

## Antimicrobial Activity of *Styrax tonkinensis* Essential Oil *in vitro* and *in situ*

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Among the genera in this family, *Styrax* is unique in that it yields benzoin resin, a resinous substance. Usually, when sharp items lacerate the bark, this resin is secreted. Because of its fragrant qualities, it has been used for centuries in cosmetics and fragrances all over the world. Additionally, *Styrax* species have long been employed in herbal remedies for a variety of ailments. The antibacterial characteristics of *Styrax tonkinensis* essential oil (STEO) were the focus of this study. The antimicrobial activity was compared to bacteria, both Gram-positive (G<sup>+</sup>) and Gram-negative (G<sup>-</sup>), using inhibition zones in agar media, minimum inhibitory concentration (MIC) bioassays and in vapour phase on fruits and vegetables model. The findings showed that STEO was very successful in inhibiting bacteria that were G<sup>+</sup> (*Bacillus subtilis* subsp. *Spizizenii* CCM 1999, *Bacillus thuringiensis* CCM 19, and *Priestia (Bacillus) megaterium* CCM 2007) as well as G<sup>-</sup> (*Citrobacter koseri* CCM 2535, *Enterobacter aerogenes* CCM 2531, *Escherichia coli* CCM 3954). The range of maximal inhibition zones and MIC values was determined to be 4.67 to 8.33 mm and 3.49 to 7.71 mg.mL<sup>-1</sup>, respectively. Furthermore, *B. thiriangensis*, *P. megaterium*, and *E. coli* were all susceptible to the antimicrobial effects of the (STEO) on the fruit and vegetable model. According to research findings, STEO is a valuable source of organic chemicals that have the potential to be innovative antibacterial agents against microbes.

**Keywords:** *Styrax* benzoin, antimicrobial activity, vegetable, fruit, *in vitro*, *in situ*

### 1 Introduction

The plant *Styrax tonkinensis* (Pierre) Craib ex Hartw is a member of the Styracaceae family, which is found in tropical and subtropical climates. The Chinese provinces of Yunnan, Guizhou, Guangxi, Guangdong, Fujian, Hunan, and Jiangxi are among those where it is most prevalent (Wang et al., 2020). The genus *Styrax* yielded numerous chemicals that were isolated, such as flavonoids (Braguine et al., 2012), lignans (Min et al., 2015), triterpenes (Pauletti et al., 2002), saponins (Yayla et al., 2002), coumarins (Pauletti et al., 2002), phenolic acid (Timmers et al., 2015), and glycoprotein (Lee et al., 2016).

Numerous *Styrax* species contain significant amounts of lignans and triterpenes. Nonetheless, there are distinctions and commonalities across the various *Styrax* species, as evidenced by the dissimilar chemical

composition of each species in the genus. Although Hu et al. (2019) also identified eight lignans from *S. tonkinensis* leaves, the majority of *S. tonkinensis* study has been concentrated on the plant's resin. Benzoin resin, sometimes called Siam benzoin balsam (Burger et al., 2016), is the resin of this plant and has been used for thousands of years in traditional Chinese medicine (Wang et al., 2016) as a medication for analgesia and blood circulation. As of right now, benzoin resin has yielded 11 triterpenes (Wang et al., 2015; Wang et al., 2006a; Zhen-feng, 2012), 11 phenylpropanoids (Burger et al., 2016; Huang et al., 2009; Wang et al., 2006b; Zhen-feng, 2012), and a few aromatics (Wang et al., 2020; Wang et al., 2012).

Fresh vegetables are a potential source of food-borne illnesses and disease outbreaks since they are typically

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consumed raw or very little cooked to retain their nutrients and flavour (Mir et al., 2018). Fresh fruit and vegetable consumption is rising worldwide, but a growth in microbial contamination poses a serious threat to this. On the other hand, current information regarding the epidemiology of microbial contamination, as well as the origins and routes of contamination in fruits and vegetables, is scarce (Snyder & Worobo, 2018). The USFDA has classified EOs, which are used as antimicrobial additives in food, as GRAS (Generally Recognized as Safe). These EOs are rich in bioactive compounds and have some exceptional antioxidant and antimicrobial properties, making them desirable as preservatives in the food industry (Atarés & Chiralt, 2016; Wrona et al., 2015).

