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Antibacterial effects of cinnamon oil against carbapenem resistant nosocomial *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates

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ABSTRACT

Widespread existence of drug-resistant pathogens poses a threat to the successful treatment of bacterial diseases and increases the need for new antibacterial agents. Natural products are the basic source of antibacterial therapeutic agents for now, and will remain so in the future. Therefore, the aim of this study was to determine the antibacterial activity of cinnamon oil against carbapenem-resistant nosocomial isolates of *Acinetobacter baumannii* (111) and *Pseudomonas aeruginosa* (136). The essential oil composition of cinnamon oil was analyzed by GC, GC/MS and the antimicrobial effect of cinnamon oil was determined by disk diffusion method. The observed zone diameters were compared with carbapenem breakpoints (CLSI standard) and it was found that only one of the *P. aeruginosa* isolates was within resistance limits. Thus, cinnamon oil has antimicrobial activity with potential use as an antimicrobial agent in the pharmaceutical industry and an additive in the food industry.

1. Introduction

Pseudomonas aeruginosa and *Acinetobacter baumannii* are frequent causes of nosocomial infections because they have higher survival rates for extended period of time in hospitals and both of them are associated with multiple drug resistances due to a long term exposure to antibiotic treatments. Such nosocomial infections prolong hospital stay, require intensive antibiotic therapy, and lead to escalation in cost and mortality. Additionally, the development of pan resistant strains has reduced further treatment options (Mendes and Turner, 2001). Carbapenems are broad spectrum antibiotics of the beta-lactam class that show rapid bactericidal effect. They are widely used in the treatment of aerobic and anaerobic bacterial infections caused by various microorganisms (Sydnor and Perl, 2011). Bacterial resistance to carbapenems, which are commonly used in the empiric treatment of infections, is predominantly caused by the presence of the Extended Spectrum Beta Lactamases (ESBL) in these bacteria; this has enabled their world wide spread (Braun et al., 2014). Due to constant increase in antibiotic resistance, There is an increasing interest on compounds and especially essential oils which have antimicrobial activity. Essential oils are one of the most important secondary metabolites of medicinal and aromatic plants, and have been used as flavor and taste modifiers in food, cosmetic, and perfume industries for many years. They are also used by pharmaceutical industries for their antibacterial, antifungal and antioxidant properties (Anwar et al., 2009a,b; Burt, 2004; Hammer et al., 2008; Sánchez-González et al., 2011). Even, analgesic, antiseptic, antimicrobial, and antioxidant effects of essential oils have been shown in several studies, these effects seem to vary according to the type of the essential oil used in the studies (Bakkali et al., 2008; Miguel, 2010).

Therefore, the aim of this study was to investigate the antibacterial activity of cinnamon oils against carbapenem-resistant nosocomial isolates of *A. baumanni* and *P. aeruginosa*.

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Table 1
Composition of the cinnamon oils by GC and GC/MS.

	Compound	Cinnamon 1 area%	Cinnamon 2 area%	Cinnamon 3 area%
1	Benzaldehyde	1.04	n.d.	0.24
2	Linalool	n.d.	1.49	n.d.
3	Benzyl alcohol	n.d.	3.95	n.d.
4	Cinnamaldehyde	76.89	10.26	99.54
5	Triacetin (glycerol)	22.07	n.d.	n.d.
6	Cinnamaldehyde propylene glycol acetal (isomer)	n.d.	38.97	n.d.
7	Cinnamaldehyde propylene glycol acetal (isomer)	n.d.	30.95	n.d.

n.d. = Not detected.

2. Materials and methods

2.1. GC and GC/MS analysis of cinnamon oil

Cinnamon oil of three different brands was purchased from herbalists and coded as Cinnamon 1–3. Composition of the oil was analyzed by GC and GC–MS. GC analysis of the essential oils was performed on an Agilent 6890N Network GC system, using a HP Innowax Capillary column with dimensions 60.0 m × 0.25 mm × 0.25 μm. Oven temperature was programmed as follows: the column was initially held at 60 °C for 10 min after injection, then increased to 220 °C with a 4 °C/min heating ramp for 10 min, further increased to 240 °C with a 1 °C/min heating ramp, and finally, held at 240 °C for 10 min. The injector and detector (FID) temperatures were maintained at 250 °C. Helium was used as the carrier gas with a split ratio: 50:1 and 2.0 μL of sample was injected. The oil was also analyzed by GC–MS using the Agilent 6890N Network GC system combined with an Agilent 5973 Network mass selective detector. The GC conditions were as given above except that the assay was run with a column flow rate of 1.2 mL/min. MS conditions were as follows: 70 eV ionization energy with mass range of 34–450 atomic mass units.

