2 hours at 80 °C in order to remove impurities and microbial cells from membrane. BCMs were then repeatedly washed with distilled water to obtain neutral pH in washed liquid.

2.2 Preparation of ethanolic AS extracts

AS were detached from the ripe avocado fruits and washed with water. AS were cut into little pieces and dried at room temperature. Dried AS were then ground and subjected to Soxhlet extraction method. Approximately 25 g of dried AS were extracted using 150 mL of ethanol as a solvent in Soxhlet extractor. Extractions were carried out for approximately 6 hours or until four recycles were completed. Rotary evaporator at reduced pressure and 40 °C was used in order to evaporate the solvent. Obtained AS extracts were stored at -20 °C until their further usage.

2.3 Enrichment of BCMs with AS extract

The Soxhlet AS extract solutions were prepared in concentrations of 10 and 100 mg/mL, respectively. The BCMs were immersed in prepared AS extract solutions for 48 hours. In order to facilitate the diffusion of the extract into the BCMs, the magnetic stirrer with 150 rpm was used. After adsorption process, enriched BCMs with AS extract were soaked over a filter paper for removal of excess extract solution.

2.4 Antibacterial activity

Antibacterial effectiveness of prepared BCMs was evaluated against Gram-negative bacterium *E. coli* and Gram-positive bacterium *S. aureus*. The agar diffusion method was used, following the protocol detailed in our previous study (Kupnik et al. 2021) with some adjustments. Briefly, 100 µL of prepared bacterial suspension was spread evenly on suitable agar plates, according to selected bacteria. Next, 1×1 cm pieces of BCMs were laid on the inoculated agar plates. Prepared agar plates with samples were incubated at optimal growth conditions for *E. coli* and *S. aureus* (37 °C, 24 hours). Pure BCM was used as a negative control. The indicator of the antibacterial effectiveness was the inhibition zone (measured in mm) formed around sample.

3 Results

BCMs were produced in HS medium and harvested after 21 days of fermentation. Procedure and final BCMs enriched with AS extract are presented in Figure 1.

A result of immersing pure BCMs in prepared AS extract solutions were orange-colored BCMs. This is due to perseorangin, a pigment present in AS, which is a result of a polyphenol oxidase-dependent reaction (Hatzakis et al. 2019).

Furthermore, BCMs enriched with AS extract were assessed for their antibacterial activity using agar diffusion method. The collected results are presented in Table 1.

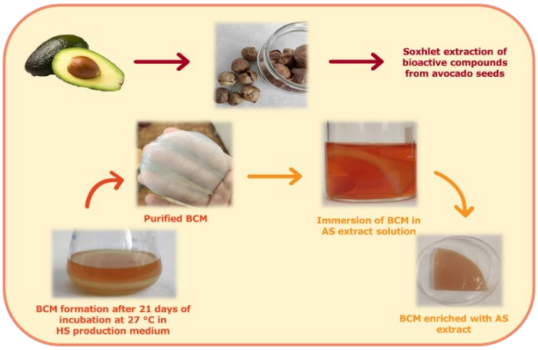

Figure 1: Procedure of production and enrichment of BCMs with AS extract
Source: own.

Table 1: Zone inhibition of enriched BCMs against *E. coli* and *S. aureus*

	1. BCM + 10 mg/mL AS extract	2. BCM + 100 mg/mL AS extract
E. coli	17 ± 3 mm	22 ± 2 mm
S. aureus	17 ± 1 mm	21 ± 1 mm

As expected, BCMs immersed in AS extract solution with a higher concentration (100 mg/mL) showed better antibacterial effect than BCMs immersed in AS extract solution with a concentration of 10 mg/mL. Enriched BCMs inhibited the growth of *E. coli* by an average of 17-22 mm, and *S. aureus* by 17-21 mm.

Figure 2 shows inhibition zones of BCMs against *E. coli*.

The result of the research showed prosperous development of antibacterially effective BCMs enriched with AS extracts.

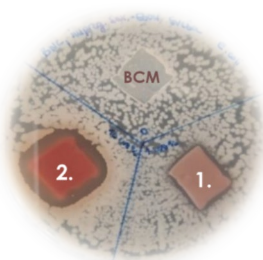

Figure 2: Inhibition zones against *E. coli*
Source: own.

4 Conclusions

Based on obtained results, the BCMs enriched with AS extracts, with exceptional antibacterial activity, showed a great possibility for their further use in biomedicine, and in the field of food packaging, cosmetic, and pharmacy.

Noteworthy, the utilization of waste avocado seeds enables and encompasses a broader strategy towards a circular economy as the volume of waste could be greatly reduced. Additionally, a renewable resource could be exploited for conversion into the presented sustainable value-added antibacterial biocomposites.

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