Antibacterial Activity of Essential Oil of Cinnamon and Trans-cinnamaldehyde against *Staphylococcus* spp. Isolated from Clinical Mastitis of Cattle and Goats

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**ABSTRACT**

**Background:** Milk production plays a role as a source of protein and constitutes an important socioeconomic factor for small farms. The lack of planning of the creators, together with the lack of specialized technical assistance, and the precarious hygienic and sanitary management, are barriers that hinder the development of the activity. Bovine and caprine mastitis is an inflammation process of the mammary gland caused by microorganisms. *Staphylococcus* spp. is the main causative agent of mastitis, with higher prevalence in cases of clinical and subclinical manifestations. The most common treatment is based on intramammary infusion of antibacterial agents. However, cure rates obtained with such drugs are not always effective, because it may determine the emergence of resistant bacteria as well increase amounts of antibiotic residues in milk. Nevertheless, the treatment of bovine subclinical mastitis caused by *Staphylococcus* spp. in the lactation can be economically unviable. Alternative treatments of bovine mastitis with plant derived compounds have been described. The investigation of its antimicrobial activity against bacterial agents of mastitis is justifiable.

**Materials, Methods & Results:** This study evaluated the antimicrobial activity of essential oil (EO) of *Cinnamomum zeylanicum* (cinnamon) and the fraction *trans*-cinnamaldehyde (TC) against 65 isolates of *Staphylococcus* spp. from mastitis of cows (n = 32) and goats (n = 33). The cinnamon EO was extracted from the leaves and was composed majority by isoeugenol (96%), while the TC was acquired commercially. The antimicrobial susceptibility tests were performed according to CLSI M31-A3 agar diffusion method. Based on these tests, the isolates were divided into subgroups according to the resistance profiles. Subsequently, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution technique CLSI M31-A3. The MICs (geometric mean, GM) for cinnamon EO were 2032 mg/L and 2102 mg/L, respectively, for cattle and goats, while the majority fraction TC was most active with MICs (GM) of 598 mg/L and 875 mg/L, respectively, for cattle and goats.

**Discussion:** Some differences between these results and those reported by other authors can be attributed to the composition of ESs or techniques employed. In this study, we used the broth microdilution technique based on the document M31-A3, from CLSI, for the determination of MICs and MBCs. The lack of an internationally standardized technique for evaluating ESs and plant extracts that allow different protocols undermines the comparisons of results. The results of this study confirmed the antimicrobial activity of cinnamon EO and TC against clinical *Staphylococcus* spp. independent of the antibacterial susceptibility profile. The best antimicrobial activity was observed with TC than in the cinnamon EO, which deserves further research investigating the use of these compound in the treatment of mastitis. The TC can be found in high concentration in cinnamon bark. However, the essential oil used in this work was extracted from the leaves, and the major component was isoeugenol. The cinnamon EO composition can be varied depending on the part of the plant where it is extracted.

**Keywords:** medicinal plants, essential oil, phytotherapy, antibacterial activity, *Staphylococcus* spp., mastitis, antimicrobial alternatives.
INTRODUCTION

Bovine mastitis is an inflammation process of the mammary gland caused by microorganisms such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. dysgalactiae*, *S. uberis* and *Escherichia coli* [2,9]. This disease results in a decrease of lactation and consequently, economic losses for the dairy industry. Worldwide, economic losses due to mastitis have been estimated at $35 billion [13]. *Staphylococcus* spp. is the main causative agent of bovine mastite, with higher prevalence in cases of clinical and subclinical manifestations [7].

The most common therapeutic is usually based on intramammary infusion of antibacterial agents. However, cure rates obtained with such drugs are not always effective, may determine the emergence of resistant bacteria [14] and increase amounts of antibiotic residues in milk [6]. Nevertheless, the treatment of bovine subclinical mastitis caused by *S. aureus* in the lactation can be economically unviable [6].

Alternative treatments of bovine mastitis with bacteriocins [9] and plant derived compounds [1,5,8] has been described. Considering that essential oils (EOs) are classified as GRAS (generally regarded as safe), show antibacterial proprieties and that resistance are not been reported after prolonged exposure, the investigation of its antimicrobial activity against bacterial agents of mastitis is justifiable.

In this context, this study aimed to: a) evaluate the *in vitro* antimicrobial activity of EOs from cinnamon (*Cinnamomum zeylanicum*) and trans-cinnamaldehyde; b) compare the activity of EOs against clinical *Staphylococcus* spp. isolates from mastitis cases with multiple profiles of susceptibility to penicillin, erythromycin, ampicillin, cephalothin and tetracycline antibiotics.

