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Antibacterial activity of the Piper aduncum oil and dillapiol, its main constituent, against multidrug-resistant strains

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 13, núm. 6, 2014, pp. 517-526

Universidad de Santiago de Chile
Santiago, Chile

Available in: http://www.redalyc.org/articulo.oa?id=85632545002
Artículo Original | Original Article

Antibacterial activity of the *Piper aduncum* oil and dillapiole, its main constituent, against multidrug-resistant strains

[Actividad antibacteriana del aceite de *Piper aduncum* y dillapiole, su componente principal, frente a cepas resistentes a múltiples fármacos]

Maria Angélica B BRAZÃO¹, Fabio V BRAZÃO², José Guilherme S MAIA¹ & Marta C MONTEIRO¹

¹Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Pará, Belém, PA, Brazil;
²Laboratório Ruth Brazão, 66060-220 Belém, PA, Brazil.

Contactos | Contacts: Marta C MONTEIRO - E-mail address: martachagas2@yahoo.com.br

Abstract: The study aimed to evaluate the bactericidal activity of oil essential and dillapiole from *P. aduncum* against standard and multidrug-resistant strains of *Staphylococcus spp*. The oil showed antimicrobial action against these strains, but better results were obtained for the standards strains of *S. epidermidis* and *S. aureus*, with MIC of 250 and 500 μg/mL, respectively. Dillapiole was less effective than the oil against the same standard and multi-drug resistant strains (MIC = 1000 μg/mL). However, when dillapiole was tested in combination with myristicin, another component of the oil, it increased its bactericidal activity and showed a synergistic action.

Keywords: *Piper aduncum*, *Piperaceae*, dillapiole; myristicin; piperitone; antimicrobial activity; multidrug-resistant strains

Resumen: El objetivo del estudio fue evaluar la actividad bactericida de los aceites esenciales y dillapiole de *P. aduncum* contra cepas estándar y multirresistentes de *Staphylococcus spp*. El aceite mostró acción antimicrobiana frente a estas cepas, pero se obtuvo mejores resultados para las cepas de *S. epidermidis* y *S. aureus*, con MIC de 250 y 500 μg/mL, respectivamente. Dillapiole fue menos eficaz que el aceite contra cepas estándar y multirresistentes (MIC = 1000 μg/mL). Sin embargo, cuando dillapiole fue probado en combinación con la miristicina, otro componente del aceite, que aumentó su actividad bactericida y mostró una acción sinérgica.

Palabras clave: *Piper aduncum*, *Piperaceae*, dillapiole, miristicina, piperitone, actividad antimicrobiana, las cepas multirresistente

INTRODUCTION

Piper is the largest genus in the Piperaceae with about 1,000 species and among them there are about 170 species occurring in Brazil (Yuncker, 1972). Piper aduncum L. is a widespread shrub growing wild in the Amazon, known as “pimento-de-macaco” [syn. Artanthe adunca (L.) Miq., A. elongata (Vahl) Miq., Piper aduncifolium Trel., P. angustifolium Ruiz & Pav., P. elongatum Vahl, Steffensia adunca (L.) Kunth, S. elongata (Vahl) Kunth, among many other]. With respect the popular use, the leaf tea of P. aduncum is indicated in the treatment of pyelitis, cystitis, erysipelas, and for wound healing (Vieira, 1991; Vanden Berg, 1993; Coimbra 1994).

Previously, from the leaves of P. aduncum, with occurrence in the Brazilian Amazon, was seen a significant essential oil yield (1.5% to 3.5%) and a high content of dillapiole (31.5% to 97.3%) (Gottlieb et al., 1981; Maia et al., 1998), a phenylpropene derivative. Dillapiole was also the main constituent in the oil of P. aduncum existing in Cuba (82.2%), Malaysia (64.5%), Costa Rica (61.8%) and Eastern Ecuador (45.92%) (Jantan et al., 1994; Ciccio & Ballester, 1997; Pino et al., 2004; Guerrini et al., 2009). On the other hand, specimens of P. aduncum occurring in the Brazilian Atlantic Forest are rich in terpene compounds as linalool and (E)-nerolidol, as well as, 1,8-cineole, (E)-caryophyllene and aromadendrene in specimens existing in Bolivia and Panamá (Pino et al., 2004; Vila et al., 2005; Oliveira et al., 2006; Navickiene et al., 2006; Guerrini et al., 2009). In this respect, the constituents of volatile oils can vary in accordance with the climate, type and management of soil, sunlight, temperature, and the humidity of different ecosystems (Almeida et al., 2009).

