

***In vitro* assessment of antifungal and antistaphylococcal activities of *Cinnamomum aromaticum* essential oil against subclinical mastitis pathogens**

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Abstract

The objective of this study was to assess the *in vitro* antibacterial and antifungal activities of *Cinnamomum aromaticum* essential oil against bacteria and yeasts isolated from the cows with subclinical mastitis. The broth microdilution method was employed to determine the antibacterial and the antifungal activities against 7 yeasts (*Candida albicans*, *Candida lambica*, *Candida tropicalis*, *Candida zeylanoides*, *Cryptococcus albidus*, *Cryptococcus laurentii* and *Rhodotorula glutinis*) and 10 *Staphylococcus spp.* strains (*Staphylococcus aureus*, *Staphylococcus chromogenes* and *Staphylococcus xylosus*) with different antibiotic resistance profile isolated from the cows with subclinical mastitis. The results showed that the tested essential oil exhibited a satisfactory antimicrobial activity against all tested bacteria with minimum inhibitory concentration value in the concentration of 0.625 µl/ml, and against all tested fungi in the concentration range of 0.625 µl/ml to 2.5 µl/ml. The minimum bactericidal concentration values ranged between 2.5 µl/ml and 10 µl/ml, and minimum fungicidal concentration values were in the range of concentration from 2.5 µl/ml to 10 µl/ml. This study revealed that *Cinnamomum aromaticum* essential oil exhibited strong antibacterial and antifungal activities, and it may be indicated as an alternative solution to minimize the risk of fungal mastitis, especially for the treatment of subclinical staphylococcal mastitis during lactation and the dry-off period.

Keywords: Mycotic mastitis, Staphylococcal mastitis, *Cinnamomum aromaticum* essential oil, minimum inhibition concentration

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Introduction

Staphylococci are one of the most common and dangerous pathogens responsible for bovine intramammary infections. The management of mastitis caused by bacteria belonging to this genus continues to be a great challenge. This is mainly due to the evolved mechanisms of resistance to antimicrobial chemotherapeutic agents (Barkema et al., 2006).

Overuse and untargeted antibiotic prescribing have led to the emergence of resistant gram-positive bacteria, which represents a major challenge for antimicrobial therapy, increasing consequently the incidence of clinical and subclinical mastitis (Bradley and Green, 2006). The use of antibiotics is not only limited to the treatment of bovine mastitis, and has even been applied for preventive purposes. In fact, the use of antibiotic dry cow therapy and the treatment of intramammary infections at drying off are a common strategy of staphylococcal mastitis management and control (Bradley, 2002). Nevertheless, even if these measures have contributed to the decrease

in the prevalence of contagious mastitis pathogens (Bradley and Green, 2006), they favored the emergence of environmental mastitis including mycotic mastitis (Krukowski et al., 2001, da Costa et al., 2012).

The emergence of the secondary form of mycotic mastitis can be explained by the use of antibiotics that reduce the incidence of bacterial infections, and such measure influences indirectly fungi reproduction due to microbial interaction. In fact, by killing bacteria, the antibiotics reduce the production of many bacterial metabolites that act as antagonists and inhibitors of yeast growth (Moretti et al., 1998). Moreover, according to Mehnert et al. (1964), some yeast species use tetracycline and penicillin as nitrogen sources for growth, so it may explain the risk of mycosis mastitis outbreaks consecutively to antibiotic applications in mastitis therapy for curative or prophylactic purposes.

Despite demonstrating biological activity *in vitro*, treating mycotic mastitis with antifungal chemotherapeutic agents is difficult giving that most antifungal agents are generally toxic to the mammary parenchyma, so they

may be more harmful than beneficial in the treatment of mycotic mastitis (Timoney et al., 1988).

In light of the aforementioned problems and concerns, it is necessary to develop alternative methods to control bacterial mastitis, and to limit the occurrence of secondary mycotic mastitis consecutive to mastitis antibiotherapy.

