Journal of Science and Technology – Engineering and Technology for Sustainable Development
Volume 36, Issue 3, July 2026, 046–053

Antibacterial Effect of Essential Oils (*Cinnamon cassia, Chenopodium ambrosioides,* and *Litsea cubeba*) Against Bacteria Isolated from Bovine Mastitis in Northern Vietnam

Chinh-Nghia Nguyen[1], Hai-Van Nguyen[1], Dinh Thu Thuy[2], Tran Kim Tuyen[2], Le Quang Hoa[1], Thu-Trang Vu[1,*]

[1] *School of Chemistry and Life Sciences, Hanoi University of Science and Technology, Ha Noi, Vietnam*
[2] *Institute of Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam*
[*] *Corresponding author email: trang.vuthu@hust.edu.vn*

Abstract

Bovine mastitis is one of the most common diseases causing losses to the dairy industry. To reduce the use of antibiotic for treatment of this disease, essential oils (EOs) have high potential to apply in the field without any harmful chemical sanitation for raw milk. In this study, the antibacterial effect of Cinnamon cassia, Chenopodium ambrosioides, and Litsea cubeba EOs against bacteria isolated from bovine mastitis were investigated. Among the three EOs, C. cassia EO showed the strongest antimicrobial activity against all tested bacteria. Moreover, the combination of C. cassia-C. ambrosioides and C. cassia-L. cubeba showed a significant synergistic effect with the total FIC value from 0.5 to 0.75, lower the MIC by 2 to 4 times. Those results suggested that EOs could be used to reduce lower the concentration used, minimum effective dose of the drugs and to replace the antibiotic treatment for bovine mastitis in the future.

Keywords: Bovine mastitis, *Cinnamon cassia, Chenopodium ambrosioides,* essential oils, *FIC*, *Litsea cubeba*, *MBC, MIC*.

1. Introduction

Lactose is the main sugar in milk and in dairy Bovine mastitis is one of the most common cattle diseases that causes significant losses to the global dairy industry through the elimination of infected milk, reduction of milk production, drug for treatment, and even animal death [1]. Besides, mastitis leads to alterations in the physicochemical characteristics of milk, resulting in low productivity and depreciation in nutritional and sensorial values of dairy products. The milking must be done every day, but the milk could or be collected until 15 days after the cow recovered by antibiotic treatment to eliminate the antibiotic contamination in the dairy products.

Dairy cow mastitis is mainly caused by microorganisms [1-3]. Among these microorganisms, *Staphylococcus* spp. (including *Staphylococcus aureus*), *Streptococcus uberis, Escherichia coli,* and Enterobacteria are frequently identified from diseased cows worldwide [1, 4-6]. These pathogens can be contagious or environmental that cause 2 types of mastitis: clinical and subclinical infections, based on their primary reservoir and mode [7] of transmission [8]. In reality, subclinical mastitis leads to more serving losses than clinical mastitis [9].

In Vietnam, most dairy farmers are smallholders with less than 10 cows/farm. They are trained in bovine farming and disease but unserious preparation and milking procedure lead to the difficulty in mastitis disease control. These days, the antibiotic drug is used as main treatment for bovine mastitis. However, the use of antibiotics in dairy farming could lead to an increase of antibiotic resistance of bacteria, a potential harmful effect on human health influent [10]. Regarding the problem of antibiotics residues, consumers are interested more in organic production of dairy products. In this context, natural products, including essential oil (EO), have high potential to prevent mastitis disease by sterilizing the teat before milking without any harmful chemical sanitation contaminated in the milk products; or to be alternative of antibiotics to control bovine mastitis, thus the milking and milk collection should be processed as soon after the cow was recovered. EOs are classified as GRAS (generally regarded as safe), show antimicrobial proprieties and resistance has not been reported after prolonged exposure [11]. When applied at appropriate concentrations or in combination with other hurdles, EOs can significantly reduce bacterial loads in raw milk and delay spoilage, although their effectiveness is strongly influenced by the milk matrix, target species, and potential sensory impacts [12]. Thus, there are many studies investigating the antimicrobial activity of EOs against microorganisms causing bovine mastitis [13-19]. *Cinnamon cassia* is a traditional plant in Indonesia, Laos, China, and Vietnam [20]. For long time, this plant has been used alone or formulated with other herbs, in food and in medicine. *C. cassia* main components: cinnamaldehyde, cinnamic acid, and coumarin, have been demonstrated to play an important

p-ISSN 3093-3242
e-ISSN 3093-3579
https://doi.org/10.51316/jst.191.etsd.2026.36.3.7
Received: Oct 22, 2025; Revised: Dec 5, 2025;
Accepted: Jan 14, 2026; Online: Jun 4, 2026

role in its antibacterial and antifungal activity [20-22]. *Litsea cubeba,* belonging to the Lauraceae family, is widely found in Southern China and South-East Asia regions. Different parts of this plant (root, stem, leaves, fruit, and flowers) have been used to produce EO and these EOs are described to exhibit antibacterial activity thanks to its main components: 1,8-cineole, sabinene, linalool, and neral [23-26]. *Chenopodium ambrosioides* is a medical plant growth in tropical and subtropical regions of America, Africa, and Europe. Constituent of ascaridole, carvacrol and caryophyllene oxide, EO of this plant show an effective activity against fungi and protozoa, and its mix with other agents was reported to have antibacterial activity [27-30].

