Efficacy of plant essential oils against *E. coli* O157:H7, *Salmonella enterica* and *L. monocytogenes* in fruit juices

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Abstract: The antimicrobial and health benefits of essential oils (EOs) have been known for years, however, the research studies about their effectiveness and optimum concentrations against food pathogens is scarce. This study investigate the effectiveness of five EOs: lemongrass, cinnamon leaf, oregano, rosemary, and sage oils for control of growth and survival of *E. coli* O157:H7, *Salmonella enterica* and *L. monocytogenes*. Minimum inhibitory concentration (MIC) was measured using agar dilution method, which showed that oregano oil exhibited the highest MIC level (1µl/ml for all bacteria), followed in close levels by lemongrass and cinnamon, then rosemary and the weakest effect was shown from sage oil. Due to the un-satisfying results of sage oil, all other EOs were selected to determine their minimum bactericidal concentrations (MBC) against the bacteria in culture media (three fruit juices: apple, orange, strawberry juices and tryptic soy broth "TSB") using broth dilution method. Statistical analysis showed significant differences ($P < 0.05$) among EO concentrations and culture media. A concentration of 1µl/ml from oregano was required to inactivate *E. coli* in all juices, while 2µl/ml was required for inactivation of both *Salmonella enterica* and *L. monocytogenes*. In TSB, however, higher concentrations were required to inactivate the bacteria, reaching up to 4 µl/ml for *L. monocytogenes*. These studies provide information about EOs as possible natural alternative for food additives to promote the safety and quality of commercial fruit juices.
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1. Introduction

Outbreaks of diseases associated with fruit juice have been increasingly a public health problem since the early 1990s, such that the U.S. Food and Drug Administration (FDA) implemented strict control measures to regulate the production of fruit juice. The final juice regulation became effective in 2004, which required that juice manufacturers comply with a hazard analysis and critical control point (HACCP) plan. From 1995 through 2005, 21 outbreaks associated with juice in the USA were reported to the Centre for Disease Control (CDC, 1999), 10 of which are implicated apple juice or cider, 8 were linked to orange juice, and 3 linked to other types of fruit juice; the total number of illnesses were 1,366 cases. Among the 13 outbreaks of known etiology, 5 were caused by *Salmonella,* 5 by *Escherichia coli* O157:H7 (Vojdani *et al*., 2008). Bacteria may contaminate the juice by using fruits that have touched the soil, by using animal manure for plant fertilization, or by inadequate washing before processing (Buchanan *et al*., 1999). Even though the use of physical treatments (heat or irradiation) as a juice sanitizer can kill the contaminating bacteria, they also induce unacceptable changes in the fruit composition (Ekasari *et al*., 1989). Such changes in juices along with the increasing demand for unpasteurized juices, especially those designated organic juices, suggest the need to develop additional effective, food compatible antibacterial agents to protect the juice and the consumer against contamination by pathogens.

Essential oils, the volatile aroma compounds extracted from plant origin, have been shown to have antimicrobial activities against a wide range of microorganisms in juices (El-Shazly *et al*., 2002; Kalemba & Kunicka, 2003; Valero and Salmeron 2003; Helal *et al*., 2006; Knight and McKellar 2007, and Tajkarimi *et al*., 2010) and other foods (Hsieh *et al*., 2001; Mau *et al*., 2001; Mejlholm and Dalgaard 2002; Tayel & El-Tras 2009; Salem *et al*., 2010). EOs are regarded as alternatives to chemical preservatives, and are designated as Generally Regarded As Safe (GRAS) in the United States (Burt, 2004). Despite the safety and human healthy aspects of EOs, spices, and herbs, their industrial use as food preservatives is still scarce compared to the synthetic chemicals because of three main reasons: limited studies about their effects in food, strong odor and high cost (Tajkarimi *et al*., 2010).

The purpose of this study was to examine and quantify the antimicrobial activities of five plant EOs against the food borne pathogens namely, *E. coli*

O157:H7, *Salmonella enterica*, and *L. monocytogenes* in liquid TSB media and in different fruit juices.

