Germicidal efficacy of disinfectant based on sodium hypochlorite and essential oils

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Summary

The use of natural substances with antimicrobial activity in combination with conventional antimicrobial agents is a promising strategy in the control of pathogenic bacteria. The aim of this study was to examine the antimicrobial activity of a disinfectant based on sodium hypochlorite and lavender, tea tree, peppermint and rosemary essential oils against Gram-positive (*Bacillus cereus* ATCC 11778, *Staphylococcus epidermidis* ATCC 12228) and Gram-negative (*Pseudomonas aeruginosa* ATCC 10145, *Salmonella* Typhimurium ATCC 14028) bacteria. Further, to evaluate the antimicrobial activity of essential oils and the disinfectant mixed in various combinations in order to detect synergistic or antagonistic effects and discuss these effects on the basis of an improved checkerboard technique. The results showed a synergistic and additive effect for most combinations of the disinfectant and essential oils with fractional inhibitory concentrations index (*FICI)* values of 0.375 and 1, respectively. The effectiveness of the disinfectant in combination with essential oils was also determined using the quantitative suspension test and results indicated the maximum germicidal effect after the exposure time of 5 min. Taking into account increased interest in the use of natural antimicrobial agents in recent years, the application of a combination of disinfectant with essential oils represents a significant strategy in the control of pathogenic bacteria.

Keywords

antibacterial activity; sodium hypochlorite; essential oil

Insufficient hygiene of food contact surfaces and medical devices often leads to contamination with bacteria that cause diseases in humans, which is a growing public health problem and causes large economic losses, despite modern improvements in food industry and medicine. In various bacteria, most commonly *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, multidrug resistance has been developed [1, 2]. In the last decade, a large number of cases of diseases caused by bacteria of the genus *Salmonella* have been documented. Although progress has been made in their control, *Salmonella* spp. continue to be the most common cause of foodborne diseases, as well as the second pathogen responsible for hospitalization of adults [3, 4]. On the other hand, *P. aeruginosa* has been classified as a priority pathogen by the World Health Organization. The excessive use of antibiotics during treatment and the actions of host immune effectors led to widespread adaptive and acquired resistance in *P. aeruginosa* strains, causing morbidity and mortality in patients with cystic fibrosis and immunocompromised individuals [5].

Numerous studies indicated the effectiveness of various disinfectants in controlling the presence and growth of pathogenic bacteria. The effectiveness of disinfectants is determined by factors such as contact time, temperature, concentration, pH, produce-to-water ratio, water hardness and the ability of bacteria to adhere to the contact surfaces [6–9]. Devitalization of most bacteria is relatively easy due to their sensitivity to heat and chemical components. However, many

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microorganisms secrete an extracellular polysaccharide matrix and form biofilms that protect individual cells that are then quite difficult to remove from surfaces. Bacteria can develop resistance to disinfectants over time [10, 11] and their alternating use is recommended as a preventive measure. Due to the growing resistance of bacteria to conventional antimicrobial agents, unconventional antimicrobial agents have become increasingly interesting in recent years. The use of natural substances with antimicrobial activity, such as essential oils, is an environmentally friendly and effective way to control the presence of potentially pathogenic bacteria. Also, these agents are increasingly used in combination with conventional antimicrobial agents. Such strategies are currently being developed and are attracting increasing attention and interest from the scientific community [12–14].

The first aim of this study was to examine the antimicrobial activity of a disinfectant based on sodium hypochlorite (Aqualor H1000, Sigma, Crvenka, Serbia) and essential oils from *Lavandula officinalis* (lavender), *Melaleuca alternifolia* (tea tree), *Mentha piperita* (peppermint) and *Rosmarinus officinalis* (rosemary) against two Gram-positive bacteria (*Bacillus cereus* ATCC 11778 and *Staphylococcus epidermidis* ATCC 12228) and two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 10145 and *Salmonella* Typhimurium ATCC 14028). The second aim was to evaluate the antimicrobial activity of disinfectant and essential oils mixed in different combination in order to detect synergistic or antagonistic effects and discuss these effects on the basis of an improved checkerboard technique. Further, the germicidal effect of the disinfectant based on sodium hypochlorite in combination with essential oils was examined.

