

BACTERIAL CELLULOSE MEMBRANES LOADED WITH CINNAMON ESSENTIAL OIL

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At present, essential oils (EOs) are of great interest to the medical field, coming as an alternative to the usual medicines and especially to antibiotics. Bacterial cellulose (BC) was qualified as a support for these EOs due to its multiple properties and an attempt was made to load this support with different concentrations of cinnamon essential oil (CEO). Infrared characterization and thermal analysis confirmed the strong interaction between BC and CEO. The good hydrophilicity of this material was highlighted by the hydrating capacity of this support. Results confirmed the presence of cinnamon oil in the structure of BC.

Keywords: cinnamon essential oil, bacterial cellulose, drug delivery

1. Introduction

Essential oils are a mixture of bioactive components, terpenes, terpenoids and phenolic compounds with specific biological activity. The antimicrobial properties of essential oils are due to these bioactive compounds [1, 2]. The mechanism of action is based on the irreversible destruction of the bacterial cell wall and membrane. This mechanism causes essential oils to be used to treat infections in the body [3] and to be used in various antimicrobial applications [4-

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6]. The active constituents of cinnamon oil responsible for antifungal activity and antimicrobial activity are cinnamaldehyde, cinnamon cinnamate and benzyl cinnamate [7]. Studies to date have shown that CEO reduces oxidative stress and microbial infections [8, 9]. Cinnamon has excellent antimicrobial properties [10], antitumor activity and antioxidant capacity which is explained by the presence of phenolic compounds in its composition. Studies to date have shown that a cinnamon content of up to 5% provides increased dressing activity without altering the biocompatibility [11]. The mechanisms involved in the antifungal effect of cinnamon oil are cytoplasmic granulation, rupture of the cytoplasmic membrane and inactivation of intracellular and extracellular enzymes [12]. Banu *et al.* made an antiviral membrane containing cinnamon and proved its activity against *Candida albicanis* and *non-albicanis* strains, which is very effective in inhibiting the biofilms of the Candida strain. The EO compound responsible for antibacterial activity in the study by Banu *et al.* is linalool [13]. In Fig. 1 are presented the properties of CEO.

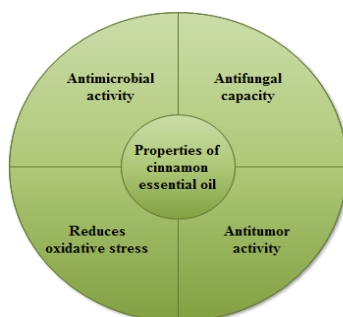


Fig. 1. Properties of cinnamon essential oil

BC has high purity and good biocompatibility [14], making it widely used in the medical field. It is currently used in the treatment of wounds due to its permeability, water retention capacity and flexibility. Its three-dimensional structure makes it good support for retaining drugs and active substances and releasing them into the wound [15-17]. Although BC is biocompatible with the human body, it serves primarily as a mechanical barrier that protects the wound surface from rapid drying and infection. To prevent infection, it must be modified in order to benefit from the antibacterial activity. Although BC does not possess intrinsic antimicrobial properties, can absorb and slowly release an antimicrobial agent [18]. Other distinctive properties of BC include: large surface area, porosity, and high mechanical strength (i.e. 118 GPa Young's modulus, in a range similar to that of steel), high water content > 90% and well-defined biocompatibility. These properties make BC an excellent biopolymer for the development of artificial skin

and blood vessels, implants, hemostatic and scaffolding structures in tissue engineering [19].

In order to be able to take advantage of the properties of CEO, in the long-term, the use of a support material is needed and in this case, BC could be a good candidate. The aim of this study was to load the BC membranes with different concentrations of cinnamon oil to make dressings for the treatment of wounds. The combination of the BC and CEO was done to develop a drug delivery support with excellent properties for skin injury treatment.