2. Material and Methods

Bacterial strains and inocula preparation

Three bacterial strains were used for this study: *E. coli* O157:H7 ATCC 43888 (human feces isolate), *Salmonella enterica* serovar Agona (isolated from alfalfa seeds), and *L. monocytogenes* LCDC (isolated from raw cabbage). All strains were provided by Dr. M.P. Doyle, University of Georgia, USA. *E. coli* and *Salmonella enterica* were maintained on nutrient agar slant medium at 5ºC, whereas *L. monocytogenes* was in tryptic soy agar (TSA) slant medium at 5ºC. To prepare the inocula of *Salmonella enterica* and *E. coli*, they were grown on TSB at 37ºC for 11 hrs and 120 rpm (cell in early stationary phase), whereas *L. monocytogenes* was grown on TSB plus 0.6% of yeast extract (Biokar Diagnostics) at 35ºC for 18 hrs and the same shaking speed to reach the early stationary phase. The cultures were diluted using saline peptone water to reach a concentration of 10^2 to 10^3 in agar dilution method, and 10^6 cfu/ml in broth dilution assay.

Test essential oils.

The following plant essential oils were purchased from Sigma (St. Louis, Mo, USA). Test essential oils, mainly, lemongrass (*Cymbopogon citrates*), cinnamon (*Cinnamomum zeylanicum*) leaf, oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), and sage (*Salvia officinalis*).

Determination of MIC of EOs

The MIC of the tested EOs were determined against *E. coli, S. enterica* , and *L. monocytogenes* using the agar dilution method (Davidson and Parish, 1989) in TSA with some modifications as the following: A short heating (5 min at 100 °C) was applied after mixing the EO with TSA to facilitate the dispersion of the oil in the media. The final concentrations of EOs in the media were 1, 3, 5, and 10 µl/ml, and the strains were grown on TSA without EO as control. Bacteria were grown in tryptic soy broth (TSB) overnight at 37ºC, then adjusted to 10^2-10^3 cfu/ml using phosphate-buffered saline (PBS). A 100 µl of the diluted bacterial culture were spread on the TSA plates, and incubated at 35ºC for 24 hrs. The MIC was considered to be the lowest concentration to maintain or reduce the inoculum level.

Determination of MBC

Based on the results of the MIC assay, four EOs that showed a high level of bacterial inhibition: oregano, lemongrass, cinnamon, and rosemary, were used for the MBC assay in TSB and different juices (apple, orange, and strawberry juice). Broth dilution method reported by Davidson and Parish (1989) was used to determine the MBC with some modifications as follows: before autoclaving the media (TSB and different juices), a 2% (v/v) Tween 80 (Scharlau Chemie) was added in order to facilitate dispersion of the EOs. Lambert *et al*. (2001) reported that MIC or MBC are affected by the dispersion agent used, and that their values might be lower if this agent is absent.

In order to prepare the juice, the fruits were washed, peeled, cut into pieces, and blended (model No: CH-1, Ultimate Chopper, China), followed by centrifugation (Avanti J-25 centrifuge, Beckman Coulter, USA) at 12000 rpm for 15 min. After filtrating and autoclaving the supernatant, a 500µl aliquot of bacterial suspensions at a concentration of 10^6 cfu/ml and each of the different EOs with a final concentration of 0.5, 1, 2, 4, 6, 8, or 10µl/ml were added to each tube containing 450 µl of sterile TSB, apple, orange, or strawberry juices with 2% Tween 80. The control was prepared using the different media with 2% Tween 80 without EOs. All treatments were incubated at 35ºC for 24 hrs in order to mimic the abuse conditions by consumers in the handling such fruit juices. A 100µl aliquot was spread on TSA plates to determine bacterial count. The MBC was defined as the EO concentration that did not allow any growth of the tested bacteria on the plate. These experiments were performed in duplicate and replicated twice ($n = 4$).

Determination of pH

The pH values of apple, orange, strawberry juices and TSB media were determined with a pH meter (Orion 720, Thermo-Scientific, USA).

