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Research Article

Antimicrobial Potential of *Cedrus deodara* Essential Oil to Preservative Effect for the Vegetables and Fruits

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A unique species of pine, *Cedrus deodara* is known for its wood oil. Its traditional therapeutic use is mainly antibacterial and anti-inflammatory. The aim of this study was to investigate the antibacterial properties of *Cedrus deodara* essential oil (CDEO) obtained from the crushed wood. The antimicrobial activity of CDEO was evaluated against Gram-negative (G^-) bacteria which included *Pseudomonas aeruginosa* CCM 1595, *Salmonella enterica* subs. *enterica* CCM 3807 and Gram-positive (G^+) bacteria *Yersinia enterocolitica* CCM 5671. *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subs. *aureus* CCM 2461 and *Streptococcus consellatus* CCM 4043 *in vitro* and *in situ*. The best antimicrobial activity for the disc diffusion method ranged from 4.67 to 9.67 mm and the minimum inhibitory concentration ranged from 1.48 to 5.44 mg.mL^{-1}. The most effective antimicrobial effect was found against *S. aureus* and *L. monocytogenes*. The vapour phase used showed the best antimicrobial effect against *P. aeruginosa* in the kiwifruit model and *L. monocytogenes* in the banana model at a lower CDEO concentration of 62.5 µg.L^{-1} and against *P. aeruginosa* in the potato model and *Y. enterocolitica* in the cucumber model at a higher CDEO concentration of 500 µg.L^{-1}. CDEO showed good antimicrobial activity against bacteria on vegetable and fruit model and may be a new preservative for storage of vegetables and fruits.

Keywords: antimicrobial activity, Gram-positive, Gram-negative bacteria, *in situ, in vitro*

1 Introduction

Large populations of bacteria have been found to be present in fresh produce such as apples, grapes, lettuce, peaches, peppers, spinach, kale and tomatoes (Badosa et al., 2008; Oliveira et al., 2010; Ponce et al., 2008; Rastogi et al., 2012). However, our knowledge of the diversity of these communities is still evolving. We are aware that important human pathogens such as *Salmonella* spp., *E. coli* and *L. monocytogenes* are associated with food. As fresh produce is often consumed raw, these pathogens have the potential for widespread disease epidemics (Critzer & Doyle, 2010; Fatica & Schneider, 2011). Microorganisms in produce also have the potential to affect human health in less obvious ways in addition to directly causing disease. The development of allergies can be influenced by exposure to non-pathogenic microbes associated with plants (Chan, 2013), and the consumption of raw foods can be an important route of entry for new commensal bacterial lineages into the human digestive tract. A large proportion of microorganisms found on kitchen surfaces appear to originate from food sources, and bacteria associated with produce in general can have a significant impact on food spoilage rates (Betts, 2006; Flores et al., 2013).

In addition to consumer perceptions of the use of natural antibacterial compounds being positive in terms of food

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safety, consumers generally view the use of natural antibacterial compounds as a suitable substitute for chemical sanitizers (Barbosa et al., 2009; Gachkar et al., 2007; Saleem, 2014; Song & Feng, 2023; Souza et al., 2007). A number of researches have been conducted in this area. Most of the treatments are aimed at killing microbes and pathogens that cause spoilage of vegetables (Goodburn & Wallace, 2013; Martin-Diana et al., 2006). Plants, such as essential oils (EOs), are the main suppliers of these naturally occurring antibacterial chemicals (Gyawali & Ibrahim, 2014; Rizzello et al., 2005). The antibacterial properties of herbs and spices have been used for food preservation since antiquity (Burt, 2004; Oussalah et al., 2006; Tian et al., 2011); current research in this area has revived interest (Patrignani et al., 2015). As EOs are natural compounds that are generally well tolerated and have fewer adverse effects than other preservatives or sanitizers, they have been used in recent decades as innovative replacements for standard antibacterial agents in foods. EOs are extracted from aromatic and medicinal plants.

