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EVALUATION OF ANTIMICROBIAL ACTIVITY OF CURCUMIN AND CAPSAICIN-LOADED SOLID LIPID NANOPARTICLE[1]

AVALIAÇÃO DA ATIVIDADE ANTIMICROBIANA DE NANOPARTÍCULA LIPÍDICA SÓLIDA CONTENDO CURCUMINA E CAPSAICINA

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ABSTRACT

In recent research curcumin and capsaicin have been used as an antimicrobial agent; in addition, curcumin is considered a photosensitizer in antimicrobial photodynamic therapy (aPDT). However, both have low aqueous solubility, a characteristic that makes it difficult to use them in clinical trials, indicating a small use. To circumvent these problems, incorporating them into solid lipid nanoparticle (NLS) may allow for more efficient delivery. This study aims to evaluate the antimicrobial activity of solid lipid nanoparticles containing curcumin and capsaicin (NCC). For this evaluation, the antimicrobial activity analysis was performed through the minimum inhibitory concentration (MIC), diffusion disc and TFA. For MIC and aPDT, triphenyl tetrazolium chloride (TTC) was used as an indicator of oxidation, which differentiates metabolically active tissues from those that are not active. Several gram-negative and gram-positive bacteria were evaluated. Based on the results obtained from the three evaluations, it is concluded that NCC did not produce an antimicrobial effect on either gram-negative or gram-positive bacteria evaluated here.

Keywords: antimicrobial photodynamic therapy, minimum inhibitory concentration, diffusion disc.

RESUMO

Nas pesquisas recentes a curcumina e a capsaicina tem sido utilizado como agente antimicrobiano, além disso, a curcumina é considerada fotossensibilizador na terapia fotodinâmica antimicrobiana (TFA). Entretanto, ambos apresentam baixa solubilidade aquosa, característica que dificulta utilizá-los em ensaios clínicos, indicando um pequeno aproveitamento. Para contornar esses problemas, incorporá-los em nanopartícula lipídica sólida (NLS) poderá permitir uma entrega mais eficiente. Este estudo tem como objetivo avaliar a atividade antimicrobiana da nanopartículas lipídicas sólida contendo curcumina e capsaicina (NCC). Para esta avaliação foi realizada análise da atividade antimicrobiana através da metodologia de concentração inibitória mínima (CIM), disco de difusão e TFA. Para o CIM e TFA foi utilizado cloreto de trifenil tetrazolio (TTC) como indicador de oxirredução, que diferencia tecidos metabolicamente ativos daqueles não ativos.

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Foram avaliadas várias bactérias gram-negativa e gram-positivas. Com base nos resultados obtidos das três avaliações, conclui-se que as NCC não produziram efeito inibitório sobre bactérias gram-negativas e gram-positivas testadas aqui.

Palavras-chave: *terapia fotodinâmica antimicrobiana, concentração inibitória mínima, disco de difusão.*

INTRODUCTION

In recent decades the global burden of antibiotic resistance has revived interest in antimicrobial properties from plants. Curcumin and capsaicin independently have antimicrobial activity (MARINI *et al*., 2015; GUTIERREZ *et al*., 2017), anti-inflammatory (KUMAR; MANJUNATHA; RAJESH, 2017), anticarcinogenic (CLARK; LEE, 2016; EL-FAR *et al*., 2019), antioxidants (GÓMEZ-ESTACA *et al*., 2017; SANDOVAL-CASTRO *et al*., 2017) among others (ZHENG *et al*., 2017, 2018). Capsaicin potentiates tissue permeability for curcumin according to Manjunatha and Srinivasan (2006). Thus, combining them into solid lipid nanoparticles (SLN) solves the problems of aqueous solubility and consequent clinical application, as it increases the bioavailability of these compounds.

SLN has unique properties that make it a promising tool for releasing antimicrobial compounds, leading to some cosmetic and pharmaceutical products for skin care applications. SLNs contain occlusive excipients which, upon application to the skin, rapidly form a thin film to reduce evaporation of water, improving hydration. They are stable in water and dermal cream and therefore can be readily incorporate into cosmetics and skin care products (WISSING; MÜLLER, 2003). In addition to topical applications, NLSs, in the form of tablets, capsules and pellets, may be used by oral administration (POUTON, 2000). Another prominent example of drug delivery, based on NLSs, is the pulmonary administration of antimicrobials, since they enter the lungs and are phagocytized by alveolar macrophages and subsequently transported to lymphoid tissues (GELPERINA *et al*., 2005). Although, the developmental history of SLN-based antimicrobial delivery system is relatively new, when compared to other nanoparticle systems such as liposomes and polymer nanoparticles, blank nanoparticle demonstrate great therapeutic potentials. In this context, this study has as general proposal to evaluate the antimicrobial activity of curcumin and capsaicin-loaded solid lipid nanoparticle.