Previously, there were some studies investigating microflora in cow's milk with mastitis in Vietnam [31, 32]. However, the research on these EOs in bacteria related to bovine mastitis has not been reported yet. The aim of this study was to evaluate the antimicrobial activity of several traditional medicine plant EOs against bacteria isolated from mastitis cow's milk in the North of Vietnam, and to evaluate the antimicrobial activity of their mixtures to detect synergistic or antagonistic effects against these pathogens.

2. Materials and Methods

2.1. Materials

Fruit of *Litsea cubeba (Lour.) Pers (L. cubeba),* medicinal plants were obtained from Phu Tho province, Vietnam (21°24'25''N;105°04'05''E); branches, bark, leaves of *C. cassia* and stem, leaves of *C. ambrosioides* plants were randomly selected from different sites in Phu Tho province (20°47'21''N; 105°21'20''E), and transported to the laboratory. The EOs were obtained by hydro-distillation method following the standard procedure of Institute of Chemistry, Vietnam Academy of Science and Technology, and then stored in the dark bottle at 4°C until use.

A total of 27 strains including eleven strains of *E. coli*, ten strains of *Staphylococcus* spp. and six strains of *S. uberis* were isolated from milk samples of subclinical bovine mastitis collected from 25 small farms in Phu Dong village, Gia Lam district, Hanoi, Vietnam. The microorganisms were identified using specified primers according to Riffon and Sakai [33, 34], and then frozen at -80°C in glycerol at Center for Research and Development in Biotechnology (CRDB), Hanoi University of Science and Technology (HUST).

2.2. Methods

2.2.1. Analysis of essential oils

Gas chromatography (GC) analyses of the EOs were carried out on an Agilent Technologies HP7890A GC equipped with a flame ionization detector (FID) and a mass spectrum detector (MSD) Agilent Technologies HP5975C and two HP-5MS columns. The GS analysis was performed once with the MSD and once with the FID. The dimensions of the columns are 60 m x 0.25 mm x 0.25 µm. The injector was set at 250 °C. The temperature program was 60 °C ramp of 4 °C /min up to 240 °C. The carrier gas was Helium at a flow rate of 1 mL/min. The split ratio was 100:1 and 1 µL of EO was injected into the GC. The MSD conditions were: full scan mode under electron impact ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35–450 amu under full scan.

Retention time indices RI of each component was determined relative to the retention times of a homologous n-alkane series with the same GC program. The relative amounts of individual components were calculated based on the GC peak area (FID response) without correction.

2.2.2. Essential oil constituent identification

The EOs constituent identification was performed by MassFinder 4.0 using their mass spectra and retention indices (RI). Further identification was performed by comparing their RI and their mass spectrum with those from HPCH1607, W09N08 libraries and NIST Chemistry WebBook database (http://webbook.nist.gov/chemistry/).

2.2.3. Disc diffusion assay

Disc diffusion method was carried out to assess antimicrobial activity of EOs according to Sheng and Zhu (2014) [35]. Tested bacteria were activated in Mueller-Hinton broth (MHB) culture for 8 h, and then sub-cultured at 1:1000 in MHB for another 14h at appropriate temperatures (Table 1). Cultures were adjusted to $1x10^6$ CFU/mL with MHB (OD_{600nm} = 0.001) and spread over the Mueller-Hinton agar (MHA) plate using 100µL of suspension. Under aseptic conditions, sterile paper discs (Whatman, 6 mm diameter) were placed on the agar surface, and *C. cassia, D. ambrosioides* and *L. cubeba* EOs at absolute concentration were applied onto the corresponding paper discs with 5µL of each. Control paper disc was loaded with 5µL MHB. Two standard antibiotics, Gentamycin (30 µg/disc) and Cephalosporin (30 µg/disc), were used as reference antibiotics for the tested bacteria. The plate was left for 30 min at 4 °C before incubated at appropriate temperature for 24h. The inhibition zone diameters were measured in millimeters and performed in three biological replicates.

2.2.4. Minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) is determined as the lowest concentration of EOs without visible growth (no change in turbidity) of bacteria. Minimum bactericidal concentration (MBC) is defined as the lowest concentration that inhibits bacterial growth on the MHA plates (less than 10 CFU/plate was regarded as no growth) [35].

