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Assessment of synergistic effects on antimicrobial activity in vapour- and liquid-phase of cinnamon and oregano essential oils against *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is an important human pathogen. In the present work, the bacterial inhibition efficacy of cinnamon and oregano essential oils (EOs) was evaluated and compared by using a disc volatilisation assay and a 96-well microplate assay. The comprehensive synergistic effects of their combinations were also indicated for eleven possible mixtures of cinnamon and oregano EOs at six dilution concentrations in both assays. The inhibition zones in the vapour-phase at the highest test concentration of EOs (160 µL/L air) varied from 31.67 ± 0.58 mm to 44.67 ± 0.58 mm. The minimum inhibitory concentration (MIC) in the volatilisation assay was indicated at 5 µL/L air when the ratios of cinnamon and oregano EOs were 8:2 and 9:1, which also showed strong synergistic activities (FIC = 0.25). *S. aureus* was more sensitive to EOs in the vapour-phase than in the liquid-phase due to lower MICs in the vapour-phase. Time kill assays indicated that *S. aureus* was inhibited by oregano EO after exposure at 30 µL/L for 120 min. The optimal combinations of the EOs in the vapour phase have potential for applications in medical development or food preservation.

Introduction

As a bacterial pathogen, *Staphylococcus aureus* causes many health problems from localised infections such as skin infections, cellulitis, myositis, and pneumonia, to systemic infections such as bacteraemia and sepsis (Kurlenda and Grinholc, 2012). In terms of foodborne illness, *S. aureus* is the leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of staphylococcal enterotoxins formed in food (Loir *et al*., 2003). Antibiotic resistant strains, specifically methicillin resistant ones, make medical treatment ineffective and cause problems for medicinal therapy (Ito and Hiramatsu, 2003). In terms of food safety, chemical preservatives have been employed to prevent spoilage and foodborne pathogens. In recent years, however, concerns about using synthetic compounds as preservatives in foods have been expressed by consumers. Therefore natural antimicrobials, e.g. medicinal plant extracts and essential oils (EOs), have recently attracted great scientific interest. The EO of cinnamon (*Cinnamomum zeylanicum* Blume) contains 66.28%–81.97% *trans*-cinnamaldehyde (Li *et al*., 2013) that contributes to its antimicrobial activity against a wide range of microbes (Gill and Richard, 2004; Ooi *et al*., 2006; Sanla-Ead *et al*., 2006). Carvacrol (26.7%), *p*-cymene (15.2%) and γ-terpinene (15.1%) are the main components of oregano (*Origanum vulgare* L.) EO (Inouye *et al*., 2001). Cinnamon EO has strong inhibitory effects against *Haemophilus influenzae*, *Streptococcus pyogenes, Streptococcus pneumonia, Escherichia coli,* and *S. aureus* at minimum inhibitory doses of 3.13, 6.25, 3.13, 12.5 and 6.25 mg/L air, respectively (Inouye *et al*., 2001). By studying the antibacterial effect of lavender and oregano EOs, Martucci *et al*. (2015) found that *S. aureus* was more sensitive to the selected EOs than *E. coli*. Cinnamon and oregano

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have been applied in the food industry to prevent contaminated spoilages and pathogens (Burt, 2004).

The antimicrobial activity of EOs in direct-contact assays such as dilution or diffusion methods are presented in several reviews (Burt, 2004; Holley and Patel, 2005). However, the activities of essential oils may decrease due to the effects of emulsifiers and solvents (Remmal *et al*., 1993; Yadav *et al*., 2012). Furthermore, high hydrophobic and volatile components of EOs cause problems for direct-contact assays to assess the antimicrobial activity of EOs accurately. A vapour-contact assay seems to be a promising method for the study of the antimicrobial ability of EOs.

