DOI:10.22059/IJVM.2024.372443.1005517 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554`

The Chemical Composition and Antibacterial Effect of Essential Oils of Rosemary and Basil in Milk

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Abstract

Background: According to the diverse side effects of antibiotics, new and natural antibacterial substances are needed to treat bacterial diseases and one of these substances is the essential oils (EOs) of medicinal plants. Milk fat and protein may reduce the antimicrobial impact of plant EOs.

Objectives: This study investigated the antibacterial activity of rosemary and basil EOs in comparison with lincospectinomycin antibiotic on three mastitis causing-bacteria including *Streptococcus agalactiae*, *Staphylococcus aureus,* and *Escherichia coli* bacteria in the milk media.

Methods: Chemical compounds of EOs were identified by gas chromatography. The minimum bactericide concentration (MBC) and the minimum inhibitory concentration (MIC) of essential oils were studied by the tube dilution method and the growth curve of bacteria was studied at 0, 6, 10, and 24 hours.

Results: The most important compounds of rosemary were carene (45.11%) and eucalyptus (20.62%), and those of basil were estragol (70.42%) and carene (17.99%). MIC and MBC of rosemary were lower than lincospectinomycin and those of basil were the same as lincospectinomycin. At 6-h, the bacterial reduction of *E. coli* and *S. agalactiae* bacteria was significant and population reduction of rosemary was significant for *S. aureus*. At 24 h, rosemary and basil significantly diminished the bacterial count of *S. aureus*, as well as, basil significantly decreased the *S. agalactiae*.

Conclusion: In general, the antibacterial effect of the EOs was acceptable, and clinical studies are recommended for the treatment of other diseases, including mastitis.

Keywords: Antibacterial effect, basil, mastitis, milk, rosemary,

Introduction

Bovine mastitis is a disease that affects dairy cows and is a major economic threat to the dairy industry across the world. It is also a potential public health concern. The most common causes of this disease are *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus agalactiae* (Zhu *et al*., 2016). For many years, administering antibacterial agents directly into the udder has been the primary approach for treating and preventing mastitis in dairy animals. The emergence of antibiotic-resistant bacteria due to their use in animals is a major concern with significant public health implications (Alekish *et al*., 2017). Currently, there is a global concern regarding the widespread of bacteria that are resistant to multiple drugs and the low effectiveness of newly developed antibiotics. Therefore, there is a strong emphasis on the utilization of natural resources, particularly essential oils, to discover new antibacterial agents (Sharifi-Rad *et al*., 2020).

Ocimum basilicum (basil) especially its aromatic leaves, has good medicinal effects and it has been used in traditional systems of medicine as a vermifuge, tonic, antispasmodic, diuretic, and for the treatment of infections of upper respiratory tract (Al Abbasy *et al*., 2015). Several researchers around the world explored the potent antimicrobial properties of basil essential oil. Basil EO showed potent antibacterial effects on both gram-negative and gram-positive bacteria (Rezzoug *et al*., 2019; De Martino *et al*., 2021; da Silva *et al*., 2022).

The aerial parts of *Rosmarinus officinalis*, also known as rosemary, contain essential oils and phenolic compounds that have various pharmacological effects including antibacterial, anti-inflammatory, and antiviral properties. The key constituents of the EO are 1,8-cineole, camphor, borneol, and β-caryophyllene. The composition of the essential oil can vary depending on the season, climate, land, soil, and developmental stages (Oliveira *et al*., 2019; Rathore *et al*., 2022).

The fat, starch, and albumin of milk may interact with antibacterial compounds and decrease the bioavailability of essential oils (Burt, 2004). Hence, it is crucial to evaluate the effectiveness of essential oils in killing bacteria in milk before using them as an intramammary infusion in mastitis treatment. Assessing the antibacterial properties of EOs in milk is more challenging compared to laboratory mediums. Although rosemary and basil are rich in essential oils and numerous studies have been conducted about their antibacterial effects in laboratory mediums, research on their antibacterial effects in milk is scarce. Therefore, this study aims to investigate the antibacterial activity of these EOs in milk, which simulates the udder environment.

Materials and methods

Rosemary and basil Essential oils were purchased from Dorrin Golab Company, Kashan, Iran.

