ORIGINAL ARTICLE

EVALUATION OF THE ANTIBIOFILM ACTIVITY OF ESSENTIAL OILS CINNAMOMUM CASSIA AND EUGENIA CARYOPHYLLUS MICROENCAPSULATED BY SPRAY DRYING

Guilherme de Ataides Ribeiro [1], Elton Clementino da Silva [2]
Silvia Ribeiro de Souza[3], Naira da Silva Campos[4]
Joanlise Marco de Leon Andrade[5], Monica Valero da Silva [6]

Highlight: (1) Presence of biofilms in food, cosmetics and pharmaceutical industries. (2) Microparticles used as carriers for essential oils. (3) Essential oils with antimicrobial and antibiofilm activity.

PRE-PROOF
(as accepted)
This is a preliminary, unedited version of a manuscript that was accepted for publication in Revista Contexto & Saúde. As a service to our readers, we are making this initial version of the manuscript available, as accepted. The article will still be reviewed, formatted and approved by the authors before being published in its final form.

http://dx.doi.org/10.21527/2176-7114.2026.51.16868

[1] Universidade de Brasília – UnB. Faculdade de Ciências da Saúde. Departamento de Farmácia. Brasilia/DF, Brazil. https://orcid.org/0009-0002-3530-8366

[2] Universidade de Brasília – UnB. Faculdade de Ciências e Tecnologias em Saúde. Ceilândia/DF, Brazil. https://orcid.org/0000-0002-3217-5222

[3] Universidade de Brasília – UnB. Faculdade de Ciências da Saúde. Departamento de Farmácia. Brasilia/DF, Brazil. https://orcid.org/0000-0001-8189-199X

[4] Universidade de Brasília – UnB. Faculdade de Ciências da Saúde. Departamento de Farmácia. Brasilia/DF, Brazil. https://orcid.org/0000-0002-7753-1059

[5] Universidade de Brasília – UnB. Instituto de Exatas. Departamento de Estatística. Brasilia/DF, Brazil. https://orcid.org/0000-0002-3984-3799

[6] Universidade de Brasília – UnB. Faculdade de Ciências da Saúde. Departamento de Farmácia. Brasilia/DF, Brazil. https://orcid.org/0000-0002-6096-2442

EVALUATION OF THE ANTIBIOFILM ACTIVITY OF ESSENTIAL OILS CINNAMOMUM CASSIA AND EUGENIA CARYOPHYLLUS MICROENCAPSULATED BY SPRAY DRYING

How to cite:

Silva MV da, Ribeiro G da A, Campos N da S, Silva EC da, Andrade JM de L, Souza SR de. Evaluation of the antibiofilm activity of essential oils *Cinnamomum cassia* and *Eugenia caryophyllus* microencapsulated by spray drying. Rev. Contexto & Saúde. 2026;26(51):e16868

ABSTRACT

Biofilm-forming microorganisms are considered a challenge in terms of eradication in the areas of food, cosmetics, and health, as they are resistant to antibiotics and sanitizers available on the market. From this problematization, the essential oils (EOs) *Cinnamomum cassia* (CCEO) and *Eugenia caryophyllus* (ECEO) encapsulated in microparticles by the spray drying technique was used to obtain a stable dry emulsion and evaluate their antimicrobial action against biofilms of the microorganisms *Staphylococcus aureus, Escherichia coli*, and *Candida albicans.* The EOs were commercially obtained from different brands, being evaluated by the agar diffusion technique for preliminary evaluation of the antimicrobial action against the challenged microorganisms. Microencapsulation was performed in spray drying, employing gum arabic and xanthan gum to obtain a dry emulsion with varying concentrations of the EOs. The microparticles were characterized for morphology, physicochemical parameters, and encapsulation efficiency (EE), and were also evaluated for anti-biofilm action. The results obtained showed relevant antimicrobial activity of the essential oils in non-encapsulated form and antibiofilm activity when they were microencapsulated, obtaining a significant logarithmic reduction concerning the initial load challenged, reducing between 3 to 4 logarithmic cycles. It was concluded that microparticles with encapsulated EOs showed promise in improving the stability of essential oils, with significant results when challenged against planktonic and sessile cells.

Keywords: Biofilm; microparticles; spray-drying; essential oils; antibiofilm.

EVALUATION OF THE ANTIBIOFILM ACTIVITY OF ESSENTIAL OILS CINNAMOMUM CASSIA AND EUGENIA CARYOPHYLLUS MICROENCAPSULATED BY SPRAY DRYING

AVALIAÇÃO DA ATIVIDADE ANTIBIOFILME DE ÓLEOS ESSENCIAIS CINNAMOMUM CASSIA E EUGENIA CARYOPHYLLUS MICROENCAPSULADOS POR SPRAY DRYING

RESUMO

Microrganismos formadores de biofilmes são considerados um desafio quanto a erradicação nas áreas de alimentos, cosméticos e saúde, pois são resistentes aos antibióticos e sanitizantes disponíveis no mercado. A partir dessa problematização empregou-se os óleos essenciais (OEs) *Cinnamomun cassia* (CCOE) e *Eugenia caryophyllus* (ECOE) encapsulados em micropartículas pela técnica spray drying com o objetivo de obter uma emulsão seca estável e avaliar sua ação antimicrobiana contra a biofilmes dos microrganismos *Staphylococcus aureus, Escherichia coli* e *Candida albicans.* Os OEs foram obtidos comercialmente de diferentes marcas, sendo avaliados pela técnica de difusão em ágar para avaliação preliminar da ação antimicrobiana frente aos microrganismos desafiados. A microencapsulação foi realizada em *spray drying*, empregando gomas arábica e xantana para obter uma emulsão seca com concentrações variadas dos OEs. As micropartículas foram caracterizadas quanto a morfologia, parâmetros físico-químicos e eficiência de encapsulação (EE), sendo também avaliadas quanto a ação anti-biofilme. Os resultados obtidos mostraram atividade antimicrobiana relevante dos óleos essenciais na forma livre e atividade anti-biofilme quando os mesmos foram microencapsulados, obtendo redução logarítmica significativa em relação a carga inicial desafiada, reduzindo entre 3 a 4 ciclos logarítmicos. Concluiu-se que as micropartículas com OEs encapsulados se apresentaram promissoras quanto a aprimorar a estabilidade dos óleos essenciais, apresentado resultados significativos quando desafiados frente a células planctônica e sésseis.

Palavras chaves: Biofilme; micropartículas; *spray-drying*; óleos essenciais; anti-biofilme.

INTRODUCTION

Biofilm is an array of microorganisms with extracellular substances that can be formed on different surfaces. These surfaces may be animal tissue (meat, fish products), catheter, teeth, stainless steel, plastic, glass, teflon, rubber, and wood, among others. They can occur in different industrial and medical settings. A range of 65-80% of infections are related to biofilms[1].

The microorganisms *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* are biofilm-forming strains, commonly investigated for their presence in hospital, food, and cosmetic and drug manufacturing areas. Several studies have been carried out to combat the formation of biofilm derived by both fungi, Gram-positive and Gram-negative bacteria[2-5].

In the food and pharmaceutical industries, microorganisms can adhere to organic and inorganic materials present on the surface of equipment and tools[6]. Sessile cells, present in the biofilm, are more resistant to the disinfection process. The cells can detach and contaminate the products that go through the equipment, which can cause economic losses and increase the risk of infections[7].