Cedrus deodara (Roxb. ex D. Don) is a conifer that grows in the Himalayan regions of India, Pakistan and Nepal at altitudes ranging from 1,200 to 3,000 m. This evergreen tree, which belongs to the family Pinaceae, grows extensively along the Himalayan mountains and forms forests. Locals use this plant extensively for medicinal purposes including treatment of fever, intestinal parasites, sinusitis, diarrhoea, dysentery, rheumatism, diabetes, cancer, ulcers, antifungal, tuberculous glanditis, and as an insecticidal and larvicidal agent against stored pests and house flies (Chaudhary et al., 2011; Kumar et al., 2019; Saab et al., 2018). The plant is traditionally used by people for covering lawns, for shelters, furniture making and as firewood.

The aim of our study was to investigate the antimicrobial activity of *Cedrus deodara* essential oil (CDEO) *in vitro* against bacteria and the preservative effect on fruits and vegetables in the vapour phase.

2 Material and Methods

2.1 Tested Essential Oil

The essential oil (EO) used in this research was produced by distillation of crushed *Cedrus deodara* (CDEO) wood and was obtained from Hanus s. r. o. (Nitra, Slovakia). The wood was purchased from India. It was kept at 4 °C in the dark for analysis.

2.2 Bacteria Tested in the Study

The assessed antibacterial efficacy of EO was tested using the following bacterial strains: Gram-negative (G^-) bacteria included *Pseudomonas aeruginosa* CCM 1595, *Salmonella enterica* subs. *enterica* CCM 3807 and *Yersinia enterocolitica* CCM 5671. Gram-positive (G^+) bacteria included *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subs. *aureus* CCM 2461 and *Streptococcus consellatus* CCM 4043. The Czech Collection of Microorganisms (CCM), located in Brno, Czech Republic, provided all G^+ and G^- bacterial species. Bacterial inocula were grown in Mueller Hinton broth (MHB, Oxoid, Basingstoke, UK) overnight at 37 °C prior to analysis. The optical density of the bacterial inoculum was set at 0.5 McFarland standard on the day of the experiment.

2.3 Disc Diffusion Method

A disk diffusion susceptibility test was performed using previously identified microbial strains. Bacterial strains were inoculated onto Mueller Hinton agar (MHA) and cultured in Mueller Hinton broth (MHB). Blank discs were dipped in 10 µL of CDEO evaluated on the agar surface. Millimetres were used to measure and record the inhibitory activity after 24 h incubation at 37 °C. The antibiotic cefoxitin and gentamicin (30 µg.disc.$^{-1}$) were supplied by Oxoid, Basingstoke, UK, as positive controls for G^- and G^+ bacteria. The whole experiment was performed in triplicate to ensure accuracy and consistency (Kačániová et al., 2023).

2.4 Minimal Inhibition Concentration

The minimum inhibitory concentration (MIC) values, namely MIC_{50} and MIC_{90}, were calculated according to the usual protocols as reported by Kačániová et al. (2020). In summary, 50 µL of microbial inoculum was added to each well of the 96-well microtiter plate. Different amounts of Mueller Hinton Broth (MHB) were combined with CDEO ranging from 10 mg.mL^{-1} to 0.00488 mg.mL^{-1}. The positive control wells contained MHB with inoculum for maximum development, while the negative control wells had MHB with CDEO at the recommended dose. After the incubation period, absorbance was measured at 570 nm using a spectrophotometer (Glomax, Promega Inc., Madison, WI, USA). MIC_{50} indicated the lowest concentration of CDEO that inhibited 50% of bacterial growth, while MIC_{90} indicated the lowest concentration that inhibited 90% of bacterial growth. To ensure consistency and reliability, the entire study was conducted in triplicate (Kačániová et al., 2023).