MATERIALS AND METHODS

Strains and growth conditions

Antibacterial activity of the essential oils and the disinfectant based on sodium hypochlorite (Aqualor H1000, Sigma, Crvenka, Serbia; pH 7.2, active chlorine 4 g·l^{-1}) was tested against *B. cereus* ATCC 11778, *Staph. epidermidis* ATCC 12228, *P. aeruginosa* ATCC 10145 and *S.* Typhimurium ATCC 14028. All strains were obtained from the Culture Collection of the microbiological laboratory at the Institute of Food Technology (University of Novi Sad, Novi Sad, Serbia). Reference strains of bacteria were preserved in tryptone soya broth (TSB, Oxoid, Basingstoke, United Kingdom) supplemented with 40 % glycerol at –80 °C and revitalized from frozen stocks by cultivation on tryptone soya agar (TSA, Oxoid) plates for 24 h at 37 °C before performing the assays. The disinfectant used is intended for the disinfection of surfaces in contact with food, healthcare settings and public places.

Essential oils

Four essential oils from *L. officinalis* (lavender), *M. alternifolia* (tea tree), *M. piperita* (peppermint) and *R. officinalis* (rosemary) were purchased from local market in Novi Sad, Serbia (the producer not shown). The main compounds commonly found in essential oils, namely, carvacrol, eugenol and thymol were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Antimicrobial activity determination

The minimum inhibitory concentration (*MIC*) was determined using the broth microdilution method according to M27-A3 protocol of the Clinical and Laboratory Standards Institute (CLSI) [15]. Briefly, the tested antimicrobial agents were first dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and incorporated into Mueller Hinton broth (MHB, HiMedia, Mumbai, India). Then, they were two-fold serially diluted to make a concentration range from 0.625 mg·ml^{-1} to 320 mg·ml^{-1} in 96-well microtiter plates. The final concentration of DMSO did not exceed 5 g·l^{-1}. Subsequently, 100 μl of the working inoculum suspension (1 × 10^{6} CFU·ml^{-1}) was added to each well and the inoculated plates were incubated for 24 h at a temperature of 37 °C except for *B. cereus*, which was incubated at a temperature of 30 °C. In all experiments, a positive control (assay medium without the antimicrobial agent and with reference strains) and a negative control (growth medium without reference strains) were included. Assays were carried out in four independent replicates for each tested microorganism. After 24 h of incubation, 20 μl of 0.1 g·l^{-1} resazurin solution (7-hydroxy-3H-phenoxazin-3-one 10-oxide, HiMedia) was added as an indicator of bacterial growth to each well of the microtiter plate and incubated for 24 h to determine a colour change. The lowest concentration at which the blue color of resazurin did not change into pink was considered as the *MIC* value for that individual essential oil and disinfectant.

The minimum bactericidal concentration (*MBC*) was determined by seeding 10 μl from wells where no visible growth was observed in triplicate on Mueller Hinton agar (MHA, HiMedia) plates and incubation for 24 h at 37 °C except for *B. cereus*, which was incubated at 30 °C. The lowest concentration of essential oils and compounds that did not yield any growth on the solid medium after the incubation period was recorded as *MBC*.

Checkerboard assay

The combined effect of the disinfectant and essential oils was evaluated by the microdilution checkerboard method [16–18]. Assays were performed in 96-well microtiter plates on the basis of *MIC* values previously obtained. Combinations of the disinfectant and essential oils were made by adding each of the tested antimicrobial agents to microtiter plate wells, with final concentrations of the disinfectant ranging from 1/8 × *MIC* to 4 × *MIC* and concentrations of essential oils ranging from 1/32 × *MIC* to 1 × *MIC*. After preparing the combinations of antimicrobial agents in microtiter plates, the bacterial suspension (1 × 10^6 CFU·ml^{-1}) was added to each well and inoculated plates were incubated for 24 h at a temperature of 37 °C except for *B. cereus*, which was incubated at 30 °C.

The optical density (*OD*) was measured at 630 nm using a microplate reader Multiskan FC (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Assay was performed at least in triplicate.

