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Cardamom Pickering emulsions stabilized by cellulose nanostructures as disinfection agents against bacteria and SARS-CoV-2

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ABSTRACT

This study introduces a new disinfectant agent utilizing cardamom essential oil stabilized by cellulose nanomaterials (CN). The Pickering emulsion technique prevented oil degradation and extended its storage duration. Cellulose nanocrystals (CNC) or cellulose nanofibers (CNF) were utilized as stabilizers, and the emulsions were evaluated for surface tension, rheology, morphology, antimicrobial and antiviral activity against *S. enterica*, *S. aureus*, *P. aeruginosa*, *E. coli*, and SARS-CoV-2. Surface tension measurements indicated an electrostatic stabilization mechanism in CNC emulsions, exhibiting fluid behavior confirmed by rheological tests. On the other hand, CNF emulsions displayed drop packaging, characteristic of shear-thinning behavior, as confirmed by the Ostwald-de-Waele model. The emulsions exhibited antibacterial activity against *S. aureus* and *P. aeruginosa*, particularly the CNC samples, suggesting that nanocellulose alters the regulation of oil migration into the medium. Testing against SARS-CoV-2 revealed that cardamom essential oil required 30 min of contact for viral protein denaturation, while CNC and CNF emulsions took 60 and 50 min, respectively. This indicates that CN acts as an encapsulating agent, prolonging the oil's release time and necessitating longer contact periods with the virus. These findings demonstrate the potential of these emulsions in developing new antimicrobial and antiviral products.

1. Introduction

The COVID-19 pandemic has raised global problems regarding the hygiene of environments highly contaminated with pathogenic microorganisms [1,2]. Thus, hospitals, clinics, and environments with a high circulation of people are places of potential disease contamination and require special attention regarding cleaning and disinfection [3]. Furthermore, sanitation is one of the most critical processes associated with all industrial sectors, consisting of the cleaning and sanitizing steps. While cleaning is carried out with detergents, sanitization is a process that uses, for example, chemical compounds to reduce or eliminate the presence of microorganisms on surfaces. These compounds are generally divided into oxidizing agents based on chlorine compounds and surface-active compounds to destabilize the membrane of microorganisms and induce their death, or even iodophors, resulting in cell death. Among the most common components, hypochlorite stands out, with high efficiency but high toxicity and potential for surface corrosion, and quaternary ammonium salts, which are less toxic but have low efficiency [3,4].

Since surfaces can be a suitable substrate for the development and persistence of viruses and bacteria, being a potential source of contamination, there is a continuous and constant search for new active agents that act in the disinfection of surfaces. New strategies have been investigated with high antimicrobial potential, low toxicity, and biodegradability [5]. The search for new materials occurs because of society's growing negative perception of synthetic materials, aiming at natural alternatives [6,7]. The literature highlights organic acids, antimicrobial peptides, and essential oils among the three main antimicrobial organic options [8].

Essential oils (EOs) can be considered an innovative alternative for this application due to their biological activity against a series of pathogenic microorganisms, such as bacteria, viruses, and fungi [9]. EOs have complex chemical compositions, with various chemical components, such as terpenes, phenylpropanoids, aldehydes, ketones, alcohols,

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esters, phenols, and ethers [10–13]. Despite the excellent antimicrobial activity of essential oils against pathogenic microorganisms, their use is limited due to their low water solubility and low stability to environmental factors such as heat, humidity, and oxygen. Instability results in the volatilization and degradation of chemical compounds present in the oil, limiting its effectiveness [14,15].

Cardamom is a famous botanical species and has been used worldwide for hundreds of years. The traditional pharmaceutical uses of this spice are mainly attributed to the presence of volatile oils and several bioactives already mentioned throughout the work, such as phenolics, 1,8-cineole, limonene, linalool, terpinolene, and myrcene. Currently, it has been highlighted for use in diseases such as flu infection, asthma, bronchitis, heart disease, and diarrhea, among others [16–18].

Among the most common stabilization techniques, nanoencapsulation and nanoemulsification stand out [11,19]. An emulsion is a mixture of two immiscible liquids where the drops are considered the dispersed phase, and the liquid around is the continuous phase. The dispersion from one phase to another, aiming to obtain a homogeneous system, occurs through amphiphilic molecules capable of decreasing the interfacial tension between the phases, resulting in high stability. Emulsifiers are essential to forming stable emulsions with appropriate shelf life and functional attributes [20]. Many emulsifiers currently used industrially to stabilize oil-in-water emulsions are synthetic surfactants. However, a counterpoint to this process is that synthetic emulsifiers increase toxicity and environmental problems caused by their low biodegradability [21]. Therefore, there is an interest in natural and environmentally correct nanoproducts based on social and environmental concerns about synthetic products [21]. In this sense, a trend is towards replacing synthetic surfactants with solid particles of natural origin that are considered “friendly,” that is, new stabilizers, such as biopolymers [22,23]. These emulsions stabilized by solid particles are known as Pickering emulsions.

