Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2014, 4 (2):6-15**
(http://scholarsresearchlibrary.com/archive.html)

Journal of Natural Product and Plant Resource
Scholars Research Library

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Antimicrobial activity of essential oils against pathogens isolated from Bovine Mastitis

Perini S.[1], Piccoli R. H.[2], Nunes C. A.[2], Bruhn F. R. P.[1], Custódio D. A. C.[1] and Costa G. M.[1*]

[1]Department of Veterinary Medicine, Federal University of Lavras, Lavras/MG, Brazil
[2]Department of Food Sciences, Federal University of Lavras, Lavras/MG, Brazil

---

ABSTRACT

Treatments for bovine mastitis based on antibiotic therapy are not always effective which may lead to faults in recovery of diseased animals, selection of resistant bacteria and the presence of antimicrobial residues in milk. In this context the use of essential oils configures as an alternative to control mastitis pathogens in dairy cows because they have antibacterial action against gram-positive and gram-negative bacteria and no diverse effects on human health. The objective of this study was to determine, by the method of broth microdilution, the minimum inhibitory concentration (MIC) of essential oils of Salvia sclarea, Eugenia caryophyllata, Thymus vulgaris, Cymbopogon winterianus, Elettaria cardamomun, Cymbopogon flexuosus, Rosmarinus officinalis and Cinnamomum cassia for isolates of Staphylococcus aureus and Streptococcus agalactiae associated with clinical and subclinical mastitis. C. cassia, T. vulgaris, C. flexuosus, E. caryophyllata, C. winterianus essential oils presented high antibacterial action against both the pathogens. The S. sclarea and R. officinalis oils did not show significant antibacterial activity against both the microorganisms. High synergism in antimicrobial action against S. aureus and S. agalactiae was observed in the combination of C. cassia, C. flexuosus and E. caryophyllata essential oils. Moderate synergism in antimicrobial effect was also observed in associations involving the C. cassia essential oil. The results indicated the prospect of using essential oils to control bovine mastitis caused by S. aureus and S. agalactiae.

Keywords: MIC, antibacterial, natural compounds, mastitis treatment, phytotherapics.

INTRODUCTION

Bovine mastitis remains as the most costly infectious disease and the most frequent reason for antibacterial use in dairy cattle worldwide [1-3]. The costs of the disease result from reduced milk production from affected animals, drug expenditures and discarded milk of animals in treatment, and replacement cows and eventually the death of animals. Besides these costs, mastitis causes alterations in the physicochemical characteristics of milk, causing low industrial productivity and depreciation in nutritional value and sensory characteristics of dairy products [4-5].

Different microorganisms are involved in the etiology of the bovine mastitis, however *S. aureus*, *S. agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* cause about 80% of cases [6]. *S. aureus* is one of the pathogens most frequently associated with intramammary infections of dairy cattle on all continents and this agent alone determines the greatest losses in dairy farming [3, 7-8] The mastitis caused by *S. agalactiae* are generally associated with elevation in bulk tank somatic cell counts (BTSCC) and global bacterial count (GBC) and a decrease in the quantity and quality of milk produced by the herd infected [9-10]. According [11], the infection rates by *S. agalactiae* vary widely among herds with high morbidity found in farms where control measures are neglected.

The treatment of clinical cases and dry cow therapy employing systemic antibiotics or intrammamary infusions are commonly used alternatives for the control of bovine mastitis [4, 12]. However, these control measures are no always effective, and there have been wide variations in treatment efficiency for different causative agents of mastitis [13], especially when it is performed during the lactation period. Furthermore, the use of antibiotics can lead to the selection of resistant bacteria and residues in milk, which creates great concern to the industry and for public health [14]. Because of these limitations in conventional strategies for bovine mastitis treatment, there is a continuous search for new therapeutic methods.

Essential oils are volatile secondary metabolites of low molecular weight derived from plants. These have antibacterial properties, with no reports of resistance after prolonged exposure to gram-positive and gram-negative bacteria, and no side effects on human health which makes them a potential weapon against bacterial diseases [15].
Due to the antibacterial and antifungal characteristics of essential oils and their main components, they are increasingly studied for the control of microorganisms [16]. It was demonstrated [17] that the lipophilic components of lemon grass essential oil play an important role on the lipid layer of the bacterial cell membrane, causing loss of its structural organization and integrity. In another study [15] the antibacterial activity of essential oils of *Origanum vulgare, Thymus vulgaris, Lippia graveolens, Zingiber officinale, Salvia officinalis, Rosmarinus officinalis, Ocimum basilicum* and its majority fractions, carvacrol and thymol, was demonstrated against *Staphylococcus* spp. isolated from cases of bovine mastitis.

In the search for new alternatives to combat the agents involved in the etiology of bovine intramammary infections), in this study it was evaluated the antimicrobial activity of some essential oils against *S. aureus* and *S. agalactiae* isolates from cases of bovine mastitis.

MATERIALS AND METHODS

Essential oils used in the experiment

The minimum inhibitory concentration of essential oils of *Salvia sclarea, Eugenia caryophyllata, Thymus vulgaris, Cymbopogon winterianus, Elettaria cardamomun, Cymbopogon flexousus, Rosmarinus officinalis* and *Cinnamomum cassia* was evaluated against *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae* (*S. agalactiae*) isolated from mammary glands of cattle suffering from mastitis. The essential oils were commercially purchased and their major components are listed in Table 1.