Essential oils have attracted interest to be used as active excipients in formulations developed to decrease and/or eradicate the development of biofilms on surfaces. They have metabolites in their composition, such as terpenes, phenylpropanoids, alcohols, ketones, aldehydes, and esters[8,9]. *Eugenia caryophyllus* is a plant of the *Myrtaceae* family whose essential oil obtained from the leaves has in its composition mainly eugenol and eugenol acetate that has anti-inflammatory, healing, and analgesic effects that successfully combat and decrease the bacteria present in the mouth and decrease the growth of *Escherichia coli* and other bacteria in the storage of juices, milks, and teas[10-12]. *Cinnamomum cassia* is a shrub species, of the *Lauraceae* family, popularly known as Chinese cinnamon and which has in its leaves, bark and stalk an essential oil with antimicrobial activity, being considered very effective, it has a high content of cinnamaldehyde, between 60 to 80%, in addition to cinnamic acid and cinnamic aldehyde derivatives[13-15].

The mechanism of action of essential oils is based on damaging the cell wall and membranes of microorganisms, changing the morphology, and coagulating the

cytoplasmic material[1]. As the permeability of the microorganism's cell membrane increases, there is also extravasation of the cytoplasm content and consequently cell death[9].

Microencapsulation is a technique used in both the food and pharmaceutical industries, which aims to increase the efficiency of encapsulated active substances by improving the physical and chemical stability of substances that change with heat. As essential oils are volatile and sensitive to oxygen and heat, microencapsulation contributes to their stability. Spray drying is one of several microencapsulation techniques, widely used to encapsulate volatile products such as flavors and essential oils, plant extracts, and vegetable oils[2, 16-20].

The spray drying process consists of transforming a solution, suspension or emulsion into a dry powder. The encapsulation efficiency is mainly linked to the wall materials, a proper selection will ensure a good morphology, stability, and functionality[21]. There are several types of encapsulation material, among which gum arabic and xanthan gum are commonly employed options, considering the low cost and water solubility. Gum arabic has emulsifying power and low viscosity, while xanthan gum is an anionic polysaccharide produced by strains of *Xanthomonas campestris*, being considered natural polymers[20, 22-24].

The objective of this work was to encapsulate *Cinnamomun cassia* (CCEO) and *Eugenia caryophyllus* (ECEO) essential oils in natural polymer microparticles without undergoing common changes during the manufacturing and storage process, such as volatilization and oxidation, acting as effective germicides against biofilm-forming bacteria and fungi, such as *S. aureus, E. coli,* and *C. albicans*.

MATERIALS AND METHODS

Essential oils

The essential oils *Cinnamomum cassia* (CCEO) and *Eugenia caryophyllus* (ECEO) were purchased from Ferquima (Industry and Commerce Limited), Vargem Grande/ São Paulo that employ a steam distillation extraction process. Ferquima's almond vegetable oil was used as a vehicle. The essential oils were kept in their original primary packaging

and stored at room temperature, in order that such packaging do not constitute interference variables regarding possible contamination and degradation of components.

Microorganisms

The strains used were *Candida albicans* (INCQS 40178), *Escherichia coli* (INCQS 00379), and *Staphylococcus aureus* (INCQS 00402) donated by the Oswaldo Cruz Foundation – National Institute for Quality Control in Health (INCQS).

Pharmaceutical Excipients

The natural polymers gum arabic and xanthan gum were purchased from Pryme Foods – LLC, Alto de Pinheiros/ São Paulo. The surfactant Tween 80 (polysorbate 80) was purchased from Dinamica (Brazil). Eugenol and Cinnamaldehyde standards for *Eugenia caryophyllus* and *Cinnamomum cassia* essential oils were purchased from Sigma Aldrich.

Preparation of the emulsions

For the preparation of the emulsions, the concentrations with the best response regarding antimicrobial activity were considered. The components of the aqueous phase (AP) (xanthan gum, gum arabic, and distilled water) were stirred for 15 minutes on an overhead stirrer (Fisatom) at 1200 rpm at room temperature, separately from the oily phase (OP) (vegetable oil and essential oil). Stirring of the oil phase was started, under the same conditions, and AP was added over OP maintaining stir for another 15 minutes, adding tween 80 dropwise over the two phases under stirring. Several O/W emulsions were prepared with different concentrations of essential oils, as shown in Table 1.

Table 1. Composition of F1 to F10 emulsions in percent (w/v) prepared with essential oils

Formulation	GA	XG	OP	VO	CCEO	ECEO	TW80	DW qsp (ml)
WF	5.9	0.1	-	5.0	-	-	0.5	200
F1	5.9	0.1	2	50	50	-	0.5	200
F2	5.9	0.1	2	50	50	-	1.0	200
F3	5.9	0.1	2	50	50	-	-	200
F4	5.9	0.1	5	50	50	-	0.5	200
F5	9.9	0.1	10	50	50	-	0.5	200
F6	5.9	0.1	10	80	20	-	0.5	200
F7	5.9	0.1	10	20	30	50	0.5	200
F8	5.9	0.1	10	10	40	50	0.5	200
F9	5.9	0.1	10	30	-	70	0.5	200
F10	5.9	0.1	10	20	-	80	0.5	200

WF: White formulation; GA: Gum arabic; XG: Xanthan gum; OP: Oily phase; VO: Vegetable oil; CCEO: *Cinnamomum cassia* essential oil; ECEO: *Eugenia caryophyllus* essential oil; TW80: Tween 80: DW: Distilled water.

Spray drying microencapsulation

The emulsions obtained were transferred to spray drying (model MSDi 1.0, LabMaq of Brazil, Ribeirão Preto, Brazil) and atomization was performed using a 0.7 mm diameter nozzle. The obtained emulsions were kept under stir on a magnetic stirrer plate (model 753A – Fisatom) at 980 rpm until the end of the spray drying process. The drying process variables were: air inlet temperature of 140°C and outlet air temperature around 100°C with drying air flow rate of 4 m^3/min, feed flow rate 0.5L/h and atomization air flow rate 30L/min[25,26].

Scanning electron microscopy

The morphology of microparticles was assessed by scanning electron microscopy (SEM). Considering the obtained formulations, some of them were separated for visualization by SEM. A random amount of each formulation was attached to a double-sided strand and placed in stubs. The samples were covered with gold in a vacuum metallizer (LEICA EM SCD 500 High Vacuum Sputter Coater) and analyzed with the scanning electron microscope (SEM Jeol JSM-7000F), in the Microscopy and

Microanalysis Laboratory of the Institute of Biological Sciences at the University of Brasília (UnB).

Spectrophotometric analysis and encapsulation efficacy

The efficacy of encapsulation (EE) was determined by UV-VIS spectroscopy[27,28] with adaptations, being performed in a UV-Visible Bel Engineering UV-M51 Spectrophotometer at wavelengths of 296 and 361 nm, to evaluate the contents of the essential oils *Eugenia caryophyllus and Cinnamomum cassia*, respectively. The chemical characterization of the EOs, obtained by steam distillation, as specified by the supplier (Ferquima), were for ECEO - yellow color, free of impurities, density (20°C): 1.046, refractive index (20°C): 1.535, main component eugenol (87%), and for CCEO - yellow color, free of impurities, density (20°C): 1.053, refractive index (20°C): 1.612, main component cinnamaldehyde (80%).

