Atividade antibacteriana de micropartículas de prata encapsuladas com méis de *Apis mellifera* e *Scaptotrigona bipuncatata*
/
Antibacterial activity of silver microparticles encapsulated with honeys from *Apis mellifera* and *Scaptotrigona bipunctata*

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RESUMO
Mesmo ante a presença mundial de antibacterianos, complicações clínicas e veterinárias causados por bactérias multirresistentes requerem novas alternativas. Vários estudos atestam que o mel é um substrato de fácil uso no processo de síntese de nanopartículas de prata cujo uso não resulta em impurezas, uso que resulta em um arranjo de prata menos tóxico, e logo um de maior potencial clínico. Baseado na literatura presente, este estudo analisou a síntese de nanopartículas de prata usando duas amostras de mel através de diferentes protocolos como forma de comparar a morfologia

e propriedades antibacterianas de doze tipos de microcompostos com nanopartículas de prata. As amostras de mel usadas foram coletadas do meliponario da Universidade Estadual de Londrina (UEL) e da Unidade de Conservação Monte Sinai (Mauá da Serra-PR, Brasil); diluídas 50% (v/v) em água e esterilizadas por filtragem usando filtros de 0.22 µm. Depois, as amostras de mel diluídas foram misturadas na proporção de 1:1 com uma solução de água contendo 5 mM de $AgNO_3$ e alíquotas foram separadas em diferentes tubos. O conteúdo de cada tubo foi submetido a um processo de síntese diferente: exposição à luz solar alcalinização até pH 5 ou alcalinização até pH 10. Após a síntese, amostras de cada tubo foram coletadas e submetidas a banho-maria a 60^{o}C por 30 minutos. O formato e tamanho das partículas de prata dentro dos microcompostos foram observadas por Microscopia Eletrônica de Varredura (MEV), que revelaram que todos os protocolos resultaram em micropartículas de prata de cujas médias de tamanho em relação ao método de síntese variaram dentre 100 e 150 ηm. A investigação quanto às propriedades antibacterianas foram feitas por meio ensaios de Concentração Inibitória Mínima (CIM), comparação de curvas de crescimento e morte e análise de alterações morfológicas em superfícies bacterianas após aplicação de tratamentos por MEV. Ensaios de CIM mostraram que as concentrações CIM variaram dentre 15.625 and 500 µM. Ademais, as curvas de crescimento e morte mostraram que *Escherichia coli* (bactéria Gram negativa) foi mais sensível aos tratamentos com os microcompostos que *Staphylococcus aureus* (bactéria Gram positiva) e que micropartículas produzidas por alcalinização até pH 10 possuem uma ação antibacteriana mais limitada. Imagens obtidas por MEV mostraram que bactérias *E. coli* expostas a micrcompostos de prata obtidos por luz solar ou basificação até pH 5 silver possuem danos em suas superfícies, o que n]ão é visto quando elas são tratadas com o microcomposto obtidos por basificação até pH 10. Em conclusão, os estudos realizados mostram o potential clínico de méis com micropartículas de prata na forma de micro compostos e a importância da futura otimização da síntese de micro compostos.

Palavras-chave: Antibacteriano, Mel, Microcompostos, Prata.

ABSTRACT

Even in face of the worldwide presence of modern antibacterial substances, veterinarian and clinical issues caused by multidrug-resistant bacteria demand new alternatives. Several studies report honey to be a clean and easy to use substrate in the synthesis of silver nanoparticles trough several methodologies, which results in a less toxic silver arrangement, and thus one with a higher clinical potential. Based on present data, this study analyzed the synthesis of silver microparticles using two honey samples and through different protocols in order to compare the morphology and antibacterial properties of 12 types of micro compounds with silver nanoparticles. The honey samples used were collected from the Londrina State University Meliponary and from the Unidade de Conservação Monte Sinai (Mauá da Serra-PR, Brazil), diluted to 50% (v/v) in water and sterilized by filtration in 0.22 µm filters. Afterwards, the diluted honey samples were mixed 1:1 with a water solution containing 5 mM $AgNO_3$ and aliquots were separated in different tubes. The content of each tube underwent a synthesis process: exposure to sunlight, basification to pH 5 or basification to pH 10. After synthesis, samplings from each sample were submitted to a heat treatment of water bath at 60^{o}C for 30 minutes. The shape and size of silver particles inside the micro compounds were observed by images obtained through Scanning Electron Microscopy (SEM), which revealed that all protocols resulted in silver microparticles which average sizes range between 100 and 150 nm. Investigation regarding the antibacterial properties was performed by determination of Minimal Inhibitory Concentration (MIC) assays, time-kill curve comparison and morphological alterations of bacteria surface after treatment aplication by SEM. The MIC assays showed that the silver microparticles MIC varied between 15.625 and 500 µM. Additionally, the time-kill curve revealed that *Escherichia coli* (Gram-negative bacteria) to be more sensitive than *Staphylococcus aureus* (Gram-positive bacteria) and that the silver microparticles produced by basification to pH 10 have

a more limited antibacterial action. SEM images showed that *E. coli* exposed to silver microparticles synthesized by sunlight or basification to pH 5 were damaged in the surface, which is not seen when the micro compounds applied is the one produced with basification to pH 10. In conclusion, the studies performed show the clinical potential of these honeys with silver microparticles in the form of micro compounds and the importance of future synthesis optimization of micro compounds.