Two-fold microdilution broth method was used to determine *MIC/MBC* of EOs against tested strains in 96-well microplates [35]. Mueller-Hinton Broth (MHB) culture and 0.2% sterilized Tween 80 (v/v) was used to dilute EOs to different concentrations: *C. cassia* EO from 3.125 µL/mL to 0.024 µL/mL; *D. ambrosioides* and *L. cubeba* EOs from 50 µL/mL to 0.39 µL/mL. Overnight bacterial cultures were diluted to approximately 1 x 10^6 CFU/mL in MHB culture. 20µL of different concentration of EOs and 180 µL of suspension containing 1x10^6 CFU/mL of indicator strain was inoculated in each well. Wells containing 200 µL of uninoculated MHB were used as blanks. Wells containing 20µL of respective bacterial culture in MHB without EO were used as positive control. The plates were incubated at appropriate temperature for 24h.

2.2.5. Synergy studies by checkerboard method

The checkerboard method was performed using 96-well microplates to obtain the fractional inhibitory concentration (FIC) index between EOs [13]. Plates consisted of columns containing 10 µL of EO (A) diluted two-fold in MHB and 0.2% sterilized Tween 80 along the *x* axis as well as rows with the same amount of EO (B) diluted two-fold in the same media along the y axis. Subsequently, 180 µL of the suspension bacterial overnight culture in MHB containing 1x10^6 CFU/mL of the indicator strain were added to all wells to obtain total of 200 µL. Plates were then incubated at appropriate temperature for 24 h.

The *FIC* indices were calculated as

$$\sum FIC = FIC_A + FIC_B \quad (1)$$

where:

$$FIC_A = \frac{MIC_A combination}{MIC_A alone} \quad (2)$$

$$FIC_B = \frac{MIC_B combination}{MIC_B alone} \quad (3)$$

The results were interpreted as synergy ($FIC \leq 1$), addition ($FIC = 1$), indifference ($1 < FIC \leq 2$) or antagonism ($FIC > 2$) [13]. Experiments were performed in three biological replicates.

2.2.6. Statistical analysis

All experiments were performed in triplicate. The obtained results were statistically analysed at the significance level *p* equal 0.05 using Microsoft Excel software. Mean values and standard deviations were calculated; the values were compared by ANOVA method.

3. Results and Discussion

3.1. Chemical Composition of Essential Oils

The percentage of the identified compounds was 99.99%, 99.73%, and 97.97% for *C. cassia*, *L. cubeba*, and *D. ambrosioides* EOs, respectively (Table 1).

Table 1. Major chemical composition of *C. cassia*, *C. ambrosioides* and *L. cubeba* EOs

Compounds	*C. cassia*	*C. ambrosioides*	*L. cubeba*
Benzaldehyde	1.57%	-	-
Cinnamaldehyde <Z->	0.71%	-	-
Cinnamaldehyde <E->	85.71%	-	-
Cinnamyl alcohol <E->	0.34%	-	-
Cinnamyl acetate <E->	5.5%	-	-
Coumarin	1.35%	-	-
Cymene <o->	-	14.32%	-
Limonene	-	0.15%	13.84%
Cineole 1,8	-	0.15%	1.5%
Ascaridole	-	0.38%	-
Ascaridole <iso->	-	19.47%	-
2,3-Dehydro-1,4-cineol	-	59.7%	-
Menth-2-en-1,4-diol	-	2.83%	-
6-Methylhept-5-en-2-one	-	-	2.68%
Myrcene	-	-	1.45%
Linalool	-	-	3.69%
Citronellal	-	-	1.45%
Sabinene	-	-	0.39%
Citronellol	-	-	0.39%
Neral	-	-	28.89%
Isoneral	-	-	1.55%
Geraniol	-	-	1.47%
Isogeranial	-	-	2.25%
Geranial	-	-	35.74%
Other compounds	4.81%	0.97%	4.44%
Total	99.99%	97.97%	99.73%

The other compounds detected in trace but not summarized in the table: i) for C. cassia EO: Styrene, Pinene<a->, Salicyaldehyde, Phenyl ethyl alcohol <b->, Benzenpropanal, Benzofuran <2-Methyl->, Phenylethyl acetate <2->, Copaene <a->, Caryophyllene <E->, Muurolene <g->, Bisabolene <b->, Cadinene <d->, Nerolidol <E->, Methoxycinnamaldehyde <(E)-o->; ii) for D. ambrosioides EO: 4-Hydroxy-4-methylcyclohex-2-enone, Cymen-8-ol <m->; iii) for L. cubeba EO: Pinene<a->, Camphene, Pinene <b->, 2,3-Dehydro-1,8-cineol, Lavandulol, Terpinen-4-ol, Terpineol <a->, Nerol, Caryophylene <E->. All the compounds mentioned were utilized for calculating the total percentage of each class of constituents