However, due to their potent antibacterial and antioxidant properties, the use of EOs as food additives has garnered attention recently (Calo et al., 2015). The fundamental antibacterial processes of EOs remain poorly known, despite the fact that their antimicrobial effects are widely acknowledged and supported by numerous research studies (Amiri et al., 2020). It is commonly known that G<sup>+</sup> bacteria are more sensitive to EO than G<sup>-</sup> bacteria (Frangos et al., 2010; Kumar Sharma et al., 2016; Lima et al., 2004; Lucera et al., 2012; Mith et al., 2014). This difference in bacterial cell wall composition may be the cause of this susceptibility (Burt, 2004).

Certain authors (Amiri et al., 2020) claim that the antibacterial processes of EO target multiple sites, making it challenging to forecast a microorganism's susceptibility to a specific EO. However, the main cause of the EO's overall antimicrobial activity is thought to be its hydrophobicity, which enables it to pass through the lipid layer of cell membranes and eventually cause changes in permeability and disruption, which in turn cause the release of ions and intracellular components and ultimately cause cellular death (Mehani & Ladjel, 2012). A thorough study of EO's general antibacterial processes can be found elsewhere (Amorati & Foti, 2017). Terpenes and other chemicals, such as ketones and phenols, are the primary ingredients of EOs and are responsible for their antibacterial properties (Pandey et al., 2017).

The aim of this paper was to study the antimicrobial activity of *Styrax tonkinensis* *in vitro* against G<sup>+</sup> and G<sup>-</sup> bacteria and *in situ* on the vegetable and fruit model.

## 2 Material and Methods

### 2.1 Essential Oil

The essential oil (EO) utilized in this study was obtained from Hanus s.r.o. (Nitra, Slovakia) and was made by alcoholic extraction of hand-picked *Styrax tonkinensis* tree resin. France was where the fruits were acquired. For analysis, they were stored in the dark at 4 °C.

### 2.2 Bacterial strains for antimicrobial application

The following bacterial strains were used to test the evaluated EO antibacterial efficacy: *Citrobacter koseri* CCM 2535, *Enterobacter aerogenes* CCM 2531, *Escherichia coli* CCM 3954 were among the Gram negative (G<sup>-</sup>) bacteria found. The following bacteria were identified as Gram positive (G<sup>+</sup>): *Bacillus subtilis* subsp. Spizizenii CCM 1999, *Bacillus thuringiensis* CCM 19, and *Priestia (Bacillus) megaterium* CCM 2007. All G<sup>+</sup> and G<sup>-</sup> bacterial species were procured from the Brno, Czech Republic-based Czech Collection of Microorganisms (CCM). Before analysis, the bacterial inoculums were cultivated for a full day at 37 °C in Mueller Hinton Broth (MHB, Oxoid, Basingstoke, UK). On the day of the experiment, the bacterial inoculum's optical density was established at 0.5 McFarland standard.

### 2.3 Disc Diffusion Method

The microbial strains that were previously indicated were used in the disk diffusion susceptibility test. Bacterial strains grown in Mueller Hinton Broth (MHB) were used to inoculate Mueller Hinton Agar (MHA). On the agar surface, blank discs were soaked in 10 µL of the evaluated *Styrax tonkinensis* essential oil (STEO). After a 24-hour incubation period at 37 °C, the inhibitory activity was quantified and noted in millimetres. Oxoid, Basingstoke, UK provided cefoxitin and gentamicin antibiotics (30 µg.disc<sup>-1</sup>) as positive controls for G<sup>-</sup> and G<sup>+</sup> bacteria. To ensure accuracy and consistency, the entire experiment was carried out in triplicate (Kačániová et al., 2023).