Chemical composition identification of the essential oils. The analysis was conducted using an Agilent 7890B gas chromatograph. The chromatograph was coupled to a mass spectrometer (Model 5977A, Agilent Technologies, USA). A specific column, HP-5MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm ID 0.25 um, Agilent Technologies), was used to separate the compounds in the sample. The injector temperature was set at 270°C. The oven temperature program started at 60°C and increased to 200°C at a rate of 5°C per minute. Helium was used as the carrier gas, which helps move the sample through the column. The injection volume was 1 microliter. The mass spectrometer scanned a range of 35 to 500 m/z while the interface temperature was set to 280°C.

Bacterial strain. The effectiveness of essential oils in combating three major mastitis bacteria, namely *Streptococcus agalactiae* (ATCC 13813), *Escherichia coli* (ATCC 25922), and *Staphylococcus aureus* (ATCC 9144) was tested. The lyophilized culture containing bacteria was obtained from the Persian Type Culture Collection in Tehran, Iran (PTCC). Tubes containing 10ml Tryptic Soy Broth (TSB) (Biolife, Milano, Italy) were incubated for 18-20 hours at 37°C, twice for growth. The cultures were mixed with sterile glycerin in a 1:5 ratio and then stored at a temperature of -20 ºC. Twice culturing in TSB at 37 ºC for 20 hours were used to obtain fresh bacteria. The cultures were stored at 4 ºC after the streaking on TSA slants (Biolife, Milano, Italy) and incubation (Basti *et al*., 2007).

Inoculum Preparation. Cells transferred from working cultures to TSB tubes and incubated at 35 ºC for 18 hours to obtain bacterial inoculum. Subcultures were prepared and incubated for 18 hours at 35 ºC. A Biochrom Ltd. spectrophotometer (Libra S12, Cambridge, London) was utilized to adjust cultures to OD 0.1 at 600 nm. This resulted in a cell concentration of 4.1×10^7 cfu/ml for *S. agalactiae*, 1.2×10^8 cfu/ml for *S. aureus*, and 3.6×10^6 cfu/ml for *E. coli*. The counting of cells in the suspensions was performed with duplicate plating and incubating from tenfold serial dilutions on TSA (Basti *et al*., 2007). Finally, 1:500 dilutions of the primary inoculum were used as working inoculum.

Milk. Free antibiotic raw milk autoclaved for 15 min at 121 ºC.

MIC and MBC. Dimethylsulphoxide (DMSO) (DMSO, Sigma, Germany) was used for dilution of essential oils at a ratio of 1:1. This dilution was then passed through a filter to sterilize it and was used for the antibacterial analysis. MIC and MBC were determined with a modified protocol for broth dilution testing (CLSI, 2015). The growth medium used was whole autoclaved milk. For the determination of MIC, twofold serial dilutions of the oil dilution (10, 5, 2.5, 1.25, 0.625, 0.312, and 0.156%) were performed in milk. Thereafter, 100 µl of bacterial inoculum was added and after overtaxing, the vials were incubated for 24 h at 37°C. For enumeration of inoculated bacteria, 100 µl of each vial was plated on a TSA plate and incubated for 24 h at 37°C. The lowest concentration without visible growth was taken as the minimum bactericidal concentration (MBC) and the following concentration was defined as the minimum inhibitory concentration (MIC). To make sure that the autoclaving process was successful, a negative control (culturing milk alone) was employed. To document bacterial growth in milk, milk- containing bacteria was taken as a positive control. For evaluation of the possible antibacterial effect of this solvent, DMSO was the vehicle control.

Bactericidal kinetics of the oils. The experiment involved inoculating sterile milk with different pathogens and exposing them to sub-MIC (minimum inhibitory concentration) of EOs, just like in the MIC tests. Inoculated milk but without EO were control samples. After incubation at 37 ºC for 24 hours, the bacterial population was counted at 0, 6, 10, and 24 hours of incubation following 0.1 mL plating of the nine serial dilutions (1:10 in normal saline). All treatments were performed twice for accuracy. Growth curves were plotted by recording bacterial count (measured in $\log_{10}$ cfu/ml) against the elapsed time (measured in hours).

Statistical analysis. Experiments were conducted in duplicate. Data were analyzed using SPSS 18 statistical software (IBM Corp., Armonk, NY, USA) with analysis of variance (ANOVA) and Tukey's test at a P-value of less than 0.05.

