

# ANTIBACTERIAL AND ANTIBIOFILM EFFECT OF WINTERGREEN AND IMMORTELLE ESSENTIAL OILS AGAINST STAPHYLOCOCCUS AUREUS

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## ABSTRACT

Biofilms are highly-structured communities of cells that produce an extracellular matrix and adhere to abiotic or biological surfaces, therefore they can contaminate foods as well. *Staphylococcus aureus* is a common bacterium with biofilm-producing character. Foods that are not cooked after handling, such as sliced meats, puddings, pastries, and sandwiches, are especially risky if are contaminated with *S. aureus* [1]. The essential oils (EOs) and their components are becoming increasingly popular as anti-biofilm agents. *Gaultheria procumbens* L. (wintergreen) and *Helichrysum italicum* Roth. (immortelle) are aromatic medicinal plants. They are traditionally used as choleric, diuretic and expectorant and in bacterial infections [2, 3]. These EOs have antibacterial effect, but their anti-biofilm activity has not been proved yet.

GC-MS analysis revealed that the main compound of wintergreen EO was methyl salicylate and the main component of immortelle EO is neryl acetate.

The MIC [Minimum Inhibitory Concentration] was determined with broth macrodilution test (wintergreen: 0.40 mg/mL; immortelle: 0.07 mg/mL) against *S. aureus*. The bacterial biofilm was created in 96-well microtiter plates. After incubation, the Tween80 solution of the EOs was added to the biofilm in MIC/2 concentration (wintergreen: 0.2 mg/mL, immortelle: 0.03 mg/mL). After a second incubation, the adherent cells were fixed with methanol and stained with 0.1% crystal violet, and dissolved in 33% acetic acid. The absorbance was measured at 595 nm with plate reader.

Our results showed that the wintergreen and immortelle oils have anti-biofilm activity against *S. aureus*, because the EOs reduced the biomass of the bacterial biofilm. It is important to highlight that the immortelle EO was more effective (inhibitory rate: 69.5%) than the wintergreen oil (inhibitory rate: 58.9%), compared to the control (untreated bacterial biofilm).

In this study, the anti-biofilm effect of wintergreen and immortelle were investigated against *S. aureus*. We conclude that the biofilm formation of *S. aureus* was more sensitive to immortelle EO. After toxicological experiment, the application of this oil against food-borne pathogens in food industry might be supposed.

[1] Bencardino D., Amagliani G., Brandi G. (2021): Carriage of *Staphylococcus aureus* among food handlers: An ongoing challenge in public health. *Food Cont.* 130:1-12.

[2] Nikolic M., Markovic T., Mojovic M., Pejcin B., Savic A., Peric T., Mojovic D., Stevic T., Sokovic M. (2013): Chemical composition and biological activity of *G. procumbens* L. essential oil. *Ind. Crops Prod.* 49: 561-567.

[3] Nincevic T., Grdisa M., Satovic Z., Dujakovic M. (2019): *Helichrysum italicum* Roth G. Don: Taxonomy, biological activity, biochemical and genetic diversity. *Ind Crop Prod.* 138: 111-487.

*Support: „SUPPORTED BY THE ÚNKP-21-4-I NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY FOR INNOVATION AND TECHNOLOGY FROM THE SOURCE OF THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND.”*

