BIOLOGIJA. 2011. Vol. 57. No. 2. P. 92–97

Antimicrobial activity of *Mentha arvensis* L. and *Zingiber officinale* R. essential oils

Rūta Mickienė[1]*,

Ona Ragažinskienė[2],

Bronius Bakutis[1]

[1] *Department of Food Safety and Animal Hygiene, Veterinary Academy of Lithuanian University of Health Science, Tilžės 18, LT-3022 Kaunas, Lithuania*

[2] *Kaunas Botanical Garden of Vytautas Magnus University, Z. E. Žilibero 6, LT-3018 Kaunas, Lithuania*

The aim of the study was to evaluate the antimicrobial effects of essential oils *in vitro* for a possible application to reduce the content of microorganisms in the air of animal farms. The essential oils *Mentha arvensis* L. and *Zingiber officinale* R. were screened against bacteria *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout. The minimal inhibitory concentrations of the active essential oils were tested using broth dilution assay at concentrations ranging from 0.1–50.0%. The oils showed a wide spectrum of antibacterial activity: concentrations of 0.1–0.8% of *Mentha arvensis* L. reduced the total bacterial counts of *Proteus mirabilis* Hauser and *Candida albicans* Berkhout. The dilution method revealed that essential oil *Zingiber officinale* R. only at high bacteriocidal concentrations was able to stop the bacterial growth. *Zingiber officinale* R. at 50.0% completely inhibited the growth of *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout.

Key words: bacteria, essential oil, antimicrobial activity, minimal inhibitory concentrations

INTRODUCTION

Aromatic plants have great importance for food, cosmetics and pharmaceutical industries. They have been used since ancient times, and despite many of them were substituted by synthetic ones, the demand for natural products is increasing. Secondary metabolites are present in all higher plants, usually in a great structural diversity. Many metabolites have been found to protect plants against viruses, bacteria, fungi. The secondary metabolities, such as cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes also act as allelochemicals, influencing the growth and development of the neighbouring plants [22]. For example, monoterpene limonene has shown to possess deterrent and insecticide properties and carvone is used as a sprout inhibitor [12, 1]. Essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids and aromatic compounds. Hydrocarbons are almost always present in monoterpenes [4]. Essential oils accumulate in all types of vegetative organs: flowers (bergamot tree), leaves (mint, eucalyptus), barks (cinnamon), woods (sandalwood), roots (vetiver), rhizomes (ginger), fruit (anise), and seeds (nutmeg). Essential oils are usually associated with a specialized storage in plants [4]. Although they are comprised of many types of compounds, the major ones are monoterpenes [20]. The synthesis and accumulation of essential oil structures are located near the surface, glandular trichomes, secretory cavities or secretory canals of the plants [4]. The impact of the environmental factors, such as temperature, relative humidity, irradiance, photoperiod and cultivation practices influence the composition of essential oils. The influence of the method of extraction on oil composition and the lability of the con-

* Corresponding author. E-mail: mickiene@lva.lt

stituents of essential oil explain the reason why the composition of the product, obtained by steam distillation, is most often different from that which is initially present in the secretary organs of the plant [4].

The exposure to particle material in the air has been claimed to evoke many adverse health effects. However, the concept of exposure is a complex one. Particles from animals, plants, microorganisms and soil derived material are usually called bioaerosols. Several health disorders and respiratory diseases: allergic alveolitis, asthma and organic dust toxic syndrome have been associated with the exposure to organic dust in work environments, such as animal farms, where airborne microbe concentrations are usually high. Airborne microbial concentrations are an important environmental risk factors for cardiopulmonary diseases and lung cancer [17].

Inhalation of airborne micro-organisms and their toxins has been recognized as an important factor in the prevalence of respiratory diseases within the farming community. One major problem here is the inhalation of airborne bacteria, in particular their endotoxins.

Microorganisms have developed resistance to many antibiotics and as a result, an immense clinical problem in the treatment of infectious diseases has been created [8]. The resistance of the organisms has increased due to the indiscriminate use of commercial antimicrobial drugs commonly applied for the treatment of infectious diseases. This situation forced the researchers to search for a new antimicrobial substance from various sources including medicinal plants. Essential oils from several plant species are able to control the Gram-negative and Gram-positive bacteria [18].

Recently, an extensive research on the antimicrobial activity of essential oils against pathogens, seeking natural and safer means for hygiene has been carried out [5].

