Effects of oregano, cinnamon, and sweet fennel essential oils and their blends on foodborne microorganisms


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Abstract

Essential oils (EO) have stood out for their potential application as antimicrobial agents, playing an important role in ensuring food quality and safety. In addition, the synergistic effect of EO blends can improve action spectrum for more effective applications. The purpose of this research was to evaluate the in vitro antimicrobial activity of oregano, sweet fennel, and cinnamon EOs, and their blends, against the pathogenic bacteria Escherichia coli and Staphylococcus aureus, and the fungus Penicillium spp. The antimicrobial activity was determined by the disk-diffusion method, at both microbial optimal growth temperature and refrigeration temperature. Different EO volumes were investigated, and an effective inhibition was observed for the concentration of 3 μL. Oregano EO has proven to be effective in all assays, when compared to the EO combinations.

Keywords

Food safety
Natural antimicrobials
Foodborne microorganisms
Antimicrobial activity
Commercial essential oils
Synergistic effect

Introduction

Food is normally susceptible to contamination by several pathogens, including bacteria and fungi. Many studies have shown the effectiveness of various essential oils (EO) against foodborne microorganisms (Viuda-Martos et al., 2012; Dussault, Vu and Lacroix, 2014; Szczepanski and Lipski, 2014). Furthermore, EO extracted from spices has been recognized as GRAS (Generally Recognized as safe) by the FDA (Burt, 2004). There is a growing interest in the industry and scientific community to study herbs and medicinal plants due to their antimicrobial, antioxidant, and aromatic properties (Bakkali et al., 2008).

Essential oils are volatile compounds synthesized in several plant organs, including flowers, buds, seeds, leaves, stems, or bark (Dvaranauskaite et al., 2009; Wannes et al., 2010; Lv et al., 2012; Hill et al., 2013), being rich in terpenoids and phenolic compounds (Rodriguez et al., 2007), which are responsible for their biological activity. Several antimicrobial agents, including EO, can be used in food or as active materials for food packaging, thus increasing the shelf life and safety of the products, by reducing or preventing growth of pathogenic and spoilage microorganisms.

The synergistic effects of the EO components have been widely studied, which can broaden the action spectrum for more effective applications, besides improving food safety. Thus, EO blends can enhance the antimicrobial effect against a wide range of microorganisms (Lopez et al., 2007). Therefore, the present study aimed to evaluate the antimicrobial effectiveness of cinnamon, oregano, and sweet fennel EOs, alone or in combination, against E.coli, S. aureus, and Penicillium spp.

Materials and Methods

Organisms and growth conditions

The microbial cultures were obtained from the American Type Culture Collection (ATCC) at Federal University of Viçosa, Minas Gerais, Brazil. The following organisms were studied: the gram-negative strain Escherichia coli (ATCC 1122), gram-positive strain Staphylococcus aureus (ATCC 6538), and fungal strain of the genus Penicillium spp. The microbial cultures were activated in brain heart infusion broth (BHI) for 24h at 36±2°C and 25±2°C for bacteria and fungi, respectively. After, the cultures were incubated in Plate Count Agar (PCA) (bacteria) and Potato-Dextrose-agar (BDA) (fungi), for 48h at 36±2°C, and 5 days at 25±2°C,
respectively. Inoculum was prepared by diluting cultures in peptone water.

**Essential oils**

Three commercial essential oils from plants were purchased from Ferquima® (Ferquima Indústria e Comércio Ltda, Vargem Grande Paulista, São Paulo). The characteristics of the EOs, including common name, major components, plant variety, and plant part used in the extraction are presented in Table 1. All EOs selected for this study were produced by distillation, and kept at room temperature in an amber glass bottles.

In vitro **antimicrobial activity**

Agar disk-diffusion method (CLSI 2003) was used to determine the antimicrobial activity of the EOs (cinnamon, sweet fennel, and oregano) against E.coli, S.aureus, and Penicillium spp.

A 100 µL suspension containing 10⁴ CFU/mL of bacteria and 10⁴ spores/mL of fungal spores was spread over the surface of Mueller-Hinton agar plates. Then, a sterile paper disk (Whatman, 10 mm in diameter) was placed on the agar surface, and EO was placed at the center of each disk. A sterile paper disk without addition of EO was used as negative control.

Different EO volumes were tested, as follows: 60, 20,15,10,8,5, and 3 µL. The plates inoculated with bacteria and fungal spores were incubated at 35 ±2°C for 48h, and 25 ±2°C for 5 days, respectively. In addition, plates inoculated with bacteria and fungi were both incubated at 8±2°C to simulate the refrigerated storage. After incubation, microbial growth was observed on the plates, and the inhibition zone diameters (colony-free perimeter) were measured with a caliper. Data were expressed as inhibition zones (cm), not including the disk area. Each experiment was performed in triplicate.