Table 1 List of the main components present in the essential oils under study

Essential oils	Majoritary components
Salvia sclarea	Linalyl acetate, linalool
Eugenia caryophyllata	Eugenol
Thymus vulgaris	Thymol
Cymbopogon winterianus	Citronellal
Elettaria cardamomun	Alpha-terpineol, eucalyptol
Cymbopogon flexousus	Geranial, neral
Rosmarinus officinalis	1,8 Cineole, alpha-pinene, camphor
Cinnamomum cássia	Cinamaldehyde

Microorganisms

In this study, we used 100 strains of *S. aureus* and 100 strains of *S. agalactiae* isolated from clinical cases (50% of strains) and subclinical cases of bovine mastitis (50% of strains) from 26 dairy herds of Southern Minas Gerais State / Brazil. The microorganisms were previously characterized by phenotypic tests according [18]. Bacterial strains that were frozen at -70°C in maintenance medium were thawed and subcultured on Müller Hinton broth incubated at 37°C for 24 hours for execution of the tests.

The medium used in culture and MIC assays for *S. agalactiae* was supplemented with 5% horse serum. Prior to carrying out the tests, the cultures of both microorganisms were assessed for purity by using smears stained by Gram. Only strains in pure cultures were used for the tests.

The microdilution method was employed to determine the minimum inhibitory concentration according [19]. Bacterial suspensions were prepared in saline (0.85% w/v) and turbidity adjusted to 0.5 by McFarland scale, resulting in a suspension containing approximately 10^8 CFU.ml^{-1} for MIC assays. Essential oils were diluted volume to volume in dimethylsulphoxide (DMSO) to facilitate solubility in the culture medium and serial dilutions were performed for each essential oil in sterile microplates, resulting in concentrations (v/v) of 2.5%, 1.25%, 0.63%, 0.31%, 0.16%, 0.08%, 0.04%, 0.02%, 0.01% and 0.005%. After serial dilution of oils, 10µL of the standardized culture of each microorganism to be tested were added aseptically into each dilution of oil and the plates were sealed

and incubated at 37°C for 18-24 hours, after which the test readings were conducted. All assays were performed in duplicate and the reading was performed visually based on the formation of turbidity and bacterial deposit. The MIC value was determined as the higher dilution of the essential oil that completely inhibited the bacterial growth.

The occurrence of synergism/antagonism in antibacterial action among the essential oils of *C. cassia*, *C. flexousus* and *E. caryophyllata* was evaluated against 30 strains of *S. aureus* and another 30 strains of *S. agalactiae*. Both pathogens were randomly selected. For this purpose, the essential oils were mixed volume to volume, as indicated in Table 2.

Table 2 Essential oils and DMSO volumes of used in serial dilution assays for evaluation of synergism/antagonism

Assays	*Cinnamomum cassia*	*Cymbopogon flexousus*	*Eugenia caryophyllata*	DMSO
1	5 µL	5 µL	___	10 µL
2	5 µL	___	5 µL	10 µL
3	___	5 µL	5 µL	10 µL
4	5 µL	5 µL	5 µL	15 µL

Statistical analysis

The Mann-Whitney test was applied to investigate possible differences in minimal inhibitory concentrations (measured on an ordinal scale) of essential oils on bacterial growth of isolates associated with clinical and subclinical mastitis.

The MIC results of essential oils were compared using the Friedman Test followed by multiple comparisons of averages by LSD Test [20]. All statistical analyzes were performed using SPSS 20.0 and a minimum significance level of 5% was considered ($p<0.05$). Additionally, to examine synergism/antagonism of associations among the essential oils, the MIC results were subjected to multivariate analysis by Principal Component Analysis (PCA), using the Chemoface Software version 1.4 [21].

RESULTS AND DISCUSSION

Minimum inhibitory concentration of essential oils

The results of MIC for both microorganisms were compiled in graphs shown in Figures 1 and 2 that follow.

According to the Figure 1A, the strains of *S. aureus* were more sensitive to the *Cinnamomum cassia* essential oil, whose MIC median was 0.04%. The essential oils of *Thymus vulgaris*, *Cymbopogon flexousus*, *Cymbopogon winterianus* and *Eugenia caryophyllata* also showed good antimicrobial activity with MIC values of 0.16%, 0.16%, 0.31% and 0.31%, respectively. The oils from *Rosmarinus officinalis*, *Salvia sclarea* and *Elettaria cardamomun* showed low antibacterial effect compared to other oils tested, with MIC values ≥ 2.5%. The essential oil of *Rosmarinus officinalis* exhibited no variation in the MIC distribution, with equal values (>2.5%) for most of the isolates.

Using agar diffusion technique to evaluate the action of the essential oil of *Cinnamomum cassia* on *S. aureus* [22], found an MIC of 0.62%, antimicrobial action lower than that found in the present study. The antimicrobial activity of essential oil of *Cinnamomum cassia* is attributed to the presence of cinnamaldehyde, which interacts with the cell membrane by altering the proton motive force and evolving to cell lysis [23-24].

There were no significant differences in antimicrobial activity of essential oils tested against *S. aureus* isolated from clinical (Fig.1B) or subclinical mastitis (Fig.1C), verifying only slight variations in the MIC distributions. The MIC value distributions were similar for most essential oils, with the exception of essential oil of *Cymbopogon winterianus*, which showed constant MIC values for strains from subclinical mastitis and severe values for some strains.

The distribution of MICs of each essential oil on isolates of *S. agalactiae* is shown in Figure 2A. The essential oil of *Cinnamomum cassia* showed the best inhibitory effect (median MIC = 0.02%) compared to the other oils tested, which showed median MICs of 0.08% for *Eugenia caryophyllata*, *Cymbopogon flexousus* and *Thymus vulgaris* and 0.16% for *Cymbopogon winterianus* and 0.62% for *Elettaria cardamomun*.