In this report, we evaluated the antimicrobial effects of essential oils *in vitro* for a possible application to reduce the content of microorganisms in the air of animal farms.

MATERIALS AND METHODS

Essential oils. Essential oils *Mentha arvensis* L. (menthol mint), *Zingiber officinale* R. (ginger), were obtained from a commercial source (Sensient Essential Oils Germany GmbH). For producing the essential oils, the steam distillation method was used.

Microorganism species. The specific bacterial cultures: *Staphylococcus aureus* Rosenbach DSM-No. 799 (*St. aureus*), *Enterococcus faecium* Schleifer and Kilpper-Bälz DSM-No. 2918 (*E. faecium*), *Pseudomonas aeruginosa* Migula DSM-No. 939 (*Ps. aeruginosa*), *Escherichia coli* Castellani and Chalmers DSM-No. 1077 (*E. coli*), *Proteus mirabilis* Hauser DSM-No. 788 (*P. mirabilis*) and the yeast *Candida albicans* Berkhout DSM-No. 1386 (*C. albicans*) were used in this study. The microorganism species were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany). These five germs are commonly used for the evaluation of disinfectant agents [3].

Determination of the minimum inhibitory concentration by broth dilution assay. The broth dilution method was used to determine the minimal inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards 2001. All tests were performed in 0.1% sterile peptone water (Oxoid, Basingstoke, UK).

The microorganisms were suspended in sterile peptone water 0.1% with the turbidity visually corresponding to 0.5 Mc Farland (approximately 108 CFU mL^{-1}). A dilution of the essentials oils was prepared in the concentration range of 0.1–50.0%, in 0.1% sterile peptone water. Standardized microorganism suspensions were inoculated into microwells. 50 µl of microorganism suspension and 50 µl of essential oil (different concentrations of 0.1–50.0%) were added to individual wells and incubated at 37 °C for 24 h. Then, 100 µl of microorganism-peptone water-essential oil suspension was dissolved in 9.9 ml tubes with 0.02% Tween 80 (Sigma). Then, 100 µl of aliquot suspension was added onto agar plates: *St. aureus* Rosenbach, *E. faecium* Schleifer and Kilpper-Bälz, *E. coli* Castellani and Chalmers, *Pr. mirabilis* Hauser were cultivated on Blood Agar Base N. 2 (Oxoid, UK) at 37 °C for 24 h. *Ps. aeruginosa* Migula was cultivated on Blood Agar Base N. 2 at 30 °C for 24 h. *Candida albicans* Berkhout were cultivated on Malt Extract-Agar (Oxoid, UK) at 25 °C for 48 h.

The MIC (minimum inhibitory concentration) is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate any visible growth. All experiments were carried out five times.

Statistical analysis. The data were analysed using the "SPSS for Windows", version 12.0 and Microsoft Office Excel 2003 calculating the mean of values (X), standard deviation (Sx) and the coefficient of variation (CV). The P value of 0.05 was set as a limit for a statistically significant difference in the studies.

RESULTS AND DISCUSION

The results show a wide variation in the antimicrobial properties of plant essential oils. The essential oils: *Mentha arvensis* L. and *Zingiber officinale* R. tested for antimicrobial activity were active against six bacterial strains. The antimicrobial activity of the essential oil for *Mentha arvensis* L. was much stronger than the *Zingiber officinale* R. The most potent inhibitory activity of *Mentha arvensis* L. was found for *P. mirabilis* Hauser and yeast *C. albicans* Berkhout with the MIC of 0.8%.The inhibitory effect of *Zingiber*

officinale R. on the growth of microorganism *E. coli* Castellani and Chalmers, *E. faecium* Schleifer and Kilpper-Bälz, *St. aureus* Rosenbach, *P. aeruginosa* Migula, *P. mirabilis* Hauser, *C. albicans* Berkhout in broth dilution is presented in Fig. 1.

