

## **Matricaria recutita extract associated with norfloxacin or cephalexin enhances the antimicrobial activity of these drugs against *Staphylococcus aureus***

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### **Resumo**

**Extrato de *Matricaria recutita* associado com norfloxacin e cefalexina aumenta a atividade antimicrobiana destes medicamentos contra *Staphylococcus aureus*.** O surgimento de infecções bacterianas, incluindo aquelas associadas com *Staphylococcus aureus*, traz à tona uma necessidade de buscar novas estratégias mais eficazes para tratamento clínico. O uso de plantas medicinais associados com os antibióticos convencionais pode ser uma opção terapêutica. Atualmente, estudos evidenciam o efeito sinérgico alcançado através da combinação de extratos vegetais com antibióticos. Nosso objetivo foi avaliar a atividade antimicrobiana e cinética bacteriana *in vitro* do extrato de *Matricaria recutita* (camomila) e sua associação com cefalexina e norfloxacin sobre isolados clínicos de *S. aureus* de origem bovina, caracterizada como resistente. Os ensaios foram realizados pelo método da diluição em meio sólido para a determinação da Concentração Inibitória Mínima (CIM). Em ambas as associações do extrato de *M. recutita* com os antibióticos norfloxacin e cefalexina,

foi observada CIM na diluição 1:64 o que correspondeu a 8µg/mL dos antibióticos e 13.43 µg/mL do extrato. A associação Cefalexina com extrato de camomila produziu um efeito sinérgico em 75% das amostras na sua CIM. A combinação com produtos naturais frequentemente utilizados pela população e os antibióticos aqui ensaiados, poderiam representar uma opção terapêutica para o tratamento de infecções causadas por *S. aureus*, como também para prevenção do desenvolvimento crescente de resistência.

**Palavras-chave:** Antibióticos; Camomila; Microrganismos resistentes; Produtos naturais

## Abstract

The emergence of bacterial infections, including those related to *Staphylococcus aureus*, has resulted in the need to search for new and more effective clinical treatment strategies. The use of medicinal plants associated with conventional antibiotics may be a therapeutic option. Currently, studies have shown the synergistic effect of combining plant extracts with antibiotics. The present study evaluated the *in vitro* antimicrobial activity and bactericidal kinetics of a *Matricaria recutita* (chamomile) extract, in association with cephalexin and norfloxacin, on clinical isolates of *S. aureus* of bovine origin, which is characterized as resistant. The tests were performed by dilution in a solid medium to determine the minimum inhibitory concentration (MIC). For both combinations of the *M. recutita* extract, with the norfloxacin and cephalexin antibiotics, we observed an MIC at a 1:64 dilution, corresponding to 8µg/mL of the antibiotic and 13.43 µg/mL of the extract. When evaluating the MIC, cephalexin associated with the chamomile extract produced a synergistic effect in 75% of the samples. The combination of natural products frequently used by the population with the antibiotics tested in this study could be a therapeutic option for the treatment of infections caused by *S. aureus*, as well as prevent an increase in resistance.

**Key words:** Antibiotics; Chamomile; Natural products; Resistant microorganisms

## Introduction

*Staphylococcus aureus* is a major nosocomial pathogen in hospital-acquired infections and is responsible for inactivating several antibiotics (CUNHA; CUNHA, 2007). Moreover, this microorganism varies widely in its sensitivity to broad-spectrum antimicrobials, making the multi-drug resistance a public health problem (STRATTON, 2000). Glycopeptides, such as vancomycin and teicoplanin, are the drugs of choice for the treatment of infections caused by methicillin-resistant *S. aureus*, which is usually resistant to β-lactam antibiotics, cephalosporins and carbapenem (OTEO; BELÉN, 2015). According to Stratton (2000), the mechanism of resistance may occur due to overproduction of β-lactamase, for strains known as BORSA (borderline oxacillin-resistant *S. aureus*), and alterations in penicillin-binding proteins (PBPs 1, 2 and 4), for strains known as MODSA (modified penicillin-binding protein *S. aureus*).