### 2.4 Broth Dilution Method

Following the standard protocols described by Kačániová et al. (2020), the minimum inhibitory concentration (MIC) values, specifically MIC<sub>50</sub> and MIC<sub>90</sub>, were calculated. To sum up, a 96-well microtiter plate was filled with 50 µL of microbial inoculum in each well. Mueller Hinton Broth (MHB) was mixed with STEO at different concentrations, ranging from 10 mg.mL<sup>-1</sup> to 0.00488 mg.mL<sup>-1</sup>. MHB with an inoculum for maximum growth was present in positive control wells, while MHB with EO at the prescribed concentration was present in negative control wells. Following incubation, a spectrophotometer (Glomax,

Promega Inc., Madison, WI, USA) was used to detect absorbance at 570 nm. The lowest EO concentration that inhibited 50% of bacterial growth was represented by MIC<sub>50</sub>, while the lowest concentration that inhibited 90% of bacterial growth was represented by MIC<sub>90</sub>. For uniformity and dependability, the entire experiment was carried out in triplicate (Kačániová et al., 2023).

### 2.5 Vapour Phase Antimicrobial Activity

Using apples, pears, carrots, and kohlrabi as substrates, the antibacterial activity of STEO's vapour phase was evaluated against a variety of bacterial strains. Both fruits and vegetables were chopped into 0.5 mm pieces, dried, and cleaned as part of the experimental protocol, which was similar to that of a prior study (Kačániová et al., 2021). Agar was then used to introduce bacteria to Petri plates. STEO was applied to sterile filter paper at different concentrations (500, 250, 125, and 62.5 mg.L<sup>-1</sup>) after being dissolved in ethyl acetate; control sheets, on the other hand, were merely exposed to ethyl acetate. Following a minute for the evaporation of ethyl acetate, Petri dishes were sealed and incubated for seven days at 37 °C. *In situ* measurements of bacterial growth were made using ImageJ and conventional techniques. With the aid of the supplied formulas, the volume density of bacterial colonies (vv) and the percentage of bacterial growth inhibition (BGI) brought about by the EOs vapour phase treatment were computed (Kačániová et al., 2021).

### 2.6 Statistical Analyses

The outcomes of the experimental evaluations, which were carried out in triplicate, are shown as mean values with matching standard deviations (SD). Using ANOVA calculator version 6.0, statistical analyses were performed at a significance level of  $p < 0.05$ , including one-way ANOVA and the Tukey's HSD test.

## 3 Results and Discussion

The antibacterial activity of STEO is shown in Table 1. *Bacillus thuringiensis* (8.33 mm) showed the strongest antimicrobial action against G<sup>+</sup> bacteria, followed

by *Bacillus subtilis* (7.67 mm) and *Priestia megaterium* (6.67 mm). Every examined G<sup>+</sup> bacteria showed signs of sensitivity to the study's medicines. *Citrobacter koseri* and *Enterobacter aerogenes* (5.33 mm) had the maximum sensitivity among G<sup>-</sup> bacteria, followed by *Escherichia coli* (4.67 mm). Interestingly, *B. subtilis* (30.67 mm) was the most antibiotic-sensitive G<sup>+</sup> bacteria and *E. coli* (29.33 mm) was the most antibiotic sensitive G<sup>-</sup> bacteria.

The usage of *Styrax* EO in scientific research and traditional medicine has led to the discovery of a variety of biological effects. Using the agar diffusion method, a prior study examined *Styrax* EO's antibacterial properties against a variety of bacterial species. Certain bacterial strains respond differently to different balsam concentrations. Its antimicrobial activity was investigated in one of the most thorough studies. *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium xerosis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescent*, and *Staphylococcus aureus* were all successfully combatted by the 10% balm concentration. 0.4% balm concentration proved to be effective against *P. vulgaris* and *E. aerogenes* (Büyükkiliç et al., 2022).

Calculating the MIC<sub>50</sub> and MIC<sub>90</sub> involved using the broth microdilution method. For *B. subtilis* and *P. megaterium*, low MIC<sub>50</sub> values (3.49 resp. 4.62 mg.mL<sup>-1</sup>) and MIC<sub>90</sub> values (3.82 resp. 4.89 mg.mL<sup>-1</sup>) were noted. The mean MIC<sub>50</sub> (5.52 resp. 6.59 mg.mL<sup>-1</sup>) and MIC<sub>90</sub> (5.88 resp. 6.89 mg.mL<sup>-1</sup>) for *E. coli* and *E. aerogenes* were ascertained. For *C. koseri*, the MIC<sub>50</sub> and MIC<sub>90</sub> values were 7.71 mg.mL<sup>-1</sup> and 7.95 mg.mL<sup>-1</sup>, respectively. Table 2 displays the outcomes of the minimal inhibitory dosages that were obtained.