2.5 Antimicrobial Activity in situ

The antibacterial activity of vapour phase CDEO was evaluated against a range of bacterial strains using kiwifruit, banana, potato and cucumber as substrates. Similar to the previous study, the experimental technique involved cutting both fruits and vegetables into 0.5 mm

pieces, drying and cleaning them (Kačániová et al., 2021). The bacteria were then added to Petri plates using agar. After dissolving in ethyl acetate, CDEO was applied to sterile filter paper at different concentrations (500, 250, 125 and 62.5 mg.L^{-1}); in contrast, control were exposed to ethyl acetate only. After evaporation of ethyl acetate for one minute, the Petri dishes were closed and cultured at 37 °C for seven days. Measurements of bacterial growth were made *in situ* using ImageJ and conventional methods. The volumetric density of bacterial colonies (vv) and the percentage of bacterial growth inhibition (BGI) caused by the evaporative phase treatment of EO was calculated using the provided formulas (Kačániová et al., 2021).

2.6 Statistical Method

Experimental evaluations were conducted in triplicate and the mean values and corresponding standard deviations (SD) are presented as results. The ANOVA calculator version 6.0 was used to perform statistical analyses such as one-way ANOVA and Tukey's HSD test at the significance level of p <0.05.

3 Results and Discussion

The epidemiology of foodborne infectious diseases has changed drastically over the past three decades, with fruits and vegetables acting as new vectors in which pathogens "emerge" (Holden et al., 2009). These new minimally processed (MP) foods come in a variety of forms, including freshly cut fruits and vegetables, and are packaged and sold as ready-to-eat meals for convenience and ease of use (De Corato, 2020; Juneja, 2002). The emergence of MP foods can be attributed to a recent trend in the market: there is a growing need for efficient preservation methods that do not require chemical preservatives (Santos & Oliveira, 2012). A specific subset of the MP food business is represented by MP fruits and vegetables, which have attracted great public interest because they are considered healthier than processed foods. A minimally processed fruit or vegetable (MPV) is defined as any fresh vegetable or fruit that has undergone little processing – usually chopped, peeled, crushed and cleaned – and packaged in a ready-to-eat state while still remaining fresh. As EOs are natural compounds that are generally well tolerated and have fewer adverse effects than other preservatives or disinfectants, they have been used in recent decades as innovative replacements for standard antibacterial agents in foods. EOs are obtained from aromatic and medicinal plants (Patrignani et al., 2015; Tian et al., 2011).

Table 1 shows the antibacterial activity of CDEO. The strongest antibacterial activity against G$^+$ bacteria was shown by *S. aureus* subs. *aureus* (9.67 mm), *S. consellatus* (8.67 mm) and *L. monocytogenes* (8.33 mm). All G$^+$ bacteria examined showed signs of susceptibility to CDEO. The G$^-$ bacteria with the highest susceptibility were *S. enterica* subs. *enterica* (5.67 mm), followed by *Y. enterocolitica* (5.33 mm) and *P. aeruginosa* (4.67 mm). Interestingly, *L. monocytogenes* (29.67 mm) was the most sensitive G$^+$ bacteria to antibiotics, while *Y. enterocolitica* (29.67 mm) was the most sensitive G$^-$ bacteria to antibiotics.

As reported in the study of Zeng et al. (2012) CDEO, it produced inhibitory zones and shown considerable inhibition against tested yeast (*S. cerevisiae*), fungi (*A. niger*, *P. citrinum*, *R. oryzae*, and *A. flavus*), bacteria (*E. coli*, *S. aureus*, *B. subtilis*, and *B. cereus*). Pine needle EO demonstrates significant inhibitory and sterilizing effect against common food-borne bacteria tested, according to the results of *in vitro* antimicrobial studies. The found antibacterial activity could be ascribed to the existence of some bioactive substances, namely linalool and eugenol.

According to the results of Majid et al. (2015), CDEO demonstrated excellent inhibitory effects against *E. coli*, *S. typhimurium*, *P. aeruginosa*, *E. faecalis*, and *B. subtilis*. *E. coli* showed 32 mm and 24 mm and an 18 mm zone of inhibition, *B. subtilis* showed 25 mm and 13 mm and an 18 mm zone of inhibition using the well and disc diffusion method, among other bacteria. Our study showed more less inhibition potential.