Keywords: Antibacterial, Honey, Micro compounds, Nanoparticles, Silver.

1 INTRODUCTION

The "Golden age" of antibiotics, which began with the discovery of penicillin, brought forward several health benefits, their inappropriate usage and presence in fomites such as water by improper, resulted in a selective pressure which favored multidrug-resistant (MDR) bacterial strains. They are ever-increasingly present in clinical cases which persists to this day; and infections caused by bacteria resistant to novel antibiotics them claim 700,000 lives every year according to the WHO, making the one of the antibacterial resistance is one of the biggest threats to global health (Singh, 2006; Ferreira et al., 2008; Silver, 2011; Todar, 2011; WHO, 2015; O' Neill, 2016; WHO, 2016). In response to this alarming scenario, many studies have been conducted in search of new therapies.

Among the articles published, many focus on the modern usage of antibacterial substances used since Antiquity. One among these is silver, which is already employed in products intended for dental care and burns and wounds healing (as silver sulfadiazine) and presents antibacterial mechanisms such as membrane disruption and DNA disrepair; however, it is recommended its formulation in the form of nanoparticle before usage in order to improve its antibacterial effect and avoid adverse ones (Kim et al., 2007; Chernousova & Epple, 2012; Gibson et al., 2014; Wang et al., 2014; Gupta et al., 2016; Gupta et al., 2019). Another substance studied for its antibacterial properties is honey, known today to have hydrogen peroxide, which causes oxidative stress; as well as to other antibacterial molecules obtained from the bee (like bee-defesin-1) and its floral source (such as flavonoids) (Bogdanov, 1996; Ganz, 2003 Kwakman et al., 2010; Bizerra er al., 2012; Kwakman & Zaat, 2012; Nolan et al., 2019). The diversity in antibacterial components tends to not favor resistant bacterial strains and has allowed for products such as Manuka© and Revamil© to be licensed for usage against bacterial infections (Molan & Bets, 2004; Kwakman et al., 2011; Carnwath et al., 2014).

The fact that silver and honey can be used as antibacterial effectively is interesting because silver nanoparticles can be obtained by basification or sunlight exposure of honey solutions with silver nitrate (Bar et al., 2009, Kwakman et al., 2010; Philip, 2010; Mittal et al., 2013; Madhu et al.,

2019). Thus, this article reports the development of micro compounds with silver nanoparticles synthesized using honey as a capping and reducing agent through different methods.

2 MATERIAL AND METHODS

ACQUISITION OF HONEY SAMPLES AND MICRO COMPOUND SYNTHESIS USING HONEY

Honeys used in for the synthesis of silver particles were obtained during the years 2018 and 2019 from hives of two bee species: the Africanized *A. mellifera* Latreille (Hymenoptera: Apidae) honeybee and the *S. bipunctata* Lepeletier 1836 (Hymenoptera: Apidae: Meliponinae) stingless bee. They were collected from a meliponary (Universidade Estadual de Londrina, Londrina-PR, Brazil) using glass syringes and metal spatulas, put in plastic tubes. Before to the conduction of synthesis protocols, they diluted 1:1 in deionized water at sterilized through filtration with 0.22 μm filters (Millipore®); and stored at 4°C. The three different methods of silver particles production were performed through adaptations from the protocols described by Tagad et al. (2013), Priz (2014) and González et al. (2016)..

Three prepared samples of "HAM" ("honey from *Apis mellifera*") solution and three of "HSB" ("honey from *Scaptotrigona bipunctata*") solution were in tubes mixed (50% vv^{-1}) with a solution of AgNO3 (Sigma-Aldrich®, Brazil) at 5 mM and then one tube of each honey sample was exposed to sunlight for 10 minutes ("ML" – "microparticles by light"). Another pair of solutions were basified by adding a 1M NaOH solution until pH was 5.0 (forming "ML"), and the last pair had their pH adjusted to 10 by similar fashion ("MAB" – microparticles by aggregative basification). All solutions were mixed and left in ambient temperature for 5 minutes before storage at 4°C for 24 hours. The number of samples obtained from these protocols was six, which totalized twelve after the production of the "heated" variants through the protocol described in "microdilution assay" section.

BACTERIAL STRAINS

The Micro dilution assays (see below) were performed against the reference bacterial reference strains: *Escherichia coli* ATCC 8739 and ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027 and ATCC 27853, *Salmonella enterica* serovar Enteritidis ATCC 13076, *S. enterica* serovar Typhimurium UK-1, *Staphylococcus aureus* ATCC 25923 and ATCC 29213, and *Staphylococcus epidermidis* strain 1E4248. Among those strains, *S. aureus* ATCC 29213 and *E. coli* ATCC 8739 were selected as Gram-positive and Gram-negative reference strains for the antibacterial effect comparison. All strains were stored at a temperature of -20°C in Brain Heart Infusion (BHI) broth (Oxoid® Brazil) containing 20% (v/v) glycerol (Merck® Brazil).