The combinations of certain EOs or their major compositions are reported to display synergistic effects on microbial inhibition (Sukatta *et al*., 2008; Goñi *et al*., 2009; Pei *et al*., 2009). Goñi *et al*. (2009) found that a combination of cinnamon and clove EOs showed a synergistic effect on the inhibition of *Listeria monocytogenes*, *Bacillus cereus*, and *Yersinia enterocolitica* when the maximal inhibition concentrations of the EOs were used. Furthermore, MICs of EO components including eugenol, cinnamaldehyde, thymol, and carvacrol decreased to 400, 100, 100 and 100 mg/L, respectively, by means of combination (Pei *et al*., 2009). It is therefore vital to evaluate the synergistic effects of EOs since the applied concentrations could be reduced significantly by using an optimal combination.

The present work was aimed to screen the antimicrobial activities of cinnamon and oregano EOs by adapting a microdilution assay and a disc volatilisation assay. The synergistic properties of the EOs were identified by producing eleven different combinations. Comparisons and explanations of antimicrobial activities and synergistic effects between the vapour- and liquid-phases of EOs were also examined in the present work.

Materials and methods

Microorganisms and inoculum preparation

Staphylococcus aureus DMST 8840 (ATCC 25923) was obtained from the Department of Medical Science, National Institute of Health, Ministry of Public Health, Nonthaburi, Thailand. The *S. aureus* was subcultured twice in Müeller Hinton broth (HiMedia, Mumbai, India) at 37°C for 18−24 h. Cells were harvested by centrifuging at 6,000 g for 3 min and washed once with sterile NaCl solution (0.85%). Sodium phosphate buffer (0.1 mol/L) was used to dilute the cell suspension to obtain a cell concentration of 10^8 CFU/mL.

Chemicals and essential oils

Cinnamon EO (Sri Lanka) and oregano EO (Spain) were obtained from Botanic Essence Co., Ltd. (Bangkok, Thailand). Müeller Hinton agar (MHA), Müeller Hinton broth (MHB) (HiMedia), resazurin (BDH Laboratory Supplies, Leuven, Belgium) and Whatman No. 1 filter paper were used in the antimicrobial assays.

Disc volatilisation assay

The antimicrobial activity of EOs in the vapour-phase was determined by employing a modified disc volatilisation assay (López *et al*., 2005). Briefly, MHA plates were prepared, sterilised, inoculated with 10^8 CFU/mL bacterial suspension, and spread evenly using a sterile cotton swab. Eleven combinations of cinnamon–oregano EOs namely 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 and 10:0 (Sukatta *et al*., 2008) were diluted in ethyl acetate to obtain two-fold serial dilutions. Next, 10 µL each dilution was completely impregnated onto a 10-mm diameter sterile blank filter disc which was then placed at the centre of inoculated MHA plates. The EO concentration in the headspace varied from 5 to 160 µl/L. A filter disc containing 10 µL ethyl acetate served as control. After the discs were dried under aseptic conditions for 1 min, the inoculated plates were sealed with sterile adhesive tape and incubated at 37°C for 24 h. The minimum inhibitory concentrations (MICs) were determined following incubation, which showed the amount of EOs per litre of the headspace atmosphere above the agar surface that could inhibit *S. aureus* and create an apparent inhibition zone. The diameter of inhibition zones was also recorded.

Following incubation, the antimicrobial atmosphere was removed by replacing the lid of a plastic dish with a blank one to check whether the effects were bacteriostatic or bactericidal. After incubation at 37°C for 14 d, if bacterium started to grow from the clear zone, the effect was bacteriostatic, whereas if no growth was observed, the effect was bactericidal (the Minimum Bactericidal Concentrations, MBCs, were recorded). The test was performed in three replicates.