The findings in a different study showed that STEO is very successful in suppressing the growth of bacteria, both G<sup>+</sup> (*Listeria monocytogenes*, *Micrococcus luteus*, and *Staphylococcus aureus*) and G<sup>-</sup> (*Escherichia coli*,

**Table 1** Disc diffusion method antimicrobial activity (mm)

Microorganism	Inhibition zone	ATB
<i>Bacillus subtilis</i> subsp. <i>Spizizenii</i> CCM 1999	7.67 ± 0.58 <sup>a</sup>	30.67 ± 0.58 <sup>a</sup>
<i>Bacillus thuringiensis</i> CCM 19	8.33 ± 0.58 <sup>a, b</sup>	27.67 ± 0.58 <sup>c, b, d, e</sup>
<i>Priestia megaterium</i> CCM 2007	6.67 ± 0.58 <sup>a, c, d, e</sup>	28.67 ± 0.58 <sup>b, c, e</sup>
<i>Citrobacter koseri</i> CCM 2535	5.33 ± 0.58 <sup>c, d</sup>	26.67 ± 0.57 <sup>b, d</sup>
<i>Enterobacter aerogenes</i> CCM 2531	5.33 ± 0.58 <sup>a, c</sup>	28.33 ± 0.58 <sup>b, c, e</sup>
<i>Escherichia coli</i> CCM 3954	4.67 ± 0.58 <sup>d, e</sup>	29.33 ± 0.58 <sup>a, c, e</sup>

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 *Styrax tonkinensis* essential oil (STEO) in mg.mL<sup>-1</sup>

Microorganism	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>Bacillus subtilis</i> subsp. <i>Spizizenii</i> CCM 1999	3.49 ±0.06 <sup>a</sup>	3.82 ±0.05 <sup>a</sup>
<i>Bacillus thuringiensis</i> CCM 19	5.59 ±0.16 <sup>b</sup>	5.82 ±0.16 <sup>b</sup>
<i>Priestia megaterium</i> CCM 2007	4.62 ±0.12 <sup>c</sup>	4.89 ±0.10 <sup>c</sup>
<i>Citrobacter koseri</i> CCM 2535	7.71 ±0.27 <sup>d</sup>	7.95 ±0.05 <sup>d</sup>
<i>Enterobacter aerogenes</i> CCM 2531	6.59 ±0.06 <sup>e</sup>	6.89 ±0.10 <sup>e</sup>
<i>Escherichia coli</i> CCM 3954	5.52 ±0.13 <sup>b</sup>	5.88 ±0.09 <sup>b</sup>

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

*Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). It was discovered that the maximal inhibition zones and MIC values ranged from 12.67 to 19.33 mm and 3.9 to 62.5 µL.mL<sup>-1</sup>, respectively. Furthermore, *Aspergillus flavus*, *Botrytis cinerea*, and *Candida albicans* were all susceptible to the antifungal effects of the STEO. According to research findings, STEO is a valuable source of organic chemicals that have the potential to be innovative antibacterial agents against microorganisms (Čmiková et al., 2023).

Using apples, pears, carrots, and kohlrabies as food models, an *in situ* antimicrobial analysis was carried out to thoroughly evaluate the antibacterial qualities of the STEO. The same bacterial strains that were used to calculate the MIC<sub>50</sub> and MIC<sub>90</sub> values were also utilized in this assessment. The results of this evaluation are shown in Table 3. The development of *B. subtilis* on apple *in situ* was evaluated by STEO, and the results showed that the concentration of 500 µg.L<sup>-1</sup> had the greatest inhibitory impact (67.25%). The development of *B. thuringiensis* and *P. megaterium* in apples was significantly inhibited by the highest tested STEO concentration (65.74 vs. 55.37%). STEO showed strong antibacterial efficacy against G<sup>-</sup> bacterial species, particularly *E. coli*, at the highest dose tested (65.70%). The results showed that *B. thuringiensis* growing on pears in a treatment with the highest dose applied (76.70%) and *P. megaterium* in a treatment with the highest concentration utilized (76.73%). The results of antimicrobial activity in vapour phase of STEO against G<sup>-</sup> showed the lowest efficacy of STEO in suppressing the growth of *E. coli*. On vegetable model the best antimicrobial activity of STEO was found against *P. megaterium* on carrots with lowest concentration of STEO (86.08%). The best antimicrobial activity was found in carrot model against *C. koseri* in lowest concentration (73.37%). On the kohlrabi model against *B. thuringiensis* (93.48%) the best antimicrobial activity in lower concentration was found.