2. Materials and Methods

2.1 Materials

For this study we used CEO, (*Oleum Cinnamon ceylanici*), produced by Roth. In making the emulsions we used Tween 80, produced by Roth, ethanol produced by Sigma Aldrich and glycerin from Sigma-Aldrich, for a better malleability of the samples. The antimicrobial assessments were performed using Nutrient Broth No. 2 and Agar with a microbiological grade, which are purchased from Sigma-Aldrich (Germany, Darmstadt). All strains tested in this study provide from the Microorganisms Collection of the Department of Microbiology, Faculty of Biology & Research Institute of the University of Bucharest.

2.2 Methods

2.2.1 Synthesis of Bacterial Cellulose

BC membranes were obtained in static culture using the average Hestrin-Schramm containing 3% fructose after 7 days. *Acetobacter sp.*, the strain used in this study, was isolated from traditional fermented vinegar in the Microbiology Laboratory of the Department of Chemical and Biochemical Engineering of the University POLITEHNICA of Bucharest. The gel films were purified by boiling in 0.1 N aqueous NaOH for 1h and rinsed with deionized water several times until the pH of the effluent solution became neutral. NaOH treatment was used to kill bacteria by cell lysis. NaOH concentrations higher than 5% are not recommended as this could change the crystal structure of BC from cellulose I to cellulose II [20].

2.2.2 Loading cinnamon essential oil into the BC membrane

To load CEO into the BC membrane structure, emulsions of this oil were made in concentrations of 1, 3 and 5%. The emulsion was made in 25 ml of ethanol, in which was added the stable concentration of oil, 1 ml of Tween 80 as a surfactant, which lowers the surface tension of liquids, favoring dispersion, and glycerin for a malleable structure of this dressing membrane used. for skin wounds. BC membranes were immersed in CEO emulsions for 24 h and then kept in an oven at a temperature of 37 °C for 48 h. The technological flow of loading BC membranes with CEO is shown in the Fig. 2.

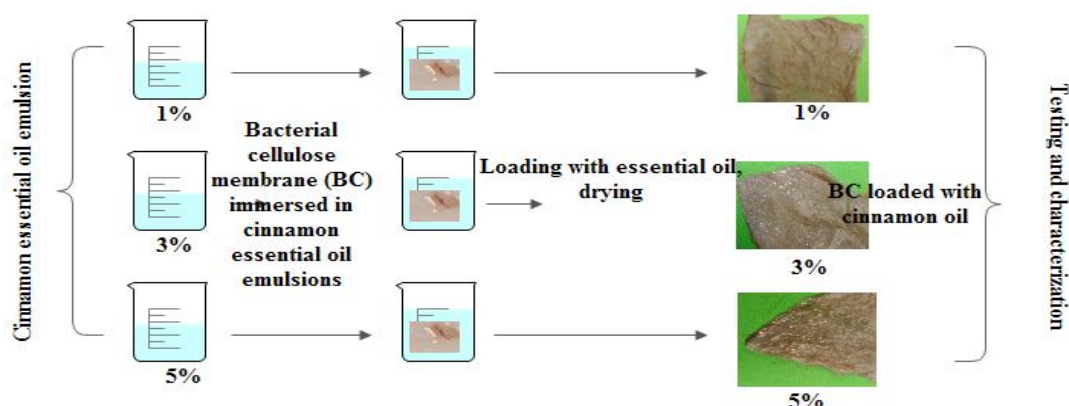


Fig. 2. The technological flow of loading BC membranes with cinnamon essential oil

2.2.3 FTIR Analysis

The synthesized samples were characterized by FTIR using a Nicolet iS50 FT-IR (Nicolet, City, MA, USA) spectrometer equipped with a DTGS detector, which provides information with a high sensitivity in the range of 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} . All spectra were obtained by co-adding 32 scans, with the scanning time being 47 s. FTIR 2D maps were recorded with a Nicolet iS50R FTIR microscope with DTGS detector, in the wavenumber range 4000–600 cm^{-1} . The 2D FTIR maps were used to obtain information about the spatial distribution of the components (homogeneity/heterogeneity).