MICRO DILUTION ASSAYS WITH AND WITHOUT HEATING

Determination of the Minimal inhibitory concentrations (MICs) for each micro compound against the varied bacterial strains tested was based on the Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines through the microdilution method after serial dilution in 96-well plates. Thermal stability of the antibacterial property of the compounds was verified by obtaining "heated" variants (‘‘δ’’) trough heating samplings from each type at 60 ºC for 30 minutes in water bath before the microdilution and assays.

Each bacterial strain prior to the assay was cultivated in Muller Hinton (MH- Difco®) agar 24 hours before the experiment and then suspended in a sterile saline solution (NaCl 0.85%, Sigma-Aldrich® Brazil) at a concentration of 1.5 x 10^8 $CFUmL^{-1}$ (0.5 on the McFarland scale). Aliquots of 10 µL were transferred to microtubes containing 990 µL MH (Difco®) broth. From those microtubes, aliquots containing 50 µL were plated in wells containing 50 µL MH (Difco®) broth with a sample serially diluted (for a final bacterial concentration of 7.5 x 10^5 $CFUmL^{-1}$). The micro compounds concentrations tested ranged from 15.625 to 500 µM; and wells without any treatment (bacterial viability control) and without bacteria (sterility control) and assays were performed in triplicate per honey samples per bacterial strain. The 96-well plates were incubated at 37 ºC for 24 hours and; after incubation, the optical density values at 600 nm were determined using a Bio-Rad® Microplate Reader (model 3550), and the MIC_{50} values were detected.

ANTIBACTERIAL EFFECT COMPARISON IN RELATION TO MICRO COMPOUND TYPE AND BACTERIA

The effect of the synthesized micro compounds on bacterial growth was compared by a time-response growth curve assay. In this assay, *E. coli* ATCC 8739 and *S. aureus* ATCC were previously cultivated in MH (Difco®) agar plates for 24 hours at 37 ºC and then suspended in sterile saline (NaCl 0.85% - Sigma-Aldrich®), resulting in two saline solutions containing 1.5 x 10^8 colony forming units (CFU) per mL (0.5 on the McFarland scale) of either *E. coli or S. aureus*. From each solution, three samples of 10 µL inoculated in a separate microtube containing 990 µL MH (Difco®) broth solutions and with a type of micro compound at the chosen concentration of 125 µM.

The microtubes were incubated at a temperature of 37°C for 7 hours. At four time points (0, 2, 4, and 7 hours after incubation), a portion of 100 µL from each sample was collected and serially diluted ten-fold in microtubes with MH (Difco®) broth. From these dilutions, three samples of 10 µL from each were inoculated in MH (Difco®) agar media plates. Lastly, those plates were cultivated at 37ºC for 18 hours and formed colonies formed were counted.

SCANNING ELECTRON MICROSCOPY OF SAMPLE NANOPARTICLES AND BACTERIA AFFECTED BY NANOPARTICLE TREATMENT

Before preparation of the microscopy slides, 1 mL of selected nanoparticle solutions (HAM ML, HAM MAB, HSB ML and HSB MAB) had their excess honey removed by centrifugation for one hour at 24ºC and 3,000 g. The resulting pellets were then resuspended in 1 mL of deionized water. For the preparation of the bacterial samples slides to be analyzed by SEM, 10 µL *S. aureus* aliquots at 1,5 x 10^8 CFU concentration were inoculated in microtubes with 990 µL solution of MH broth with selected nanoparticles (both heated and non-heated) solution at 125 µM. Afterwards, those samples were incubated for 4 hours at 37ºC, centrifuged four times at 1,000 g, 3,000 g and twice at 7,000 g (each 5 minutes long and all at 24ºC), and resuspended 100 µL of deionized water. From each sample, 10 uL were collected and deposited in different polylysine-coated (1%) glass slide inside wells and submitted to the four stages of slide preparation: fixation, post-fixation, dehydration and critical point drying.

The first step, fixation, was performed right after the sample dried in the slide by immersing the slide overnight at 4ºC in a 0.1 M cacodylate buffer solution with 2% glutaraldehyde and 2% paraformaldehyde. In the next morning, for the post-fixation stage, the buffer was removed and replaced by 1% OsO_4 solution and left in room temperature for two hours. Afterwards, the slides were dehydrated through submersion in gradient ethanol solution (70, 80, 90, and 100ºGL). Lastly, the samples were submitted critical point dehydration using CO_2 BALTEC CPD 030 Critical Point Dryer) and then coated in gold using the Baltec SCD Super Cotter. The ensuing slides were observed with a scanning electron microscope (FEI Quanta 200) and images taken were analyzed with Image J.

STATISTICAL ANALYSIS

Data collected from the time-kill curve assay were analyzed by one-way ANOVA and differences between CFU logarithm means of the same bacteria submitted to different treatments were determined using Tukey's range test or the Chi-square test ($\alpha = 5\%$). Comparison between the CFU among *S. aureus* and *E. coli* treated with the same HAM micro compound was made in similar manner, however the logarithm CFU average of either initial timestamp (time= 0) were subtracted from logarithm of CFU averages from each subsequent timestamps; followed by paired t-test analysis. All tests were performed with the statistical programs GraphPad Prism version 6.02 and BioEstat version 5.3.