The combined use of antimicrobials, such as cephalosporins and fluoroquinolones, could possibly suppress the emergence of resistant mutant strains, and

produce a synergistic *in vivo* effect (ZUCARELLI et al., 1988; PEREIRA et al., 2008). However, it is debatable whether conventional antimicrobials become enhanced when combined with other compounds, such as natural products.

Plants with therapeutic properties are of great importance for human and veterinary medicine worldwide (TOMAZZONI et al., 2006; SILVA et al., 2012), especially in developing countries, due to the high cost of conventional drugs (MINJA, 1994). Chamomile (*Matricaria recutita* Linn) belongs to the family Asteraceae (LINS et al., 2013). The pure extract of *M. recutita* is used to treat gastrointestinal disorders, oral infections, and dermatitis. In addition, it has sedative, anti-inflammatory, antifungal and antibacterial activities (ALBUQUERQUE et al., 2010a). The effects of *M. recutita* are attributed to azulene, an essential oil composed of sesquiterpene alcohol, alpha-bisabolol, and chamazulene that inhibit *in vitro* the chemical mediators of the inflammatory process, as well as flavonoids that inhibit the release of histamine (HILI et al., 1997; BEDI; SHENEFELT, 2002). According to Brehm-Stecher and Johnson (2003), the high concentration of alpha-

bisabolol in the dry extract of *M. recutita* is related to antibacterial activity, and a concentration of 1mg/ml presented bactericidal activity against *S. aureus*.

Currently, the combined use of herbal extracts or essential oils with commercial antimicrobials has become an alternative for fighting multi-resistant strains (BARRETO et al., 2014). Studies have been conducted in order to test combinations of plant extracts with conventional antibiotics, such as fluoroquinolones, against bacteria of clinical importance (MACHADO et al., 2003; MUSUMECI et al., 2003; PEREIRA et al., 2008). When associated, the effects can be enhanced, and this results in a more efficient interaction against microorganisms (ELBASHITI et al., 2011; HUSSIN; EL-SAYED, 2011; BARRETO et al., 2014). In this regard, the aim of this study was to evaluate the *in vitro* antimicrobial activity and bactericidal kinetics of an *M. recutita* extract, associated with cephalixin (cephalosporin) and norfloxacin (fluoroquinolone), on clinical isolates of *S. aureus* of bovine origin.

## Material and Methods

### Plant material and extraction

Flowers of *Matricaria recutita* were identified at the Laboratory of Toxicology, Department of Pharmaceutical Sciences, Universidade Federal de Pernambuco (UFPE), Recife, and archived under number 898 in the Arruda Câmara herbarium at the Universidade Estadual da Paraíba. The extraction was made at the Laboratory of Pharmaceutical Technology, Department of Pharmacy, UFPE. This procedure was conducted using the continuous-flow leaching method at room temperature, with an extraction solution of alcohol (80% v/v) that was constantly restored over a 24 h period. We obtained 500 mL of extract with a final concentration of 860.00 µg/mL (PEREIRA et al., 2010a; 2010b).

### Acute toxicological assay of the *Matricaria recutita* extract

Forty (40) male Swiss mice were used, which were 06-08 weeks old, had an average weight of 28g, and were obtained from the Sector Biotherium at the Health and

Rural Technology Center – CSTR, Universidade Federal de Campina Grande – UFCG, Campus de Patos. For the toxicological assay, the animals were allocated into 4 groups of 10 mice each, and treated with 0.25 mL of the plant extract, intraperitoneally (IP) in a single dose, according to the protocol established by the World Health Organization (WHO 1990-1991). Each group was treated with the extract diluted in autoclaved distilled water at concentrations of 430 µg/mL, 215 mg/mL, 107.5 mg/mL and 53.75 mg/mL, respectively. The groups were observed during a 24-hour period, maintained in an environment with a temperature of 25±3°C, relative air humidity between 30 and 70%, and 12h/12h light-dark cycle. The animals received commercial feed and drinking water *ad libitum*, in order to determine the LD<sub>50</sub>.