The essential oil of *Rosmarinus officinalis* and *Salvia sclarea* showed low antibacterial effectiveness, with MIC values of 1.25% and higher than 2.5%, respectively. In the MIC distribution, a greater dispersion was observed for oils of *Elettaria cardamomun*, *Cymbopogon winterianus* and *Salvia sclarea* and lower dispersion for *Rosmarinus*

officinalis, *Thymus vulgaris* and *Cymbopogon flexousus*. *Cymbopogon flexousus* presented higher frequency of severe values in relation to the other essential oils.

No difference in MIC was observed for the different essential oils tested against *S. agalactiae* isolated from clinical (Fig. 2B) or subclinical mastitis (Fig. 2C), except for *Elettaria cardamomun* and *Salvia sclarea* that showed the highest antimicrobial activity against isolates from subclinical mastitis. Besides these differences, there was considerable variation in the distribution of MIC for microorganisms isolated in both types of mastitis, especially for essential oil of *Cymbopogon flexousus*. There were no differences in MIC median for essential oils of *Salvia sclarea* and *Cymbopogon winterianus*, however, these essential oils showed different MIC distributions among strains obtained from clinical and subclinical mastitis.

The differences in MIC related to presentation form of mastitis for *S. agalactiae* may reflect population diversity among the population tested, considering that strains tested were obtained from 26 different dairy herds and that several studies indicate that there is wide diversity in the population of *S. agalactiae* associated with bovine mastitis [25-27].

Antimicrobial activity of *Cymbopogon flexousus* essential oil against pathogens causing bovine mastitis was previously reported [17]. The researchers demonstrated that although the main components of essential oils possess antibacterial activity against *S. aureus* and *S. agalactiae*, the pure components were less effective and that only the essential oil showed bactericidal activity against all the bacteria tested.

The essential oil of *Thymus vulgaris* showed a median MIC switching from 0.16% for *S. aureus* to 0.08% for *S. agalactiae*. In another approach [28], the technique of microdilution in broth was used for evaluation of MIC of the *Thymus vulgaris* essential oil against *S. aureus.* The MIC value found was 0.31%, higher than the value observed in our study.

Antibacterial effects of the *Eugenia caryophyllata* essential oil against *S. aureus* were evaluated [29]. The researchers observed a MIC (0.3 $mg.mL^{-1}$) much higher than the value observed in this study. The eugenol, being the major constituent, may be responsible for the antibacterial effect of *Eugenia caryophyllata* essential oil. This compound is capable of denaturing proteins and it reacts with the phospholipids of the cell membrane, changing their permeability [30].

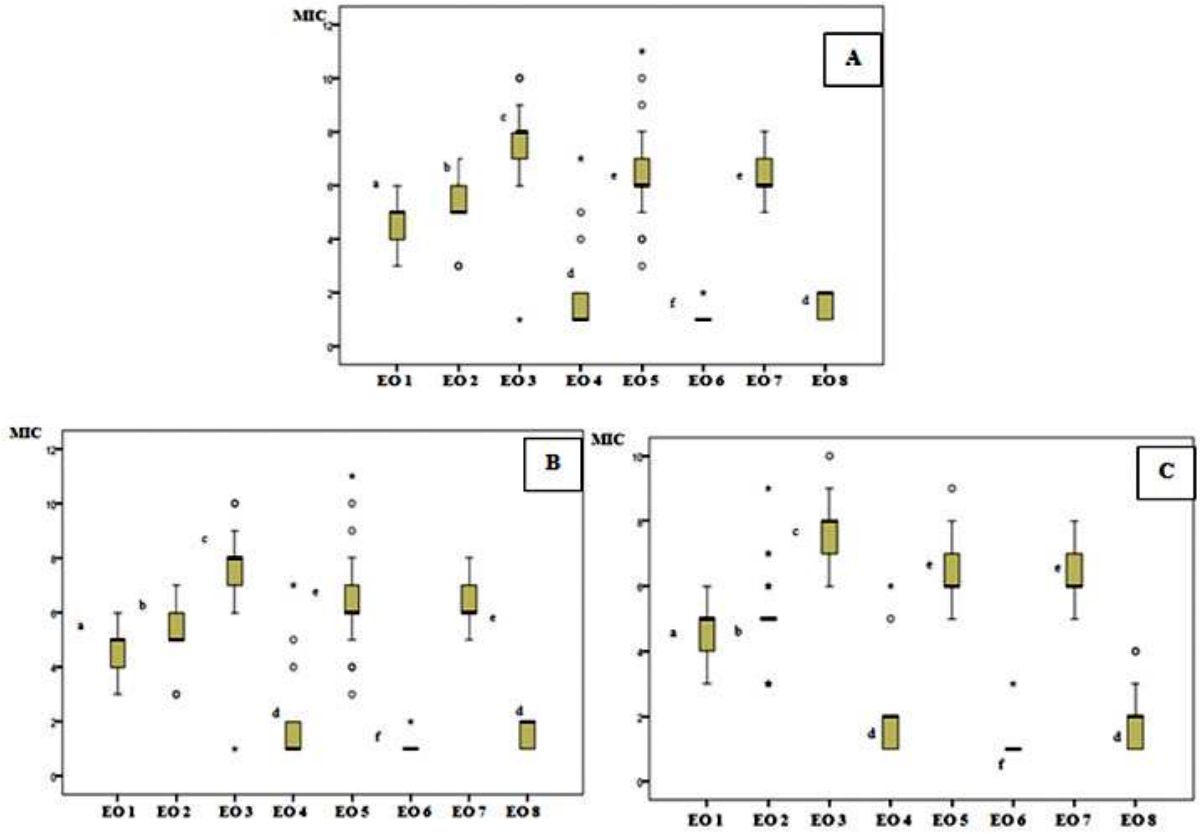
The antibacterial activity of *Cymbopogon winterianus* essential oils against the foodborne pathogen *S. aureus* was studied using the agar diffusion technique [31]. Higher MIC values than to those observed in this study were observed.