Broth microdilution assay

A broth microdilution method modified from Sarrazin *et al*. (2012) was used to test the antimicrobial effects of EOs by using 96-well microplates covered with lids with condensation rings to prevent cross-contamination of vapour of EOs, and the lid of the microplate was sealed with Parafilm after covering. Eleven combinations of cinnamon–oregano EOs including 0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2,

9:1 and 10:0 (Sukatta *et al*., 2008) were diluted in 1% Tween 80 to obtain two-fold serial dilutions. EO concentrations in the wells were 0.25, 0.5, 1, 2, 4, and 8 µL/mL. Each well contained 100 µL EOs at a specific concentration and 100 µL MHB inoculated with *S. aureus* (the final concentration was approximately 5×10^4 CFU/mL). The sterility control wells contained 200 µL sterile MHB, and no microorganisms. The positive solvent control wells were filled with 100 µL 1% Tween 80 instead of EO solution. The 96-well microplates were incubated at 37°C for 24 h. Following incubation, bacterial growth was indicated by adding 20 µL resazurin solution and incubation for 3 h. A change of solution colour from blue to pink showed the presence of *S. aureus*. Minimum inhibitory concentrations were the lowest concentrations of EO that could prevent the colour change of the resazurin to pink (Sarrazin *et al*., 2012).

The MBCs of EOs were determined by culturing onto MHA. Total suspension (approximately 220 µL) from the wells showing a blue colour was centrifuged at 6,000 g for 2 min. The supernatant was discarded and the bacterial cells were suspended in NaCl solution (0.85%). This step was carried out to remove the EOs. The suspension (5 µL) was then aliquoted onto MHA plates at three different points and incubated at 37°C for 24 h. The bactericidal concentration was the lowest concentration of EOs that could cause the microorganism to fail to grow on MHA. The test was conducted with three replicates.

Synergistic analysis

In order to determine the synergistic effect of the combination of cinnamon (A) and oregano (B) EOs, a fractional inhibition concentration index (FIC) was estimated. FIC for each combination was calculated by the following equations (Davidson and Parish, 1989):

$$FIC_A = \frac{(\text{MIC of A in presence of B})}{(\text{MIC of A})}$$

$$FIC_B = \frac{(\text{MIC of B in presence of A})}{(\text{MIC of B})}$$

$$FIC_{index} = FIC_A + FIC_B$$

$FIC_{index} < 1$: synergistic effect

$FIC_{index} = 1$: additive effect

$FIC_{index} > 1$: antagonistic effect

The effects of all combinations were graphically described by plotting isobologram using the mean FIC values from the triplicated experiments. FIC_A values were plotted (on the vertical axis) versus FIC_B (on the horizontal axis). In the isobologram, points of combination on straight line indicated additive effect, above the line antagonistic effect, and below the line synergistic effect (Ocana *et al*., 2012).

Time kill assay

A disc volatilisation assay was performed to determine the time kill of EOs in the vapour-phase (López *et al*., 2005). The strain was exposed to EOs (30 µL/L) of combinations 8:2, 9:1 and individual EO for 60, 120, 180, 240, 360, 420 and 1,440 min. The antimicrobial atmospheres were removed by changing the lids containing filter disc for a sterile lids. After the incubation period, kill times were determined as the shortest time resulting in a visible bacteria growth inhibition. The diameters of the inhibition zones were also recorded. The test was performed in triplicate.

Statistical analyses

The analyses were carried out in triplicate and results were expressed as mean ± standard deviation. Analyses of variance (ANOVA) were conducted, and differences among samples means were analysed by Duncan's multiple range test ($p < 0.05$) by using SPSS (IBM SPSS Statistics 19.0, IBM Corp. Armonk, NY., USA).

Results

Inhibition zones

The inhibition effects of different EO combinations in vapour-phase against *S. aureus* are presented in Table 1. All combinations showed antimicrobial activity against the pathogen as they were able to cause apparent halos on the surface of MHA. Generally, the diameters of the inhibition zones increased with increasing EO concentration. The values for the inhibition zone diameters ranged from 31.67 ± 0.58 mm to 44.67 ± 0.58 mm at 160 µL/L of EO, and were significantly different from the ones of other concentrations of EOs, except for the combination ratios 5:5, 7:3, 8:2, and 9:1.