Fractional inhibitory concentrations index (*FICI*) values were calculated using the following formulae:

$$FICI = FIC_{\mathrm{A}} + FIC_{\mathrm{B}} \quad (1)$$

$$FIC_{\mathrm{A}} = \frac{MIC_{\mathrm{AX}}}{MIC_{\mathrm{AY}}} \quad (2)$$

$$FIC_{\mathrm{B}} = \frac{MIC_{\mathrm{BX}}}{MIC_{\mathrm{BY}}} \quad (3)$$

where FIC_{A} is fractional inhibitory concentration of the disinfectant, sodium hypochlorite, FIC_{B} is fractional inhibitory concentration of an essential oil, MIC_{AX} is the minimum inhibitory concentration of the disinfectant, sodium hypochlorite, in the presence of the essential oil, MIC_{AY} is the minimum inhibitory concentration of the disinfectant, sodium hypochlorite, alone, MIC_{BX} is the minimum inhibitory concentration of the essential oil in the presence of the disinfectant, sodium hypochlorite, MIC_{BY} is the minimum inhibitory concentration of the essential oil alone.

FICI values were interpreted following the model suggested by Mulyaningsih et al. [17]. The results were interpreted as synergistic effect (S) ($FICI \leq 0.5$), additive effect (Ad) ($0.5 < FICI \leq 1$), indifferent effect (I) ($1 < FICI \leq 4$) or antagonistic effect (A) ($FICI > 4$).

Germicidal effect

The effectiveness of the combination of the disinfectant based on sodium hypochlorite and essential oils from *L. officinalis, M. alternifolia, M. piperita* and *R. officinalis* in the ratio 6 : 1 : 1 : 1 : 1 was determined using the quantitative suspension test according to Aarnisalo et al. [6]. Briefly, 8 ml of the combination of the disinfectant based on sodium hypochlorite with the concentration of 600 g·l^{-1} with essential oils and 1 ml of sterile water were added to test tubes, followed by 1 ml bacterial suspension ragned from 3 × 10^6 CFU·ml^{-1} to 1 × 10^7 CFU·ml^{-1}. The activity was tested at a contact time of 1 min and 5 min at a temperature of 21 °C. Further, 1 ml of the test solution was transferred to tubes containing 8 ml of neutralization solution (30 g·l^{-1} Tween 80, 1 g·l^{-1} L-histidine and 5 g·l^{-1} Na-thiosulfate in TSB medium) and 1 ml of sterile water. After neutralization, a series of dilutions was prepared and 1 ml of the solution was transferred from each test tube onto TSA plates. As a control, pure bacterial suspensions were serially diluted and plated on TSA plates. The plates were incubated for 24 h at 37 °C except for *B. cereus*, which was incubated at 30 °C. The germicidal effect of the disinfectant alone was also examined at the concentration of the disinfectant of 1 000 g·l^{-1}.

The germicidal effect (*GE*) of the combination of the disinfectant based on sodium hypochlorite and essential oils was calculated according to the formula:

$$GE = \log N_{\mathrm{C}} - \log N_{\mathrm{D}} \quad (4)$$

where N_{C} are the counts of microorganisms in the control and N_{D} are the counts of microorganisms in the suspension after the effect of the combination of the disinfectant and essential oils, and disinfectant alone.

Efficacy, the effect of the disinfectant based on sodium hypochlorite in combination with essential oils or alone on the growth of bacteria was expressed in percent.

RESULTS AND DISCUSSION

The continued increase in antimicrobial resistance to chemically synthesized agents that have harmful impacts on the human health and environment have encouraged research for novel sources of antimicrobial agents. Essential oils as natural antimicrobial agents have long been recognized to exhibit significant and promising activity against pathogenic microorganisms [14, 19, 20].

The obtained *MIC* and *MBC* values (Tab. 1)

Tab. 1. Antimicrobial activity of the agents.

