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

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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 metabolites, 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 channels 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-
constituents 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 secretory 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 (Sensent 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 disinfectants [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 9.5 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 Berkhou 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 DISCUSSION

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 Pr. 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, antitumorogenic, antioxidative, hypoglycemic hepatoprotective, diuretic, and hypcholesterolemic 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 concentration 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),
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*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.

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References

MINTHA ARVENsis L. IR ZINGIBER OFFICINALE R.
ETERINIŲ ALIEJŲ ANTIMIKROBINIS AKTYVUMAS

Santrauka

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