MATERIALS AND METHODS

**Microorganisms**

Sixty-five coagulase positive *Staphylococcus* spp. isolates from mastitis of cows (n = 32) and goats (n = 33), provided by the Laboratório de Bacteriologia Veterinária, Universidade Federal de Santa Maria (UFSM), RS, Brazil, were studied. *S. aureus* ATCC 29213 was employed as reference strain.

**Antimicrobial susceptibility test**

The antimicrobial susceptibility tests were performed according to CLSI M31-A3 agar diffusion method [4] and included ceftiofur (30 µg) (CEF), penicillin (10U) (PEN), ampicillin (10 µg) (AMP), cephalothin (30 µg) (CEPHA), oxacillin (1 µg) (OXA), erythromycin (15 µg) (ERY) and tetracycline (30 µg) (TET). Based on these tests, the isolates were divided into subgroups according to the resistance profiles (Table 1).

**Essential oils and majority compounds**

It was evaluated the essential oils (EO) from cinnamon (*Cinnamomum zeylanicum*) and trans-cinnamaldehyde (TC). Cinnamon EO was extracted from leaves and was composed majority by isoeugenol (96%), as previously analyzed [10]. The TC1 was acquired from Acros Organics.

**Determination of EO minimal inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)**

Essential oils were first diluted with methanol to achieve the concentration of 640 mg/L (Solution I) and then diluted to 1:100 in Mueller-Hinton broth, resulting in the concentration of 6400 mg/L (Solution II). The MICs were determined in Mueller-Hinton broth. The MICs were determined after incubation at 35°C/24 h in aerobic conditions. These tests were performed in triplicate.

The MBCs were determined from wells without visible bacterial growth after 24 h incubation. A volume of 10 µL was inoculated in each well containing essential oil incorporated into Mueller-Hinton broth. The MBCs were determined after incubation at 35°C/24 h in aerobic conditions. These tests were performed in triplicate.

The MBCs were determined from wells without visible bacterial growth after 24 h incubation. A volume of 10 µL was transferred into the surface of Mueller-Hinton agar, which was incubated at 35°C/24 h. Then bacterial growth was observed, registering the lowest concentration of cinnamon EO or TC that did not show bacterial growth. These tests were performed in triplicate.

**Statistical Analysis**

The Mann-Whitney test was used to compare two independent samples in order to observe whether the di-
different study groups had similar patterns of susceptibility or not, face a particular essential oil or major compounds.

**RESULTS**

The geometric means (GM) of the MICs and MBCs of EO against *Staphylococcus* spp. were [GM-MIC/GM-MBC]: *Cinnamomum zeylanicum* EO [2032 mg/L / 4263 mg/L] and [2102 mg/L / 3786 mg/L] against the isolates from cows and goats, respectively; trans-cinnamaldehyde [598 mg/L / 2238 mg/L] and [875 mg/L / 2468 mg/L] against the isolates from cows and goats, respectively (Table 1).

Table 1. Antimicrobial activity (mg/L) of the essential oil of cinnamon and trans-cinnamaldehyde (TC) against *Staphylococcus* spp. isolated from cattle and goat mastitis.

<table>
<thead>
<tr>
<th></th>
<th>MIC Range</th>
<th>GM</th>
<th>MIC50 Range</th>
<th>GM</th>
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<tbody>
<tr>
<td><strong>Staphylococcus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cl. from cattle</td>
<td>800 - 3200</td>
<td>2032^A</td>
<td>1600</td>
<td>1600 - 6400</td>
</tr>
<tr>
<td>TC</td>
<td>400 - 800</td>
<td>598^B</td>
<td>800</td>
<td>400 - 6400</td>
</tr>
<tr>
<td>cl. from goat</td>
<td>1600 - 3200</td>
<td>2102^A</td>
<td>1600</td>
<td>1600 - 6400</td>
</tr>
<tr>
<td>TC</td>
<td>800 - 1600</td>
<td>875^B</td>
<td>800</td>
<td>800 - 6400</td>
</tr>
</tbody>
</table>

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration, MIC50 = minimum inhibitory concentration able to inhibit 50% of the isolates; Geometric Mean = GM, where the same letters in the same column indicate similar antimicrobial activity. For comparisons between the MICs and MBCs of GM was used the Mann-Whitney test ($P < 0.05$).

Table 2. Relationship between the different profiles of resistance of *Staphylococcus* isolates against antibacterial agents and the essential oil of *Cinnamomum zeylanicum* (Cz) and trans-cinnamaldehyde (TC) susceptibility.