In another previous study, the broth dilution technique was used to evaluate the antimicrobial activity of *Elettaria cardamomun* essential oil and its components against several gram positive and negative bacteria [32]. Corroborating ours findings, *S. aureus* showed small sensitivity to the essential oil, showing inhibition only in high concentrations. The antimicrobial activity of essential oil of *Elettaria cardamomun* is associated with its major components (alpha-terpineol, eucalyptol) [33].

The antimicrobial activity of essential oils of *Rosmarinus officinalis* and *Salvia sclarea* was previously studied in bacteria causing human or animal respiratory tract diseases [34]. Similar to the results obtained in this study, the essential oil of *Salvia sclarea* showed no activity against *S. aureus* and low activity against *S. agalactiae*, while the essential oil of *Rosmarinus officinalis* showed reduced antimicrobial for both pathogens.

The differences observed in MIC of essential oils verified in different studies may be explained by differences in the composition of the essential oils. These variations may be caused by several factors, including changes of geographic location, soil characteristics, seasonal factors and plant picking time [35]. Essential oil samples of the same type of plant, but from different places, present different chemical compositions [36]. Another cause for variations can be attributed to differences among strains. The susceptibility of pathogens to essential oils may be influenced by the origin (country, host) of the bacteria strains used in the different studies. Several studies pointed great diversity in *S. aureus* [3, 37-40] and *S. agalactiae* [10, 27, 41] populations in function of geographic location and host of origin of isolates.

Figure 1. Minimum inhibitory concentration (MIC) observed for eight essential oils (box-plot) against *Staphylococcus aureus* isolated from bovine clinical and subclinical mastitis (A), clinical mastitis (B) and subclinical mastitis (C)



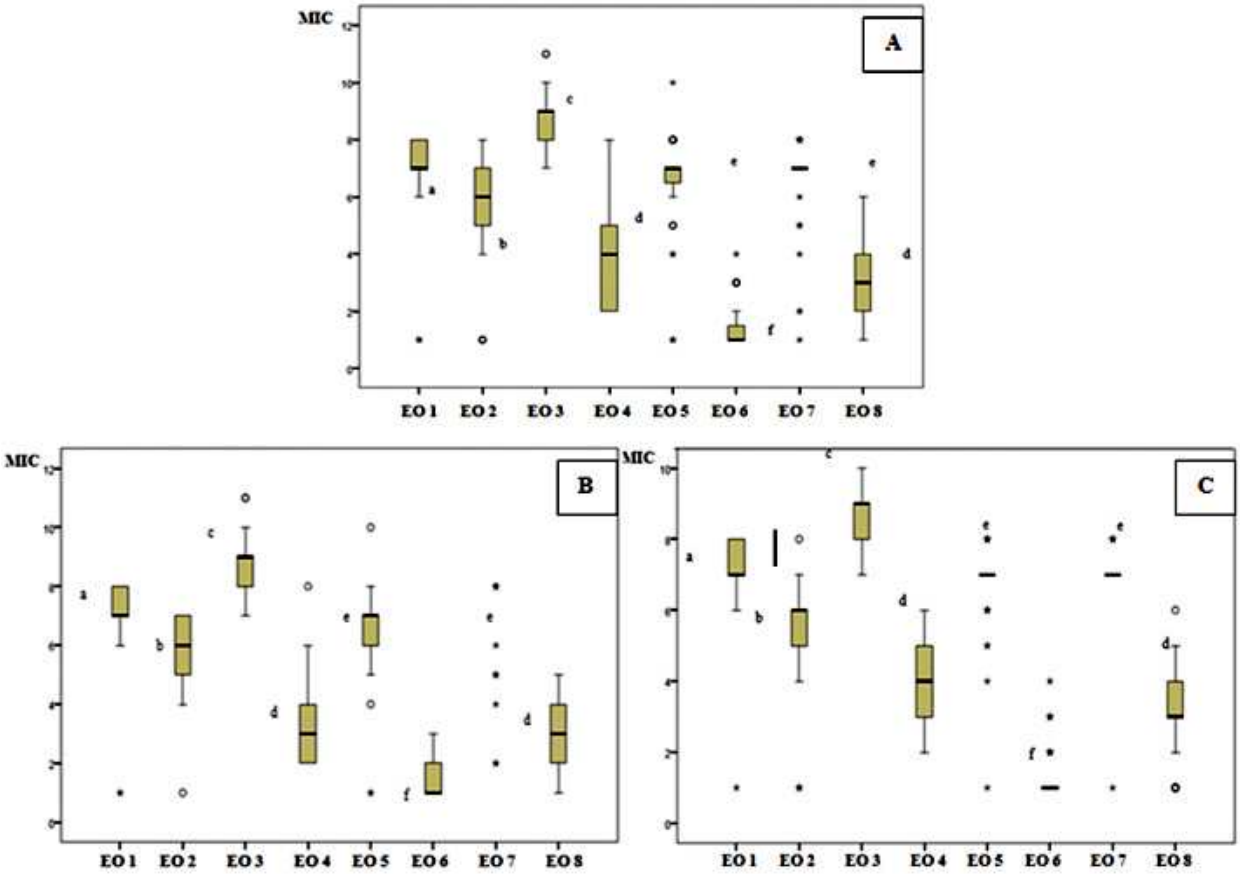
Minimum inhibitory concentration (MIC): 1: >2.5%; 2: 2.5%, 3: 1.25%; 4: 0.62%; 5: 0.31%, 6: 0.16%; 7: 0.08%; 8: 0.04%, 9: 0.02%; 10: 0.01%; 11: 0.005%; 12: >0.005%

Distributions followed by the same letter do not differ by the LSD test ($p > 0.05$).