MATERIALS AND METHODS

Curcumin and Capsaicin-Loaded Solid Lipid Nanoparticle

Curcumin and capsaicin loaded-solid lipid nanoparticle (NCC) suspensions were prepared using a high shear mixer (RAFFIN *et al*., 2012). The NCC was previously characterized and

validated by our research group (NISHIHIRA *et al*., 2019). Briefly, the physicochemical characteristics of suspensions results of NCC and nanoparticle without curcumin and capsaicin (NB) are presented in table 1.

Table 1 - The results of the physicochemical characterization of NCC and NB.

	Diameter (nm)	PDI	Zeta potential (mV)	pH
NCC	112 ± 4	0.242 ± 0.00	- 5.62 ± 0.89	5,83 ± 0.32
NB	111 ± 1	0.216 ± 0.01	- 5.83 ± 0.25	5.80 ± 0.06

Fonte: NISHIHIRA *et al*. (2019)

Strains and Culture conditions

For the analysis of antimicrobial properties, the compounds were analyzed by the microdilution technique to obtain the minimum inhibitory concentration (MIC) against the following bacterial isolates: *Staphylococcus aureus* (ATCC 25923)*, Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 27212), *Proteus vulgaris* (clinical isolate/UFN), *Escherichia coli* (ATCC 25922)*, Serratia liquefaciens* (clinical isolate/UFN), *Enterobacter cloacae* (clinical isolate/UFN), *Aeromonas media* (clinical isolate/UFN), *Salmonella paratyphi* (clinical isolate/UFN), *A. veronii* (clinical isolate/UFN), *Salmonella enterica* (clinical isolate/UFN) and *coagulase-negative staphylococci* (clinical isolate/UFN). All the strains were stored as frozen stocks with 15% glycerol at -80 °C.

Bacterial suspensions were standardized from an overnight culture in Mueller-Hinton broth (MHB), then incubated at 37 °C for 24 h in the shaker at 120 rpm. After dilution was carried out in MH broth until the turbidity reached an optical density between 0.08 - 0.100 (1.5×10^8 CFU/mL) corresponding to 0.5 McFarland, measured by spectrophotometry at the wavelength of 625 nm.

Disc-Diffusion Assay

The disc-diffusion assay was performed according to the methodology of Shahverdi *et al.* (2007). First, bacterial inoculums at 0.5 McFarland were inoculated on the surface of MH agar plates. Then, 10 µL of NCC, NB, CC (curcumin and capsaicin only) and T (Tween 80) were dispensed onto sterile filter paper disks and left to dry at room temperature. These discs were then placed on MH agar plates, using a sterile forceps to avoid contamination and gently pressed to guarantee full contact with the surface of the agar. Plates were incubated at 37 °C for 24 hours, and the antimicrobial potential of the samples was evaluated based on the mean diameter of the zone of inhibition, around the discs, measured in millimeters.

Minimum Inhibitory Concentration (MIC)

MIC is represented by the lower concentration where an agent with antimicrobial activity prevents the visible growth of a microorganism (ANDREWS, 2001) and minimum bactericidal concentrations (MBCs. A broth microdilution method was used to determine the MIC of NCC, NB, CC, and T, by the method described by Mathers (2015). Briefly, the test was done using 96-well flat-bottomed microplate, with an assay volume of 200 µL/well, where each well received 100 µL of MHB, followed by the addition of 100 µL of NCC reaching the final concentrations of 5, 50, 150 µg/mL for curcumin and 0.17, 1.6, 5 µg/mL of capsaicin. NB, CC, and T were used as control groups in a single concentration equivalent to 150 µg/mL of curcumin and 5 µg/mL of capsaicin of the NCC. Positive and negative groups were represented by MH broth plus microorganisms and MH broth only, respectively. The plates were then inoculated with a 10 µl/well of MHB bacterial cultures, with a concentration of 10^6 CFU/mL, reaching the final bacterial cell concentration in the wells of 10^5 CFU/mL. Then, the microplates were incubated for 24 h at 37 °C. After this time, 10 µL of 2,3,5-triphenyltetrazolium chloride (TTC) solution (2%) were added to all wells and then incubated for two hours at 37 °C. Finally, the red color formation, indicative of cell viability, was optically evaluated, being TTC used as an indicator of oxirreduction, which differentiates metabolically active from non-active cells based on the enzymatic reduction of 2,3,5-triphenyletrazolium (colorless) to 1,3,5-triphenylformazan (reddish color) in living cells (GABRIELSON *et al*., 2002). The MIC was defined as the concentration of the substance that will inhibit the visible growth of a microorganism after 24 hours of incubation.