Alternative treatment of bovine mastitis with essential oils and plant-derived antimicrobials has been assessed *in vitro* on major bacterial and fungal mastitis pathogens (Baskaran et al., 2009, Dal Pozzo et al., 2011, Ksouri et al., 2017). In fact, essential oils are generally regarded as safe and, unlike antibiotics, no resistance has been reported after a prolonged exposure of bacteria to essential oils (Dal Pozzo et al., 2011). Furthermore, synergism between plant metabolites and antibiotics has been described by Hemaiswarya et al. (2008), which suggests the use of essential oils as adjuvants.

The antibacterial activity of the *Cinnamomum aromaticum* essential oil against the tested bacteria causing mastitis was already well-documented (Zhu et al., 2016). Nevertheless, as far as we know, its potential antifungal activity against mycotic mastitis pathogens has not yet been assessed.

In this context, the aim of the present study was to evaluate the *in vitro* antimicrobial activity of essential oil from cinnamon bark (*Cinnamomum aromaticum*) for a possible use as an alternative treatment during lactation, or as a dry cow therapy against yeasts and *Staphylococcus spp.* with different antibiotic resistance profile, and isolated from milk of the cows with subclinical mastitis.

Material and methods

Microorganisms

The antimicrobial activity of *Cinnamomum aromaticum* essential oil was evaluated against 10 isolates of *S.aureus* (n=8), *S. chromogenes* (n=1) and *S. xylosus* (n=1), and 7 yeast isolates (*Candida albicans*, *Candida lambica*, *Candida tropicalis*, *Candida zeylanoides*, *Cryptococcus albidus*, *Cryptococcus laurentii* and *Rhodotorula glutinis*). The microbial strains were isolated from the same herds during a survey on mastitis in the region of Tiaret (Western Algeria).

Briefly, Chapman agar was used for the detection of presumptive *Staphylococcus spp.* strains. Incubation was carried out aerobically at 37°C for 24 h. Subsequently, coagulase activity was measured by a tube test. Coagulase-positive isolates were presumptively defined as *Staphylococcus aureus*. Coagulase-negative isolates were subjected to further biochemical characterization using API Staph test (bio-Mérieux™, France) in order to identify the coagulase-negative *Staphylococcus* isolates to the *species level*.

Yeast strains were cultured in Sabouraud dextrose agar/chloramphenicol and incubated aerobically for 48–72 h at 27°C. Identification was performed based

on morphological, physiological and biochemical characterization as described by Pincus et al. (2007). Bovine serum was used to identify the germ tube produced by *Candida albicans*. Urea hydrolysis test was performed for screening on *Cryptococcus neoformans*. Growth in a medium containing cycloheximide 0.1%, growth in Rice cream medium, growth in different temperatures (27°C and 37°C) and the auxanographic characters (API 20C Aux, bio-Mérieux™, France) were used for further investigations.

Preparation of inoculum

Prior to the experiment, the bacterial strains were inoculated onto the surface of Chapman agar media and the inoculum suspensions were obtained by taking five isolated colonies from 24 h cultures. The colonies were suspended in 5 ml of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard.

Fungal inoculum suspensions were prepared from 48 h cultures on the surface of Sabouraud chloramphenicol media, and the turbidity was adjusted to 0.5 McFarland Standard.

Testing the susceptibility of *Staphylococcus spp.* isolates to antibacterial chemotherapeutic agents

Antibiotic susceptibility was investigated using the disk diffusion method on Mueller-Hinton agar. The tested antibiotics and their corresponding disc concentrations were as follows: amoxicillin/clavulanic acid (20/10 µg), chloramphenicol (30 µg), cephalotin (30 µg), erythromycin (15 µg), kanamycin (30 µg), oxacillin (1 µg), penicillin (10 µg), streptomycin (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), and vancomycin (30 µg). The inhibitory zone diameters were measured and obtained results were interpreted according to the standards determined for antimicrobial susceptibility testing in the veterinary medicine at the national level based on the World Health Organization recommendations (MoARD, 2011). The sensitivity to the corresponding antibiotic was classified by the diameter of the inhibition halos as: Resistant (R), Intermediate (I), or Sensitive (S).

Essential oil used in the experiment

The essential oil used in the present study was provided by the laboratory for research on local animal products, Ibn-Khaldoun University, Tiaret.

