Original Article

Chemical composition and antibacterial activity of essential oils against pathogens often related to cattle endometritis

Renan Braga Paiano¹, Jeannine Bonilla², Ricardo Luiz Moro de Sousa³, Andrea Micke Moreno⁴, Pietro Sampaio Baruselli¹

¹ Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil
² Departamento de Engenharia de Alimentos, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, Brazil
³ Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, Brazil
⁴ Departamento de Medicina Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil

Abstract

Introduction: Endometritis is a condition marked by inflammation of the endometrium that affects dairy cows from 21 days after parturition, causing damage to herd fertility and economic losses on farms. The use of active compounds obtained from plant sources has gained importance as disease treatment agents in farm animals due to the high resistance rates currently observed against traditional antibiotics commonly used. The study was carried out to examine the chemical composition and to investigate the antibacterial activity of rosemary, cinnamon, cloves, eucalyptus, lemon, oregano and thyme essential oils against the reference strain of Escherichia coli (ATCC 25922), Fusobacterium necrophorum (ATCC 25286), Trueperella pyogenes (ATCC 19411) and Staphylococcus aureus (ATCC 29213), considered as typical bacteria causing endometritis.

Methodology: The chemical composition of the seven essential oils were analyzed by GC-MS and their antibacterial activity was evaluated by the disc diffusion method.

Results: Thirty-six components were identified in total using GC-MS analyzes. The main compounds were cinnamaldehyde (86.5% for cinnamon essential oil), eugenol (85.7% for clove essential oil), 1,8-cineol (80% for eucalyptus and 47.8% rosemary essential oils), limonene (65.5% for lemon essential oil), carvacrol (72.1% for oregano essential oil) and thymol (48.8% for thyme essential oil). The disc diffusion assay revealed that cinnamon, clove, oregano, and thyme essential oils showed the best results compared to the other three essential oils, showing the largest zone of inhibition against all bacteria evaluated.

Conclusions: These findings indicated that essential oils are a potential agent to be used as an alternative for bovine endometritis treatment.

Key words: Bovine endometritis; essential oils; antimicrobial activity; phytotherapy.

J Infect Dev Ctries 2020; 14(2):177-183. doi:10.3855/jidc.12076

(Received 04 October 2019 – Accepted 03 december 2019)

Copyright © 2020 Braga Paiano et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction

Uterine diseases cause profound economic losses in the dairy sector, mainly due to costs related to decreased milk production, increased use of medicines to treat diseases, discarding milk through antibiotics, and the damage caused by death or early culling of the cows [1,2]. Among uterine diseases, endometritis is one of the most important, being characterized by inflammation of the endometrium from 21 days after parturition [3-5], with purulent or mucopurulent uterine discharge [6].

The prevalence of endometritis reported in Brazil was 28.4% in 338 cows evaluated [6]. The bacteria most often described causing endometritis are Trueperella pyogenes, Escherichia coli and Fusobacterium necrophorum [1,7]. The use of antibiotics is the most used therapy against endometritis [1]. However, indiscriminate use of antibiotics may contribute to increased resistance of pathogenic bacteria, compromising the success of therapy, and may cause low efficacy of the drugs [8].

In this sense, the use of products of natural origin has become an alternative to reduce the use of antibiotics in dairy cows. Thus, essential oils are volatile substances naturally produced by plants as secondary metabolites, and are known for their
antibacterial, antifungal, and antiviral properties, among others [9]. They can be extracted from various parts of plants, such as roots, leaves, bark, seeds, and fruits [10,11]. Their components include two classes of separate biological origin: the prime group consists of terpenes and terpenoids, and the second of aliphatic and aromatic components [12]. According to Pauli and Schilcher, [13], the antimicrobial activity of essential oils can be witnessed by in vitro tests, being the most three important ones are the agar diffusion, the agar or broth dilution and the vapor phase test.

The use of essential oils in cattle has increased in recent years, and the action in the treatment of diarrhea in calves [14] and mastitis [11] has been reported. However, there is little information on the use of essential oils as a therapy for endometritis. In this context, the aim of the present study were to characterize the chemical composition and to investigate the antibacterial properties of rosemary (Rosmarinus officinalis), cinnamon (Cinnamomum cassia), clove (Eugenia caryophyllus), eucalyptus (Eucalyptus globulus), lemon (Citrus limon), oregano (Origanum vulgare) and white thyme (Thymus vulgaris) essential oils against four bacteria’ strains causing endometritis.

