Activity of *Origanum vulgare* Essential Oil in Liquid and Vapor Phase against Pathogenic Microorganisms

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Abstract
The analysis was aimed to evaluate the antimicrobial activity of the essential oil *Origanum vulgare* against various gram-positive and gram-negative bacteria and yeast. To test its suitability in the vapor phase for the elimination of biofilm-forming bacteria on fruits and vegetables. To evaluate its potential for use in the storage of vegetables and fruits. The tested essential oil *Origanum vulgare* showed strong antimicrobial activity against various types of microorganisms. Due to the high antimicrobial activity against diverse group of microorganisms, it could also be a suitable option for the post-harvest treatment of crops in the future. In evaluating the inhibitory activity in the vapor phase, an effect on the biofilm-forming bacteria *S. maltophilia* and *B. subtilis* was demonstrated. Against *S. maltophilia*, the highest efficacy was found at a concentration of 500 µL/L by 91.27% inhibition of bacteria on potatoes. Against *B. subtilis*, the most effective concentration was 250 µL/L by 63.24 % of bacterial inhibition on carrots. Relatively stable growth inhibition was found in the apple sample at all concentrations tested. Due to the vaporous application, it would be possible to use lower concentrations of essential oils to achieve the antimicrobial activity. The impact on the sensory properties of food would be reduced.

Keywords: *Origanum vulgare*, biofilm, vapor phase, *ex situ*

1. Introduction

Origanum vulgare is a widespread species native to the Mediterranean area usually called “oregano”. It is most often used as a spice in the culinary sphere. Oregano has very good effects in folk medicine for various digestive problems, suffocating coughs as well as skin problems [1]. It is one of the most frequently studied essential oils due to its antioxidant and antimicrobial activity [2].

Essential oils from plant extracts are natural antimicrobials. Essential oils from herbs and spices have been shown to have strong antibacterial effects against foodborne pathogens [3].

The high content of water and nutrients in fruits and vegetables makes these products vulnerable to spoilage during storage. For this reason, they are an ideal substrate for pathogenic microorganisms. The use of natural compounds such as volatile organic compounds and essential oils is a suitable alternative to synthetic sprays. Essential oils and organic compounds are natural secondary metabolites produced by plants and are generally considered safe for fruit and vegetable storage applications [4].

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Ability to form a biofilm is one of the important factors that enable spreading of bacterial contamination. Biofilm is resistant to antibacterial substances, which poses a great risk to the safety of stored vegetables. Therefore, it is necessary to seek an effective antibacterial agent to reduce the risk of biofilm contamination [5].

The analysis was aimed to evaluate the antimicrobial activity of the essential oil *Origanum vulgare* against various gram-positive and gram-negative bacteria. To test its suitability in the vapor phase for the elimination of biofilm-forming bacteria on fruits and vegetables. To evaluate its potential for use in the storage of vegetables and fruits.

2. Materials and methods

Origanum vulgare essential oil was purchased from Hanus, s.r.o. (Nitra, Slovakia). It was prepared by steam distillation of fresh stalk. The manufacturer states that the main components of the essential oil are carvacrol 70.8 %, thymol 3.9 %, p-cymene 5.6 %, γ-terpinene 4.8 %, β-caryophyllene 2.16 %, α-terpinene 1.06 %, β-myrcene, and α-pinene. It was stored in the dark at 4 °C throughout the analyzes.

Microorganisms (*Bacillus subtilis* CCM 2772, *Pseudomonas aeruginosa* CCM 1959, *Staphylococcus aureus* subsp. *aureus* CCM 2461, *Yersinia enterocolitica* CCM 5671, *Enterococcus faecalis* CCM 4224, *Salmonella enteritidis* subsp. *enteritidis* CCM 4420, *Candida krusei* CCM 8271, *Candida albicans* CCM 8186, *Candida tropicalis* CCM 8223, *Candida glabrata* CCM 8270) for analyzes were obtained from the Czech collection of microorganisms. The bacteria forming the biofilm *Bacillus subtilis* and *Stenotrophomonas maltophilia* were obtained from the dairy industry. They were identified by 16S rRNA sequencing and MALDI-TOF MS Biotyper.