The samples were analyzed according to the methodology of Sansone et al.[28], with adaptations. Aliquots of each formulation (15 mg) were resuspended in dichloromethane (JTBaker) (15 mL), sonicated in ultrasound (Unique) for 30 minutes, and then centrifuged (Spinplus Titan) at 4500 rpm for 10 minutes. The supernatant was filtered through a 0.45 µm sterile filter (Kasvi) and then analyzed by UV-VIS.

The absorbance of these solutions was obtained, having as white the solvent used in the preparation of the solutions (dichloromethane). Analytical curves were prepared in triplicate with the standards of Eugenol (ECEO) and Cinnamaldehyde (CCEO), with concentrations between 0.3135 and 10 µg/mL. The linearity of the analytical curves was evaluated using the determination factor (R^2), obtaining values equal to 0.9910 and 0.9989 for Eugenol and Cinnamaldehyde, respectively.

Encapsulation efficacy (EE) was determined using equation 1:

$$EE\ (\%) = \frac{\text{Total amount of loaded EO}}{\text{Initial amount of EO}} \times 100 \quad (1)$$

Characterization of microparticles

The pH of the formulations was evaluated using a model 1500 potentiometer from Gehaka. Aliquots of 0.25 g of each formulation were transferred to 30 mL of distilled water, stirring for better homogenization of the mixture, then the pH was measured.

The measurements of zeta potential, polydispersity index (PDI), and size were evaluated using the Zetasizer Nano Series equipment (model ZEN3690, Malvern Instruments®, United Kingdom) of the Morphology Laboratory of the Institute of Biological Sciences at the University of Brasília (UnB). The formulations were weighed in an approximate amount of 0.05 g and diluted in distilled water to 2500 µL, from this dilution 10 µL was transferred and diluted with distilled water and ethyl alcohol to a volume of 1000 µL, and each value was measured with an average of 10 runs and the associated uncertainty was given by the equipment.

Evaluation of antimicrobial activity by disk diffusion of essential oils

The CCEO and ECEO were evaluated for antimicrobial activity using concentrations of 10 to 100%, ranging from 10 to 10%, diluted in almond vegetable oil and stored in sterile Eppendorf. The agar diffusion technique using 10 mm paper disc was employed using 10^8 CFU/mL inoculum standardized with the 0.5 McFarland scale ($1.5x10^8$ Colony Forming Units - $CFU.mL^{-1}$) for *C. albicans* and for the standardization of *E. coli* and *S. aureus* the 1.0 McFarland scale ($3.0x10^8$ Colony Forming Units - $CFU.mL^{-1}$) (Probac of Brazil) was employed. A volume of 25 mL of each culture medium, Sabouraud Dextrose Agar – SDA (Kasvi, Brazil) for yeast and Mueller Hinton Agar - MHA (Kasvi, Brazil) for bacteria, was transferred to duplicate sterile Petri dishes (100x20 mm), after solidification agar plates are inoculated with a standardized inoculum of the test microorganism 10^8 CFU/mL was spread on the plate surface with the aid of sterile swab (Kasvi, Brazil). Then, the disks were positioned in the plate duplicates, one of them being the standard. For yeasts, 10 µL of miconazole nitrate at a concentration of 2% (20 mg/mL) of the Vodol brand was used, and for bacteria, Gentamicin 30 disks of the DME brand were used. In the samples disks, 10 µL of the essential oil diluted in vegetable oil was placed before transferring to the surface of the culture medium. With sterile forceps, the disks were positioned equidistantly and after 30 minutes of diffusion

at room temperature, the plates were transferred to an oven (SolidSteel) 32.5±2.5°C/24 hours. The halos were measured with the aid of a ruler and the arithmetic mean was made[29,30].

Minimum inhibitory and bactericidal concentrations of essential oils

The minimum inhibitory concentration (MIC) test was performed using the broth dilution technique, with some modifications[31,32]. From a fresh inoculum of each bacterium, *E. coli* and *S. aureus*, an aliquot of each microorganism with bacteriological loop was removed and transferred to Mueller Hinton Broth (MHB) (Kasvi, Brazil) incubating at 32.5±2.5°C/48 hours. After this period, the microorganisms were standardized using the 0.5 McFarland scale for *C. albicans* and 1.0 McFarland scale for *E. coli* and *S. aureus*, and from this standardized suspension 40 µL was transferred to test tubes, in triplicate, with MHB and 1.0% Tween 80 (Dinamica, Brazil), then placing each essential oil at concentrations of 0.0; 0.01; 0.03; 0.06; 0.12; 0.25; 0.50 and 1.0% (v/v). A negative control of the MHB with the 1.0% Tween 80 was used for comparison. It was incubated at 32.5±2.5°C/48 hours and the MIC value (lower concentration than the antimicrobial agent inhibited growth) was determined macroscopically.

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined from the MIC results. Aliquots of 80 µL were transferred from each tube that showed no visible growth to plate triplicates with MHA medium, incubated at 32.5±2.5°C/48 hours. MBC and MFC were considered the lowest concentration where no microbiological growth was observed after exposure to essential oils (*Eugenia caryophyllus* and *Cinnamomum cassia*) at different concentrations. The same was done with *C. albicans* yeast, changing only the culture media for Sabouraud Dextrose Agar and Sabouraud Dextrose Broth (Kasvi, Brazil).

Use of microparticles against biofilms

Preparation of stainless steel coupons (washing/sanitizing/sterilization)

According to Oliveira et al.[14] and Millezi et al.[33], the preparation technique was based on washing the stainless steel coupons (2.0x2.0 cm) initially with distilled water and enzymatic detergent (Dinamicatec D27-Dinamica), then rinsing with distilled water.

After this first washing, the process of sanitization and sterilization began. Several sets of 10 stainless steel coupons were sanitized in a controlled environment using 10 mL of acetone P.A (Dinamica); they were rinsed with sterile distilled water, dried, washed again with 70% alcohol (Qhemis), washed with sterile distilled water and transferred for drying in an oven at 70°C/2 hours in Petri dishes (150x20 mm), then sterilized at 121°C/15 min.

Challenge against microorganisms in sessile form

The stainless steel coupons were challenged by adding, to each set of 10 coupons in triplicate of Petri dishes (150x20 mm), 60 mL of TSB (Tryptic Soy Broth) or SDB (Sabouraud Dextrose Broth), previously inoculated with 1.0 mL of the standardized suspension of bacteria (*E. coli* or *S. aureus*) or the same amount for *C. albicans*, then incubating at 32.5±2.5°C/48 hours, under static conditions. After the 48-hour incubation period for biofilm formation, the biofilms were treated. The coupons were washed three times with 0.9% saline, and after washing, the treatment was added using 0.5 g of the microcapsule samples shown in Table 1, solubilized in 60 ml of 0.9% saline solution with 0.5% Tween 80, in triplicate. The treatment remained for 20 minutes and was then discarded, and three more washes were performed with 0.9% saline. After this process, the swab was passed over the surface of the coupons and transferred to 10 mL of 0.1% peptone water and vortexed for 2 minutes. From this suspension, serial dilutions from 10^{-1} to 10^{-4} were made, and 0.5 mL of each dilution was transferred to triplicate Petri dishes by surface spreading over the TSA for bacteria and SDA for fungi. The plates were incubated at 32.5±2.5°C for 24 hours, and the CFU/mL were counted[14]. For comparative effect it was done using a treatment with sanitizer, 50% benzalkonium chloride (Dinamica) at 0.5% (v/v).