Methodology

Essential oils

The essential oils of rosemary (Rosmarinus officinalis), cinnamon (Cinnamomum cassia), clove (Eugenia caryophyllus), eucalyptus (Eucalyptus globulus), lemon (Citrus limon), oregano (Origanum vulgare) and white thyme (Thymus vulgaris) were obtained from Ferquimica® (Vargem Grande Paulista, São Paulo, Brazil).

Gas chromatography/mass spectrometry (GC-MS) analysis

The essential oils chemical components were identified by gas chromatograph coupled to mass spectrometry (GC-MS). GC analyses were performed using a Shimadzu GC-2010 gas chromatograph, equipped with a GCMS-QP2010 Ultra mass spectrometer (Shimadzu, Suzhou, China). A split/splitless injector was used. Sample (1 μl) was injected into the injector with a split ratio of 1:10. Oven temperature was 40 °C for 3 min, then programmed heating from 40 to 280 °C at a rate of 8 °C/min. Injector temperature was 250 °C. Helium was used as carrier gas with 14 mL/minute flow rate. The volatile compounds were identified by comparison with mass spectra with those recorded in the National Institute of Standards and Technology database.

Bacterial strains

The evaluated bacterial strains in this study were Escherichia coli (ATCC 25922), Fusobacterium necrophorum (ATCC 25286), Trueperella pyogenes (ATCC 19411) and Staphylococcus aureus (ATCC 25923). All microorganisms were cultured in BHI broth (Brain Heart Infusion, Acumedia, Lansing, MI, USA), being incubated at 37 °C for 24 hours (E. coli and S. aureus strains) or 48 hours (T. pyogenes strains).

Disc diffusion assay

After incubation period the cultures were diluted in sterile saline solution and the turbidity adjusted to the 0.5 standard McFarland scale (~10⁸ CFU/mL). With the use of a sterile cotton swab, surface of plates containing Mueller-Hinton agar (MHA; Difco) were inoculated with the bacterial suspension. To test T. pyogenes strain, the MHA was supplemented with 5% sheep blood and to test F. necrophorum strain, the medium used was Brucella agar (Acumedia, Lansing, MI, USA), supplemented with 5% sheep blood, hemine (Interlab Difco, São Paulo, Brazil) and vitamin K1 (Interlab Difco, São Paulo, Brazil).

Paper disks with 6 mm diameter (Whatman nº 3) soaked with 20 μL of each pure essential oil were laid on the surface of inoculated agar. Discs of ceftriaxone (30 μg, Cefar Diagnósticos Ltda., São Paulo, Brazil) were used as positive control. A paper disc soaked with 20 μL of solution consisting of phosphate buffered saline (PBS, Sigma, São Paulo, Brazil) with 0.5% (v/v) polysorbate 80 (Tween 80) was used as negative control and loaded in each tested plate.

The plates were incubated at 37 °C for 24 hours (E. coli and S. aureus) or 48 hours (T. pyogenes) in aerobic conditions, or 37 °C for 48 hours in anaerobic condition (F. necrophorum). Anaerobic conditions were maintained by using an anaerobic jar with anaerobic atmosphere generator (Anaerobac, Probac, São Paulo, Brazil). After incubation, the inhibition zone diameter (IZD) was measured in accordance with the Clinical and Laboratory Standards Institute guidelines [15] and all experiments were carried out in three independent replicates.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and the differences between the means and
standard error were tested by Tukey test. Statistical significance is considered as \( P < 0.05 \).

**Results**

*Chemical composition of the essential oils*

The volatile compounds for all studied essential oils are listed in Table 1. The major chemical constituent found in cinnamon essential oil was cinnamaldehyde (86.5\%), and in clove essential oil was eugenol (85.7\%). The eucalyptus essential oil was particularly rich in 1,8-cineol (80.0\%), while the essential oil of lemon contained a high percentage of limonene (65.5\%). In oregano essential oil the most abundant compound was carvacrol (72.1\%), and in rosemary essential oil was 1,8-cineol (47.8\%). Thymol (48.8\%) and \( p \)-cymene (26.4\%) were the main compounds identified in the thyme essential oil.