The dilution method revealed that essential oil *Zingiber officinale* R., having hydrocarbon as the major component, exhibited the weakest activity against microorganisms. 6-Gingerol, 6-shogaol, 8-gingerol, and 10-gingerol have been identified as the principal pungent components of ginger [11, 16]. Gas chromatography / mass spectrometry identified 66 compounds in the essential oil of ginger, the major compounds of which are camphene, b-phellandrene and 1,8-cineol. Other constituents included (–)-a-zingiberen, (–)-b-bisabolen, (+)-ar-curcumen, (–)-b-sesquiphellandren and acyclic afarnesen. The ratio of (+)-arcurcumen and (–)-a-zingiberen plus (–)-b-sesquiphellandren and the viscosity of the essential oil allowed to get information on the quality of the herbal preparation. The oil also contains sesquiterpenes and sesquiterpene alcohols, the latter having an impact on the smell of ginger. The taste of ginger is mainly affected by monoterpenes (camphen, limonen, myrcen, b-phellandren and a-pinen, borneol, 1,8-cineol, citronellol, geranial, geraniol, geranylacetate, linalool, neral and others. The high content of neral and geranial corresponds to the lemon smell ginger. Pharmacological studies indicate that *Zingiber officinale* R. exerts anti-inflammatory, antipyretic, antimicrobial, antischistosomal, antitumorigenic, antioxidative, hypoglycemic hepatoprotective, diuretic, and hypocholesterolemic effects *in vitro* activities [2, 6, 10, 14, 15].

Only high bacteriocidal concentrations of *Zingiber officinale* R. essential oil were able to stop the bacterial growth. *Zingiber officinale* R. at 50.0% concentration completely inhibited the growth of *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani and Chalmers, *Proteus mirabilis* Hauser and the yeast *Candida albicans* Berkhout.

The result showed that *Zingiber officinale* R. at the inhibitory concentration, equal to 0.5%, was the most active against *Staphylococcus aureus* Rosenbach, *Pseudomonas aeruginosa* Migula and *Proteus mirabilis* Hauser, and the percentage of growing bacteria was the following: *St. aureus* 20.0 ± 17.8% ($p > 0.05$), *P. aeruginosa* 40.0 ± 24.6% ($p > 0.05$), *P. mirabilis* 23.44 ± 5.9% ($p < 0.05$). The results of antibacterial test with *E. coli, C. albicans* and *E. faecium* were similar – the inhibition percentage was low and the percentage of growing bacteria was the following: *E. coli* 91.6 ± 15.3 ($p < 0.05$), *C. albicans* 86.5 ± 14.7 ($p < 0.05$) and *E. faecium* 80.7 ± 17.7 ($p < 0.05$), *Escherichia coli* Castellani and Chalmers demonstrated different responses to *Zingiber officinale* R. that can probably be attributed to their different membrane structures. The essential oil molecules attach to bacterial cell membrane structures, causing a breakdown in the membrane permeability and an increased susceptibility to essential oils.

The inhibitory effect of *Mentha arvensis* L. on the growth of microorganism *E. coli, E. faecium, St. aureus, P. aeruginosa, P. mirabilis, C. albicans* in broth dilution is presented in Figs. 2 and 3.

The results show that *Mentha arvensis* L. had a more expressed antimicrobial activity for all the microorganism species than *Zingiber officinale* R. A concentracion of 2.0% was already sufficient to inhibit *St. aureus* and *E. coli*. Minimal bactericidal concentration of *Mentha arvensis* L. essential oil to inhibit growth of all four bacteria, *E. coli* (5.6 ± 5.6) ($p > 0.05$), *E. faecium* (50.9 ± 17.7) ($p < 0.05$),