The use of EOs in vapour phase, which may be more successful than EOs in liquid phase, is a more modern notion with potential for food fumigation

and preservation (Inouye, 2003; Netopilova et al., 2021; Tyagi & Malik, 2011). Since EOs are volatile, they can be used in the vapour phase without coming into contact with food; nevertheless, further testing is still necessary to determine their toxicity and allergenicity. Since heat can change the components of EOs (Laird & Phillips, 2012; Su et al., 2007) and using water-based media, like agar or broth, can lessen their antibacterial activity (Houdkova & Kokoska, 2020; Laird & Phillips, 2012), EO vapours should be distributed by natural evaporation. Over time, the antibacterial activity may also decrease (Fisher et al., 2009; Su et al., 2007). Romeo et al. (2010) discovered that sealed plastic bags were less effective at preventing oxygen diffusion and bacterial survival when applied to grated carrots treated with essential oils of lemon verbena, cypress, and lemon balm. The antibacterial action of EO vapours may be enhanced by high aw or a saturated damp environment (Houdkova & Kokoska, 2020).

Obaidat & Frank (2009) assessed the effects of carvacrol vapour and cinnamon aldehyde at 20, 40, and 80 µL.L<sup>-1</sup> of air on whole and chopped spinach and lettuce that were sealed in jars at high relative humidity levels against *E. coli* O157:H7. All leaf tissues showed a reduction in pathogen populations after being exposed to the EO vapours, with intact lettuce showing the highest inactivation. The action of cinnamaldehyde, carvacrol, and allyl isothiocyanate (a chemical found in horseradish and mustard) against a five-strain cocktail of *Salmonella* and a four-strain cocktail of *E. coli* O157:H7 on sliced and whole tomatoes was also investigated by Obaidat & Frank, 2009. The lowest concentration of each antimicrobial agent deactivated *Salmonella* on whole tomatoes to levels below detection. At 25 °C, carvacrol and cinnamondehyde showed very modest effects on sliced tomatoes but inactivated pathogens on entire tomatoes, indicating that larger quantities of these compounds were needed at higher temperatures. In our study the highest concentration (500 µg.L<sup>-1</sup>) was most effective against bacterial strains on fruits model and lowest concentration (62.5 µg.L<sup>-1</sup>) on vegetable model.