**Antibacterial activity**

The *in vitro* antibacterial activities of seven essential oils against several bacteria strains were qualitatively and quantitatively assessed by the measuring of IZD using the agar disc diffusion method as shown in Table 2.

The results obtained with ceftiofur against *E. coli* ATCC 25922 (27.86 mm of IZD) and *S. aureus* ATCC 25923 (29.00 mm of IZD) strains were within the expected values according to [15] (Table 2). These results revealed that the cinnamon oil presented the greater IZD that varied from 29.67 to 38.33 mm. The larger IZD was observed in *S. aureus* (38.33 mm), and

| Compounds          | Cinnamon | Clove | Eucalyptus | Lemon | Oregano | Rosemary | Thyme |
|--------------------|----------|-------|------------|-------|---------|----------|-------|
| Benzaldehyde       | 2.40     | -     | -          | -     | -       | -        | -     |
| Borneol            | 0.95     | -     | -          | -     | 0.90    | 2.70     | 0.33  |
| Bornyl acetate     | -        | -     | -          | -     | 0.90    | -        | -     |
| \( \delta \)-Cadinene | -    | 0.10  | -          | -     | -       | -        | -     |
| Camphene           | -        | -     | -          | -     | 4.50    | 0.92     | -     |
| Camphor            | -        | -     | -          | -     | 11.90   | -        | -     |
| Carvacrol          | -        | -     | 72.12      | -     | -       | -        | 2.88  |
| \( \alpha \)-Caryophyllene | - | 1.81  | -          | -     | -       | -        | 0.11  |
| \( \beta \)-Caryophyllene | - | 11.50 | -         | -     | 3.03    | 3.50     | 1.21  |
| Caryophyllene oxide | -     | 0.17  | -          | -     | -       | -        | 0.08  |
| 1,8-Cineol         | -        | -     | 80.04      | -     | 47.80   | -        | -     |
| \( \rho \)-Cymene  | -        | -     | 2.96       | -     | 4.81    | 1.40     | 26.43 |
| Cinnamaldehyde     | 86.50    | -     | -          | -     | -       | -        | -     |
| Cinnamyl alcohol   | 0.91     | -     | -          | -     | -       | -        | -     |
| Coumarin           | 2.11     | -     | -          | -     | -       | -        | -     |
| Decanol            | -        | -     | 0.04       | -     | -       | -        | -     |
| \( \alpha \)-Farnesene | -    | 0.08  | -          | -     | -       | -        | -     |
| Eugenol            | -        | -     | 85.73      | -     | -       | -        | -     |
| Geranyl acetate    | -        | -     | 0.12       | -     | -       | -        | -     |
| \( \alpha \)-Humulene | -     | -     | -          | -     | 1.01    | -        | -     |
| Isoborneol         | -        | -     | -          | -     | -       | -        | 0.29  |
| Limonene           | -        | -     | 9.02       | 65.59 | 0.83    | 2.20     | 1.05  |
| Linalool           | -        | -     | 0.13       | 3.03  | -       | 4.51     | -     |
| Myrcene            | -        | -     | 1.55       | -     | 1.50    | 1.04     | -     |
| Neral              | -        | -     | 0.60       | -     | -       | -        | -     |
| Neryl acetate      | -        | -     | 0.21       | -     | -       | -        | -     |
| \( \alpha \)-Pinene | -    | 3.97  | 2.34       | 0.30  | 11.40   | 3.43     | -     |
| \( \beta \)-Pinene | -        | -     | 15.06      | -     | 7.80    | 0.54     | -     |
| Sabine             | -        | -     | 1.76       | -     | 0.10    | -        | -     |
| Salicylaldoxyde    | 1.85     | -     | -          | -     | -       | -        | -     |
| Styrene            | 2.34     | -     | -          | -     | -       | -        | -     |
| \( \alpha \)-Terpineol | -   | -     | -          | 0.70  | 1.70    | 0.48     | -     |
| \( \gamma \)-Terpineol | -  | 4.01  | 7.93       | 4.81  | 0.70    | 6.05     | -     |
| \( \gamma \)-Terpineol | -    | -     | -          | -     | -       | 0.14     | -     |
| Terpinen-4-ol      | -        | -     | -          | 0.83  | 0.90    | -        | -     |
| Thymol             | -        | -     | 2.04       | -     | 48.80   | -        | -     |
smaller IZD was observed in *T. pyogenes* (29.67 mm). Clove essential oil produced an IZD varying from 9.67 to 21.67 mm, being the smaller IZD observed against *E. coli* (9.67 mm) and the larger IZD observed against *F. necrophorum* (21.67 mm). Eucalyptus essential oil produced an IZD varying from 8 to 8.33 mm, the smaller IZD was observed against *S. aureus* (8 mm) and the larger IZD against *E. coli* (8.33 mm) and no effect were observed on *F. necrophorum* and *T. pyogenes*. The lemon essential oil presented no IZD against any strains tested. The IZD produced by oregano essential oil varied from 20.67 to 36 mm, the smaller IZD was observed against *S. aureus* (8 mm) and the larger IZD showed against *E. coli* (8.33 mm) and no effect were observed on *F. necrophorum* and *T. pyogenes*. Rosemary essential oil presented no IZD against any strains tested. The IZD produced by oregano essential oil varied from 20.67 to 36 mm, being the smaller IZD observed against *E. coli* (20.67 mm) and the larger IZD showed against *S. aureus* (36 mm). Rosemary essential oil produced an IZD varying from 8 to 10.67 mm, the smaller IZD was observed against *E. coli* (8 mm) and the larger IZD was seen against *S. aureus* (10.67 mm) with no inhibition effects being observed against *F. necrophorum* and *T. pyogenes*. The inhibition zone produced by the thyme essential oil varied from 24.67 to 36 mm, the smaller IZD was observed against *F. necrophorum* (24.67 mm), whereas the larger IZD was seen showed against *S. aureus* (36 mm).

