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Research article

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF TWO DIFFERENT ESSENTIAL OILS AGAINST MASTITIS ASSOCIATED PATHOGENS

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Mastitis is one of the most common and costly diseases affecting dairy cows worldwide. Since antibiotic resistance has become a global threat to both animal and human health, it is becoming more urgent to continuously search for new therapeutical alternatives for the control and treatment of bovine mastitis. Hence, our research aimed to test the therapeutic use of two essential oils (EOs) based on their chemical composition, antibacterial and antioxidant potential. The present study was conducted by collecting milk samples from the cows diagnosed with clinical or subclinical mastitis with the aim of isolating and identifying bacterial strains. The antioxidant potential of essential oils of *Menthae piperitae* (MP) and *Melissa officinalis* (MO) was evaluated in several *in vitro* assays. In the MP EO, a total of 38 compounds were identified, with menthol as the dominant compound, whereas in MO EO 51 compounds were identified. Furthermore, the values of minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) have been used to quantitatively measure the antibacterial activity of each essential oil. In accordance with which, MP EO samples exhibited a higher degree of antibacterial activity than MO EO. Thus, EOs have been shown to be promising alternatives to antibiotics because of their availability, biodegradability, and lower risk of side effects as compared with conventional, antimicrobial treatment. Nevertheless, further clinical studies are needed to test the potential role of EOs in treating mastitis in dairy cows.

Key words: antibacterial activity, antimicrobials, essential oil, mastitis, menthol

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INTRODUCTION

Mastitis is recognized as having a negative impact on animal welfare and a global problem causing huge losses to a country's national income [1-3]. It is said to be a multi-etiological complex disease caused by bacterial pathogens, in most cases, whereas the most common mammary gland pathogens are streptococci, staphylococci, and coliform organisms. [4]. Thus, mastitis is the primary reason for antibiotic use in dairy production systems, [5,6] as well as for the additional costs arising from the presence of antibiotic residues in milk that could be the cause of potential antimicrobial-resistance consequences, which may be partly responsible for the low cure rates [4,5,7].

Under these circumstances, there is a growing need for identifying alternatives to antibiotics as a preparation for the approaching post-antibiotic era [8]. This trend has increased interest in phytotherapy [9] with a large amount of research that has been focused on antibacterial effects of different herbs [10]. Essential oils (EOs) are highly concentrated and complex mixtures of chemical compounds extracted from aromatic plants and may have significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal properties [11,12]. Accordingly, EOs are included in the "generally recognized as safe" (GRAS) list [7] with limited opportunity for developing resistance after prolonged exposure [4,13,14]. Hence, the use of EOs in protecting livestock from infections mainly in organic farms have become common practice [15].

Peppermint (*Mentha x piper*ita L.) and lemon balm (*Melissa officinalis* L.) of the *Lamiaceae* family are traditional medicinal plants, widely used in the food and pharmaceutical industries. Some of the main active constituents of both plants are present in EOs. Besides, various phenolic compounds are present, among which rosmarinic acid is considered one of the most important. Both EOs have shown numerous pharmacological activities, such as antimicrobial, antioxidant, antispasmodic, sedative and neuroprotective [16-18].

Thus, the aim of the present study was to evaluate the *in vitro* antimicrobial activity, chemical composition and antioxidative potential of *Melissa officinalis* (MO) and *Menthae piperitae* (MP) EOs against strains belonging to mastitis-associated pathogens in Serbia.

MATERIAL AND METHODS

Sampling procedure and ethical approval

The experimental protocol was approved by the Animal Ethics Committee of the Ministry of Agriculture, Forestry and Water Management - Veterinary Directorate (9000-689/2, 06.07.2020.) The milk samples were collected according to standard procedures, clean dry teats wiped with alcohol, foremilk 2, 3 milk stikes, and the collected milk samples from five dairy farms located in Serbia were stored in sterile tubes. The number of cows per farm varied between twenty and three hundred. All