As described previously (Selles et al., 2017), the essential oil was extracted from the bark of *Cinnamomum aromaticum* by hydrodistillation technique using Clevenger apparatus. The average yield of the extracted *Cinnamomum aromaticum* essential oil was 1.46±0.05% (w/w). The obtained oil was analyzed by a gas chromatography-mass spectrometric and gas chromatography/ flame ionization detector. In total, analyses resulted in the identification of 89 compounds, with e-cinnamaldehyde (94.67%) being the major component followed by coumarin (0.88%) and cinnamyl acetate (0.74%).

Minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration of *Cinnamomum aromaticum*

The broth microdilution method employed for determining antimicrobial activities of the essential oil was performed according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1999). The minimum inhibitory concentration determining was performed by a serial dilution method in 96 well microtiter plates. The starting concentration of the essential oil solutions was 10 µl/ml for *Cinnamomum aromaticum* essential oil. Further stock solutions of the essential oil were prepared in 10% aqueous Tween 20, and then double serial dilutions of the oil were made. The inoculum was added to all wells and the plates were incubated at 37°C during 24 h for bacteria and 48 h at 27°C for yeasts. The bacterial/fungal growth was visualized by adding 20 µl of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution (Radulovic et al., 2011). Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the oil that inhibited visible growth (red-colored pellet at the bottom of the wells after the addition of TTC), while the minimum bactericidal concentration (MBC) was defined as the lowest concentration that killed 99.9% of bacterial cells. To determine MBC /or MFC (minimum fungicidal concentration), the broth was taken from each well without visible growth and inoculated on Mueller-Hinton agar for 24 h at 37 ° C for bacteria, or Sabouraud chloramphenicol agar for 48 h at 27 ° C for yeasts. Positive growth controls and sterility controls were included. The experiments were done in triplicate.

The ratios MBC/MIC and MFC/MIC were calculated to determine the nature of antimicrobial effect of the essential oil. *Cinnamomum aromaticum* essential oil was considered bactericidal (or fungicidal) if the ratio of the MBC (or MFC) to MIC did not exceed a value of 4. With a ratio greater than 4 and less than 32, the *Cinnamomum aromaticum* essential oil was considered bacteriostatic (or

fungistatic). The microbial strain was considered tolerant to the *Cinnamomum aromaticum* essential oil if the ratio MBC/MIC (or MFC/MIC) is higher or equal to 32 (Cutler et al., 1994).

Results

Most of the *Staphylococcus spp.* strains isolated from the cow milk samples were found to be highly resistant to most of the recommended chemotherapeutics usually used in the treatment of intramammary infections (Table 1).

All tested strains exhibited sensitivity towards cephalixin, kanamycin and trimethoprim / sulfamethoxazole.

Only one strain of *S. aureus* was susceptible to all antibiotics. The remaining *Staphylococcus spp.* strains (9/10) were resistant to penicillin and tetracycline. Of these nine strains, eight *Staphylococcus spp.* strains exhibited a resistance towards amoxicillin/ clavulanic acid.

The results clearly indicated that *Cinnamomum aromaticum* essential oil exhibited satisfactory antibacterial activity at the tested concentrations against all the *Staphylococcus spp.* pathogens (Table 2).

All tested staphylococcal isolates presented the same MIC value. Concentration of 0.625 µl/ml was sufficient to inhibit the growth of the tested coagulase-positive and coagulase-negative *Staphylococcus mastitis* pathogens.

MBC values ranged between 2.5 µl/ml and 10 µl/ml for *Staphylococcus aureus*, 1.25 µl/ml for *Staphylococcus xylosus* and 10 µl/ml for *Staphylococcus chromogenes*.

The MBC/MIC ratios for the *Cinnamomum aromaticum* essential oil ranged between 2 to 16 for *Staphylococcus spp.* strains. This oil exhibited bactericidal activity against 7 bacterial strains (6 strains of *Staphylococcus aureus* and *Staphylococcus xylosus*), whereas it is qualified as bacteriostatic against the 3 remaining *Staphylococcus spp.* strains (2 strains of *Staphylococcus aureus* and *Staphylococcus chromogenes*).