Interestingly, the EO components were very different in three essential oils. *C. cassia* EO showed a very high percentage of Cinnamaldehyde <E-> (85.71%), followed by Cinnamyl acetate <E-> (5.5%), and Coumarin (1.35%). Besides, the highest percentage compound found in *C. ambrosioides* EO was monoterpenoid, in which the first place was 2,3-Dehydro-1,4-cineol (59.7%), followed by Ascaridole <iso-> (19.47%), and Cymene <o-> (14.32%). In *L. cubeba* EO, terpenoid compounds exhibited high concentration. Monoterpenoid (Geranial and Neral) were the dominant compounds (35.74% and 28.89%, respectively). On the contrary, the monoterpene constituents showed a lower concentration, and Limonene was the compound with the highest percentage in this group (13.84%).

This result suggested us investigate the combination effects of EOs against bacteria isolated from mastitis bovine milk because of the increasing number of individual components in the mixture.

3.2. The Antimicrobial activities of EOs by Disc Diffusion Assay

From milk samples collected of subclinical bovine mastitis, eleven strains of *E. coli*, ten strains of *Staphylococcus* spp., and six strains of *S. uberis* were isolated. Antimicrobial activity of EOs against these bacteria was investigated by disc diffusion assay (Table 2). The larger inhibition zone gave the meaning of higher antibacterial activity of the EOs on the tested microbial species. Among the three EOs, *C. cassia* EO showed the strongest antimicrobial activity against all tested bacteria, followed by *C. ambrosioides* EO, and the lowest antimicrobial activity was *L. cubeba* EO. The inhibition zone of *C. cassia* EO ranged from 27.3 ± 2.2 mm, 23.2 ± 3.4 mm, and 28.1 ± 1.4 mm against *E. coli*, *Staphylococcus* spp., and *S. uberis*, respectively. The inhibition zone of *C. ambrosioides* and *L. cubeba* EOs against tested bacteria were much lower than *C. cassia* EO (from 10.5–14.2 mm). Moreover, *Staphylococcus* spp. was less sensitive to EOs, with smaller inhibition zone value than those of *E. coli* and *S. uberis*.

In our work, *C. cassia* showed higher inhibitory zone against bacteria causing bovine mastitis than *D. ambrosioides* and *L. cubeba* (Table 2). In *C. cassia* EO, the main component is Cinnamaldehyde, an aldehyde (Table 1). In *C. ambrosioides* and *L. cubeba* EOs, the main components are terpenoid, including alcohol and ethers. Many studies reported that antimicrobial activity of EOs is conditioned by the activity of their components, in which the activity rank of EO components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons [36, 37]. Thus, *C. cassia* EO showed a better effect against pathogenic bacteria than these two others.

Table 2. Inhibition zone of EOs against *E. coli, S. uberis* and *Staphylococcus* spp.

Essential oils	*E. coli*	*Staphylococcus* spp.	*S. uberis*
C. cassia	27.3 ± 2.2	23.2 ± 3.4	28.1 ± 1.4
C. ambrosioides	14.2 ± 2.0	11.7 ± 1.7	13.4 ± 0.7
L. cubeba	10.8 ± 1.6	10.5 ± 1.9	13.3 ± 1.1
Gentamycin	29.1 ± 1.7	-	-
Cephalosporin	-	35.3 ± 3.3	31.3 ± 4.5
Blank control (MHB)	ND	ND	ND

Values are mean diameter of inhibitory zone (mm) ± standard deviation of strains from the same species; "-": no tested; ND: no diameter.

Table 3. Inhibitory effect of EOs (*MIC* and *MBC* value) against *E. coli, Staphylococcus* spp. and *S. uberis* (*MIC* and *MBC* values in µL/mL)

Essential oils	*C. cassia*			*C. ambrosioides*			*L. cubeba*		
	MIC	*MBC*	*MBC/MIC*	*MIC*	*MBC*	*MBC/MIC*	*MIC*	*MBC*	*MBC/MIC*
E. coli	0.196	0.80	4.1	1.56	6.24	4.0	1.56	3.12	2.0
Staphylococcus spp.	0.39	1.20	3.1	3.12	12.48	4.0	3.12	12.48	4.0
S. uberis	0.39	1.20	3.1	3.12	6.24	2.0	3.12	6.24	2.0

Table 4. Interaction effect of EOs (*FIC* values) on the isolated strains in bovine mastitis