In particular, cellulose nanomaterials, including cellulose nanocrystals (CNC) and cellulose nanofibrils (CNF), have attracted much interest as an interfacial stabilizer for encapsulating phase-change material due to their nanoscale size, inherent amphiphilic structure, abundance, sustainability, and thermal stability [24–26]. According to Bergfreund, Jotam, et al. 2019 Pickering emulsions stabilized by CNC particles exhibit preferable stability due to their ability to lower interfacial tension and irreversible adsorption at the oil/water (O/W) interface compared to those stabilized by small molecule synthetic surfactants [27,28]. Phosanam et al. (2023) stabilized ginger essential oil with cellulose nanocrystals and reported that the CNC concentration affects the size of emulsified oil droplets [29].

This work developed and characterized Pickering emulsions containing cardamom EO, stabilized with cellulose nanostructures, i.e., CNC or CNF, aiming at applications as a sanitizing agent. Surface tension and rheology characterized emulsions for their physical properties. The emulsions were tested to verify their antimicrobial activity against gram-positive and gram-negative bacteria and the SARS-CoV-2. The microbial inactivation mechanisms were investigated to develop a biologically active material for disinfection, being of high biotechnological interest.

2. Materials and methods

2.1. Materials

Cellulose nanocrystals (CNC) (L/D = 15, and zeta potential of −33.0 ± 1.0 mV, at neutral solution (pH ~ 7) resulting from the hydroxyls from cellulose’ structure, meaning that the samples have stable electrostatic charges) were prepared and characterized according to our previous works [30,31]. Cellulose nanofibrils (CNF) (L/D = 60, zeta potential of −33.2 ± 3.2 mV, and 12 % hemicellulose composition) were kindly donated by Suzano Papel e Celulose (São Paulo, Brazil) [32]. The morphology of the nanocelluloses can be found in our previous works. Ferquima Indústria e Comércio Ltda. (São Paulo, Brazil) provided the Cardamom essential oil (*Elettaria cardamomum*, CAS Number 8000-66-6). The essential oil’s main characteristics are density (20 °C) between 0.92 and 0.94, refraction index between 1.460 and 1.467, soluble in alcohol, and insoluble in water.

2.2. Emulsion preparation

Emulsions were previously selected based on previous work [33], in which process parameters were investigated to select stable emulsions. This work investigated four emulsions: two CNC-stabilized and two CNF-stabilized. The CNC-stabilized emulsions were prepared with 0.5 wt% of cellulose nanocrystals and 30/70 oil/water proportion; samples were named PE-CNC1 (3 min and 10,000 rpm for homogenization), and PE-CNC2 (7 min and 12,000 rpm for homogenization), which PE is Pickering Emulsion. The CNF-stabilized emulsions were prepared with 1 wt% of cellulose nanofibers and 12,000 rpm homogenization speed; samples were named PE-CNF1 (3 min of homogenization 30/70 oil/water proportion) and PE-CNF2 (7 min of homogenization and 20/80 oil/water proportion).

The tests were conducted at room temperature to avoid the volatility of the essential oil, since the literature indicates that this EO is subject to the volatility of its components in high temperatures, often above 149 °C [34]. So, considering the literature, there was no significant risk of oil volatilization during our experiment. Ambient temperature is insufficient to cause volatilization of the main components of cardamom essential oil, ensuring its properties remain stable throughout the study.

Fig. 1a shows the schematic illustration of the adopted methodology, and Fig. 1b shows a scheme representing the mixture of cellulose and cardamom essential oil systems from a chemical perspective.

The oil concentration was selected by extrapolating the MIC and MBC results of the cardamom essential oil against *Salmonella enterica* (ATCC n° 10708) (MIC: 3.125 % and MBC: 6.25 %); *Staphylococcus aureus* (ATCC n° 6538) (MIC: 12.5 % and MBC: 25 %); *Pseudomonas aeruginosa* (ATCC n° 10145) (MIC: 3.125 % and MBC: 6.25 %); *Escherichia coli* (ATCC n° 11229) (MIC: 3.125 % and MBC: 6.25 %).

2.3. Characterization

2.3.1. Surface tension

The surface tension was measured using an optical tensiometer Theta Lite (dpUnion, Brazil). A sample drop (8 μL) remained suspended at the tip of a 14-gauge plastic needle. The drop’s surface tension relative to air was measured for 30 s and then calculated. The experiments were conducted five times, always keeping the same drop volume.

2.3.2. Rheology

Rheological measurements were performed using a rotational rheometer, model Viscotester IQ – Thermo HAAKE (Thermo Fisher Scientific, Germany), equipped with a double-cone rotor and a stationary plate surrounded by a cylindrical wall. The curves were obtained in Controlled Rate (CR) mode by increasing the shear rate from 0 to 1000 s^{-1}, 600 s, maintaining at 1000 s^{-1} for 60 s, and returning to 0 s^{-1} in 600 s. All rheological parameters were obtained from the flow curves executed in Controlled Rate (CR) mode by HAAKE software RheoWin 4.63.0003.