Combinations of EOs exhibited higher antimicrobial potential than individual EOs. Individual cinnamon and oregano oils produced inhibition zones at 20 µL/L, and similar effects were observed in two combination ratios (2:8 and 1:9). An increase in cinnamon oil percentage in the other combinations showed a stronger action against *S. aureus* since the pathogen was inhibited at a lower concentration (10 µL/L).

Table 1 Diameter of inhibition zones ± standard deviation (mm) as evaluated by disc volatilization assay; Cin = cinnamon, Ore = oregano.

Ratios (v/v)		Concentrations (µL/L air)					
Cin	Ore	5	10	20	40	80	160
0	10	0.00^{e}	0.00^{e}	12.76 ± 0.58^{d}	34.67 ± 0.58^{c}	36.67 ± 1.53^{b}	44.67 ± 0.58^{a}
1	9	0.00^{e}	0.00^{e}	10.67 ± 1.16^{d}	30.00 ± 0.00^{c}	32.00 ± 0.00^{b}	35.00 ± 0.00^{a}
2	8	0.00^{e}	0.00^{e}	10.00 ± 0.00^{d}	25.33 ± 0.58^{c}	30.00 ± 0.00^{b}	31.67 ± 0.58^{a}
3	7	0.00^{f}	10.00 ± 0.00^{e}	24.67 ± 0.58^{d}	30.00 ± 0.00^{c}	33.67 ± 0.58^{b}	35.00 ± 0.00^{a}
4	6	0.00^{f}	10.67 ± 0.58^{e}	24.33 ± 1.16^{d}	28.00 ± 0.00^{c}	30.00 ± 0.00^{b}	35.33 ± 1.16^{a}
5	5	0.00^{e}	17.00 ± 0.00^{d}	27.33 ± 2.89^{c}	32.00 ± 0.00^{b}	32.67 ± 0.58^{ab}	34.67 ± 0.58^{a}
6	4	0.00^{f}	17.00 ± 0.00^{e}	29.33 ± 1.16^{d}	34.67 ± 0.58^{c}	36.00 ± 0.00^{b}	37.00 ± 0.00^{a}
7	3	0.00^{d}	22.67 ± 2.31^{c}	28.00 ± 0.00^{b}	34.33 ± 0.58^{a}	35.00 ± 0.00^{a}	36.00 ± 0.00^{a}
8	2	17.00 ± 1.73^{c}	24.67 ± 0.58^{d}	29.33 ± 1.16^{c}	33.33 ± 1.16^{b}	34.67 ± 0.58^{ab}	35.33 ± 0.58^{a}
9	1	19.00 ± 1.73^{d}	24.00 ± 1.73^{c}	27.00 ± 1.73^{b}	34.00 ± 1.73^{a}	34.33 ± 1.16^{a}	35.67 ± 0.58^{a}
10	0	0.00^{e}	0.00 ^{e}	20.67 ± 1.16^{d}	30.00 ± 0.00^{c}	36.67 ± 0.58^{b}	38.00 ± 0.00^{a}

Means ± SD followed by a different superscript letter within a row are significantly different ($p < 0.05$).

It is possible to state that cinnamon EO was more effective than oregano EO in the inhibition of *S. aureus*. In particular, when cinnamon and oregano were employed at the ratios 8:2 and 9:1, these combinations could render a halo on the surface of agar medium inoculated with *S. aureus* at a lower concentration of 5 µL/L. Figure 1 shows the apparent inhibition zones caused by these combinations of EOs. Therefore, the combinations of these EOs enhanced antimicrobial activity in a concentration-dependent manner.