farms housed Holstein-Friesian cattle and the samples were taken from lactating cows with clinical and subclinical mastitis. Clinical mastitis was diagnosed according to the presented changes in the udder and milk by veterinary practitioners, while subclinical mastitis was confirmed by using milk samples for somatic cell count. The study was conducted from October 2020 to May 2021 by taking milk samples from all animals during morning milking. Prior to milking, the udder skin was cleaned, washed, and dried. The samples were then stored in sterile tubes labeled with an ID number. All milk samples were kept at 4°C and tested in the Laboratory for Milk Hygiene at the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad. The samples were inoculated on 2% blood agar, using a platinum loop (0.01 mL), followed by incubation of the samples for 48 h at 37 °C. Besides, biochemical and cultural characteristics of the grown microorganisms were used as a mean of their identification. Isolation and identification of bacterial strains was done using the collected milk samples. A loopful of milk sample was streaked on blood agar (Oxoid) and then subcultured on the following selective media: Mannitol Salt Agar, Edwards Agar, Salmonella-Shigella Agar, and MacConkey Agar. Furthermore, the isolation of mastitis - associated pathogens was assessed by the method described by Kovačević et al. [19].

Essential Oils

EOs of peppermint (*Mentha x piperita* L., Lamiaceae) (MP) and lemon balm (*Melissa officinalis* L., Lamiaceae) (MO) evaluated in the present study were purchased from a certified manufacturer (Pharmanais LLC, Serbia). Row plant material (*Menthae piperitae folium* and *Melissae folium*) was sampled before being distilled by the manufacturer and, after confirmation of identity, voucher specimens (MP-01/2021 and MO-01/2021, respectively) deposited at the Herbarium of the Laboratory of Pharmacognosy, Department of Pharmacy, Faculty of Medicine, University of Novi Sad. According to the certificate obtained from the manufacturer, both EOs were isolated using the internal steam distillation technique (Cellkraft AB, Sweden).

Analysis of EOs' chemical composition

The qualitative and quantitative analysis of EOs was carried out on HP-5MS capillary column (30 m x 0.25 mm; 0.25 μm film thickness) on the Agilent 6890B GC-FID instrument coupled with the Agilent 5977 MSD. The samples were injected in splitless mode, at inlet temperature of 220°C. The oven temperature was set at 60°C and increased to 246°C at a 3°C/min rate. Helium was used as the carrier gas at a rate of 1 mL/min while the temperature of the MSD transfer line was set at 230°C. Mass spectral data were collected in scan mode ($m/z = 50 – 550$), while compound identification was performed using the NIST (v14) mass spectral database and by comparison to relative retention indices (RT), as well as literature data [20].

Evaluation of antioxidant potential

The antioxidant potential of MO and MP EOs was evaluated using several *in vitro* assays. The potential of EOs to neutralize 2, 2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl (OH) and nitroso (NO) radicals was assessed by previously described spectrophotometric methods. The liposome emulsion containing Fe2+/H2O2 induced lipids was used as a model system of biological membrane for testing the lipid peroxidation (LP) inhibition capacity. Also, the potential of EOs to reduce Fe3+ (Ferric reduction antioxidant potential - FRAP test) was assessed by a method described by Kovačević et al. [19]. As a positive control of antioxidant capacity of the investigated EOs, ascorbic acid (AA), propyl gallate (PG) and synthetic antioxidants, such as butylated hydroxytoluene (BHT) were tested under the same experimental conditions. Each EO sample and antioxidants used as control substances were analyzed in four replicates in all test systems.

The determination of EOs' effectiveness against mastitis-associated bacteria

To determine minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of EOs, a modified resazurin microtitre-plate assay was used, as reported by Čabarkapa et al. [21]. Briefly, EOs were dissolved in Muller–Hinton Broth (MHB) supplemented with 0.5% Tween 80 (Polyoxyethylenesorbitan monooleate, HiMedia Laboratories Pvt. Ltd., Mumbai, India), and diluted to concentrations ranging from 1000 to 0.9 mg/mL. Twenty microliter (μL) aliquots of each tested EOs were added to 96-well microtiter plates. Subsequently, aliquots of 160 μL of MHB were added into each well. As the final step, 20 μL of the standardized bacterial suspension was inoculated into each well. The test was performed in a total volume of 200 μL with final EOs' concentrations ranging from 100 to 0.09 mg/mL, while the final microbial concentration was 107 CFU/mL. The plates were incubated at 37 °C for 24 h. simultaneously, the same tests were performed for growth control (MHB + test organism), negative control (MHB + solvent + test organism), and sterility control (MHB + test oil). At the end of incubation time, 10 μL of the resazurin solution (0.01%) (Sigma-Aldrich, St Louis, MO, USA) was added to each well.