Statistical analysis

Results of the MBC were analyzed by multifactor analysis of variance (ANOVA), using SPSS 10.0 (SPSS Inc., Chicago, Ill.). The results of each microorganism were analyzed independently, and the evaluated factors were the EO concentrations (0.5, 1, 2, 4, 6, 8, and 10 µl/ml) and the type of culture media (apple, orange, strawberry juices and TSB).

3. Results and Discussion

Minimum Inhibitory Concentration

The five EOs used in this study have shown different levels of growth inhibition against the bacterial pathogens (Table 1) with most of the EOs showing an MIC between 1-3 µl/ml. In general, oregano oil showed the highest MIC level (1µl/ml for all bacteria), followed in close levels, were lemongrass and cinnamon, then rosemary, and the weakest effect was shown from sage oil. *L. monocytogenes* tended to be more sensitive to the EOs compared to *E. coli* and *Salmonella enterica*, especially with lemongrass which showed MIC of 1

µl/ml for *L. monocytogenes*, while showing an MIC of 3 µl/ml for both *E. coli* and *S. enterica*. Hammer *et al*. (1999) reported similar concentration (2.5 µl/ml) of lemongrass in order to inhibit *Salmonella typhimurium*. Also, similar results were found by Elgayyar *et al*. (2001) who determined the EO inhibition using the paper disc agar diffusion method and found that both *E. coli* O157 and *S. typhimurium* showed inhibition zones of 87mm, while *L. monocytogenes* showed an inhibition zone of only 53 mm. On the other hand, Duan and Zhao (2009) reported in his inhibitory study of EOs that the growth of *E. coli* O157:H7 and *S. enterica* **ser.** Enteritidis was completely inhibited when the concentration of lemongrass was increased to 3 µl/ml. In addition, Table (1) did not show much difference between *E. coli* and *S. enterica* in their response to the EOs, except for cinnamon, where the MIC were 3 µl/ml for *E. coli*, but only 1µl/ml for *S.* enterica. While these results are in agreement with those reported by Elgayyar *et al*.(2001) regarding the MIC of oregano and rosemary, Gutierrez *et al*. (2008) showed that the MIC of oregano against *E. coli* was clearly lower (350 ppm) than that of *S. enterica* (550 ppm). The obvious difference between the previously reported data might be a result of the experimental conditions, specific strains, microorganism resistances, or the EO's nature and manufacturers. For instance, Gutierrez *et al*. (2008) did his assay using absorbance-based microtiter plate method, while Elgayyar *et al*. (2001) used a filter paper disc diffusion method. On the other hand, researcher have used different solvents to be added with the EOs in the medium. Hammer *et al*. (1999) used Tween 20 to facilitate the miscibility of EOs within the medium, while Pattnaik *et al*. (1996) used sodium taurocholate. In addition to the previous differences, the MIC definition used by different researchers is a factor that may complicate the comparison among the published data. Some researchers define the MIC as the lowest concentration resulting in maintenance or reduction of inoculum viability, while others define it as the lowest concentration leading to a significant decrease (more than 90%) of the inoculum viability (Cosentino *et al*., 1999).

Minimum Bactericidal Concentration

The obtained results showed that the sage oil did not show effective inhibition against the tested microorganisms, therefore, it was excluded from the MBC assay, while the other EOs (oregano, lemongrass, cinnamon, and rosemary) were tested. Similarly to the results of the MIC assay, differences have been observed among the efficiency of oregano, lemongrass, cinnamon, and rosemary against the three microorganisms (Tables 2-4). Statistical analysis was performed independently for each microorganism, and revealed significant differences ($P < 0.05$) among EO concentrations,

