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ANTIMICROBIAL EFFECT OF IMMORTELLE ESSENTIAL OIL AND *LACTOBACILLUS RHAMNOSUS* CELL-FREE SUPERNATANT ON *BACILLUS CEREUS*

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INTRODUCTION

The presence of pathogenic and toxigenic microorganisms in food is still a problem of modern food production. On the other hand, research points to the high efficiency of natural antimicrobial agents, such as essential oils (EO) of plants and metabolites of Lactic Acid Bacteria (LAC), such as lactic acid and bacteriocin, against pathogenic microorganisms - food contaminants. In addition, the application of these compounds favorably affects oxidative and sensory properties of food. Thus, much attention is currently being paid to the biopreservation of food with natural antimicrobial compounds. For this reason, the aim of this research was to determine individual and synergistic effect of EO immortelle (*Helichrysum arenarium* L.) and *Lactobacillus rhamnosus* cell-free supernatant on *Bacillus cereus, in vitro*.

MATERIAL AND METHOD

To test antimicrobial activity of cell-free supernatant of BMK, the species *L. rhamnosus* ATCC 7469 was selected, and the test microorganism was *B. cereus* ATCC 11778. The antimicrobial activity of immortelle EO and *L. rhamnosus* cell-free supernatant against *B. cereus* was tested by disc diffusion (determination of zones inhibition) and by microdilution method (determination of minimum inhibitory, MIC, and minimum bactericidal concentration, MBC) individually and in a mixture (in ratio 1:1). After determining the MIC and MBC of oil, *L. rhamnosus* cell-free supernatant, and mixture of oil and *L. rhamnosus* cell-free supernatant, Fractional Inhibitory Concentration Index (FIC_{index}) was determined for the mixture. The chemical composition of immortelle EO was determined by GC-MS analysis.

DISCUSSION

GC-MS analysis revealed that the main component of immortelle EO is α-pinene (20.33%). The results of the disc-diffusion method show that EO immortelle showed the best antimicrobial effects when it was applied in an amount of 20 µL (inhibition zone 21 mm). The MIC for EO immortelle was 14.20 µL/mL and MBC was 28.41 µL/mL. The cell-free supernatant of *L. rhamonosus* showed a weaker antimicrobial effect against *B. cereus*, compared to EO immortelle, with MIC of 113.64 µL/mL and MBC of 227.27 µL/mL. The tested mixture of EO immortelle and *L. rhamnosus* cell-free supernatant (in ratio 1:1) showed a synergistic effect against *B. cereus* with FIC_{index} of 0.56.

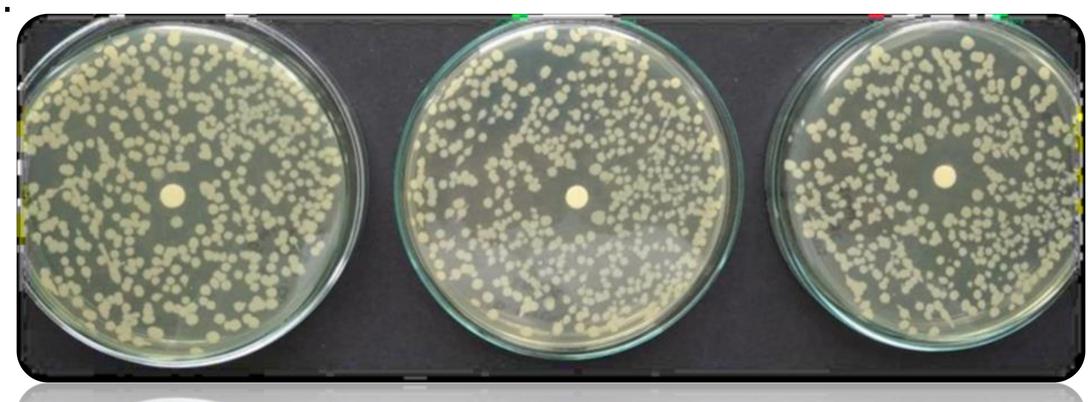


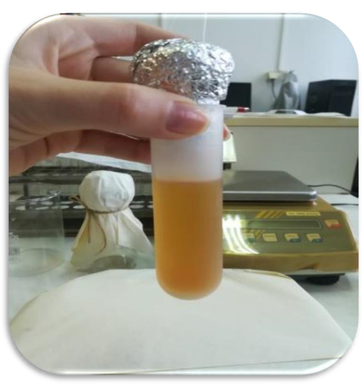
Fig. 1. Zone inhibition by disc-diffusion method for *B. cereus*

Table 1. Chemical composition of immortelle EO

Compaunds	Share (%)
α-pinene	20.33
kamfene	0.55
β-pinene	0.46
p-cimene	0.66
limonene	3.81
1,8-cineol	0.31
γ-terpinene	0.11
linalol	1.07
terpinen-4-ol	0.26
α-terpineol	0.32
nerol	0.72
neril acetate	14.23
kopen	2.42
β-curcumen	3,45
α-farnesen	1.10
caryophyllene	4.24
α-bergamoten	1.05
β-farnesen	0.19
longipinen	0.39
γ-curcumen	3.13
α-curcumen	6.90
β-selinen	10.00
α-selinen	5.30
α-murolen	0.28
β-bisabolen	0.46
γ-cadinene	0.34
δ-cadinene	0.73
α-bisabolen	0.14
caryophylilene oksid	1.40
guaiol	0.12
Total identified (%)	84.46
Not identified (%)	15.54

Table 2. MIC and MBC

Sample	MIC (µL/mL)	MBC (µL/mL)
EO immortelle	14.20	28.41
L. rhamnosus cell-free supernatant	113.64	227.27
Mixture (1:1)	14.20	28.41



CONCLUSION

The obtained research indicates a significant antimicrobial potential of EO immortelle and *L. rhamnosus* cell-free supernatant, as well as their mixture, and represents a contribution to the development of a new concept of alternative methods in protecting food from microbiological contamination.

Key words: *antimicrobial potential, immortelle, L. rhamnonosus cell-free supernatant*

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