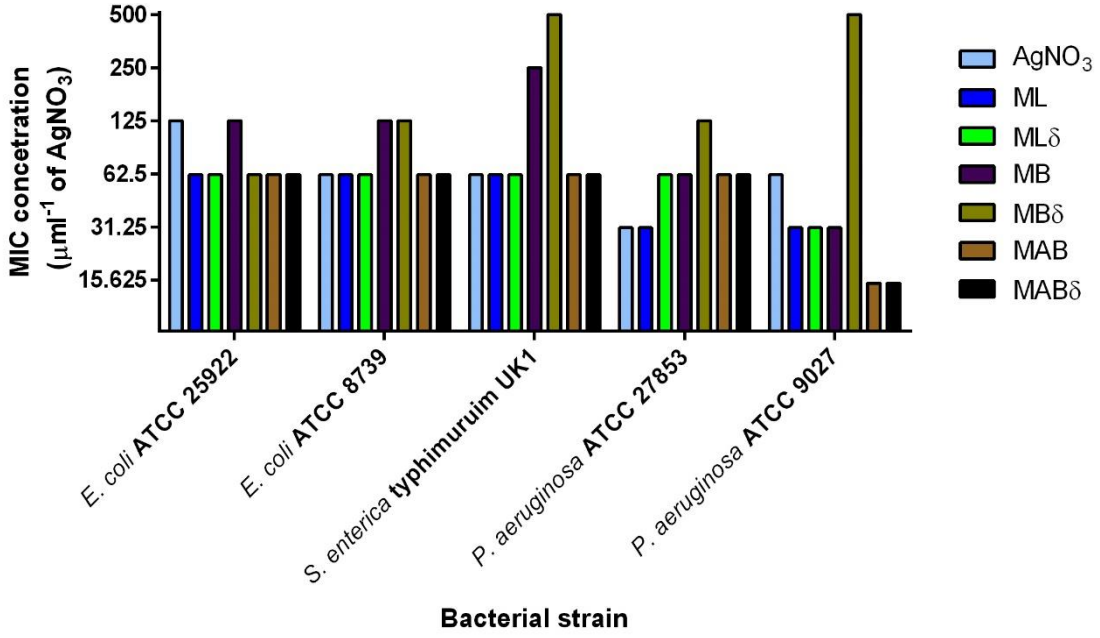
3 RESULTS

MIC ASSAY OF MICRO COMPOUNDS

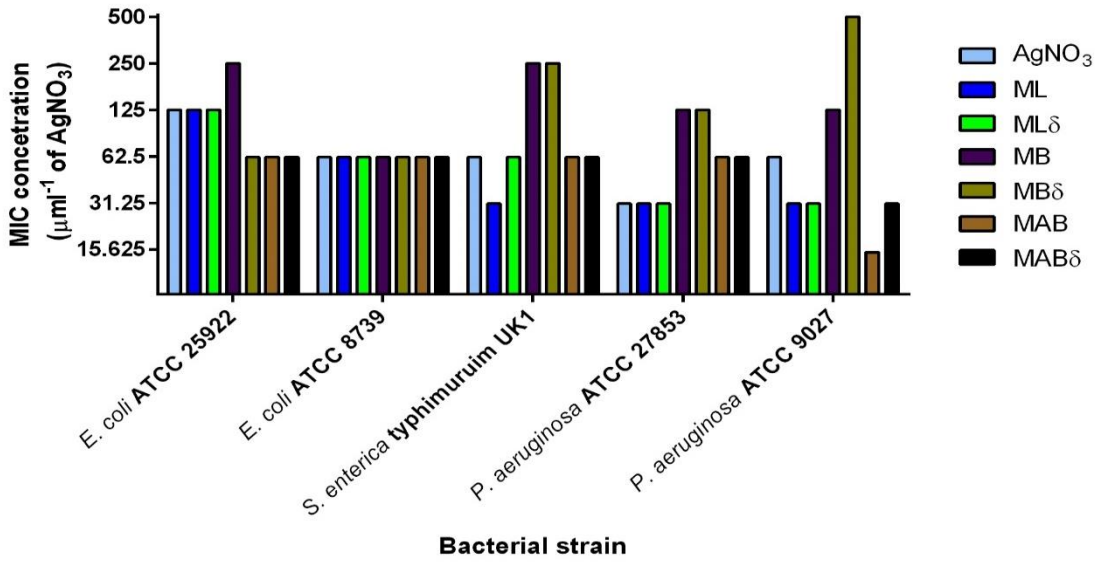
Figures 1A and 1B display Minimal Inhibitory Concentrations (MICs) of HAM and HSB micro compounds against Gram negative bacterial ATCC strains compared to treatment with $AgNO_3$, while figures 1C and 1D display the MIC concentrations of the same compounds against Gram positive strains. The MICs obtained varied between 31 to 250 µM, and the concentration of 62.5 µM was the modal MIC. Mean MIC of HAM micro compounds tested against the Gram-negative strains was 98.6 µM; while mean MIC for HSB micro compounds was 98.1 µM. Against the Gram-positive strains, the average MIC of HAM and HSB micro compounds were 96.9 µM and 124.8 µM, respectively. Mean MIC for treatments with silver nitrate ($AgNO_3$) against the Gram-negative strains calculated was 56.0 µM; and 124.7 µM against the Gram-positive ones.