Table 1. Antibiotics susceptibility testing against *Staphylococcus spp.* isolated from bovine subclinical mastitis

Strain No.	Species	Antibiotics										
		AUG	C30	CN	E15	K30	OX1	P10	S10	SXT	TE30	VA
1	<i>Staphylococcus aureus</i>	R	R	S	S	S	S	R	S	S	R	S
2	<i>Staphylococcus aureus</i>	R	S	S	R	S	S	R	S	S	R	S
3	<i>Staphylococcus aureus</i>	R	S	S	S	S	S	R	R	S	R	S
4	<i>Staphylococcus aureus</i>	R	S	S	R	S	R	R	S	S	R	S
5	<i>Staphylococcus aureus</i>	S	R	S	R	S	S	R	R	S	R	S
6	<i>Staphylococcus aureus</i>	R	S	S	R	S	S	R	R	S	R	S
7	<i>Staphylococcus aureus</i>	R	S	S	R	S	S	R	R	S	R	S
8	<i>Staphylococcus aureus</i>	S	S	S	S	S	S	S	S	S	S	S
9	<i>Staphylococcus chromogenes</i>	R	S	S	I	S	R	R	S	S	R	I
10	<i>Staphylococcus xylosus</i>	R	S	S	R	S	R	R	S	S	R	S

With MIC values ranging between 0.625 and 1.25 µl/ml, the antifungal activity of the *Cinnamomum aromaticum* essential oil seems to be heterogeneous comparing to its antibacterial activity against *Staphylococcus spp.* isolates (Table 3).

The highest MIC value (1.25 µl/ml) was exhibited by two major mastitis pathogens: *Candida albicans* and *Candida tropicalis*.

Candida albicans, *Candida tropicalis*, *Candida zeylanoides* and *Cryptococcus albidus* exhibited the highest MFC value with the concentration of 2.5 µl/ml. The lowest MFC value was exhibited by *Candida lambica*, *Cryptococcus laurentii* and *Rhodotorula glutinis* strain at the concentration of 1.25 µl/ml.

Some shift of MFC value of the *Cinnamomum aromaticum* essential oil has been observed in comparison to the value of concentration, which effectively inhibited the growth of the yeasts (MIC). The MFC/MIC ratios ranged between 2 and 4. The highest number of shifts of MFC values in comparison to MIC values was obtained for *Candida zeylanoides* and *Cryptococcus albidus*.

Discussion and conclusions

The most well-known and important antibiotic resistance of *Staphylococcus aureus* mastitis pathogens is the resistance to penicillin (Barkema et al., 2006). In the present study, most of the tested *Staphylococcus aureus* and coagulase-negative staphylococci strains were found penicillin-resistant. This high-rate resistance to penicillin may explain the difficulties in the treatment of staphylococcal subclinical mastitis, giving that the probability of cure of penicillin-resistant *Staphylococcus aureus* is lower than for penicillin-sensitive *Staphylococcus aureus* (Barkema et al., 2006). Moreover, it has been reported that penicillin-resistant *Staphylococcus aureus* strains are more likely to be resistant to non-penicillin antibiotics usually used for the treatment of mastitis than are penicillin-sensitive strains (Ziv and Storper, 1985, Sol et al., 1997). According to Ito et al., (2003), such condition may be explained by the coexistence on pathogenicity islands of genes encoding penicillin resistance and virulence factors, for example, genes encoding the production of biofilms. The obtained results are of great importance if we consider that all

Table 2. Antimicrobial activity of the *Cinnamomum aromaticum* essential oil against *Staphylococcus spp.* isolated from bovine subclinical mastitis

Species	<i>Cinnamomum aromaticum</i> essential oil		
	MIC (µl/ml)	MBC (µl/ml)	MBC/MIC
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus aureus</i>	0.625	10	16
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus aureus</i>	0.625	5	8
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus aureus</i>	0.625	2.5	4
<i>Staphylococcus chromogenes</i>	0.625	10	16
<i>Staphylococcus xylosus</i>	0.625	1.25	2

Table 3. Antifungal activity of the *Cinnamomum aromaticum* essential oil against yeast strains isolated from bovine subclinical mastitis.