2.2.4 SEM Analysis

The SEM-EDS characterization was performed using a QUANTA INSPECT F50, FEI Company, Eindhoven, The Netherlands scanning electron microscope equipped with field emission gun electron-FEG (field emission gun) with 1.2 nm resolution and an energy dispersive X-ray spectrometer (EDS) with an MnK resolution of 133 eV.

2.2.5 Thermal Analysis

The thermal analysis TG-DSC for the samples was performed with a Netzsch STA 449C Jupiter apparatus. Amounts of 10 mg of each sample were placed in an open crucible made of alumina and heated with 10 $\text{K} \cdot \text{min}^{-1}$ from room temperature up to 900 $^{\circ}\text{C}$, using dried air (50 mL/min) as a sweeping gas. An empty alumina crucible was used as the reference. The evolved gases were analyzed with a FTIR Tensor 27 from Bruker (Bruker Co., Ettlingen, Germany), equipped with a thermostated gas cell.

2.2.6 Water Absorption Capacity

The water absorption capacity of BC samples loaded with CEO, in concentrations of 1, 3 and 5%, was evaluated by determining the change in sample

weight during adsorption in distilled water for 72 h. The measurements were performed at different time intervals: 5, 10, 20, 30 and 45 min, and 1, 2, 4, 6, 8, 12, 24, 48 and 72 h.

2.2.7 Antibacterial Activity

The antibacterial activity was evaluated against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 by quantitatively determining the ability of selected strains to adhere to the surface of functionalized materials using the method described in the previous study [20]. The biological assessments were performed in three independent determinations and the data results were statistically analysed using the GraphPad Prism program for Windows 64-bit, version 9.3.1 (471), developed by GraphPad Software, San Diego, CA (USA). We compared the resulting data using analysis of variance (ANOVA), and Dunnett's multiple comparisons test where a p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) is particularly useful for analyzing material morphology. SEM images of BC-Control sample are presented in Fig. 3. From these images it can observe a three-dimensional structure and a high porosity of the analyzed material, in agreement with other experimental observations [21, 22]. The high porosity and the nature of this material lead to a very high absorption capacity, thus being suitable for the absorption of exudate even in high exuding wounds. At the same time, thanks to this molecular and micro-structure, CEO could be absorbed [23, 24].

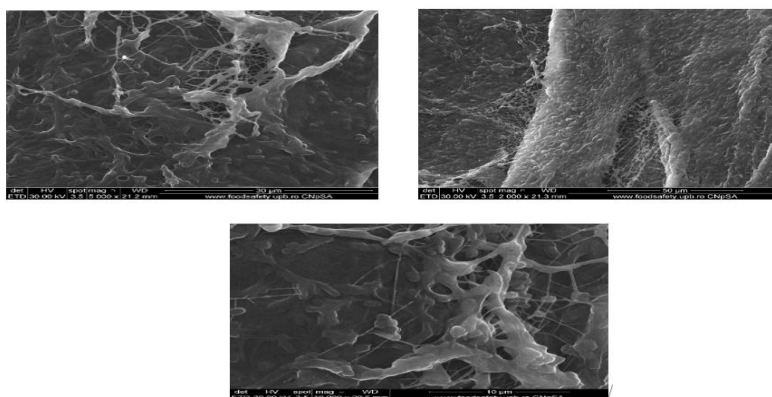


Fig 3. SEM images showing the structure of the BC-Control sample at different magnitudes (5,000x, 2,000x and 10,000x)

3.2 FT-IR Analysis

The samples as well as the CEO were characterized by FT-IR spectroscopic analysis. Fig. 4 shows the spectra for BC-Control, BC-Cinnamon 1%, BC-Cinnamon 3%, BC-Cinnamon 5% and CEO. The control analysis of the BC-Control sample showed its characteristic chemical bonds.