The antimicrobial activity of *O. vulgare* essential oil was determined using the disk diffusion method. The microorganisms were recultivated on suitable nutrient media at optimal temperatures and growth conditions for the individual species of microorganisms. The inoculum was prepared to an optical density of 0.5 McF. One hundred μL of inoculum was inoculated into a Petri dish with Mueller Hinton agar (MHA). Six mm blank discs were placed on the plates. Ten μL of *O. vulgare* essential oil was applied to each disc. The prepared PDs were incubated with bacteria aerobically at 37 °C for 24 h and yeasts at 25 °C for 24 h. After incubation, the diameters of the inhibition zones were measured. Criteria for inhibitory activity were determined, with an inhibition zone diameter above 5 mm being considered as weak inhibitory activity, above 10 mm as moderate inhibition, and above 15 mm as very strong inhibition. Each test was repeated 3 times.

Ex situ antimicrobial analysis of the vapor phase of *O. vulgare* essential oil was tested on *S. maltophilia* and *B. subtilis* biofilm-forming bacteria. Carrots, potatoes, and apples were used as model foods. The samples were washed with distilled water and cut into slices (5 mm). The inoculum was prepared by recultivating of the microorganisms on Tryptone soya agar (TSA) at 37 °C for 24 h. MHA was poured into 60 mm diameter Petri dishes (PD). MHA was also poured into the lid of the PD. Individual slices of model food were placed on solidified nutrient medium in PD. Three inoculum stabs were applied into the slice. *O. vulgare* essential oil was diluted in ethyl acetate to a final volume of 500, 250, 125, and 62.5 μL/L. Using a micropipette, the individual concentrations were applied to a 55 mm round sterile filter paper. The filter paper with the appropriate concentration of essential oil was placed in the PD lid. To evaporate the remaining ethyl acetate, the filter paper was dried for 1 minute. PDs were hermetically sealed with a lid containing solidified agar. Petri dishes were incubated at 37 °C for 7 days.

Ex situ bacterial growth was determined using stereological methods. In this concept, the volume density (Vv) of bacterial colonies was firstly estimated using ImageJ software counting the points of the stereological grid hitting the colonies (P) and those (p) falling to the reference space (growth substrate used). The volume density of bacterial colonies was consequently calculated as follows: $Vv (\%) = P / p$. The antibacterial activity of the essential oil was expressed as percentage of bacterial growth inhibition (BGI) which was calculated using the formula: $BGI = [(C – T) / C] * 100$, where C is bacterial growth (expressed as Vv) in the control group and T is the growth in the treatment one (with addition of the essential oil).

3. Results and discussion

The results of the disk diffusion method showed a strong antimicrobial effect. Inhibition of

microorganism growth was observed in all species tested. Weak inhibitory activity was observed in *Y. enterocolitica*. Moderate inhibitory activity was detected in three *Candida* species. The other species tested showed very strong inhibition of the growth of microorganisms (
Table 1).

Table 1. Antimicrobial activity of *O. vulgare* essential oil

Microorganism	Zone inhibition (mm)	Effectiveness of inhibition
Bacillus subtilis	20.33 ± 1.53	***
Pseudomonas aeruginosa	28.67 ± 1.53	***
Staphylococcus aureus subsp. Aureus	18.33 ± 1.53	***
Yersinia enterocolitica	5.33 ± 0.58	*
Enterococcus faecalis	20,00 ± 1,00	***
Salmonella enteritidis subsp. enteritidis	18. 00 ± 2.00	***
Candida krusei	18.67 ±1.53	***
Candida albicans	13.00 ± 2.00	**
Candida tropicalis	14.00 ± 1.00	**
Candida glabrata	13.67 ± 1.53	**
Biofilm *Bacillus subtilis*	30.00 ± 1.00	***
Biofilm *Stenotrophomonas maltophilia*	20.67 ± 0.58	***