For *E. coli*, essential oil of cinnamon and thyme had the larger (P < 0.05) IZD compared to the other essential oils. For *T. pyogenes* and *F. necrophorum*, cinnamon essential oil had the larger (P < 0.05) IZD compared to the other essential oils. Against the *S. aureus*, essential oil of cinnamon, oregano and thyme had the highest (P < 0.05) IZD compared to the other essential oils and positive control.

### Discussion

Goñi *et al.* [16] showed that the major components of the essential oil of cinnamon is cinnamaldehyde. The content of cinnamaldehyde (86.5%) identified for cinnamon essential oil in our results is similar to Li *et al.* [17] (66.2-81.9), higher than Lv *et al.* [18] (77.3%) and lower than Zhang *et al.* [19] (92.4%). Our results highlighted the significant higher activity (P < 0.05) of cinnamon essential oil when compared to ceftiofur for *S. aureus* and *T. pyogenes*. *T. pyogenes* is considered one of the most important pathogens causing endometritis in dairy cows and cephalosporin-based drugs are most commonly used as treatments in cows with endometritis [1,20]. In addition, it is important to emphasize that cinnamon essential oil showed significantly higher antibacterial activity (P < 0.05) than the other essential oils (clove, eucalyptus, lemon, oregano, rosemary and thyme) investigated in the present study against the pathogenic species of *F. necrophorum* and *T. pyogenes*. Studies regarding antibacterial activity of essential oils in relation to these two pathogenic species causing endometritis have not been found. This is the first study to show the in vitro activity of essential oils in potentially endometritis-causing bacteria. According to our results, it was demonstrated the promising potential of cinnamon essential oil as therapy in cows with endometritis.

