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In vitro antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp.

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Abstract

This study evaluated the antimicrobial activities of an essential oil of *Origanum minutiflorum* (O. Schwarz and P.H. Davis) against ciprofloxacin-resistant *Campylobacter* spp., by broth microdilution and agar well-diffusion methods. Moreover, *O. minutiflorum* oil was analyzed by gas chromatography/mass spectrometry (GC/MS). Twenty-nine components were identified, representing 98.7 of the oil. The oil yield from the plants was 4.0–4.4% v/w. The major components of *O. minutiflorum* oil were carvacrol (73.9%) and *p*-cymene (7.20%). The oil has lower contents of carvacrol methyl ether (0.05%), heptadecanol (0.06%) and carvacryl acetate (0.06%). Twenty-one *Campylobacter* spp. (12 *C. jejuni*, 5 *C. lari* and 4 *C. coli*) strains using in this study were selected among 300 isolates according to their resistance to ciprofloxacin. The minimum inhibitory concentration (MIC) values for bacterial strains, which were sensitive to the essential oil of *O. minutiflorum*, were in the range of 7.8–800 μg/ml. The essential oil obtained showed strong antimicrobial activity against all of the tested ciprofloxacin-resistance *Campylobacter* spp. These results suggest that the essential of *O. minutiflorum* may be used as a natural preservative in food against food-born disease, such as Campylobacteriosis.

Keywords: Antimicrobial activity; *Origanum minutiflorum*; *Campylobacter* spp.; GC/MS analysis

1. Introduction

Turkey is regarded as an important gene-centre for the family *Lamiaceae*. The leafy parts of plants, such as oregano, thyme and savory, belonging to the *Lamiaceae* family, have been added meat, chicken and food products for many years (Baydar, Sagdic, Ozkan, & Karadogan, 2004). Members of the genus *Origanum* (*Lamiaceae* family) are among the most important aromatic plants worldwide. Twenty-four species and 27 taxa are found in the flora of Turkey and the East Aegean Islands, 16 of them being endemic (Aligiannis, Kalpoutzakis, Mitaku, & Chinou, 2001; Davis, Mill, & Tan, 1984; Davis, Mill, & Tan, 1988). Many *Origanum* plants are characterized by a wide range of volatile secondary metabolites and by the existence of chemical differences with respect to both essential oil content and composition. *Origanum* plants are widely used in the flavouring of food products and alcoholic beverages, as well as in perfumery, because of their spicy fragrance. Moreover, in particular, owing to the antioxidant and antimicrobial activities of their essential oils, *Origanum* species have recently been of great interest, in both academia and the food industry as potential natural additives, to replace synthetic products (Tepe, Daferera, Sokmen, Polissiou, & Sokmen, 2004). Therefore there is an increasing demand for accurate knowledge of the minimum inhibitory concentration (MIC) of essential oils to enable a balance between the sensory acceptability and antimicrobial efficacy (Lambert, Skandamis, Coote, & Nychas, 2001).

Campylobacter spp., especially *Campylobacter jejuni* and *C. coli*, have emerged worldwide as leading causes of acute bacterial gastroenteritis (Threfall, Ward, Frost, & Willshaw, 2000; WHO/FAO, 2001). Several studies have identified chicken as the main source of this infection. *Campylobacter* spp. are part of normal enteric flora in

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animals (chicken, pigs and cattle) and can be transmitted to humans through contaminated foods (Atanassova & Ring, 1999; Dominguez, Gomez, & Zumalacarregui, 2002). Most *Campylobacter* enteric infections are self-limited and do not require antimicrobial drug treatment. However, severe or long-lasting infections do occur and may justify antimicrobial drug therapy. In these cases, erythromycin or fluoroquinolones (e.g. ciprofloxacin) are often the drug of choice (Engberg, Aarestrup, Taylor, Gerner-Smidt, & Nachamkin, 2001). But, antimicrobial resistance, among *Campylobacter* spp., to drugs used in the treatment of human infection is increasing. It is not surprising, therefore, that several countries have reported a rise in ciprofloxacin-resistant *C. jejuni* in human infections (Gaudreau & Gilbert, 2003). Similarly, there has been an increase in the prevalence of ciprofloxacin-resistant *C. jejuni* in human infections, emphasizing the potential to acquire gastroenteritis due to ciprofloxacin-resistant *Campylobacter* from consumption of chicken. (Endtz et al., 1991). Furthermore, some studies have demonstrated an association between ciprofloxacin-resistant *Campylobacter* infections and a longer duration of illness (Engberg, Neimann, Moller Nielsen, Aarestrup, & Fussing, 2004; Nelson et al., 2004). This situation has forced scientists to search for new antimicrobial substances from various sources, such as medical plants (Şahin et al., 2003).

