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IN VITRO AND *IN SITU* ANTIBACTERIAL POTENTIAL OF *CITRUS AURANTIFOLIA*

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Abstract: *This study explores the antibacterial properties of Citrus aurantifolia essential oil against plant pathogenic bacteria, aiming to address the need for new antimicrobials due to bacterial resistance. Two methods were used to measure antibacterial activity, including in vitro disc diffusion and in situ surface application on carrots. Results revealed significant antibacterial effects of C. aurantifolia essential oil, particularly against Bacillus subtilis and Xanthomonas arboricola. These findings underscore the potential of natural compounds as alternatives to synthetic antibacterial agents in combating bacterial resistance.*

• Introduction

Citrus aurantifolia, a member of the Rutaceae family, is renowned for its wide-ranging biological activities attributed to its rich array of secondary metabolites. With increasing concerns over synthetic antimicrobials in food preservation, natural alternatives like citrus-derived compounds, including essential oils, are gaining attention. Our research aims to explore the antibacterial properties of *Citrus aurantifolia* against plant pathogens, both in vitro and *in situ*, with implications for enhancing food quality and shelf life.

• Material and method

Citrus aurantifolia essential oil (CAEO) was sourced from cold-pressed pericarp and stored at 4 °C. Bacterial strains, including both Gram-positive (*Bacillus subtilis* CCM 2217, *Priestia* (*Bacillus*) *megaterium* CCM 2007, and Gram-negative), and Gram-negative (*Xanthomonas arboricola* CCM 1441, *Pectobacterium carotovorum* CCM 1008, and *Pseudomonas putida* CCM 7156) species were obtained from the Czech Collection of Microorganisms. The disc diffusion method was employed to evaluate antibacterial activity, while antimicrobial efficacy in the vapor phase was assessed using carrots as a substrate. Statistical analyses were performed using one-way ANOVA and Tukey's HSD test ($p < 0.05$).

• Results and discussions

The antibacterial activity of CAEO ranged from 4.33 to 9.33 mm. The best antimicrobial activity of CAEO was found against *B. subtilis* from the group of G+ bacteria and against *P. carotovorum* from group of G- bacteria. Examining the inhibitory effects on G+ bacterial strains in the carrot model, it was discovered that CAEO was most effective against *P. megaterium* at concentrations of 125 µg/L (66.47 %), while *B. subtilis* showed the highest levels of suppression at concentrations of 500 µg/L (64.00 %). Significantly, the vapor phase of CAEO demonstrated the highest effectiveness against G- bacteria at the lower dosage (62.5 µg/L), with reported inhibitory effects of 96.40 % against *X. arboricola* in the carrot model.

• Conclusions

In conclusion, the packaging industry's interest in improving food safety and extending shelf life has led to the integration of antimicrobial compounds into food packaging materials, reflecting a shift towards natural and renewable resources. While direct addition of natural compounds to foods is common, alternative methods like dipping, spraying, and coating with active solutions are gaining momentum. Further research on diverse food models and storage conditions is recommended to maximize the potential of *Citrus aurantifolia* essential oil as a natural preservative substitute.

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