### Skin irritation test

Twenty-four hours before the test, the mid-dorsal region of the animals was shaved (area 1.5 cm x 1.5 cm) to apply the extract and observe for possible reactions. Three mice were selected for each dilution of the tested product (430 µg/mL to 13.43 µg/mL). We applied 0.5 mL of the dilutions on the intact skin. The application sites were covered with cotton pads attached to the skin with hypoallergenic tape. At the end of this period, the patches were removed, and excess product was removed using sterile saline. Records were obtained after observations at 3 min, 1, 4, 24, 48 and 72 h after removal, registering the skin reactions for erythema and edema, according to the calculation of the irritation index. Thus, they were classified according to the obtained index in OECD Guide nº 404 (OECD, 2002) for a sensitivity and irritation test, as recommended by the Ministério da Saúde (2004).

### Strains of resistant *Staphylococcus aureus*

Milk samples and nasal swabs were collected at the Semi-arid Research Center (NUPEÁRIDO), Patos county, PB, property of the Universidade Federal de Campina Grande (UFCG) (07°03'27" to 07°03'39"S, 37°16'21" to 37°16'38"W). Samples were obtained from 30 naturally infected lactating bovines. After washing the teat with soap and water, drying it with paper towels and disinfecting the ostium with 70° GL ethyl alcohol,

we aseptically collected approximately 5 mL of milk from each mammary quarter reactive to the “California Mastitis Test” (CMT), using a slant tube in horizontal position (CAVALCANTI-DANTAS et al., 2016).

Nasal swabs were collected and all samples were stored in sterile threaded tubes, identified and sent under refrigeration in isothermal boxes, to the Laboratory of Genetics and Microbiology at the Universidade Federal da Paraíba – CCEN, for microbiological examination and identification of *S. aureus*.

### Biochemical identification of *Staphylococcus aureus*

Serial dilutions were made until a concentration of  $10^{-5}$ . An aliquot of 0.1 mL was distributed on petri plates containing the selective medium Baird-Parker agar, by a plating method that used a Drigalski handle. Subsequently, the plates were incubated at 37°C for 24-48 h. The selected colonies were inoculated into test tubes with approximately 2 mL of brain heart infusion broth and incubated at 37°C for 24 h. The suspected colonies were purified and confirmed by Gram stain, catalase, coagulase, thermonuclease, growth in 10% NaCl and anaerobic consumption of mannitol (BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY, 1994; SIMÕES et al., 2013). This assay was performed in triplicate.

In this study, 8 isolated strains of *S. aureus* were used: two standard samples (ATCC 29213 and ATCC 25925), one from nasal fossa (FN 121), three from lactating udders (U102, U122, U250) and two from milk (L146, L311). In order to identify the resistant strains, we previously evaluated the sensitivity to three different classes, with antibiotics, through the disk diffusion test on Petri plates containing Muller Hinton Agar (MHA) (YOUN et al., 2011). The strains were tested against  $\beta$ -lactam antibiotics (ampicillin and penicillin), aminoglycosides (neomycin and gentamicin) and erythromycin.

### Sensitivity test

The *Matricaria recutita* extract was serially diluted in order to obtain concentrations ranging from 3.350

$\mu\text{g/mL}$  to 860.00  $\mu\text{g/mL}$ . In association, a scale of antibiotics with increasing concentrations of cephalexin and norfloxacin, ranging from 0.01565 to 512  $\mu\text{g/mL}$ , were individually added to the bacterial cultures of *S. aureus*. The bacteria were previously cultivated in BHI (Brain Heart Infusion, Sigma™) medium, at 37°C for 24 h, and the suspensions were adjusted according to turbidity pattern corresponding to 0.5 of the McFarland nephelometric scale. This corresponds to approximately  $1.5 \times 10^8$  CFU/mL (colony forming unit/mL), which was diluted in BHI broth until obtaining a concentration of  $1.5 \times 10^5$  CFU/mL.