Essential Oil 1 (EO1): *Eugenia caryophyllata*; EO2: *Cymbopogon winterianus*; EO3: *Cinnamomum cassia*; EO4: *Elettaria cardamomun*; EO5: *Cymbopogon flexousus*; EO6: *Rosmarinus officinalis*; EO7: *Thymus vulgaris*; EO8: *Salvia sclarea*

★ *Severe extreme value*
○ *Moderate extreme value*

Figure 2. Minimum inhibitory concentration (MIC) observed for eight essential oils (box plot) against *Streptococcus agalactiae* recovered from bovine clinical and subclinical mastitis (A), clinical mastitis (B) and subclinical mastitis (C)



Minimum inhibitory concentration (MIC): 1: >2.5%; 2: 2.5%, 3: 1.25%; 4: 0.62%; 5: 0.31%, 6: 0.16%; 7: 0.08%; 8: 0.04%, 9: 0.02%; 10: 0.01%; 11: 0.005%; 12: >0.005%

Distributions followed by the same letter do not differ by the LSD test ($p > 0.05$).

Essential Oil 1 (EO1): *Eugenia caryophyllata*; EO2: *Cymbopogon winterianus*; EO3: *Cinnamomum cassia*; EO4: *Elettaria cardamomun*; EO5: *Cymbopogon flexousus*; EO6: *Rosmarinus officinalis*; EO7: *Thymus vulgaris*; EO8: *Salvia sclarea*

* *Severe extreme value*
○ *Moderate extreme value*

Other variables that may explain the differences in MIC may be related to the bacterial sensitivity evaluation methods used (diffusion in agar, broth dilution in tubes, microdilution in plates), microorganism growth evaluation form in the presence of the antimicrobial agent, oil solubility or its components in the culture medium used and the type and amount of emulsifier [42-43]. Furthermore, the emulsifying agent can interact with essential oils under evaluation, interfering with antibacterial activity, or it can possess antibacterial activity itself. These effects may be accentuated or minimized, depending upon the preparation manner of the emulsifier agent solution [42]. It is possible that emulsifying agents such as DMSO or tween could influence the bacterial growth or cell membrane permeability, acting antagonistically or synergistically with the essential oil active components [44]. In the present study, previous assays showed no inhibitory or stimulatory effects of DMSO in the microorganisms studied.

MIC for combination of essential oils

The MIC values for different essential oil combinations are listed in Table 3. Figure 3 presents plotted distributions of MIC values for associations of essential oils of *Eugenia caryophyllata, Cinnamomum cassia* and *Cymbopogon flexousus* for *S. aureus* (Fig. 3A) and *S. agalactiae* (Fig. 3B).

According data shown in Table 3 and Figure 3, all combinations of essential oils tested resulted in synergism in inhibitory activity against *S. aureus*, except the association of essential oils of *Cinnamomum cassia* and *Eugenia caryophyllata*, whose MIC remained unmodified. The only associations that resulted in synergism against *S. agalactiae* were *Eugenia caryophyllata* and *Cymbopogon flexousus* and the mixture of the three oils (*Eugenia caryophyllata*, *Cinnamomum cassia* and *Cymbopogon flexousus*). The MIC distributions for essential oil associations were relatively uniform for *S. aureus* and highly variable for *S. agalactiae.* The antimicrobial action resulting from the association of the three essential oils was highly potentiated for both the pathogens, characterizing intense synergism among them.

Principal component analysis (PCA) (Fig. 4) shows, graphically, the occurrence of synergism pointed out in Table 3. Vectors pointing in the same direction 1/MIC indicate synergism between combinations and the power of combinations is inversely proportional to the MIC value. The PCA reinforce results highlighted in Table 3, where the combination involving the mixture of the three essential oils resulted in major antimicrobial effect against *S. aureus* (Fig. 4A) and *S. agalactiae* (Fig. 4B).

Table 3. Median MIC values obtained from the combination of *Eugenia caryophyllata*, *Cinnamomum cassia* and *Cymbopogon flexousus* essential oils for *S. aureus* and *S. agalactiae* isolated from bovine mastitis