Figures 1A and 1B Minimal Inhibitory concentrations (MICs) of $AgNO_3$ micro compounds produced with honey collected from *Apis mellifera* honeybees (A) or *Scaptotrigona bipunctata* stingless bees (B) against American Type Culture Collection (ATCC) Gram-negative bacterial strains

1A



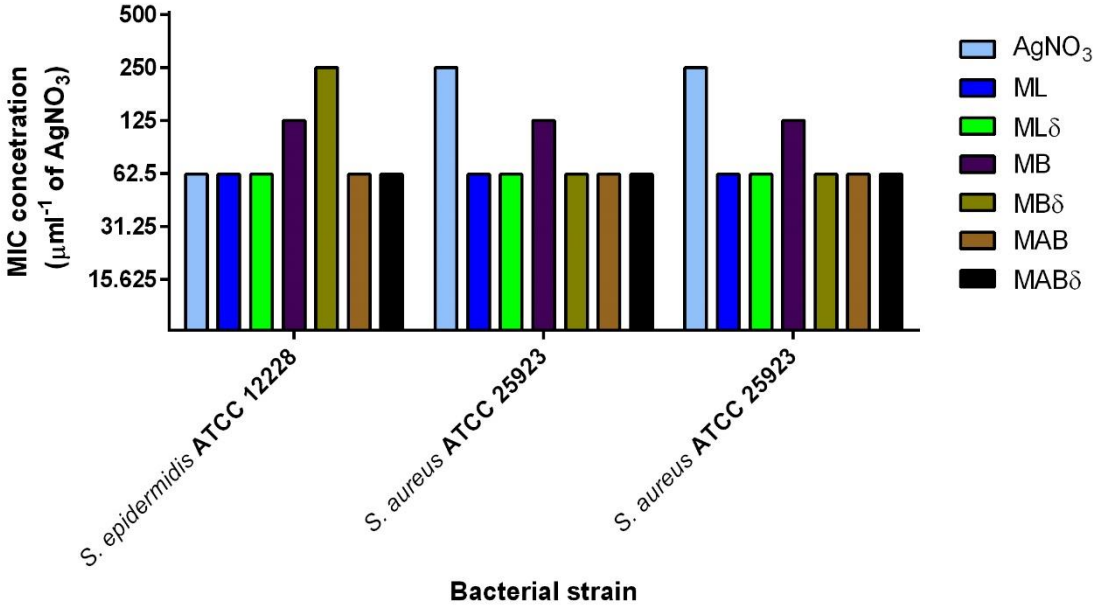
1B



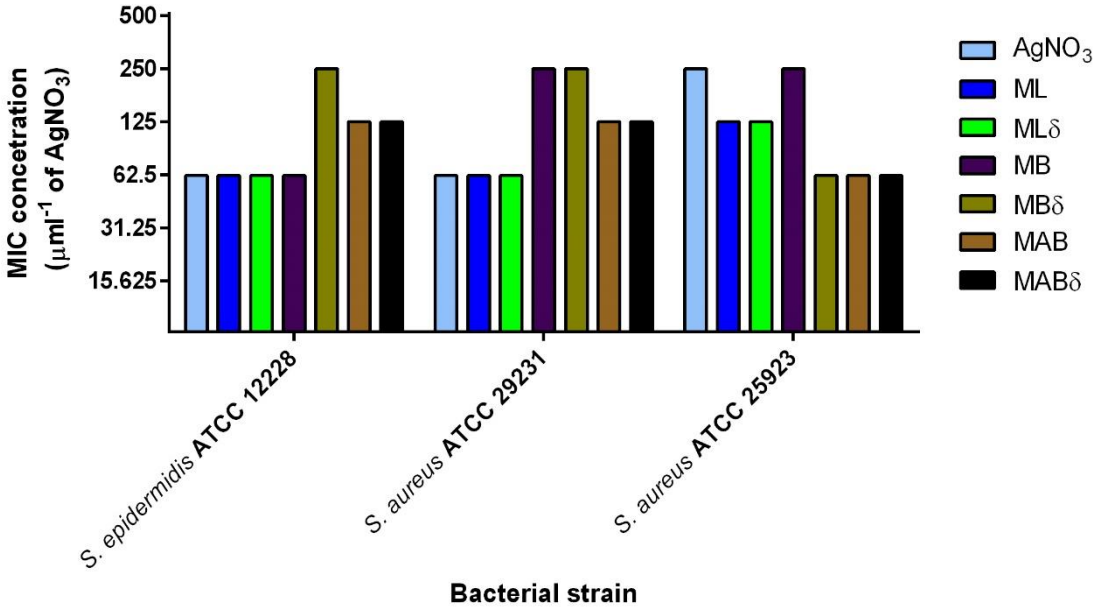
Notes: MIC concentrations are separated by according to the combinations bacteria and microparticles type tested. Abbreviations: $AgNO_3$, Silver nitrate; ATCC, American Type Culture Collection; HAM, Honey samples collected from *Apis mellifera* honey honeycombs; HSB, Honey samples collected from *Scaptotrigona bipunctata* honeycombs; ML, microparticles obtained by light exposure; MB, microparticles obtained by adjusting pH to 5; MAB, micro compound obtained by by adjusting pH to 10*;* µM, micromolar, δ, heated variant of micro compound.

