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Development and characterization of bacterial cellulose membrane incorporated with Witch hazel extract

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ABSTRACT

Bacterial cellulose (BC) has stood out in the biomedical field for its biocompatibility, non-toxicity and high liquid absorption capacity. Thus, studies have been conducted aiming at the functionalization of BC with substances that add properties, such as anti-inflammatory and antimicrobial action. *Hamamelis virginiana* plant extract is known for its astringent, anti-inflammatory and antimicrobial properties. Thus, the present work aimed to incorporate aqueous (AE) and glycolic (GE) extracts of witch hazel in different concentrations to BC, aiming at its application as a curative. BC membranes incorporated with the extracts were characterized by thermogravimetric analysis (TGA), Fourier transforms infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and antimicrobial activity. The TGA results indicated a reduction in thermal stability and the appearance of a new stage of degradation in the samples that underwent incorporation. Furthermore, the FTIR showed the presence of aromatic compounds not found in the BC, corroborating the TGA. The micrographs revealed that the incorporation of the extracts resulted in the formation of a film on the surface of the membranes, covering the nanofibers. These results show that incorporating the aqueous and glycol extracts in the BC membrane was successful. However, despite the crude extracts' antimicrobial potential, antimicrobial activity in the functionalized BC samples was not observed.

Keywords: Bacterial cellulose; Therapeutic plant; *Komagataeibacter hansenii*; *Hamamelis virginiana*.

1. INTRODUCTION

Cellulose ($C_6H_{10}O_5$) is the most abundant natural biopolymer in the world since it can be obtained from plants and synthesized by fungi and bacteria. Thus, interest in cellulose and its use is increasingly frequent due to its wide availability. Bacterial cellulose (BC), produced by different genera of bacteria, has the same chemical structure as vegetable cellulose. However, it has high purity due to the absence of hemicellulose, pectin, and lignin, in addition to an ultrafine nanofiber network structure, well organized and capable of retaining large amounts of water [1]. Furthermore, its biodegradability, biocompatibility and non-cytotoxicity make BC a versatile biomaterial of commercial interest [2].

BC membranes can be applied in several areas, such as cosmetics, bioremediation, filtration membrane, packaging, clothing, and dressings [2]. In the area of dressings, when comparing traditional dressings, such as gauze and sponges, with dressings based on BC, it appears that those produced with BC are easier and painless at the time of removal, not damaging the skin [3], moreover, due to its high water retention capacity, it allows a moist atmosphere at the wound site, which is critical in healing, while the absorbent properties allow the wound exudate to be removed from the site [4, 5]. Despite its many advantages, BC does not have antibacterial properties, which are crucial to prevent possible infections during wound healing [5–7].

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However, its microporous structure and large surface area allow the retention of many active compounds, this resource also directly influences the slow release of these compounds at the wound site, promoting a more lasting effect [8]. In addition, the presence of hydroxyls on its surface gives it high hydrophilicity [9], thus, it has a natural affinity for polar and water-soluble molecules, allowing the absorption of hydrophilic compounds. Therefore, several studies have been conducted to incorporate natural substances into BC that add specific properties according to the desired application to improve BC properties and increase them. For health-oriented applications, one can cite the use of herbal medicines, which, because they are natural, do not have as many side effects as other synthetic medicines and make the production cost cheaper [10, 11].

Plant extracts have been used for wound healing for many decades. Some medicinal plants such as *Curcuma longa (L.)*, *Terminalia arjuna* and *Aloe barbadensis* have proven healing activity and are effective in treating wounds [12]. In addition to the substances already evaluated, there is an infinity of plants with antimicrobial activity, such as *Hamamelis virginiana*.

Hamamelis virginiana or witch hazel, native to eastern North America, is an autumn flowering shrub. It is known for its astringent, antiseptic, antimicrobial, anti-inflammatory, and antioxidant properties [11], and its main applications are for treating hemorrhoids, superficial skin wounds and skin inflammations. Witch hazel extracts are obtained from leaves, branches, and houses, each of which has a specific therapeutic effect [13]. Its antimicrobial activity was tested by PEREIRA JÚNIOR *et al.* [14], proving that the hydroalcoholic extract of the plant has bacteriostatic properties from a concentration of 10% and bactericidal from 20%. The authors HUGHES-FORMELLA *et al*. [15] investigated the anti-inflammatory action of witch hazel extract. The research evaluated the effectiveness of an after-sun lotion with 10% witch hazel and compared it to other lotions without the extract. The test showed that the lotion containing witch hazel was more effective. Studies of extract properties are generally conducted using pure extract. Thus, associating witch hazel extract, which has proven excellent properties, with biomaterials is very promising.

NEVES *et al.* [16] successfully explored the association between plant extracts and BC membranes in his studies. In your studies, membranes incorporated with Barbatimão extract showed antimicrobial activity against *Escherichia coli* and *Pseudomonas aeruginosa*, microorganisms usually found in wounds. Thus, the combination of these materials has great potential, despite this, the incorporation of witch hazel extract with BC has been little explored.

Thus, considering that the 10% concentration of witch hazel in the reported works showed both anti-inflammatory and bacteriostatic action, the purpose of this work is to associate these properties with BC, which can incorporate natural substances, developing a biomaterial with potential for biomedical applications, such as dressings for treating inflammation and wounds. Like this, the present work aimed to develop bacterial cellulose membranes incorporated with aqueous (AE) and glycolic (GE) extracts of witch hazel. In addition to the 10% concentration of witch hazel, other concentrations were tested, including 20% for the glycolic extract, since this concentration showed bactericidal properties.

2. MATERIALS AND METHODS

Figure 1 presents the experimental design with the steps taken.

2.1. Synthesis of BC membranes

The BC membranes were synthesized by the bacteria *Komagataeibacter hansenii* (ATCC 23769), preserved in conical tubes with glycerol solution and maintained at a temperature of –20 °C. For cell reactivation and membrane production, a culture medium containing 5 g/L of yeast extract, 5 g/L of peptone and 20 g/L of mannitol, previously sterilized at 121 °C for 15 minutes, was used. The cells were reactivated in an Erlenmeyer flask containing 100 mL and incubated at a temperature of 30 °C for seven days. Subsequently, 10 mL of the inoculum was transferred to other Erlenmeyer flasks containing 40 mL of culture medium. Flasks were incubated at 30 °C for 11 days for BC membranes to grow.

2.2. Purification of BC membranes

The membranes were previously washed with distilled water to remove the residual culture medium and heated in a 0.1M NaOH solution at 80 °C for 1 hour to dissolve the bacterial cells. After the heat treatment, the membranes were submerged in distilled water and changed daily until reaching pH 7. Subsequently, the membranes were submerged in distilled water and sterilized at 121 °C for 15 min to be stored in the refrigerator until use.

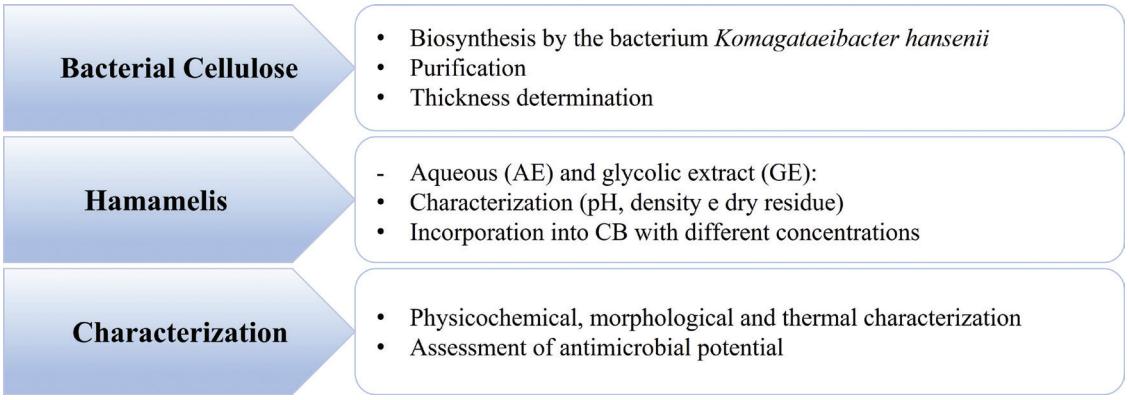

Figure 1: Flowchart representing the steps of the methodology.