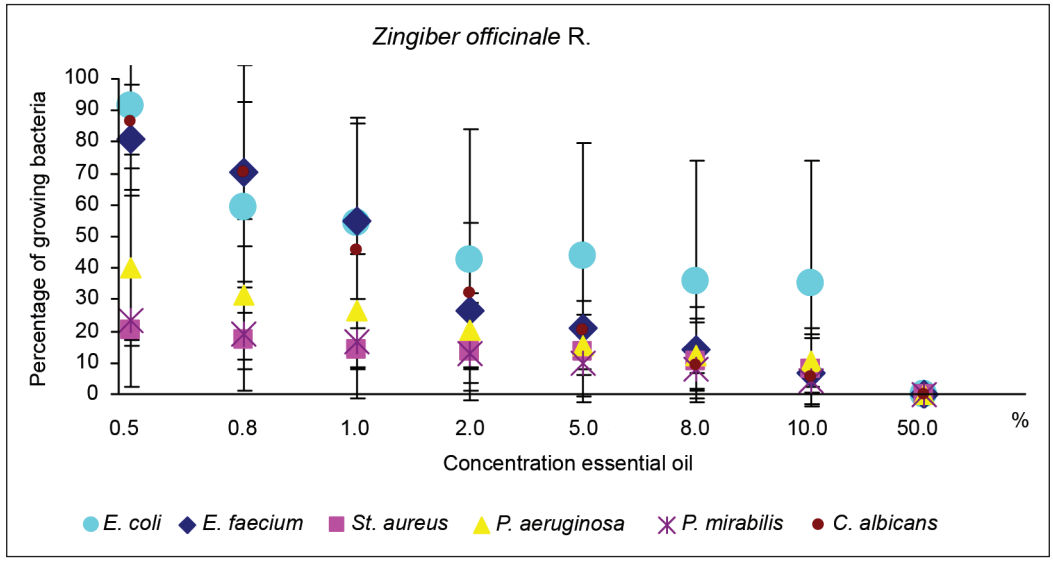

Fig. 1. Effect of *Zingiber officinale* R. on *E. coli, E. faecium, St. aureus, P. aeruginosa, P. mirabilis, C. albicans*

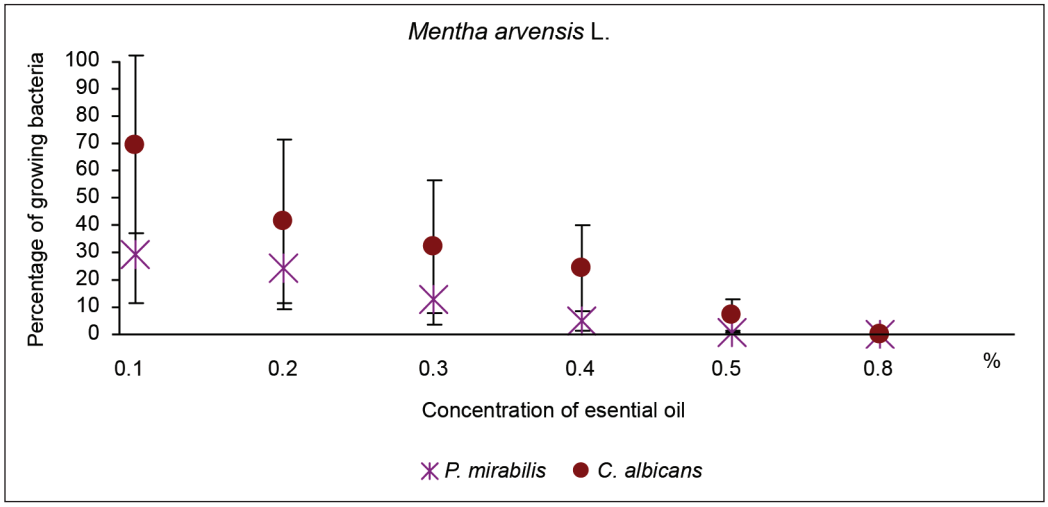

Fig. 2. Effect of *Mentha arvensis* on *P. mirabilis*, *C. albicans*

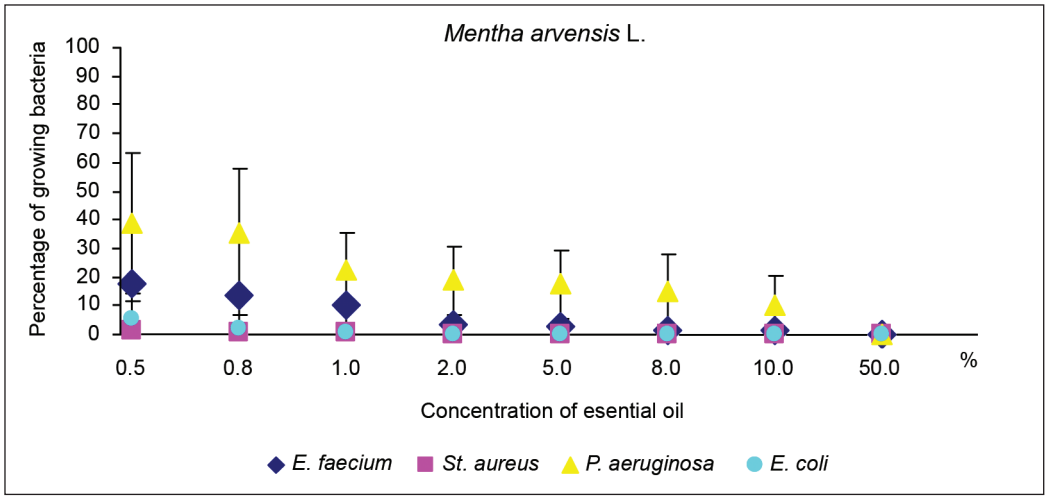

Fig. 3. Effect of *Mentha arvensis* L. on *E. faecium*, *St. aureus*, *P. aeruginosa*, *E. coli*