Figures 1C and 1D Minimal Inhibitory concentrations (MICs) of $AgNO_3$ micro compounds produced with honey collected from *Apis mellifera* honeybees (C) or *Scaptotrigona bipunctata* stingless bees (D) against American Type Culture Collection (ATCC) Gram-positive bacterial strains.

1C



1D

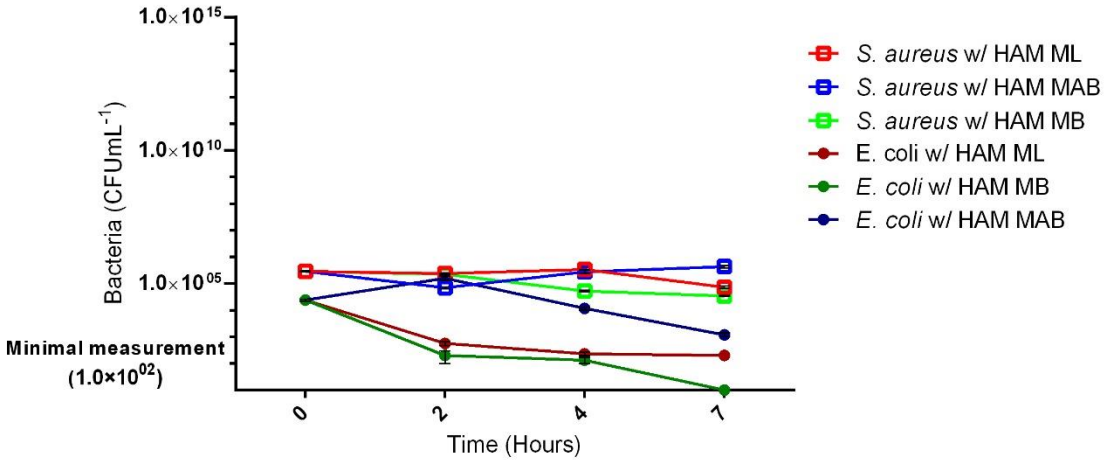


Notes: MIC concentrations are separated by according to the combinations bacteria and microparticles type tested. Abbreviations: $AgNO_3$, Silver nitrate; ATCC, American Type Culture Collection; HAM, Honey samples collected from *Apis mellifera* honey honeycombs; HSB, Honey samples collected from *Scaptotrigona bipunctata* honeycombs; ML, micro compound obtained by light exposure; MB, micro compound obtained by adjusting pH to 5; MAB, micro compound obtained by by adjusting pH to 10*;* µM, micromolar, δ, heated variant of micro compound.

ANTIBACTERIAL EFFECT COMPARISON IN RELATION TO SYNTHESIS TYPE AND BACTERIA

The effects of HAM micro compounds on the bacterial growth kinetics of *E. coli* ATCC 8739 and *S. aureus* ATCC 29213 up to 7 hours are shown in the figure 2. Effects are analyzed based on CFU counts in determined timestamps and results are displayed in number of colony forming units per milliliter ($CFUmL^{-1}$). The Gram-negative strain (*E. coli* ATCC 8739) demonstrated a higher sensitivity than the Gram-positive one (*S. aureus* ATCC 29213), as the corrected CFU average of *E. coli* was significantly lower than the corrected *S. aure*us CFU, expect for the HAM MAB micro compound after two hours of incubation. In relation to the comparison between treatments, as shown in tables 1 and 2, usage of ML micro compounds resulted the significantly lowest CFU counts; while MAB microparticles presented a more limited antibacterial effect.

Figures 2A Time-kill curves of *Staphylococcus aureus* ATCC 29231 and *Escherichia coli* ATCC 8739 exposed to HAM microparticles.



Notes: Time-kill curves are nominated after the bacteria exposed to antibacterial treatment, honey samples used in micro compound synthesis and method of synthesis. All micro compound treatments were at 125 µM .
Abbreviations: CFU, colony forming units; HAM, Honey samples collected from *Apis mellifera* honey honeycombs; HSB, Honey samples collected from *Scaptotrigona bipunctata* honeycombs; MAB, micro compound obtained by adjusting pH to 10; MB, micro compound obtained by adjusting pH to 5; ML, microparticles obtained by light exposure.

Tables 1A and 1B ANOVA rankings of CFU obtained from *Escherichia coli* ATCC 8738 (A) and *Staphylococcus aureus* ATCC 29231 (B) submitted to different HAM microparticles.
(A)

	Time (in hours)		
Synthesis	2	4	7
ML	B	B	B
MB	C	B	C
MAB	A	A	A

(B)