Results

Chemical composition of the essential oils. During the GC/MS analysis, it was found that the essential oil of rosemary contained 3-carene as the major constituent with a concentration of 45.11%, followed by eucalyptol (1,8-cineol) at 20.62% and levoverbenone at 5.91%. In the basil oil, estragol (70.42%), 3-carene (17.99%), and eucalyptol (8.61%) were the main ones (Tables 1 and 2).

MBC and MIC. The effect of rosemary and basil on *S. aureus* and *E. coli* and the effect of basil on *S. agalactiae* were similar to the effect of lincospectinomycin but the effect of rosemary on *S. agalactiae* was higher than lincospectinomycin (Table 3).

Time kill assay. Figures 1-3 depict the impact of rosemary and basil on milk bacteria. At 6-h, the population of *S. agalactiae* and *E. coli* bacteria was significantly reduced and population reduction of rosemary was significantly for *S. aureus*. At 24 h, rosemary and basil significantly diminished the bacterial count of *S. aureus*, as well as, basil significantly decreased the *S. agalactiae*.

Discussion

Antimicrobial efficacy of different essential oils frequently is assessed by the broth dilution method (Hood *et al*., 2003), but in the present study for simulation of the udder environment milk was used instead of broth. The presence of lipophilic molecules including lipids in milk, due to their hydrophobic nature, may pose a challenge to the antibacterial activity of EOs against mastitis pathogens (Burt, 2004).

This work showed that the major components of rosemary EO were carene, eucalyptol, and Levoverbenone. Gachkar *et al*. (2007) showed high antibacterial effect of rosemary from Iran against *Listeria monocytogenes, S. aureus,* and *E. coli* (MBCs: 2-4 µg/ml) which was attributed to camphor, verbenone, and borneol (Gachkar *et al*., 2007). In another study from Iran, the most compounds of 7 rosemary populations were eucalyptol (5.63-26.89%), camphor (66.1-24.82%), and alpha-pinene (14.69-20.81%) (Bajalan *et al*., 2017). A study reported a moderate antimicrobial activity of rosemary oil from Turkey (MBCs ranging from 2.5 to 20 μg/mL). This was attributed to the high content of 1,8-cineol (Celiktas *et al*., 2007). The main components of rosemary EO from Spain and Morocco were reported camphor, alpha-pinene and eucalyptol (Diass *et al*., 2021; Melero-Bravo *et al*., 2022). Alpha pinene (75.4 - 18.2%) and eucalyptol (15.6 - 3.5%), were the most constituents in all periods of samplings of rosemary (Serralutzu *et al*., 2020). In the present study and the above studies, eucalyptus was reported as one of the main compounds, but camphor and alpha-terpinene, which were reported in most of the studies, were not seen in the present study.

In the present study, the major constituents of basil EO were stragole (methyl chavicol), carene, and eucalyptol. The main components of an Iranian basil EO were found methyl chavicol, linalool, and epi-α-cadinol in purple cultivar and methyl chavicol, geranial, and neral in green cultivar (Sajjadi, 2006). In another study from Armenia, the major constituents of basil were methyl chavicol and linalool (Avetisyan *et al*., 2017). The composition of basil oil from Italy is affected by the season in which the plants are harvested. For example, the essential oil obtained from plants harvested in May is mainly composed of linalool, whereas the October sample contains eugenol as the main constituent. Various factors may cause chemical differences in different geographic regions. These factors may include solar radiation, shading, soil quality, temperature, and other factors that may influence metabolic pathways or genes responsible for producing volatiles and terpenes (da Silva *et al*., 2022).

