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ORIGINAL ARTICLE

Evaluation of the antimicrobial efficacy of *Minthostachys verticillata* essential oil and limonene against *Streptococcus uberis* strains isolated from bovine mastitis

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Essential oil;
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Abstract Bovine mastitis is a disease that causes great economic losses per year, being *Streptococcus uberis* the main environmental pathogen involved. The aim of the present study was to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *Minthostachys verticillata* essential oil and limonene for *S. uberis* strains isolated from bovine mastitis. In addition, the effect of MIC on biofilm formation was analyzed. MIC values for the essential oil ranged from 14.3 to 114.5 mg/ml (1.56–12.5% v/v) and MBC between 114.5 and 229 mg/ml (12.5–25% v/v). MICs for limonene ranged from 3.3 to 52.5 mg/ml (0.39–6.25% v/v) and MBC was 210 mg/ml (25% v/v). Both compounds showed antibacterial activity and affected the biofilm formation of most of the strains tested. In conclusion, these compounds could be used as an alternative and/or complementary therapy for bovine mastitis caused by *S. uberis*.

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PALABRAS CLAVE
Streptococcus uberis;
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Limoneno;
Mastitis bovina;
Actividad antibacteriana

Evaluación de la eficacia antimicrobiana del aceite esencial de *Minthostachys verticillata* y limoneno contra cepas de *Streptococcus uberis* aislados de mastitis bovina

Resumen La mastitis bovina es una enfermedad que causa grandes pérdidas económicas por año, *Streptococcus uberis* es el principal patógeno ambiental involucrado. El objetivo del presente estudio fue determinar la concentración inhibitoria mínima (CIM) y la concentración bactericida mínima (CBM) del aceite esencial de *Minthostachys verticillata* y del limoneno sobre cepas de *S. uberis* aisladas de mastitis bovina. Además, se analizó el efecto del aceite esencial y el limoneno en la CIM determinada en caso sobre la formación de *biofilm* de estas cepas. Los valores de CIM del aceite esencial oscilaron entre 14,3 y 114,5 mg/ml (1,56%-12,5% v/v) y los de CBM entre 114,5 y 229 mg/ml (12,5%-25% v/v). Las CIM del limoneno oscilaron entre 3,3 y 52,5 mg/ml (0,39% - 6,25% v/v) y la CBM fue de 210 mg/ml (25% v/v). Ambos compuestos mostraron actividad antibacteriana y afectaron la formación de *biofilm* de la mayoría de las cepas. En conclusión, estos compuestos podrían ser utilizados como terapia alternativa o complementaria para la mastitis bovina causada por *S. uberis*.

Introduction

Mastitis is a worldwide disease of dairy cattle that is caused by a wide variety of organisms that affect milk quality and yield, resulting in major economic losses. In many countries it is the most costly disease in dairy milk production[18]. *Streptococcus uberis* is an important pathogen implicated in bovine mastitis, which is predominantly associated with subclinical and clinical intramammary infections in both lactating and non-lactating cows. This species is particularly problematic due to the fact that it is ubiquitous in the dairy environment. A potential virulence factor, possibly linked to the ability of *S. uberis* to adhere to cells, would be the formation of biofilm[33]. It is important to forestall the formation of biofilm in order to treat and prevent intramammary infections.

The ineffectiveness of the different procedures to reduce the rate of new infections has directed research studies toward the search for alternative control methods[21,27]. Within this context, the search for new effective natural prototypes for the treatment of bovine mastitis does not compromise the milk quality that is important for a better quality of dairy farming and food production. Alternative treatments with medicinal plants may be a safe, efficient and a low-cost option for treating bovine mastitis[23]. Essential oils classified as GRAS (Generally Regarded As Safe), show antibacterial properties and resistance has not been reported after prolonged exposure. Therefore, the investigation of their antimicrobial activity against bacterial agents of mastitis is justifiable[9].

Minthostachys verticillata (Griseb) Epling (Lamiaceae), commonly referred to as ''peperina'', is an ethnobotanical aromatic herb with various uses and properties. This species is distributed in South American countries such as Colombia, Venezuela, Brazil, Ecuador, Peru, Bolivia, and in the northwest and central regions of Argentina[24,29]. According to folk traditional medicine it is used as a digestive, sedative, antispasmodic, stimulant, and also to alleviate respiratory illnesses, bronchitis, and asthma[10].

Moreover, numerous *in vitro* studies have described the antiviral, antibacterial and antifungal properties of *M. verticillata* essential oil (EO)[2,11,14,20,26].

In addition, the lack of toxic effect of *M. verticillata* EO and its main compounds, both *in vitro* as *in vivo*, has been demonstrated[5,7,12,13,32]. In a previous assay, we demonstrated that EO obtained from this species and from limonene, one of its main compounds, showed antimicrobial activity against the major bovine mastitis pathogens such as *Staphylococcus aureus*, *S. uberis*, *Escherichia coli* and coagulase negative *Staphylococcus* (CNS) by the disk diffusion method[6].

The aim of the present work was to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *M. verticillata* EO and limonene for *S. uberis* strains isolated from bovine mastitis. In addition, the effect of MIC on biofilm formation was analyzed.

Materials and methods

Plant material

Green leaves and thin stems from *M. verticillata* were collected in Villa Larca city, province of San Luis, Argentina in April, 2013. The voucher specimens were deposited in the herbarium of Universidad Nacional de Río Cuarto (Río Cuarto city, province of Córdoba, Argentina).

Essential oil extraction

Essential oil was obtained from the aerial parts of the plant, composed of leaves and parts of the stem. To prepare the EO, 60 grams of ground material were hydrodistilled using a Clevenger-type apparatus for 3 h. The oil was separated