2.3. The thickness of BC membranes

The thickness of each hydrated membrane was determined using a caliper. The measurement was taken at four points on the membrane, and based on these values, the arithmetic means the thickness of each membrane was obtained. The calculation for the final average was based on the average of all membranes.

2.4. Characterization of witch hazel extracts

The witch hazel extracts were characterized according to their color by visual analysis, determining the pH, density, and dry residue content. The glycolic extract (GE) was obtained from the manipulation pharmacy, and, for its production, propylene glycol was used as a solvent. The aqueous extract (AE) was purchased from the company M.A.S Nunes Essências.

2.4.1. Determination of pH

The determination was performed with 5 mL samples in duplicate.

2.4.2. Density of witch hazel extracts

Density was determined from 5 mL samples in duplicate. The volume was measured using a 10 mL cylinder, and the mass was on an analytical balance. The extract´s mass was determined through the difference between the mass of the test tube with 5 mL of extract and the empty test tube. Then, the density was determined by dividing the extract´s mass by the volume.

2.4.3. Dry residue content

The methodology described in the Brazilian Pharmacopoeia [17] was used to determine the dry residue content. With an automatic pipette, 2 mL of the extract was transferred to a Petri dish, measuring approximately 5 cm in diameter and 2 cm in height. The initial mass was determined using an analytical balance. The sample was evaporated to dryness in a water bath. Then, it was dried in an oven at 100 °C for 3 h and stored in a desiccator to cool. The mass was measured again on the analytical balance. The amount of dry residue was calculated by the difference between the initial and final mass, and the residue content was calculated in percentage m/v.

2.5. Incorporation of witch hazel extracts

The plant extracts were incorporated by ex situ technique, in which the membranes were immersed in the extract and left for 24 h, being turned over every 2 h. Furthermore, to enable the adsorption of extracts on the surface of BC membranes, these were subjected to a mechanical process to remove excess water. Incorporation was carried out with glycolic and aqueous extracts of witch hazel. After incorporation, the membranes were dried in an oven at 30 °C for 24 h and stored in a desiccator.

2.5.1. Test with different concentrations of witch hazel extracts

In order to determine which concentration of witch hazel extract would be necessary to inhibit microbial growth, concentrations of 2.5, 5 and 10% (v/v) were used for the aqueous extract (AE) and 2.5, 5, 10 and 20% (v/v) for

glycolic (GE). The incorporation at 20% (v/v) was not carried out with the aqueous extract because this was purchased with an initial concentration of 10% (v/v), while the glycol was purchased pure.

2.6. Characterization

The pure BC membranes and incorporated with witch hazel extracts were characterized by thermogravimetric analysis (TGA), Fourier transforms infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and antimicrobial activity.

2.6.1. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was carried out to confirm the incorporation of the extracts and to evaluate their influence on the thermal stability of the BC. The samples were heated from 25 to 500 °C at 10 °C/min under an inert N_2 atmosphere. The analysis was carried out in TA Instruments equipment, model TGA-Q50. To adjust the parameters and graphically represent the results, the TA - Universal Analysis software was used.

2.6.2. Fourier Transform Infrared Spectroscopy (FTIR)

The spectroscopy analysis in the infrared region was performed to identify the membranes' functional groups and determine their chemical composition. FTIR analysis was also performed on pure extracts to identify the presence and verify its incorporation into the membrane. The analysis was performed on Perkin Elmer Frontier equipment. 32 scans were performed per sample, from 500 to 4000 cm^{-1}, in attenuated total reflectance (ATR) mode.

2.6.3. Scanning Electron Microscopy (SEM-FEG)

Scanning electron microscopy analysis was performed to evaluate the surface characteristics of the membrane and detect the presence of the extract. The membranes were fixed on metallic support, covered with carbon, and observed in the Zeiss MEV-FEG DSM 940A microscope with 5 kV of accelerating voltage.

2.6.4. Antimicrobial activity

Based on the Kirby-Bauer method [18], the membranes were submitted to the Antimicrobial Sensitivity Test. The technique consists of inoculating a standardized amount of bacteria of the same species on a Petri dish containing Müller-Hinton agar. For the assay, the gram-negative bacterium *E.coli* (ATCC 8739), the gram-positive *S. aureus* (ATCC 25923) and the mild-durable *Candida albicans* (ATCC 10231). Each microorganism was inoculated separately in each plate and the membranes, previously sterilized and with a diameter of 6 mm, were distributed over the plate. The plates were stored in an oven at 30 °C for 24 hours for later evaluation of the formation of inhibition halos, measured with a caliper.

3. RESULTS AND DISCUSSION

3.1. Synthesis of BC membranes

The membranes obtained had an average thickness of 2.5 mm (± 0.17) and an average mass of 9.03 g (± 0.289). Before purification, a yellowish color was observed in the membranes due to the medium in which they were grown. After purification, the color became less intense as the soaking water was changed. Upon reaching pH 7, the purification process was completed, and the membrane showed a whitish color.

Figure 2 shows the appearance of the membrane before and after the purification process. Figure 2a shows the membrane after 11 days of cultivation, and in Figure 2b, the membrane underwent heat treatment and the purification process with water until it reached pH 7.

3.2. Characterization of witch hazel extracts

Visual analysis shows that the glycolic extract has an intense brown color (Figure 3a). In contrast, the aqueous one has a soft yellow color (Figure 3b), suggesting differences in its composition. Both extracts had a pH close to 4.5 and a density of 1.055 kg/L (± 0.077) for the glycolic extract (GE) and 1.040 kg/L (± 0.028) for the aqueous extract (AE).

It is known that the polarity and molecular structure of the solvent affects the type of compound that can be extracted, as well as the amount [19, 20]. This is because medicinal plants are composed of several substances

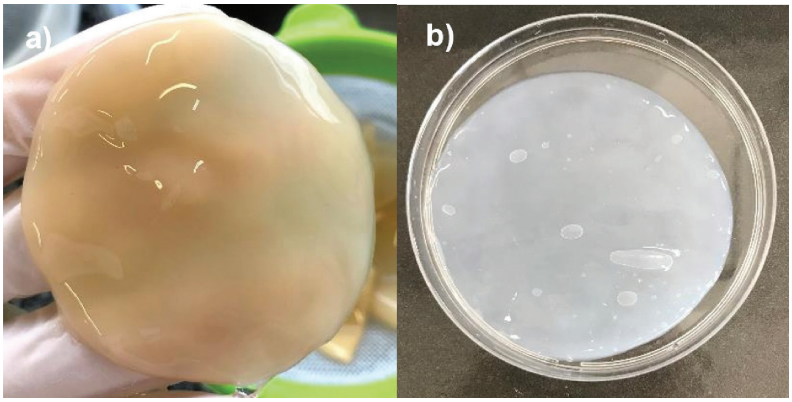

Figure 2: Bacterial cellulose membranes a) before and b) after purification.

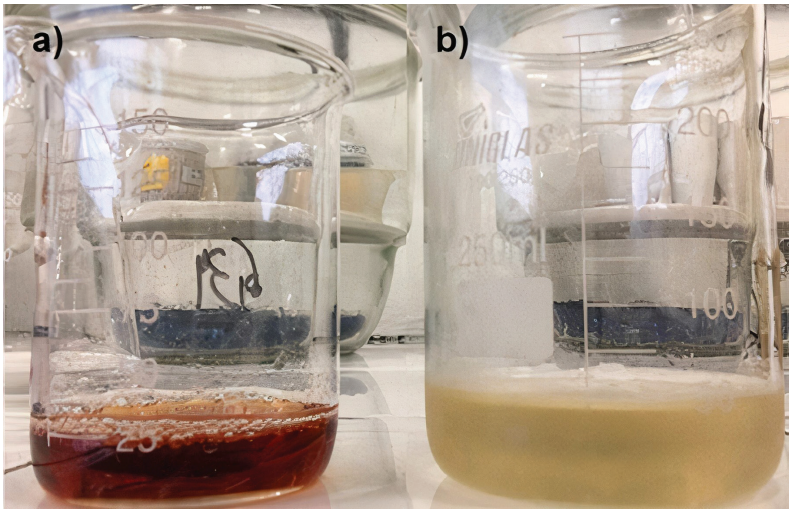

Figure 3: The appearance of the a) glycolic and b) aqueous extracts used.

that present structural and polarity differences. Consequently, each substance has a different affinity with each type of solvent and the greater the affinity, the greater the concentration of this substance in the extract. Thus, polar solvents extract phenolic compounds, their glycosides, and saponins, while non-polar solvents extract fatty acids and steroids [20]. There is no solvent capable of solubilizing all the plant's active compounds [19]. For this reason, the incorporation and bactericidal potential of the two different extracts were tested.