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs)

Table 2 shows the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and fractional inhibition concentration (FIC) index of different combinations of EOs against *S. aureus* in the vapour- and liquid-phases. In general, *S. aureus* seemed to be more sensitive to the vapour of the EOs than liquid EOs. The MICs in the vapour-phase were considerably lower than those in the liquid-phase although the definition and the unit of MIC in the two methods are different. MICs in the vapour contact assay varied from 5 µL/L to 20 µL/L air while those in the microdilution assay ranged from 0.5 µL/L to 4 µL/L solution. The EOs in both phases shared the same trend of antimicrobial inhibition on *S. aureus*, although the microorganism seemed to be more sensitive to the combined EOs when the percentage of cinnamon oil was increased. However, the lowest MIC in the vapour-phase was obtained with the combination of 8:2 and 9:1 (MIC = 5 µL/L) and the ones in the liquid-phase were observed with the combinations of 9:1 and 10:0 (MIC = 0.5 µL/L). MBCs in the volatilisation assay were equal to MICs because the inhibition effects remained for at least 14 d after removing the antimicrobial atmosphere. For the microdilution assays, MBCs were higher than MICs in almost all combinations, except for the ratios 0:10, 1:9, 2:8 and 8:2. Hence, it could be inferred that the antimicrobial activity of the selected EOs in the vapour-phase could be achieved with a smaller amount of EO than that in the liquid-phase.

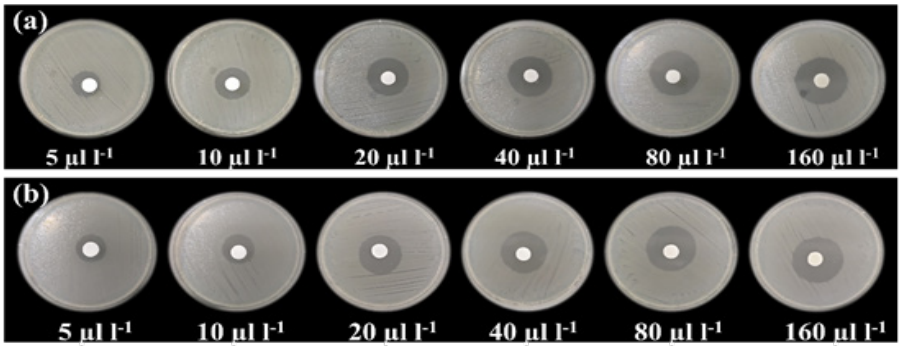

Figure 1 Inhibition zones against *S. aureus* with filter paper discs containing EOs of cinnamon and oregano at ratio (a) 8:2 and (b) 9:1.

Table 2 Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and fractional inhibition concentrations (FICs) index as evaluated by disc volatilization and broth microdilution assay; Cin = cinnamon, Ore = oregano.

Ratios (v/v)		MIC		MBC		FIC	
Cin	Ore	Vapour-phase (µL/L)	Liquid-phase (µL/L)	Vapour-phase (µL/L)	Liquid-phase (µL/L)	Vapour-phase	Liquid-phase
0	10	20	4	20	4	1	1
1	9	20	4	20	4	1	1.7
2	8	20	4	20	4	1	2.4
3	7	10	2	10	4	0.5	1.55
4	6	10	2	10	4	0.5	1.9
5	5	10	1	10	2	0.5	1.13
6	4	10	1	10	2	0.5	1.3
7	3	10	1	10	2	0.5	1.48
8	2	5	1	5	1	0.25	1.65
9	1	5	0.5	5	1	0.25	0.91
10	0	20	0.5	20	1	1	1

Synergistic effects

Davidson and Parish (1989) classified and described the synergistic effect of EOs and their antimicrobial components when they are combined. A synergistic effect is shown when the effect of the combination is higher than the sum of the effects of the individual components. If these values are equal, the combination shows an additive effect. Finally, an antagonistic effect is observed when the combination of EOs shows a lower antimicrobial effect in comparison with that of individual EOs.