Identity of the individual components in the oils was assigned by comparison of their retention times and mass spectra with previously published data (Adams, 2001) and by comparison of their mass spectra in the Wiley and NIST libraries. Percentage of the components was calculated from the GC peak area using the normalization method.

2.2. Microorganisms

Isolates of *P. aeruginosa* (136) and *A. baumannii* (111) strains (total 247), identified as causative agents of nosocomial infections in various hospitals in Turkey between January 2011 and December 2011, were used in the study. Second strains isolated from same patients were excluded. Meropenem and imipenem susceptibilities of these strains were evaluated using the *E*-test (AB Biodisk, Solna, Sweden) according to CLSI criteria. As a control *P. aeruginosa* ATCC 27853 strain was included in the study.

2.3. Antimicrobial activity test

Antibacterial activity of the essential oils was determined using the disk diffusion assay (Bauer et al., 1966). For this purpose, isolates were cultured at 37 °C for 24 h in Mueller Hinton Agar (MHA) and bacterial suspensions were prepared with Mueller Hinton Broth (MHB) to match McFarland standard No. 0.5 turbidity. A hundred microlitre of this suspension was spread on a MHA plate. Sterile paper discs (6 mm diameter) were impregnated with 15 μL of the essential oils and placed on the surface of the MHA plates. These plates were subsequently incubated at the appropriate temperature for 24 h and the diameter (mm) of the zone of inhibition was measured. Antimicrobial activity of cinnamon oil against *P. aeruginosa* ATCC 27853 was determined using disk diffusion and macro dilution methods. All the experiments were carried out in duplicates and the results are average of these values.

Table 2
Zone diameters (mm) of isolates.

Zone diameter (mm)	*Pseudomonas aeruginosa* (*n*)	*Acinetobacter baumannii* (*n*)
≤15 mm	1	n.d.
16–18 mm	38	2
19–21 mm	38	4
>22 mm	59	105
Total (*n*)	136	111

n.d. = Not detected.

3. Results

3.1. GC and GC/MS results of cinnamon oils

In this study, three different brands of cinnamon oil were subjected to GC and GC/MS analyses to identify their composition (Table 1).

It was found that cinnamon 2 contained cinnamaldehyde propylene glycol acetal isomers, which are synthetic compounds used as flavoring agents, especially in chewing gums (Jhonson and Greenberg, 1992). Thus, Cinnamon 2 was found to be artificial. Cinnamaldehyde was the major component of both Cinnamon 1 (76.89%) and Cinnamon 3 (99.54%). Since cinnamaldehyde is responsible for the antibacterial activity of cinnamon oil, Cinnamon 3, which had a higher amount of cinnamaldehyde, was chosen for further bioactivity testing (Hamidpour et al., 2015).

3.2. Antimicrobial activity test results

The antimicrobial activity of Cinnamon 3 was evaluated using the disk diffusion method and the results are shown in zone diameters (Table 2).

For interpretation of the observed zone diameters of cinnamon oil, the antibiotic sensitivity limits of the carbapenem group of antibiotics given in the CLSI standards were used (CLSI, 2014). Sensitivity limits of meropenem, imipenem and doripenem for *P. aeruginosa* and *A. baumannii* are given in Table 3 (CLSI, 2014).

The highest zone diameters were determined as 44 mm and 35 mm for *A. baumannii* and *P. aeruginosa,* respectively. It is important that, only one *P. aeruginosa* isolate was found to be resistant to the antibacterial activity of Cinnamon 3, when the susceptibility breakpoints of carbapenems in CLSI standard were used as reference. Minimal Inhibition Concentration (MIC) and inhibition zone diameter of cinnamon oil against *P. aeruginosa* ATCC 27853 was determined 0.0019 mL/mL and 21 mm zon diameter for cinnamon oil 3.