2.3.3. Antimicrobial tests

Bacterial isolates were obtained from the Center for Interdisciplinary Procedures of Microorganisms Collection Center of Adolfo Lutz Institute (Brazil). Affiliated to World Federation Culture Collections (WFCC) #282, Depository Collection – #017/09-SECEX/CGEN/MMA: *Salmonella enterica* (ATCC n° 10708); *Staphylococcus aureus* (ATCC n° 6538); *Pseudomonas aeruginosa* (ATCC n° 10145); *Escherichia coli* (ATCC n° 11229).

The microorganisms were received from ATCC and reconstituted as

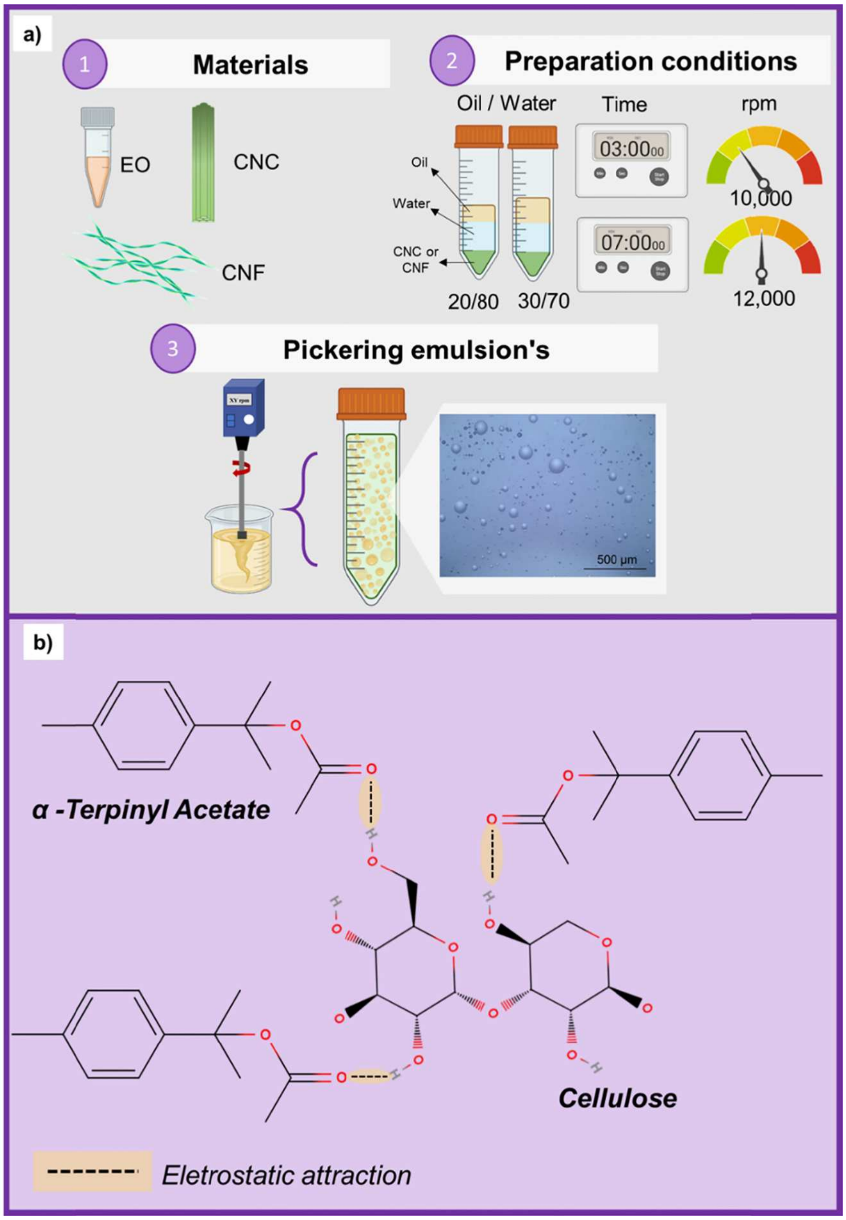

Fig. 1. a) Illustrative representation of the methodology adopted for preparing the Pickering emulsion b) Illustration of the synthesis/manufacturing from a chemical perspective of the cardamom/cellulose system.

directed. Stock cultures were cultivated in Mueller-Hinton broth with glycerol 15 % and stored at −70 °C. Stock cultures were cultivated in Mueller-Hinton agar (Oxoid, Thermo Fisher Scientific, USA) and incubated at 37 °C for 24 h. Then, it was inoculated in culture broth to obtain a turbidity equivalent to 0.5 Mc Farland, a concentration equivalent to 1.5×10^8 CFU.mL^{-1} [33,35]. Then, to carry out the tests, 30 mL of agar were melted and cooled to 48 ± 1 °C. Then, 500 μL of the microorganisms at 1.5×10^8 CFU.mL^{-1} were added, and this mixture was transferred to a petri dish. After solidification, a 0.9 cm diameter hole made in the middle of the plates was completed with 0.2–0.5 mL of emulsion. Incubation was carried out at 36 ± 1 °C for 24 h [36,37]. The appearance of inhibition halos around the orifice with the product was evaluated. These halos have been measured, and the appearance of a zone of inhibition of any dimension indicates that the product under analysis has properties. The zone of inhibition's size depends on the emulsion's diffusion in the agar preparation. As a control, we use widely used sanitizers such as hypochlorite and Lysoform®.