Fraction inhibitory concentration indexes can be estimated and classified in different approaches (Bassolé and Juliani, 2012). In the present work, the FIC values of cinnamon and oregano EOs were calculated based on the MICs and these were plotted in isobolograms (Figure 2). Table 2 also shows the FIC index of the combinations of EOs. A synergistic effect was clearly obtained for the inhibition of *S. aureus* in the vapour-phase of EOs. Regardless of individual EO treatments, there were two combinations showing an additive effect (FIC index was equal to 1) including 1:9 and 2:8, while the remaining combinations contributed significantly to the improvement of the inhibition effects. A particularly strong synergistic activity was found for the combinations 8:2 and 9:1 (FIC = 0.25). Conversely, the combination of cinnamon and oregano EOs in the liquid-phase indicated antagonistic effects, and only the 9:1 combination gave a slightly synergistic inhibition (FIC = 0.91).

Cinnamon EO showed a higher contribution to the antimicrobial ability of the combined EOs in both methods since an increase in cinnamon ratios provided a higher synergistic effect. However, the vapour-phase was a more effective approach to combine these EOs for the inhibition of *S. aureus*.

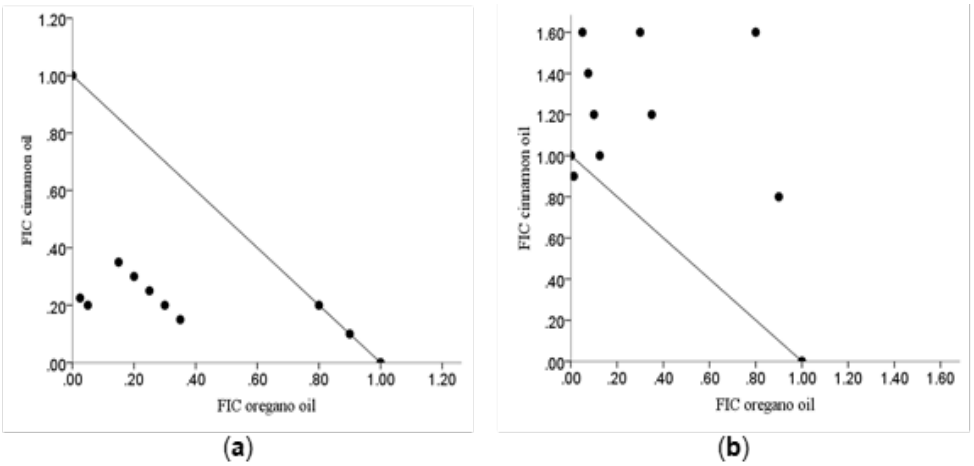

Figure 2 Isobolograms of cinnamon and oregano oils against *Staphylococcus aureus* in (a) vapour and (b) liquid phase.

Kill time assay

Since the combination ratios 8:2 and 9:1 exhibited high inhibition effects on *S. aureus*, it is essential to determine the optimum time of exposure of these combined EOs against the pathogen. Figure 3 shows that the kill time (KT) obtained at 30 µL/L for combined EOs and individual cinnamon EO was the same at 180 min, whereas *S. aureus* was inhibited at a shorter time (120 min) by exposure to oregano EO. Reducing the treatment time of EOs is vital, especially in the vapour-phase application, since it is complicated to maintain the volatility of EO compounds for a long time in real life.

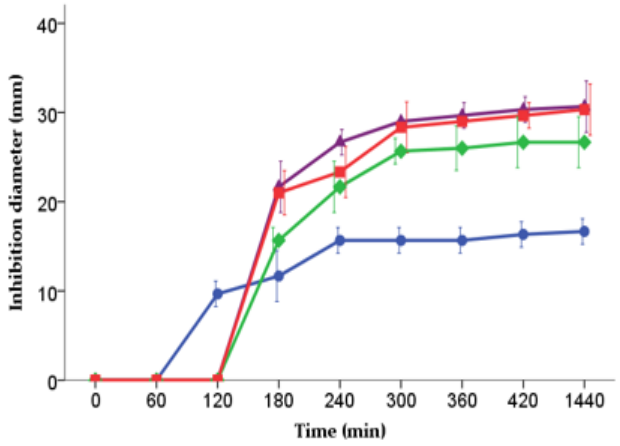

Figure 3 Inhibition diameter obtained by time kill assay at combined ratios of cinnamon:oregano oils ◆ (0:10); ■ (1:9); ▲ (2:8); and ● (10:0).