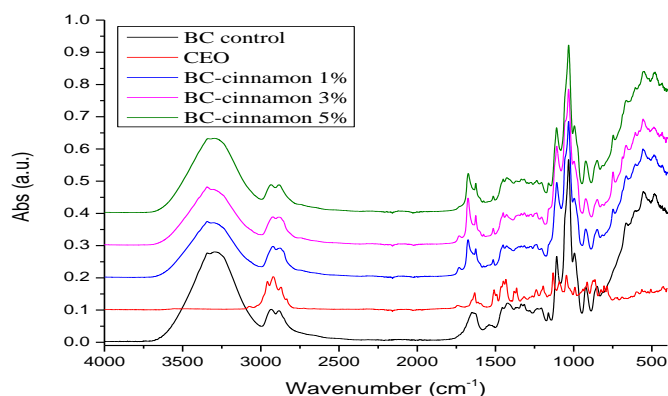


Fig. 4. FTIR spectra of BC-Control, BC-Cinnamon 1%, BC-Cinnamon 3% BC-Cinnamon 5%, and Cinnamon essential oil.

The FT-IR spectrum of CEO shows its chemical composition. The peaks observed at 3020, 2815.7, 1670.7, 1625.0 and 1575.6 cm^{-1} are representative for C-H sp^2 and C-H sp^3 stretches, aldehyde C-H bonds, C = O aromatic aldehyde bonds. The bands recorded in the range 1449.6 - 687.5 cm^{-1} are representative of the off-plane bending C - H for CH_3 , C - O stretch for alcohols C - H bending sp^3 , C - H bending sp^2 aromatic and absorption of the alkyne group. These results confirm the chemical structure of this essential oil [11]. BC FT-IR control spectrum presents its characteristic chemical bonds. It can be seen that at 3292.8 cm^{-1} , there is a stretching vibration of OH groups, and at 2933.52 cm^{-1} , a stretching vibration of CH_2 and the CH group. The HOH binding vibration of the crystallization water is identified at 1647.1 cm^{-1} and at 1253 cm^{-1} , the OH deformation vibration can be also observed. The peak at 1221 cm^{-1} is assigned CH vibration deformation. The asymmetric tensile vibrations of C-C are identified at 1161.49 cm^{-1} . In table 1 can be observed the assignment of relevant IR absorption bands of CEO, BC-Cinnamon 1%, BC-Cinnamon 3%, BC-Cinnamon 5% composites. Fig. 5 presents some of the most relevant FTIR maps recorded at 3519, 1693 and 1030 cm^{-1} for the BC, BC-CEO1%, BC-CEO3% and BC-CEO5%. Based on these images, it can conclude that all the samples have good homogeneity at microscale level.