Statistical analysis

The obtained results were processed by Microsoft Office Excel v2019. The experimental measurements were perfomed in triplicate or quadruplicate, whereas the results were expressed as mean values corrected by the standard deviation. For the purpose of determination of antioxidant potential expressed as concentration required for neutralization of 50% of generated free radicals / lipid peroxidation process the linear regression model was utilized.

RESULTS

Prevalence and isolation of mastitis associated pathogens

Bacteriological testing was performed on a total of 77 milk samples, while pathogens were isolated in 49 (63.63%) of them. The isolated pathogens were the most common mastitis pathogens, including *Streptococcus* spp. *heamoliticus* (Strep_bh), *E. coli* (E_c), *Enterobacter sakazakii* (E_s), *Klebsiella oxytoca* (K_o), *Staphylococcus aureus* (Staph_a), *Staphylococcus* spp. *coagulase-negative* (Staph_cn), *Streptococcus dysgalactiae* (Strep_d), *Streptococcus* spp. (Strep), and *Streptococcus uberis* (Strep_u). Among them, the most common was *Streptococcus* spp., identified in seventeen samples (22.07%), followed by ten (12.98%) samples with *E. coli*, and 8 samples *Streptococcus* spp. *heamoliticus* (10.38%). Besides, *Staphylococcus aureus* was found in 6 (7.79%) samples, *Staphylococcus* spp. *coagulase negative* in 5 (6.49%), while *Streptococcus uberis* was found in 3 samples (3.86%). *Streptococcus dysgalactiae, Klebsiella oxytoca*, and *Enterobacter sakazakii* were found in one sample each (1.29%) (Figure 1).

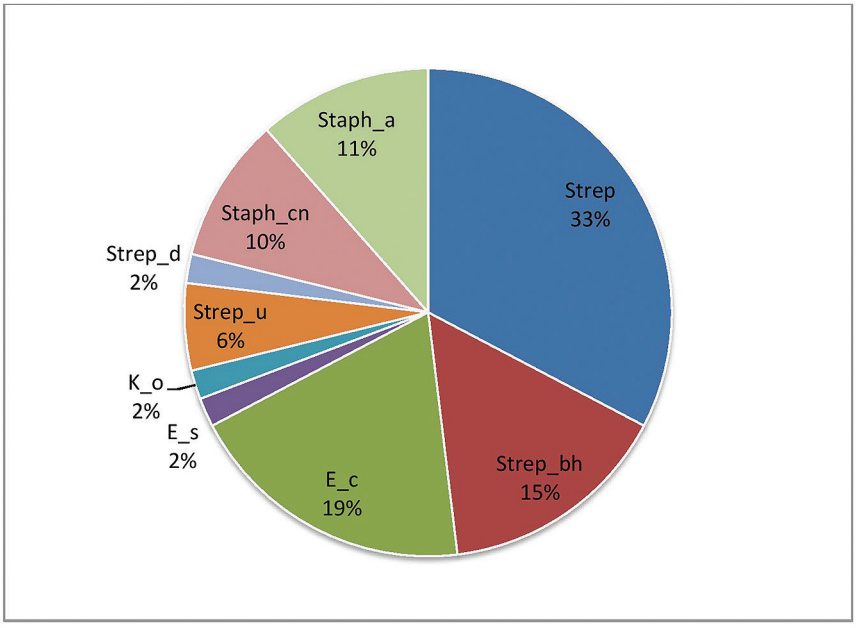

Figure 1. Proportion (%) of the evaluated bacterial strains in the collected samples. *Streptococcus* spp. (Strep), *Streptococcus* spp. β heamoliticus (Strep_bh), *E. coli* (E_c), *Enterobacter sakazakii* (E_s), *Klebsiella oxytoca* (K_o), *Streptococcus uberis* (Strep_u), *Streptococcus dysgalactiae* (Strep_d), *Staphylococcus* spp. coagulase negative (Staph_cn) and *Staphylococcus aureus* (Staph_a)

Chemical composition and antioxidant potential of EOs

The detailed chemical composition of tested *Melissa officinalis* (MO) EOs and *Menthae piperitae* (MP) is listed in Table 1. In the MP EO, the total of 38 compounds (99.41%)

were identified with the oxygenated monoterpenes as a major group of constituents (91.96%), whereas the main compounds were menthol (37.69%), menthone (9.76%) isomenthone (23.84%) and menthofuran (9.23%), all from menthane class of monoterpenes (Table 1).