2.3.4. SARS-CoV-2 inactivation tests

First, SARS-CoV-2 positive human biological samples, identified in the laboratory's routine diagnostic assay by the real-time RT-PCR method and stored in the Adolfo Lutz Institute – Santo André Regional Center biorepository, were used. For this, 100 μL of the sample was previously analyzed by a Rapid Antigen Test to confirm viral viability (sensitivity of 96.7 %) [38]. After obtaining a positive result, a mixture of 100 μL of the SARS-CoV-2 positive sample and 100 μL of the emulsion was prepared, and different contact times were tested. The initial testing time was 30 min, and if the emulsion resulted in a positive test, i.e., there was no virus protein denaturation, the new contact times were higher (50 and 60 min). Tests were performed in duplicate. The tests were performed following the standard normative approved by the Research Ethics Committee of the Universidade Federal do ABC and Adolfo Lutz Institute CAAE: 49573421.2.3001.0059, and the Scientific-Technical Council of Adolfo Lutz Institute, CTC 18-N/2021. All procedures followed Adolfo Lutz Institute's biosafety standards at the Santo André Regional Center.

3. Results

3.1. Surface tension

The surface tension (ST) of PE-CNC1, PE-CNC2, PE-CNF1, and PE-CNF2 were 45.7 ± 0.2, 45.2 ± 0.4, 33.9 ± 0.1, and 32.4 ± 0.1 mV/m, respectively (Fig. 2). The CNC emulsions showed similar ST values, which can be associated with the cellulose nanocrystal's structure, that presents free hydroxyls in their structure that favor their adsorption at the oil/water interface and, at the same time, generate repulsive forces between the oil droplets. The electrostatic stabilization mechanism resulted in similar stability for both CNC emulsions and, consequently, similar physicochemical characteristics, resulting in statistically similar ST values.

On the other hand, the PE-CNF1 showed a higher ST value than the PE-CNF2, indicating that for the second sample, probably occurred nanofibers' accumulation and assembly at the liquid–air interface [39]. Besides, according to Berg et al., CNC and CNF differences influence their contact angle and surface tension [40]. The nanocellulose structures show different interactions in a fluid–fluid interface, such as DLVO interactions, capillary forces, hydrophilic behavior, and monopolar and dipolar interactions. Since the L/D of CNCs and CNFs are different, it is expected that higher particles, e.g., CNF, can show gravity-induced flotation capillary forces due to their weight [41].

CNCs can show immersion capillary forces depending on their position at the water surface droplet and surface chemistry. Thus, the intensity of surface tension depends on the forces of interactions associated with the morphology of the nanocellulose, size, and states of dispersion. In this way, the lower ST values verified for the CNF emulsions can also be attributed to the nanofibers morphology, emulsions dispersion system, and the combination of electrostatic and steric forces to stabilize the Pickering emulsions.

Considering the slightly lower values for PE-CNF2 could indicate better CNF adsorption at the oil droplets interface [42]. According to Zhou et al., low ST values suggest that the cellulose nanomaterials are easily adsorbed at the interface, affecting the particles' packing and forming an interfacial structure, a critical characteristic for Pickering emulsion's physicochemical properties [43].

3.2. Rheology

Considering a Pickering emulsion system prepared for commercial applications, its flowability is crucial for preparing and storing the final product. Fig. 3 shows the shear viscosity of the cardamom EO and its Pickering emulsions. The PE-CNC emulsions showed a slight increase in viscosity at low shear rates, followed by a Newtonian flow during all the tests.

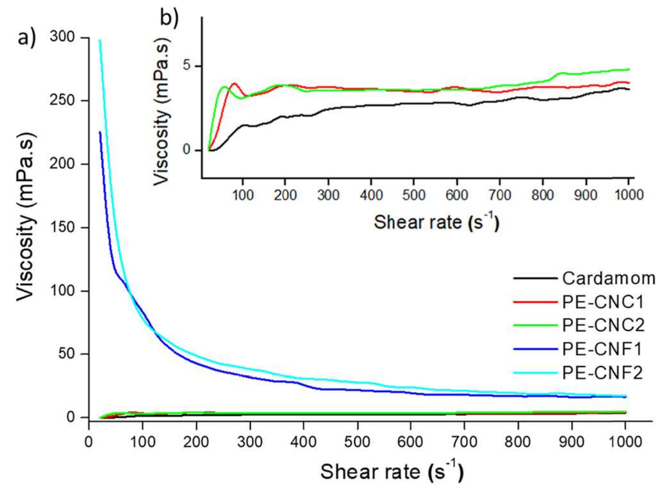

Fig. 3. Viscosity versus shear rate curves of cardamom essential oil and its Pickering emulsions a) complete graphs and b) zoom in the samples at low viscosity (cardamom, PE-CNC1, and PE-CNC2).

The initial slight increase in viscosity is associated with the morphology of CNCs, which tend to organize themselves to generate a percolation network that connects CNCs with distributed interfaces [29]. The percolation plays a crucial role, representing the emulsion at rest. Low shear rates can even help the emulsion stability through system self-organization in which the nanocrystals have already been aligned on the surface of the oil droplets [44,45]. According to Niu et al. (2023), low CNC concentrations can lead to Newtonian behavior, while higher contents could alter the rheological properties and show a typical shear-thinning behavior [46].