**Combination of essential oils**

The essential oils were blended in equal parts, as follows: oregano + sweet fennel (OF); oregano + cinnamon (OC); cinnamon + sweet fennel (CF); and cinnamon + sweet fennel + oregano (CFO). A volume of 3 µL of EO blends was used in the experiments, once it was the lowest effective dosage as determined in preliminary tests, thus reducing costs with EO.

**Statistical analysis**

The experiment was carried out in a completely randomized design. All determinations were carried out in three replicates, and the results were expressed as mean of each replicate. Differences between the results were evaluated by analysis of variance (ANOVA) using the F-test, and Tukey’s test was used to compare means at 5% probability, using the SAS® software 9.0 (SAS Institute Inc., NC, USA).

**Results and Discussion**

In general, the oregano EO exhibited the highest antimicrobial effect, followed by cinnamon EO and sweet fennel EO. Biological activities are dependent on the composition of essential oil. Some authors studied different components present in EO and found higher antimicrobial activity in carvacrol followed by eugenol and anethole (major component of sweet fennel EO) (Sleha et al., 2014; Wieczynska et al., 2016). Studies have also shown that EOs containing phenols as major components, such as carvacrol (major component of oregano EO) or eugenol (major component of cinnamon EO) presented the highest antimicrobial activity (Bassolé, and Juliani, 2012).

**Volumes of EO tested: 60 and 20 µL**

The volumes of EO of 60 and 20 µL have poured to the culture medium, thus it was not possible to determine the inhibition zones to confirm the antimicrobial effect against the bacteria and fungi for these volumes.

**Volumes of EO tested: 5, 10, and 15 µL**

The antimicrobial activity of the EO was evaluated using the disc-diffusion method (Table 2). The higher the volume of EO, the greater the inhibition zone, thus the volumes of 10 and 15 µL EO showed higher antimicrobial effectiveness when compared to 5 µL EO.

Both oregano and cinnamon EOs exhibited antimicrobial activity against all microorganisms in the temperatures tested. In general, oregano...
EO presented the highest effectiveness, followed by cinnamon EO, while the sweet fennel EO was less effective, as can be seen in Table 2. Similar results were observed by Dussault, Vu and Lacroix (2014), who studied the antibacterial properties of commercial essential oils (oregano > cinnamon > sweet fennel).

Refrigeration is probably the most popular form of food preservation, and, in this study, EO in combination with refrigeration temperatures prevented the growth of \textit{S. aureus} and \textit{Penicillium} spp, and decreased growth of \textit{E. coli} at 8 ± 2°C for 10 days. A greater antimicrobial effect was observed against Gram-positive bacteria as \textit{S. aureus}, when compared with the gram-negative strains as \textit{E. coli}, as also reported by several authors (Zinoviadou, Koutsoumanis and Biliaderis, 2009; Viuda-Martos et al., 2012; Teixeira et al., 2013). The lower antimicrobial activity against the gram-negative strains may be due to a higher resistance of these microorganisms provided by the external lipopolysaccharide wall surrounding the peptidoglycan cell wall (Zinoviadou, Koutsoumanis and Biliaderis, 2009). In this study, the cinnamon EO inhibited both the fungal growth and the production of spores, which was also observed by Nielsen and Rios (2000), and Xing et al. (2010) for \textit{Aspergillus flavus}.

### Table 2. Mean inhibition zone diameters (cm) for the different dosages (5, 10, and 15 μL) of essential oils against \textit{E. coli}, \textit{S. aureus}, and \textit{Penicillium} spp. at both optimum and refrigeration temperatures. Disc diameter is not included.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Temperature</th>
<th>Cinnamon EO</th>
<th>Sweet fennel EO</th>
<th>Oregano EO</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. coli}</td>
<td>35 ± 2°C</td>
<td>1.5</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>\textit{S. aureus}</td>
<td>35 ± 2°C</td>
<td>1.4</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>\textit{Penicillium} spp</td>
<td>25 ± 2°C</td>
<td>3.4</td>
<td>5.0*</td>
<td>5.0*</td>
</tr>
</tbody>
</table>

*Volumes of EO tested: 3, 5, and 8 μL

Whereas no significant (p > 0.05) differences were observed for lower volumes of EO (3, 5, and 8 μL) (data not shown), the lowest effective volume (3 μL) was used throughout the experiment, once similar results can be achieved at lower costs. Oregano EO was more effective against the microorganisms evaluated, followed by cinnamon EO and sweet fennel EO (Table 3), with no significant differences (p > 0.05) between cinnamon and sweet fennel essential oils against \textit{S. aureus} and \textit{Penicillium} spp. All EO exhibited low antimicrobial activity against \textit{E. coli}, except sweet fennel EO that had no activity against this microorganism. Souza and others (2013) and Goñi and others (2009) studied the inhibition of \textit{Aspergillus flavus} and \textit{Aspergillus niger}, and \textit{E. coli} and \textit{S. aureus} by oregano EO, respectively, and found similar results of this study.