Table 1. Chemical composition (expressed as percentage) of EOs of *Mentha x piperita* (MP) and *Melissa officinals* (MO)

Peack No.	Compound	RI*	*M. piperita*	*M. officinalis*
Monoterpene Hydrocarbons			**2.82**	**4.67**
1	α-Pinene	937	0.86	0.56
2	Camphene	952	0.08	0.12
3	β-Pinene	978	0.76	0.98
4	β-Myrcene	991	0.05	0.19
5	α-Phellandrene	1005	0.14	0.22
6	α-Terpinene	1017	0.21	0.24
8	Limonene	1030	0.24	1.31
10	γ-Terpinene	1060	0.48	1.05
Aromatic Monoterpene Hydrocarbons			**0.22**	**0.67**
7	p-Cymene	1025	0.22	0.67
Oxygenated Monoterpenes			**91.96**	**58.72**
9	1.8-Cineole	1032	1.45	0.47
11	Linalool	1099	0.25	0.73
12	*trans*-Verbenol	1143	0	0.34
13	Isopulegol	1146	0.27	0.21
14	*trans*-Chrysanthemal	1152	0	0.47
15	Citronellal	1153	0	9.39
16	Menthone	1153	9.76	0
17	Menthofuran	1159	9.23	0
18	Isomenthone	1164	23.84	0
19	Isoneral	1170	0	0.54
20	Menthol	1174	37.69	0
21	Terpinen-4-ol	1177	0.38	0
22	Isomenthol	1183	0.79	0
23	Isogeranial	1185	0	0.82
24	α-Terpineol	1189	0.65	0
25	Citronellol	1228	0	1.59
26	Neral	1240	0	15.81
27	Pulegone	1237	0.79	0
28	Carvone	1242	0.15	0

29	Geraniol	1253	0	1.63
30	Piperitone	1253	1.45	0
31	Geranial	1270	0	21.83
33	Menthyl acetate	1295	0.89	0
34	Isomenthyl acetate	1305	4.37	0
35	Geranic acid methyl ester	1324	0	0.63
36	Citronellyl acetate	1353	0	1.64
37	Neryl acetate	1362	0	0.97
38	Geranyl acetate	1382	0	1.65
Sesquiterpene Hydrocarbons			**3.77**	**32.44**
39	α-Cubebene	1351	0.09	0.12
40	α-Copaene	1376	0	0.86
41	(-)-β-Bourbonene	1384	0.45	0.36
42	β-Cubenene	1388	0.18	0.34
43	*cis*-β-Caryophyllene	1406	0	0.31
44	*trans*-β-Caryophyllene	1419	1.43	19.18
45	β-Copaene	1432	0	0.14
46	γ-Elemene	1433	0.12	0.22
47	*trans*-α-Bergamotene	1435	0	0.49
48	Aromandendrene	1440	0.24	0.18
49	*cis*-β-Famesene	1443	0	0.23
50	Humulene	1454	0.28	1.35
51	*trans*-β-Famesene	1456	0.17	0.05
52	allo-Aromandendrene	1461	0	0.12
53	γ-Muurolene	1477	0	0.21
54	Germacrene D	1482	0.23	5.81
55	β-Selinene	1486	0	0.11
56	α-Muurolene	1499	0.37	0.52
57	δ-Cadinene	1524	0.21	1.84
Oxygenated Sesquiterpenes			**0.53**	**2.89**
58	Caryophyllenyl alcohol	1572	0.05	0.12
59	Caryophyllene oxide	1581	0.48	1.94
60	epi-α-Muurolol	1642	0	0.42
61	α-Cadinol	1653	0	0.41
Aliphatic Compunds			**0.11**	**0.4**
5	3-Octanol	994	0	0.18
32	1-Decanol	1273	0.11	0.22
TOTAL OF IDENTIFIED COMPOUNDS			**99.41**	**99.79**

*Retention indices

The free radical scavenging capacity (RSC) of tested MO and MP EOs, as well as positive control substances evaluated in a series of *in vitro* tests is presented in Table 2. All results, except those obtained in FRAP test, are presented as the IC50 values, which represent the concentrations of EOs and positive controls that caused 50% of neutralization, determined by linear regression analysis. The FRAP test is a different model of antioxidant potential evaluation tests, which correlates with the neutralization of hypochlorite and peroxynitrite anion [22]. Therefore, results are presented as AA equivalents (Table 2).