St. aureus (1.19 ± 1.6) ($p > 0.05$), *P. aeruginosa* (38.9 ± 24.5) ($p > 0.05$) was 50.0%. The menthol content of different mint origin varied between 10.0% to 63.0% and menthone content from 12.0 to 76.0%. The content of some mint origins was found to be even higher than stated in the the European Pharmacopoeia standard (30.0–55.0%) [9]. Menthol is a terpenoid, found in the essential oils of the mint family (*Mentha* spp.). Menthol ($C_{10}H_{20}O$) is solid at room temperature, forming long crystals that have a fatty touch. There exist several isomers of menthol, some with a menthol smell, others without. Normally occurring menthol, with the strongest smell, is (–)-menthol.

In our study, the essential oil *Mentha arvensis* L. having menthol as the major component exhibited most potent activity against *P. mirabilis, C. albicans* and a strong antimicrobial activity against *E. coli* and *St. aureus. P. mirabilis* and *C. albicans* were much more sensitive to the *Mentha arvensis* L. essential oil and only a minimal (0.8%) bactericidal concentration for these two germs was needed. At a concentration of 0.1%, *P. mirabilis* (29.5 ± 18.0) ($p > 0.05$), *C. albicans* (69.6 ± 32.4) ($p < 0.05$) were still growing (Fig. 3).

Mentha arvensis L. essential oil presented 50.0% minimal bactericidal concentration for four bacteria: *Enterococcus faecium* Schleifer and Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Staphylococcus aureus* Rosenbach, *Escherichia coli* Castellani and Chalmers and 0.8% for *Proteus mirabilis* Hauser and yeast *Candida albicans* Berkhout. Menthol from *Mentha arvensis* L. not only affected sensory parameters, but also expressed antibacterial and antifungal activities. *Mentha arvensis* L. have been shown to be active against a variety of microorganisms, including both gram-positive and – negative bacteria, as well as fungi [13, 19, 21]. Such properties are exploited in diverse products, including dental root canal sealers, antiseptics, food preservatives, and feed supplements. The toxic effects on the membrane structure and function have generally been used to explain

the antimicrobial activity of mint oil and menthol, although the exact mechanism of action is not fully understood [21]. Antimicrobial effect of menthol might have resulted, at least partially, from a perturbation of the lipid fraction of microorganism plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials. Furthermore, Schelz et al. [19] demonstrated that menthol displayed an antiplasmid activity. The toxic effects on membrane structure and function have generally been used to explain the antimicrobial activity of menthol, although the exact mechanism of action is not fully understood [21].

CONCLUSIONS

From this study, we can conclude that the essential oils *Mentha arvensis* L. and *Zingiber officinale* R. exhibited the antimicrobial activity to varying degrees against all separately tested strains. The maximum antimicrobial activity was observed against *P. mirabilis* Hauser and yeast *Candida albicans* Berkhout using the *Mentha arvensis* L. essential oil. This study confirms that the *Mentha arvensis* L. oil can be a good source of antibacterial agents for a possible application to reduce the content of microorganisms in the air of animal farms.