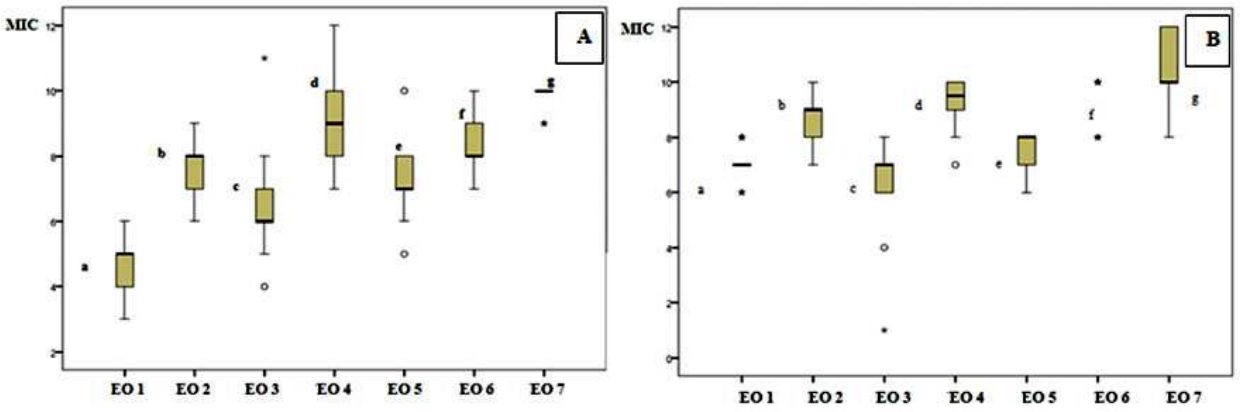
Essential oils associations	Median MIC	
	S. aureus	*S. agalactiae*
Only *Eugenia caryophyllata*	0.31%	0.08%
Only *Cinnamomum cassia*	0.04%	0.02%
Only *Cymbopogon flexousus*	0.16%	0.08%
Eugenia caryophyllata plus *Cinnamomum cassia*	0.02%	0.02%
Eugenia caryophyllata plus *Cymbopogon flexousus*	0.08%	0.04%
Cinnamomum cassia plus *Cymbopogon flexousus*	0.04%	0.04%
Eugenia caryophyllata plus *Cinnamomum cassia* plus *Cymbopogon flexousus*	0.01%	0.01%

Synergism in antibacterial effects among essential oils had been the subject of several studies. In one study, the antimicrobial effect of associations of essential oils of *Cymbopogon flexousus*, *Origanum vulgare* and *Syzygium aromaticum* was evaluated using the agar dilution method [45], but no synergism was observed. In another approach [46] the synergism between the essential oils of *Lavandula officinales*, *Melaleuca alternifolia*, *Juniperus virginiana*, *Thymus vulgaris* and *Eugenia caryophyllata* was investigated by the broth microdilution method. In agreement ours results, this study pointed out an expressive increase in antibacterial activity in the mixture of essential oils as compared to oil alone.

The biological characteristics of the essential oils can be the result of a synergy of all the molecules or only of the interactions of the components present in higher concentrations. Generally, the main components reflect in their biophysical and biological characteristics and the extension of effects depends on their concentration when tested alone or included in essential oils [47].

There are some generally accepted interaction mechanisms that produce antimicrobial synergism such as inhibition of a common biochemical pathway of protective enzymes in microorganism, as well as combinations of active agents that disrupt cell walls and facilitate the absorption of other antimicrobial agents [48].

Figure 3. Minimum inhibitory concentration (MIC) observed for combinations of essential (box-plot) oils against *Staphylococcus aureus* (A) and *Streptococcus agalactiae* (B) isolated from bovine mastitis



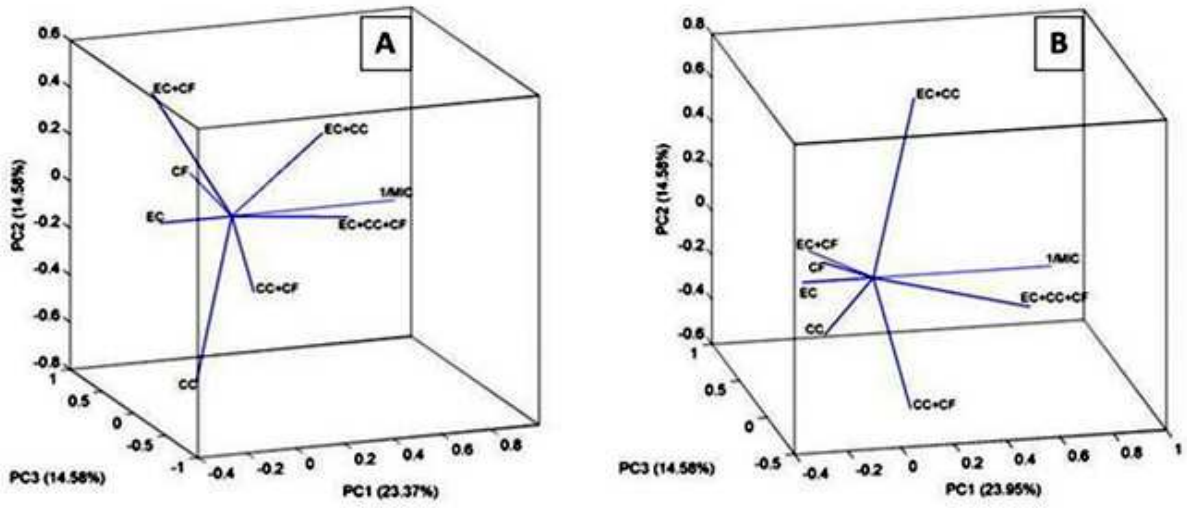
Minimum inhibitory concentration (MIC): 1: >2.5%; 2: 2.5% , 3: 1.25%; 4: 0.62%; 5: 0.31%; 6: 0.16%; 7: 0.08%; 8:0.04%; 9: 0.02%; 10: 0.01%; 11: 0.005%; 12: < 0.005%

Distributions followed by the same letter do not differ by LSD test (p > 0.05)

Essential Oil 1 (EO 1): *Eugenia caryophyllata*; **EO 2**: *Cinnamomum cassia*; **EO 3**: *Cymbopogon flexousus*; **EO 4**: *Eugenia caryophyllata + Cinnamomum cassia*; **EO 5**: *Eugenia caryophyllata + Cymbopogon flexousus*; **EO 6**: *Cinnamomum cassia + Cymbopogon flexousus* ; **EO 7**: *Eugenia caryophyllata + Cinnamomum cassia + Cymbopogon flexousus*

* Severe extreme value
○ Moderate extreme value

Figure 4. Principal Components Analysis (PCA) of combinations of essential oils of *Cinnamomum cassia* (CC), *Eugenia caryophyllata* (EC), *Cymbopogon flexousus* (CF) on *S. aureus* (A) and *S. agalactiae* (B) isolated from bovine mastitis



The concept of synergism has been the central theme of many discussions in phytochemistry [45]. The perspective of synergism between essential oils represent an alternative to the potentiating of their antimicrobial effects, and reduction of their therapeutic concentration, minimizing costs and side effects in humans and animals.