Table 2. Antioxidant potential of tested EOs of *Mentha x piperita* (MP) and *Melissa officinalis* (MO) and positive control substances. All of the measurements were performed in quadruplicate.

Samples	Assay				
	DPPH IC_{50}	OH IC_{50} (µg/mL)	NO IC_{50} (µg/mL)	LP IC_{50} (µg/mL)	FRAP (mg AAE**/ mL EO)
	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD	$\bar{X}$±SD
M. piperita	8439 ± 13.05	n.d.*	n.d.*	1656 ± 11.92	11.00 ± 0.21
M. officinalis	1083 ± 10.85	n.d.	n.d.	140 ± 5.02	49.15 ± 0.05
AA***	/	2030 ± 8.39	/	/	/
PG****	0.76 ± 0.02	8.75 ± 0.29	/	/	/
BHT*****	/	0.03 ± 0.01	/	7.18 ± 0.36	/

*Not detected; **Ascorbic acid equivalents; ***Ascorbic acid; ****Propyl gallate; *****Butylated hydroxytoluene

EOs effectiveness against Mastitis-Associated Bacteria

Minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of *Melissa officinalis* (MO) and *Menthae piperitae* (MP) EOs against mastitis-associated pathogens are presented in Table 3. MO EO exhibited a lower degree of antibacterial activity against tested mastitis-associated pathogens (MIC/MBC values were >100/>100 mg/ml for all tested bacteria) than MP EO. Furthermore, MP EO sample exhibited a higher degree of antibacterial activity than MO EO. MIC for the tested bacterial species ranged from 0.39 to >100 mg/ml, and the lowest MIC values were found for the tested *Streptococcus spp. ß - heamoliticus* strain. The obtained MIC and MBC values of MP EO indicate that gram-positive strains (*Streptococcus* spp. *and Staphylococcus* spp.) are more susceptible than gram-negative (*E. coli*, *Enterobacter sakasakii* and *Klebsiella oxytoca).*

Table 3. Minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of Melissa officinalis (MO), Menthae piperitae (MP) EOs against mastitis-associated pathogens.

Sample	MO (MIC/MBC)	MP (MIC/MBC)
E.coli	>100/>100	>100/>100
E.coli	>100/>100	>100/>100
E.coli	>100/>100	>100/>100
E.coli	>100/>100	100/>100
Klebsiella oxytoca	>100/>100	100/>100
Enterobacter sakasakii	>100/>100	100/>100
Streptococcus spp. ß - heamoliticus	>100/>100	0.78/1.56
Streptococcus spp. ß - heamoliticus	>100/>100	0.39/0.78
Streptococcus spp. ß - heamoliticus	>100/>100	0.78/1.56
Streptococcus spp.	>100/>100	12.5/25
Streptococcus spp.	100/>100	0.78/1.56
Streptococcus spp.	>100/>100	1.56/3.125
Staphylococcus spp.	>100/>100	6.25/12.5
Staphylococcus spp. coagulase negative	>100/>100	>100/>100

DISCUSSION

Numerous publications have presented data on the antibacterial potential of different EOs [23-25]. Pharmacological characteristics of the active principles represent potentially the most serious alternative to antimicrobials [26]. This potential of EOs could be used for the control of different livestock diseases, such as mastitis in dairy cows. Besides acquiring knowledge regarding antibacterial activity against mastitis-related bacteria, development of such strategies requires detailed evaluation of chemical composition and antioxidant potential of EOs. Hence, the results related to the chemical composition of MP EO obtained in this study are in accordance with the previously published data [18,27,28], with menthol as the dominant compound which is similar to the requirements prescribed by the Ph. Eur. 10 (2020) [29]. Fifty –one compounds were identified in MO EO (amounting to 99.79%), being classified as monoterpenes, with a predominance of oxygenated monoterpenes (58.72%). Apart from the notable quantities of sesquiterpene hydrocarbons (32.44%), dominant compounds in MO EO were citrals geranial (21.83%) and neral (15.81%), together with citronellal (9.39%) and trans-β-caryophyllene (19.18%), which is also in line with the previously published data [30,31]. Generally, it has been proven that plants possess a significant antioxidant potential, mainly due to the presence of different aromatic, phenolic and especially flavonoid compounds in the aglycone form. However, in most of the assayed systems both of the tested EOs either did not exhibit or exhibited notably weaker free radical scavenging effects (RSC), which is similar to the results