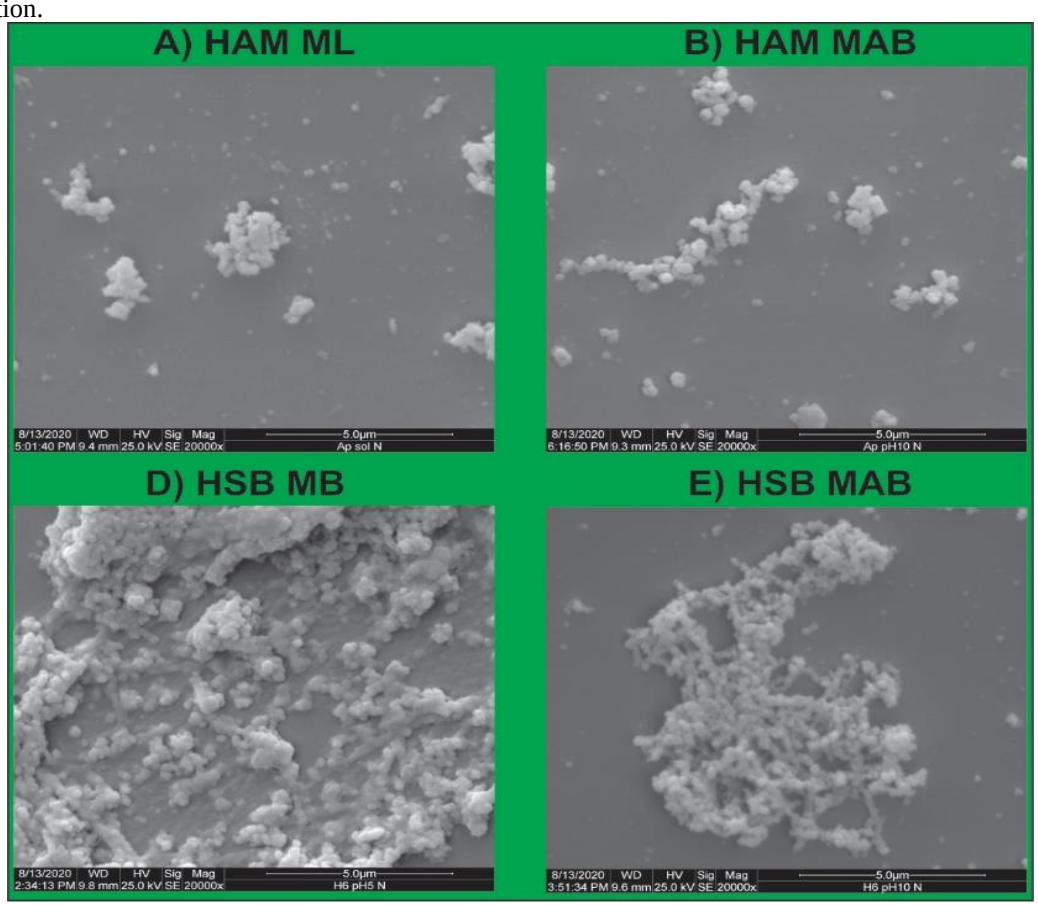
	Time (in hours)		
Synthesis	2	4	7
ML	A	A	B
MB	A	B	C
MAB	B	A	A

Notes: ANOVA analysis results of CFU exposed to micro compound treatments are displayed in the form of ranks. It was attributed to the highest CFU average in a given timestamp the rank ‘‘A’’, and to each significantly lower mean was attributed the next letter as its rank. All micro compound treatments were at 125 µM.
Abbreviations: MAB, micro compound obtained by by adjusting pH to 10; MB, micro compound obtained by adjusting pH to 5; ML, micro compound obtained by light exposure.

SEM IMAGES OF CENTRIFUGED PARTICLES INSIDE MICRO COMPOUNDS AND MEASUREMENTS

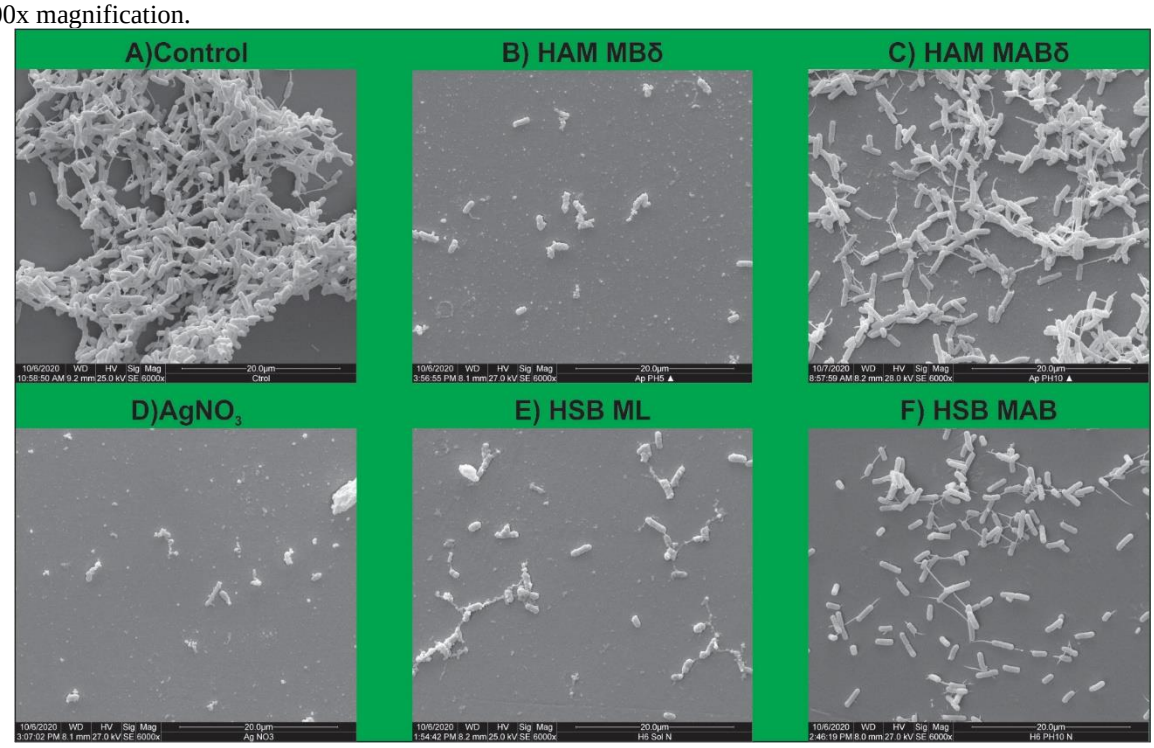
From the SEM images taken at a magnification of 20,000 x, silver microparticles formation inside the micro compounds obtained trough different methods can be seen in the panel depicted in figure 3. Average size (established as the equivalent circular diameter) among the silver particles inside all types of micro compounds varied between 100 and 150 nm**.** Thus the particles inside the micro compounds can be considered a mixture nanoparticles and fine particles according to international organizations (EU, 2011; FDA 2014). The other panel (figure 4) show bacteria presenting blebs after being growth in media containing ML or MB micro compounds, regardless of which honey samples was used in the synthesis process.