Design of Experiment and Statistical Analysis

A 3x4 completely randomized factorial design was conducted considering essential oil formulations (F4, F5, F10) and control (benzalkonium chloride) and microbial species (*E. coli*, *C. albicans*, and *S. aureus*) as factors and the Log reductions as the response variable. Three repetitions were used for each treatment combination.

An analysis of variance (ANOVA) was performed to test the effects of the use of essential oil formulations and bacterial species on the Log reductions. Tukey HSD test was used for post-hoc comparisons. Differences were considered statistically significant if p-value < 0.05.

All statistical analyses were performed using the R software (R Core Team, 2021[34]), version 4.3.2.

RESULTS AND DISCUSSION

Characterization of microparticles

Microparticles that showed the best anti-biofilm results were evaluated in relation to physicochemical characteristics. The Scanning electron microscopy (SEM) of the formulations (F4, F5, and F10) was made as well as the WF, without essential oil. The microparticles presented a lumpy appearance with white coloration, characteristic of the wall materials used. In the SEM, it was observed, according to Figures 1 and 2, that the particles were spherical, with a regular shape and with a rough surface (small cavities) for the samples containing essential oil. Such characteristics were already expected, due to the predominant use of gum arabic as a wall material[35]. The morphology of the microparticles that did not contain essential oil in their composition presented a surface with invaginations and wrinkling, that is, irregular spherical morphology, as shown in Figure 2, when compared to the microparticles that contained the essential oil. The morphology in this case is probably attributed to the drying process and the polymers used[36].

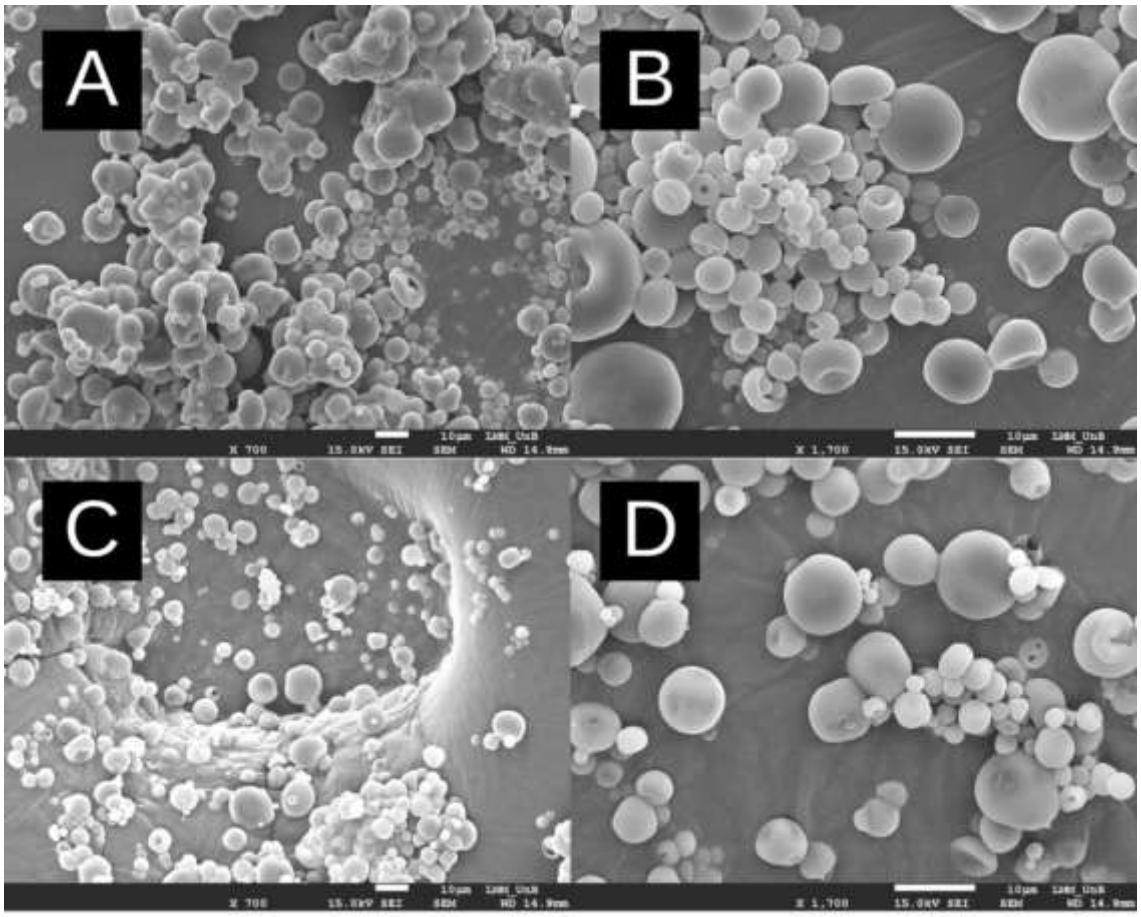

Figure 1: Scanning electron photomicroscopy of formulations F4 700X (A), F4 1700X (B), F5 700X (C), and F5 1700X (D)

