

Extracts of *Tagetes minuta* L. front of bacteria regarding bovine mastitis

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Abstract

Bovine mastitis is a problem in the context of animal health. However medicinal plants are a management strategy in agroecological production systems. In this context, the plant *Tagetes minuta* L. plays a leading role, due to its recognition in the popular pharmacopeia. Thus, the objective of this work was to assess the effect of hydroalcoholic extracts of *T. minuta* from different collection sites compared to ten bacteria related to bovine mastitis. The antimicrobial evaluation was done through the microdilution technique in syrup, in 96 well microplates in triplicate, for the determination of Minimal Bacterial Concentration (MBC) (%). In the microplates, the extracts of *T. minuta* and the inoculums of the bacteria were placed in addition to the growth control of the bacteria and the control of the extracts, which were incubated for 72 hours at 37°C in an oven. After this time, aliquots of 5µL of each orifice were transferred to plates containing 5% equine defibrinated blood agar and maintained in incubation for 24 hours at the same previous temperature. With this, the growth readings were performed, with the subsequent interpretation of the results by the geometric mean of the MBC (%). The results indicate that there is a difference in sensitivity to microorganisms by the extracts as a function of the collection sites and both present antimicrobial action.

Keywords: agroecology, antimicrobial, bioactive plants, chinchilla, milk production

Introduction

Folk knowledge about the use of medicinal plants is strongly tied to the culture of the population. The use of medical plants in the veterinary therapeutics is feasible, safe, secure to obtain, and has low costs (Marinho et al., 2007).

In this context, the species *Tagetes minuta* L., which is being used by farmers in the context of animal health (Wanzala et al., 2012), is a valuable resource to be studied for management in agroecosystems (Lovatto et al., 2011).

Tagetes minuta is an aromatic plant, native to South America, belonging to the Asteraceae family, with characteristics of being an erect sub-shrub, with little branching, of 1-2 m

of height, with composite leaves, imparipinnate and flowers in chapters colored yellow, popularly known as chinchilla and among others, with a robust biocidal potential (Chamorro et al., 2008; Gakuubi et al., 2016). Its main compounds are the essential oils, mainly terpenes, and flavonoids, with corroborated bactericide potential (Cornelius & Wycliffe, 2016; Shahzadi & Shah, 2015). The composition of the active compounds of chinchilla may vary, depending on the plant organ that produces them, the collection site and also throughout the development cycle of the plant (Chamorro et al., 2008; López et al., 2009).

In the context of dairy production, bovine

mastitis, the inflammation of the mammary glands, is the most common disease among adult cows. Bacterial infection is the leading causes of the onset of this disease (Peres Neto & Zappa, 2011).

Thus, in order to ensure animal health, through the asepsis of equipment and the udder of the animals, the use of medicinal plants displayed potential in the prevention and treatment of bovine mastitis in agro-ecological production systems (Schuch et al., 2008).

Therefore, the objective of this work was to assess the effect of hydroalcoholic extracts of *T. minuta* from different collection sites against ten bacteria related to bovine mastitis.

Materials and Methods

Leaves and inflorescences of *T. minuta* were collected in full bloom in two distinct locations, in Canguçu, RS, Brazil, at coordinates 31° 26' 36" S 52° 38' 19" W (Location 1) and in Pelotas, RS, Brazil, at coordinates 31° 37' 22" S 52° 31' 32" W (Location 2). The materials were collected in may 2008, in the morning, after successive days without rain. After collection, the samples were dried at room temperature until they were brittle at the pressure exerted by the fingers.

For the preparation of the hydroalcoholic extract, the ratio of 1:10 (100 g of plant/1L of 70° GL cereal alcohol) was used. The material was immersed in alcohol for 15 days in amber glass, with two daily shaking. Afterward, the samples were pressed and stored in the dark at room temperature until the time of use in the experiment.

Microbiological tests were performed at the Laboratory of Infectious Diseases of the Federal University of Pelotas (UFPel). For this purpose, the extracts were prepared by solvent extraction using a rotary evaporator, at 30 RPM, 50°C and negative pressure of 600 mm/Hg, and finally, the initial volume of the solution was filled with sterile distilled water. The dilutions used in the experiment were 50%, 25%, 12.5%, and 6.25%.

Bacteria tested were *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 12600,

Staphylococcus aureus, *Staphylococcus hyicus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus intermedius* and *Streptococcus uberis*. The inoculums were prepared at the concentration of 10⁵ UFC.mL⁻¹ in medium containing saline and 2X BHI broth (brain heart infusion).

The antimicrobial evaluation was done through the microdilution technique in broth, in 96-well microplates in triplicate, in which the extracts of *T. minuta* and the inoculums of the bacteria were placed, as well as the growth control of the bacteria and the control of the extracts, which were incubated for 72 hours at 37°C. After this period, aliquots of 5µL of each orifice were transferred to plates containing 5% equine defibrinated blood agar and maintained in incubation for 24 hours at the same previous temperature. With this, the qualitative readings of bacterial growth [with growth (+); without growth (-)] with subsequent interpretation of the results by the geometric mean of the Minimal Bacterial Concentration (MBC) (%) (Mota et al., 2013, Oyarzabal et al., 2011, Prestes et al., 2008).