The Newton model adjusted the cardamom essential oil, PE-CNC1, and PE-CNC2 emulsions (Fig. 3b) with viscosities values of 3.430, 3.702, and 4.513 mPa.s, respectively. The higher viscosity values are attributed to the CNCs. Besides, thixotropy values were 8.9, 177.5, and 12.7 $Pa.s^{-1}$ for cardamom EO, PE-CNC1, and PE-CNC2 samples, respectively. These values are low, indicating good uniformity of behavior over time, except for the sample PE-CNC1, which probably shows a more heterogeneous structure, and its structural relaxation depends on the time [44]. According to Yuan et al. (2021), emulsions stabilized with cellulose structures commonly show a thixotropic behavior due to the cellulose's ability to self-assemble and continuously form new structures [45].

The PE-CNF emulsions showed a completely distinct behavior from the CNC emulsions with typical shear-thinning behavior, where the apparent viscosity decreased with the shear rate increase, similar to

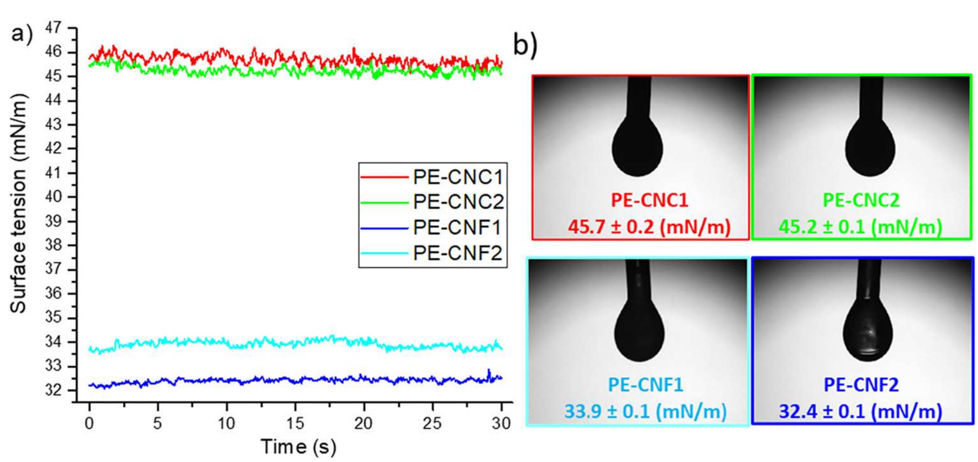

Fig. 2. a) Surface tension measurements over time, and b) obtained surface tension average values.

those reported by Lei et al. [47]. As Paximada et al. (2016, 2020) described, this rheological behavior is justified by the nanofibrils' entangled network breakdown and the orientation of the fibrils along flow lines upon the shear force [48,49]. In detail, at low shear rates, the droplets have a random distribution because of their Brownian motion, and the droplets are maintained together due to the insufficient hydrodynamic forces to disrupt the bonds between the droplets, resulting in relatively high viscosity. According to Liu et al. (2019), the high viscosity at low shear rate restrains the flavor release of the emulsions, being positive for applications where the flavor can be a limitation [50]. With the increase in the shear rate, the hydrodynamic forces were enough to disrupt the bonds and deform the CNF network and oil droplets, forming cellulose nanofiber layers. Consequently, the viscosity decreased until reaching a constant value at a high shear rate [20,51].

The negative values observed for the thixotropy values indicate reversible increases in the shear strength with time, i.e., the viscosity increases with time, and similar results were reported by Ferreira et al. (2023) [52]. It is a phenomenon associated with structure regeneration, usually verified for systems with nanofibers [51,53].

For these emulsions, the Ostwald-de-Waele model was used to evaluate the flow type, following Eq. (1), where γ is the shear rate, K is the consistency index, and n is the flow behavior index. The emulsions showed an R^2 higher than 0.99, confirming a good fit for the model. Table 1 shows the obtained values after the Ostwald-de-Waele model fitting.

$$\eta_a(\gamma) = K\gamma^{n-1} \quad (1)$$

The n values less than 1 confirm the emulsions' non-Newtonian pseudoplastic behavior, which is the most common nonideal behavior exhibited for most commercial emulsions since low n values facilitate the oil dispersion and improve its bioactivity [32,54]. The low K values confirm low apparent viscosity [53,55]. According to Yuan et al., the rheology results suggested a system with a high capacity for bridging flocculation [56].

3.3. Antimicrobial tests

Figs. 4 and 5 show the antimicrobial results of cardamom essential oil and its emulsions stabilized with CNC or CNF against gram-positive and gram-negative bacteria tested. The EO showed bacterial inhibition against *E. coli,* and *P. aeruginosa*, with greater activity against *S. aureus (gram-positive)*. The differences are possibly due to the distinct cell wall structures between gram-positive and gram-negative bacteria, with the outer membrane in gram-negative acting as a barrier to many environmental substances [57,58].