* Weak inhibition; ** Moderate inhibition; *** Very strong inhibition

Oniga et al. [6] found *O. vulgare* antimicrobial activity by disk diffusion for *S. enteritidis* 18.00 ± 0.00 mm and for *S. aureus* 16.00 ± 1.00 mm. Benedec et al. [7] determined zones of inhibition for *S. aureus* 12 ± 0.50 mm and for *C. albicans* 12 ± 1.00. Teles et al. [8] measured zones of inhibition for *S. aureus* 15.67 ± 0.577 mm and for *P. aeruginosa* 12 ± 0.00 mm. Enayati Ward et al. [9] found inhibition zones for *S. aureus* 16.8 ± 0.60 mm and for *C. albicans* 10.8 ± 0.3 mm. Bhat et al. [10] evaluated the inhibitory effect of *O. vulgare* against the genus *Candida* and measured the zones of inhibition for *C. tropicalis* 14.00 mm and for *C. albicans* 10.00 mm. Sarikurkcu et al. [11] found inhibition zones for *E. faecalis* 22.33 ± 0.57 mm, *P. aeruginosa* 16.33 ± 0.57 mm, *B. cereus* 20.33 ± 0.57 mm, *S. aureus* 26.67 ± 1.15 mm and *C. albicans* 24.67 ± 0.57 mm. Şahin et al. [12] measured inhibition zone for *B. subtilis* 29 mm. The findings of these authors are consistent with our findings and all of them confirmed the strong antimicrobial activity of *O. vulgare* essential oil. *O. vulgare* essential oil could find an application as a natural antimicrobial substance in the food industry. Due to its high antimicrobial activity against diverse group of microorganisms, it could also be a suitable supplement for the post-harvest treatment of crops in the future.

The results of the *Ex situ* vapor phase analysis on the carrot sample (Table 2) showed inhibition at all tested concentrations. The most significant inhibition against *B. subtilis* was at concentration 250 µL/L and *S. maltophilia* at 500 µL/L. In the potato sample (Table 3), inhibition of *B. subtilis* occurred at all tested concentrations. The highest inhibition rate of essential oil was 17.44 % which was recorded at concentration of 500 µL/L. Inhibition of oregano essential oil against *S. maltophilia* was showed at concentration 500 µL/L with inhibition rate 91.27 %. In the apple sample (

Table 4), both biofilm-forming microorganisms were inhibited at all concentrations. *B. subtilis* was the most effectively inhibited at 125 µL/L with inhibition rate 47.29 %. *S. maltophilia* was inhibited by 47.17 % at concentration 500 µL/L.
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Table 2. *Ex situ* antimicrobial activity of *O. vulgare* vapor phase on carrots

	BGI [%]			
Microorganisms	**62.5 µL/L**	**125 µL/L**	**250 µL/L**	**500 µL/L**
B. subtilis	13.10	27.01	63.24	18.29
S. maltophilia	15.31	1.17	13.85	57.47

Table 3. *Ex situ* antimicrobial activity of *O. vulgare* vapor phase on potatoes

	BGI [%]			
Microorganisms	**62.5 µL/L**	**125 µL/L**	**250 µL/L**	**500 µL/L**
B. subtilis	5.52	10.58	13.53	17.44
S. maltophilia	-2.41	1.15	17.90	91.27

Table 4. *Ex situ* antimicrobial activity of *O. vulgare* vapor phase on apples

	BGI [%]			
Microorganisms	**62.5 µL/L**	**125 µL/L**	**250 µL/L**	**500 µL/L**
B. subtilis	29.04	47.29	32.18	19.79
S. maltophilia	41.30	45.23	44.77	47.13

Nedorostova et al. [13] concluded that *O. vulgare* essential oil has shown high efficacy in the vapor phase and could potentially be used in a fight against foodborne bacterial pathogens. Doran et al. [14] found out that several essential oils are effective in the vapor phase against bacteria and even against *Staphylococcus aureus* (MRSA). Laird and Phillips [15] stated in their findings that essential oils such as tea tree, bergamot, lavender, and eucalyptus in the vapor phase have an antimicrobial effect against bacteria and fungi. We consider the vapor phase of essential oils to be a current topic of research. Due to the vaporous application, it would be possible to use lower concentrations of essential oils to achieve the antimicrobial activity. The impact on the sensory properties of food would be reduced.

4. Conclusions

The tested essential oil *Origanum vulgare* showed strong antimicrobial activity against gram-positive, gram negative bacteria, and against yeasts. The high antimicrobial activity against diverse group of microorganisms, could also improve the post-harvest treatment of crops in the future. Evaluation of the inhibitory activity in the vapor phase demonstrated visible effect against the biofilm-forming bacteria. The highest inhibition rate against *S. maltophilia*, was found at 500 µL/L of oregano essential oil with inhibition value 91.27 % on potatoes. *B. subtilis*, was the most vulnerable against concentration 250 µL/L of essential oil by 63.24 % inhibition on carrots. Relatively stable growth inhibition was found in the apple sample at all concentrations tested. Application of the vapor phase allows the use of lower concentrations of oregano essential oils to achieve the antimicrobial activity. The impact on the sensory properties of food would be reduced in positive way.

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