### Table 3. Mean inhibition zone diameters (cm) for the antimicrobial activity of 3 μL EOs against \textit{E. coli}, \textit{S. aureus}, and \textit{Penicillium} spp. Disc diameter is not included.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Essential Oils</th>
<th>Cinnamon</th>
<th>Sweet fennel</th>
<th>Oregano</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Escherichia coli}</td>
<td>0.36*</td>
<td>0.06*</td>
<td>2.17*</td>
<td></td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>3.03*</td>
<td>3.66*</td>
<td>5.33*</td>
<td></td>
</tr>
<tr>
<td>\textit{Penicillium spp.}</td>
<td>4.53*</td>
<td>3.60*</td>
<td>6.20*</td>
<td></td>
</tr>
</tbody>
</table>

*Means within columns followed by the same superscript lower case letter, and means within rows followed by the same superscript uppercase letter are not significantly different from each other (p > 0.05), by Tukey test.
exhibited an improved effect against *E. coli*, while the blend containing sweet fennel EO + oregano EO showed lower effect against *Penicillium* spp.

The positive and negative interactions of EO may depend on both the microorganisms studied and the ratio of the blend components (Harris *et al.* 2002; Radulović *et al.* 2007; Bassolé and Juliani, 2012). When analyzing the effects of EOs, either alone (Table 3) or in combination (Table 4), on the microbial growth, although the best results were found for oregano EO against all microorganisms, lower antimicrobial effects were observed for the blends containing this essential oil, i.e., an antagonistic effect may occur when oregano EO is combined with sweet fennel EO and/or cinnamon EO.

The cinnamon EO exhibited less or no effect against the microorganisms studied when combined with sweet fennel EO, probably due to an antagonistic effect of these essential oils against *S. aureus* and *Penicillium* spp. An antagonistic effect occurs when the combined effect of two or more components is lower than their individual effects (Goñi *et al.*, 2009), while a synergistic effect increases the action spectrum, thus improving the effectiveness of the essential oils (Goñi *et al.*, 2009). No synergistic effect was observed between the combinations of EOs in the present study.

### Conclusion

Oregano essential oil was the most effective antimicrobial agent against bacteria and fungi, followed by cinnamon EO and sweet fennel EO. The essential oils in combination showed lower antimicrobial effect when compared to the oregano EO alone. It was observed that the dosage of 3 μL of essential oil was sufficient for inhibition of the microorganisms studied. Essential oils can be used as a substitute for synthetic chemicals in food preservation. An effective combination of essential oils against target microorganisms can lead to a reduction of the pronounced aroma of EO, without affecting the quality of the products.

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### References


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Table 4. Effect of the essential oils blends (1:1 or 1:1:1) on the microbial growth. Mean inhibition zone diameters (cm) for the antimicrobial activity of 3 μL EO blends against *E. coli*, *S. aureus* and *Penicillium* spp. Disc diameter is not included.

<table>
<thead>
<tr>
<th>Blends</th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Penicillium</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregano + Cinnamon</td>
<td>0.54*</td>
<td>1.53*</td>
<td>2.63*</td>
</tr>
<tr>
<td>Oregano + sweet fennel</td>
<td>0.50*</td>
<td>3.67*</td>
<td>0.8*</td>
</tr>
<tr>
<td>Oregano + Cinnamon + sweet fennel</td>
<td>0.57*</td>
<td>2.15*</td>
<td>3.41*</td>
</tr>
<tr>
<td>Cinnamon + sweet fennel</td>
<td>0.08*</td>
<td>0.08*</td>
<td>0.44*</td>
</tr>
</tbody>
</table>

*Means within columns followed by the same superscript lower case letter are not significantly different from each other (p> 0.05)


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Sleha, R., Mosio, P., Vyrchalova, M., Jantovska, A., Bostikova, V. and Mazurova, J. In vitro antimicrobial activities of cinnamon bark oil, anethole, carvacrol, eugenol and guaiazulene against *Mycoplasma hominis* clinical isolates. 2014. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslavik Republic 158: 208-211.