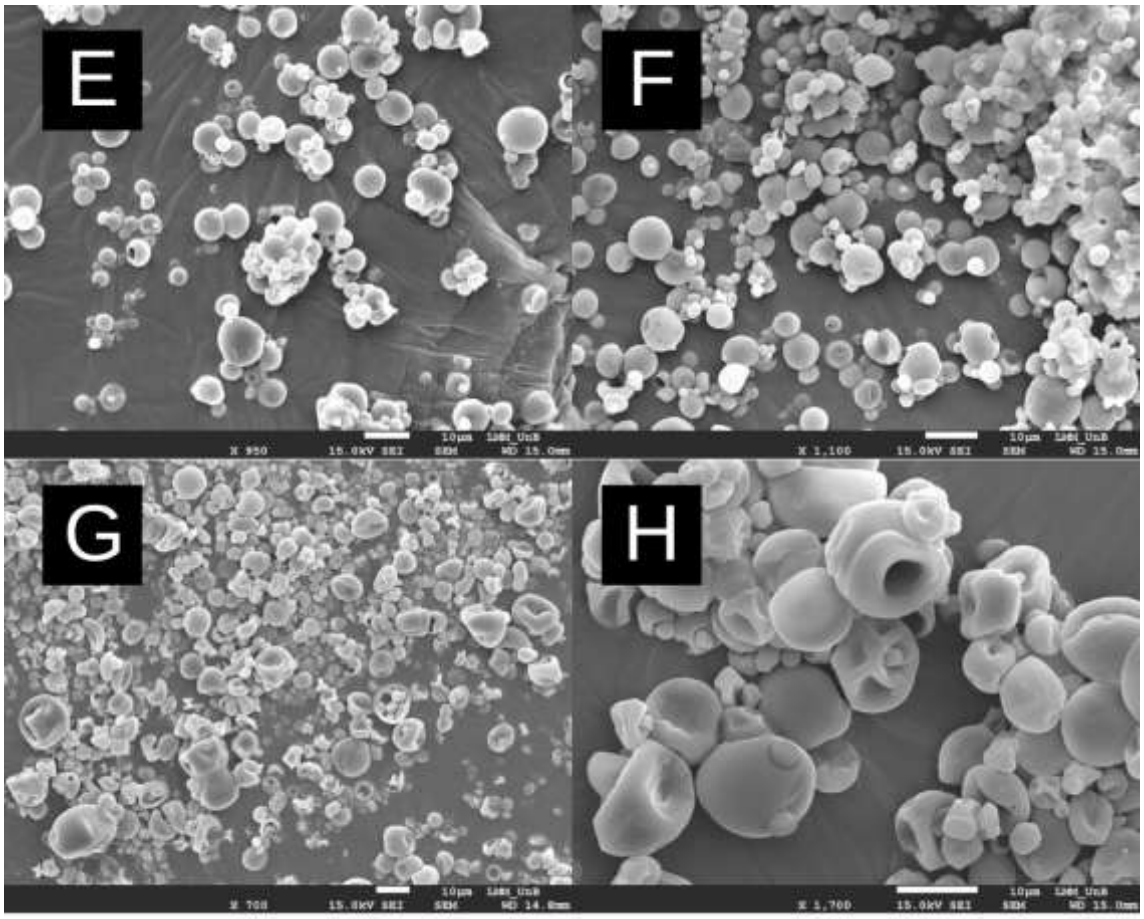

Figure 2: Scanning electron photomicroscopy of formulations F10 950X (E), F10 1700X (F), WF 700X (G), and WF 1700X (H)

Size, Zeta potential, PDI (Polydispersity Index), and pH

Microparticles with and without essential oil were evaluated for hydrogen potential (pH), Zeta potential, size, and PDI, which are physicochemical characteristics that assist in the evaluation of the stability of pharmaceutical formulations. Table 2 shows the average values of the readings referring to pH, Zeta potential, size, and PDI.

Table 2: Mean values referring to the characterization parameters of the microparticle formulations

Formulation	pH	ZP (mV)	Size (nm)	PDI
WF	5,0	-0,384	272,93	0,217
F4	4,5	-26,56	267,56	0,283
F5	5,0	-28,13	535,46	0,671
F10	5,8	-0,416	603,86	0,709

WF = White formulation; pH = hydrogen potential; ZP= Zeta potential; PDI= Polydispersity index

The negative values of the Zeta potential are desirable, confirming that the microparticles were dispersed, something relevant to remain stable when it comes to pharmaceutical dispersions, since the dispersion of particles with expressive negative values results in greater stability of the formulations avoiding their aggregation[37]. However, when the value of the Zeta potential is close to zero, there are fewer repulsion forces between the particles, so they tend to aggregate[18], this is confirmed in formulations F10 and WF, with essential oil and without essential oil, respectively.

Regarding size and PDI, the values obtained by the Zetasizer Nano presented values of approximately 200 to 600 nanometers and PDI in the range of 0.217 to 0.709. Some formulations had PDI values above the range of 0.3 showing that the formulation is polydisperse with respect to size, i.e., more than one population of particles. The graphs obtained in Zetasizer confirmed the existence of more than one population of microparticles present in formulations F5 and F10. The presence of particles with varying sizes is common in pharmaceutical dispersions, this can be confirmed by the SEM presented in Figures 1 and 2.

The pH reading was also within expected values considering the presence of the surfactant Tween 80 that contributed with acidic pH values. The pH also showed that there was no variation in the amount of microencapsulated essential oil, with a reading around 4.5 to 5.8. The formulation without essential oil did not present relevant variations in relation to the others with essential oil, so the presence of essential oil did not interfere with the pH values.

Spectrophotometric analysis and encapsulation efficacy of microparticles

The microparticles obtained with the essential oils (*Cinnamomum cassia* and *Eugenia caryophyllus*) showed EE values, according to calculations obtained using equation (1). The analyses showed a low EE for the F5 (14.90±0.002), but the formulations F4 (63.18±0.01) and F10 (40.66±0.006) obtained values above 40% of EE for CCEO and ECEO, respectively.

The encapsulation efficiency values are directly related to the type of polymer used to obtain the microparticles and the size of the particles obtained[18,38]. Another factor that should be considered when it comes to the use of essential oils as an active ingredient for encapsulation is their volatility, which can interfere with encapsulation efficiency. According to the results obtained, it is possible to verify that the encapsulation efficiency of the CCEO was higher for formulation F4 when compared to the other formulations, which can be explained by the fact that the concentration of gum arabic is lower when compared to F5, and by the fact that the encapsulated essential oil (CCEO) had a higher encapsulation affinity to this polymeric material, than the essential oil (ECEO) in formulation F10. However, another relevant factor must be considered when evaluating the two formulations F4 and F5 whose essential oil and polymers used are the same, the amount of gum arabic (9.9 g) and the proportion of the oil phase (10%) of F5 is higher concerning F4 (Table 1), which corroborates the fact that the increase in the amount of polymer and the proportion of the oil phase did not influence the proportional increase in encapsulation efficiency. So, as mentioned by Estevinho et al.[18], the choice of the polymer may interfere with the encapsulation rate, so it is possible that the CCEO may present a higher encapsulation rate using other types of polymers as an encapsulation material. The same may occur for the ECEO because the encapsulation rate was lower in relation to the other essential oil using the same polymers. However, the result of 63.18% encapsulation in F4 is considered a good result compared to the others.

Evaluation of the antimicrobial activity of essential oils

The agar disk diffusion bioassay to analyze the antimicrobial activity of essential oils (*Eugenia caryophyllus* and *Cinnamomum cassia*) obtained significant microbial growth inhibition values, as shown in Table 3.

Table 3: Mean values of microbial growth inhibition halos (mm) obtained using concentrations of 10 to 100% of the essential oils *Eugenia caryophyllus* and *Cinnamomum cassia*

%ECEO	*C. albicans*	*E. coli*	*S. aureus*	%CCEO	*C. albicans*	*E. coli*	*S. aureus*
10	17.0	0	0	**10**	35.0	11.0	12.0
20	1.5	0	11.5	**20**	38.0	19.0	23.0
30	16.0	0	13.0	**30**	44.0	23.0	22.0
40	26.0	13.5	16.5	**40**	41.0	19.0	31.0
50	38.5	13.5	19.5	**50**	43.0	25.0	30.5
60	28.5	21.5	19.5	**60**	50.0	24.5	31.0
70	35,5	24.5	22.5	**70**	48.0	29.5	33.0
80	40.0	23.5	21.0	**80**	49.5	32.0	34.5
90	31.5	24.5	24.5	**90**	47.5	34.0	35.5
100	31.5	26.0	22.0	**100**	40.0	31.0	39.0
GT	-	30.0	25.0	**GT**	-	28.4	23.0
MZ	17.0	-	-	**MZ**	20.2	-	-

GT: Gentamicin; MZ: Miconazole; ECEO: *Eugenia caryophyllus essential oil*; CCEO: *Cinnamomum cassia essential oil.*

The arithmetic means of the inhibition halos obtained in the agar disk diffusion bioassay is represented in Table 3, and from these results a selection of some proportions of each EO was made to prepare the microparticles obtained by spray drying, using vegetable oil as an essential oil carrier vehicle. The values of inhibition diameter less than 15.0 mm were disregarded because they did not present significant inhibitory activity, according to Abrantes et al.[4] The EOs *Eugenia caryophyllus* and *Cinnamomum cassia* presented mean inhibition halo diameter with values above the standard used and also close to the standard. The growth inhibition response against *C. albicans* was superior to the miconazole nitrate standard with both essential oils used. The best microbial growth inhibition response, when using bacteria (*E. coli* and *S. aureus*), was obtained with CCEO, values higher than the Gentamicin standard.