Species	<i>Cinnamomum aromaticum</i> essential oil		
	MIC (µl/ml)	MFC (µl/ml)	MFC/MIC
<i>Candida albicans</i>	1.25	2.5	2
<i>Candida lambica</i>	0.625	1.25	2
<i>Candida tropicalis</i>	1.25	2.5	2
<i>Candida zeylanoides</i>	0.625	2.5	4
<i>Cryptococcus albidus</i>	0.625	2.5	4
<i>Cryptococcus laurentii</i>	0.625	1.25	2
<i>Rhodotorula glutinis</i>	0.625	1.25	2

penicillin-resistant *Staphylococcus aureus* infections are ineligible for treatment (Barkema et al., 2006).

Recourse to essential oils for the treatment of staphylococcal infections has been suggested in order to reduce the use of antibiotics in the treatment of staphylococcal mastitis (Piotr et al., 2018), and could be considered as a solution to decrease the risk of the secondary form of mycotic mastitis.

With MIC values of 0.625 µl/ml, all tested staphylococcal strains including coagulase-positive and coagulase-negative strains were sensitive to cinnamon oil. MIC of *Cinnamomum aromaticum* essential oil against *Staphylococcus aureus* was higher than that of *Cinnamomum verum* essential oil (Bouhdid et al., 2010), much higher than that of *Cinnamomum zeylanicum* essential oil (Unlu et al., 2010), and 10-fold higher than that of *Cinnamomum aromaticum* essential oil (Zhu et al., 2016). Nevertheless, MIC values within *Staphylococcus spp.* were very similar, and no differences between coagulase-positive and coagulase-negative staphylococci were observed. The similarity in the susceptibility to the essential oil constituents expressed by different *Staphylococcus aureus* strains was not associated with the antibiotic resistance profile. This independence toward the antibacterial susceptibility profile was reported by Dal Pozzo et al. (2012), who found that there were no significant differences in *Staphylococcus aureus* susceptibility to cinnamon essential oil among strains with different antibiotic resistance profiles.

The MBC/MIC ratios for some tested bacteria were extremely high, indicating that *Cinnamomum aromaticum* essential oil is bacteriostatic rather than bactericidal for the *Staphylococcus sp.* strains commonly involved in bovine mastitis.

The antifungal activity of the *Cinnamomum aromaticum* essential oil was previously analyzed by the group of Ooi et al. (2006), and the observed MIC values were definitely lower (in the range from 0.0006 to 0.0097 µl/ml) in comparison to the results obtained in the present investigation (between 0.625 µl/ml and 1.25 µl/ml). As reported previously for *Candida albicans* (Almeida et al., 2016), the difference in MIC values might be due to the variability of cinnamon essential oil composition. The MFC values ranged between 1.25 and 2.5 µl/ml. These results indicate that the *Cinnamomum aromaticum* essential oil have a strong anti-yeast activity against mastitis pathogens including *Candida albicans*, one of the most common pathogens of the clinical and subclinical mastitis.

The variability in MICs and MFCs values among the tested yeasts may be due to the strain susceptibility, including the antifungal susceptibility profile. Indeed, in a previous publication, fluconazole-resistant *Candida spp.* strains were less susceptible to cinnamon essential oil than their fluconazole-susceptible counterparts (Pozzatti et al., 2008).

As a key point result, it is worthy to mention that at the highest MBC concentration (10 µl/ml), all tested yeast strains including *Candida albicans*, were highly sensitive

to *Cinnamomum aromaticum* essential oil. These obtained results could be of a great interest for combating recalcitrant mastitis involving the yeasts. Indeed, the *in vitro* effectiveness of some essential oils against the bovine mastitis strains has also been confirmed previously with the aim to develop an alternative treatment for mycotic mastitis. For this purpose, Ksouri et al. (2017) tested the antifungal effects of oils from *Origanum floribundum* Munby, *Rosmarinus officinalis* and *Thymus ciliatus* against *Candida albicans* isolated from bovine clinical mastitis.