CONCLUSION

The essential oils of *Cinnamomum cassia*, *Eugenia caryophyllata*, *Cymbopogon flexousus*, *Thymus vulgaris* and *Cymbopogon winterianus* showed potent inhibitory activity against *S. aureus* and *S. agalactiae*, all presenting as alternatives to be evaluated *in vivo* for the treatment of intramammary infections caused by these agents in cattle.

The associations of the essential oils of *Cinnamomum cassia*, *Eugenia caryophyllata* and *Cymbopogon flexousus* generally resulted in enhance of antimicrobial action against *S. aureus* and *S. agalactiae*, mainly the association between the three essential oils.

No difference in MIC was observed for the different essential oils tested against *S. aureus* in relation to strains isolated from clinical or subclinical mastitis. *Elettaria cardamomun* and *Salvia sclarea* showed the highest antimicrobial activity against strains of *S. agalactiae* isolates from subclinical mastitis.

REFERENCES

[1] Bradley AJ **2002** Bovine mastitis: an evolving disease. *Veterinary Journal*, **164**, 116-128.
[2] Benedette, M. F., Silva, D., Rocha, F.P.C., Costa, E.A.D.A. & Avanza, M.F.B. (**2008**). Mastite bovina. *Revista Científica Eletrônica de Medicina Veterinária*, **6**, 1-5.
[3] Peton, V. & Le Loir, Y. (**2014**) *Staphylococcus aureus* in veterinary medicine. *Infection, Genetics and Evolution*, 21, 602-615.
[4] Santos, M.V; Fonseca, L.F.L. (**2007**) *Estratégias para controle da mastite e melhoria da qualidade do leite.* São Paulo: Editora Manole Ltda, 1ª Ed., 314p.
[5] Akers, R. M. & Nickerson, S.C. (**2011**) *Journal of Mammary Gland Biology and Neoplasia*, **16**, 275–289.
[6] Ranjan, R., Swarup, D., Patra, R.C. & *Nandi, D.* (**2006**) *Perspectives in Agriculture, Veterinary Sciences, Nutrition and Natural Resources*, **1**, 1-7.
[7] Schlegelová, J., Dendis, M., Benedík, J., Babák, V., & Rysánek, D. (**2003**) *Veterinary Microbiology*, **92**, 327-334.
[8] Vasudevan, P., Nair, M.K.M., Annamalai, T.A. & Venkitanarayanan, K.S. (**2003**) *Veterinary Microbiology*, **92**, 179-185.
[9] Zafalon, L.F., Amaral, L.A., Nader Filho, A., Oliveira, J.V., Resende, F.D. & Oliveira, J.A. (**1999**) *Revista NAPGAMA*, **2**, 4-6.
[10] Merl, K., Abdulmawjood, A.,Lammler, C. & Zschock, M. (**2003**) *Fems Microbiology Letters*, **226**, 87-92.
[11] Duarte, R.S., Miranda, O.P., Bellei, B.C., Brito, M.A.V.P. & Teixiera, L.M. (**2004**) *Journal of Clinical Microbiology*, **42**, 4214-4222.
[12] New reference about strategies of control mastitis=12
[13] Lago, A., Godden, S.M., Bey, R., Ruegg, P.L. & Leslie, K. (**2011**) *Journal of Dairy Science*, **94**, 4457-4467.
[14] Zafalon, L. F., Filho, N.A., Oliveira, J.V. & Resende, F.D. (**2007**) Mastite subclínica causada por *Staphylococcus aureus*: custo-benefício da antibioticoterapia de vacas em lactação. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **59**, 577-585.
[15] Pozzo, M. D., Viégas, J., Santurio, D., Rossatto, L., Soares, I. H., Alves, S. & Costa, M. (**2011**) Activity of essential oil from spices against *Staphylococcus* spp. isolated from bovine mastitis. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **63**, 1229-1232.
[16] Andrade, M. A., Cardoso, M.G., Batista, L.R., Mallet, A.C.T & Machado, S.M.F. (**2012**) Óleos essenciais de *Cymbopogon nardus*,*Cinnamomum zeylanicium* e *Zingiber officinale*: composição,atividades antioxidantes e antibacteriana. *Revista Ciência Agronômica*, **43**, 3999-3408.
[17] Aiemsaard, J., Aiumlamai, S., Aromdee, C., Taweechaisupapong, S. & Khunkitti, W. (**2011**) *Research in Veterinary Science*, **91**, 31–37.
[18] Chair, S.P.O., González, R.N., Hogan, J.S., Jayarão, B.M. & Owens, W.E. (**2004**) Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality. In: NMC - *National Mastitis Council.* Verona – USA, 4ª ed. 46p.
[19] CLSI (**2008**). Performance Standards for Antimicrobial Disk and Dilution Tests for Bacteria Isolated from Animals, Approved Standard CLSI Document M31-A3, 3ª ed. *Clinical and Laboratory Standards Institute*, Wayne, PA.
[20] Marôco, J. (**2010**) *Análise estatística com o PASW Statistics.* 953p.
[21] Nunes, C. A. (**2012**) Chemoface software: versão 1.4. Lavras: UFLA, Software.
[22] Nimje, P. D., Garg, H., Gupta, A., Srivastava, N., Katiyar, M. & Ramalingam, C. (**2013**) *Der Pharmacia Lettre*, **5**, 53-59.
[23] Chang, S. T., Chen, P. F. & Chang, S. C. (**2001**) *Journal of Ethnopharmacology*, **77**, 123-127.
[24] Gill, A. O. & Holley, R. A. (**2004**) *Applied and Environmental Microbiology*, **70**, 5750–5755.