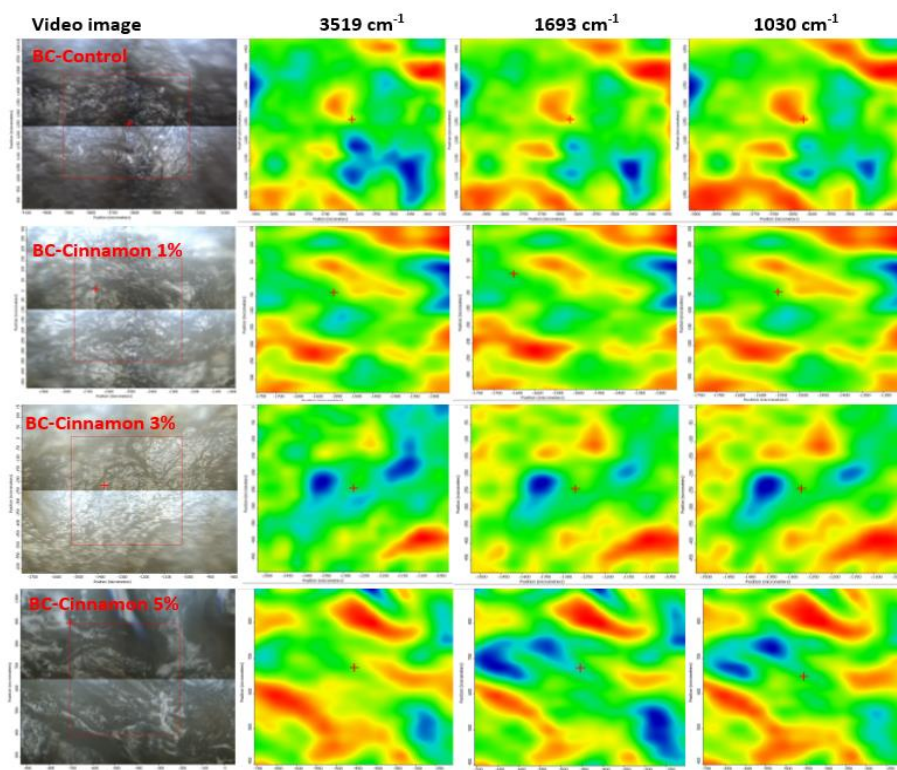


Fig. 5. FTIR microscopy images recorded for the BC-Control, BC-Cinnamon 1%, BC-Cinnamon 3% and BC-Cinnamon 5%

Table 1

Assignment of relevant IR absorption bands of Cinnamon essential oil, BC-Cinnamon 1%, BC-Cinnamon 3%, BC-Cinnamon 5%

Assignment	Wavenumbers (cm ⁻¹)			
	CEO	BC-Cinnamon 1%	BC-Cinnamon 3%	BC-Cinnamon 5%
Phenolic O-H stretches	1670.17	1675.17	1675.16	1675.22
Aldehyde C-H bonds, C = O aromatic aldehyde bonds	1624.97	1626.17	1625.98	1625.92
Plane bending C - H for CH ₃ , C - O stretch for phenol hydroxyl, C- O stretch for alcohols	1449.62	1450.89	1450.43	1450.11
Stretching vibrations of C-O and the C-OH deformation vibration.	1032.66	1032.36	1032.38	1032.09
C-H bending vibration absorption	917.02	923.07	922.93	922.79

3.3 Thermal Analysis

The BC presents several distinct mass loss steps, corresponding to the water elimination, dehydration, decomposition and oxidation (Fig. 6). In the first step, up to 160°C the water molecules, physically adsorbed by the cellulosic structure, are eliminated. This accounts for 23.51% of the initial mass. The process is accompanied by an endothermic effect with the minimum at 94.6°C. The hydrogen bonds between water and cellulose chains are responsible for the higher temperature needed to eliminate the water [25].

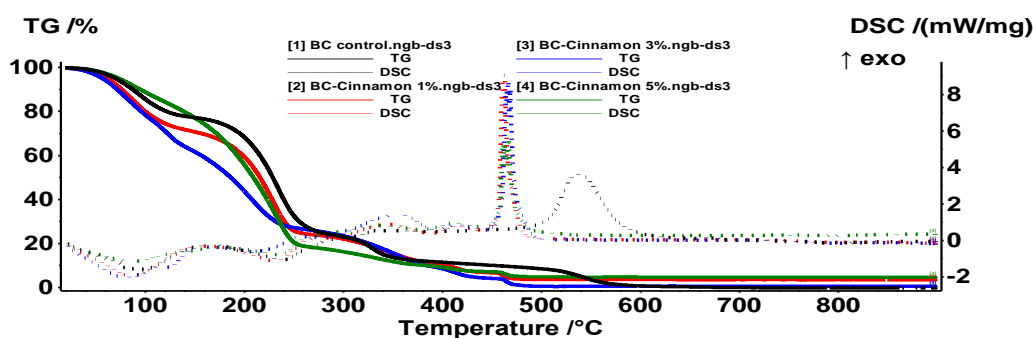


Fig 6. TG-DSC curves for BC and CEO loaded BC samples

In the second process, between 160-300°C, the cellulosic fibers are starting to decompose [26]. This is the main mass loss step with a value of 53.51%. An endothermic effect with minimum at 240.1°C accompanies this process. The FTIR spectra recorded for the evolved gases indicate the presence of water and carbon dioxide molecules in principal, but also some traces of carbon monoxide and hydrocarbons fragments. Table 2 centralizes the TG-DSC data for the BC and CEO loaded BC samples.