The dry residue content test was carried out to determine the amount of witch hazel substances that became soluble by the extraction liquid. The analysis also allows the evaluation of the extraction potential of the extracting liquid [21]. The experiment was performed in duplicate, and the results showed a residue content of 4.38% m/v (± 0.005) for the aqueous extract and 2.75% m/v (± 0.003) for the glycolic extract. The test was performed with the original samples without dilution. It is observed that, despite the glycolic extract being more concentrated, the aqueous extract had a higher residue content, suggesting that, compared to propylene glycol, water as a solvent can extract a greater number of substances contained in this plant species. However, the extraction method and the part of the plant must also be considered so that it can be stated with certainty that extraction with water is more efficient. However, the details of the manufacturing process of the extracts were not made available by the providers.

3.3. Incorporation of witch hazel extracts

After incorporation, there was a change in the tonality of the membranes (Figure 4), and the higher the concentration of extracts, the more intense the color of the membrane. Furthermore, the remaining liquid in the glassware after incorporation showed a light color, almost transparent, proving that the extract was incorporated into the membrane.

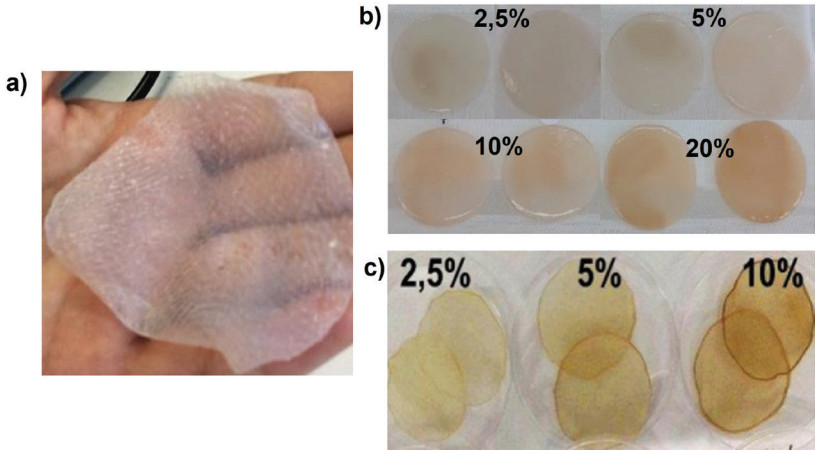

Figure 4: a) Pure BC membranes after drying and b) after incorporation and drying with extract glycolic and c) aqueous extract.

3.4. Characterization

3.4.1. Thermogravimetric Analysis (TGA)

Figure 5 shows the TG and DTG curves for pure BC and BC incorporated with an aqueous extract (Figures 5a and b) or glycolic extract (Figures 5c and d) of witch hazel at different concentrations.

The degradation profile of pure BC is marked by the occurrence of three stages of mass loss, indicated by the gray arrows in Figure 5. The first stage occurs from 30 °C to 150 °C and corresponds to the loss by evaporation of adsorbed water [22]. Although the membranes are dry, the hygroscopic character of BC causes the water in the structure to remain in the sample. The second stage is characterized by an accentuated loss of mass, corresponding to the degradation of cellulose, which includes depolymerization, decomposition of glucose units and dehydration [23]. This stage was marked by an extrapolated starting temperature of degradation ($T_{onset\ 3}$) of 333 °C and maximum degradation temperature ($T_{peak\ 3}$) of 356 °C, demonstrating that the biopolymer has good thermal stability. The third stage presented a mass loss of 16.35% at around 643 °C, referring to the degradation of carbonaceous residues [24], with no residue at the end of the analysis. The results obtained for the pure and incorporated sample are presented in Table 1.

For membranes that underwent incorporation with an aqueous extract (BC/AE) (Figures 5a and 5b), in addition to the profile observed in pure BC, there was the appearance of a new mass loss event, indicated by the red arrow, with maximum degradation temperature ($T_{peak\ 2}$) around 207 °C. Because it occurs at an intermediate temperature between water evaporation and cellulose degradation, the event was attributed to the degradation of the incorporated extract. Other observed changes were the reduction of the thermal stability of the membrane, reaching a difference of 20 °C for BC/AE 10%, and the presence of residue for all samples of BC/AE. The presence of residues can be explained due to the analysis methodology, which used a nitrogen atmosphere [25]. As it is inert, nitrogen prevents the complete oxidation of the organic matter present.

BC membranes with added glycolic extract (BC/GE) also showed four stages of mass loss, except for the most concentrated sample (BC/GE20%), which showed only three, since it was not possible to separate the first stage degradation attributed to evaporation loss of adsorbed water second stage attributed to extract degradation. However, the first stage of degradation of BC/GE20% showed a percentage of mass loss of 36%, higher than the others. Thus, as the membrane had been dried before analysis, it can be inferred that the loss of 36% of the total mass is not due to water evaporation alone, suggesting the presence of other compounds, such as the extract. Furthermore, this event occurred at a maximum temperature ($T_{peak\ 2}$) of 119 °C, close to the degradation temperature of the extracts in the other samples.

The new stage of mass loss, related to the glycolic extract, presented a maximum degradation temperature ($T_{peak\ 2}$) between 112 and 159 °C. When comparing with the BC/AE membranes, it can be noted that the degradation of the glycolic extract occurred at a lower temperature, reaching a difference of 94 °C. The incorporation of GE also affected the thermal stability of the BC membrane, reducing the cellulose degradation temperature by up to 21 °C. In addition, the samples also showed residues at the end of the analysis, attributed to the incomplete degradation of organic matter.

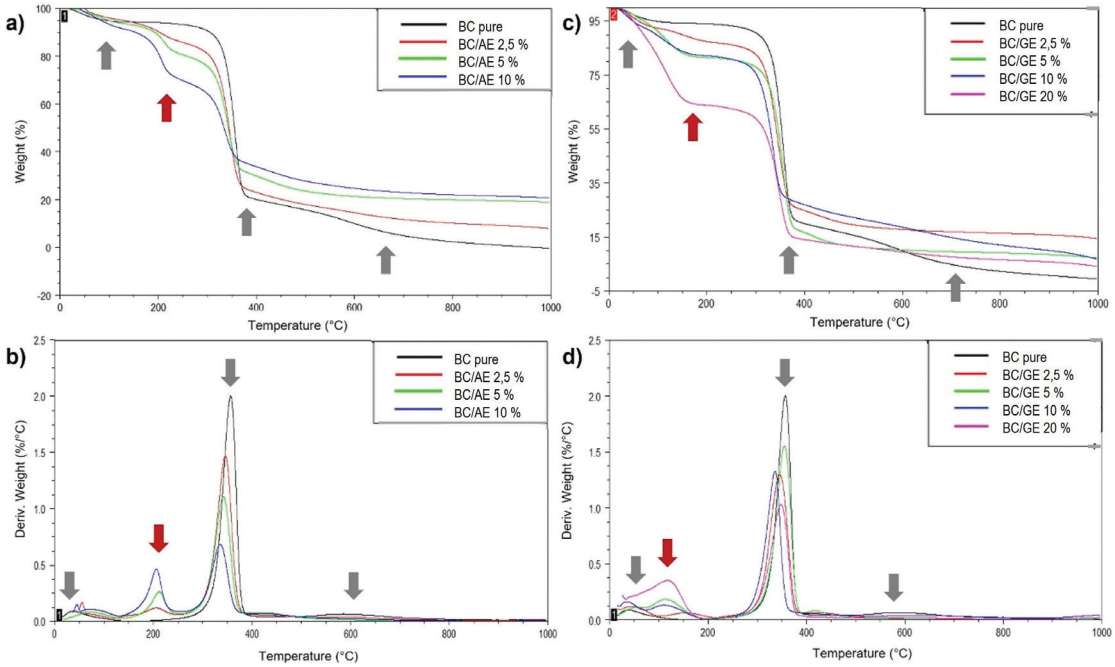

Figure 5: TG curves for pure BC samples and a) BC incorporated with different concentrations of witch hazel aqueous extract; b) DTG curve for BC incorporated with different concentrations of aqueous extract of witch hazel; c) TG curve for BC incorporated with different concentrations of witch hazel glycolic extract; d) DTG curve for BC incorporated with different concentrations of witch hazel glycolic extract.