Received 24 April 2011
Accepted 31 August 2011

References

1. Aflatuni A. The use of plant origin substances against *Galerucella sagitaria*. Proceedings of the Nordic Association of Agricultural Scientists 22nd Congress "Nordic Agriculture in Global. Perspective", July 1–4 2003, Turku, Finland.
2. Bandara BMR, Fernando IHS, Hewage CM, Karunaratne V, Adikaram NKB, Wijesundara DSA. Antifungal activity of some medicinal plants of Sri Lanka. J of the National Science Council of Sri Lanka 1989; 17: 1–13.
3. Bodenschatz W. Desinfektion–Rechtsvorschriften und Materialien. Behr's Verlag GmbH & Co, Loseblattwerk 1997; 10.
4. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publ Londres, New York, Paris 1995: 405–66.
5. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol 2004; 94: 223–53.
6. Chrubasik S, Pittler MH, Roufogalis BD. Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. Phytomedicine 2005; 12: 684–701.
7. Chrubasika S, Pittlerc MH, Roufogalisb DB. Phytomedicine 2005; 12: 684–701.
8. Davies J. Inactivation of antibiotic and the dissemination of resistance genes. Science 1994; 264: 375–82.
9. European Pharmacopeias. 3rd ed. Strasbourg, France: Council of Europe 1997: 1298–300.
10. Haraguchi H, Kuwata Y, Inada K, Shingu K, Miyahara K, Nagao M, Yagi A. Antifungal activity from *Alpinia galanga* and the competition for incorporation of unsaturated fatty acid in cell growth. Planta Medica 1996; 62: 308–13.
11. He X., Bernart M., Lian WL, Lin L. High-performance liquid chromatography–electrospray mass spectrometric analysis of pungent constituents of ginger J Chromatogr 1998; 327–796.
12. Ibrahim AM, Kainulainen P, Aflatuni A, Tiilikkala K, Holopainen J. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: With special reference to limonene and its suitability for control of insect pests. Agricultural and Food Science in Finland 2001; 10: 243–59.
13. Iscan G, Kirimer N, Kurkcuoglu M, Baser KH, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem 2002; 50: 3943–6.
14. Iwu MM, Anyanwu BN. Phytotherapeutic profile of Nigeria herbs 1: Anti inflammatory and antiarthritic agents. J Ethnopharmacology 1982; 6: 263–74.
15. Jitoe A, Masuda T, Tengah IGP, Suprapta DN, Gara IW, Nakatani N. Antioxidant activity of tropical ginger extracts and analysis of the contained curcuminoids. J Agricultural Food Chemist 1992; 40: 1337–40.
16. Nakazawa T, Ohsawa K. Metabolism of [6]-gingerol in rats. Life Sci 2002; 70: 2165–75.
17. Pope CA, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. J Am Med Assoc 2002; 287(9): 1132–41.
18. Rehder VLG, Duarte MCT, Alves A, Melo LV, Delarmelina C. Antimicrobial activities of extracts and isolated substances of *Mikania laevigata* Schultz Bip and *Mikania glomerata.* Int Symp on the Chem of Nat Prod 2000, PPA 104.
19. Schelz Z, Molnar J, Hohmann J. Antimicrobial and antiplasmid activities of essential oils. J Fitoterapia 2006; 77: 279–85.
20. Seigler DS. Plant Secondary Metabolism. Kluwer Academic Publishers. Boston / Dordrecht / London 1998: 759.
21. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C. Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005; 49: 2474–8.
22. Wink M. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 2003; 64: 3–19.

Rūta Mickienė, Ona Ragažinskienė, Bronius Bakutis

MENTHA ARVENSIS L. IR *ZINGIBER OFFICINALE* R. ETERINIŲ ALIEJŲ ANTIMIKROBINIS AKTYVUMAS

Santrauka

Tyrimo tikslas – *Mentha arvensis* L. ir *Zingiber officinale* R. eterinių aliejų antimikrobinių savybių nustatymas, siekiant įvertinti jų tinkamumą mažinti gyvūnų aplinkos užterštumą mikroorganizmais. *Mentha arvensis* L. ir *Zingiber officinale* R. eteriniai aliejai testuoti naudojant *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer ir Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani ir Chalmers, *Proteus mirabilis* Hauser bakterijų kultūras, taip pat mieles *Candida albicans* Berkhout. Nustatytos minimalios tirtų eterinių aliejų slopinančios koncentracijos siekė nuo 0,1 iki 50,0 %. Eterinio aliejaus *Mentha arvensis* L. minimali slopinanti koncentracija siekė nuo 0,1 iki 0,8 % *Proteus mirabilis* Hauser ir *Candida albicans* Berkhout bakterijų kultūroms. *Zingiber officinale* R. eterinio aliejaus minimali slopinanti koncentracija siekė 50,0 % šioms bakterijų kultūroms: *Staphylococcus aureus* Rosenbach, *Enterococcus faecium* Schleifer ir Kilpper-Bälz, *Pseudomonas aeruginosa* Migula, *Escherichia coli* Castellani ir Chalmers, *Proteus mirabilis* Hauser ir *Candida albicans* Berkhout mielėms.

Raktažodžiai: bakterijos, eteriniai aliejai, antimikrobinis aktyvumas, minimali slopinanti koncentracija