The outer membrane could protect the Salmonella species in this scenario since no inhibition halo was observed [59]. According to Cui et al. (2020), cardamom EO probably alters the cell walls of gram-positive bacteria, inhibiting their metabolic activity and the formation of extracellular polymers, leading to bacterial death [60].

Regarding Pickering emulsions, none of the samples showed bioactivity against *E. coli* or *Salmonella* bacteria, both gram-negative. For the Salmonella species, these results were expected to follow the oil trend. The emulsification results about *E. coli* can be consequent to the EO dilution, as described in section 2.2, resulting in insufficient bactericidal groups. Additionally, the CNFs can decrease the oil vehiculation due to the interactions with cellulose, affecting the antimicrobial activity, as described by Chevalier et al. (2024) [61].

Table 1
Calculated parameters for emulsions using the Ostwald-de-Waele model, using Eq. (1).

Sample	K (consistency coefficient)	n (flow behavior index)	Thixotropy ($Pa.s^{-1}$)
PECar-CNF1	1.865	0.2938	−1390.0
PECar-CNF2	2.962	0.2305	−571.8

The emulsions had smaller inhibition halo diameters than the EO against *S. aureus* and *P. aeruginosa*. According to Agaoglu et al. (2006), the protective outer membrane of gram-negative bacteria serves as an efficient barrier against specific hydrophilic solutes and macromolecules [62]. Another important aspect is that cellulose, the stabilization particle used in the emulsification mechanism, probably affects the oil release [8,63]. Even with this aspect, considering a possible application of our material as a sanitizer, we considered that the emulsion is a good option and could be applied easily. Of course, other methodologies and systems could be used to improve the oil activity, aiming to achieve other applications with different requirements [64].

PE-CNF samples did not show inhibition against *P. aeruginosa*. This result is associated with the morphology of nanocellulose and its interaction with the EO, in addition to the different regulations of oil migration to the medium. According to the literature, the CNFs create a solid and dense three-dimensional network that results in a more tortuous path for the oil and its volatile components to reach the surface, as shown in Fig. 6. This structure reduces the oil's permeability, influencing the diffusion of the EO in the medium [65].

Considering both systems, the results indicate that CNC-stabilized emulsions (PE-CNC1 and PE-CNC2) can form new organic anti-infective drugs to neutralize microorganisms resistant to multiple drugs or as alternatives to chemical antimicrobial preservatives in the food industry to increase the shelf life of food products as previously suggested [58,66].

3.4. SARS-CoV-2 inactivation tests

The cardamom EO and its emulsions were tested against the SARS-CoV-2, and the Rapid Antigen Test was applied to check the protein denaturation as an indicator of virus viability, as presented in Fig. 7. This test was used because, after contact with Cardamom Pickering Emulsions, the virus can continue or not with its integral protein structure capable of being detected in the rapid antigen test, and after how long of contact, it loses its integrity. Viral inactivation assays with electron microscopy are excellent for good visualization of the antiviral effect. However, SARS-CoV-2 requires Biosafety Level 3 (BLS-3) facilities, which we don't have. The rapid antigen test was previously compared with the viral viability assay and was shown to be equivalent [38]. The cardamom EO showed antiviral activity after 30 min in contact with the virus, probably due to the 1,8-cineole compound [67]. The active compounds in the EO are expected to damage the virus capsid and inhibit human contamination by touching contaminated surfaces [68].

Based on the antimicrobial results, samples PE-CNC2 and PE-CNF1 were selected to be tested against the virus. None of the emulsions showed negative results after 30 min of contact, indicating that the emulsification reduced the bioavailability of the active components and, consequently, required longer times for the denaturation of viral proteins. Or, as hypothesized in the antimicrobial tests, the lower concentration of the EO in the emulsion could compromise the antiviral activity. The PE-CNC2 sample required 60 min of contact to inactivate the virus, while the PE-CNF1 sample took 50 min. These results are similar to those observed for tests against bacteria, in which nanocellulose interacted with EO, changing the regulation of oil migration and its availability for interaction with microorganisms. Despite the long time for inactivation via protein denaturation, these results indicate the potential of EO for application in new antimicrobial formulations, and possibly, the oil could be more efficient by analyzing other inactivation mechanisms.