Table 2

The TG-DSC data for the BC and CEO loaded BC samples

Sample	Mass loss			Thermal effects		
	RT-160°C	160-300°C	300-600°C	Endo I	Endo II	Exo
BC	23.51%	53.51%	23.27%	94.6°C	240.1°C	335.6/539.8°C
BC-Cinnamon 1%	30.43%	47.51%	18.55%	87.6°C	230.9°C	345.8/462.5°C
BC-Cinnamon 3%	40.97%	35.62%	22.87%	79.3°C	206.7°C	356.1/466.4°C
BC-Cinnamon 5%	25.86%	57.86%	11.59%	86.8°C	234.6°C	337.1/467.0°C

Strong exothermic effects are recorded after 300°C, indicating the start of oxidation processes. The sample is partially oxidized up to 440°C, with a recorded mass loss of 12.60%, and the residual carbonaceous mass is burned up to 600°C, with a mass loss of 10.67%. The main component of the evolved gases, after 300°C is the CO₂, with some traces of H₂O and C-H fragments observable mainly under 440°C.

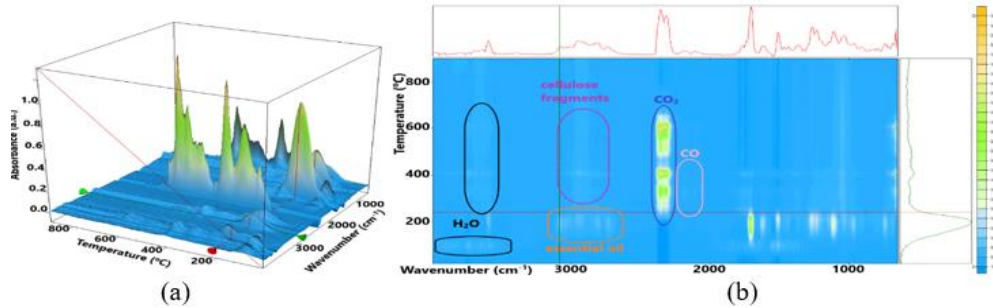


Fig.7. The evolved gases FTIR 3D diagram for the BC-Cinnamon 5% sample (a) and its 2D projection with assigned identification / temperature intervals (b)

Similar behavior is recorded for all the BC-Cinnamon samples, the principal numeric data being presented in the Table 2. The presence of the CEO components in the evolved gases is indicated by the FTIR analysis (Fig. 7). The 3D FTIR plot (Fig. 7a) presents the evolution of the FTIR spectrum vs temperature. By projecting this map in 2D space (wavenumber vs temperature) we can easily identify the components and temperature intervals when they are eliminated from the sample (Fig. 7b). Therefore, the essential oil components could be identified as being eliminated around 200°C mark.

3.4 Kinetic of Water Absorption

The use of a material as a wound dressing must meet several conditions, such as: accelerating the wound healing process and preventing damage to newly formed skin, having the ability to absorb exudate from the wound, being able to release active substances and being easily and painlessly removed [20]. BC is often used as a wound dressing material, having all of the above characteristics. Cinnamon oil has been preferred for its antibacterial, antiseptic properties, thus protecting against infections and contributing to rapid tissue repair [8].

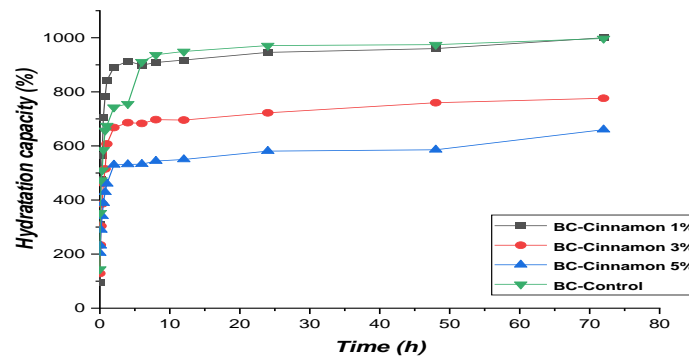


Fig. 8. Absorption kinetics of samples BC-Control, BC-Cinnamon 1%, BC-Cinnamon 3%, BC-Cinnamon 5%