and culture media. There were significant difference ($P < 0.05$) in MBC among the used media, as well as the bacterial counts resulted from the different concentrations of the same EO. Bactericidal concentration significantly differed ($P < 0.05$) among culture media for the same strain, where higher concentrations of EOs were required to eradicate the growth of each strain in TSB, and to less extent in apple media as compared to orange and strawberry media (Tables 2 through 4). Cinnamon exhibited efficiency at a concentration of 2 µl/ml in apple juice for the three microorganisms, except for *L. monocytogenes*, where the effective concentration was 4 µl/ml. These results are in accordance with those reported by Ceylan *et al*. (2004), who evaluated the antimicrobial activity of cinnamon with sodium benzoate or potassium sorbate against *E. coli* O157:H7 in apple juice and showed that cinnamon exhibited significant antimicrobial activity against *E. coli* O157:H7 in apple juice at concentrations of 1, 2, and 3 µl/ml. It's worthy of note that the three juices showed a bactericidal activity to different extents without EO (control) as compared to the TSB, with the average bacterial count for each medium with the different bacteria being 4.27, 3.33, 2.94 and 8.73 for apple, orange, strawberry and TSB, respectively. Such effect seems to be a consequence of the pH differences in the different media (apple, 4.2; orange, 3.85; strawberry, 3.53; TSB, 7.5). Raghubeer *et al*. (1994) showed that although viable cells of the inoculated *E. coli* O157:H7 were detectable in pH 4.51 salad dressing stored for 17 days at 4 °C, none was detectable in the pH 3.9 mayonnaise after 96 hrs storage at 22 °C, which highlights the clear effect of little pH change in the media on the microbial growth. Even though the pH effect on microorganisms is well known, other factors such as the fruit components may play a role as bactericidal agents.

Rosemary has shown the least bactericidal effect against all three microorganisms, requiring as high as 6 µl/ml to inactivate *E. coli* in all three juices (Table 2). In contrary, oregano exhibited the highest effect requiring only 1 µl/ml to inactivate *E. coli* in all three juices (Table 2), and *S. enterica* in orange and strawberry juices (Table 3), and as low as 0.5 µl/ml to inactivate *L. monocytogenes* in orange and strawberry juices (Table 4). Cinnamon and lemongrass were comparable in their effect on the three bacterial strains, showing medium bactericidal concentration between oregano and rosemary (Tables 2-4). Gutierrez *et al*. (2008) demonstrated a similar order of strength when he studied the inhibitory

effect of oregano and rosemary among other essential oils. They reported that the inhibitory concentrations of oregano and rosemary against *E. coli* were 350 and 12500 ppm, and against *Salmonella typhimurium* were 550 and 12500 ppm, respectively.

Table (2) showed that cinnamon was stronger (2 µl/ml) than rosemary (6 µl/ml) in destroying *E. coli* in apple juice as well as other media. This is supported by the findings of Friedman *et al*. (2004) when they determined the bactericidal activities (BA50 values) of several EOs against *E. coli* O157:H7 and *S. enterica* in apple juice. They found that the BA50 for cinnamon bark oil and lemongrass on *E. coli* O157:H7 added to apple Juice and incubated for 1hr at 37ºC were 0.089, and 0.079, respectively. Their data, however, differed with the present study when comparing the effect on *S. enterica* (Table 3), where they found that the BA50 values for cinnamon bark oil and lemongrass were 0.014 and 0.010, respectively. It's important to highlight that their experimental settings were different from the present study, as they measures the percentage of the oil that resulted in a 50% decrease in the number of bacteria (BA50) under the tested conditions. Additionally, the incubation period was only 60 min at 37ºC.

L. monocytogenes (Table 4) showed much lower MBC for oregano when compared with rosemary in the three juices (i.e., 2, 0.5, and 0.5 µl/ml for oregano, in contrast with 6, 4, and 4 µl/ml for rosemary in apple, orange, strawberry juices, respectively). Similar trend of EO strength was found by Gutierrez *et al*. (2008) who showed that the inhibitory concentrations of oregano and rosemary against *L. monocytogenes* were 75 and 4500 ppm, respectively. This work offers a contribution to the knowledge of MIC and MBC of lemongrass, cinnamon leaf, oregano, rosemary and sage necessary to eliminate the pathogenic bacteria such as *E. coli, Salmonella* and *Listeria* from unpasteurized fruit juices. Results suggest that EOs could be possible natural food additives that enhance the microbiological quality of fruit juices, while avoiding the possible risks of the synthetic preserving chemicals.