Overall, the antimicrobial potential of the EOs was exposure-time dependent since the diameter of inhibition increased with the extension of the exposure time. Unsurprisingly, the curve of the 9:1 combination ratio virtually overlapped with the one of cinnamon EO because the dominant component of this combination is cinnamon EO. It was observed that when the period of exposure was long enough, the inhibition zone reached a certain threshold. Although oregano EO was faster at producing an inhibition effect, cinnamon EO and combined EOs caused larger inhibition zones. Besides, the inhibition diameter for oregano EO reached the maximum value at 240 min while those of the other treatments were not reached in this time. The results demonstrated that oregano EO was more effective in terms of exposure time, but cinnamon presented a higher potential to inhibit *S. aureus* according to the larger diameters of the corresponding inhibition zones, which correlated well with the previous assay. The optimum combinations of EOs, however, did not present any advantages although they showed highly synergistic effects in the previous test. Therefore, further study on KT should be conducted to utilise these good combinations.

Discussion

The increase in *S. aureus* antibiotic resistant strains is a concern for medicinal therapy. In recent years, concerns about using synthetic compounds as preservatives in foods have been expressed by consumers, while EOs are classified as Generally Recognized As Safe (GRAS) substances by the U.S. FDA (FDA, 2013). Furthermore, the antimicrobial and antioxidant properties of EOs have been demonstrated in many studies (Friedman *et al*., 2002; Burt, 2004). Therefore, EOs could be alternatives to synthetic preservatives to prevent microbial contamination and prolong the shelf-life of foods. The present work shows a very strong activity of cinnamon EO, oregano EO and their combinations against a standard *S. aureus* strain since low MIC values were recorded. In a vapour-phase assay, these values ranged from 5 to 20 µL/L while the growth of *S. aureus* was inhibited by the liquid-phase oils at higher concentrations from 0.5 to 4 µL/L. Moreover, the vapour assay indicated that MBCs were equal to MICs in all combinations, suggesting an advantage in using EOs in the vapour-phase. These findings are in agreement with other studies which reported that certain EOs including oregano and cinnamon in the vapour-phase had a lethal effect on *S. aureus* and *Pseudomonas aeruginosa*, even in small amounts (Pibiri, 2006) and lemongrass oil had an inhibitory effect against *E. coli* in the vapour- and liquid-phases (Tyagi and Anushree, 2012). In addition, López *et al*. (2005) reported that their selected EOs had bactericidal effects on the test microorganisms in a disc volatilisation assay. Although Tween 80 used as emulsifier did not show any antimicrobial inhibition of *S. aureus* in the positive control of the liquid-phase test, the emulsifier might have some effect on the activity of EOs. In fact, the emulsification and solubilisation of EOs could cause their diminished antimicrobial ability (Remmal *et al*., 1993; Yadav *et al*., 2012). The findings obtained in the present work demonstrate the potential of EOs in vapour-phase applications. In term of food preservation, the effectiveness of EOs decreases when applied directly on food surfaces by dipping, spraying or powdering due to their binding to food components and affinity with water (Avila-Sosa *et al*., 2012). Therefore, active packaging containing EOs could avoid this problem and the results of the present work strongly support the application of EOs in active food packaging.