Table 1: Data obtained from the TGA analysis for membranes of pure BC and incorporated with aqueous or glycol extract of witch hazel.

SAMPLES	MASS LOSS 1 (%)	WEIGHT LOSS 2 (%)	$T_{peak\ 2}$ (°C)	$T_{onset\ 3}$ (°C)	WEIGHT LOSS 3 (%)	$T_{peak\ 3}$ (°C)	$T_{onset\ 4}$ (°C)	WEIGHT LOSS 4 (%)	RESIDUE (%)
BC	5.75	–	–	333	72.99	356	643	16.35	0
BC/AE 2.5%	4.71	9.52	204	323	61.01	346	497	13.05	7.94
BC/AE 5%	6.56	12.88	211	319	48.56	342	504	11.58	18.95
BC/AE 10%	8.72	21.58	206	313	33.59	335	510	13.13	20.69
BC/GE 2.5%	7.63	5.4	159	319	60.71	345	495	8.86	2.48
BC/GE 5%	5.2	13.32	117	330	63.49	355	475	8.3	7.36
BC/GE 10%	7.41	10.39	112	311	52.9	336	489	14.35	7.02
BC/GE 20%	36.16		119	319	48.84	348	495	7.26	4.28

3.4.2. Fourier Transform Infrared Spectroscopy (FTIR)

Figure 6 presents the infrared absorption spectroscopy (FTIR) of BC samples and pure liquid glycolic (GE) and aqueous (AE) extracts.

The FTIR spectrum of the pure BC membrane presents a profile characteristic of that presented in the literature [26]. It is known that the BC membrane is formed from glucose molecules joined by glycosidic bonds and intramolecular and intermolecular hydrogen bonds. Therefore, it is expected to find bonds between carbon and hydrogen of aliphatic, carbon and oxygen, and carbon and OH.

The prominent bands identified in the FTIR spectrum for the pure membrane were at 3343 cm^{-1} (1.A), referring to stretching of the hydroxyl group, at 2910 cm^{-1}, related to CH stretching and asymmetric CH_2 stretching of aliphatic, at 1640 cm^{-1} (1.C), associated with OH deformation, at 1424 cm^{-1} (1.D) referring to CH_2, deformation, angular deformation in the OH plane at 1335 cm^{-1} (1.D) and 1052 cm^{-1} (1.E), referring to the symmetrical stretching Us(C-O)/(C-C) [27–29]. It can be attested that the sample analyzed was bacterial cellulose since the spectrum showed the characteristic bonds of this biomaterial.

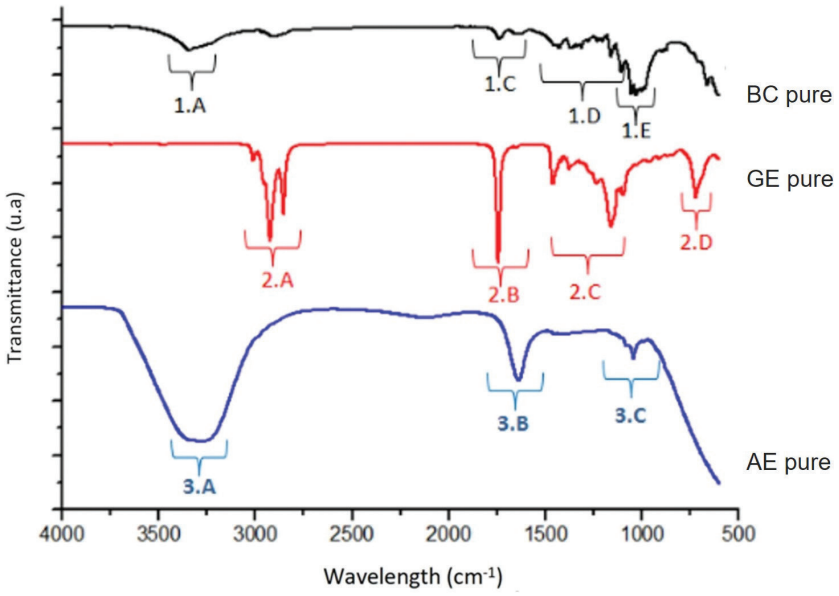

Figure 6: FTIR spectra were obtained for pure BC membrane and glycolic and aqueous extracts.

In order to understand the spectrum presented by the pure extracts and confirm their presence in the membranes, it is necessary to observe their composition. Plant extracts are a mixture of different substances, from polyphenols to essential oils. Generally, the compounds found in witch hazel extracts have aromatic rings and some radicals such as aldehydes, ketones and esters. Thus, it is expected to find in the spectrum characteristic bands of bonds between C-H of aromatics, C=O, C=C and C-OH bonds.

The profile obtained for the pure aqueous extract of witch hazel (AE) (Figure 6) shows three characteristic bands. The first band (3.A), at 3300 cm^{-1}, is attributed to the axial deformation of O-H in intermolecular hydrogen bonding [30]. The band is wide and strong due to the presence of water, which was used as a solvent in the preparation of the extract. The second band is presented at 1638 cm^{-1} (3.B), and may be related to the C=C stretching of the aromatic group [30, 31]. The third (3.C), at 1046 cm^{-1}, may be related to C-O axial deformation vibrations [30, 32].

The pure witch hazel glycolic extract (GE), Figure 6, presents four expressive regions in its profile: the first region, represented by 2.A, corresponds to the axial deformation of C-H and presents a band at 3002 cm^{-1}, referring to the deformation in aromatic rings, another in 2923 cm^{-1} symmetrical deformation of CH_3 in aliphatics and another in 2854 cm^{-1}, referring to asymmetrical deformation of CH_2. In the second region (2.B), there is an intense band at 1744 cm^{-1}, which may be related to the axial deformation of C=O. Region 2.C presents two more evident bands, one at 1464 cm^{-1} attributed to angular deformation of C-H, and another at 1159 cm^{-1}, which may be associated with deformation of CH in the plane of aromatic rings, and region 2.D shows a band located at 722 cm^{-1}, referring to the asymmetric angular deformation in the methylene plane [30].

Despite the extracts being produced from plants of the same species, the spectra did not show similar behavior, showing differences in their composition. This result corroborates the visual analysis previously discussed and demonstrates that the composition of the extract can change due to changes in variables such as solvent, part of the plant used, and extraction method, among other factors.

Figure 7 presents the spectrum of membranes functionalized with an aqueous extract (a) and glycolic extract (b) at different concentrations.

The spectra of the incorporated samples showed characteristic bands for pure BC and pure extracts.

For samples with AE, the presence of the extract is confirmed by the band at 1641 cm^{-1}, attributed to the C=C binding of aromatics [32]. Furthermore, the bands close to 3300 cm^{-1}, 2900 cm^{-1} and 1000 cm^{-1} showed greater intensity with the addition of the aqueous extract. According to PICCHI [33] the band's broadening at 3300 cm^{-1}, referring to the C-OH bond, may be related to forming a hydrogen bond between witch hazel and cellulose.