4. Conclusions

Recently, there has been a growing search for new antimicrobial

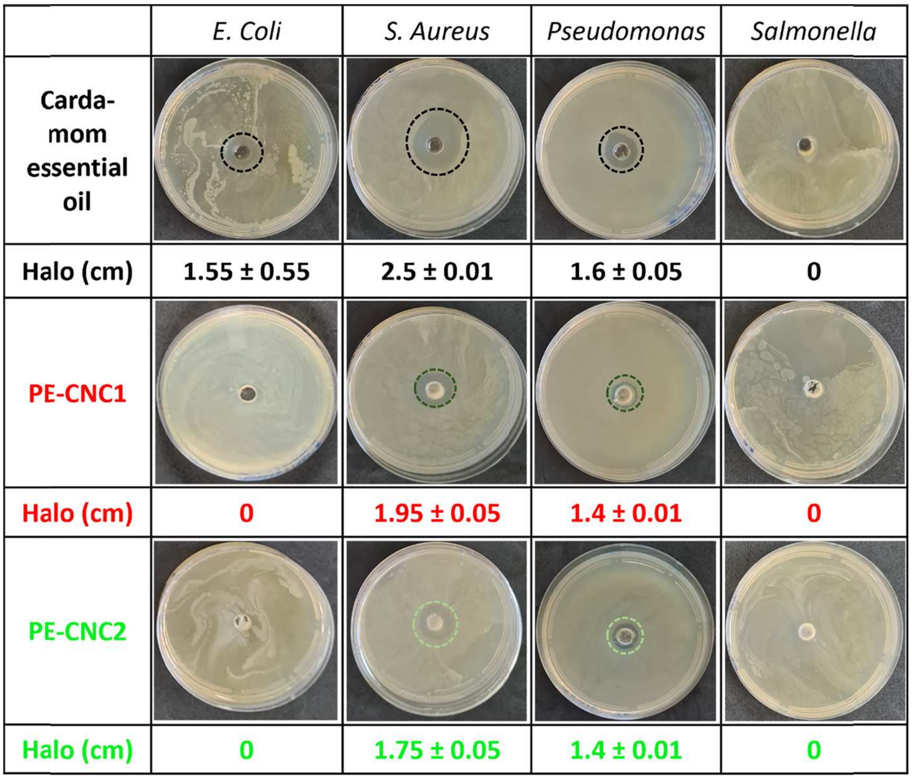

Fig. 4. Results of antimicrobial tests using cardamom essential oil and its emulsions stabilized with CNC.

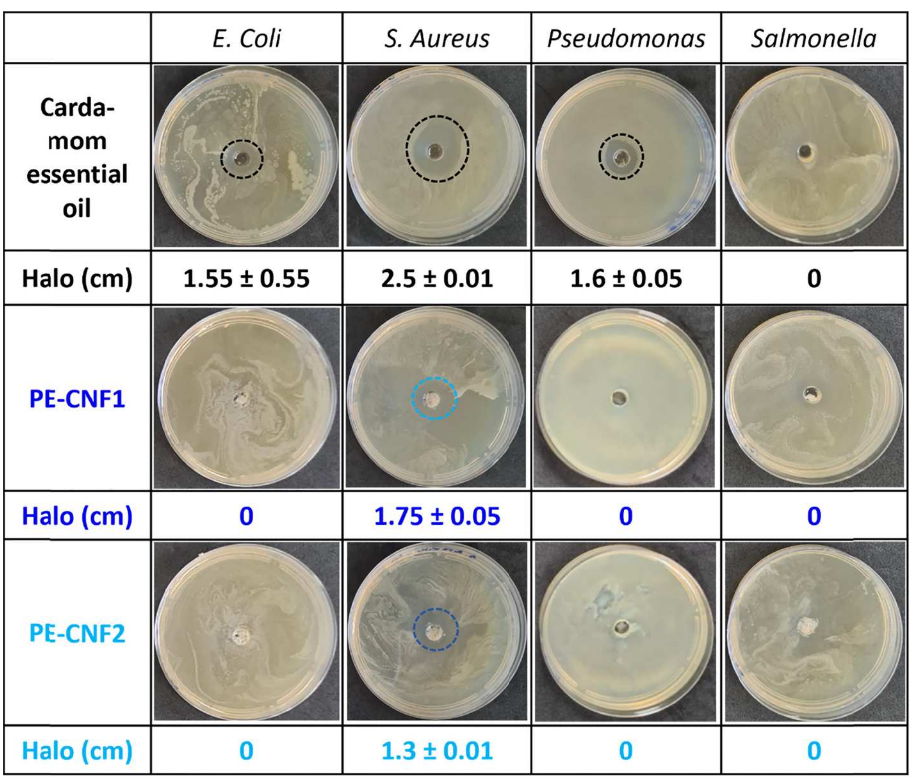

Fig. 5. Results of antimicrobial tests using cardamom essential oil and its emulsions stabilized with CNF.

materials with an environmentally friendly character. This work successfully prepared cardamom Pickering emulsions using cellulose nanomaterials as a solid phase. The emulsions showed a distinct stabilization mechanism for each nanoparticle morphology: the cellulose nanocrystals (CNC) stabilized by electrostatic interactions, maintaining a Newtonian fluid behavior, where the oil droplets were encapsulated inside the CNCs. On the other hand, the cellulose nanofibers (CNF) formed a gel-like structure that trapped the oil droplets inside the pores