Essential oil (A)	Essential oil (B)	Bacteria	∑*FIC* value[1]	Interaction
C. cassia	*C. ambrosioides*	*E. coli*	0.5-0.75	Synergistic
		Staphylococcus spp.	0.75	Synergistic
		S. uberis	0.5-0.75	Synergistic
C. cassia	*L. cubeba*	*E. coli*	0.5	Synergistic
		Staphylococcus spp.	0.75	Synergistic
		S. uberis	0.5-0.75	Synergistic
C. ambrosioides	*L. cubeba*	*E. coli*	1	No difference
		Staphylococcus spp.	2	No difference
		S. uberis	1	No difference

[1]*: FIC value was calculated according to (2.1) and (2.2) formula.*

3.3. Determination of MIC and *MBC* of Essential Oils

To identify the concentration of EOs inhibiting all isolated strains, *MIC* and *MBC* values were determined for strains showed the lowest inhibitory zone diameter. The results of *MIC* and *MBC* of EOs against *E. coli, Staphylococcus* spp., and *S. uberis* were represented in Table 3. *C. cassia* EO possessed a strong bactericidal effect on all tested strains with all *MIC* and *MBC* values below 1.0 µL/mL. *C. ambrosioides* and *L. cubeba* EOs showed similar antimicrobial effect, where MIC and *MBC* values were less than 3.5 µL/mL and 12.5 µL/m, respectively. In most cases, considering the *MBC/MIC* was lower than 4, the tested EOs showed the bactericidal effect against isolated strains in bovine mastitis except for *C. cassia* against *E. coli*.

Furthermore, *E. coli* was reported to be the most sensible bacteria faced to three tested EOs, with *MIC* and *MBC* values less than these two other species.

Overall, *MIC* and *MBC* values of *C. cassia* EO were slightly higher than in other studies. Zhu *et al.* (2016) reported that *MIC* and *MBC* values of *C. cassia* oil were 0.025% and 0.1% (v/v) for *E. coli,* and 0.0125% and 0.05% (v/v) for *S. aureus*, respectively [17]. In other work, these values of *C. cassia* EO against *E. coli* also remained low (0.125 µl/ml and 0.25 µl/ml, respectively) [38]. However, these values of *L. cubeba* and *C. ambrosioides* for *E. coli,* and *S. aureus* were lower than in other works [23, 24, 26, 27, 29]. Furthermore, strains from Gram positive bacteria: *Staphylococcus* spp. and *S. uberis* showed less susceptibility against all tested EOs compared to *E. coli* (Gram negative bacteria). Our finding indicated that *MIC* of three EOs against *Staphylococcus* spp. and *S. uberis* were two-fold higher than that of *E. coli*. In general, higher antibacterial activity of EOs is observed on Gram positive bacteria [39,40]. In Gram negative bacteria, the presence of an outer membrane surrounding the cell wall limits the diffusion rate of hydrophobic compounds through its lipopolysaccharide layer, leading to its resistance against EOs [7]. In contrast, lipophilic character present in Gram positive bacteria cellular walls increases membrane fluidity, that facilitates the penetration of hydrophobic compounds of EOs [41]. The main reasons for these differences might be due to the variability of EO composition and the geographical origin of pathogenic bacteria.

3.4. Synergy Study of the Combination Effect of EOs against Isolated Strain in Bovine Mastitis

In this study, *FIC* value range for each type of interaction used for evaluating the synergy between EOs is in alignment with the value ranges provided by the paperwork of Fratini *et al*., 2017 [19]. Overall, no antagonist effect was observed, and synergistic effect was showed between *C. cassia*-*C. ambrosioides* and *C. cassia*-*L. cubeba* with the total *FIC* value from 0.5 to 0.75, lower the *MIC* by 2 to 4 times. When using a combination of *L. cubeba* and *C. ambrosioides* EOs against the tested bacteria, indifferent effects were observed with ∑*FIC* value = 1. The synergistic effect observed against strains from *E. coli* and *S. uberis* was the most significant with ∑*FIC* value = 0.5 in 4/6 tests and 3/6 tests, respectively, and in case of *Staphylococcus* spp., the ∑*FIC* value was 0.75 in all experiments.

After identifying the *MIC* and *MBC* value of EOs, the synergistic interaction between different EOs mixtures was investigated. The synergistic effect was reported between *C. cassia*-*C. ambrosioides* and *C. cassia*-*L. cubeba* EOs against all studied bacteria, while the combination between *C. ambrosioides*- *L. cubeba* showed an indifferent effect. Previously, the mixture of Cinnamon EO with thyme oil, clove oil or with some plants' EOs was demonstrated to display an additive effect against bacterial species, including *B. subtilis, B. cereus, S. aureus*, compared to the pure EO [42, 43]. However, antimicrobial effect of mixture containing *L. cubeba* or *C. ambrosioides* EOs was scarcely reported. In a study

focused on some bacterial strain supporting livestock mastitis, Fratini *et al.* (2014) indicated that the mixture of carvacrol/thymol/ p-cymene EO presented a strong inhibition against *S. aureus* and *S. sciuri* [13]. Besides, research on combination between some EO and antibiotic agents was carried out. Synergic effects against pathogenic bacteria were observed when oregano oil was mixed with gentamycin or cinnamon oil with streptomycin [15,22,44].