Aliquots of 50  $\mu\text{L}$  were taken from these tubes and seeded in Petri dishes containing Muller Hinton Agar (MHA). Then, using a 6 mm diameter glass tube, we made wells in the culture medium, which were filled with 25  $\mu\text{L}$  of tannin and cephalexin diluted in distilled water. The initial concentration for the serial dilution was 500 mg/mL for the extract and 512  $\mu\text{g/mL}$  for the antibiotics. This concentration was selected according to the results obtained by the CLSI (2010). These plates were maintained in an incubator at 37°C for 24 h. The MIC was considered the lowest concentration of the drug that completely inhibited bacterial growth (PEYRET et al., 1990). The diameter of the halos of bacterial growth inhibition (CALEGARI-JACQUES, 2003; SHELBURNE et al., 2004) formed around each well was measured in millimeters. We used a qualitative method, which allowed us to classify the bacterial sample as susceptible and resistant to the association used (PEYRET et al., 1990). Based on preliminary studies (VIEIRA et al., 2009; SANTOS et al., 2011), we considered strains with halos above 10 mm to be sensitive.

### Bactericidal kinetics of the *Matricaria recutita* extract

The bactericidal curve of the *M. recutita* extract was evaluated by a known method (PEYRET et al., 1990). Samples of *S. aureus* were inoculated in BHI nutrient broth, incubated at 37°C for 18 to 20 h, and subcultivated in Mueller Hinton broth for 1 h, obtaining an inoculum of  $10^5$  CFU/mL. A 9 mL aliquot of the bacterial culture was added to 1 mL of extract, equivalent to 10 times the

MIC value, according to results of previous studies of our group. Another 9 mL aliquot of the bacterial culture was added to 1 mL of ethanol. The tubes were maintained in an incubator at 37°C for 24 h; aliquots were taken after 2, 4, 6, 8 and 24 h of incubation and plated on blood agar. The plates were read after incubation for 48 h at 37°C, using the standard plate count method. Previous studies considered the kinetics satisfactory when there was a reduction greater than or equal to  $2 \log^{10}$  CFU/mL, for a period less than or equal to 24 h (SHELBURNE et al., 2004).

### Statistical analysis

The sensitivity test was performed in triplicate and assessed with an analysis of variance (ANOVA), using the Kruskal-Wallis test, followed by Dunn's test, in order to compare the central tendencies of inhibiting bacterial growth (CALEGARI-JACQUES, 2003). Tests were considered significant when the p value was lower than 5%. The data were also recorded in a database using the software SPSS (*Statistical Package for Social Sciences*) for Windows, version 15.0, and analyzed by descriptive and inferential statistics.

### Results

The toxicity evaluation was performed with the *M. recutita* extract applied to mice. We observed that there were no manifestations of hypersensitivity reaction, itching or any other clinical signs of intoxication associated with possible hypersensitivity to the extract. The concentrations used did not kill the mice when administered intraperitoneally.

The resistance profile against *S. aureus* strains was initially checked and 12.6% were resistant to ampicillin, 11.2% to penicillin, 12.3% to neomycin, 12.6% to gentamicin and 47.5% to erythromycin.

These strains were considered resistant to the tested classes, corroborating analyses conducted in others studies (BARRETO et al., 2014). The protocol used in our study allowed us to determine the antimicrobial activity of the *M. recutita* extract associated with norfloxacin or cephalexin on samples of *S. aureus*

of bovine origin, based on the minimum inhibitory concentration (MIC). The initial concentration was 512 µg/mL for antibiotics and 860.00 µg/mL for the extract.