Much of the inhibitory effect was similar to that demonstrated by Marchese et al.[39], who attributed the antimicrobial effect to the high rate of terpenes found in ECEO, mostly to eugenol, which has bactericidal activity. Escobar[40], proposes three possible mechanisms of action exerted by eugenol on cells: (a) imbalance in ion homeostasis; (b)

changes in the cell membrane; and (c) generation of oxidative stress. This corroborates the results obtained in the tests in which the essential oil *E. caryophyllus* thanks to these mechanisms may be an ally in the fight against bacteria, Gram-negative or Gram-positive.

The results presented in Table 3, for the three microorganisms, show that the antimicrobial response didn´t always increase with increasing essential oil concentration, as there was more of a correlation between the microorganism's sensitivity and the essential oil used. This was observed with ECEO against *Candida albicans* even with increasing concentration, there was not always an increase in the size of the inhibition zone. However, when CCEO was used against *Candida albicans*, there was generally an increase in the size of the inhibition zone as the CCEO concentration increased.

Based on the results obtained, it is noteworthy that the CCEO was able to inhibit all tested microbial strains, when challenged in a 1:1 (EO:VO) ratio. Results that emphasize the antimicrobial action of this essential oil were mentioned in studies by Unlu et al.[41], Clemente et al.[42], and Vasconcellos et al.[43], who also sought to determine the antimicrobial mechanism of *Cinnamomum cassia* and reported that CCEO inhibits the sensing quorum of the biofilm; stimulates the inhibition of cell division, inhibits ATPase, forms porins in the biofilm membrane, and alters bacterial mobility. Thus, the choice of both CCEO and ECEO to prepare the microparticles in order to evaluate their antibiofilm action was based on the promising results of the use in non-encapsulated form with inhibition halos above 20.0 mm.

Minimum inhibitory and bactericidal concentrations of essential oils

The results of MIC, MBC and MFC the CCEO employing the microorganisms *E. coli* (0.06/0.12), *S. aureus* (0.03/0.25), and *C. albicans* (0.01/0.03), confirmed that the CCEO showed lower MIC, MBC and MFC values when challenged against the three microorganisms, something that was also observed when the agar diffusion technique was used to evaluate the antimicrobial action of this essential oil against the same microorganisms. The results of ECEO showed MIC, MBC and MFC values to *E. coli* (0.12/0.25), *S. aureus* (0.25/0.50), and *C. albicans* (0.25/1.0), respectively. The equivalence of the results against two different techniques, but with similar objectives, reinforce that CCEO has better antimicrobial properties than ECEO when using the same

microbial strains. It should be noted that the CCEO is more sensitive to Gram-positive bacteria in relation to Gram-negative, and these microbial strains form biofilms, as well as the yeast *C. albicans.* The results obtained for MIC and MBC were similar to the study by Liang et al.[44], which used CCEO in non-encapsulated form and in nanoemulsion to evaluate its antibacterial activity against 6 microbial strains, of which *S. aureus* and *E. coli* were also evaluated.

Essential oil microparticles as a treatment against biofilms

Means and standard deviations of Log reductions by bacterial species and treatment are presented in Table 4. The F5 treatment showed the highest average reduction (4.27 ± 0.70), followed by F4 and F10. Regarding bacterial species, the greatest average reduction was observed for surfaces by *E. coli* (4.19 ± 0.30) and the lowest by *S. aureus*.

Means between groups were compared by two-way ANOVA. The interaction effect was not significant in the initial model (p-value = 0.41). Therefore, a model with just the main effects was chosen, showing significance for comparisons between levels of both microbial species (p-value = $3x10^{-6}$) and treatment (p-value = 0.001). Diagnostic plots did not reveal any violations of ANOVA assumptions (not shown).

Tukey HSD tests were used to identify specific differences between factor levels, showing that surfaces contaminated by bacterial species *S. aureus* presented significantly lower average reduction than the other 2 species and treatment F5 differs significantly from the F10 and control treatments (Table 4). Compact letter display (CLD) was used to indicate significant differences between levels of bacterial species and treatments, considering a significance level $\alpha = 0.05$.

Table 4. Log reduction averages ± standard deviations (and significance) of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* adhered to stainless steel obtained after exposure to treatments (F4, F5, F10, and control)

Factors	**Means ± SD$^{(CLD)\ 1}$**	**% [2]**
Microbial species		
C. albicans	3.91 ± 0.65^{a}	93
E. coli	4.19 ± 0.30^{a}	100
S. aureus	3.30 ± 0.47^{b}	79
Treatments		
Control	3.47 ± 0.58^{a}	81
F4	3.81 ± 0.36^{ab}	89
F5	4.27 ± 0.70^{b}	100
F10	3.66 ± 0.51^{a}	86

Control: benzalkonium chloride; F4 and F5: CCEO; F10: ECEO

The Log reduction was obtained by the difference between log (10^{8}) (the log of initial cell count) and the log of survival cell count following antimicrobial treatments.

[1] Compact letter display (CLD), presented along with averages and standard deviations (SD), is used to indicate significant differences between levels of bacterial species and treatments given by Tuckey HSD test, followed by 2-way ANOVA. Averages that were not statistically different share the same letter considering a significance level $\alpha = 0.05$.

[2] % Refers to percentage decrease compared to the highest value (references with % equals 100) per species and per treatment.

Based on Table 4, it was observed that the most resistant biofilm to treatments was *S. aureus*, with a log reduction of 3.30±0.47. The other challenged biofilms were more sensitive to treatments F4, F5, and F10. From the results obtained, it is possible to verify that there were log reduction responses for all treatments, but the CCEO had a better log reduction response than the ECEO, confirming the results obtained when the non-encapsulated essential oils were used, that is, the antimicrobial action of the CCEO remained even when it was inserted into microparticles. Logarithmic reductions in the range of 3 to 4 logarithmic cycles when it comes to biofilm is considered a significant response considering the difficulty that is eradicating these types of microbial systems. A relevant aspect to be emphasized is that the three treatments presented values above the control (benzalkonium chloride) regarding logarithmic reduction.

CONCLUSION

The use of essential oils for antimicrobial and antibiofilm purposes showed significant results in the challenges, especially for the *Cinnamomum cassia* essential oil. The encapsulation of essential oils in microparticles prepared by spray drying confirmed that the antimicrobial activity was not altered, thus being an alternative to keep essential oils protected from oxidation reactions, common during their exposure to air and light. The challenge against biofilms of the microorganisms *S. aureus*, *E. coli*, and *C. albicans* showed values of relevant logarithmic reductions, confirming the antibiofilm action of both *Cinnamomum cassia* and *Eugenia caryophyllus* essential oils with greater sensitivity to *E. coli* and *C. albicans.* The resistance of the *S. aureus* strain in relation to other microorganisms was maintained in all experiments, confirming what has already been published in other scientific studies.