EOs are volatile compounds and could be safe to use in food industry [37]. Our result suggests an encouraging of combining *C. cassia* and *L. cubeba* EOs to enhance the antimicrobial effects against bovine mastitis isolates, lower the concentration used, minimum effective dose of the drugs and to replace the antibiotic treatment for bovine mastitis in the future.

4. Conclusion

The findings of current study displayed strong antibacterial activity of *C. cassia, C. ambrosioides* and *L. cubeba* EOs. Moreover, the results also figured out a promising combination of *C. cassia-C. ambrosioides* and *C. cassia-L. cubeba* EOs, that had an additive antibacterial effect on pathogenic bacteria. In general, *C. cassia* and *L. cubeba* EOs have been used for long time not only in medicinal activity but also in food industry [24,25]. The ability of EOs against pathogenic bacteria suggested that the EOs could be used to sterilize the cow breast to reduce the concentration of drug used for the treatment of bovine mastitis. More studies related to the interaction between EOs and milk, and *in vitro* research are required before applying this method to dairy industry.

References

[1] Ruegg P. L., A 100-year review: Mastitis detection, management, and prevention, Journal of Dairy Science, vol. 100, iss. 12, pp. 10381–10397, Dec. 2017. https://doi.org/10.3168/jds.2017-13023

[2] Gomes F. and Henriques M., Control of bovine mastitis: Old and recent therapeutic approaches, Current Microbiology, vol. 72, pp. 377–382, 2016. https://doi.org/10.1007/s00284-015-0958-8

[3] Vliegher S. De, Fox L. K., Piepers S., Mcdougall S., and Barkema H. W., Invited review: Mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control, Journal of Dairy Science, vol. 95, iss. 3, pp. 1025–1040, Mar. 2012. https://doi.org/10.3168/jds.2010-4074

[4] Pumipuntu N., Tunyong W., Chantratita N., Diraphat, P., Pumirat, P., Sookrung, N., Chaicumpa, W., and Ondrawattana N., *Staphylococcus* spp. associated with subclinical bovine mastitis in central and northeast provinces of Thailand. PeerJ, vol. 7, Mar. 2019, e6587. https://doi.org/10.7717/peerj.6587

[5] Boireau C., Cazeau G., Jarrige N., Calavas D., and Madec J., Antimicrobial resistance in bacteria isolated from mastitis in dairy cattle in France, 2006 – 2016, Journal of Dairy Science, vol. 101, pp. 1–12, 2018. https://doi.org/10.3168/jds.2018-14835

[6] Hoque M. N., Das Z. C., Rahman A. N. M. A., Haider M. G., and Islam M. A., Molecular characterization of Staphylococcus aureus strains in bovine mastitis milk in Bangladesh. International Journal of Veterinary Science and Medicine, vol. 6, pp. 53–60, 2018. https://doi.org/10.1016/j.ijvsm.2018.03.008

[7] Burt S., Essential oils: Their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology, vol. 94, pp. 223– 253, 2004. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[8] Bogni C., Odierno L., and Raspanti C., War against mastitis: current concepts on controlling bovine mastitis pathogens. Microbial Pathogens, pp. 483–494, 2011.

[9] Chockalingam A., Zarlenga D. S., and Bannerman D. D., Antimicrobial activity of bovine bactericidal permeability–increasing protein–derived peptides against gram-negative bacteria isolated from the milk of cows with clinical mastitis. American Journal of Veterinary Research, vol. 68, pp. 1151–1159, 2007. https://doi.org/10.2460/ajvr.68.11.1151

[10] Gonggrijp M. A., Santman-Berends I. M. G. A., Heuvelink A. E., Prevalence and risk factors for extended-spectrum β-lactamase- and AmpC-producing Escherichia coli in dairy farms. Journal of Dairy Science, vol. 99, pp. 9001–9013, 2016. https://doi.org/10.3168/jds.2016-11134

[11] Abboud M., R E. R., Jammal B., and Sleiman M., In Vitro and In Vivo Antimicrobial Activity of two Essential Oils Thymus Vulgaris and Lavandula Angustifolia against Bovine Staphylococcus and Streptococcus Mastitis Pathogen, Middle East Journal of Agriculture Research, vol. 4, pp. 975–983, 2015.