Antimicrobial agent	Gram-positive bacteria				Gram-negative bacteria			
	Bacillus cereus		*Staphylococcus epidermidis*		*Pseudomonas aeruginosa*		*Salmonella* Typhimurium	
	MIC [mg·ml^{-1}]	*MBC* [mg·ml^{-1}]	*MIC* [mg·ml^{-1}]	*MBC* [mg·ml^{-1}]	*MIC* [mg·ml^{-1}]	*MBC* [mg·ml^{-1}]	*MIC* [mg·ml^{-1}]	*MBC* [mg·ml^{-1}]
Disinfectant	0.50	0.25	0.25	0.25	0.50	0.50	0.25	0.25
Lavandula officinalis	20.00	20.00	80.00	40.00	40.00	40.00	80.00	80.00
Melaleuca alternifolia	40.00	40.00	5.00	5.00	20.00	20.00	20.00	20.00
Menthae piperita	10.00	10.00	40.00	40.00	40.00	40.00	10.00	10.00
Rosmarinus officinalis	20.00	20.00	80.00	80.00	80.00	80.00	160.00	160.00
Carvacrol	0.16	0.16	0.16	0.16	0.63	0.63	0.16	0.31
Eugenol	0.31	0.31	0.16	0.31	2.50	2.50	0.63	0.63
Thymol	0.16	0.31	0.16	0.31	0.63	0.63	0.08	0.16

Values represent the mean value obtained in four independent replicates.
MIC – minimum inhibitory concentration, *MBC* – minimum bactericidal concentration.

indicated the fact that the microbial strains were highly sensitive to the tested antimicrobial agents, as well as that they were significantly more sensitive to the applied disinfectant compared to the essential oils. The results showed that the essential oils of *M. alternifolia* and *M. piperita* exhibited the strongest antimicrobial effect with *MIC* and *MBC* values in the range of 5 mg·ml^{-1} to 40 mg·ml^{-1}, while the essential oil of *R. officinalis* had the weakest effect with *MIC* and *MBC* values of 20 mg·ml^{-1} to 160 mg·ml^{-1}.

Numerous studies were conducted to determine the antibacterial activity of essential oils and concluded that Gram-positive bacteria are more sensitive to the effects of essential oils than Gram-negative bacteria [21–24]. In our study, Gram-positive bacterium *B. cereus* was the most sensitive microorganism tested. The lower sensitivity of Gram-negative bacteria can be explained by the difference in the structure of the cell wall, since they possess an outer membrane surrounding the cell wall, which limits the diffusion of hydrophobic compounds through its lipopolysaccharide covering [25, 26]. Since essential oils consist of a large number of chemical components, components of essential oils may act independently or synergistically on the structures of the bacterial cell. Major compounds of essential oils with antimicrobial activity include terpenoids, such as the phenols thymol, carvacrol and linalool, terpenes such as pinene, phenylpropenes, such as eugenol and cinnamaldehyde [13]. Phenolic compounds containing a hydroxyl group, such as carvacrol, thymol or eugenol, are responsible for damaging the cell membrane and exhibit a broad spectrum of antimicrobial activity against food-borne bacteria [19, 27]. It can be seen that among the compounds, thymol and carvacrol exhibited strongest antimicrobial activity with *MIC* values ranging from 0.078 mg·ml^{-1} to 0.63 mg·ml^{-1}, while eugenol showed weakest activity with *MIC* values from 0.16 mg·ml^{-1} to 2.50 mg·ml^{-1}. The Gram-negative bacterium *P. aeruginosa* showed significant resistance to the tested compounds, in particular to eugenol with *MIC* value of 2.50 mg·ml^{-1}.

In order to reduce the minimum effective dose of conventional antimicrobial agents and thereby reduce potential side effects and toxicity, as well as prevent the spread of antimicrobial resistance, the effect of the combination of disinfectant based on sodium hypochlorite and essential oils *L. officinalis, M. alternifolia, M. piperita* and *R. officinalis* was examined. The *FIC* and *FICI* values are shown in Tab. 2. The results showed that for the most combinations of the disinfectant and essential oils, a synergistic or additive effect was detected in all tested bacteria. Gram-positive bacteria *B. cereus* and *Staph. epidermidis* showed higher frequency of synergistic and additive effects than Gram-negative bacteria. Combinations of the disinfectant at a concentration of 1/4 × *MIC* and lavender essential oil at concentrations of 1/4 × *MIC* to 1/8 × *MIC* indicated the existence of a synergistic effect in Gram-positive bacteria, while an additive effect was found in Gram-negative bacteria (*FIC* = 1). *FICI* values of the disinfectant and tea tree essential oil for *B. cereus, Staph. epidermidis* and *S.* Typhimurium (*FICI* = 0.38) were lower than 0.50, which is the value indicating a synergistic interaction.