The samples incorporated with glycolic extract also showed more intense bands, at 3345 cm^{-1}, 2970 cm^{-1} and 1032 cm^{-1}. The spectrum also showed the presence of a discrete band at 3002 cm^{-1}, referring to the binding

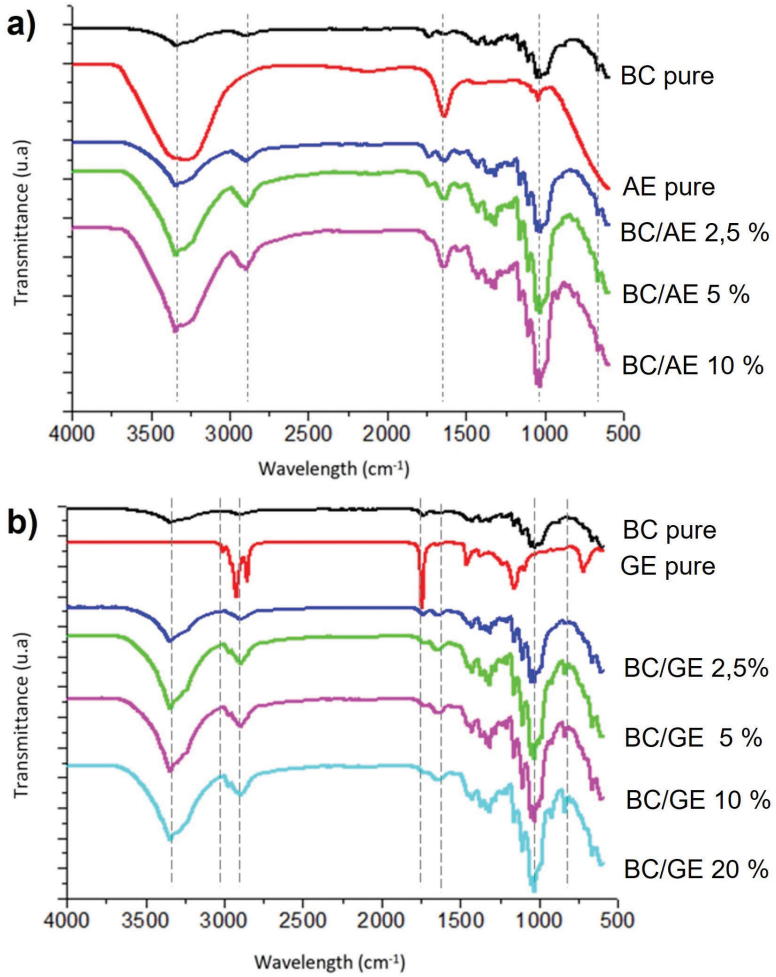

Figure 7: FTIR spectra were obtained for the pure membrane and the membranes incorporated with a) aqueous extract and b) witch hazel glycolic extract in different concentrations.

Table 2: Data from FTIR analysis for BC and BC membranes incorporated with aqueous and glycol extracts of witch hazel.

TYPE OF BONDS	NO OF CHARACTERISTIC WAVE	LITERATURE	BC/AE	BC/GE
υ(O-H) cellulose I (3-OH…O-5)	3343	[27]	3345	3345
U(CH)	2968	[30]	–	2970
υ(CH); υa(CH_2)	2910	[29]	2895	2894
δ(C=C)	1640	[32]	1641	1641
δ(C=C)	1540	[30]	1539	–
δa C=C of the ring	1455	[30]	–	1455
δ(HCH, OCH) on the plane	1424	[28]	1429	1428
δ(OH) on the plan	1335	[28]	1336	1337
Ua(C-O-C) C-2…O-2	1110	[30]	1108	1108
Us(C-O)/C-C	1052	[27]	1054	1054
δ(C-O) of alcohols	1039–1023	[30, 31]	–	1032
δ(C-H) of isopropyl	922–919	[30]	–	921
δ(CH_2) on the plan	900–700	[26]	–	838
δ(C-O-H), off plan	662	[26]	663	663

U = stretch; Us = symmetric stretching; Ua = asymmetric stretching; δ = angular strain.

of CH to aromatics, confirmed by the presence of a C=C bond at 1455 cm^{-1}, in addition to the discrete band at 1744 cm^{-1}, attributed to the C=O bond. The other bands shown in the spectra are shown in Table 2.

3.4.3. Scanning Electron Microscopy (SEM)

The micrographs of the membrane surface of pure BC and functionalized with GE and AE are shown in Figures 8 and 9.

The pure BC membrane (Figure 8a) and the other membranes incorporated with extract showed a network of nanofibers with an average diameter of 60 nm randomly distributed, characteristic morphology of BC. It is also possible to notice that the functionalized membranes presented a denser matrix, making the nanofibers less visible. The adsorption of the extract on the membrane has created a kind of film on its surface, filling the pores and covering the BC nanofibers. This behavior was also observed by PICCHI [33] in his studies involving the incorporation of propolis into the BC membrane.

BC samples incorporated with glycolic extract also showed a network of randomly distributed nanofibers with an average diameter of 61 nm. Unlike membranes with aqueous extract, increasing the concentration did not change the morphology of the membranes, except for the sample functionalized with 20% extract (Figure 9e). The nanofibers were also recoated in this membrane by the glycolic extract, confirming its adsorption to the membrane. It is essential to consider that microscopy analysis is performed on a small sample fraction and that the membrane morphology may not be uniform throughout its length. Furthermore, as shown in Figure 4, it is assumed that the extract was not uniformly incorporated into the BC, as it is possible to perceive regions of more intense color in the membranes.

The interaction between the compounds and BC can occur physically by adhesion or chemically through hydrogen bonds between the hydroxyl groups of the BC chains with the added materials [34, 35]. Studies

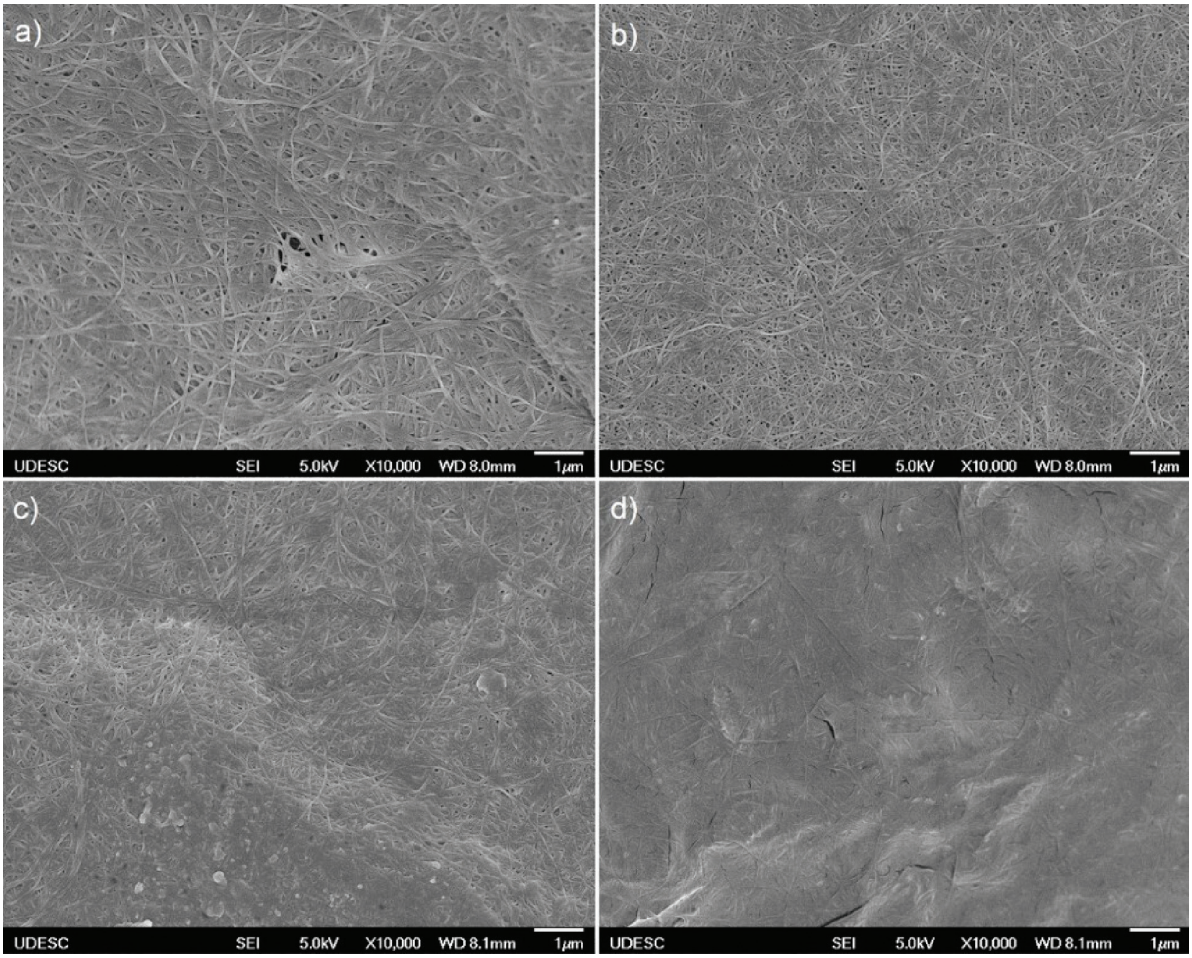

Figure 8: Surface electron microscopy of a) pure BC and BC functionalized with aqueous extract at concentrations of b) 2.5%, c) 5%, and d) 10%.