[12] Mishra A. P., Devkota H. P., and Nigam M., Combination of essential oils in dairy products: a review of their functions and potential benefits, LWT, vol. 133, 2020, Art. no. 110116. https://doi.org/10.1016/j.lwt.2020.110116

[13] Fratini F., Casella S., and Leonardi M., Fitoterapia Antibacterial activity of essential oils, their blends and mixtures of their main constituents against some strains supporting livestock mastitis, Fitoterapia, vol. 96, pp. 1–7, 2014. https://doi.org/10.1016/j.fitote.2014.04.003

[14] Montironi I. D., Cariddi L. N., and Reinoso E. B., Evaluation of the antimicrobial efficacy of Minthostachys verticillata essential oil and limonene against Streptococcus uberis strains isolated from bovine mastitis, Revista Argentina de Microbiología, vol. 48, pp. 210–216, 2016. https://doi.org/10.1016/j.ram.2016.04.005

[15] Maia N. L., de Barros M., and de Oliveira L. L., Synergism of plant compound with traditional antimicrobials against Streptococcus spp. isolated from bovine mastitis. Frontiers in Microbiology, vol. 9, pp. 1–10, 2018. https://doi.org/10.3389/fmicb.2018.01203

[16] Abdalhamed A. M., Zeedan G. S. G., and Zeina H. A. A. A., Isolation and identification of bacteria causing mastitis in small ruminants and their susceptibility to

antibiotics, honey, essential oils, and plant extracts. Veterinary World, vol. 11, pp. 355–362, 2018. https://doi.org/10.14202/vetworld.2018.355-362

[17] Zhu H., Du M., Fox L., and Zhu M. J., Bactericidal effects of Cinnamon cassia oil against bovine mastitis bacterial pathogens. Food Control, vol. 66 pp. 291–299, 2016. https://doi.org/10.1016/j.foodcont.2016.02.013

[18] Grzesiak B., Kołodziej B., Głowacka A., and Krukowski H., The effect of some natural Essential oils against bovine mastitis caused by prototheca zopfii isolates in Vitro, Mycopathologia, vol. 183, pp. 541–550, 2018. https://doi.org/10.1007/s11046-018-0246-9

[19] Fratini F., Mancini S., and Turchi B., A novel interpretation of the fractional inhibitory concentration index: The case Origanum vulgare L., and Leptospermum scoparium J . R . et G., forst essential oils against Staphylococcus aureus strains, Microbiological Research, vol. 195, pp. 11–17, 2017. https://doi.org/10.1016/j.micres.2016.11.005

[20] Ooi L. S. M., Li Y., Kam S.-L., Wang H., Wong E. Y. L., and Ooi V. E. C., Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb cinnamomum cassia blume, The American Journal of Chinese Medicine, vol. 34, pp. 511–522, 2006. https://doi.org/10.1142/S0192415X06004041

[21] Gucwa K., Milewski S., Dymerski T., and Szweda P., Investigation of the antifungal activity and mode of action of thymus vulgaris, citrus limonum, pelargonium graveolens, cinnamomum cassia, ocimum basilicum, and eugenia caryophyllus essential oils. Molecules, vol. 23, 2018, Art. no. 1116. https://doi.org/10.3390/molecules23051116

[22] Atki Y. El, Aouam I., and Kamari F. El, Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics, Journal of Advanced Pharmaceutical Technology and Research, vol. 10, pp. 63–67, 2019. https://doi.org/10.4103/japtr.JAPTR_366_18

[23] Nguyen H. V, Caruso D., and Lebrun M., Antibacterial activity of Litsea cubeba (Lauraceae, May Chang) and its effects on the biological response of common carp Cyprinus carpio challenged with Aeromonas hydrophila, Journal Appl Microbiol, vol. 121, pp. 341–351, 2016. https://doi.org/10.1111/jam.13160

[24] Nguyen H. V, Meile J.-C., Lebrun M., Caruso D., Chu-Ky S., and Sarter S., Litsea cubeba leaf essential oil from Vietnam: chemical diversity and its impacts on antibacterial activity. Letters in Applied Microbiology, vol. 66, pp. 207–214, 2018. https://doi.org/10.1111/lam.12837

[25] Thielmann J., Muranyi P., and Kazman P., Screening essential oils for their antimicrobial activities against the foodborne pathogenic bacteria Escherichia coli and Staphylococcus aureus. Heliyon, vol. 5, 2019, e01860. https://doi.org/10.1016/j.heliyon.2019.e01860

[26] She Q. H., Li W. S., Jiang Y. Y., Wu Y. C., Zhou Y. H., and Zhang L., Chemical composition, antimicrobial activity and antioxidant activity of Litsea cubeba essential oils in different months. Natural Product Research, pp. 1–4, 2019. https://doi.org/10.1080/14786419.2018.1557177

[27] Monzote L., García M., Pastor J., Essential oil from Chenopodium ambrosioides and main components: Activity against Leishmania, their mitochondria and other microorganisms. Experimental Parasitology, vol. 136, pp. 20–26, 2014. https://doi.org/10.1016/j.exppara.2013.10.007