<table>
<thead>
<tr>
<th>Groups (n)</th>
<th>Resistance profile</th>
<th>GM MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(18)</td>
<td>Sensible</td>
<td>Cz 2136.2^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 892.4^A</td>
</tr>
<tr>
<td>2(6)</td>
<td>TET-resistant</td>
<td>Cz 1984.5^A</td>
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<tr>
<td></td>
<td></td>
<td>TC 800.0^A</td>
</tr>
<tr>
<td>3(15)</td>
<td>TET-PEN-resistant</td>
<td>Cz 2032.8^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 778.9^A</td>
</tr>
<tr>
<td>4(6)</td>
<td>ERY-TET-resistant</td>
<td>Cz 2146.6^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 800.0^A</td>
</tr>
<tr>
<td>5(4)</td>
<td>ERY-PEN-resistant</td>
<td>Cz 2340.2^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 800.0^A</td>
</tr>
<tr>
<td>6(8)</td>
<td>ERY-PEN-TET-resistant</td>
<td>Cz 2260.4^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 800.0^A</td>
</tr>
<tr>
<td>7(8)</td>
<td>PEN-ERY-AMP-CEPHA-TET-resistant</td>
<td>Cz 1896.9^A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TC 796.8^A</td>
</tr>
</tbody>
</table>

TET = tetracycline; PEN = penicillin, ERY = erythromycin; OXA = oxacillin, AMP = ampicillin, cephalothin = CEPHA; GM MIC = Geometric Mean of Minimum Inhibitory Concentrations, (n) number of isolates. For the comparisons between the MG MICs was used the Mann-Whitney test ($P > 0.05$).

**DISCUSSION**

In this study, *Cinnamomum zeylanicum* EO and trans-cinnamaldehyde showed bacteriostatic and bactericidal activity at the tested concentrations against the *Staphylococcus* spp. isolates evaluated. The antimicrobial activity of cinnamon EO and TC was independent of the antibacterial susceptibility profile, which is in agreement with Chang et al. [3], who observed inhibition of multiresistant and sensitive bacteria to antibiotics, when tested the antimicrobial activity of essential oil of cinnamon containing 76% cinnamaldehyde (MIC = 250-500 mg/L) in contrast with the absence of growth inhibition when the essential oil of cinnamon was composed of 1.8 cineole.
(11.32%), linalool (9.83%), neral (12.82%), cinnamyl acetate (9.04%) and cinnamaldehyde (8.35%). These authors also observed inhibition of bacteria when evaluating the majority fraction cinnamaldehyde (MIC = 250 to 1000 mg/L). However, this was not observed up to highest concentration tested (1,000 mg/L) for the eugenol fraction. Against Candida spp. sensitive and resistant to fluconazole, was reported antifungal activity of cinnamon EO regardless of the antifungal susceptibility profile of yeasts [10].

The EO extracted from cinnamon bark, composed by cinnamaldehyde (68.95%) and eugenol (2.77%) showed antibacterial activity (MIC = 2,000 mg/L) against Staphylococcus aureus ATCC 29213 [12]. The EO of cinnamon leaf, composed majority by eugenol (73.27%) against S. aureus ATCC 6538 showed antibacterial activity (MIC = 1.25 mg/L and MBC = 20 mg/L) [11]. However, Baskaran et al. [1] reported that the majority fraction of cinnamaldehyde was more effective than eugenol against five species of bovine mastitis pathogens. Against strains of S. aureus (DTSL-35, 17, and 38) the TC showed antibacterial activity (MIC = 0.1% and MBC = 0.45%).

The antimicrobial activity of essential oil extracted from leaves of cinnamon and TC was confirmed in this study, agreeing with the above mentioned authors. The best antimicrobial activity was observed with TC than in the cinnamon EO. Some differences between these results and those reported by other authors can be attributed to the composition of EO or techniques employed. In this study was used the broth microdilution technique based on the document CLSI M31-A3 for determination of MICs and MBCs. However, there is a lack of an internationally standardized technique for evaluating EO and plant extracts that allow the comparison of the different protocols used.

CONCLUSIONS

It was verified that the antimicrobial activity of cinnamon EO and TC were independent of susceptibility profile of the antibiotics tested, which reinforces the status of these essential oils as an alternative in the treatment of mastitis in cattle and goats. Thus, it was concluded: a) The TC was more active than the cinnamon EO tested; b) The susceptibility of Staphylococcus spp. isolated was similar, regardless of source (cattle or goats); c) in subgroups with antimicrobial resistance profiles, there were no significant differences in susceptibility to EO cinnamon or TC.

SOURCE AND MANUFACTURER

Trans-cinnamaldehyde (Acros Organics), New Jersey, USA.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES


