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Evaluation of Bacterial Resistance to Essential Oils and Antibiotics After Exposure to Oregano and Cinnamon Essential Oils

Raquel Becerril,[1] Cristina Nerín,[1] and Rafael Gómez-Lus[2]

Abstract

Essential oils (EOs) are excellent antimicrobial agents sometimes used in active food packaging. This work studies the susceptibility of 48 clinical isolates and 12 reference strains of Gram-negative bacilli to oregano essential oil, cinnamon essential oil, and combinations of both. Furthermore, the tendency of the clinical isolates to develop resistance to these EOs and to different antibiotics after sequential oregano or cinnamon exposure was studied. For this purpose, antibiotic susceptibility (through disk diffusion assays and minimum inhibitory concentration [MIC] determination) and oregano and cinnamon susceptibility (through MIC and minimum bactericidal concentration [MBC] determination) were compared after 50 passages in the presence or absence of subinhibitory concentrations of oregano and cinnamon essential oils. The results showed that all strains were susceptible to both EOs and their combination independently of the antibiotic resistance profile. In addition, neither synergistic nor antagonistic effects were observed between oregano and cinnamon essential oils at the concentrations tested. After the sequential exposure to both EOs, only *Serratia marcescens*, *Morganella morganii*, and *Proteus mirabilis* treated with oregano changed their antibiotic resistance profile and/or increased their resistance to this EO. However, the changes in antibiotic and oregano resistance were not related.

Introduction

Despite exceptional medical advances in the development of antibiotics, bacterial infections remain an important healthcare concern due to the emergence of increasing bacterial resistance and the corresponding increases in healthcare costs and mortality rates (Giske *et al*., 2008). In recent years, considerable efforts have been made to control the spread of pathogens with various strategies, including the use of alternative antimicrobial compounds (Jones *et al*., 1998; Hamilton-Miller, 2004).

Essential oils (EOs) are natural products obtained from plants with proven antimicrobial properties against a wide range of microorganisms (Burt, 2004; Becerril *et al*., 2007; Tajkarimi *et al*., 2010). Due to these properties, EOs have traditionally been used to protect food against microbial deterioration (Davidson, 1997; Draughon, 2004). However, EOs give quite a strong taste and odor, and they are not suitable as additives in some food products. To minimize the effects produced on organoleptic properties, one smart solution is to incorporate the EOs in the packaging material, which would result in very low concentrations of EOs in the food, since the amount of EOs released from the packaging material is very low.

Antimicrobial active packaging is nowadays considered as an innovative solution to extend the shelf life of foodstuffs while maintaining its quality (Appendini *et al*., 2002; Suppakul *et al*., 2003). New active packaging materials containing EOs as antimicrobial substance have been demonstrated to prevent the proliferation of pathogenic and spoilage microorganisms in food, ensuring consumer safety and extending the shelf life (López *et al*., 2007a; Rodríguez *et al*., 2008; Gutiérrez *et al*., 2009; Rodriguez-Lafuente *et al*., 2010). Due to their volatility, EOs incorporated in food packaging material are able to create a protective atmosphere around the food, preventing microorganism proliferation (López *et al*., 2007b; Tunc *et al*., 2007; Goñi *et al*., 2009).

Besides displaying high antimicrobial activity against a wide range of microorganisms, an ideal antimicrobial substance should not induce the development of antimicrobial resistance, or at least, it should maintain its effectiveness over a long period of use. In active packaging applications, the

[1]Departamento de Química Analítica, Instituto de Investigación en Ingeniería de Aragón (I3A), Grupo GUIA, and [2]Departamento de Microbiología Medicina Preventiva y Salud Pública, Instituto de Investigación de Ciencias Ambientales (IUCA), Universidad de Zaragoza, Zaragoza, Spain.

appearance of strains resistant to the antimicrobial substance incorporated into the material seriously reduces its usefulness since the safety for the consumer cannot be guaranteed. For this reason, evaluation of the risk of the appearance of resistant strains is essential to the development of new antimicrobial agents.

In recent years, there has been a remarkable increase in the use of antimicrobial substances for different purposes such as food preservation. However, there are no studies about the consequences that their use could have in the development of bacterial resistance to other antimicrobials such as antibiotics. It is well known that bacteria may develop mechanisms of resistance to antimicrobials that also increase their resistance to antibiotics (Russell *et al*., 1998; Poole, 2002). Despite the importance and widespread use of EOs as antimicrobial substances, very little is known about the consequences of their use on bacterial resistance.

Gram-negative bacilli have a high pathological importance. The *Enterobacteriaceae* alone represent 50% of clinically significant isolates in humans (Joklik *et al.,* 1992). Most Gram-negative bacilli are opportunistic pathogens and are difficult to treat due to antibiotic resistance or multiresistance (Pumarola *et al*., 1987). However, other species such as certain strains of *Escherichia coli* are pathogenic strains and produce severe infections. The habitat of Gram-negative bacteria is ubiquitous, since they can survive in soil, water, or the intestines of animals. For this reason, they can contaminate food causing its degradation or, in some cases, infections in consumers.

This work studies the susceptibility of several clinical strains as well as some reference Gram-negative bacilli to oregano and cinnamon essential oils. The study also includes the tendency of clinical isolates of Gram-negative bacilli to develop resistance to these popular EOs and to different antibiotics after continuous oregano or cinnamon exposure. Such cross-resistances have been studied for the first time in this research.

Methods

Essential oils

The EO of *Cinnamomum zeylanicum* (CI; Chemical Abstracts Service [CAS] Registry Number 805-91-6) and the EO of *Origanum Vulgaris* (OR; CAS Registry Number 8007-11-2) were supplied by Argolide Química S.L. (Barcelona, Spain).

Bacteria

The EOs were tested against 60 Gram-negative bacilli. Forty-eight of them were isolated from clinical human samples and have varying susceptibility to antibiotics: one *Serratia marcescens,* one *Morganella morganii,* one *Proteus penneri,* one *Klebsiella oxytoca,* one *Salmonella enterica,* one *Enterobacter cloacae*, one *Citrobacter freundii*, one *Pseudomonas. aeruginosa,* 10 *Klebsiella pneumoniae* (three extended-spectrum beta-lactamase producer), 10 *Escherichia coli* (2 extended-spectrum beta-lactamase producer), 10 *Acinetobacter baumannii,* and 10 *Proteus mirabilis.* The rest (12 strains) were reference strains: *Acinetobacter baumanii* CECT 452 (Colección Española de Cultivos Tipo), *K. pneumonie* ATCC 13883 (American Type Culture Collection), *E. coli* ATCC 25922, *S. marcescens* ATCC 8100, *C. freundii* ATCC 8090, *K. oxytoca* CECT 860, *P. aeruginosa* ATCC 27853, *M. morganii* CECT 173, *P. penneri* CECT 864, *E. cloacae* ATCC 23355, *S. enterica* subsp. *enterica* CECT 556, and *P. mirabilis* CECT 4168.

Bacteria were isolated and identified in the clinical microbiology laboratory at the Hospital Clínico Universitario Lozano Blesa (Zaragoza, Spain). Strains isolated from different sources (urine 30%, blood 45%, respiratory tract 12.5%, and others 10%) were identified using a commercially available microdilution system (WIDER I, Francisco Soria-Melguizo, Madrid, Spain).

Assays

EO susceptibility

MIC and MBC determination. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of oregano, cinnamon, and combinations of both were determined for all strains by the broth dilution method used by Becerril *et al.* (2007). Briefly, twofold serial dilutions of oregano, cinnamon, and combinations of both (75% oregano [OR] and 25% cinnamon [CI], 75% CI and 25% OR, and 50% CI and OR) were prepared in ethanol (75%), and 10-μL aliquots of these solutions were mixed with 1 mL of broth medium containing 100 μL of inoculum solution (106 colony forming unit [CFU]/mL). After the incubation period, the bacterial growth was determined by measuring the optical density at 625 nm. Subsequently, the corresponding decimal dilutions were made and plated for counting the number of CFUs. Controls were carried out with no EO and 10 μL of ethanol.

The effect of the EO combinations was evaluated by calculating the fractional inhibitory concentration (FIC) index using the following formula: FIC_{OR} = MIC of OR in combination/MIC of OR alone; FIC_{CI} = MIC of CI in combination/MIC of CI alone; FIC index = $FIC_{OR} + FIC_{CI}$. Synergy between the two agents was defined as an FIC index of ≤ 0.5 and antagonism as an FIC index of > 4.0 (White *et al*., 1996).

Study of the tendency of bacteria to develop resistance

Continuous treatment with EOs. In order to determine the influence of a continuous treatment with EOs on the strains, Petri dishes with Muller-Hinton agar were inoculated with *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas aeruginosa,* or *Serratia marcescens*. Subsequently, 10 μL of cinnamon or oregano essential oil were added to a 10-mm sterile blank filter disk placed on the center of the inoculated Petri dish. After 24 h of incubation at 37°C, colonies growing next to the inhibition zone, and thus cultured with subinhibitory concentrations of EO, were collected, washed, and suspended in physiological saline solution. The suspension obtained was inoculated in a new Petri dish, with a new sterile blank filter disk impregnated with the same EO, as previously described. The process was repeated 50 times for each bacterium. Controls without adding EOs were carried out.

After passage number 20 and passage number 50, the collected bacteria were inoculated in EO-free Muller-Hinton agar and incubated overnight at 37°C. After the incubation period, the bacteria were collected and frozen in skimmed milk at −20°C for further analysis. The strains treated with OR for 20 or 50 passages were called 20OR and 50OR, respectively; the bacteria treated with CI for 20 or 50 passages, 20CI and 50CI, respectively; and bacteria without treatment were called CT.

To assure the correct identification of bacteria after the treatment with EOs, analytical profile index (API) testing

using 20E test strips (BioMeriex, Marcy-ÍÉtoile, France) was carried out.

EO susceptibility. The MIC and the MBC of OR and CI of treated and non-treated bacteria were determined by the broth dilution method described above.

Antibiotic susceptibility. The antibiotic susceptibility of treated and non-treated bacteria was evaluated using a disk diffusion assay carried out in accordance with the National Committee for Clinical Laboratory Standards (NCCLS, 2003b). After the statistical analysis of the results obtained, the activity of antibiotics that showed different activity for treated and non-treated bacteria was quantified using a broth microdilution method (NCCLS, 2003a).

Disk diffusion assay

Disk diffusion MH agar plates were inoculated with a bacterial suspension of 10^8 CFU/mL. Antibiotic disks containing 10 μg of ampicillin, 20/10 μg of amoxicillin/clavulanic acid, 30 μg of cefotaxime, 30 μg of cefepime, 10 μg of gentamicin, 10 μg of tobramycin, 10 μg of streptomycin, 30 μg of kanamycin, 30 μg of tetracycline, 30 μg of minocycline, 30 μg of chloramphenicol, 30 μg of nalidixic acid, 5 μg of ciprofloxacin, and 1.25/23.75 μg trimethoprim-sulfamethoxazole (Bio-Rad, Hercules, CA) were placed in MH agar plates and incubated for 20–24 h at 37°C. After the incubation period, the inhibition zone diameters (IZDs) were measured, including the antibiotic disk (in mm).

The IZDs obtained in the disk diffusion assay for exposed bacteria during 20 or 50 passages were compared to those obtained for bacteria without being exposed using the statistical SPSS software package version 13.0 (SPSS Inc., Chicago, IL). Data were compared either using Tukey's Honestly Significant Differences (HSD) test when variances were not different or using the Games-Howell test when variances were significantly different. Tests based on the Student's range distribution ensured that the chances of finding a significant difference were maintained at a α-significance level. In all cases, comparisons were performed at 95% ($\alpha = 0.05$) significance.

Broth microdilution test

A broth microdilution method was used for determining the MICs of the antibiotics that showed different activity for treated and non-treated bacteria. Microtiter plates containing serial dilutions of antimicrobial agents AMP, TET, MINO, CIP, and C were inoculated with 100 μL of a bacterial suspension in Muller Hinton broth to obtain a final inoculum size of 10^5 CFU/mL. Subsequently, the plates were incubated for 20–24 h at 37°C. The MICs were read as the lowest concentration of an antimicrobial agent at which visible growth was inhibited.

All tests were repeated at least three times.

Results

EO susceptibility: MIC and MBC determination

The antimicrobial activity of oregano and cinnamon and combinations of both was determined against a series of selected Gram-negative bacteria. The MICs and MBCs obtained are shown in Table 1.

Oregano and cinnamon essential oils were active against all the Gram-negative bacilli tested, with a MIC and MBC range of 100–800 mg/L. Oregano was slightly less active against *P. aeruginosa* ATCC 27853, with MIC and MBC reaching 800 mg/L, and slightly more active against one strain of *A. baumanii*, with MIC of 100 mg/L. Cinnamon essential oil showed MIC values of 200–400 mg/L (onefold difference), so there were no substantial differences in MIC among the tested bacteria. The MBC values oscillated between 400 and 800 mg/L, except for strains of *M. morganii* and *A. baumanii* in which MBCs were 200 mg/L. Therefore, there were no big differences in susceptibility among the strains from different or from the same species, or even among strains with different antibiotic resistance profiles, including beta-lactamase–producing bacteria.

The oregano and cinnamon combinations tested did not show synergistic or antagonistic effects, since the values obtained for the FIC index were higher than 0.5 and lower than 4 (data not shown).

Passages with EOs

Five bacteria were exposed to 50 oregano and cinnamon passages: *E. coli*, *S. marcensces*, *M. morganii*, *P. mirabilis,* and *P. aeruginosa*. *E. coli* could not be studied because it failed to grow after several oregano or cinnamon passages.

Pseudomonas aeruginosa colonies showed some morphological alterations after the treatment with EOs (Fig. 1). Colonies of 50OR and 50CI were less mucoid and showed different coloration than CT, suggesting alterations in lipopolysaccharide (LPS) content or composition and in pigment production.

EO susceptibility

MICs and MBCs of oregano were determined for CT, 20OR, and 50OR bacteria, whereas MICs and MBCs of cinnamon were determined for CT, 20CI, and 50CI bacteria. Table 2 shows the data obtained.

According to Table 2a, the serial passage with oregano increased the MIC and MBC values of *M. morganii* and the genetically closely related *P. mirabilis* from 200 to 800 mg/L. The other exposed bacteria, *S. marcenses* and *P. aeruginosa* 20OR and 50OR, showed a sensitivity very similar to that of the non-treated bacteria, presenting identical MIC values or values differing by a single dilution only. In the case of cinnamon, there was no substantial change in MIC for all the bacteria even after 50 passages, as the MIC values differed less than twofold.

Antibiotic susceptibility

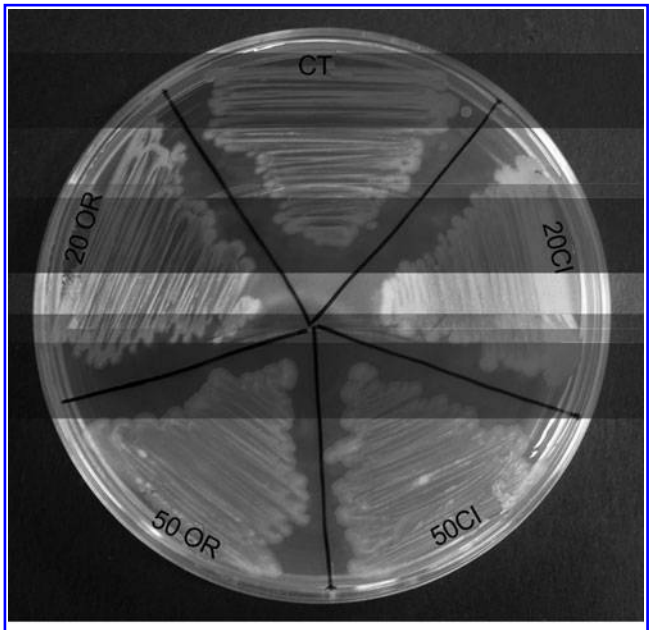
The antibiotic susceptibility to treated and non-treated bacteria was determined using a disk diffusion assay. Different classes of antibiotics were tested: beta-lactams, aminoglicosides, tetracyclines, quinolones, and chloramphenicol.

When the statistical analysis of the IZDs revealed differences between the exposed and non-exposed bacteria, the MICs were determined using a broth microdilution method. Table 3 shows the IZDs and MICs obtained in cases in which the test showed differences. After the EO exposure, the disk diffusion assay of *S. marcescens* only revealed significant differences after the passages with oregano, in particular for tetracycline, minocycline, nalidixic acid, ciprofloxacin, and

Table 1. Comparative Activity of Oregano Oil (OR), Cinnamon Oil (CI), and Their Combinations Against Gram-Negative Bacilli

		OR		*CI*		*50% OR+50% CI*		*25% OR+75% CI*		*75% OR+25% CI*	
Organism	*Type (no tested)*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
E. coli	ref (1)	200	200	200	400	200	200	200	400	200	200
	clin (10)	200–400	200–400	400	800	200–400	400	400	400–800	200–400	200–400
S. marcescens	ref (1)	200	200	400	400	400	400	400	400	200	200
	clin (1)	400	400	400	400	400	400	400	400	400	400
C. freundii	ref (1)	200	200	200	400	200	400	200	200	200	200
	clin (1)	200	200	200	400	200	400	200	200	200	200
M. morganni	ref (1)	200	200	200	200	200	200	200	200	200	200
	clin (1)	200	200	200	400	400	400	400	400	400	400
S. enterica	ref (1)	200	200	200	800	400	400	400	400	400	400
	clin (1)	200	400	400	800	400	400	400	400	400	400
K. oxytoca	ref (1)	200	200	400	800	400	400	400	400	200	200
	clin (1)	200	200	400	400	400	400	200	200	400	400
K. pneumoniae	ref (1)	200	200	400	400	400	400	200	400	200	200
	clin (10)	200	200	400	400–800	400	400	200–400	200–400	200–400	200–400
P.aeruginosa	ref (1)	800	800	400	800	800	800	400	800	800	800
	clin (1)	400	400	400	400	400	800	400	400	400	400
A. baumannii	ref (1)	200	200	200	200	200	200	200	200	200	200
	clin (10)	100–400	100–400	200–400	200–800	100–400	200–400	100–400	100–400	100–400	200–400
E. cloacae	ref (1)	400	400	200	400	400	400	400	400	400	400
	clin (1)	400	400	400	800	400	400	400	400	400	400
P. mirabilis	ref (1)	200	200	400	400	400	400	400	400	400	400
	clin (10)	200–400	200–400	200–400	400–800	400	400	400	400	400	400–800
P. penneri	ref (1)	200	200	200	400	200	200	200	200	200	200
	clin (1)	200	400	400	400	400	800	400	400	400	400
Range		100–800	100–800	200–400	200–800	100–800	200–800	100–400	100–800	100–800	200–800

MICs and MBCs (obtained for 12 clinical strains) are shown in mg/L.
MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; ref, reference; clin, clinical.



CT, bacterial without treatment; 20CI, bacterial treated with cinnamon oil for 20 passages; 50CI, bacterial treated with cinnamon oil for 50 passages; 20OR, bacterial treated with oregano oil for 20 passages; 50CI, bacterial treated with oregano essential oil for 50 passages.

FIG. 1. Morphological alterations of *Pseudomonas aeruginosa* colonies after the treatment with oregano oil (OR) and cinnamon oil (CI).

Table 2. MICs and MBCs (mg/L) Values of Cinnamon Oil (CI) (a) and Oregano Oil (OR) (b) Obtained for Bacteria Before and After Passaging at Subinhibitory Concentrations of Cinnamon or Oregano Oil

(a) Oregano oil

	MIC			*MBC*		
	CT	*20OR*	*50OR*	*CT*	*20OR*	*50OR*
M. morganii	200	800	800	200	800	800
S. marcescens	400	400	400	400	400	400
P. mirabilis	200	800	800	200	800	800
P. aeruginosa	400	400	800	400	800	800

(b) Cinnamon oil

	MIC			*MBC*		
	CT	*20CI*	*50CI*	*CT*	*20CI*	*50CI*
M. morganii	200	400	400	400	400	400
S. marcescens	400	400	400	400	400	400
P. mirabilis	400	400	400	800	800	800
P. aeruginosa	400	400	400	400	800	800

CT, bacterial without treatment; 20CI, bacterial treated with cinnamon essential oil for 20 passages; 50CI, bacterial treated with cinnamon essential oil for 50 passages; 20OR, bacterial treated with oregano essential oil for 20 passages; 50CI, bacterial treated with oregano essential oil for 50 passages; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

Table 3. IZDs and MIC of Tetracycline (TET), Minocycline (MINO), Nalidixic Acid (NAL), Ciprofloxacin (CIP), Chloramphenicol (C), and Ampicillin (AMP) Against *Serratia marcescens* (a) and *Proteus mirabilis* (b) After 20 (20R) or 50 (50R) Passages in the Presence of Oregano Oil

(a) *Serratia marcescens*

	TET		*MINO*		*NAL*		*CIP*		*C*	
	IZD (SD)	*MIC*	*IZD (SD)*	*MIC*	*IZD (SD)*	*MIC*	*IZD (SD)*	*MIC*	*IZD (SD)*	*MIC*
NT	15 (3.7)	16	20.8 (2.3)	32	26.5 (0.7)	2	27 (1.4)	0.2	24.5 (1.7)	8
20OR	8.7 (2.1)	64	14 (1.4)	64	21.3 (1.2)	16	23.3 (3.2)	0.5	17 (2.3)	16
50OR	7.7 (1.2)	>64	12 (0.8)	64	17.3 (2.5)	16	17.3 (2.5)	0.5	16.7 (1.5)	16

(b) *Proteus mirabilis*

	AMP	
	IZD (SD)	*MIC*
NT	8.8 (3.4)	>64
20OR	19 (2.8)	64
50OR	19.3 (2.1)	8

IZDs, inhibition zone diameters; MIC, minimum inhibitory concentration; SD, standard deviation.

chloramphenicol, and therefore MICs for these antibiotics were determined. According to these results, treated *S. marcescens* slightly increased their MIC to minocycline, chloramphenicol, and ciprofloxacin (onefold increase). In the cases of nalidixic acid and tetracycline, MICs increased from 2 to 16 mg/L and from 16 to 64 mg/L, respectively, after 20 passages of oregano, indicating an increase in the resistance of *S. marcescens* to these antibiotics.

P. mirabilis exposed to oregano showed a progressive increase of susceptibility to ampicillin, with a variation of MIC from >64 to 64 mg/L after 20 passages and to 8 mg/L after 50 passages. However, there was no evidence of antibiotic susceptibility variation in the *P. aeruginosa* and *M. morganii* strains.

Discussion

The spread of drug-resistant microorganisms and the search for natural antimicrobial substances for use in food preservation has increased the interest in EOs, which have been demonstrated to have strong antimicrobial properties (Davidson, 1997; López *et al*., 2005; Manso *et al*., 2010).

The present study confirms that both oregano and cinnamon essential oils have a high antimicrobial activity against reference strains and clinical isolates of Gram-negative bacilli. The results indicated that this activity does not depend on the antibiotic susceptibility pattern, even in bacteria with high antimicrobial resistance rates including extended-spectrum beta-lactamase producers. This same conclusion was obtained by other authors studying different EOs, including oregano and cinnamon (Opalchenova *et al*., 2003; Mayaud *et al*., 2008; Doran *et al*., 2009). MICs and MBCs similar to those found by other authors were obtained in some cases in this study, but different values were found in other cases. For example, Mayaud *et al*. (2008) obtained similar MICs against Gram-negative bacilli for cinnamon, while they obtained different values for oregano. The disparity in the results could be attributed to variations in the chemical composition of EOs obtained from the same plant species (Kalemba *et al*., 2003; Burt, 2004), since the chemical composition of EOs can differ in different parts of a plant, the stage of plant development, the growth conditions (e.g., temperature, soil, fertilizers), the drying system, and the extraction procedure.

Because EOs are composed of a large number of chemical constituents, it is not surprising that different combinations of them show synergistic or antagonistic effects (Burt, 2004; Goñi *et al.*, 2009; Tajkarimi *et al*., 2010). However, according to the results obtained in the present study, there is no interaction observed between cinnamon and oregano. As far as we know, no interactions have been described in the literature between cinnamaldehyde and carvacrol (Michiels *et al*., 2007; Pei *et al.*, 2009) which are, according to the results obtained by López *et al*. (2006), the major compounds of cinnamon (90%) and oregano (80%), respectively.

It is well documented that bacteria can develop resistance to antimicrobials due to continuous and prolonged exposure to antimicrobial agents. The frequency of resistance acquisition depends on the type of antimicrobial and bacteria. We observed that resistance development was only detected for two out of four bacteria species tested to oregano essential oil, but not cinnamon. Other natural substances such as honey or tea tree oil have also been studied, and the results also varied depending on both the substance and the bacteria (McMahon *et al.*, 2007; Cooper *et al*., 2010).

Due to the complex composition of EOs, it is likely that their antibacterial activity is due to different mechanisms of action that implies several targets in the cell (Burt, 2004). For this reason, it is expected that bacteria rarely develop a resistance mechanism for EOs. In this work, this hypothesis is supported for cinnamon, since after 50 passages with cinnamon, an increase in resistance was not detected. However, the development of resistance to oregano was observed after exposure to this EO for 50 passages in the cases of *M. morganii* and *P mirabilis*.

The complexity of EO composition could also determine the resistance mechanisms that bacteria develop. It is expected that resistant organisms will show general resistance mechanisms instead of specific methods that would imply target site

mutations. In fact, it has been observed that a general resistance mechanism, such as efflux pumps, is involved in bacterial resistance to some EOs and chemical constituents, for example, pine oil, tea tree oil, or terpine-4-ol (Moken *et al*., 1997; Papadopoulos *et al*., 2008). Further studies should be carried out to investigate the molecular mechanisms that increase bacterial resistance to oregano essential oil.

The use of a specific antimicrobial substance can contribute to the increase in resistance to other antimicrobials, such as antibiotics (Russell *et al*., 1998; Fernandes *et al*., 2003). It has been described, for example, that the use of chlorine for purifying water and the use of quaternary ammonium in hospital disinfection have been related to the appearance of bacteria resistant to antibiotics (Russell *et al*., 1998). According to the results obtained in this study, the sequential exposure to oregano increased resistance to antibiotics in *Serratia marcensens*, especially to tetracycline and nalidixic acid. However, these resistance increases are not related to an increase in resistance to oregano, as occurs with other antimicrobial substances used as biocides, for example, chlorhexidine, pine oil, or tea tree oil, where cross-resistance with antibiotics in Gram-negative bacteria has been observed (Moken *et al*., 1997; Carsenti-Etesse *et al*., 1999; McMahon *et al*., 2007).

This study has shown that only one of two EOs tested, cinnamon, does not increase bacterial resistance to antibiotics or to cinnamon itself after continuous treatment. Therefore, cinnamon could be a better candidate than oregano for use as an antimicrobial agent, since its use does not produce a fast development of bacterial resistance and it exhibits a high antimicrobial activity. In fact, cinnamon EO has been applied successfully to prevent the proliferation of microorganisms in food (Rodríguez *et al*., 2008; Gutierrez *et al*., 2009).

Conclusion

The results from this study confirm that cinnamon and oregano are efficient antimicrobial agents at low concentrations against Gram-negative bacilli with varying sensitivity to antibiotics. In addition, repeated use of cinnamon does not significantly change bacterial susceptibility to this EO or to antibiotics, but oregano could increase or decrease the resistance to some antibiotics. These results should be considered in further applications of EOs as antimicrobial agents.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
C. Nerín, Ph.D.
Departamento de Química Analítica
Instituto de Investigación en Ingeniería de Aragón (I3A)
Grupo GUIA
Universidad de Zaragoza
M[a] de Luna 3
50018 Zaragoza, Spain

E-mail: cnerin@unizar.es

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