Based on the results of the clove essential oil components, our results are in accordance with literature data, which show that eugenol (> 85%) is the major component identified [21,22]. 1,8-cineole (> 80%) was the main component identified in eucalyptus essential oil, corroborating the results of previous study [23]. According to the identification of the lemon essential oil components, the main constituents identified were limonene (65.5%) and β-pinene (15%). which is in agreement with the study by Hsouna *et al.* [24] (39.7% and 25.44, respectively). Carvacrol (>70%) was the major component of oregano essential oil, similar results were described by Ebani *et al.* [25] (65.90%) and Fratini *et al.* [26] (65.94%). The main constituent of the rosemary essential oil was 1,8-cineole (47%) and is similar to that described by Yang *et al.* [27] (46%). The main compound identified in thyme

---

### Table 2. Inhibition zone diameter identified by disc diffusion method with essential oils tested.

| Bacteria                      | Cinnamon | Clove | Eucalyptus | Lemon | Oregano | Rosemary | Thyme | Positive control | Negative control |
|-------------------------------|----------|-------|------------|-------|---------|----------|-------|-----------------|------------------|
| *Escherichia coli* ATCC 25922 | 32.33 ± 1.29a | 9.67 ± 1.29a | 8.33 ± 1.29c | 6.00 ± 1.29c | 20.67 ± 1.29a | 8.00 ± 1.29a | 26.67 ± 1.29a | 27.86 ± 1.29a | 6.00 ± 1.29c |
| *Truerrorella pyogenes* ATCC 19411 | 29.67 ± 0.31 | 15.00 ± 0.31d | 6.00 ± 0.31c | 6.00 ± 0.31a | 21.00 ± 0.31c | 6.00 ± 0.31c | 25.00 ± 0.31b | 21.33 ± 0.31b | 6.00 ± 0.31c |
| *Fusobacterium necrophorum* ATCC 25286 | 32.00 ± 1.13a | 21.67 ± 1.13b | 6.00 ± 1.13c | 6.00 ± 1.13b | 22.00 ± 1.13a | 6.00 ± 1.13c | 24.67 ± 1.13b | 37.33 ± 1.13a | 6.00 ± 1.13c |
| *Staphylococcus aureus* ATCC 25923 | 38.33 ± 0.75a | 15.33 ± 0.75c | 8.00 ± 0.75d | 6.00 ± 0.75e | 36.00 ± 0.75a | 10.67 ± 0.75b | 36.00 ± 0.75a | 29.00 ± 0.75a | 6.00 ± 0.75e |

*Inhibition zone diameter, values represent mean of three replicates ± standard error. Different letters in the same line represent statistical difference (P < 0.05) in the size of inhibition zones including diameter of disc 6 mm formed under the paper disc by each essential oil.
essential in oil in this study was thymol (48%), our results are in agreement with those described by Sokovic et al. [28] (48%) and Ebani et al. [25] (52%). Different growing environments such as altitude, hours of sunshine, temperature, rainfall, and parts of the plant extracted for the supply of essential oil may contribute to the difference between the percentages of identified active components [29,30].

The results of this study shown that the essential oils tested have different activity against the bacteria evaluated considering the IZD observed. To date, there have been no reports in the literature on the use of essential oils against strains of *T. pyogenes* and *F. necrophorum* strains.

Several authors have reported the antibacterial activity of *Cinnamom cassia* essential oil [19,31-34]. Our results of IZD of cinnamon essential oil against *E. coli* (32 mm) are similar to those described by Nimje et al. [31] (32 mm), Melo et al. [32] (30 mm) and Zhu et al. [34] (30 mm), and larger than those described by Zhang et al. [19] (19 mm). Based on the results observed in the present study the IZD (38 mm) of cinnamon essential oil against *S. aureus*, our results are in agreement with those described by Melo et al. [32] (40 mm) and Cieslak et al. [33] (35 mm), and larger than those described by Zhu et al. [34] (29 mm), Zhang et al. [19] (28 mm) and Nimje et al. [31] (21 mm). The main component of *Cinnamom cassia* oil used in this study was cinnamaldehyde (86%). The antibacterial activity of *Cinnamom cassia* essential oil is mainly due to the cinnamaldehyde component, that have hydrophobic properties, and can react with bacterial cell membranes, contributing to damage the membrane, another action is the ability to inhibit bacterial peptide and protein synthesis, thus having gram-positive and gram-negative bacteria action [34,35].