The significant antibacterial and antifungal effects of *Cinnamomum aromaticum* essential oil can be attributed to the cinnamon bark components including cinnamaldehyde, which represents the major compound of the used essential oil (Selles et al., 2017). In fact, in addition to the previously mentioned antibacterial and antifungal effects, it has been proven that cinnamaldehyde possesses strong antibiofilm activity against *Staphylococcus aureus* recovered from the cases of subclinical bovine mastitis (Budri et al., 2015). The antibiofilm activity of cinnamaldehyde against clinical and reference strains of *Candida albicans* has been investigated by (Khan and Ahmad, 2011), and it has been indicated that cinnamaldehyde exhibited not only an antibiofilm activity against *Candida albicans*, but even increased susceptibility of both sessile and planktonic *Candida albicans* cells to antimicrobial agents including amphotericin B and fluconazole. Nevertheless, in the current study and in the absence of relevant results, it is more accurate to admit that the antimicrobial activity of the *Cinnamomum aromaticum* essential oil could be attributed not only to the effect of cinnamaldehyde alone, but also to the synergetic effect between different compounds of the essential oil including minor constituents.

The mode of action of the *Cinnamomum aromaticum* essential oil and its antimicrobial compounds against Gram-positive bacteria and fungi seems to be similar. At MIC level, by targeting the cell membrane, *Cinnamomum aromaticum* essential oil impaired the membrane integrity of *Staphylococcus aureus* (Zhu et al., 2016). In another study, the same mode of action was reported against *Candida albicans* with the cell membrane being the target site of cinnamaldehyde in both sessile and planktonic cells (Khan and Ahmad, 2011).

In conclusion, the satisfactory antimicrobial activity of the *Cinnamomum aromaticum* essential oil against *Staphylococcus spp.* and yeast strains suggests that this oil may be used as an alternative treatment for subclinical mastitis, limiting consequently the expansion of the secondary form of mycotic mastitis consecutive to antibiotic treatment of staphylococcal mastitis during lactation, or at the dry-off period. However, before any *in vivo* application of the *Cinnamomum aromaticum* essential oil or its derived antimicrobials, it will be necessary to conduct toxicity tests on mammary cell cultures.

Conflict of interest

We declare that we have no conflict of interest.

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***In vitro* ispitivanje antifungalne i antistafilokokne aktivnosti esencijalnog ulja *Cinnamomum aromaticum* protiv uzročnika subkličkog mastitisa**

Sažetak

Cilj ovog istraživanja jeste procjena *in vitro* antibakterijske i antifungalne aktivnosti esencijalnog ulja *Cinnamomum aromaticum* protiv bakterija i gljivica izoliranih iz krava sa subkličkim mastitisom. Da bi se odredila antibakterijska i antifungalna aktivnost protiv 7 gljivica (*Candida albicans*, *Candida lambica*, *Candida tropicalis*, *Candida zeylanoides*, *Cryptococcus albidus*, *Cryptococcus laurentii* i *Rhodotorula glutinis*) i 10 sojeva *Staphylococcus spp.* (*Staphylococcus aureus*, *Staphylococcus chromogenes* i *Staphylococcus xylosus*) koji su nakon izolacije iz krava sa subkličkim mastitisom pokazale različit profil antibiotske rezistencije korištena je mikrodiluciona metoda antibiograma.

Rezultati su pokazali da je testirano esencijalno ulje imalo zadovoljavajuću antimikrobnu aktivnost protiv svih testiranih bakterija sa minimalnom vrijednošću inhibitorne koncentracije od 0.625 ul/ml i svih testiranih gljivica u koncentracijama od 0.625 µl/ml do 2.5 µl/ml. Minimalne baktericidne koncentracijske vrijednosti su se kretale između 2.5 µl/ml i 10 µl/ml, a minimalne fungicidne koncentracijske vrijednosti između 2.5 µl/ml i 10 µl/ml. Ovo istraživanje je dokazalo da esencijalno ulje *Cinnamomum aromaticum* posjeduje jaku antibakterijsku i antifungalnu aktivnost te se može smatrati alternativnim načinom preveniranja rizika od nastanka mastitisa, te posebice u terapiji subkličkog stafilokoknog mastitisa za vrijeme laktacije i zasušivanja.

Ključne riječi: mikotični mastitis, stafilokokni mastitis, esencijalno ulje *Cinnamomum aromaticum*, minimalna inhibitorna koncentracija