Regarding the graph from Fig. 8 of the absorption capacity of the samples it could be observed that all the samples absorb a large amount of water, between 580 and 950%, which is suitable even for the moderate/heavy exuding wounds. Based on the Fig. 8, it can conclude that all the samples reach the maximum absorption capacity within several tens of hours. The sample loaded with 5% has an absorption capacity of approximately 580%, the one loaded with 3% Cinnamon essential oil has a capacity of 650%, and the one with 1% and pure BC reaches the maximum absorption at about 950% which is justified according to the more hydrophobic nature of the CEO. It is worth to mention that 1% CEO not alter significantly the water absorption. Moreover, during the analyzed period of time, the dressing materials seems to be non-degraded and considering the time for which they are applied, seems to be suitable for the desired application.

3.5. Antibacterial Assay

BC loaded with antimicrobial agents can be used as wound dressing material due to its properties such as purity, biocompatibility, water uptake capacity, high porosity, biological activity etc. [27].

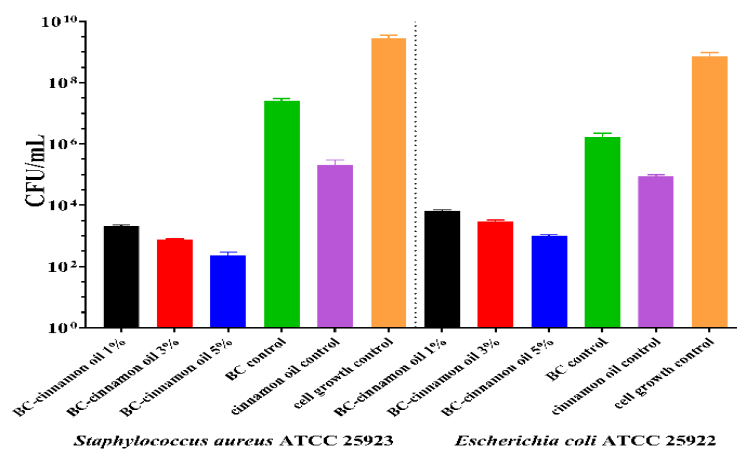


Fig. 9. The influence of BC with cinnamon oil on the capacity of the bacterial cells (*S. aureus* ATCC 25923, *E. coli* ATCC 25922) to adhere to the surface of the membranes

The impact of the antimicrobial effect of cinnamon oil within BC-Cinnamon oil samples was statistically analysed using one-way ANOVA and Dunnett's multiple comparisons tests and the results were considered statistically significant ($P < 0.05$). The BC tested in this study presented a moderate antibacterial activity and the BC-Cinnamon oil samples had a strong antimicrobial effect on the strains as seen in Fig. 9. These materials presented an inhibitory effect with at least 3 order CFU/mL compared to cinnamon oil control or BC control. *S. aureus* and *E.*

coli showed a higher sensibility to the action of the BC-Cinnamon oil samples (at all concentrations) and the BC-Cinnamon 5% highly/ strongly inhibited the adherence of the bacterial strains on the surface of the membranes. Orlando et al. [28] presented a similar study based on BC as a substrate for developing antibacterial wound dressings to facilitate the healing process.

4. Conclusions

In this study we aimed at loading the cinnamon essential oil in the bacterial cellulose membrane. Based on the results obtained, these membranes are able to absorb a large amount of water, a property useful for maintaining an optimal level of humidity for treating skin injuries. Scanning electron microscopy analysis revealed a three-dimensional structure the structure that helps encapsulate the CEO molecules within the BC membrane. By infrared analysis we demonstrated the presence of cinnamon oil in the structure of the BC membrane, by comparing the obtained spectra. The studied samples present also a strong antimicrobial effect on *S. aureus* ATCC 25923, *E. coli* ATCC 25922 stains. Looking to the future, biocompatibility assessments and antimicrobial testing of these membranes will be considered, and the proper content of the CEO will be optimized.

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