of other authors [28,31]. Although it is difficult to make generalized comparison of the results published by different laboratories due to the different experimental conditions, presentation of results, different methods for evaluation of antioxidant potential, etc., the weak RSC could be explained with the specific composition of the tested EOs and the absence of aromatic compounds such as thymol or carvacrol. It is established that these aromatic oxygenated monoterpenes exhibit the ability to achieve a resonantly stable radical structure by the hydrogen atom or electron donation to ROS and thus neutralize the cascade of free radical reactions [18]. It is possible that the antioxidant capacity is diminished by the dominant components. But, it should be stressed that comparison of antioxidant potential in the present study was performed between pure compounds with confirmed strong antioxidant capacity of EOs.

The antimicrobial activity of EOs is attributed mostly to their ability to integrate and disrupt bacterial membrane structure and function, although the exact mechanism of action is not fully understood. The results obtained in this study regarding antimicrobial activity indicate that gram-positive strains (*Streptococcus* spp. *and Staphylococcus* spp.) are more susceptible than gram-negative (*E. coli*, *Enterobacter sakasakii*, *Klebsiella oxytoca)* to treatment with MP EO. In the MP EO tested sample the most important compounds identified were menthol (37.69%), isomenthone (23.84%), menthone (9.76%) and menthofuran (9.23%), which are well known for their antimicrobial effects. Testing mechanisms of menthol antibacterial activity showed that gram-positive strains (*Staphylococcus aureus* MIC 0.62 mg/mL) are more susceptible than gram-negative (*E. coli* MIC 2.5mg/mL) [32].

In MO EO the most important compounds identified were geranial (21.83%), neral (15.81%), citronellal (9.39%) and trans-caryophyllene (19.18%), which are well known for their antimicrobial effects. In this study MO EO did not exhibit antimicrobial activity against tested bacteria. Previously, Mimica-Dukic et al. [31] reported antimicrobial effects of the MO EO on bacterial (*P. aeruginosa*, E. *coli, S. aureus, S. epidermidis, Shigella sonnei, Sarcina lutea*, *Micrococcus flavus, Bacillus subtilis, Salmonella enteritidis and S. typhi*) and six fungi strains *in vitro.* Moreover, they reported that the most effective antimicrobial properties were expressed on a multi-resistant strain of *S. sonnei.* The differences between antibacterial activities of the reported ones and MO EOs may be attributed to different chemical composition since in EOs it is highly affected by genotype, phenological development (raw material collected prior to, while, or after blooming), drying method, as well as the range of ecological factors that influence habitat [18,27,31].

CONCLUSION

As mentioned above, *Menthae piperitae* (MP) EO exhibited potent antioxidant effects and better antimicrobial activity against tested mastitis-associated pathogens than *Melissa officinalis* (MO) EO. Unlike MO EO, the use of MP EO could be considered to be included in the development of a formulation used for the prevention and

treatment of mastitis. Hence, the exploitation of EOs as a potential replacement for mastitis therapy could represent 'a new era for phytopharmaceuticals. Additionally, the extent to which bacteria might acquire resistance to EO components has yet to be systematically and extensively investigated.