A number of authors mentioned the antimicrobial activity and studied the mechanism of action of essential oils [14, 19, 27]. There are some generally accepted mechanisms of the antimicrobial interaction for synergy such as sequential inhibition of a common biochemical pathway, inhibition of protective enzymes, combinations of cell wall active agents and use of cell wall active agents to enhance the uptake of other antimicrobials [26]. The combination of the disinfectant with an essential oil consisting of various biochemical components may positively influence and increase the antimicrobial efficacy. Our results indicated an additive effect in Gram-positive bacteria when the disinfectant was combined with peppermint and rosemary essential oils, with *FICI* values ranging from 0.75 to 1.00 (Tab. 2). Additive interactions were also observed with *S.* Typhimurium when the disinfectant was combined with essential oils of *L. officinalis* and *R. officinalis*. Synergistic and additive interactions of antimicrobial drugs may be the basis for strategies of controlling resistance evolution, since the administration of multiple drugs may disrupt several bacterial functions and thus minimize the selection of resistant strains [28]. In addition, an indifferent effect was detected in *P. aeruginosa* for the combination of the disinfectant with peppermint and rosemary essential oils. No antagonism was observed for any of the combinations evaluated. These results are in accord with the fact that combined antimicrobial agents are often used to provide a broader antibacterial spectrum and to minimize toxicity as well as emergence of resistant bacteria [29].

The results on the germicidal effect of combinations of the disinfectant and essential oils are shown in Tab. 3. The germicidal effect of the disinfectant alone was also examined and the results are shown in Tab. 4. The maximum germicidal effect of a combination of the disinfectant and essential oils was achieved after exposure for 5 min for all tested microorganisms. The weakest effect was recorded against *B. cereus* and *S.* Typhimurium at the exposure time of 1 min, while the stronger effects were achieved against *Staph. epidermidis* and *P. aeruginosa*.

Numerous studies showed high effectiveness of the disinfectant based on sodium hypochlorite against microorganisms in the food industry despite the increasing availability of other

Tab. 2. Interaction effects of combinations of agents.

Bacteria	*MIC* [mg·ml^{-1}]		*FIC*		*FICI*	Interaction effect
	Disinfectant	Essential oil	Disinfectant	Essential oil		
Combination of disinfectant with lavender essential oil						
Bacillus cereus	0.13	2.50	0.25	0.13	0.38	S
Staphylococcus epidermidis	0.03	20.00	0.13	0.25	0.38	S
Pseudomonas aeruginosa	0.25	20.00	0.50	0.50	1.00	Ad
Salmonella Typhimurium	0.13	40.00	0.50	0.50	1.00	Ad
Combination of disinfectant with tea tree essential oil						
Bacillus cereus	0.13	5.00	0.25	0.13	0.38	S
Staphylococcus epidermidis	0.03	1.25	0.13	0.25	0.38	S
Pseudomonas aeruginosa	0.25	10.00	0.50	0.50	1.00	Ad
Salmonella Typhimurium	0.03	5.00	0.13	0.25	0.38	S
Combination of disinfectant with peppermint essential oil						
Bacillus cereus	0.25	5.00	0.50	0.50	1.00	Ad
Staphylococcus epidermidis	0.13	20.00	0.50	0.50	1.00	Ad
Pseudomonas aeruginosa	0.25	40.00	0.50	1.00	1.50	I
Salmonella Typhimurium	0.13	2.50	0.50	0.25	0.75	Ad
Combination of disinfectant with rosemary essential oil						
Bacillus cereus	0.25	10.00	0.50	0.50	1.00	Ad
Staphylococcus epidermidis	0.13	20.00	0.50	0.25	0.75	Ad
Pseudomonas aeruginosa	0.25	80.00	0.50	1.00	1.50	I
Salmonella Typhimurium	0.13	40.00	0.50	0.25	0.75	Ad

MIC – minimum inhibitory concentration, *FIC* – fractional inhibitory concentration, *FICI* – fractional inhibitory concentrations index
Interaction effect: S – synergistic, Ad – additive, I – indifferent.

Tab. 3. Germicidal effect of combinations of the disinfectant based on sodium hypochlorite and essential oils.