Table 1: Inhibitory effect of some EOs against *E. coli, Salmonella enterica,* and *L. monocytogenes*

6	Conc. (µl/ml)	Microorganisms (cfu/ml)		
		E. coli	***Salmonella enterica***	***L. monocytogenes***
Oregano	0	$8.30x10^2$	$9.60x10^2$	$2.75x10^3$
	1	$4.65x10^2$	$3.25x10^2$	$4.05x10^2$
	3	0	0	0
	5	0	0	0
	10	0	0	0
Lemongrass	0	$8.30x10^2$	$9.60x10^2$	$2.75x10^3$
	1	$5.45x10^2$	$3.70x10^2$	$2.80x10^2$
	3	$6.25x10^1$	$2.30x10^1$	0
	5	0	0	0
	10	0	0	0
Cinnamon	0	$8.30x10^2$	$9.60x10^2$	$2.75x10^3$
	1	$4.95x10^2$	$5.45x10^2$	$6.85x10^1$
	3	$4.50x10^1$	0	0
	5	0	0	0
	10	0	0	0
Rosemary	0	8.30x102	9.60x102	2.75x103
	1	6.10x102	6.65x102	1.90x103
	3	1.25x102	2.70x102	7.45x102
	5	3.30x101	4.55x101	3.60x102
	10	0	0	0
Sage	0	8.30x102	9.60x102	2.75x103
	1	8.20x102	8.95x102	1.15x103
	3	7.55x102	6.60x102	7.80x102
	5	6.85x102	3.30x102	2.35x102
	10	4.70x102	7.45x101	0

[a] EOs: Essential oils

Table 2: Effect of different concentrations of some EOs on *E. coli* in apple, orange, and strawberry juices, or TSB after incubation for 24hrs at 35ºC

EO [a]	Conc. (µl/ml)	Media [b] (juices and TSB) Apple	Orange	Strawberry	TSB
Oregano	0	4.94 A X	3.87 A X Y	3.79 A Y	8.85 A Z
	0.5	2.51 B X	2.18 B X	2.25 B X	3.22 B X
	1	<1 C X	<1 C X	<1 C X	2.43 B Y
	2	<1 C X	<1 C X	<1 C X	<1 C X
	4	<1 C X	<1 C X	<1 C X	<1 C X
	6	<1 C X	<1 C X	<1 C X	<1 C X
	8	<1 C X	<1 C X	<1 C X	<1 C X
	10	<1 C X	<1 C X	<1 C X	<1 C X
Lemongrass	0	4.94 A X	3.87 A X	3.79 A X	8.85 A Y
	0.5	2.72 B X	2.4 B X	1.83 B X	3.7 B X
	1	2.65 B X	2.06 B X	<1 C Y	3.52 B C X
	2	<1 C X	<1 C X	<1 C X	2.74 C Y
	4	<1 C X	<1 C X	<1 C X	1.68 D Y
	6	<1 C X	<1 C X	<1 C X	<1 E X
	8	<1 C X	<1 C X	<1 C X	<1 E X
	10	<1 C X	<1 C X	<1 C X	<1 E X
Cinnamon	0	4.94 A X	3.87 A X Y	3.79 A Y	8.85 A Z
	0.5	2.93 B X	2.44 B X	2.47 B X	3.62 B Y
	1	2.28 B X	1.45 C X	1.76 B X	3.3 B Y
	2	<1 C X	<1 D X	<1 C X	2.34 C Y
	4	<1 C X	<1 C X	<1 C X	<1 D X
	6	<1 C X	<1 C X	<1 C X	<1 D X
	8	<1 C X	<1 C X	<1 C X	<1 D X
	10	<1 C X	<1 C X	<1 C X	<1 D X
Rosemary	0	4.94 A X	3.87 A X Y	3.79 A Y	8.85 A Z
	0.5	2.86 B X	2.6 B X	2.43 B X	4.74 B Y
	1	2.57 B X	2.52 B X	2.27 B X	4.32 B C Y
	2	2.2 B C X	2.14 B X	2.04 B C X	3.65 C Y
	4	1.53 C X	1.28 C X	1.37 C X	2.61 D Y
	6	<1 D X	<1 D X	<1 D X	2.19 D Y
	8	<1 D X	<1 D X	<1 D X	1.2 E Y
	10	<1 D X	<1 D X	<1 D X	<1 F X

[a] EOs: Essential oils

[b] values are means of plate counts from two experiments, each in duplicate (n _ 4), expressed as log cfu per milliliter. Different capital letters (A through F) represent significant differences ($P < 0.05$) among EO concentrations for each medium. Different letters (X through Z) represent significant differences ($P < 0.05$) among culture media for the same EO.