Antimicrobial, molluscicidal and cytotoxic properties of extracts and essential oil from Piper aduncum have been reported. Prenylated p-hydroxybenzoic acid derivatives, dihydrochalcones and chromenes isolated from its leaf alcoholic extract were active against protozoa parasites and diverse microorganisms, including pathogenic bacteria and fungi (Nair & Burke, 1990; Orjala et al., 1993; Orjala et al., 1994; Okunade et al., 1997; Lentz et al., 1998; Kloucek et al., 2005; Guerrini et al., 2009). The ethanol extract from aerial part of P. aduncum were more active against Gram-positive than against Gram-negative bacteria (Kloucek et al., 2005).

The essential oil of P. aduncum proved to be toxic and insecticide for some insect pests, especially against Anopheles marajoara and Aedes aegypti, the mosquitoes that transmit malaria and dengue, as well as against Solenopsis saevissima, the fire ant which has caused serious problems in Northern Brazil. The biological effects observed for the oil of P. aduncum were attributed to dillapiole, a phenylpropene derivative and its main constituent (Bernard et al., 1995; Almeida et al., 2009; Souto et al., 2012). Dillapiole was also reported as a synergist of various natural insecticides, including carbamates, organochlorides, pyrethrum, tenulin, and azadirachtin. Furthermore, it was speculated that dillapiole exhibits synergistic action with other volatiles occurring in the oil of the P. aduncum, increasing its stability and insecticide effect (Hand & Dewan, 1974; Tomar et al., 1979a; Tomar et al., 1979b; Bernard et al., 1990). The oil of P. aduncum and dillapiole showed also fungicidal activity against Crinipellis pernicioso and Fusarium solani f. sp. Piperis, two phytopathogenic fungi, which infects the cocoa and pepper crops in the Brazilian Amazon (Bastos, 1997; Benchimol et al., 2001). The toxicological evaluation for the oil of P. aduncum showed low toxicity in mice (LD₅₀ 2.400 ± 191.7 mg/kg) (Sousa et al., 2008).

In the surveillance studies have been seen an increase in the resistance for Gram positive pathogens, which are dangerous nosocomial infection agents. About 60% of all strains are methicillin-resistant Staphylococcus aureus (MRSA) and Staphylococcus coagulase negative (SCN). These strains are resistant to most beta-lactam antibiotics, causing an increase in mortality of patients and high health-care costs (Rice, 2006; Martins & Cunha, 2007; Fessler et al., 2010). Moreover, it has been reported that bacteria resistant to beta-lactam antibiotics are more sensitive to oils and extracts of plants, as well as their isolated compounds (Ríos & Recio, 2005).

Above, it was seen that P. aduncum extracts have antimicrobial, molluscicidal and cytotoxic properties while its essential oil and dillapiole showed insecticide and fungicide effects. The aim of this study was to evaluate the antibacterial activity of the oil of P. aduncum and dillapiole against multi-resistant isolates, in order to analyze their potential use as inhibitors of hospital infection.
MATERIAL AND METHODS

Plant Material and processing
The aerial parts (leaves and thin stems) of *P. aduncum* have been obtained from an experimental station located in the municipality of Santo Antonio do Tauá, Pará state, Brazil. Vouchers were deposited in the herbarium of Emilio Goeldi Museum, city of Belém, state of Pará, Brazil. The plant was dried in an air circulating oven (24 h, 35 ºC) to reduce the moisture to 15% and then subjected to hydrodistillation (300g, 3 h) using a Clevenger-type apparatus. The resulting oil was dried over anhydrous sodium sulfate, and its percentage content was calculated on the basis of the plant dry weight. The moisture amount of the sample was calculated after phase separation in a Dean-Stark trap (5 g, 60 min), using toluene.