The inhibition halos of the *M. recutita* extract and norfloxacin had diameters ranging from 44 to 10 mm in all dilutions, demonstrating 100% effectiveness until a 1:512 dilution (Table 1). Growth inhibition was homogeneous, according to the concentration level of the diluted extract in association with norfloxacin. The concentration of the extract (µg/mL) and norfloxacin (µg/mL) that was  $32 + 53.75$  µg/mL (dilution 1:16) presented no significant difference ( $p = 0.10$ ) in the mean diameter of the inhibition halos of *S. aureus* growth. However, it was significant ( $p < 0.05$ ) when compared with the mean diameter of the halos for the concentrations (norfloxacin/extract) of (16/26.87), (8/13.43) \*; the latter concentration is considered the MIC (Table 1). In the latter concentration, all strains presented sensitivity to the association, in view of their respective MICs and inhibition halos, when used alone.

Regarding the association of the *M. recutita* extract and cephalexin, the results were similar to those obtained with the combination of the extract with norfloxacin, where we obtained an MIC at a dilution of 1:64, corresponding to 8 µg/mL of the antibiotic and 13.43 µg/mL of the extract. For this MIC, we observed an effect for the combination in 75% of samples, i.e., only 2 samples were resistant to the association (Table 2). It is important to emphasize that the antimicrobials, when tested individually, presented halos of 28 mm for norfloxacin and 29 mm for cephalexin, for the initial concentration of 512 µg/mL with MICs of 64µg/mL (norfloxacin) and 32µg/mL (cephalexin). On the other hand, the pure extract of *M. recutita* presented halos of 22 mm for the concentration of 860.00 µg/mL with an MIC of 430 µg/mL, but when combined, the halos ranged from 31-46 mm, demonstrating that the *in vitro* antimicrobial action against these microorganisms was enhanced when using the pure extract of *M. recutita* and the evaluated antimicrobials. Thus, we observed a correlation between the decrease in tannin and cephalexin concentrations and the diameter of the inhibition halos.

For this association, we observed that the extract has a significant pharmacological effect when associated with the antibiotics evaluated in the study, enhancing the *in vitro* activity of the antibiotics against these microorganisms.

The bactericidal kinetics of *M. recutita* extract were tested on a sample of *S. aureus* of bovine origin obtained from an udder (102), in which bactericidal activity was observed in the first 2 h of contact. In

Table 3, death kinetics for *S. aureus* were observed. In the analysis between the control and the extracts, all results were significant with decreased bacterial growth, from  $5.88 \times 10^8$  CFU/mL at time zero to  $6.68 \times 10^7$  CFU/mL within the first hour, where partial death of bacteria occurred. In both samples there was a reduction of  $2 \log_{10}$  CFU/mL in the initial number of bacteria ( $p < 0.05$ ) (Table 3). In the analysis of the control and gentamicin, inhibition of drug was not significant.

TABLE 1: Minimum inhibitory concentration (MIC) and diameter of the inhibition halos (mm) in solid medium of the association of the *Matricaria recutita* (chamomile) extract (EP) with norfloxacin (Nor) against *Staphylococcus aureus*.

Microorganism	Concentration in mg/mL+ µg/mL						
	860 + 512	430 + 256	215 + 128	107.5 + 64	53.7 + 32	26.8 + 16	13.4 + 8
	Dilutions						
	EP+Nor	1:2	1:4	1:8	1:16	1:32	1:64*
FN121	33	32	30	27	23	18	10
U102	44	38	36	34	31	29	14
U122	42	39	38	35	32	28	13
U250	39	38	36	32	29	25	12
L146	31	43	40	37	34	27	13
L311	34	39	35	33	31	25	12
ATCC 29213	40	39	37	31	28	24	12
ATCC 25925	44	40	39	34	30	25	10
<b>Mean ± SD</b>	$38.3 \pm 5^a$	$38.5 \pm 3^a$	$36.3 \pm 3^a$	$32.8 \pm 2.9^a$	$29.7 \pm 3.2^a$	$25.1 \pm 3.3^{ab}$	$12.0 \pm 1.4^{cb}$

Means followed by the same letter were not significantly different. Kruskal-Wallis test; EP = Pure extract; FN = Nasal Fossa; U = Udder; L = milk; Nor= Norfloxacin;

\* MIC = 8 µg/mL (antibiotic) and 13.43 µg/mL (extract).