The encapsulation rate of the essential oils was considered good, but more studies need to be carried out changing the types of polymers to verify if this will bring better results regarding the encapsulation efficiency rate and antibiofilm response.

DISCLOSURES

The authors declare the absence of conflict of interest associated with the publication of this work.

ACKNOWLEDGEMENTS

The authors thank the collaboration of the Microscopy and Microanalysis Laboratory and the Morphology Laboratory, both from the Institute of Biological Sciences at the University of Brasília. This work was funded by the Federal District Research Support Foundation.

REFERENCES

1 Kerekes E, Vidács A, Jenei JT, Gömöri C, Takó M, Chandrasekaran M, et al. Essential oils against bacterial biofilm formation and quorum sensing of food-borne pathogens and spoilage microorganisms. In: Méndez-Vilas A (ed) The battle against microbial pathogens: basic science, technological advances and educational programs, 2015;429–437.

2 Garcia LGS, Rocha MG, Lima LR, Cunha AP, Oliveira JS, Andrade APC, Ricardo NMPS, Pereira-Neto W.A, Sidrim JCC, Rocha MFG, Vieira RS, Brilhante RSN. Essential oils encapsulated in chitosan microparticles against *Candida albicans* biofilms. Int. J. Biol. Macromol. 2021;166:621–632. https://doi.org/10.1016/j.ijbiomac.2020.10.220.

3 O'Toole G, Kaplan HB and Kolter R. Biofilm formation as microbial development. Annu. Rev. Microbiol. 2000;54:49–79. https://doi.org/10.1146/annurev.micro.54.1.49.

4 Abrantes MR, Lima EO, Araújo M, Medeiros P, Menezes CP, Guerra FQS, Milan EP. Atividade antifúngica de óleos essenciais sobre leveduras *Candida* não *albicans*. Rev. Bras. Farm. 2013;94(3):227-233.

5 De Vita D, Friggeri L, D`Auria FD, Pandolfi F, Piccoli F, Panella S, Palamara AT, Simonetti G, Scipione L, Di Santo R, Costi R, Tortorella S. Activity of caffeic acid derivatives against *Candida albicans* biofilm. Bioorg. Med. Chem. Lett. 2014;24:1502-1505. https://doi.org/10.1016/j.bmcl.2014.02.005.

6 Pinheiro RR, Sabagh BP, Vidal LMR, Veras JFC, Vieira VV, Souto ASS, Pereira DCR, Neves MC, Villas-Boas MHS. Avaliação da contaminação microbiana em produtos desinfetantes de uso geral. Rev. Inst. Adolfo Lutz. 2013;72(2):170-4. https://doi.org/10.18241/0073-98552013721560

7 Oliveira, MMM, Brugnetra DF, Piccoli RH. Biofilmes microbianos na indústria de alimentos: uma revisão. Rev. Inst. Adolfo Lutz. 2010;69(3):277-84. https://doi.org/10.53393/rial.2010.v69.32626

8 Vergis J, Gokulakrishnan P, Agarwal RK, Kumar A. Essential Oils as Natural Food Antimicrobial Agents: A Review. Crit. Rev. Food Sci. Nutr. 2013;55(10):1320–1323. https://doi.org/10.1080/10408398.2012.692127.

9 Silva BD, Rosário DKA, Weitz DA, Conte-Júnior CA. Essential oil nanoemulsions: Properties, development, and application in meat and meat products. Trends Food Sci. Techn. 2022;121:1-13. https://doi.org/10.1016/j.tifs.2022.01.026

10 Mouhoub A, Guendouz A, Alaoui-Talibi ZEl, koraichi SI, Delattre C, Modafar CEl. Evaluation of different characteristics and bioactivities of chitosan-based films incorporating *Eugenia caryophyllus* and *Cinnamomum zeylanicum* essential oils. Mater. Chem. Phys. 2023;307:1-8. https://doi.org/10.1016/j.matchemphys.2023.128201

11Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol. 2000; 31**:**247-256. https://doi.org/10.1590/S1517-83822000000400003

12 Mau JL, Chen CP, Hsieh PC. Antimicrobial effects of extracts from Chinese chive, cinnamon and corni fructus. J. Agric. Food Chem. 2001;49(1):183-188. https://doi.org/10.1021/jf000263c

13 Farias KS, Alves FM, Santos-Zanuncio VS, Sousa Jr PT, Silva DB, Carollo CA. Global distribution of the chemical constituents and antibacterial activity of essential oils in Lauraceae family: A review. S. Afr. J. Bot. 2023;155:214-222. https://doi.org/10.1016/j.sajb.2023.02.028

14 Oliveira MMM, Brugnera DF, Nascimento JA, Piccoli RH. Control of planktonic and sessile bacterial cells by essential oils. Food Bioprod. Process. 2012;90:809-818. https://doi.org/10.1016/j.fbp.2012.03.002

15 Ooi LSM, Li Y, Sheung-Lau K, Wang H, Wong EYL, Ooi VE. Antimicrobial Activities of Cinnamon Oil and Cinnamaldehyde from the Chinese Medicinal Herb *Cinnamomum cassia* Blume. Am. J. Chin. Med. 2006;34(3): 511–522. https://doi.org/10.1142/S0192415X06004041

16 Van CK, Nguyen PTN, Nguyen TTT, Bach LG. Microencapsulation of Citrus latifolia peel essential oil by spray-drying using maltodextrin: Characterization, antimicrobial activities, and release profile. LWT- Food Sci. Technol. 2024;197: 1-12. https://doi.org/10.1016/j.lwt.2024.115825

17 Shafiei A, Safaei-Ghomi J, Masoomi R. Microencapsulation of *Ducrosia anethifolia* essential oil: Optimization characterization, and stability. Appl. Food Res. 2024;4:1-10. https://doi.org/10.1016/j.afres.2024.100598

18 Estevinho BMAN, Rocha FAN, Santos LMS, Alves MAC. Using water-soluble chitosan for flavour microencapsulation in food industry. J. Microencapsul. 2013;30(6): 571–579. https://doi.org/10.3109/02652048.2013.764939.

19 Castel V, Rubiolo AC, Carrara CR. Brea gum as wall material in the microencapsulation of corn oil by spray drying: Effect of inulin addition. Food Res. Int. 2018;103:76–83. https://doi.org/10.1016/j.foodres.2017.10.036

20 Outuki PM, Belloto de Francisco LM, Hoscheid J, Bonifácio KL, Barbosa DS, Cardoso MLC. Development of arabic and xanthan gum microparticles loaded with an extract of *Eschweilera nana* Miers leaves with antioxidant capacity. Colloids Surf. A: Physicochem. Eng. Asp. 2016;499:103–112. https://doi.org/10.1016/j.colsurfa.2016.04.006

21 Drusch S, Mannino S. Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids. Trends Food Sci. Technol. 2009;20:237-244. https://doi.org/10.1016/j.tifs.2009.03.007

22 Al-Hamayda A, Abu-Jdayil B, Ayyash M, Tannous J. Advances in microencapsulation techniques using Arabic gum: A comprehensive review. Ind. Crop. Prod. 2023;205:1-10. https://doi.org/10.1016/j.indcrop.2023.117556