Oil-composition analysis
The analysis of the oil was carried out on a THERMO DSQ II GC-MS instrument, under the following conditions: DB-5ms (30 m x 0.25 mm; 0.25 µm film thickness) fused-silica capillary column; programmed temperature, 60-240 ºC (3 ºC/min); injector temperature, 250 ºC; carrier gas, was helium adjusted to a linear velocity of 32 cm/s (measured at 100 ºC); injection type, splitless (2 µL of a 1:1000 hexane solution); split flow was adjusted to yield a 20:1 ratio; septum sweep was a constant 10 ml/min; EIMS, electron energy, 70 eV; temperature of ion source and connection parts, 200 ºC. The quantitative data regarding the volatile constituents were obtained by peak-area normalization using a FOCUS GC/FID operated under conditions similar to those in GC-MS, except for the carrier gas, which was nitrogen. The retention index was calculated for all the volatiles constituents using an *n*-alkane (C₈-C₂₀, Sigma-Aldrich) homologous series.

Individual components were identified by comparison of both mass spectrum and GC retention data with authentic compounds, which were previously analyzed and stored in the data system, as well as with the aid of commercial libraries containing retention indices and mass spectra of volatile compounds commonly found in essential oils (NIST, 2005; Adams, 2007).

Oil-fractionation
The oil (5 g) was submitted to column chromatography (CC, 80 x 3cm) over silica gel (SiO₂, 70 g, 230-400 mesh), with hexane as solvent, and the separated fractions were monitored by TLC (visualization with vanillin/H₂SO₄, 110 ºC). The content of dillapiole in the oil and in the purified fractions was determined by GC/FID.

Determination of antibacterial activity

Test organisms
Antibacterial activity was evaluated for the standard strains of *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228, which were obtained from the INCQS/FIOCRUZ (Instituto Nacional de Controle da Qualidade da Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil). Furthermore, two multi-drug resistance (MDR) clinic isolates (*S. epidermidis* and methicillin-resistant *Staphylococcus aureus*, MRSA) were also applied as test organisms and obtained from cultures of patient samples existing in the Public Hospital (Bacteriological Laboratory) of Belém city, Pará, Brazil. These isolates were identified at species level, using conventional microbiological methods and *in vitro* susceptibility, when tested by disk diffusion in the automated system Vitek 2 (BioMérieux, France). MDR isolates were defined according to an international expert proposal in view to establish standard definitions for acquired resistance, supported by the European Centre for Disease Prevention and Control (ECDC, Stockholm, Sweden) and the US Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). The drug resistance profiles of these isolates were described in Table 1. According to this definition, a MDR isolate should have acquired resistance (non-sensitivity) to more than one agent in three or more antimicrobial classes (Magiorakos et al., 2012).
Table 1

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Source</th>
<th>Antibiotic resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>Surgical wound</td>
<td>Pen, Oxa, Met, Gen, Cip, Nor, Eri, Clin, Rif, TMP/SMX</td>
</tr>
<tr>
<td>MDR <em>Staphylococcus epidermidis</em></td>
<td>Surgical wound</td>
<td>Pen, Oxa, Met, Gen, Cip, Mox, Nor, Eri, Clin, TMP/SMX</td>
</tr>
<tr>
<td>MDR <em>Staphylococcus lentus</em></td>
<td>Surgical wound</td>
<td>Pen, Oxa, Met, Gen, Cip, Mox, Nor, Eri, Clin</td>
</tr>
</tbody>
</table>

Pen, Penicillin; Oxa, Oxacillin; Met, Methicillin; Gen, Gentamicin; Cip, Ciprofloxacin; Mor, Moxifloxacin; Nor, Norfloxacin; Eri, Erythromycin; Clin, Clindamycin; Rif, Rifampicin; TMP/SMX = trimethoprim/sulfamethoxazole; MDR - Multidrug-resistant

**Broth micro-dilution assay for minimum inhibitory concentration (MIC)**

For bacterial inocula preparation, strains were grown to exponential phase in Mueller–Hinton broth (Merck, German) at 37 ºC, for 18 h, and adjusted by diluting fresh cultures to turbidity, equivalent to 0.5 McFarland scale (approximately of 2 x 10⁸ CFU/mL) as described by Clinical and Laboratory Standards Institute (CLSI, 2012). MIC assay was performed by using the broth microdilution method in Mueller-Hinton broth (MHA) as described by CLSI (2012). Separately, the oil and dillapiole were dissolved in tween 20 to the highest concentration (2000 μg/mL) to be tested and, then, a serial two-fold dilution was made in a concentration range from 100 to 1000 μg/mL in 1 mL sterile test tubes containing MHA. The inoculum (100 μL) containing 5x10⁸ CFU/mL was added to each well and 100 μL from their serial dilutions were transferred into continuous wells. The final volume in each well was 200 μL. The last well containing MHA (100 μL) in Tween 20 without the oil and inoculum (100 μL) was used as negative control.