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Authors' contributions

ZK and BB conceived the study and carried out the conceptualization, participated in its design and coordination and helped to draft the manuscript. NČ, DT and MM participated in the design of the study and performed the data analysis. DT, MR, IČ and MM carried out the study and developed the design and assisted in data collection and evaluation. All authors were writing- review & editing the manuscript and have read and agreed to the published version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Sharun K, Dhama K, Tiwari R, Gugjoo MB, Yatoo MI, Patel SK, Pathak M, Karthik K, Khurana SK, Singh R, Puvvala B, Amarpal, Singh R, Singh KP, Chaicumpa W: Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. Vet Q 2021, 41: 107-136.
2. Cheng WN, Han SG: Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments—A review. Asian-Australas J Anim Sci 2020, 33: 1699-1713
3. Suvajdžić B, Teodorović V, Vasilev D, Karabasil N, Dimitrijević M, Đorđević J, Katić V: Detection of icaA and icaD genes of Staphylococcus aureus isolated in cases of bovine mastitis in the Republic of Serbia. Acta Vet-Beograd 2017, 67: 168-177.
4. Gomes, F, Henriques, M: Control of bovine mastitis: old and recent therapeutic approaches. Curr Microbiol 2016, 72: 377-382.
5. Krömker V, Leimbach S: Mastitis treatment—reduction in antibiotic usage in dairy cows. Reprod Domest Anim 2017, 52: 21-29.
6. Saini V, McClure J T, Léger D, Keefe G P, Scholl DT, Morck DW, Barkema HW: Antimicrobial resistance profiles of common mastitis pathogens on Canadian dairy farms. J Dairy Sci 2012, 95: 4319-4332.
7. Dal Pozzo M, Santurio DF, Rossatto L, Vargas AC, Alves SH, Loreto ES, Viegas J: Activity of essential oils from spices against Staphylococcus spp. isolated from bovine mastitis. Arq Bras Med Vet Zootec 2011, 63: 1229-1232.

8. Tamminen LM, Emanuelson U, Blanco-Penedo I: Systematic review of phytotherapeutic treatments for different farm animals under European conditions. Front Vet Sci 2018, 5: 140.
9. Blanco-Penedo I, Lundh T, Holtenius K, Fall N, Emanuelson U: The status of essential elements and associations with milk yield and the occurrence of mastitis in organic and conventional dairy herds. Livest Sci 2014, 168: 120-127.
10. Alekish MO, Ismail ZB, Awawdeh MS, Shatnawi S: 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. Vet world 2017, 10: 895-900
11. Bakkali F, Averbeck S, Averbeck D, Idaomar M: Biological effects of essential oils– a review. Food Chem Toxicol 2008, 46: 446-475.
12. Bassolé NHI, Juliani RH: Essential oils in Combination and Their Antimicrobial Properties. Molecules 2012, 17: 3989-4006.
13. Swedzinski C, Froehlich KA, Abdelsalam KW, ChaseC, Greenfield T J, Koppien-Fox J, Casper DP: Evaluation of essential oils and a prebiotic for newborn dairy calves. Transl Anim Sci 2020, 4: 75-83.
14. Cho TJ, Park SM, Yu H, Seo GH, Kim HW, Kim SA, Rhee MS: Recent Advances in the Application of Antibacterial Complexes Using Essential Oils. Molecules 2020, 25: 1752.
15. Fratini F, Casella S, Leonardi M, Pisseri F, Ebani VV, Pistelli L: Antibacterial activity of essential oils, their blends and mixtures of their main constituents against some strains supporting livestock mastitis. Fitoterapia 2014, 96: 1-7.
16. Singh R, Shushni MA, Belkheir A: Antibacterial and antioxidant activities of Mentha piperita L. Arab J Chem 2015, 8: 322-328.
17. Shakeri A, Sahebkar A, Javadi B: Melissa officinalis L. - A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2016, 188: 204-228.
18. Mimica-Dukic N, Bozin B: Essential oils from Lamiaceae species as promising antioxidant and antimicrobial agents. Nat Prod Commun 2007, 2: 445 -452.
19. Kovačević Z, Radinović M, Čabarkapa I, Kladar N, Božin B: Natural agents against bovine mastitis pathogens. Antibiotics 2021, 10: 205.
20. Adams RP : Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured publishing corporation, 2007.
21. Čabarkapa I, Čolović R, Đuragić O, Popović S, Kokić B, Milanov D, Pezo L: Anti-biofilm activities of essential oils rich in carvacrol and thymol against Salmonella Enteritidis. Biofouling 2019, 35:361-375.
22. MacDonald-Wicks LK, Wood LG, Garg ML: Methodology for the determination of biological antioxidant capacity *in vitro*: a review. J Sci Food Agric 2006, 86: 2046-2056.
23. Szweda P, Zalewska M , Pilch J , Kot B , Milewski S: Essential oils as potential anti-staphylococcal agents. Acta Vet-Beograd 2018, 68: 95-107.
24. Mullen KAE, Lee AR, Lyman RL, Mason SE, Washburn SP, Anderson KL: An *in vitro* assessment of the antibacterial activity of plant-derived oils. J Dairy Sci 2014, 97: 5587-5591.
25. Motlagh KM, Kazemi M, Ghasemi A., Farahani KHA, Yahyaei M, Reyaei M, Rensis De F, Taddei S: Antibacterial effect of medicinal plant essence (Thymus vulgaris) on major bacterial mastitis pathogen *in vitro*. Int J Adv Biol Biomed Res 2014, 2: 286-294.