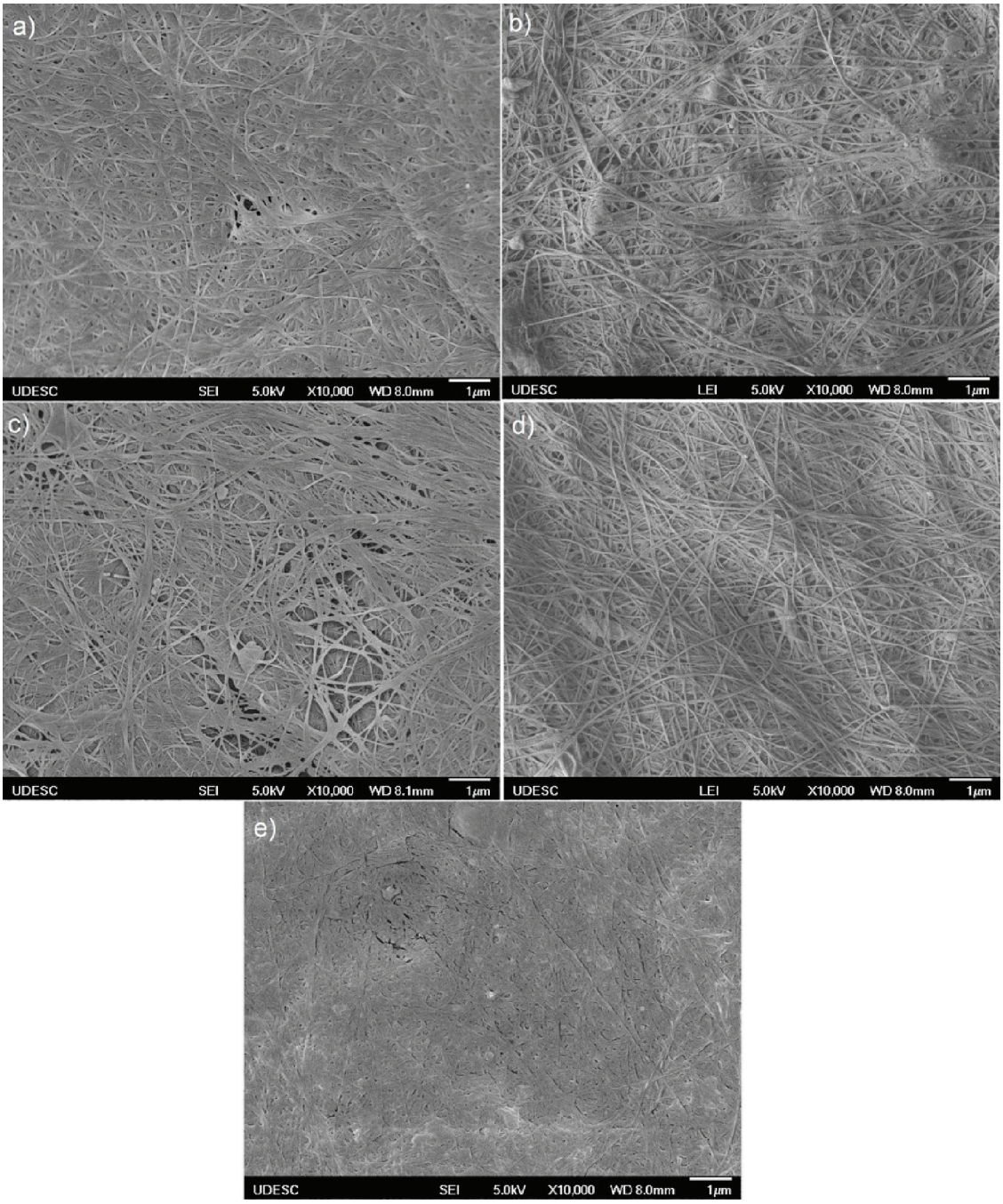

Figure 9: Surface electron microscopy of a) pure BC and BC functionalized with glycolic extract at concentrations of b) 2.5%, c) 5%, d) 10% and e) 20%.

performed by JAHED *et al.* [36] incorporated essential oil into chitosan and found through SEM that the incorporation of oil resulted in a more homogeneous film than the control. In addition, the possible formation of hydrogen bonds between the hydroxyl group of chitosan and the hydroxyl groups of the oil caused a decrease in the porosity and roughness of the film. This behavior was observed for the membranes incorporated with EA and the membrane incorporated with 20% GE, in which there was a decrease in the pores, suggesting that chemical interactions between the hydroxyls of the solvents and the BC may have occurred. On the other hand, membranes with lower concentrations of GE did not show many changes in their morphology, suggesting that chemical adhesion did not occur. However, again there is the issue of non-uniformity of extract incorporation.

Another point is that when using polar solvents such as water, more carbohydrates and phenolic compounds will be present in the extract [37]. In contrast, solvents such as glycol promote greater yields in extracting

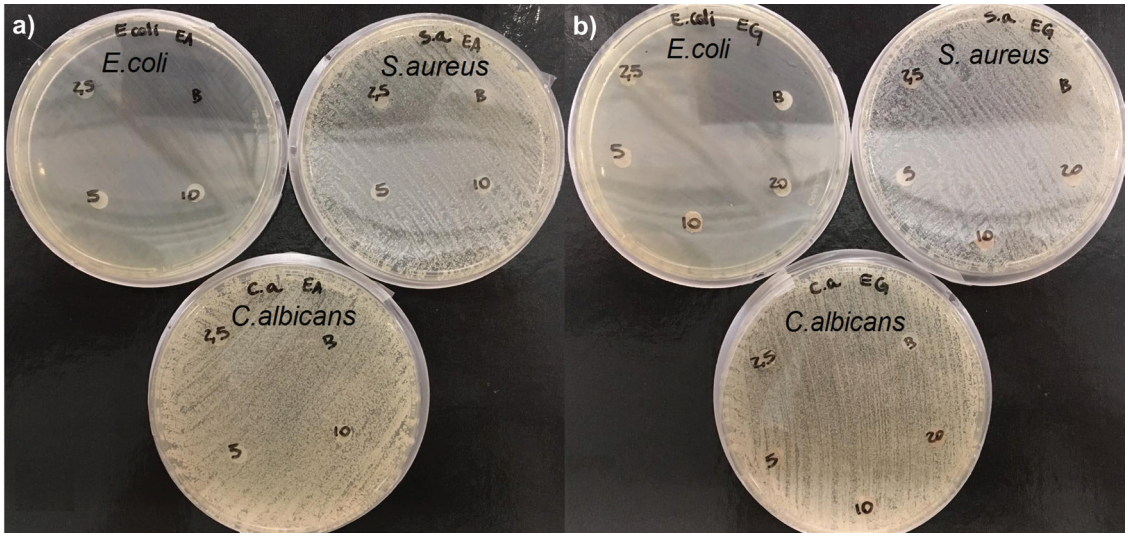

Figure 10: Antibiogram results of samples with a) BC/AE and b) BC/GE for all concentrations.

substances such as tannins and flavonoids [38]. Thus, the two extracts have the potential to form hydrogen bonds with the membrane. However, the interaction between them depends on several factors, mainly on the composition and concentration of these compounds in the extracts, which determine the interaction between the BC hydroxyls and the extract.