MIC and MBC of rosemary and basil EOs against bacteria have not been reported in milk, but different values have been reported in laboratory synthetic media. In a study, MIC and MBC of 156 mg/ml were reported for rosemary essential oil against multidrug-resistant *S. aureus* (Esmael *et al*., 2020). In another research, the MBC and MIC of rosemary EO against multidrug-resistant *S. aureus* were 0.03% and 0.1%, respectively, and against *Escherichia coli* was 0.3% and 0.5% (Jiang *et al*., 2011). MBC and MIC of basil essential oil against *E. coli* and *S. aureus* in a study was 128 µg/ml (Rezzoug *et al*., 2019). In another study from Armenia, the MIC of two varieties of basil against *S. aureus* was 3.125 and 6.25 µl/ml and against *E. coli* 13 and 26 µl/ml (Avetisyan *et al*., 2017). In another study in Italy, the MIC of basil essential oil collected in May and October was reported as 6 mg/ml against *E. coli* and 4 and 5 mg/ml against *S. aureus* (De Martino *et al*., 2021). Different studies have reported different values for MIC and MBC, which could be due to different bacterial strains and different essential oil compounds. To classify the antibacterial power of plant extracts, MIC (µg/ml) obtained by macrodilution or microdilution method is used and divided into very effective (less than 100 µg/ml), effective (100-500 µg/ml), moderate (500-1000 µg/ml), low effect (1000-2000 μg/ml) and ineffective (more than 2000 μg/ml) (Sharifi-Rad *et al*., 2020). According to this classification, the essential oils of the present study were effective on bacteria.

In the present study, the MIC and MBC of basil on three bacteria and those of rosemary on *S. aureus* and *E. coli* were similar to lincospectinomycin antibiotic. The MIC and MBC of rosemary on *S. agalactiae* were lower than lincospectinomycin. These results showed the good antibacterial effect of rosemary and basil EOs.

Another noteworthy point in the present study was the stronger antibacterial effect of essential oils on *S. agalactiae* and *S. aureus* (Gram-positive) than on *E. coli* (Gram-negative) bacteria, which was expected by us and has been confirmed in other studies. The presence of lipopolysaccharides (LPS) (hydrophilic) in the outer membrane of Gram-negative bacteria is a major obstacle for EOs, which primarily consist of hydrophobic constituents (da Silva *et al*., 2022). Hydrophobic properties allow association with bacterial membranes and mitochondria, disrupting cell structure and leading to cell death by molecule and ion leakage from the cell (da Silva *et al*., 2022).

Regarding the growth curve, at 6-h and 24-h the EOs had some antibacterial effects against bacterial populations that were higher against Gram-positive than Gram-negative bacteria. Using sub-MIC concentrations of essential oils can lead to limited impact against Gram-negative bacteria due to the LPS barrier hindering penetration and if the inhibitory concentration or even multiple inhibitory concentrations were used, the antibacterial effects would be much stronger.

The antibacterial effects of the EOs are mostly attributed to their main components (Burt, 2004). Attributing essential oil activity to a single component is like attributing the success of a play to just one actor. The true magic lies in the interplay of major and minor components, and their synergistic and antagonistic interactions (Bajalan *et al*., 2017)**.**

Conflict of Interest

The authors declare that there is no conflict of interest regarding to publication of this paper.

References

Al Abbasy, D. W., Pathare, N., Al-Sabahi, J. N., & Khan, S. A. (2015). Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). *Asian Pacific Journal of Tropical Disease 5*, 645–649. https://doi.org/10.1016/S2222-1808(15)60905-7

Alekish, M. O., Ismail, Z. B., Awawdeh, M. S., & Shatnawi, S. (2017). Effects of intramammary infusion of sage (*Salvia officinalis*) essential oil on milk somatic cell count, milk composition parameters and selected hematology and serum biochemical parameters in Awassi sheep with subclinical mastitis. *Veterinary World 10*, 895–900. http://www.veterinaryworld.org/Vol.10/August-2017/10.html PMID: PMC5591475

Avetisyan, A., Markosian, A., Petrosyan, M., Sahakyan, N., Babayan, A., Aloyan, S., et al. (2017). Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. BMC Complementary and Alternative Medicine 17, 1–8. https://doi.org/10.1186/s12906-017-1587-5

Bajalan, I., Rouzbahani, R., Ghasemi, A., Maggi, F. (2017). Industrial Crops & Products Antioxidant and antibacterial activities of the essential oils obtained from seven Iranian populations of Rosmarinus o ffi cinalis. Industrial Crops & Products 107, 305–311. https://doi.org/10.1016/j.indcrop.2017.05.063

Basti, A. A., Misaghi, A., & Khaschabi, D. (2007). Growth response and modelling of the effects of *Zataria multiflora* Boiss. essential oil, pH and temperature on *Salmonella Typhimurium* and Staphylococcus aureus. *LWT - Food Science and Technology 40*, 973–981. https://doi.org/10.1016/j.lwt.2006.07.007