[28] Limaverde P. W., Campina F. F., da Cunha F. A. B., Inhibition of the TetK efflux-pump by the essential oil of Chenopodium ambrosioides L. and α-terpinene against Staphylococcus aureus IS-58. Food and Chemical Toxicology, vol. 109, pp. 957–961, 2017. https://doi.org/10.1016/j.fct.2017.02.031

[29] Morais de Oliveira-Tintino C. D., Tintino S. R., Limaverde P. W., Inhibition of the essential oil from Chenopodium ambrosioides L. and α-terpinene on the NorA efflux-pump of Staphylococcus aureus. Food Chemistry, vol. 262, pp. 72–77, 2018. https://doi.org/10.1016/j.foodchem.2018.04.040

[30] Jesus R. S., Piana M., Freitas R. B., In vitro antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from Chenopodium ambrosioides L. Brazilian Journal of Microbiology, vol. 49, pp. 296–302, 2018. https://doi.org/10.1016/j.bjm.2017.02.012

[31] Östensson K., Lam V., Sjögren N., and Wredle E., Prevalence of subclinical mastitis and isolated udder pathogens in dairy cows in Southern Vietnam. Tropical Animal Health and Production, vol. 45, pp. 979–986, 2013. https://doi.org/10.1007/s11250-012-0320-0

[32] Ngoc P. B., Identification of major bacteria causing dairy cow mastitis, their drug resistance and preventive measures, Vietnam Academy of Agriculture Sciences, 2003.

[33] Riffon R., Sayasith K., Khalil H., Dubreuil P., Drolet M., and Lagace J., Development of a rapid and sensitive test for identi cation of major pathogens in Bovine Mastitis by PCR ´, Journal of Clinical Microbiology, vol. 39, pp. 2584–2589, 2001. https://doi.org/10.1128/JCM.39.7.2584-2589.2001

[34] Sakai H., Procop G. W., and Kobayashi N., Simultaneous detection of staphylococcus aureus and coagulase-negative staphylococci in positive blood cultures by real-time PCR with two fluorescence resonance energy transfer probe sets, Society, vol. 42, pp. 5739–5744, 2004. https://doi.org/10.1128/JCM.42.12.5739-5744.2004

[35] Sheng L., Zhu M. J., Inhibitory effect of cinnamomum cassia oil on non-O157 shiga toxin-producing Escherichia coli, Food Control, vol. 46 pp. 374–381, 2014. https://doi.org/10.1016/j.foodcont.2014.05.050

[36] Tongnuanchan P., Benjakul S., Essential oils: extraction, bioactivities, and their uses for food preservation, Journal of Food Science, vol. 79, pp. 1231–1249, 2014. https://doi.org/10.1111/1750-3841.12492

[37] Kalemba D. and Kunicka A., Antibacterial and antifungal properties of essential oils, Current Medicinal Chemistry, vol. 10, pp. 813–829, 2003. https://doi.org/10.2174/0929867033457719

[38] Mith H., Duré R., Delcenserie V., Zhiri A., Daube G., and Clinquart A., Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. Food Science & Nutrition, vol. 2, pp. 403–416, 2014. https://doi.org/10.1002/fsn3.116

[39] Burt S., Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology, vol. 94, pp. 223– 253, 2004. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

[40] Tongnuanchan P, Benjakul S., Essential oils: Extraction, bioactivities, and their uses for food preservation, Journal of Food Science, vol. 79, iss. 7, pp. R1231–R1249, Jun. 2014. https://doi.org/10.1111/1750-3841.12492

[41] Cox S. D., Mann C. M., and Markham J. L., The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil), Journal of applied microbiology, vol. 88, pp. 170–175, 2000. https://doi.org/10.1046/j.1365-2672.2000.00943.x

[42] Lu F., Ding Y., Ye X., Ding Y., Antibacterial effect of cinnamon oil combined with thyme or clove oil, Agricultural Sciences in China, vol. 10, pp. 1482–1487, 2011. https://doi.org/10.1016/S1671-2927(11)60142-9

[43] Utchariyakiat I., Surassmo S., Jaturanpinyo M., Khuntayaporn P., and Chomnawang M. T., Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant Pseudomonas aeruginosa and the synergistic effects in combination with other antimicrobial agents. BMC Complementary and Alternative Medicine, vol. 16 pp. 1–7, 2016. https://doi.org/10.1186/s12906-016-1134-9

[44] Rosato A., Piarulli M., Corbo F., In vitro synergistic antibacterial action of certain combinations of gentamicin and essential oils. Current Medicinal Chemistry, vol. 17, pp. 3289–3295, 2010. https://doi.org/10.2174/092986710792231996