23 Bajac J, Nikolovski B, Loncarevi´ I, Petrovi´ J, Bajac B, Đurovi´ S, Petrovi´ L. Microencapsulation of juniper berry essential oil (*Juniperus communis* L.) by spray

drying: microcapsule characterization and release kinetics of the oil. Food Hydrocoll. 2022;125:1-10. https://doi.org/10.1016/j.foodhyd.2021.107430

24 Li H, Peng F, Lin JX, Xiong T, Huang T. Preparation of probiotic microcapsules using gelatin-xylooligosaccharides conjugates by spray drying: Physicochemical properties, survival, digestion resistance and colonization. Food Biosci. 2023;52:1-7. https://doi.org/10.1016/j.fbio.2023.102462

25 Ascheri DPR, Marquez MOM, Martucci ET. Microencapsulação de óleo essencial de laranja: Seleção de material de parede. Ciênc. Tecnol. Aliment. 2003;23(supl.):1-6. https://doi.org./10.1590/S0101-20612003000400002

26 Fernandes RVB, Carmo EL, Borges SV, Botrel DA, Silva YF, Souza HJB. Comportamento de óleo essencial de alecrim microencapsulado por spray drying em diferentes umidades relativas. Ciênc. Agríc. 2016;14(1):73-82. https://doi.org/10.28998/rca.v14i1.2469

27 Granata G, Stracquadanio S, Leonardi M, Napoli E, Consoli GMT, Cafiso V, Stefani S, Geraci C. Essential oils encapsulated in polymer-based nanocapsules as potential candidates for application in food preservation. Food Chem. 2018;269:286-292. https://doi.org./10.1016/j.foodchem.2018.06.140.

28 Sansone F, Picerno P, Mencherini T, Villecco F, D'Ursi AM, Aquino RP, Lauro MR. Flavonoid microparticles by spray-drying: Influence of enhancers of the dissolution rate on properties and stability. J. Food Eng. 2011;103:188-196. https://doi.org/10.1016/j.jfoodeng.2010.10.015

29 Farmacopéia Brasileira. 6. ed. 2019. Agência Nacional de Vigilância Sanitária. Disponível em: http://portal.anvisa. gov.br/documents/33832/259143/Volume+I+Pronto.pd f/4ff0dfe8-8a1d-46b9-84f7-7fa9673e1ee1. Acesso em: 20 jan. 2024

30 Pinto TJA, Kaneko TM, Pinto AF. Controle Biológico de Qualidade de Produtos Farmacêuticos, Correlatos e Cosméticos. 4° ed. São Paulo: Atheneu Editora; 2015.

31 Romano I, Granata G, Polia A, Finorea I, Napolib E, Geracib C. Inhibition of bacterial growth on marble stone of 18th century by treatment of nanoencapsulated essential oils. Int. Biodeter. Biodegr. 2020;148:1-5. https://doi.org/10.1016/j.ibiod.2020.104909

32 Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc. 2008;3(2):163-175. https://doi.org/10.1038/nprot.2007.521.

33 Millezi AF, Piccoli RH, Oliveira JM, Pereira MO. Anti-biofim and Antibacterial Effect of Essential Oils and Their Major Compounds. J. Essent. Oil-Bear. Plants. 2016:19 (3):624-631.https://doi.org/10.1080/0972060X.2014.960262

34 R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2021. URL https://www.R-project.org/

35 Estevinho BN, Damas AM, Martins P, Rocha F. Microencapsulation of ß-galactosidase with different biopolymers by a spray-drying process. Food Res. Int. 2014;64:134-140. https://doi.org/10.1016/j.foodres.2014.05.057

36 Mladenovska K, Cruaud O, Richomme P, Belamie E, Raicki RS, Venier-Julienne MC, Popovski E, Benoit JP, Goracinova K. 5-ASA loaded chitosan-Ca-alginate microparticles: Preparation and physicochemical characterization. Int. J. Pharm. 2007; 345:59–69. https://doi.org/ 10.1016/j.ijpharm.2007.05.059

37 Lachman L, Lieberman HA, Kaning JL. Teoria e prática na indústria farmacêutica. 2.ed. Lisboa: Fundação Calouste Gulberkian; 2010.

38 Ribeiro AM, Shahgol M, Estevinho BN, Rocha F. Microencapsulation of Vitamin A by spray-drying, using binary and ternary blends of gum arabic, starch and maltodextrin. Food Hydrocoll. 2020;108:1-13. https://doi.org/10.1016/j.foodhyd.2020.106029

39 Marchese A, Barbieri R, Coppo E, Orhan IE, Daglia M, Nabavi SF, Ajami M. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit. Rev. Microbiol. 2017;43(6):668–689. https://doi.org/ 10.1080/1040841X.2017.1295225.

40 Escobar-Garcia M, Rodríguez-Contreras K, Ruíz-Rodríguez S, Pierdant-Pérez M, Cerda-Cristerna B, Pozos-Guillén A. Eugenol Toxicity in Human Dental Pulp Fibroblasts of Primary Teeth. J. Clin. Pediatr. Dent. 2026;40(4):312-318.https://doi.org/ 10.17796/1053-4628-40.4.312.

41 Unlu M, Ergene E, Unlu GV, Zeytinoglu HS, Vural N. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from Cinnamomum zeylanicum Blume (Lauraceae). Food Chem. Toxicol. 2010;48(11):3274–3280. https://doi.org/ 10.1016/j.fct.2010.09.001.

42 Clemente I, Aznar M, Silva F, Nerín C. Antimicrobial properties and mode of action of mustard and cinnamon essential oils and their combination against foodborne bacteria. Innovative Food Sci. Emerg. Technol. 2016;36:26–33. https://doi.org/10.1016/j.ifset.2016.05.013

43 Vasconcelos NG, Croda J, Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. Microb. Pathog. 2018;120:198–203. https://doi.org/10.1016/j.micpath.2018.04.036

44 Liang D, Feng B, Li N, Su L, Wang Z, Kong F, Bi Y. Preparation, characterization, and biological activity of *Cinnamomum cassia* essential oil nano-emulsion. Ultrason. Sonochem. 2022;86:1-10. https://doi.org/10.1016/j.ultsonch.2022.106009

EVALUATION OF THE ANTIBIOFILM ACTIVITY OF ESSENTIAL OILS CINNAMOMUM CASSIA AND EUGENIA CARYOPHYLLUS MICROENCAPSULATED BY SPRAY DRYING

Submitted: December 20, 2024
Accepted: August 26, 2025
Published: January 2, 2026

Author contributions
Guilherme de Ataides Ribeiro: Investigation
Elton Clementino da Silva: Resources
Silvia Ribeiro de Souza: Investigation
Joanlise Marco de Leon Andrade: Formal analysis
Naira da Silva Campos: Investigation
Mônica Valero da Silva: Project administration
All the authors approved the final version of the text.
Conflict of interest: The authors declare no conflict of interest.
Funding: Fundação de Apoio à Pesquisa do Distrito Federal (FAPDF)
Corresponding author: Mônica Valero da Silva Universidade de Brasília – UnB Faculdade de Ciências da Saúde Departamento de Farmácia. UnB - Plano Piloto, Brasília/DF, Brazil. Zip code 70910-900
Editor-in-chief: Adriane Cristina Bernat Kolankiewicz. PhD **Editor:** Matias Nunes Frizzo. PhD