TABLE 2: Minimum inhibitory concentration (MIC) and diameter of inhibition halos (mm) in solid medium of the association of the *Matricaria recutita* (chamomile) extract (EP) with cephalixin (Cef) against *Staphylococcus aureus*.

Microorganism	Concentration in mg/mL+ µg/mL						
	860 + 512	430 + 256	215 + 128	107.5 + 64	53.7 + 32	26.8 + 16	13.4 + 8
	Dilutions						
	EP + Cef	1:2	1:4	1:8	1:16	1:32	1:64*
FN121	44	38	36	34	31	29	14
U102	46	43	41	37	32	30	16
U122	40	39	36	34	29	26	00
U250	35	32	29	26	22	19	00
L146	39	38	36	34	30	27	10
L311	37	35	32	29	28	25	14
ATCC 29213	38	36	33	29	26	23	10
ATCC 25925	42	39	36	31	29	25	13
<b>Mean ± SD</b>	$40.1 \pm 3.6^a$	$37.5 \pm 3.2^a$	$34.8 \pm 5^a$	$31.7 \pm 3.6^a$	$28.3 \pm 3^a$	$25.5 \pm 3.4^{ab}$	$9.6 \pm 6.2^{cb}$

Means followed by the same letter were not significantly different. Kruskal-Wallis test ( $p < 0.05$ ). EP = Pure extract; FN = Nasal Fossa; U = Udder; L = milk; Cef = Cephalixin; \* MIC = 8 µg/mL (antibiotic) and 13.43 µg/mL (extract).

TABLE 3: Bactericidal kinetics of the *Matricaria recutita* extract (chamomile) on strains of *Staphylococcus aureus*.

<i>S. aureus</i>	Time of Action of the Extract (minutes)/CFU/mL						
	0	30	60	240	360	840	1440
Udder T	5.88 x 10 <sup>8*</sup>	7.4 x 10 <sup>6*</sup>	6.68 x 10 <sup>7</sup>	12.84 x 10 <sup>7</sup>	9.8 x 10 <sup>8</sup>	11.24 x 10 <sup>9</sup>	7.3 x 10 <sup>10</sup>
Udder C	5.88 x 10 <sup>8</sup>	4.7 x 10 <sup>7</sup>	11.26 x 10 <sup>7</sup>	5.72 x 10 <sup>8</sup>	6.56 x 10 <sup>9</sup>	6.92 x 10 <sup>10</sup>	12.33 x 10 <sup>11</sup>

Udder T = Treated; Udder C = Control; Kruskal-Wallis Test \*p < 0.05.

## Discussion

When comparing the average of the inhibition halos produced, we found that the extract, when associated with both antibiotics tested, presented antimicrobial activity against *S. aureus*. These are relevant data, considering that the species *S. aureus* (MRSA) presents resistance to the majority of conventional antibiotics (CUNHA; CUNHA, 2007).

The anti-inflammatory, antioxidant and genotoxic activities of chamomile extracts (*M. recutita*) have been investigated in laboratory studies and with experimental models, where concentrations that had effects ranged from 47.41 mg/mL to 40 g/L (MORAIS et al., 2009; VIEIRA et al., 2009). Our results indicate the MICs were 1:64 dilutions, which correspond to 8 µg/mL of norfloxacin or cephalexin and 13.43 µg/mL of *M. recutita* extract, and were the optimal concentrations for the preparation of substances for clinical use. Traditionally, *in vitro* antimicrobial activity is assessed by measuring the MIC, which can be done by measuring halos or by determining resistance levels. However, other pharmacodynamic parameters can be used to determine antibacterial and bactericidal efficacy, such as bactericidal kinetics (SANTOS et al., 2011).