Chloramphenicol (50 μg/mL) served as the positive control in the parallel experiments. The MIC was defined as the lowest concentration of the oil and dillapiole at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity as described by Quadros et al. (2011). Each concentration reading was repeated three times. All experiments were repeated at least twice.

**Statistical analysis**

Results were expressed as mean (SEM). Statistically significant differences between groups were determined by one-way analysis of variance (ANOVA) followed by Tukey’s test (p ≤ 0.05).

**RESULTS AND DISCUSSION**

**Oil-analysis and purified fractions**

The dried aerial part (leaves and thin stems) of *Piper aduncum* were subjected to hydrodistillation, and the yield of oil was 3.0%. The volatile constituents of the oil and its column chromatography purified fractions were analyzed by GC and GC-MS. The identified constituents were listed in Table 2, totaling 95.6%. The main constituent was dillapiole comprising 76.5% of the oil. Taking into account the presence of myristicin (2.1%), apiole (1.2%) and elemicin (0.8%), three other constituents structurally related to dillapiole, the final content of phenylpropanoids reached 80.5% of the oil. The oil was subjected to column chromatography with a view to purifying dillapiole. Between the purified fractions, the higher fraction (2.8 g) showed dillapiole with a percentage of 94.6%, which then it was used in the antibacterial assays. It is also remarkable the high content of piperitone (6.1%) in the oil.
**Table 2**
Constituents identified in the oil (%) of *P. aduncum*.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Retention Index*</th>
<th>Oil %</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>934</td>
<td>0.7</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>978</td>
<td>0.5</td>
</tr>
<tr>
<td>Limonene</td>
<td>1026</td>
<td>0.8</td>
</tr>
<tr>
<td>(Z)-β-Ocimene</td>
<td>1035</td>
<td>0.2</td>
</tr>
<tr>
<td>(E)-β-Ocimene</td>
<td>1045</td>
<td>0.4</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1056</td>
<td>1.3</td>
</tr>
<tr>
<td>Terpinen-4-ol</td>
<td>1174</td>
<td>2.3</td>
</tr>
<tr>
<td>Piperitone</td>
<td>1251</td>
<td>6.1</td>
</tr>
<tr>
<td>(E)-Caryophyllene</td>
<td>1416</td>
<td>1.5</td>
</tr>
<tr>
<td>Germacrone D</td>
<td>1484</td>
<td>1.2</td>
</tr>
<tr>
<td>Myristicin</td>
<td>1519</td>
<td>2.1</td>
</tr>
<tr>
<td>Elemicin</td>
<td>1555</td>
<td>0.8</td>
</tr>
<tr>
<td>Dillapiole</td>
<td>1621</td>
<td>76.5</td>
</tr>
<tr>
<td>Apiole</td>
<td>1678</td>
<td>1.2</td>
</tr>
<tr>
<td>Sesquiterpenes, both hydrocarbons and oxygenated, not identified</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>99.2</strong></td>
<td></td>
</tr>
</tbody>
</table>

* on column DB-5ms.