Bacteria	*N*c [log CFU·ml^{-1}]	Exposure time 1 min		Exposure time 5 min	
		GE [log CFU·ml^{-1}]	Efficacy [%]	*GE* [log CFU·ml^{-1}]	Efficacy [%]
Bacillus cereus	6.78	5.12	75.5	6.78	100.0
Staphylococcus epidermidis	7.15	5.95	83.2	7.15	100.0
Pseudomonas aeruginosa	7.01	5.91	84.3	7.01	100.0
Salmonella Typhimurium	6.53	5.03	77.0	6.53	100.0

In mixture with essential oils, the concentration of the disinfectant was 600 g·l^{-1}.
N_C – initial number of bacteria (expressed per millilitre of suspension), *GE* – germicidal effect.

Tab. 4. Germicidal effect of the disinfectant based on sodium hypochlorite.

Bacteria	*N*c [log CFU·ml^{-1}]	Exposure time 1 min		Exposure time 5 min	
		GE [log CFU·ml^{-1}]	Efficacy [%]	*GE* [log CFU·ml^{-1}]	Efficacy [%]
Bacillus cereus	6.78	4.80	70.8	6.78	100.0
Staphylococcus epidermidis	7.15	5.87	82.1	7.15	100.0
Pseudomonas aeruginosa	7.01	5.96	85.0	7.01	100.0
Salmonella Typhimurium	6.53	4.98	76.3	6.53	100.0

Concentration of the disinfectant was 1 000 g·l^{-1}.
N_C – initial number of bacteria (expressed per millilitre of suspension), *GE* – germicidal effect.

disinfectants due to its easy application, excellent sterilization power and cost-effectiveness [30–32]. It was observed in our study that the bactericidal effect of the combination of this type of disinfectant and essential oils was higher compared to the effect of the disinfectant alone at the shortest exposure time of 1 min. The results showed a significantly stronger effect of the combination of the disinfectant and essential oils against *B. cereus* compared the disinfectant alone. An exposure time of 5 min was required to achieve complete germicidal effect against all tested bacteria in our experimental conditions.

CONCLUSIONS

The results obtained in this study demonstrated that the disinfectant based on sodium hypochlorite and essential oils from *L. officinalis, M. alternifolia, M. piperita* and *R. officinalis* exhibited significant antibacterial activity. Combinations of the disinfectant and essential oils showed synergistic or additive effect in most cases. An exposure time of 5 min was required to achieve full germicidal effects against all tested strains. The study also revealed that combinations of the disinfectant and selected essential oils can efficiently inhibit the growth of the tested bacteria at lower concentrations than required for the individual essential oils and the disinfectant. Therefore, they could be further developed for practical use in the control of pathogenic bacteria in food production, in clinical and public settings.

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REFERENCES

1. Tropea, A.: Microbial contamination and public health: An overview. International Journal of Environmental Research and Public Health, *19*, 2022, article 7441. DOI: 10.3390/ijerph19127441.
2. Tomičić, Z. – Čabarkapa, I. – Čolović, R. – Đuragić, O. – Tomičić, R.: Salmonella in the feed industry: problems and potential solutions. Journal of Agronomy, Technology and Engineering Management, *2*, 2019, pp. 130–137. ISSN: 2620-1755.
3. Popa, G. L. – Popa, M. I.: *Salmonella* spp. infection – a continuous threat worldwide. Germs, *11*, 2021, pp. 88–96. DOI: 10.18683/germs.2021.1244.
4. Williams, S. M. – Ebel, D. E. – Golden, J. N. – Schlosser, D. W.: Temporal patterns in the occurrence

of *Salmonella* in raw meat and poultry products and their relationship to human illnesses in the United States. Food Control, *35*, 2014, pp. 267–273. DOI: 10.1016/j.foodcont.2013.07.016.