Bactericidal kinetics is an experimental method that determines the viability of the tested organisms after exposure to the extract, or antibiotic, of interest during a given period (MAY et al., 2000). Some studies considered the kinetics satisfactory when there was a reduction greater than or equal to 2log [10] CFU/ mL, for a period shorter than or equal to 24 h (SHELBURNE et al., 2004). Other authors have demonstrated that an *M. recutita* extract has excellent *in vitro* bactericidal activity against *Pseudomonas aeruginosa* (CARVALHO et al., 2014).

Our results demonstrate that *M. recutita* caused a decrease in the growth of *S. aureus* samples at an exposure time between 30 minutes and 4 h. Other studies obtained similar results to ours when evaluating the synergistic activity of norfloxacin, tetracycline and erythromycin associated with an ethanol extract of *Mangifera indica* L. bark on *S. aureus* isolates (OLIVEIRA et al., 2011). These authors noted that the extract could potentially be an adjunctive source for antibiotics. According to several authors, ethanol extracts of medicinal plants can enhance the effect of commercial antimicrobials, providing, by synergism, a more efficient interaction against resistant microorganisms (ELBASHITI et al., 2011; HUSSIN; EL-SAYED, 2011). In an attempt to elucidate the mechanism of action of an *M. recutita* extract, regarding its antimicrobial activity, Brehm-Stecher and Johnson (2003) isolated active compounds and tested them alone. These authors observed, by diffusion in agar, that alpha-bisabolol, sesquiterpenes nerolidol, farnesol and apritone, in low concentrations (0.5 mM, 1 mM and 2 mM), modify the permeability of membranes in microorganisms and can pass through the cell membrane of bacteria (such as *S. aureus* and *E. coli*). According to these authors, the alteration in membrane permeability allows the entry of exogenous substances, such as antibiotics, to the interior of bacteria, which provides a synergistic effect of the antimicrobial potential of some antibiotics, such as erythromycin, gentamicin and vancomycin, on the growth of *S. aureus*. Thus, the authors state that the increased effectiveness of sesquiterpenes in penetrating bacterial membranes may be related to the similarity of their chemical structures with the membranes, which have long carbon chains. These data corroborate Cornwell and Barry (1994), who observed that the long carbon chains in farnesol and nerolidol molecules

play an important role in penetrating the membranes of bacterial cells, which would favor the entry of exogenous substances, such as antibiotics.

Therefore, the results obtained in this study are very significant, providing new perspectives for further research, in addition to future *in vitro* and *in vivo* clinical studies, in order to find alternative techniques for controlling bacterial infections at low concentrations with lower risk of collateral effects. Although there is a growing number of publications of *in vitro* models with natural products, more information and investigations are needed to elucidate the physiological processes involved in the detected antimicrobial response (ALBUQUERQUE et al., 2010b; LINS et al., 2013). New studies are also needed to better understand the possible risks and benefits of the combined use of natural products and conventional antimicrobials, because they may provide means to elucidate the mechanisms associated with bacterial resistance.

In this context, the optimization of the use of antibiotics associated with extracts or isolated phytochemicals should be based on pharmacodynamic principles, which have important implications in conducting antibiotic therapy, thus contributing to a decrease in the emergence of antibiotic resistance (PEREIRA et al., 2008).

In conclusion, the *M. recutita* extract promoted a synergistic effect of the antimicrobial potential of norfloxacin and cephalexin on the growth of *S. aureus*, enhancing antimicrobial activity and improving the *in vitro* effect of the drugs. This extract can be used as an important tool against antibiotic-resistant microorganisms.

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