Antimicrobial Photodynamic Therapy (aPDT)

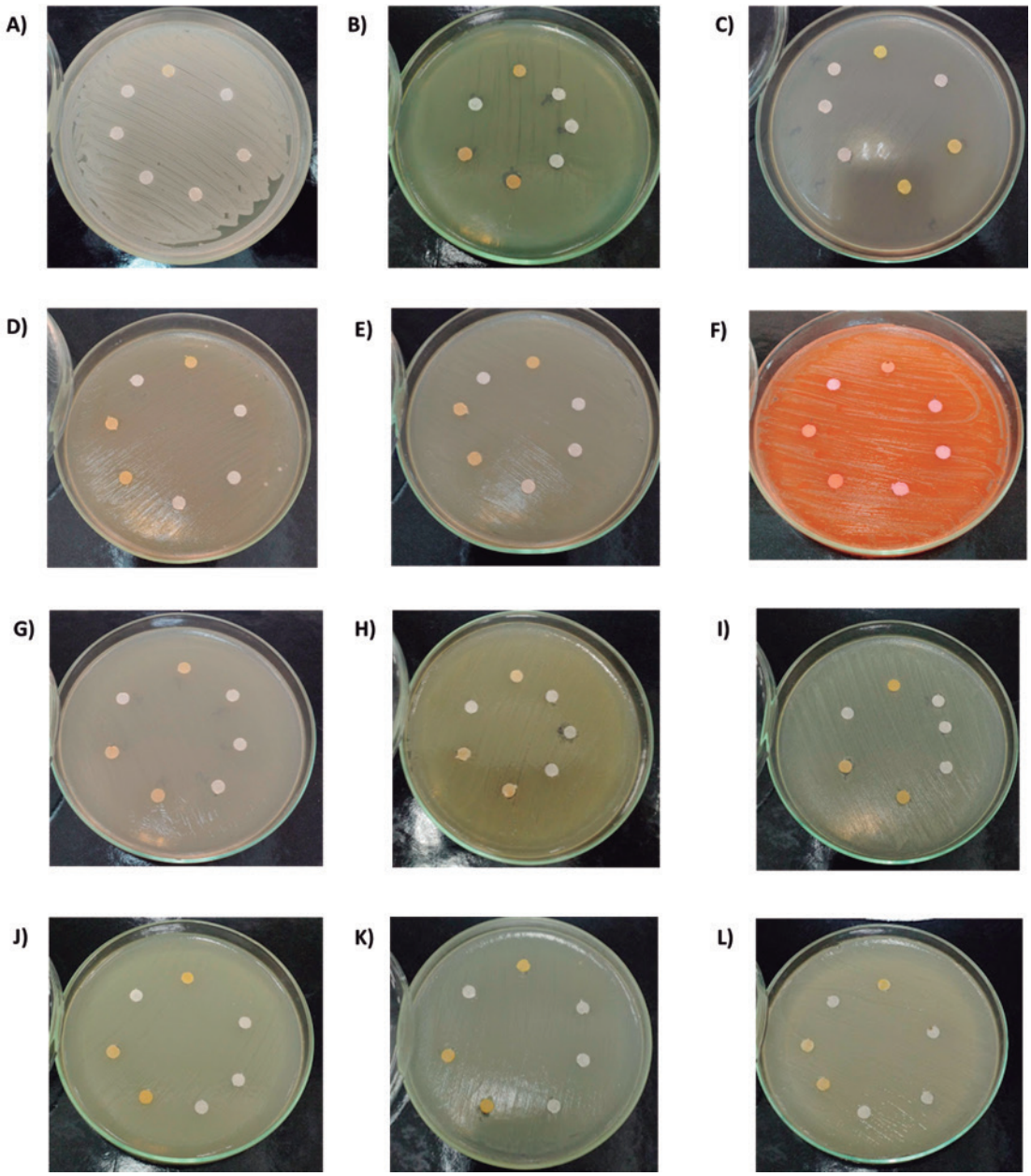
The aPDT were determined qualitatively by the microplate dilution technique described by Gutierrez *et al.* (2017), similar to the methodology described for MIC determination, where 10 µL of the bacterial suspensions and NCC were pipetted in two 96-well flat-bottomed microplates, in the concentrations of 5, 50, 150 µg/mL of curcumin and 0.17, 1.6, 5 µg/mL of capsaicin. As NCC control groups, NB and CC were used in a single concentration equivalent to 150 µg/mL and 5 µg/mL of capsaicin of the NCC. MHB and microorganism represented the positive control group, and the negative group represented only by MHB. The plate containing the strains and the treatments were incubated for one hour, with an incidence of blue LED light (440-460 nm), then incubated for 24 hours at 37 °C (HANAKOVA *et al*., 2014). After this period, 10 µL of TTC (2%) were added and incubated again for another two hours, and the red color formation was evaluated in the wells, representing bacterial growth.