Results and Discussion

Differences of results were observed for the two extracts of *T. minuta* used in this work against bacteria related to bovine mastitis, as shown in Table 1 by MBC (%), which may be related to the abiotic factors present in the different sites.

The secondary compounds present in plants are influenced by several abiotic factors, which may reflect on the composition and amount of compounds produced, such as temperature, salinity, water, solar radiation, chemical and mechanical stresses (Ramakrishna & Gokare, 2011). In this context, Cornellus & Wycliffe (2016) mention that factors such as location, development stage, plant parts, soil type, nutritional status, climate, sunlight, harvesting, and extraction methods influence the composition and amount of compounds present in the product of *T. minuta*.

Tannins, saponins, alkaloids, and flavonoids found in the composition of the methanolic extract of *T. minuta* are probably related to its antimicrobial action against

gram-positive and gram-negative bacteria (Rachuonyo et al., 2016). This effect had been already pointed out by Tereschuk et al. (1997) and Shahzadi & Shah (2015).

In this context, both extracts of *T. minuta*,

tested in the work, presented efficiency for 50% of the microorganisms. Within the other 50%, 30% were resistant to either extract and 20% to both (Table 1).

Table 1. Minimum bactericidal concentration (MBC) of the hydroalcoholic extract of *T. minuta* L. from different locations determined by the microdilution method in broth.

Microorganisms	MBC (%)	
	Location I*	Location II**
<i>Escherichia coli</i> ATCC 8739	Resistant***	Resistant
<i>Pseudomonas aeruginosa</i> ATCC 10145	Resistant	Resistant
<i>Salmonella typhimurium</i> ATCC 14028	Resistant	50
<i>Staphylococcus aureus</i> ATCC 12600	19,84	9,92
<i>Staphylococcus aureus</i>	39,68	Resistant
<i>Staphylococcus hyicus</i>	25	6,25
<i>Streptococcus agalactiae</i>	31,49	31,49
<i>Streptococcus dysgalactiae</i>	6,25	12,5
<i>Streptococcus intermedius</i>	31,49	15,74
<i>Streptococcus uberis</i>	Resistant	50

*Location I: Canguçu-RS/Brazil **Location II: Pelotas-RS/Brazil ***Resistant: without bactericidal action

Accordingly, the extract from plants of site II, showed a higher sensitivity to microorganisms. As comparing the MBC (%) of each extract against a specific bacteria strains, our results highlighted that half of the concentration of the extract derived from plants from site II was required to inhibit *S. aureus* ATCC 12600, and *S. intermedius* in comparison with the extract of the site I. This value was observed three times lower for *S. hyicus*. Just one among the strains of *S. aureus* was resistant to the extract of site II. *Salmonella typhimurium* ATCC 14028 and *S. uberis* displayed resistance against the extract derived from plants collected in Site I (Table 1).

Both extracts displayed similar effects against the strains *E. coli* ATCC 8739, *P. aeruginosa* ATCC 10145, and *S. agalactiae* microorganisms. Among these bacterial strains, only the last one was sensitive to the extracts (Table 1).

In order to meet these results, Gonçalves et al. (2013) evaluated the essential oils and hydroalcoholic extracts of different plant species of the Brazilian flora in eight bacteria related to bovine mastitis. They identified that the hydroalcoholic extract of *T. minuta* was effective for *S. aureus* at 50%, for two isolates of *Staphylococcus coagulase +* at 19% and 50% for *Staphylococcus coagulase -* at 39% and *S. uberis* at 9.9%, but resistant to *E. coli* and *P. aeruginosa*. Therefore, a similar effect observed by Schuc

et al. (2008) for the bacteria *S. agalactiae* and *S. aureus* at the time of exposure to the hydroalcoholic extract of *T. minuta* of 30 s and 30 min, respectively, and no action against *P. aeruginosa*.

Finally, we shall highlight the potential of the use of medicinal plants species in the management of bovine mastitis in the context of agroecological production. It is, however, crucial to point out that rotation among different products from medicinal plants is critical, as bacteria may develop resistance to these compounds, or even to ensure safe handling if there are variations in plant composition due to some abiotic condition. Furthermore, there is a need for studies focusing on the combination of plants. As observed by Rachuonyo et al. (2016), the mixture of extracts from different plants (among them those of *T. minuta*) can potentiate the antimicrobial action.

Conclusions

The results of the study highlight that hydroalcoholic extracts of *T. minuta* from plants collected in different sites provide different effect against bacteria commonly associated with mastitis. Even these differences in effectivity, both the extracted presented in this paper displayed antimicrobial activity.

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