26. Marjanović Đ, Bogunović, D, Milovanović M, Marinković D, Zdravković N, Magaš V, Trailović S: Antihelminic activity of carvacrol, thymol, cinnamaldehyde and p-cymen against the free-living nematode Caenorhabditis elegans and rat pinworm Syphacia muris. Acta Vet-Beograd 2018, 68: 445-456.
27. Haydari M, Maresca V, Rigano D, Taleei A, Shahnejat-Bushehri A, Hadian J, Sorbo S, Guida M, Manna C, Piscopo M, Notariale R, De Ruberto F, Fusaro L, Basile A: Salicylic acid and melatonin alleviate the effects of heat stress on essential oil composition and antioxidant enzyme activity in Mentha × piperita and Mentha arvensis L. Antioxidants 2019, 8: 547.
28. Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M: Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Med 2003, 69: 413-419.
29. EDQM. European Pharmacopoeia 10.3; The European Directorate for the Quality of Medicines & HealthCare, Council of Europe:Brusselles, Belgium 2020. pp. 1648–1650.
30. Luz JMQ, Silva SM, Habber LL, Marquez MOM: Essential oil of Melissa officinalis L. at differents seasons, systems of planting and fertilizations. Rev Bras de Plantas Medicinais 2014, 16: 552-560.
31. Mimica-Dukic N., Bozin B, Sokovic, Simin N: Antimicrobial and Antioxidant Activities of Melissa officinalis L. (Lamiaceae) Essential Oil. J Agric Food Chem 2004, 52: 2485-2489.
32. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G: Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005, 49:2474-2478.

HEMIJSKI SASTAV, ANTIOKSIDATIVNI POTENCIJAL I ANTIBAKTERIJSKA AKTIVNOST DVA RAZLIČITA ETARSKA ULJA PROTIV UZROČNIKA MASTITISA

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Mastitis je poznat kao jedna od najčešćih i ekonomski najznačajnijih bolesti koja pogađa mlečne krave širom sveta. Obzirom da rezistencija na antibiotike predstavlja pretnju za zdravlje životinja i ljudi, kontinuirana potraga za novim terapijskim alternativama u kontroli i lečenju mastitisa je hitna. Stoga je cilj našeg istraživanja bio da se ispita hemijski sastav, antibakterijski i antioksidativni potencijal dva etarska ulja (EU). Istraživanje je sprovedeno uzimanjem uzoraka mleka od krava kojima je dijagnostikovan klinički ili subklinički mastitis u cilju izolacije i identifikacije sojeva bakterija. Antioksidativni potencijal etarskih ulja *Menthae piperitae* (MP) i *Melissa officinalis* (MO) procenjen je u nekoliko *in vitro* testova. Ispitivanjem hemijskog sastava etarskog ulja MP identifikovano je ukupno 38 jedinjenja, sa mentolom kao dominantnim jedinjenjem, dok je u etarskom ulju MO identifikovano 51 jedinjenje. Antibakterijska aktivnost etarskog ulja izražena je kao minimalna inhibitorna koncentracija (MIC) i minimalna baktericidna koncentracija (MBC). Uzorak EU MP pokazivao je viši stepen antibakterijske aktivnosti od EU MO. Etarska ulja mogu da predstavljaju moguću alternativu antibioticima zbog

njihove dostupnosti, biorazgradljivosti i nižeg rizika od neželjenih efekata u poređenju sa konvencionalnom antibiotskom terapijom. Ipak, potrebno je više kliničkih studija da bi se ispitala moguća uloga EU u lečenju mastitisa kod mlečnih krava.