**Antibacterial bioassays**
The antibacterial activity of the oil of *P. aduncum* and dillapiole against ATCC strains and MDR isolates is showed in Figure 1. The oil was effective against *Staphylococcus aureus* ATCC strain at concentrations of 500 µg/mL, 750 µg/mL and 1000 µg/mL, inhibiting the bacterial growth in 50%, 90% and 100%, respectively (Figure 1, panel A). However, the oil only was able to inhibit significantly the growth of MRSA isolate at the concentrations of 750 µg/mL and 1000 µg/mL, corresponding to 10% and 90%, respectively (Figure 1, panel B). With respect to dillapiole, it was able to inhibit 100% the growth of *S. aureus* ATCC strain at concentration of 1000 µg/mL, while it was not effective against the MRSA isolate at the same concentration (Figure 1, panel A and B). Similarly, the oil also inhibited the growth of *S. epidermidis* ATCC strain at concentrations between 250 to 1000 µg/mL, while it was only effective against the MDR *S. epidermidis* isolate at concentrations of 750 to 1000 µg/mL (Figure 1, panel C and D, respectively). With respect to dillapiole, it showed a inhibition of 25% in growth of *S. epidermidis* ATCC strain and MDR *S. epidermidis* isolate, at concentration of 1000 µg/mL (Figure 1, panel C and D, respectively).
Figure 1
Antibacterial activity of the oil and dillapiole from *P. aduncum* against standard strains and multi-drug resistant isolates. (A) *S. aureus* ATCC 25923 strain; (B) methicillin-resistant *S. aureus* (MRSA) isolate; (C) *S. epidermidis* ATCC 12228 strain; and (D) MDR *S. epidermidis* isolate. *S. aureus* and *S. epidermidis* were incubated at different concentrations of oil and dillapiole, during 24 h and 37 °C. As negative control (C-) was used tween 20 and positive control (C+) was used chloramphenicol. Bacterial growth were expressed as mean (SEM) percentage (n = 3). *p< 0.001 compared with the negative control.

The MIC data for the oil and dillapiole of *P. aduncum* tested against ATCC strains and MDR isolates, and compared with literature data, were summarized in Table 3. These data showed that the oil was able to inhibit the standard strains of *S. epidermidis* and *S. aureus* ATCC with MIC values of 250 and 500 μg/mL, respectively. Even with respect to oil, it was effective to inhibition the growth of MRSA *S. aureus* and MDR *S. epidermidis* with MIC values of 750 μg/mL, while for MDR *S. lentus* it was active only with values >1000 μg/mL. Previously, the oil of *P. aduncum* had indicated a MIC value of 5240 μg/mL for the inhibition of *S. aureus* ATCC 25923 (see Table 3) (Guerrini et al., 2009).

In comparison with the oil, the dillapiole was less effective against the ATCC strains and MDR isolates, showing MIC values higher than 750 μg/mL (see Table 3). The fact that the oil was more effective than dillapiole might be interpreted by a synergistic action existing in the oil. It is likely that the presence of other volatile constituents in the oil was contributing to its greater bacterial effect. The co-occurrence of myristin and dillapiole in essential oils is frequent because dillapiole results from
enzymatic methoxylolation of myristicin. From the seeds of *Myristica fragrans* (Myristicaceae), it has been seen that myristicin exhibits high antimicrobial activity against gram-positive (*S. aureus*, MIC = 0.75 µg/mL; *Micrococcus luteus*, MIC = 0.625 µg/mL) and gram-negative bacteria (*Pseudomonas aeruginosa*, MIC = 0.60 µg/mL; *Escherichia coli*, MIC = 1.25 µg/mL) (Narasimhan & Dhake, 2006). On the other hand, others studies have shown that the myristicin was ineffective against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 9027 (Stefano et al., 2011; Torbati et al., 2013), showing that the antibacterial action of myristicin is still controversial.

Based on the facts above, the dillapiole when tested in combination with the myristicin (Fluka standard), under the same working conditions and using percentages of 1 to 2% of myristicin, showed antibacterial activity with comparable values to that previously observed for the oil and dillapiol alone, that was MIC values between 500 and 750 µg/mL (Table 3). Other important component from oil of *P. aduncum*, is the Piperitone, which it can help in bactericidal action of dilapiole and/or myristicin. Although in this study, the piperitone has not been tested with dillapiole because it was not possible to obtain a sample of this compound. However, most studies have reported that this component has a high bactericidal action against gram-positive and gram-negative bacteria. In this regards, the essential oil of *Mentha pulegium* L. rich in piperitone (38%) showed powerful antimicrobial activity against *Staphylococcus aureus* ATCC 25923 (MIC = 0.5 µg/mL), *Staphylococcus epidermidis* ATCC 12228 (MIC = 1 µg/mL), *Bacillus cereus* (MIC = 1 µg/mL), *Escherichia coli* (MIC = 2 µg/mL) (Mahboubi & Haghi, 2008). Similarly, the oil of *Tagetes patula* L. (Asteraceae) rich that also is in piperitone (33.8%) showed exhibited highly bactericidal effect against *S. aureus* ATCC 25923 (MIC = 30 µg/mL), *P. aeruginosa* (MIC = 130 µg/mL and *E. coli* (MIC = 60 µg/mL) (Rondón et al., 2006). Others researches have also reported that the essential oil of aerial parts of *Ziziphora persica* that also was detected piperitone was active against several Gram-positive and Gram-negative bacteria with MIC in the range of 7.81–250 µg/ml (Meral et al., 2002; Ozturk & Ercisli, 2006). Also, the oil of *Satureja parvifolia* (Lamiaceae) rich in piperitone (34.9%) was found to have moderate activity against *S. aureus* methicillin sensitive and *S. aureus* clinic isolate (MIC = 1630 µg/mL) (Luna et al., 2008). These data support our finding that several compounds, including myristicin and piperitone, may act synergistically in the antimicrobial activity.