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

Microorganisms	Concentration of EO ( $\mu\text{g.L}^{-1}$ )			
	62.5	125	250	500
<b>Apple</b>				
<i>Bacillus subtilis</i>	32.23 $\pm$ 0.49 <sup>a</sup>	44.40 $\pm$ 2.59 <sup>a, c, d</sup>	55.26 $\pm$ 1.61 <sup>a</sup>	67.25 $\pm$ 1.78 <sup>a</sup>
<i>Bacillus thuringiensis</i>	25.74 $\pm$ 1.80 <sup>b, c, e</sup>	34.39 $\pm$ 2.10 <sup>b, d</sup>	46.14 $\pm$ 1.19 <sup>b, e</sup>	55.37 $\pm$ 2.14 <sup>b, e</sup>
<i>Priestia megaterium</i>	25.71 $\pm$ 1.11 <sup>b, c, e</sup>	46.63 $\pm$ 1.58 <sup>a, c, d</sup>	55.07 $\pm$ 1.58 <sup>a, c, d</sup>	65.74 $\pm$ 1.10 <sup>a, c, d</sup>
<i>Citrobacter koseri</i>	34.41 $\pm$ 0.46 <sup>a, d</sup>	46.07 $\pm$ 1.63 <sup>a, c, d</sup>	55.29 $\pm$ 1.56 <sup>a, c, d</sup>	65.41 $\pm$ 2.21 <sup>a, c, d</sup>
<i>Enterobacter aerogenes</i>	25.33 $\pm$ 1.71 <sup>b, c, e</sup>	36.48 $\pm$ 1.68 <sup>b, d</sup>	44.03 $\pm$ 1.52 <sup>b, e</sup>	55.92 $\pm$ 1.67 <sup>b, e</sup>
<i>Escherichia coli</i>	33.33 $\pm$ 1.07 <sup>a, d</sup>	44.00 $\pm$ 0.59 <sup>a, c, d</sup>	54.58 $\pm$ 1.07 <sup>a, c, d</sup>	65.70 $\pm$ 2.07 <sup>a, c, d</sup>
<b>Pear</b>				
<i>Bacillus subtilis</i>	27.81 $\pm$ 0.12 <sup>a</sup>	43.63 $\pm$ 1.10 <sup>a</sup>	56.67 $\pm$ 2.06 <sup>a</sup>	67.77 $\pm$ 2.01 <sup>a</sup>
<i>Bacillus thuringiensis</i>	35.04 $\pm$ 1.56 <sup>b, c, d</sup>	45.63 $\pm$ 1.23 <sup>a, b, d</sup>	56.66 $\pm$ 1.17 <sup>a, b, d</sup>	76.70 $\pm$ 2.10 <sup>b, c</sup>
<i>Priestia megaterium</i>	34.34 $\pm$ 1.46 <sup>b, c, d</sup>	55.81 $\pm$ 1.01 <sup>c</sup>	64.03 $\pm$ 2.51 <sup>c</sup>	75.91 $\pm$ 2.69 <sup>b, c</sup>
<i>Citrobacter koseri</i>	32.56 $\pm$ 1.17 <sup>b, c, d</sup>	44.37 $\pm$ 1.12 <sup>a, b, d</sup>	53.78 $\pm$ 2.01 <sup>a, b, d</sup>	63.77 $\pm$ 1.05 <sup>a, d</sup>
<i>Enterobacter aerogenes</i>	24.11 $\pm$ 1.54 <sup>a, e</sup>	33.34 $\pm$ 1.06 <sup>e</sup>	44.11 $\pm$ 2.01 <sup>e</sup>	55.66 $\pm$ 1.02 <sup>e</sup>
<i>Escherichia coli</i>	24.96 $\pm$ 2.33 <sup>a, e</sup>	35.29 $\pm$ 2.22 <sup>e</sup>	46.65 $\pm$ 1.28 <sup>e</sup>	54.93 $\pm$ 0.68 <sup>e</sup>
<b>Carrot</b>				
<i>Bacillus subtilis</i>	75.41 $\pm$ 1.66 <sup>a</sup>	55.20 $\pm$ 2.53 <sup>a</sup>	43.70 $\pm$ 0.12 <sup>a</sup>	36.42 $\pm$ 1.57 <sup>a</sup>
<i>Bacillus thuringiensis</i>	67.18 $\pm$ 2.14 <sup>b, e</sup>	54.11 $\pm$ 0.52 <sup>a, b, d</sup>	46.78 $\pm$ 1.11 <sup>a, b</sup>	35.74 $\pm$ 1.06 <sup>a, b</sup>
<i>Priestia megaterium</i>	86.08 $\pm$ 1.65 <sup>c</sup>	67.07 $\pm$ 2.13 <sup>c</sup>	55.60 $\pm$ 1.22 <sup>c</sup>	45.96 $\pm$ 1.78 <sup>c</sup>
<i>Citrobacter koseri</i>	73.37 $\pm$ 1.53 <sup>a, d</sup>	56.85 $\pm$ 1.01 <sup>a, b</sup>	34.74 $\pm$ 2.10 <sup>d, e</sup>	16.40 $\pm$ 1.69 <sup>d</sup>
<i>Enterobacter aerogenes</i>	66.04 $\pm$ 1.63 <sup>b, e</sup>	44.70 $\pm$ 1.00 <sup>e</sup>	33.81 $\pm$ 1.71 <sup>d, e</sup>	23.51 $\pm$ 2.30 <sup>e</sup>
<i>Escherichia coli</i>	65.99 $\pm$ 1.78 <sup>b, e</sup>	56.62 $\pm$ 1.12 <sup>a, b, d</sup>	33.26 $\pm$ 1.47 <sup>d, e</sup>	14.72 $\pm$ 2.19 <sup>d</sup>
<b>Kohlrabi</b>				
<i>Bacillus subtilis</i>	86.19 $\pm$ 2.37 <sup>a</sup>	76.29 $\pm$ 1.62 <sup>a</sup>	64.89 $\pm$ 2.30 <sup>a</sup>	55.13 $\pm$ 2.62 <sup>a</sup>
<i>Bacillus thuringiensis</i>	93.48 $\pm$ 2.33 <sup>b</sup>	76.22 $\pm$ 1.70 <sup>a, b</sup>	56.62 $\pm$ 1.12 <sup>b, c</sup>	35.34 $\pm$ 1.25 <sup>b, c</sup>
<i>Priestia megaterium</i>	85.53 $\pm$ 2.08 <sup>a, c, d</sup>	67.51 $\pm$ 1.48 <sup>c, d</sup>	55.11 $\pm$ 1.50 <sup>b, c</sup>	35.37 $\pm$ 1.23 <sup>b, c</sup>
<i>Citrobacter koseri</i>	85.74 $\pm$ 1.76 <sup>a, c, d</sup>	65.57 $\pm$ 2.15 <sup>c, d</sup>	45.03 $\pm$ 1.10 <sup>d</sup>	23.03 $\pm$ 1.46 <sup>d</sup>
<i>Enterobacter aerogenes</i>	74.63 $\pm$ 1.06 <sup>e</sup>	56.44 $\pm$ 1.63 <sup>e</sup>	35.28 $\pm$ 2.37 <sup>e</sup>	14.74 $\pm$ 1.79 <sup>e</sup>
<i>Escherichia coli</i>	77.03 $\pm$ 1.52 <sup>e</sup>	54.80 $\pm$ 1.79 <sup>e</sup>	37.07 $\pm$ 1.13 <sup>e</sup>	16.85 $\pm$ 1.01 <sup>e</sup>