Table 3: Effect of different concentrations of some EOs on *Salmonella enterica* in apple, orange, and strawberry juices, or TSB after incubation for 24hr at 35ºC

EO [a]	Conc. (µl/ml)	Media [b] (juices and TSB) Apple	Orange	Strawberry	TSB
Oregano	0	4.62 A X	3.45 A Y	3.15 A Y	8.71 A Z
	0.5	2.48 B X	2.16 B X	1.8 B X	3.45 B Y
	1	1.64 C X	<1 C Y	<1 C Y	2.78 B C Z
	2	<1 D X	<1 C X	<1 C X	2 C Y
	4	<1 D X	<1 C X	<1 C X	<1 D X
	6	<1 D X	<1 C X	<1 C X	<1 D X
	8	<1 D X	<1 C X	<1 C X	<1 D X
	10	<1 D X	<1 C X	<1 C X	<1 D X
Lemongrass	0	4.62 A X	3.45 A Y	3.15 A Y	8.71 A Z
	0.5	2.47 B X	1.76 B X	1.54 B X	4.22 B Y
	1	2.28 B X	1.3 B X	<1 C Y	3.68 B C Z
	2	1.85 B X	<1 C Y	<1 C Y	3.11 C Z
	4	<1 C X	<1 C X	<1 C X	1.43 D Y
	6	<1 C X	<1 C X	<1 C X	<1 E X
	8	<1 C X	<1 C X	<1 C X	<1 E X
	10	<1 C X	<1 C X	<1 C X	<1 E X
Cinnamon	0	4.62 A X	3.45 A Y	3.15 A Y	8.71 A Z
	0.5	2.17 B X	1.45 B X	1.38 B X	3.85 B Y
	1	1.56 B X	<1 C Y	<1 C Y	3.43 B Z
	2	<1 C X	<1 C X	<1 C X	2.2 C Y
	4	<1 C X	<1 C X	<1 C X	<1 D X
	6	<1 C X	<1 C X	<1 C X	<1 D X
	8	<1 C X	<1 C X	<1 C X	<1 D X
	10	<1 C X	<1 C X	<1 C X	<1 D X
Rosemary	0	4.62 A X	3.45 A Y	3.15 A Y	8.71 A Z
	0.5	3.4 B X	2.51 B X	2.33 A B X	5.83 B Y
	1	2.88 B C X Z	2.06 B C X Y	1.54 B Y	3.65 C Z
	2	2.62 B C X	1.3 C Y	<1 C Z	2.89 C D X
	4	2.27 C X	<1 D Y	<1 C Y	2.67 D X
	6	<1 D X	<1 D X	<1 C X	2.21 D E Y
	8	<1 D X	<1 D X	<1 C X	1.81 E F Y
	10	<1 D X	<1 D X	<1 C X	1.2 F Y

[a] EOs: Essential oils

[b] values are means of plate counts from two experiments, each in duplicate (n _ 4), expressed as log cfu per milliliter. Different capital letters (A through F) represent significant differences ($P < 0.05$) among EO concentrations for each medium. Different letters (X through Z) represent significant differences ($P < 0.05$) among culture media for the same EO.