3.4.4. Antimicrobial activity

Figure 10 presents the results obtained for the membranes of BC/AE (a) and BC/GE(b) at different concentrations.

Despite the antimicrobial potential of the extract having been proven in previous studies carried out by PEREIRA *et al*. [14] and the witch hazel extract having significant antimicrobial activity against fungi [39], none of the samples provided halos of inhibition against the tested microorganisms, confirming no significant antimicrobial activity.

The plant is known to be composed of substances such as tannins, essential oils and phenolic compounds that have an inhibitory effect on microorganisms [39]. However, the extracts' composition and quality depend on several factors, such as the production method, the solvent used, and the part of the plant used. As there is no information on the extracts' composition, it is assumed that the concentration of substances with antimicrobial properties could be lower than necessary.

In addition, previous research has only evaluated the antimicrobial potential of the liquid extract without being incorporated into a support material, such as the BC membrane. The adsorption of the extract on the surface of the membrane may have interfered with the diffusion of the active ingredient in the culture medium, contacting the microorganism difficult. NASCIMENTO *et al.* [40] demonstrated that the diffusion in the medium could be influenced by the physical-chemical properties of the components, impacting the result obtained.

It is also worth noting that the concentration of the active principle, or the component responsible for the bactericidal action, directly influences its efficiency. Deficient concentrations of the active ingredient may not be effective, as in the analysis presented by PEREIRA JÚNIOR *et al*. [14], in which the 10% extract showed only bacteriostatic activity and, from 20% onwards, showed bactericidal activity. Although the concentration of the initial extract solution for incorporation is known, the exact amount of extract incorporated is unknown.

4. CONCLUSIONS

The application potential of BC and witch hazel extract is enormous, and there is still much to be researched. As expected, the membrane easily incorporated both extracts employed, resulting in a change in its color.

The thermogravimetric analysis corroborated the visual analysis, confirming the presence of extract in the membrane, as it presented a change in its degradation profile and residues at the end of the analysis. Furthermore, it was found that the incorporation of the extracts caused a decrease in the thermal stability of the BC, with variations of up to 22 °C. However, this decrease would not affect its application.

The spectrum generated by FTIR indicated the presence of characteristic bands of BC, both in the pure sample and in the functionalized samples, which added to the profile presented by the pure extracts. These membranes showed more pronounced peaks for higher extract concentrations due to the increased amount of chemical bonds characteristic of each wavelength.

Membranes functionalized with aqueous and glycolic extract of *Hamamelis virginiana* did not show antimicrobial activity against the microorganisms tested, although some active principles present in the plant show such properties. A possibility would be the low concentration of these active principles, dilution of the extract during incorporation or difficulty diffusing them in the culture medium.

This work is an initial study about incorporating different witch hazel extracts in BC membranes to develop a dressing with antimicrobial potential. The incorporation was successful, as shown by the FTIR, TGA and SEM analyses. On the other hand, as the membrane did not show antimicrobial activity, the following steps, considering its application as dressings, it is essential to test higher concentrations of extract since dilution may occur during incorporation and the extract will be diffused into the medium, in the case of injury, slowly and in smaller proportions. In addition, extracts have been widely studied pure, but only some studies have been conducted with them incorporated into other materials. Thus, despite having studied concentrations that have already proven bactericidal and bacteriostatic effects, it did not show these effects when incorporated into BC. Thus, it is interesting to study the desorption kinetics in a simulated environment, in addition to characterizing the extracts by chromatography, in order to identify the compounds present.

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6. BIBLIOGRAPHY

[1] AMORIM, J.D.P., SOUZA, K.C., DUARTE, C.R., *et al*., "Plant and bacterial nanocellulose: production, properties and applications in medicine, food, cosmetics, electronics and engineering: a review", *Environmental Chemistry Letters*, v. 18, n. 3, pp. 851–869, Mar. 2020. doi: http://dx.doi.org/10.1007/s10311-020-00989-9

[2] GREGORY, D.A., TRIPATHI, L., FRICKER, A.T.R., *et al*., "Bacterial cellulose: a smart biomaterial with diverse applications", *Materials Science and Engineering R Reports*, v. 145, pp. 100623, Jul. 2021. doi: http://dx.doi.org/10.1016/j.mser.2021.100623

[3] ZHOU, C., YANG, Z., XUN, X., *et al*., "De novo strategy with engineering a multifunctional bacterial cellulose-based dressing for rapid healing of infected wounds", *Bioactive Materials*, v. 13, pp. 212–222, Jul. 2021. doi: http://dx.doi.org/10.1016/j.bioactmat.2021.10.043. PubMed PMID: 35224303.

[4] SWINGLER, S., GUPTA, A., GIBSON, H., *et al*., "Recent advances and applications of bacterial cellulose in biomedicine", *Polymers*, v. 13, n. 3, pp. 1–29, Jan. 2021. doi: http://dx.doi.org/10.3390/polym13030412. PubMed PMID: 33525406.

[5] FAISUL ARIS, F.A., MOHD FAUZI, F.N.A., TONG, W.Y., *et al*., "Interaction of silver sulfadiazine wıth bacterial cellulose via ex-situ modification method as an alternative diabetic wound healing", *Biocatalysis and Agricultural Biotechnology*, v. 21, pp. 101332, Sep. 2019. doi: http://dx.doi.org/10.1016/j.bcab.2019.101332

[6] ŻYWICKA, A., FIJAŁKOWSKI, K., JUNKA, A.F., *et al*., "Modification of bacterial cellulose with quaternary ammonium compounds based on fatty acids and amino acids and the effect on antimicrobial activity", *Biomacromolecules*, v. 19, n. 5, pp. 1528–1538, May. 2018. doi: http://dx.doi.org/10.1021/acs.biomac.8b00183. PubMed PMID: 29579391.

[7] DI, Z., SHI, Z., ULLAH, M.W., *et al*., "A transparent wound dressing based on bacterial cellulose whisker and poly(2-hydroxyethyl methacrylate)", *International Journal of Biological Macromolecules*, v. 105, n. 1, pp. 638–644, Dec. 2017. doi: http://dx.doi.org/10.1016/j.ijbiomac.2017.07.075. PubMed PMID: 28716748.

[8] SWINGLER, S., GUPTA, A., GIBSON, H., *et al*., "Recent advances and applications of bacterial cellulose in biomedicine", *Polymers*, v. 13, n. 3, pp. 412, Jan. 2021. doi: http://dx.doi.org/10.3390/polym13030412. PubMed PMID: 33525406.

[9] ESA, F., TASIRIN, S.M., RAHMAN, N.A., "Overview of bacterial cellulose production and application", *Agriculture and Agricultural Science Procedia*, v. 2, pp. 113–119, 2014. doi: http://dx.doi.org/10.1016/j.aaspro.2014.11.017

[10] WOLFF, H.H., KIESER, M., "Hamamelis in children with skin disorders and skin injuries: results of an observational study", *European Journal of Pediatrics*, v. 166, n. 9, pp. 943–948, Sep. 2007. doi: http://dx.doi.org/10.1007/s00431-006-0363-1. PubMed PMID: 17177071.

[11] AMÊNDOLA, I., "*Avaliação da atividade antimicrobiana e anti-inflamatória do extrato glicólico de Hamamelis virginiana Linnaeus*", M.Sc. Thesis, UNESP, São José dos Campos, São Paulo, 2015.

[12] SHARMA, A., KHANNA, S., KAUR, G., *et al.*, "Medicinal plants and their components for wound healing applications", *Future Journal of Pharmaceutical Sciences,* v. 7, n. 1, pp. 1–13, Feb. 2021.

[13] ABBAS, T.F., ABBAS, M.F., LAFTA, A.J., "Antibacterial activity and medical properties of Witch Hazel Hamamelis virginiana", *Annals of Tropical Medicine and Public Health*, v. 23, n. 11, pp. 46, Jul. 2020. http://dx.doi.org/10.36295/ASRO.2020.231146

[14] PEREIRA JÚNIOR, C.L., MÁXIMO, H.J., VARZONE, J.R.M., "Perfil antimicrobiano de extratos hidroalcoólico de plantas nativas no controle de bactérias patogênicas", In: *15° Congresso Nacional de Iniciação Científica*, Ribeirão Preto, Brasil, 2015.