RESULTS AND DISCUSSION

Disc-Diffusion Method

In the test of susceptibility of the microorganisms by the disc-diffusion method, the treatments did not present zones of inhibition around the discs against the different bacterial strains (Figure 1). These results show agreement with those of the MIC, previously presented, arguing that they do not present the antibacterial effect.

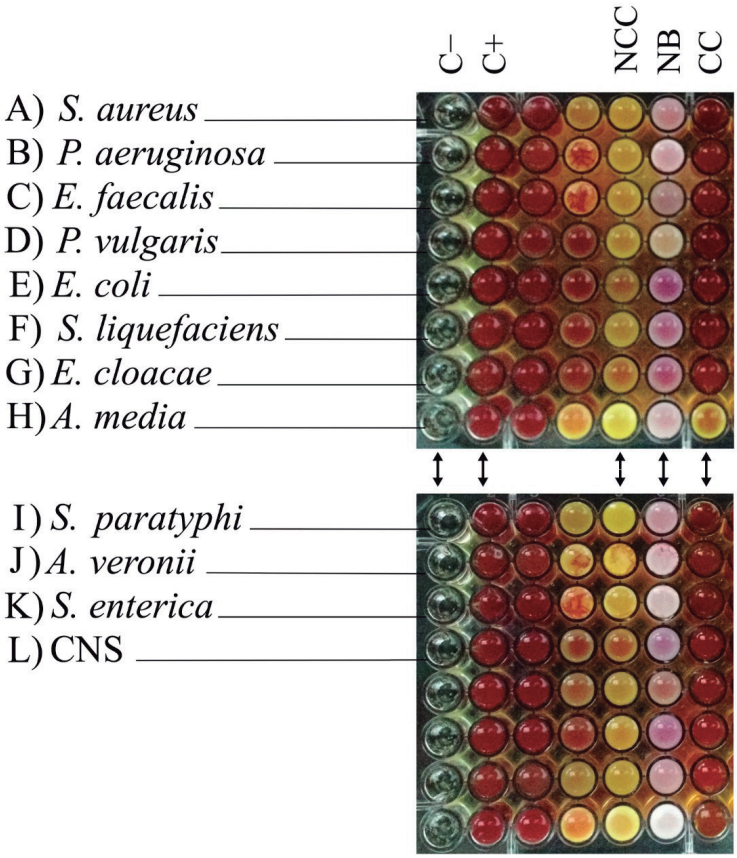
Figure 1 - Determination of the antimicrobial effect by the disc-diffusion method for: A) *S. aureus*, B) *P. aeruginosa*, C) *E. faecalis*, D) *P. vulgaris*, E) *E. coli*, F) *S. liquefaciens*, G) *E. cloacae*, H) *A. media*, I) *S. paratyphi*, J) *A. veronii*, K) *S. enterica*, and L) *coagulase-negative staphylococci* (CNS)



Minimum Inhibitory Concentration (MIC)

The microdilution method represents a low cost, simple, fast and very sensitive technique, allowing to determine the MIC of compounds, representing the lowest concentration of an antimicrobial agent, which prevents the visible growth of a microorganism (ELOFF, 1998; PALOMBO, 2011). The MIC results showed that the NCC does not present antimicrobial activity against the microorganisms tested in this study (Figure 2), confirmed by the TTC red color.