The antibacterial activity of *C. atlantica* EO against *Escherichia coli*, *B. subtilis*, *Micrococcus luteus*,

Table 1 Disc diffusion method antimicrobial activity

Microorganism	Inhibition zone	ATB
***Listeria monocytogenes* CCM 4699**	8.33 ±0.58^{a}	29.67 ±0.58$^{a, b, c, e}$
***Staphylococcus aureus* subs. *aureus* CCM 2461**	9.67 ±0.58$^{a, b, c}$	28.33 ±0.58$^{a, b, c, d, e}$
***Streptococcus consellatus* CCM 4043**	8.67 ±0.58$^{a, b, c}$	29.33 ±0.58$^{a, b, c, e}$
***Pseudomonas aeruginosa* CCM 1595**	4.67 ±0.58$^{d, e}$	27.67 ±0.58$^{b, d, e}$
***Salmonella enterica* subs. *enterica* CCM 3807**	5.67 ±0.58$^{d, e}$	28.67 ±0.58$^{a, b, c, d, e}$
***Yersinia enterocolitica* CCM 5671**	5.33 ±0.58$^{d, e}$	29.67 ±0.58$^{a, b, c, e}$

Data are the mean (±SD) of 3 samples; different letters in each column refer to significant differences (Tukey, p <0.05); ATB – antibiotics

Table 2 Minimal inhibition concentration (MIC) of *Cedrus deodara* essential oil (CDEO)

Microorganism	MIC_{50}	MIC_{90}
***Listeria monocytogenes* CCM 4699**	1.48 ±0.12[a]	1.74 ±0.22[a]
***Staphylococcus aureus* subs. *aureus* CCM 2461**	3.26 ±0.16[b]	3.57 ±0.11[b]
***Streptococcus consellatus* CCM 4043**	2.31 ±0.15[c]	2.78 ±0.10[c]
***Pseudomonas aeruginosa* CCM 1595**	5.44 ±0.12[d]	5.65 ±0.05[d]
***Salmonella enterica* subs. *enterica* CCM 3807**	4.34 ±0.03[e]	4.65 ±0.16[e]
***Yersinia enterocolitica* CCM 5671**	4.44 ±0.20[e]	4.78 ±0.10[f]

Data are the mean (± SD) of 3 samples. Different letters in each column refer to significant differences (Tukey, p <0.05)

Table 3 *In situ* analysis of the antimicrobial activity (% inhibition) of the vapour phase of CDEO in fruits and vegetables