The antibacterial effects of clove essential oil have been described in the literature [36]. Our results of IZD of clove essential oil against *E. coli* (9 mm) were smaller than those noted by Oulkheir et al. [37] (16 mm), Prabuseenivasan et al. [38] (17 mm) and Bartkiene et al. [39] (11 mm). The IZD of clove essential oil against *S. aureus* (15 mm) noted in this study were similar than those described by Prabuseenivasan et al. [38] (16 mm) and Bartkiene et al. [39] (16 mm). The main component of clove essential oil was eugenol (85%), this compound is responsible for the antibacterial effect of clove essential oil. The eugenol has the ability to denature proteins and react with cell membrane phospholipids, altering membrane permeability [36].

Our study showed least inhibitory activity of eucalyptus essential against *E. coli* (8 mm) and *S. aureus* (8 mm). Fratini et al. [26] also did not observe IZD results using eucalyptus essential oil against *S. aureus* and *E. coli*.

Hsouna et al. [24] using lemon essential oil noted IZD against the reference strain of *E. coli* (15 mm) and *S. aureus* (22 mm). However, in the present study no antibacterial activity was identified against the bacteria tested.

Previous studies showed the antibacterial activity of oregano essential oil [26,40]. Our results of IZD of essential oil of oregano against *E. coli* (20 mm) were smaller than those noted by Melo et al. [32] (38 mm), while against *S. aureus*, our results of IZD (36 mm) are larger than those described by Ebani et al. [25] (13 mm). The major constituent of oregano essential oil in this study was carvacrol (72%). The main mechanism of action of carvacrol against the bacterial cell is the collapse of the proton motor force, the depletion of the ATP pool, and may act on the phospholipid bilayer of the cell membrane, increasing the permeability and leakage of vital intracellular components, which can cause membrane disruption and contribute to cell death [25].

The antibacterial activity of rosemary essential oil has been previously reported [41]. Our results of IZD of rosemary oil against *E. coli* (8 mm) and *S. aureus* (10 mm) were smaller than those showed by Prabuseenivasan et al. [38] (17 mm and 12 mm, respectively). The differences might be related with distinct composition of the essential oils tested.

The high antimicrobial activity of thyme essential oil has been previously revealed [11]. Thyme essential oil showed a range of IZD of 24–36 mm in this study. These results are in agreement with those reported by Oulkheir et al. [37] that showed activity of thyme essential oil against *E. coli* (18 mm) and *S. aureus* (22 mm). Thymol (48%), the main compound of thyme essential oil, have been found to exhibit antimicrobial activity [42], acting on the membrane of bacteria, contributing to the release of lipopolysaccharides, increasing the permeability of the cell membrane, and increasing the loss of ATP and the leakage of vital intracellular constituents [25].

**Conclusion**

This study revealed that essential oils have antibacterial activity against the main bacteria tested causing endometritis. Therefore, essential oils have great potential as an alternative to be explored as endometritis therapy in dairy cows. Further *in vivo*
studies are recommended to evaluate the use in clinical applications.