Figure 3 Selected Scanning Electron Microscopy of micro compounds containing silver particles at 20,000x magnification.



Abbreviations: HAM, Honey samples collected from *Apis mellifera* honey honeycombs; HSB, Honey samples collected from *Scaptotrigona bipunctata* honeycombs; MAB, micro compound obtained by by adjusting pH to 10; MB, micro compound obtained by adjusting pH to 5; ML, micro compound obtained by light exposure.

Figure 4 Selected Scanning Electron Microscopy of *Escherichia coli* ATCC 8739 cultivated with micro compounds at 20000x magnification.



Notes: Images are nominated after which micro compound *Escherichia coli* ATCC 8739 was exposed to. All micro compound treatments were at 125 µM. The bacteria in the ‘‘Control’’ image were not exposed to any treatment, while bacteria in the ‘‘$AgNO_3$’’ were exposed to silver nitrate at 125 µM.
Abbreviations: $AgNO_3$, Silver nitrate; Control, bacteria not subjected HAM, Honey samples collected from *Apis mellifera* honey honeycombs; HSB, Honey samples collected from *Scaptotrigona bipunctata* honeycombs; MAB, micro compound obtained by by adjusting pH to 10; MB, micro compound obtained by adjusting pH to 5; ML, micro compound obtained by light exposure; δ, heated variant of micro compound was used.

4 DISCUSSION

The obtained MIC concentrations of all non-centrifuged micro compounds (figures 1A through 1D) varied between 15 and 250 µM even among the heated variants (which, given the methodology deployed, means 1.62-27 μgmL^{-1} of silver is present in the compounds at MIC); while the size of nanoparticles inside them varied between 100 and 150 nm (figure 3). This concentration range is similar to findings regarding MIC concentrations of nanoparticles which are described having similar sizes and are shown to be effective antibacterial in literature, and thus these results allude to a promising result for the micro compounds synthesized (Hajipour et al., 2012; Khan, 2012; Tagad et al., 2013; Priz, 2014; González et al., 2016; Escárcega-González et al., 2018). However, the time-kill curve assay reveals more nuances and differences to the antibacterial effect (figure 2).

In accordance to the MIC findings, all treatments presented for seven hours an antibacterial effect at least bacteriostatic. Additionally, the Gram-negative bacteria (*E. coli*) was more sensitive to the micro compound treatments than the Gram-positive one (*S. aureus*), which were which corroborates to current literature (Baek and An, 2011; Ashkarran et al., 2012; Hajipour et al., 2012;

Lagbas et al., 2015). Additionally, this finding advocates for micro compounds usage against bacterial infections in isolation as well as in combination because other studies have established Gram-negative bacteria to be more resistant to other antibacterial substances (including novel antibacterials) (Ashkarran et al., 2012; Lagbas, Pelisco, Riego, 2015; Nishio et al., 2015; Jimenez et al., 2016; Gupta et al., 2019; Clébis et al., 2019).

The time-kill curve assay (figure 2) also demonstrates MB micro compounds to be more effective than comparison to ML; and MAB micro compounds to be the least effective. This is corroborated in the SEM images of bacteria after micro compound treatments, which show a greater amount of cellular damage in cases which *E. coli* was subjected to treatments with ML or MB (figure 4). While the antibacterial properties described in this work differs greatly from those described for honey, high degrees of basicity also compromise stability of antibacterial molecules such as H_2O_2, and thus the data found indicate that honey might partially contribute to the antibacterial effect could be related to the of the antibacterial effect of the MAB micro compound (Nishio et al., 2015 Clébis et al., 2019; Brudzynski 2020,). Therefore, micro compounds obtained through different protocols were shown by the experiments performed to present differences in their effect against bacteria.

In conclusion, the data here describes the synthesis of micro compounds containing silver through usage of two honey samples and their clinical potential against bacterial and present antibacterial effect. The types of micro compounds obtained also demonstrated significant differences in their effect against bacteria, which warrant further studies as well as reveal new possibilities for clinical treatments.

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