Microorganisms	Concentration of EO (μg.L^{-1})			
	62.5	125	250	500
Kiwi				
Listeria monocytogenes	77.14 ±1.40[a]	55.44 ±2.17[a]	35.82 ±1.80[a]	26.21 ±1.54[a]
Staphylococcus aureus	75.81 ±1.67[a, b, c, e]	65.03 ±1.01[b, c, d]	54.77 ±2.74[b, c, d]	25.38 ±2.55[a, b]
Streptococcus consellatus	74.97 ±1.68[a, b, c, e]	65.18 ±3.08[b, c, d]	54.31 ±2.81[b, c, d]	45.08 ±1.58[c, d]
Pseudomonas aeruginosa	85.14 ±1.58[d]	63.66 ±2.27[b, c, d]	55.03 ±3.48[b, c, d]	45.80 ±1.79[c, d]
Salmonella enterica	76.81 ±2.15[a, b, c, e]	56.11 ±0.59[a, e]	34.00 ±1.15[a, e]	15.73 ±1.86[e]
Yersinia enterocolitica	66.47 ±1.68f	45.37 ±0.53f	33.43 ±2.09[a, e]	22.40 ±0.64[a, b]
Banana				
Listeria monocytogenes	84.12 ±3.42[a]	76.95 ±1.56[a]	66.94 ±0.82[a]	56.47 ±1.55[a]
Staphylococcus aureus	65.71 ±2.33[b, d]	55.69 ±0.23[b, d, e]	46.09 ±1.62[b, d]	35.08 ±2.68[b, d]
Streptococcus consellatus	74.52 ±1.00[c, e]	65.59 ±2.20[c]	54.34 ±2.55[c]	45.41 ±1.59[c]
Pseudomonas aeruginosa	65.74 ±2.57[b, d]	56.76 ±1.71[b, d, e]	44.56 ±3.83[b, d]	33.23 ±0.87[b, d]
Salmonella enterica	76.37 ±2.59[c, e]	54.40 ±0.55[b, d, e]	35.11 ±2.63[e]	25.21 ±2.53[e]
Yersinia enterocolitica	76.32 ±0.78[c, e]	65.37 ±0.45[c]	54.81 ±2.70[c]	25.53 ±2.30[e]
Potato				
Listeria monocytogenes	25.07 ±2.62[a]	33.59 ±1.00[a]	43.92 ±1.98[a]	76.66 ±1.08[a, b]
Staphylococcus aureus	23.70 ±0.98[a]	42.74 ±0.85[b, e]	54.74 ±2.00[b, d, e]	66.69 ±1.22[a, b, c, d, e]
Streptococcus consellatus	24.40 ±2.11[a]	34.96 ±2.51[a, c, d]	44.47 ±1.13[a, c]	56.32 ±1.82[b, c]
Pseudomonas aeruginosa	25.70 ±2.16[a]	34.15 ±0.80[a, c, d]	57.03 ±1.51[b, d, e]	76.92 ±0.64[a, b, d, e]
Salmonella enterica	26.08 ±2.52[a]	43.70 ±1.05[b, e]	56.85 ±1.01[b, d, e]	74.52 ±1.85[a, b, d, e]
Yersinia enterocolitica	23.00 ±1.17[a]	46.06 ±1.63[b, e]	64.37 ±3.16f	75.22 ±2.50[a, b, d, e]
Cucumber				
Listeria monocytogenes	24.29 ±2.18[a]	32.74 ±0.96[a, b]	54.11 ±2.78[a]	76.80 ±2.35[a, b, c, d]
Staphylococcus aureus	25.80 ±1.70[a]	34.23 ±0.96[a]	45.59 ±2.43[b, c]	72.07 ±2.40[a, b, c, e, d]
Streptococcus consellatus	23.74 ±0.89[a]	33.76 ±1.97[a]	45.44 ±1.56[b, c]	76.78 ±2.07[a, b, c, d]
Pseudomonas aeruginosa	26.11 ±2.26[a]	36.11 ±1.64[a]	54.92 ±2.16[a, d, e]	76.51 ±2.72[a, b, c, d]
Salmonella enterica	26.09 ±2.27[a]	34.40 ±2.55[a]	55.76 ±2.21[a, d, e]	66.88 ±2.82[b, e]
Yersinia enterocolitica	25.51 ±1.18[a]	37.48 ±1.53[a, b]	55.77 ±1.82[a, d, e]	77.69 ±0.96[a, b, c, d]

Data are the mean (±SD) of 3 samples; different letters in each column refer to significant differences (Tukey, p <0.05)

and *Staphylococcus aureus* was also determined by Satrani et al. (2006). A related study showed that at a concentration of 1/100 v/v, the essential oils extracted from wingless and winged cedar seeds could stop *Escherichia coli* from growing (Rhafouri et al., 2014).

The MIC_{50} and MIC_{90} were calculated using the broth microdilution method. *L. monocytogenes* and *S. consellatus* were found to have low MIC_{50} values (1.48 resp. 2.31 mg.mL^{-1}) and MIC_{90} values (1.74 resp. 2.78 mg.mL^{-1}). Value MIC_{50} (4.34 resp. 4.44 µL.mL^{-1}) and MIC_{90} (4.65 resp. 4.78 mg.mL^{-1}) were found for *S. enterica* and *Y. enterocolitica*. Table 2 displays the results obtained for the minimal inhibitory concentration.