5. Langendonk, R. F. – Neill, D. R. – Fothergill, J. L.: The building blocks of antimicrobial resistance in Pseudomonas aeruginosa: Implications for current resistance-breaking therapies. Frontiers in Cellular and Infection Microbiology, *11*, 2021, article 665759. DOI: 10.3389/fcimb.2021.665759.
6. Aarnisalo, K. – Salo, S. – Miettinen, H. – Suihko, M. L. – Wirtanen, G. – Autio, T. – Lunden, J. – Korkeala, H. – Sjoberg, A. M.: Bactericidal efficiencies of commercial disinfectants against *Listeria monocytogenes* on surfaces. Journal of Food Safety, *20*, 2000, pp. 237–250. DOI: 10.1111/j.1745-4565.2000.tb00302.x.
7. Banach, J. L. – Sampers, I. –Van Haute, S. – Van der Fels-Klerx, H. J.: Effect of disinfectants on preventing the cross-contamination of pathogens in fresh produce washing water. Intenational Journal of Environmenat Research and Public Health, *12*, 2021, pp. 8658–8677. DOI: 10.3390/ijerph120808658.
8. Song, X. – Vossebein, L. – Zille, A.: Efficacy of disinfectant-impregnated wipes used for surface disinfection in hospitals: a review. Antimicrobial Resistance and Infection Control, 8, 2019, article 139. DOI: 10.1186/s13756-019-0595-2.
9. Sharma, M. – Beuchat, L. R.: Sensitivity of *Escherichia coli* O157:H7 to commercially available alkaline cleaners and subsequent resistance to heat and sanitizers. Applied and Environmental Microbiology, *70*, 2004, pp. 1795–1803. DOI: 10.1128/AEM.70.3.1795-1803.2004.
10. Rozman, U. – Pušnik, M. – Kmetec, S. – Duh, D. – Šostar Turk, S.: Reduced susceptibility and increased resistance of bacteria against disinfectants: A systematic review. Microorganisms, *9*, 2021, article 2550. DOI: 10.3390/microorganisms9122550.
11. Maillard, J. Y.: Resistance of bacteria to biocides. Microbilogy spectrum, *6*, 2018, pp. 1–17. DOI: 10.1128/microbiolspec.ARBA-0006-2017.
12. Galgano, M. – Capozza, P. – Pellegrini, F. – Cordisco, M. – Sposato, A. – Sblano, S. – Camero, M. – Lanave, G. – Fracchiolla, G. – Corrente, M. – Cirone, F. – Trotta, A. – Tempesta, M. – Buonavoglia, D. – Pratelli, A.: Antimicrobial activity of essential oils evaluated in vitro against *Escherichia coli* and *Staphylococcus aureus*. Antibiotics (Basel), *11*, 2022, article 979. DOI: 10.3390/antibiotics11070979.
13. Santos, M. I. S. – Marques, C. – Mota, J. – Pedroso, L. – Lima, A.: Applications of essential oils as antibacterial agents in minimally processed fruits and vegetables – A review. Microorganisms, *10*, 2022, article 760. DOI: 10.3390/microorganisms10040760.
14. Tomičić, Z. – Tomičić, R. – Smole Možina, S. – Bucar, F. – Turek, I. – Raspor, P.: Antifungal and anti-adhesion activity of plant extracts and essential oils against *Candida* spp. and *Pichia* spp. Journal of Food and Nutrition Research, *61*, 2022, pp. 61–68. ISSN: 1336-8672. <https://www.vup.sk/download.php?bulID=2137>
15. M27-A3. Reference method for broth dilution antifungal susceptibility testing of yeasts. 3rd ed. Wayne : Clinical and Laboratory Standards Institute, 2008.
16. M7-A6. Methods for dilution susceptibility tests for bacteria that gow aerobically. 6th ed. Wayne : Clinical and Laboratory Standards Institute, 2003.
17. Mulyaningsih, S. – Sporer, F. – Zimmermann, S. – Reichling, J. – Wink, M.: Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. Phytomedicine, *17*, 2010, pp. 1061–1066. DOI: 10.1016/j.phymed.2010.06.018.
18. Tome, M. – Zupan, J. – Tomičić, Z. – Matos, T. – Raspor, P.: Synergistic and antagonistic effects of immunomodulatory drugs on the action of antifungals against *Candida glabrata* and *Saccharomyces cerevisiae*. PeerJ, *6*, 2018, article e4999. DOI: 10.7717/peerj.4999.
19. Nazzaro, F. – Fratianni, F. – De Martino, L. – Coppola, R. – De Feo, V.: Effect of essential oils on pathogenic bacteria. Pharmaceuticals (Basel). *6*, 2013, pp. 1451–1474. DOI: 10.3390/ph6121451.
20. Tomičić, Z. – Tomičić, R. – Kocić Tanackov, S. – Raspor P.: Essential oils as antimicrobial and anti-adhesion agents against bacteria *Salmonella* Typhimurium and *Staphylococcus aureus*, and yeast *Candida albicans* and *Saccharomyces cerevisiae*. Food and Feed Research, *49*, 2022, pp. 107–115. DOI: 10.5937/ffr0-37683.
21. Patterson, J. E – McElmeel, L. – Wiederhold, N. P.: In vitro activity of essential oils against Gram-positive and Gram-negative clinical isolates, including Carbapenem-resistant *Enterobacteriaceae*. Open Forum Infectious Diseases, *6*, 2019, article ofz502. DOI: 10.1093/ofid/ofz502.
22. Cho, T. J. – Park, S. M. – Yu, H. – Seo, G. H. – Kim, H. W. – Kim, S. A. – Rhee, M. S.: Recent advances in the application of antibacterial complexes using essential oils. Molecules, *25*, 2020, article 1752. DOI: 10.3390/molecules25071752.
23. Man, A. – Santacroce, L. – Jacob, R. – Mare, A. – Man, L.: Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. Pathogens (Basel), *8*, 2019, article 15. DOI: 10.3390/pathogens8010015.
24. Marino, M. – Bersani C. – Comi, G.: Impedance measurement to study antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. International Journal of Food Microbiology, *67*, 2001, pp. 187–195. DOI: 10.1016/s0168-1605(01)00447-0.
25. Burt, S.: Essential oils: their antibacterial properties and potential applications in foods – a review. International Journal of Food Microbiology, *94*, 2004, pp. 223–253. DOI: 10.1016/j.ijfoodmicro.2004.03.022.
26. Lv, F. – Liang, H. – Yuan, O. – Li C.: In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Research