[15] HUGHES-FORMELLA, B.J., BOHNSACK, K., RIPPKE, F., *et al*., "Anti-inflammatory effect of hamamelis lotion in a UVB erythema test", *Dermatology (Basel, Switzerland)*, v. 196, n. 3, pp. 316–322, 1998. doi: http://dx.doi.org/10.1159/000017904. PubMed PMID: 9621139.

[16] NEVES, E.Z., KUMINECK JUNIOR, S.R., KATRUCHA, G.P., *et al*., "Development of bacterial cellulose membranes incorporated with plant extracts", *Macromolecular Symposia*, v. 406, n. 1, pp. 2200031, 2022. doi: http://dx.doi.org/10.1002/masy.202200031

[17] AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, *Farmacopeia Brasileira*, 5 ed., Brasília, ANVISA, 2010.

[18] BAUER, A.W., KIRBY, W.M., SHERRIS, J.C., *et al*., "Antibiotic susceptibility testing by a standardized single disk method", *American Journal of Clinical Pathology*, v. 45, n. 4, pp. 493–496, Apr. 1966. doi: http://dx.doi.org/10.1093/ajcp/45.4_ts.493. PubMed PMID: 5325707.

[19] CHUO, S.C., NASIR, H.M., MOHD-SETAPAR, S.H., *et al*., "A glimpse into the extraction methods of active compounds from plants", *Critical Reviews in Analytical Chemistry*, v. 52, n. 4, pp. 667–696, Sep. 2022. doi: http://dx.doi.org/10.1080/10408347.2020.1820851. PubMed PMID: 32954795.

[20] DIRAR, A.I., ALSAADI, D.H.M., WADA, M., *et al*., "Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants", *South African Journal of Botany*, v. 120, pp. 261–267, Jan. 2019. doi: http://dx.doi.org/10.1016/j.sajb.2018.07.003

[21] BORRELA, J.C., CARVALHO, D.M.A., "Avaliação comparativa da qualidade de extratos de Calendula officinalis L. (Asteraceae) comercializados em farmácias de manipulação em Ribeirão Preto-SP", *Revista Brasileira de Farmácia*, n. 92, pp. 13–18, 2011.

[22] LIMA, L.R., SANTOS, D.B., SANTOS, M.V., *et al*., "Nanocristais de celulose a partir de celulose bacteriana", *Quimica Nova*, v. 38, n. 9, pp. 1140–1147, Nov. 2015.

[23] COSTA, A.F.S., ALMEIDA, F.C.G., VINHAS, G.M., *et al*., "Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources", *Frontiers in Microbiology*, v. 8, pp. 2027, Oct. 2017. doi: http://dx.doi.org/10.3389/fmicb.2017.02027. PubMed PMID: 29089941.

[24] ARAÚJO JÚNIOR, A.M., BRAIDO, G., SASKA, S., *et al*., "Regenerated cellulose scaffolds: preparation, characterization and toxicological evaluation", *Carbohydrate Polymers*, v. 136, pp. 892–898, Jan. 2016. doi: http://dx.doi.org/10.1016/j.carbpol.2015.09.066. PubMed PMID: 26572426.

[25] PINTO, P., BARUD, H.S., POLITO, W.L., *et al*., "Preparation and characterization of the bacterial cellulose/polyurethane nanocomposites", *Journal of Thermal Analysis and Calorimetry*, v. 114, n. 2, pp. 549–555, Feb. 2013. doi: http://dx.doi.org/10.1007/s10973-013-3001-y

[26] PECORARO, É., MANZANI, D., MESSADDEQ, Y., *et al.*, "Bacterial cellulose from *Glucanacetobacter xylinus*: preparation, properties and applications", In: Belgacem, M.N., Gandini, A. (eds), *Monomers, polymers and composites from renewable resources,* Amsterdam, Elsevier, pp. 369–383, Jan. 2007.

[27] ARAÚJO, I.M.S., SILVA, R.R., PACHECO, G., *et al*., "Hydrothermal synthesis of bacterial cellulose-copper oxide nanocomposites and evaluation of their antimicrobial activity", *Carbohydrate Polymers*, v. 179, pp. 341–349, Jan. 2018. doi: http://dx.doi.org/10.1016/j.carbpol.2017.09.081. PubMed PMID: 29111060

[28] BARUD, H. "Preparo e caracterização de novos compósitos de celulose bacteriana", M.Sc. Thesis, UNESP, Araraquara, São Paulo, 2006.

[29] BARROS, M.O., "*Propriedades de celulose bacteriana: Influência do tempo de fermentação em diferentes cepas de Komagataeibacter*", M.Sc. Thesis, UFC, Fortaleza, Ceará, 2021.

[30] SILVERSTEIN, R.M., BASSLER, G.C., MORRILL, T.C., *Identificação espectrométrica de compostos orgânicos*, 5 ed., Rio de Janeiro, LTC, 1991.

[31] OLIVEIRA, R.N., MANCINI, M.C., OLIVEIRA, F.C.S., *et al*., "FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing", *Revista Materia*, v. 21, n. 3, pp. 767–779, Jul. 2016. doi: http://dx.doi.org/10.1590/S1517-707620160003.0072

[32] STUART, B.H., *Infrared spectroscopy: fundamentals and applications*, Chichester, Wiley, 2004. doi: http://dx.doi.org/10.1002/0470011149

[33] PICCHI, J.B., "*Preparo e caracterização físico-química e biológica de um biocompósito à base de celulose associada à própolis*", M.Sc Thesis, USP, São Carlo, São Paulo, 2010. doi: http://dx.doi.org/10.11606/D.82.2010.tde-20072012-090651

[34] SHAH, N., UL-ISLAM, M., KHATTAK, W.A., *et al*., "Overview of bacterial cellulose composites: a multipurpose advanced material", *Carbohydrate Polymers*, v. 98, n. 2, pp. 1585–1598, 2013. doi: http://dx.doi.org/10.1016/j.carbpol.2013.08.018. PubMed PMID: 24053844

[35] KUMINECK JUNIOR, S.R., "*Desenvolvimento de membranas de celulose bacteriana incorporadas com óleos essenciais para uso no tratamento de feridas*", M.Sc. Thesis, UNIVILLE, Joinville, Santa Catarina, 2023.

[36] JAHED, E., KHALEDABAD, M.A., BARI, M.R., *et al*., "Effect of cellulose and lignocellulose nanofibers on the properties of Origanum vulgare ssp. gracile essential oil-loaded chitosan films", *Reactive & Functional Polymers*, v. 117, pp. 70–80, Aug. 2017. doi: http://dx.doi.org/10.1016/j.reactfunctpolym.2017.06.008

[37] MARKOM, M., HASAN, M., DAUD, W.R.W., *et al*., "Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: Effects of solvents and extraction methods", *Separation and Purification Technology*, v. 52, n. 3, pp. 487–496, 2007. doi: http://dx.doi.org/10.1016/j.seppur.2006.06.003

[38] LONGHINI, R., RAKSA, S.M., OLIVEIRA, A.C.P., *et al*., "Obtenção de extratos de própolis sob diferentes condições e avaliação de sua atividade antifúngica", *Revista Brasileira de Farmacognosia*, v. 17, n. 3, pp. 388–395, Sep. 2007. doi: http://dx.doi.org/10.1590/S0102-695X2007000300015

[39] BHAVNANI, S.M., BALLOW, C.H., "New agents for Gram-positive bacteria", *Current Opinion in Microbiology*, v. 3, n. 5, pp. 528–534, 2000. doi: http://dx.doi.org/10.1016/S1369-5274(00)00134-X. PubMed PMID: 11050454.

[40] NASCIMENTO, P.F.C., NASCIMENTO, A.C., RODRIGUES, C.S., *et al*., "Atividade antimicrobiana dos óleos essenciais: uma abordagem multifatorial dos métodos", *Revista Brasileira de Farmacognosia*, v. 17, n. 1, pp. 108–113, 2007. doi: http://dx.doi.org/10.1590/S0102-695X2007000100020