Table 4: Effect of different concentrations of some EOs on *L. monocytogenes* in apple, orange, and strawberry juices, or TSB after incubation for 24hr at 35ºC

EO [a]	Conc. (µl/ml)	Media [b] (juices and TSB) Apple	Orange	Strawberry	TSB
Oregano	0	3.25 A X	2.65 A X Y	1.87 A Y	8.62 A Z
	0.5	2.7 A X	1.68 B X	1.24 A Y	4.53 B Z
	1	2.34 B X	1.2 B Y	<1 B Z	3.43 C X
	2	1.52 C X	<1 C Y	<1 B Y	2.28 D X
	4	<1 D X	<1 C X	<1 B X	2.13 D Y
	6	<1 D X	<1 C X	<1 B X	1.85 D E Y
	8	<1 D X	<1 C X	<1 B X	1.33 E Y
	10	<1 D X	<1 C X	<1 B X	<1 F X
Lemongrass	0	3.25 A X	2.65 A X Y	1.87 A Y	8.62 A Z
	0.5	2.29 B X	1.37 B X	<1 B Y	3.56 B Z
	1	2.07 B X	<1 C Y	<1 C Y	2.04 C X
	2	<1 C X	<1 C X	<1 C X	1.34 C Y
	4	<1 C X	<1 C X	<1 C X	<1 D X
	6	<1 C X	<1 C X	<1 C X	<1 D X
	8	<1 C X	<1 C X	<1 C X	<1 D X
	10	<1 C X	<1 C X	<1 C X	<1 D X
Cinnamon	0	3.25 A X	2.65 A X Y	1.87 A Y	8.62 A Z
	0.5	2.7 A X	1.68 B X	1.24 A Y	4.53 B Z
	1	2.34 B X	1.2 B Y	<1 B Z	3.43 C X
	2	1.52 C X	<1 C Y	<1 B Y	2.28 D X
	4	<1 D X	<1 C X	<1 B X	2.13 D Y
	6	<1 D X	<1 C X	<1 B X	1.85 D E Y
	8	<1 D X	<1 C X	<1 B X	1.33 E Y
	10	<1 D X	<1 C X	<1 B X	<1 F X
Rosemary	0	3.25 A X	2.65 A X Y	1.87 A Y	8.62 A Z
	0.5	2.61 B X	2.17 A X	1.56 A X	4.74 B Y
	1	2.43 B X	1.84 A X Y	1.2 A Y	4.35 B C Z
	2	2.09 B C X	<1 B Y	<1 B Y	3.9 C Z
	4	1.66 C X	<1 B Y	<1 B Y	3.62 C Z
	6	<1 D X	<1 B X	<1 B X	3.18 C D Y
	8	<1 D X	<1 B X	<1 B X	2.37 D Y
	10	<1 D X	<1 B X	<1 B X	<1 E X

[a] EOs: Essential oils

[b] values are means of plate counts from two experiments, each in duplicate (n _ 4), expressed as log cfu per milliliter. Different capital letters (A through F) represent significant differences ($P < 0.05$) among EO concentrations for each medium. Different letters (X through Z) represent significant differences ($P < 0.05$) among culture media for the same EO.

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References

Buchanan, R. L.; Edelson, S. G.; Miller, R. L.; Sapers, G. M. (1999): Contamination of intact apples after immersion in an aqueous environment containing *Escherichia coli* O157:H7. *J. Food Prot. 62*: 444-450.

Burt S. (2004): Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol., 94: 223–53.

CDC: Centers for Disease Control and Prevention. (1999): Outbreak of *Salmonella serotype muenchen* infections associated with unpasteurized orange juice— United States and Canada, June 1999. MMWR 48:582–5.

Ceylan, E.; Fung, D. Y. C.; and Sabah, J. R. (2004): Antimicrobial activity and synergistic effect of cinnamon with sodium benzoate or potassium sorbate in controlling *Escherichia coli* O157:H7 in apple juice. J. Food Sci. 69:102–106.

Cosentino, S.; Tuberoso, C. I. G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; and Palmas, F. (1999): *In vitro* antimicrobial activity and chemical composition of Sardinian and Thymus essential oils. Lett. Appl. Microbiol., 29:130–135.

Davidson, P. M., and Parish, M. E. (1989): Methods for testing the efficacy of food antimicrobials. Food Technol. 43:148–155.

Duan, J. and Zhao, Y. (2009): Antimicrobial efficiency of essential oil and freeze–thaw treatments against *Escherichia coli* O157:H7 and *Salmonella enterica* Ser. Enteritidis in Strawberry Juice. J Food Sci. 74: 131-137.