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology 94*, 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022 PMID: 15246235

Celiktas O.Y., Kocabas, E.E.H., Bedir, E., Sukan, F.V., Ozek, T., Baser, K.H.C. (2007). Antimicrobial activities of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. *Food Chem*istry;100(2):553–559.

https://doi.org/10.1016/j.foodchem.2005.10.011

CLSI (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25, Wayne. USA.

da Silva, W. M. F., Kringel, D. H., de Souza, E. J. D., da Rosa Zavareze, E., Dias, A. R. G. (2022). Basil Essential Oil: Methods of Extraction, Chemical Composition, Biological Activities, and Food Applications. *Food and Bioprocess Technology 15*, 1–27. https://doi.org/10.1007/s11947-021-02690-3

De Martino, L., Amato, G., Caputo, L., Nazzaro, F., Scognamiglio, M. R., De Feo, V. (2021). Variations in composition and bioactivity of *Ocimum basilicum* cv 'Aroma 2' essential oils. *Industrial Crops and Products 172*, 114068. https://doi.org/10.1016/j.indcrop.2021.114068

Diass, K., Brahmi, F., Mokhtari, O., Abdellaoui, S., Hammouti, B. (2021). Biological and pharmaceutical properties of essential oils of *Rosmarinus officinalis* L. And *Lavandula officinalis* L. In *Materials Today: Proceedings* 45, 7768–7773. https://doi.org/10.1016/j.matpr.2021.03.495

Esmael, A., Hassan, M. G., Amer, M. M., Abdelrahman, S., Hamed, A. M., Abd-raboh, H. A., et al. (2020). Antimicrobial activity of certain natural-based plant oils against the antibiotic-resistant acne bacteria. *Saudi Journal of Biological Sciences 27*, 448–455. https://doi.org/10.1016/j.sjbs.2019.11.006 PMCID: PMC6933203

Gachkar, L., Yadegari, D., Rezaei, M. B., Taghizadeh, M., Astaneh, S. A., Rasooli, I. (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. *Food Chemistry 102*, 898–904. https://doi.org/10.1016/j.foodchem.2006.06.035

Hood, J. R., Wilkinson, J. M., Cavanagh, H. M. A. (2003). Evaluation of Common Antibacterial Screening Methods Utilized in Essential Oil Research. *Journal of Essential Oil Research 15*, 37–41. https://doi.org/10.1080/10412905.2003.9698631

Jiang, Y., Wu, N., Fu, Y., Wang, W., Luo, M. (2011). Chemical composition and antimicrobial activity of the essential oil of Rosemary. *Environmental Toxicology and Pharmacology 32*, 63–68. https://doi.org/10.1016/j.etap.2011.03.011 PMID: 21787731

Melero-Bravo, E., Ortiz de Elguea-Culebras, G., Sánchez-Vioque, R., Fernández-Sestelo, M. Herraiz-Peñalver, D. (2022). Variability of essential oil in cultivated populations of

Rosmarinus officinalis L. in Spain. Euphytica 218, 1–12. https://doi.org/10.1007/s10681-022-03020-0

Oliveira, J. R. De, Esteves, S., Oliveira, L. (2019). *Rosmarinus officinalis* L . (rosemary) as therapeutic and prophylactic agent. *Journal of Biomedical Science 8*, 1–22. https://doi.org/10.1186/s12929-019-0499-8 PMID: 30621719

Rathore, S., Mukhia, S., Kapoor, S., Bhatt, V., Kumar, R., Kumar, R. (2022). Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya. *Scientific Reports 12*, 1–13. https://doi.org/10.1038/s41598-022-07298-x PMID: 35228638

Rezzoug, M., Bakchiche, B., Gherib, A., Roberta, A., Guido, F., Kilinçarslan, Ö. et al. (2019). Chemical composition and bioactivity of essential oils and Ethanolic extracts of Ocimum basilicum L. and Thymus algeriensis Boiss. & Reut. from the Algerian Saharan Atlas. BMC Complementary and Alternative Medicine 19, 1–10. https://doi.org/10.1186/s12906-019-2556-y PMID: 31227024

Sajjadi, S. E. (2006). Analysis of the essential oils of two cultivated basil (Ocimum basilicum L.) from Iran. *Daru 14*, 128–130.