The oil's MBC and MIC ranged from 0.39 to 6.25 µg.mL^{-1} and 0.2 to 1.56 µg.mL^{-1}, respectively. The microbe that tested most sensitive was *R. oryzae*. When *R. oryzae* first appeared, at 0.2 µg.mL^{-1}, pine needle EO reduced its development. At 0.39 µg.mL^{-1}, it had a sterilizing effect. Out of all the bacteria that were examined, *B. subtilis* showed the highest susceptibility to pine needle EO, with MIC and MBC values of 0.39 and 0.78 µg.mL^{-1}, respectively (Zeng et al., 2012).

To fully assess the antibacterial properties of CDEO, *in situ* antimicrobial research was conducted using kiwifruit, banana, potato and cucumber as food models. The same bacterial strains used for this investigation were used to determine the values of the disk diffusion method and the broth dilution method. Table 3 shows the results of the evaluation. When the evolution of *L. monocytogenes* on kiwifruit *in situ* was investigated, the concentration of 62.5 µg.L^{-1} showed the greatest inhibitory effect (77.14%). The lowest CDEO concentration tested significantly reduced the development of *S. aureus* and *S. consellatus* (75.81 vs. 74.97%) on kiwifruit. At the highest dose tested CDEO showed significant antibacterial efficacy against G^- bacterial species (85.14%), especially *P. aeruginosa*. The findings showed that *L. monocytogenes* (84.12%) grew in the treatment with the lowest concentration used and *S. consellatus* grew on banana in the treatment with the lowest dose used (74.52%). The antibacterial activity of CDEO in vapour phase against G^- showed the highest efficacy of CDEO in inhibiting the growth of *S. enterica*. Potato with the highest concentration of CDEO showed the highest antibacterial activity of CDEO against *L. monocytogenes* (76.66%) in the vegetable model. The potato model showed the strongest antibacterial activity against *P. aeruginosa* (76.92%) at the lowest concentration. The lowest concentration of antibacterial activity against *Y. enterocolitica* (77.69%) was seen on the cucumber model.

In different studies based on the growth of *P. aurantiogriseum* (62.5, 125 and 250 µg.L^{-1}), *P. expansum* (all tested concentrations), *P. chrysogenum* (250 and 500 µg.L^{-1}) and *P. italicum* (62.5 and 500 µg.L^{-1}) on a bread model, our results demonstrated the antifungal activity of *Cedrus atlantica* EO. The strongest concentrations of EO (250 and 500 µg.L^{-1}) showed remarkable antifungal activity against all tested fungal strains when applied to carrot substrate. The lowest concentrations of EO also showed significant antifungal activity against *P. aurantiogriseum* (62.5 and 125 µg.L^{-1}), *P. expansum* (125 µg.L^{-1}) and *P. chrysogenum* (125 µg.L1). When celery was used as a food model, a very significant inhibitory effect of EO against the growth of some strains of *P. aurantiogriseum* (62.5 µg.L^{-1}), *P. expansum* (250 µ.L^{-1}) and *P. chrysogenum* (all concentrations tested) was demonstrated. The lowest concentration of EO showed weak to moderate antifungal activity against *P. italicum*, but also weakly promoted fungal growth (Kačániová et al., 2022).

According to Chaudhary et al. (2012), *Pinus roxburghii* and *Cedrus deodara* have antibacterial properties and can be used to treat infectious diseases. Further research is needed to identify the active constituents of the plants that confer them antibacterial properties.

4 Conclusions

Due to their antibacterial properties and potential use as bioactive additives or ingredients, EOs are a hot topic of research in the food industry. Therefore, the assessment of their use as a food preservative requires thorough research on their antibacterial activity. In this study, we evaluate the antibacterial activity of CDEO. In terms of food preservation, encapsulated EOs may be a more environmentally friendly and non-toxic alternative to chemicals, which have a number of negative effects on both the environment and human health. CDEO have demonstrated antibacterial activity against both G^+ and G^- bacteria. CDEO was the most effective against G^+ and G^- depending on the antibacterial activity technique used. The broad spectrum and strong antibacterial activity of CDEO suggest that it can be used for plant and crop protection as well as for extending the shelf life of food.

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