[25] Brochet, M., Couvé, E., Zouine, M., Vallaeys, T., Rusniok, C., Lamy, M. C., Buchrieser, C., Trieu-Cuot, P., Kunst, F., Poyart, C. & Glaser**,** P. **(2006**) *Microbes and Infection*, **8**, 1227-1243.
[26] Springman, A.C., Lacher, D.W., Wu, G., Milton, N., Whittam, T.S., Davies, D.H. & Manning, S.D. (**2009**) *Journal of Bacteriology*, **191**, 5419-5427.
[27] Yang, Y.C., Liu, Y.L., Ding, Y., Yi, L., Ma, Z., Fan, H. & Lu, C. (**2013**) *Plos One*, **8**, 1-8.
[28] Al-bayati, F.A. (**2008**) *Journal of Ethnopharmacology*, **116**, 403-406.
[29] Silvestri, J. D. F., Paroul, N., Czyewski, E., Lerin, E., Cansian, R.L., Mossi, A., Toniazzo, G., Oliveira, D. & Treichel, H. (**2010**) *Revista Ceres*, **57**, 589-594.
[30] Briozzo, J., Núñez, L., Chirife, J., Herszage, L. & D'Aquino, M. (**1989**) *Journal of Applied Bacteriology*, **66**, 69-75.
[31] Brugnera, D. F., Oliveira, M. M. M. & Piccoli, R. H. (**2011**) *Brazilian Journal of Food Nutrition*, **22,** 339-343.
[32] Kubo, I., Himejima, M. & Muroi, H. (**1991**) *Journal of Agricultural and Food Chemistry***, 39**, 1984-1986.
[33] Dhulap, S., Anita, M., & Hirwani, R. R. (**2008**) *Pharmacognosy Reviews Suplement*, **2,** 27-35.
[34] Fabio, A., Cermelli, C., Fabio, G., Nicoletti, P. & Quaglio, P. (**2007**) *Phytotherapy Research*, **21**, 374-377.
[35] Kamatou, G. P., Van Zyl, R.L., Van Vuuren, S.F., Figueiredo, A.C., Barroso, L.G. & Pedro, A.M. (**2008**) *South African Journal of Botany*, **74**, 230-237.
[36] Castro, H. G., Barbosa, L.C.A., Leal, T.C.A.B., Souza, C.M. & Nazareno, A.C. (**2007**) Crescimento, teor e composição do óleo essencial de *Cymbopogon nardus* (L*). Revista Brasileira de Plantas Medicinais: Brazilian Journal of Medicinal Plants*, **9**, 55-61.
[37] Smith, E. M., Green, L. E., Medley, G. F., Bird, FOX, L. K., Suhkken, Y. H., Kruze, J. V., Bradley, A. J., Zadoks, R. N. & Dowson, C. G. (**2005**) *Journal of Clinical Microbiology*, **43**, 4737-4743.
[38] Smyth, D.S., Feil, E.J., Meaney, W.J., Hartigan, P.J., Tollersrud, T., Fitzgerald, J.R., Enright, M.C. & Smyth, C.J. (**2009**) *Journal Medical Microbiology*, **58**, 1343-1353.
[39] Costa, G.M., Paiva, L.V., Figueiredo, H.C.P., Figueira, A.R., Pereira, U.P. & Silva, N. (**2012**) *Research in Veterinary Science*, **93**, 733-735.
[40] Smith, E.M., Needs, P.F., Manley, G. & Green, L.E. (**2014**) *Infection, Genetics and Evolution*, **22**, 208-215.
[41] Pereira, U.P., Mian, G.F., Oliveira, I.C.M., Benchetrit, L.C., Costa, G.M. & Figueiredo, H.C.P. (**2010**) *Veterinary Microbiology*, **140**, 86-192.
[42] Hammer, K.A., Carson, C.F. & Riley, T.V. (**1999**) *Journal of Applied Microbiology***, 86**, 985-990.
[43] Opalchenova, G. & Obreshkova, D. (**2003**) *Journal of Microbiological Methods*, **54**, 105-110.
[44] Nascimento, F. C., Nascimento, A.C., Rodrigues, C.S., Antoniolli, A.R., Santos, P.O., Júnior, A.M. & Trindade, R.C. (**2007**) *Revista Brasileira de Farmacognosia*, **17**, 108-113.
[45] Pereira, A., Cardoso, M.G., Abreu, L.R., Morais, A., Guimarães, L.G .& Salgado, A.P. (**2008**) *Ciência e Agrotecnologia*, **32**, 887-893.
[46] Novacosk, R. & Torres, R.S. (**2006**) *Revista Analytica*, **21**, 36-38.
[47] Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (**2008**). *Food and Chemical Toxicology*, **46**, 446-475.
[48] Santiesteban-Lopez, A., Palou, E. & López-Malo, A. (**2007**) *Journal of Applied Microbiology*, **102**, 486-497.