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

A combination of sweet orange (*Citrus sinensis*) and bergamot (*Citrus bergamia*) EO vapours was tested against vancomycin-resistant (VR) and vancomycin-susceptible (VS) *Enterococcus faecium* and *Enterococcus faecalis* on lettuce and cucumber in a separate study by Fisher et al. (2009) and on the quality and sensory qualities of sweet cherries in a study by Maghenzani et al. (2018) that examined the effects of thyme (*Thymus vulgaris*) and savory (*Satureja montana*) EOs at 4.5 or 18 mg.mL<sup>-1</sup>, respectively. During storage at 20 °C, EO treatment dramatically decreased rot; 4.5 mg.mL<sup>-1</sup> thyme EO proved to be the most successful treatment, reducing rotting fruit by 61.9% when compared to the control.

#### 4 Conclusions

There are arguments in favour of EOs acting as excellent disinfectants. However, due to undesirable sensory effects, only very low doses of EOs can be used. As long as foodborne pathogens on fresh produce do not exceed an organoleptic threshold that would make them unacceptable to consumers, different EOs or combinations of EOs could be useful in reducing foodborne pathogens. Instead of washing fresh foods with thinner surfaces, such as leafy vegetables, or freshly cut produce that could result in browning, whole produce with thicker skin and/or rind would be more suitable for treatment with EO wash water. Future research must therefore focus on the highest acceptable concentrations of EOs that preserve their bactericidal effects without adversely

affecting organoleptic properties. While EOs are used in combination with other therapies (the 'barrier concept'), they can be very useful in controlling pathogens, while used alone they are not. Fresh foods can be safely and effectively disinfected using small amounts of EOs, but cost considerations must also be taken into account. When applied to different bacterial strains, STEO was only somewhat effective, but when applied to both tested bacterial groups in the vegetable model at lower concentrations, it was extremely successful.

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