Figure 2 - 96-well plates with treatments and bacteria, after TTC pipetting:
A) *S. aureus*, B) *P. aeruginosa*, C) *E. faecalis*, D) *P. vulgaris*, E) *E. coli*, F) *S. liquefaciens*, G) *E. cloacae*, H) *A. media*, I) *S.paratyphi*, J) *A. veronii*, K) *S. enterica*, e L) *coagulase-negative staphylococci* (CNS)



In contrast to our results, studies have described that the aqueous extract of Curcuma longa extract shows antimicrobial effect at concentrations of 4 to 16 µg/mL against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) (NIAMSA; SITTIWET, 2009). Another study with curcumin nanoparticle of size 2 to 40 nm showed that it is more freely dispersible in water, leading to a more significant antimicrobial activity against *S. aureus, E. coli* and *P. aeruginosa* due to the reduced size of the particles and improved bioavailability (BHAWANA *et al*., 2011; SHAILENDIRAN *et al*., 2011).

However, nanocurcumin demonstrated a more remarkable activity against gram-positive than gram-negative bacteria (BHAWANA *et al*., 2011).

The potential mechanism of antibacterial activity of curcumin is due to the accumulation of intracellular ROS, which cause damage to the conformation of the bacterial cell membrane, causing cell death of *S. aureus* (SHOME *et al*., 2016). On the other hand, MOLINA-TORRES; GARCÍA-CHÁVEZ; RAMÍREZ-CHÁVEZ (1999) determined that capsaicin at concentrations up to 200 or 300 μg/mL only delayed the growth of *E. coli*. In addition, Gunes *et al*. (2016) have demonstrate that *S. aureus, E. faecalis, P. aeruginosa* and *E. coli* showed antimicrobial activities at MIC ranging between 175 and 293 μg/mL, thus showing that the concentrations used in our study were low compared to these studies.

Antimicrobial Photodynamic Therapy (aPDT)

In aPDT, each of these factors (photosensitizing agent, light, oxygen) is harmless by itself, but when combined, they can produce cytotoxic reactive oxygen species (EROs) that can selectively destroy cells (SHARMAN; ALLEN; VAN LIER JE, 1999). The mechanism of action of aPDT is briefly described by the excitation of a non-toxic photosensitizing agent (PS), which forms a long-lived excited triplet state, which then transfers energy to the surrounding molecules, generally for molecular oxygen, to form EROs highly reactive and cytotoxic, such as hydroxyl radicals and singlet oxygen (HAYEK *et al*., 2005; PASCHOAL *et al*., 2013). EROs can modify plasma membrane structures or even DNA (JORI *et al*., 2006), as well as cause cell death through several mechanisms, including lipid peroxidation, inhibition of enzyme systems and agglutination of proteins that are critical for other biological systems (OCHSNER, 1997; ANDERSEN *et al*., 2007). The aPDT may be highly selective for diseased microorganisms or tissues (HAYEK *et al*., 2005; PASCHOAL *et al*., 2013).

During aPDT, only cells with PS selective accumulation, which are also exposed to light, are killed (PFITZNER *et al*., 2004). Thus, curcumin is a good PS, due to its low toxicity, being a pure compound of organic synthesis (PABON, 1964). Its maximum absorption occurs at relatively short wavelengths (408 - 430 nm), compared to so-called second generation PS (*ie*, derivatives of porphyrins, phthalocyanines, naphthalocyanines and chlorines), which have strong absorption also in the range of 650 and 800 nm (KONAN; GURNY; ALLÉMANN, 2002; PRIYADARSINI, 2009). Most importantly, this yellow compound has many of the characteristics that make the PS formulation challenges, such as low solubility in water, possible aggregation in aqueous solutions and low stability (HEGGE *et al*., 2010). However, curcumin and capsaicin do not present any type of load, and this may be one of the possible causes of NCC not showing aPDT effect.

CONCLUSION

Both NCC and NB nanoparticles produced no inhibitory effect on gram negatives and gram positives microorganisms tested. Findings from this study demonstrate that concentrations of 150 μg/mL of curcumin and 5 μg/mL of capsaicin have no antibacterial activity on these species.

This result may be due to the low concentration of curcumin and capsaicin used in this research. However, it may be the type and concentration of surfactant used in the preparation of this NCC. As well as the average diameter of this NCC could be influencing our results. It is difficult to make a precise comparison with other research as there are currently no standards or regulations. Emphasizing that, further studies are needed to elucidate the antimicrobial effective dose of NCC.

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