International, *44*, 2011, pp. 3057–3064. DOI: 10.1016/j.foodres.2011.07.030.

27. Miklasińska-Majdanik, M. – Kępa, M. – Wojtyczka, R. D. – Idzik, D. – Wąsik, T. J.: Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. International Journal of Environmental Research and Public Health, *15*, 2018, article 2321. DOI: 10.3390/ijerph15102321.
28. Scandorieiro, S. – de Camargo, L. C. – Lancheros, C. A. C. – Yamada-Ogatta, S. F. – Nakamura, C. V. – de Oliveira, A. G. – Andrade, C. G. T. J. – Duran, N. – Nakazato, G. – Kobayashi, R. K. T.: Synergistic and additive effect of oregano essential oil and biological silver nanoparticles against multidrug-resistant bacterial strains. Frontiers in Microbiology, *7*, 2016, article 760. DOI: 10.3389/fmicb.2016.00760.
29. Wei, C. F. – Shien, J. H. – Chang, S. K. – Chou, C. C.: Florfenicol as a modulator enhancing antimicrobial activity: example using combination with thiamphenicol against *Pasteurella multocida*. Frontiers in Microbiology, *7*, 2016, article 389. DOI: 10.3389/fmicb.2016.00389.
30. Fukuzaki, S.: Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Science, 11, 2006, pp. 147–157. DOI: 10.4265/bio.11.147.
31. Byun, K. H. – Han, S. H. – Yoon, J. W. – Park, S. H. – Ha, S. D.: Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on *Salmonella* Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. Food Control, *123*, 2021, article 107838. DOI: 10.1016/j.foodcont.2020.107838.
32. Ríos-Castillo, R. B. – Fontecha Umaña, F. – Rodríguez-Jerez, J. J.: Long-term antibacterial efficacy of disinfectants based on benzalkonium chloride and sodium hypochlorite tested on surfaces against resistant Gram-positive bacteria. Food Control, *93*, 2018, pp. 219–225. DOI: 10.1016/j.foodcont.2018.06.008.
33. Peta, M. E. – Lindsay, D. – Brözel, V. S. – Von Holy, A.: Susceptibility of food spoilage *Bacillus* species to chlorine dioxide and other sanitizers: research letter. South African Journal of Science, *99*, 2003, pp. 375–380. ISSN: 0038-2353.

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