Acknowledgements
This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References
1. Leblanc SJ (2008) Postpartum uterine disease and dairy herd reproductive performance: A review. Vet J 176: 102–114.
2. Haimerl P, Arlt S, Borchardt S, Heuwieser W (2017) Antibiotic treatment of metritis in dairy cows – A meta-analysis. J Dairy Sci 100: 3783–3795.
3. Leblanc SJ, Duffield TF, Leslie KE, Bateman KG, Keefe GP, Walton JS, Johnson WH (2002) Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J Dairy Sci 85: 2223–2236.
4. Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M (2005) Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64: 1879–1888.
5. Paiano RB, Birgel DB, Birgel Junior EH (2019a) Uterine involution and reproductive performance in dairy cows with metabolic diseases. Animals 9: 93.
6. Paiano RB, Gonçalves CGP, Mendes JPG, Bonilla J, Birgel DB, Birgel Junior EH (2019b) Comparative biochemical profiles, production and reproduction status of the postpartum dairy cows with and without purulent vaginal discharge. Reprod Domest Anim 54: 1188–1194.
7. Sheldon IM, Williams EJ, Miller AN, Nash DM, Herath S (2008) Uterine diseases in cattle after parturition. Vet J 176: 115–121.
8. Liu MC, Wu CM, Liu YC, Zhao JC, Yang YL, Shen JZ (2009) Identification, susceptibility, and detection of integron-gene cassettes of Arcanobacterium pyogenes in bovine endometritis. J Dairy Sci 92: 3659–3666.
9. Bonilla J, Sobral PJA (2019) Application of active films with natural extract for beef hamburger preservation. Ciênc Rural 49: e20180797.
10. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils – A review. Food Chem Toxicol 46: 446–475.
11. Szweda P, Zalewska M, Pilch J, Kot B, Milewski S (2018) Essential oils as potential anti-staphylococcal agents. Acta Vet Beograd 68: 95–107.
12. Tariq S, Wani S, Rasool W, Shafi K, Bhat MA, Prabhakar A, Shalla AH, Rather MA (2019) A comprehensive review of the antibacterial, antifungal and antiviral potential of essential oils and their chemical constituents against drug-resistant microbial pathogens. Microb Pathog 134.
13. Pauli A, Schilcher H (2010) In vitro antimicrobial activities of essential oils. In Baser KHC, Buchbauer G, editors. Handbook of essential oils, science, technology, and application. New York: CRC Press. 353–547.
14. Katsoulos PD, Karatzia MA, Dovas CI, Filioissis G, Papadopoulos E, Kiossisa Arsenopoulos EK, Papadopoulos T, Boscos C, Karatzia H (2017) Evaluation of the in-field efficacy of oregano essential oil administration on the control of neonatal diarrhea syndrome in calves. Res Vet Sci 115: 478–483.
15. Clinical and Laboratory Standard Institute (CLSI) (2018) Performances standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 4th informational supplement. CLSI document VET08 (ISBN 978-1-68440-011-9).
16. Goñi P, López P, Sánchez C, Gómez-Luz R, Becerril R, Nerín C (2009) Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem 116: 982–989.
17. Li YQ, Kong DX, Hong W (2013) Analysis and evaluation of essential oil components of cinnamon barks using GC-MS and FTIR spectroscopy. Ind Crop Prod 41: 269–278.
18. Lv F, Liang H, Yuan Q, Li C (2011) In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int 44: 3057–3064.
19. Zhang Y, Liu X, Wang Y, Jiang P, Quek SY (2016) Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control 59: 282–289.
20. Sheldon IM, Owens SE (2017). Postpartum uterine infection and endometritis in dairy cattle. Anim Reprod 14: 622–629.
21. Chaieb K, Hajloulou H, Zmantar T, Kahla-Nakhli AB, Rouabhia M, Mahdouiani K, Bakhrouf A (2007) The chemical composition and biological activity of clove essential oil, Eugenia caryophyllata (Syzygium aromaticum L. Myrtaceae): A Short Review. Phytother Res 21: 501–506.
22. Bhuiany MNI, Begum J, Nandi NC, Akter F (2010) Constituents of the essential oil from leaves and buds of clove (Syzygium caryophyllatum (L.) Alston). Afr J Plant Sci 4: 451–454.
23. Sacchetti G, Maietti S, Muzzoli M, Seaglianti M, Manfredini S, Radice M, Bruni R (2005) Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 91: 621–632.
24. Hsouna AB, Halima NB, Smouei S, Hamdi N (2017) Citrus lemon essential oil: chemical composition, antioxidant and antimicrobial activities with its preservative effect against Listeria monocytogenes inoculated in minced beef meat. Lipids Health Dis 16: 146.