Concerning to the alcoholic extract of *P. aduncum* the antimicrobial properties observed were attributed to their isolated compounds, such as the p-hydroxybenzoic acid derivatives, dihydrochalcones and chromenes (Nair & Burke, 1990; Orjala et al., 1993; Orjala et al., 1994; Okunade et al., 1997; Lentz et al., 1998; Kloucek et al., 2005). However, as it is crude extract obtained with polar solvents, it is very likely that the essential oil components have been extracted also and, to some extent, may have contributed to the antimicrobial properties observed for the plant. To corroborate this hypothesis, a previous ethanolic extract of *P. aduncum* showed smaller inhibition than the oil tested in the present work. The inhibition values for the strains of *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were 1000 µg/mL and 2000 µg/mL in the extract while, for the oil the values were 500 µg/mL and 250 µg/mL, respectively.

**CONCLUSION**

The oil and extracts from aerial parts of *Piper aduncum* have showed numerous biological properties. In this study was seen that the oil has significant bactericidal activity against resistant strains of *Staphylococcus aureus*, *S. epidermidis* and *S. lentus*, which are dangerous nosocomial infection agents. Dillapiole, its main constituent, was less effective against these bacteria. However, when it was tested in combination with myristicin, other constituent of the oil, it had increased its bactericidal activity. Therefore, the bactericidal action of the oil is more significant due to synergistic action of its main constituents, as myristicin and piperitone.
Table 3
Antibacterial activity of the oil and dillapiole from *Piper aduncum* against standard strains and multi-drug resistant isolates (MRSA and MDR), in comparison with literature data.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>P. aduncum Oil</th>
<th>Dillapiolene</th>
<th>Dillapiole + Myristicin-rich Oil</th>
<th>Piperitone-rich Oil</th>
<th>Ethanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (μg/mL)</td>
<td>MIC Lit (1)</td>
<td>MIC (μg/mL)</td>
<td>MIC Lit (2)</td>
<td>MIC Lit (3)</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 25923</td>
<td>500</td>
<td>5240</td>
<td>500</td>
<td>NA</td>
<td>30-0.5</td>
</tr>
<tr>
<td><em>S. epidermidis</em> ATCC 12228</td>
<td>250</td>
<td>&gt;750</td>
<td>750</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> isolate</td>
<td>&gt;750</td>
<td>&gt;1000</td>
<td>&gt;750</td>
<td>NA</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Methicillin-resistant <em>S. aureus</em> (MRSA)</td>
<td>&gt;750</td>
<td>&gt;1000</td>
<td>&gt;750</td>
<td>NA</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>MDR <em>S. epidermidis</em></td>
<td>750</td>
<td>&gt;750</td>
<td>&gt;750</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>MDR <em>S. lentus</em></td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>

Lit = literature data: (1) Guerrini *et al*., 2009; (2) Narasimhan & Dhake, 2006; (3) Rondón *et al*., 2006; Luna *et al*., 2008; (4) Kloucek *et al*., 2005. NA, Not Active.

ACKNOWLEDGMENTS
We are grateful to CNPq/BIONORTE Program for the financial support and to Laboratório Ruth Brazão and Laboratório Jayme Aben Athar, from the Santa Casa de Misericórdia do Pará, for their technical support. This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), FAPESPA and Federal University of Pará/PA. J. G. S. Maia and M. C. Monteiro were recipients of fellowships from CNPq.

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