Ekasari, I.; Bonestroo, M. H.; Jongen, W. M. F.; Pilnik, W. (1989): Mutagenicity and possible occurrence of flavonol aglycones in heated orange juice. *Food Chem. 31*: 289-294.

Elgayyar, M.; Draughon, F.A.; Golden, D.A.; and Mount, J.R. (2001): Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot., 64: 1019–1024.

El-Shazly A.; Dorai, G.; and Wink, M. (2002): Composition and Antimicrobial Activity of Essential Oil and Hexane Ether Extract of *Tanacetum santolinoides*. Z. Naturforsch. 57c: 620-623.

Friedman, M.; Henika, P. R.; Levin, C. E.; and Mandrell, R. E. (2004): Bacterial activities of

plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. J. Agric. Food Chem. 52(19): 6042–6048.

Gutierrez, J.; Rodriguez, G.; Barry-Ryan, C., and Bourke, P. (2008): Efficacy of plant essential oils against food borne pathogens and spoilage bacteria associated with ready-to-eat vegetables: Antimicrobial and sensory screening. J. Food Prot. 71(9): 1846–1854.

Hammer, K. A.; Carson, C. F.; and Riley, T. V. (1999): Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86:985–990

Helal, G. A.; Sarhan, M. M.; Abu Shahla, A. N. K. and Abou El- Khair, E. K. (2006): Antimicrobial activity of some essential oils against microorganisms deteriorating fruit juices. Mycobiology. 34(4): 219-229.

Hsieh P-C, Mau JL; Huang SH. (2001): Antimicrobial effect of various combination of plant extract. Food Microbiol., 18:35–43.

Kalemba, D.; Kunicka, A. (2003): Antibacterial and antifungal properties of essential oils. Curr. Med. Chem.,10: 813-829.

Knight KP; McKellar RC. (2007): Influence of cinnamon and clove essential oils on the *D*- and *Z*-values of *Escherichia coli* O157:H7 in apple cider. J Food Prot ,70:2089–94.

Lambert, R. J. W.; P. N. Skandamis; P. J. Coote, and G. J. Nychas. (2001): A study of minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91:453–462.

Mau J.L., Chen C.P., and Hsieh P.C. (2001): Antimicrobial effects of extracts from Chinese chive, cinnamon and corni fructus. J. Agric Food Chem., 49:183–8.

Mejlholm O., and Dalgaard P. (2002): Antimicrobial effect of essential oils on the seafood spoilage microorganism *Photobacterium phosphoreum* in liquid media and fish products. Lett. Appl. Microbiol. 34:27–31.

Pattnaik, S., V. R. Subramanyam, and C. Kole. (1996): Antibacterial and antifungal activity of ten essential oils *in vitro*. Microbios 86: 237–246.

Raghubeer, E.V.; Ke, J.S.; Campbell, M.L.; Meyer, R.S., (1994): Fate of *Escherichia coli* O157:H7 and other coliforms in commercial mayonnaise and refrigerated salad dressing. J. Food Prot. 58: 13–18.

Salem, A. M.; Amin, R. A.; and Afifi, G. S. A. (2010): Studies on antimicrobial and antioxidant efficiency of some essential oils in minced beef. J. of American Science. 6(12): 691-700.

Tajkarimi, M.M.; Ibrahim, S.A.; Cliver, D.O. (2010): Antimicrobial herb and spice compounds in food. Food Control 21:1199–1218.

Tayel, A. A.; El-Tras, W.F.; (2009): Possibility of fighting food borne bacteria by Egyptian folk medicinal herbs and spices extracts. J. Eg. Public Health Assoc. 84:21-32.

Valero M., and Salmeron M.C. (2003): Antibacterial activity of 11 essential oils against *Bacillus cereus* in tyndallized carrot broth. Int J. Food Microbiol., 85:73–81.

Vojdani, J. D.; Beuchat, L.R.; and Tauxe, R.V. (2008): Juice-Associated Outbreaks of Human Illness in the United States, 1995 through 2005. J Food Prot., 71: 356–364

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