In the literature, there are several studies on antimicrobial activity and the essential oil composition of *Origanum* species, whereas the antimicrobial activity of the essential oil of *Origanum minutiflorum,* against ciprofloxacin-resistant *Campylobacter* spp., has never before been studied. Especially, wild oregano (*O. minutiflorum*) is endemic in Turkey, and so is of special importance for the study. The aims of this study were (i) to investigate the antimicrobial activity of the essential oil of *O. minutiflorum* by broth microdilution and agar well-diffusion methods against ciprofloxacin-resistant *Campylobacter* spp. and (ii) to determine the chemical composition of its hydro-distilled essential oil by GC/MS.

2. Materials and methods

2.1. Plant materials

O. minutiflorum plants were collected during the flowering stage in September, 2004, on Sögüt mountain (elevation 1684 m), Sütçüler-Isparta, where it is endemic. The identification of plant materials was confirmed by a plant taxonomist, Prof. Dr. Hayri Duman, in the Department of Biology, Gazi University, Ankara, Turkey.

2.2. Isolation of essential oil (EO)

A dried sample from the aerial parts (leaves, flowers and steams) of the plant was subjected to water distillation for 3 h in a Clevenger-type apparatus (yield 4.0–4.4% v/w). The EO was stored in the dark at 4 °C prior to further analysis.

2.3. Test microorganisms, isolation and preparation of inocula

Twenty-one *Campylobacter* spp. (12 *C. jejuni*, 5 *C. lari* and 4 *C. coli*) strains were selected among 300 isolates according to their resistance to ciprofloxacin. These strains were isolated from different parts of each of the carcasses: body or cavity. Twenty-five grams from each sample, were placed in 225 ml of pre-enrichment broth (Lab M, Lab 135) in sterile plastic bags for 4 h at 37 °C and 20 h at 42 °C. Following pre-enrichment, 100 μl of the pre-enrichment broth were cultured on *Campylobacter* blood-free agar, containing CCD-agar (charcoal cefoperazone deoxyholate agar) (Lab M, Lab 112 containing vancomycin, polimyxin and trimethoprim). CCD-agar plates were incubated at 42 °C for 48 h in a microaerobic atmosphere, using gas-generating sachets (Oxoid BR 038). *Campylobacter* species were identified by their morphological and Gram stain characteristics (Adesiyum, 1993; Fraser, Chandan, Yamazaki, Brooks, & Garcia, 1992). Isolates were identified as *C. coli*, *C. jejuni* or *C. lari*, using biotyping (api-CAMPY, bio-Merieux).

2.4. Determination of minimum inhibitory concentration (MIC)

Microdilution broth susceptibility assay was used (Koneman, Allen, Janda, Scherckenberger, & Winn, 1997). A stock solution of essential oil was prepared in 10% dimethylsulfoxide (DMSO) and then serial dilutions of essential oil were made in a concentration range from 7.8 to 800 μg/ml. The 96-well plates were prepared by dispensing, into each well, 95 μl of Mueller-Hinton broth (MHB), 100 μl of EO and 5 μl of the inoculants. The inoculums of microorganisms were prepared using 24 h cultures and suspensions were adjusted to 4 McFarland standard turbidity. The final volume in each well was 200 μl. A positive control (containing inoculum but no EO) and negative control (containing EO but no inoculum) were included on each microplate. The contents of the wells were mixed and the microplates were incubated at 42 °C for 24 h under microaerophilic conditions (BBL GasPak System). Three replicates of each microassay were carried out and the experiment was carried out twice. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. The experiment was performed in triplicate.

2.5. Inhibitory effect by the agar well-diffusion method

The determination of the inhibitory effect of EO on test bacteria was carried out by the agar diffusion method (Kalemba & Kunicka, 2003). *Campylobacter* cultures were grown at 42 °C for 48 h in MHB. The culture suspensions