Serralutzu, F., Stangoni, A. Pietro, Amadou, B., Tijan, D., Re, G. A., et al. (2020). Essential oil composition and yield of a *Rosmarinus officinalis* L. natural population with an extended flowering season in a coastal Mediterranean environment and perspectives for exploitations. *Genetic Resources and Crop Evolution 67*, 1777–1793. https://link.springer.com/article/10.1007/s10722-020-00939-y

Sharifi-Rad, J., Ezzat, S. M., El Bishbishy, M. H., Mnayer, D., Sharopov, F., Kılıç, N. (2020). Rosmarinus plants: Key farm concepts towards food applications. *Phytotherapy Research 34*, 1474–1518. **https://doi.org/10.1002/ptr.6622** PMID: 32058653

Zhu, H., Du, M., Fox, L., & Zhu, M. J. (2016). Bactericidal effects of Cinnamon cassia oil against bovine mastitis bacterial pathogens. *Food Control 66*, 291–299. DOI: https://doi.org/10.1016/j.foodcont.2016.02.013

ترکیبات شیمیایی و اثر ضدباکتریایی اسانس رزماری و ریحان در محیط شیر

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چکیده:

مقدمه: با توجه به عوارض جانبی متنوع آنتی‌بیوتیک‌ها، مواد ضدباکتری طبیعی و جدیدی برای درمان بیماری‌های باکتریایی مورد نیاز است که یکی از این مواد اسانس‌های روغنی گیاهان دارویی است. چربی و پروتئین شیر ممکن است اثر ضد میکروبی اسانس‌های روغنی را کاهش دهد.

هدف: این مطالعه به بررسی فعالیت ضد باکتریایی اسانس‌های رزماری و ریحان در مقایسه با آنتی بیوتیک لینکواسپکتینومایسین بر روی سه باکتری عامل ورم پستان شامل استرپتوکوکوس آگالاکتیه، استافیلوکوک اورئوس و اشرشیاکلی در محیط کشت شیر پرداخت.

روش‌ها: ترکیبات شیمیایی اسانس‌ها با کروماتوگرافی گازی شناسایی شدند. حداقل غلظت بازدارنده و حداقل غلظت باکتری‌کشی اسانس‌ها به روش رقیق سازی لوله‌ای و منحنی رشد باکتری‌ها در ساعت‌های 0، 6، 10 و 24 بررسی شد.

یافته‌ها: مهمترین ترکیبات رزماری کارن (45/11 درصد) و اکالیپتوس (20/62 درصد) و ریحان استراگول (70/42 درصد) و کارن (17/99 درصد) بود. حداقل غلظت بازدارنده و باکتری‌کشی رزماری کمتر از لینکواسپکتینومایسین و ریحان مشابه لینکواسپکتینومایسین بود. در ساعت 6، کاهش باکتری‌های استرپتوکوکوس آگالاکتیه و اشرشیاکلی و کاهش جمعیت رزماری برای

استافیلوکوکوس اورئوس معنی‌دار بود. در ساعت 24 ، رزماری و ریحان تعداد باکتری های استافیلوکوکوس اورئوس و ریحان تعداد استرپتوکوکوس آگالاکتیه را بصورت معنی‌داری کاهش دادند.

نتیجه گیری: به طور کلی اثر ضدباکتریایی اسانس‌های رزماری و ریحان قابل قبول بود و انجام مطالعات بالینی برای درمان سایر بیماری‌ها از جمله ورم پستان توصیه می شود.

کلمات کلیدی: اثر ضدباکتریایی، ورم پستان، ریحان، رزماری، شیر

Figure legends

Figure 1: Growth curve of *E. coli* after exposure to 0% (control, ♦) and sub-MIC of rosemary (●) and basil (■) essential oil.

[a-c] Values marked with different letters show significant (P<0.05) differences at the same time.

Figure 2: Growth curve of *S. aureus* after exposure to 0% (control, ♦) and sub-MIC of rosemary (●) and basil (■) essential oil.

[a-c] Values marked with different letters show significant (P<0.05) differences at the same time.

Figure 3: Growth curve of *S. agalactiae* after exposure to 0% (control, ♦) and sub-MIC of rosemary (●) and basil (■) essential oil.

[a-c] Values marked with different letters show significant (P<0.05) differences at the same time.