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**CITRUS NOBILIS AS ANTIMICROBIAL AGENT AGAINST PLANT
BACTERIAL PATHOGENS**

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Abstract: *Citrus essential oils (EOs), commonly derived from fruit peels, offer versatile industrial applications due to their potent antimicrobial properties. This study assessed the efficacy of Citrus nobilis EO, obtained via cold pressing of unripe pericarp, against prevalent plant diseases. Using the disc diffusion method under in vitro conditions, the antibacterial activity of C. nobilis EO was evaluated. Results demonstrated moderate antimicrobial effects, particularly against Bacillus subtilis, suggesting its potential as a natural remedy for plant diseases.*

• **Introduction**

Plant essential oils (EOs), particularly those derived from citrus fruits, are renowned for their antimicrobial properties, making them valuable in the food industry. However, concerns over the impact of heat treatments on food quality have led to the exploration of alternative preservation methods, including the use of EOs in conjunction with milder heat treatments. This study aims to evaluate the antibacterial properties of *Citrus nobilis* against plant pathogens, employing both in vitro and in situ analyses using a carrot model.

• **Material and method**

The essential oil (EO), extracted from unripe *Citrus nobilis* pericarp (CNEO), was sourced and stored at 4 °C. Five bacterial strains, including both Gram-negative and Gram-positive bacteria, were selected from the Czech Collection of Microorganisms. Antibacterial properties of CNEO were evaluated using the disc diffusion method on Mueller Hinton agar plates. Gentamicin and chloramphenicol served as positive controls. In situ assessment was conducted using carrot slices as a bacterial growth substrate, with CNEO samples applied at various concentrations. After seven days of incubation at 37 °C, bacterial growth was assessed using ImageJ software. Statistical analyses were performed using one-way ANOVA followed by Tukey's HSD test.

• **Results and discussions**

The antibacterial activity of CNEO ranged from 2.33 to 10.33 mm. The best antimicrobial activity of CNEO was found against *X. arboricola* from the group of G- bacteria and against *P. megaterium* from group of G+ bacteria. CNEO was shown to be most effective against *B. subtilis* at concentrations of 62.5 µg/L (94.52 %), while *P. megaterium* showed the maximum levels of suppression at concentrations of 250 µg/L (87.86 %). This was determined by looking at the inhibitory effects on G+ bacterial strains in the carrot model. Notably, at a lower dosage (62.5 µg/L), the vapor phase of CNEO showed the maximum efficiency against G- bacteria, with reported inhibitory effects of 66.11% against *X. arboricola* in the carrot model.

• **Conclusions**

Minimally processed fruits represent an important area of potential growth in the rapidly expanding fresh-cut produce industry. However, the level of safety achieved with the currently applied preservation methods appears to be insufficient. Interest in the potential use of natural compounds to prevent microbial growth has notably increased in response to consumer pressure to reduce or eliminate chemically synthesized additives in foods. It is advisable to conduct further studies using various food models and storage conditions to optimize the application of *Citrus nobilis* EO as a natural substitute for synthetic preservatives.

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