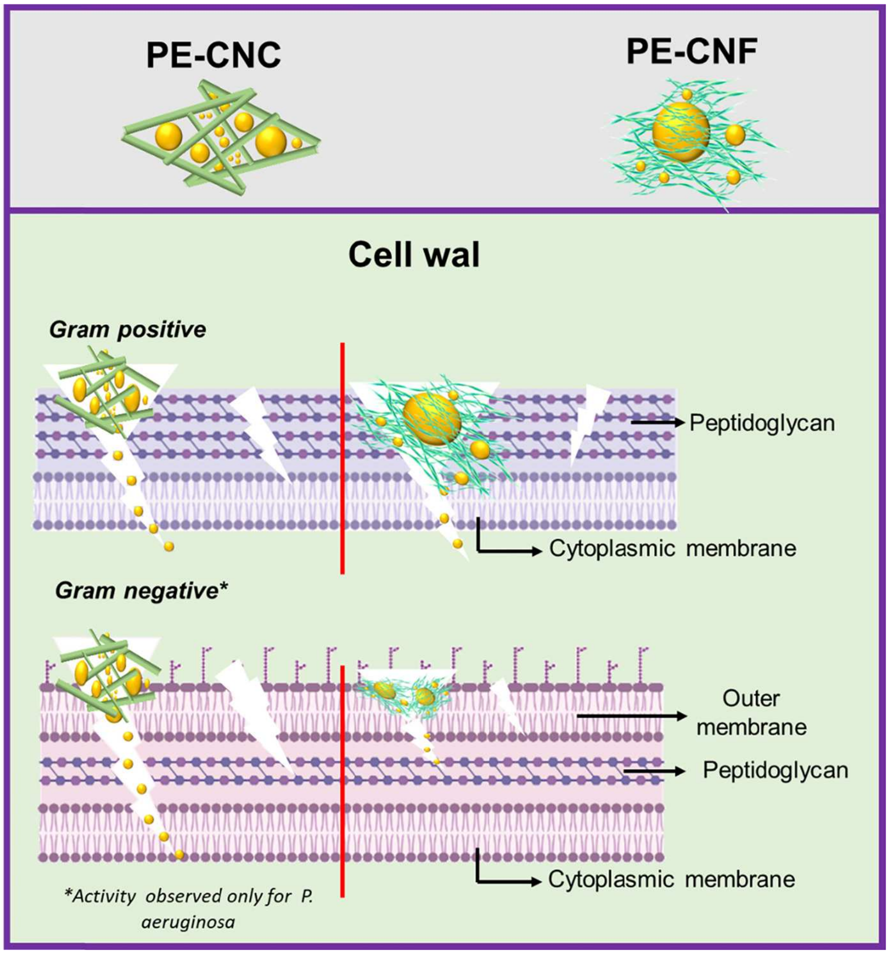

Fig. 6. Proposed mechanism for antimicrobial assay with gram-positive and gram-negative bacteria as a function of activity with PE-CNC and PE-CNF. * **Activity was only observed with *Pseudomonas aeruginosa* (ATCC n° 10145).**

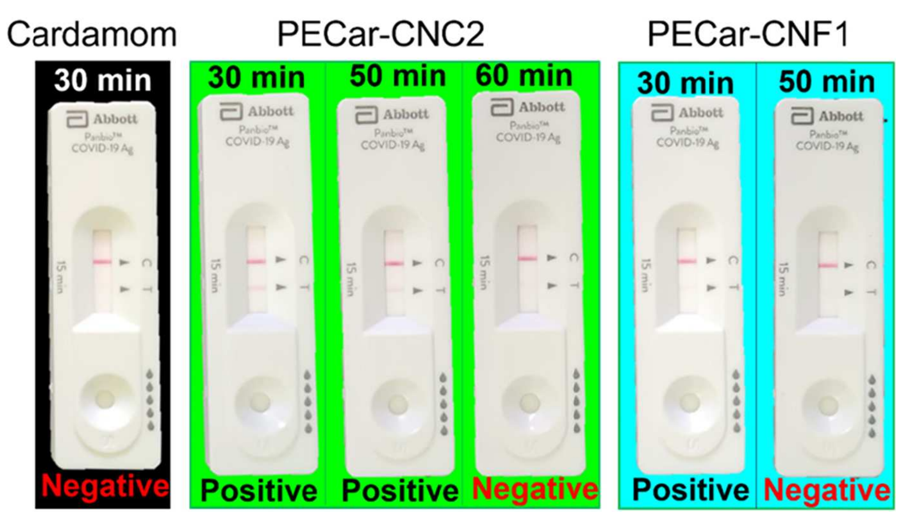

Fig. 7. The Rapid Antigen Test tested results obtained for cardamom EO and its emulsions against SARS-CoV-2 at different times.

with a shear-thinning behavior and stabilized the EO via a steric mechanism. The stabilization mechanism impacted the antimicrobial activities. The CNC emulsions showed higher antibacterial activity against *S. aureus* and *P. aeruginosa* than CNF samples, suggesting that CNF limits the oil migration to the medium. However, considering the SARS-CoV-2 assays, the results were the opposite, and the CNF emulsions required a lower time (50 min) to denature the virus protein, resulting in its inactivation. In contrast, the CNC samples required 1 h in contact with the virus. The results suggest that the developed emulsions have a good potential to be applied to nanotechnology as antimicrobial products in numerous industrial sectors, such as biomedical, pharmaceutical, and food.

CRediT authorship contribution statement

Alana Souza: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Maurício Kato:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Rafaela Reis Ferreira:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Eliana Yudice:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ivana Campos:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Luiz Setz:** Writing – original draft, Visualization, Formal analysis, Data curation. **Vijaya Rangari:** Writing – original draft, Visualization, Validation, Data curation. **Derval Rosa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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