25. Ebani VV, Nardoni S, Bertelloni F, Najar B, Pistelli L, Mancianti F (2017) Antibacterial and antifungal activity of essential oil against pathogens responsible for otitis externa in dogs and cats. Medicines 4: 21.
26. Fratini F, Mancini S, Turchi B, Friscia E, Pistelli L, Giusti G, Cerri D (2017) A novel interpretation of the fractional inhibitory concentration index: The case Origanum vulgare L. J Dairy Sci 92: 211–220.
27. Goñi P, López P, Sánchez C, Gómez-Luz R, Becerril R, Nerín C (2009) Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. Food Chem 116: 982–989.
28. Bonilla J, Sobral PJA (2019) Application of active films with natural extract for beef hamburger preservation. Ciênc Rural 49: e20180797.
29. Wang R, Wang R, Wang B (2009) Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. J Infect Dev Ctries 2020; 14(2):177-183.
30. Hossain MA, Saliha RAH, Afaf MW, Qasim AR, Jamal NS (2014) Comparison of chemical constituents and in vitro antimicrobial activities of three brands clove essential oils from Golf region. Asian Pac J Trop Dis 4: 262–268.
31. Nimje PD, Garg H, Gupta A, Srivastava N, Katiyar M, Ramalingam C (2013) Comparison of antimicrobial activity of *Cinnamomum zeylanicum* and *Cinnamomum cassia* on food spoilage bacteria and water borne bacteria. Pharm Lett 5: 53–59.
32. Melo ADB, Amaral AF, Schaefer G, Luciano FB, Andrade C, Costa LB, Rostagno MH (2015) Antimicrobial effect against different bacterial strains and bacterial adaptation to essential oils used as feed additives. Can J Vet Res 79: 285–289.
33. Cieslak E, Mack JP, Rojtman A (2016) Essential oils and methylglyoxal: a possible alternative treatment for antibiotic resistant bacterial infections. Int J Pharm Pharm Sci 8: 107–110.
34. Zhu H, Du M, Fox L, Zhu M-J (2016) Bactericidal effects of *Cinnamomum cassia* oil against bovine mastitis bacterial pathogens. Food Control 66: 291–299.
35. Rutala WA, Weber DJ, Healthcare Infection Control Practices Advisory Committee (2008) Guideline for disinfection and sterilization in healthcare facilities. Centers for Disease Control and Prevention website. Available: https://www.cdc.gov/infectioncontrol/pdf/guidelines/disinfection-guidelines-H.pdf. Accessed: 05 February 2020.
36. Perini S, Piccoli RH, Nunes CA, Bruhn FRP, Custódio DAC, Costa GM (2014) Antimicrobial activity of essential oils against pathogens isolated from bovine mastitis. J Nat Prod Plant Resour 4: 6–15.
37. Oulkheir S, Aghrouch M, Mourabit FE, Dalha F, Graich H, Amouch F, Ouzaid K, Moukale A, Chadi S (2017) Antibacterial activity of essential oils extracts from cinnamon, thyme, clove and geranium against a gram negative and gram positive pathogenic bacteria. J Dis Med Plant 3: 1–5.
38. Prabuseenivasan S, Jayakumar M, Ignacimuthu S (2006) In vitro antibacterial activity of some plant essential oils. BMC Complement Altern Med 6: 39.
39. Bartkienie E, Ruzauskas M, Lele V, Zavistanaviciute P, Bernatoniene J, Jakstas V, Ivanauskas L, Zadeike D, Klupsaite D, Viskelis P, Bendoraitiene J, Navikaite-Snipatiene V, Juodeikien G (2018) Development of antimicrobial gummy candies with addition of bovine colostrum, essential oils and probiotics. Int J Food Sci Technol 3: 1227–1235.
40. Marques JL, Volcão LM, Funck GD, Kroning IS, Silva WP, Fiorentini ÂM, Ribeiro GA (2015) Antimicrobial activity of essential oils of *Origanum vulgare* L. and *Origanum majorana* L. against *Staphylococcus aureus* isolated from poultry meat. Ind Crop Prod 77: 444–450.
41. Jiang Y, Wu N, Fu YJ, Wang W, Luo M, Zhao CJ, Zua YG, Liu XL (2011) Chemical composition and antimicrobial activity of the essential oil of *Rosemary*. Environ Toxicol Pharmacol 32: 63–68.
42. Costa CB, Sendra E, López JF, Álvarez JAP, Martos MV (2013) Chemical composition and in vitro antibacterial properties of essential oils of four Thymus species from organic growth. Ind Crop Prod 50: 304–311.

**Corresponding author**
Renan Braga Paiano
Intituição: Departamento de Reprodução Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil
Av. Professor Orlando Marques de Paiva, 87, CEP 05508010. São Paulo/SP, Brazil.
Phone: +55 11 30917674
Fax: +55 11 30322224
Email: renanpaiano@hotmail.com; barusell@usp.br

**Conflict of interests**: No conflict of interests is declared.