The present work also showed that strongly

synergistic activity in the vapour-phase could be achieved by mixing cinnamon and oregano EOs. The concentration of EOs was reduced 5-fold in the combination ratios 8:2 and 9:1 when compared with the amount of individual EOs needed to show a visible growth inhibition in the vapour-assay (5 and 20 µL/L, respectively). The MIC values of cinnamon and oregano EOs were approximately the same as those of other study which indicated that the MICs of cinnamon and oregano against *S. aureus* were 20 mg/L (Becerril *et al*., 2007). In another study, Goñi *et al*. (2009) found that the components of combined EOs in the vapour-phase were more similar to those of cinnamon vapour when mixed with cinnamon and clove EOs. This might be due to a difference in mode of action and the composition of antimicrobial agents in both assays. In liquid-phase, the antimicrobial activity is due to greater hydrophilic and less volatile substances. In vapour-phase, it depends on the volatility of compounds, and the high volatility properties of EO compounds contributes to their antimicrobial effect (Tyagi and Anushree, 2012). Therefore, the synergistic effects of the EOs could benefit the applications of EOs since lower concentrations of combined EOs could afford the same inhibition effect when compared with individual oils. This provides an effective strategy because the use of lower concentrations of EOs can reduce their negative effects on food sensory properties when applied as food preservatives.

Because each EO consists of two or three major components accounting for 20%–70% and several minor constituents, the antimicrobial potential of EOs is the result of the interaction between them (Delaquis *et al*., 2002; Burt, 2004). According to the literature, the antimicrobial activity and synergistic effects obtained from cinnamon and oregano can be explained as follows: carvacrol, a main constituent in oregano EO, was found by Ultee *et al*. (2000) to interact with the phospholipid bilayer and change the structure of *Bacillus cereus* membrane and increase bacterial cell membrane permeability (Lambert *et al*., 2001). Nowotarska *et al*. (2017) showed evidence confirming that antimicrobial compounds are targeting the cell membrane by incorporation into the monolayer, formation of aggregate/rafts of antimicrobials and lipids, and reduction in the packing effectiveness of the lipid molecules, resulting in an increase in membrane fluidity. Furthermore, cinnamaldehyde can inhibit the activity of enzymes related to cytokine interaction and cause less functional responses in cells when it is used at low concentrations. It can also inhibit the activity of ATPase at higher concentrations. At a lethal level, it is able to perturb the cell membrane (Nazzaro *et al*., 2013). The synergistic activity of thymol, cinnamaldehyde and carvacrol were studied by Zhou *et al*. (2007). The authors proposed two hypotheses explaining the synergistic activity of their combinations. First, thymol or carvacrol could increase the permeability of the cytoplasmic membrane, and probably enable cinnamaldehyde to be more easily transported into the cell. Second, thymol or carvacrol could increase the number, size or duration of pores created by cinnamaldehyde binding to proteins in the cell membrane, so that a synergistic effect is achieved when these two components are used in combination.

To the authors' knowledge, although cinnamon and oregano EOs were the topics of numerous studies on the antimicrobial activity of EOs, the antimicrobial and synergistic activity of their combinations is less known. In the present work, the comprehensive combinations of these oils were investigated in both vapour- and liquid-phases. The aim of the present work was achieved by finding the optimum combinations of cinnamon and oregano EOs in the vapour phase which showed high synergism, and these results could support the development of medicinal treatments or food preservation methods using EOs as antimicrobial agents. However, further study on reducing exposure time as well as the exact antimicrobial mechanisms is recommended to provide more information for possible applications of the EOs.

Conclusion

The present work evaluated and compared the antimicrobial activity of the combination of cinnamon and oregano essential oils at different ratios against *S. aureus* in vapour- and liquid-phases. The synergistic effects were also studied to indicate the optimal combinations of EOs for the inhibition of the pathogen. In general, the vapour-phase application was the most suitable approach to apply the combination of EOs since less concentration was required. In fact, MICs of the combination ratios 8:2 and 9:1 were very low at 5 µL/L air. Moreover, these combinations showed high synergistic effects with FIC = 0.25. The results provide an alternative approach to using natural compounds for developing medical therapies or active packaging against *S. aureus*.

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