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Short communication

Antibacterial activity of essential oils from plants of the genus *Origanum*

Michalis K. Stefanakis [a], Eleftherios Touloupakis [a], Elias Anastasopoulos [b], Dimitrios Ghanotakis [a], Haralambos E. Katerinopoulos [a,*], Pavlos Makridis [c]

[a] Department of Chemistry, University of Crete, Voutes, Heraklion 71003, Crete, Greece
[b] Department of Plant Production, Plant Biotechnology Laboratory, Technological and Educational Institute of Larissa, 41110 Larissa, Greece
[c] Institute of Aquaculture, Hellenic Centre for Marine Research, P.O. Box 2214, 71003 Heraklion, Crete, Greece

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ABSTRACT

In this study, three plant species, members of the family of *Lamiaceae* and the genus *Origanum*, namely, *Origanum vulgare* subsp. *hirtum*, *Origanum onites* L., and *Origanum marjorana* L. were studied for their chemical composition and antibacterial activity. Essential oils of these plants were received by means of micro-steam distillation and their components were analyzed by gas chromatography and mass spectrometry (GC–EIMS). The major components identified in all three species are carvacrol and thymol. The oils were assayed as potential food control antimicrobial agents. *In vitro* studies showed that the essential oils showed strong antimicrobial activity against 5 bacterial and 1 yeast strains.

1. Introduction

Essential oils (EOs) are aromatic oily liquids obtained from various plants generally localized in temperate to warm countries. In nature as secondary metabolites, EOs play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also act against herbivores (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). An estimated 3000 essential oils are known, of which about 300 are commercially available and destined mostly for the flavor and fragrances market (Van de Braak & Leijten, 1999, 116 pp.). EOs are very complex natural mixtures containing hydrocarbons (mainly terpenoids) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers). Their composition may vary considerably between plant species and varieties, and, within the same variety, from different geographical origin (Zygadlo & Juliani, 2003). EOs are widely used in medicine, in perfumes, cosmetics and bath products, as flavoring agents in food and drink, and in many other manufacturing areas. EOs can constitute a powerful tool to reduce the development and dissemination of antimicrobial resistance. Nowadays essential oils are recognized as safe substances (ESO, GRAS – 182.20) by the Food and Drug Administration (2005) and some contain compounds which can be used as antibacterial additives (Ait-Ouazzou et al., 2011; Cox et al., 2001; Muyima, Zulu, Bhengu, & Popplewell, 2002; Nerio, Olivero-Verbel, & Stashenko, 2010).

They become increasingly popular as natural antimicrobial and antioxidant agents that may be used in food preservation. Public concern about the use of antibiotics in livestock feed has increased, because of the emergence of antibiotic resistant bacteria and their possible transmission from livestock to humans. In fact, in the European Union the use of synthetic antibiotics, health and growth promoters as additives in livestock feed has been prohibited since 2006 by the European Parliament and Council Regulation (EC No.1831/2003). In this context, one of the possible solutions is the use of EOs such as those found in the genus *Origanum* and in its 3 species used for this study: *Origanum vulgare* subsp. *hirtum*, *Origanum onites* and *Origanum marjorana*.

Antimicrobials are used in the food industry for two main reasons: to control natural spoilage processes (food preservation), and to prevent the growth of micro-organisms, including pathogenic micro-organisms (food safety).

A large number of reports concerning the antioxidant and the antimicrobial ability of essential oils have already been published (Bagamboula, Uyttendaele, & Debevere, 2003; Bakkali, Averbeck, Averbeck, Zhiri, & Idaomar, 2005; Botelho et al., 2007; Castilho,

* Corresponding author. Tel.: +30 2810 545026; fax: +30 2810 545001.
E-mail address: kater@chemistry.uoc.gr (H.E. Katerinopoulos).

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Savluchinske-Feio, Weinhold, & Gouveia, 2012; Deba, Xuan, Yasuda, & Tawata, 2008; Gülçin, Elmastaş, & Aboul-Enein, 2007; Kalemba & Kunicka, 2003; Pauli, 2006; Pavela, 2009; Sacchetti et al., 2005; Saidana et al., 2008; Si et al., 2006; Thuille, Fille, & Nagl, 2003; Valero & Salmeron, 2003; Ye, Dai, & Hu, 2013). In these studies, EO exhibited very good insecticide, bactericide and fungicide effects (Kumar, Shukla, Singh, Prasad, & Dubey, 2008; Srivastava, 2008). Burt (2004) gives an overview of the studies of the antibacterial activity of essential oils in foods. As typical lipophiles, they cross through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them. In general, the cytotoxic activity of essential oils is mostly due to the presence of phenols, aldehydes and alcohols (Bruni et al., 2003; Sacchetti et al., 2005).

The aims of this study were a) to determine the chemical composition of essential oils extracted from three species of the genus *Origanum*, and b) evaluate their ability to inhibit *in vitro* bacterial strains such as *Vibrio*, isolated from aquaculture facilities, and the common microbial strains *Escherichia coli* and *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Materials

Samples of leaves, stems and/or seeds from three *Origanum* species were collected from various regions of Greece, or made available from commercial sources (Table 1).

The collection and/or identification of the plant material were carried out by Assistant Professor Ilias Anastasopoulos, at the Plant Biotechnology Laboratory of Larissa's Technological Educational Institute. Voucher specimens have been deposited at the Herbarium of the Institute. All non-commercial plants were grown from seeds, except from samples 0 and 5 which were asexually propagated from cuttings of the same plant, and therefore genetically identical. Plants were grown in a plastic greenhouse at the Technological Educational Institute, in Larissa, Greece, and received no treatment apart from watering.

2.2. Extraction methods

After collection, the plant material was air dried in the dark at room temperature (~25 °C) for 10 days. The dried plant parts underwent hydrodistillation for two hours on a Clevenger apparatus connected to a modified refrigerated essential oil receiver (European Pharmacopoeia 5.0). Refrigeration was used to reduce the byproducts of the thermal treatment. The essential oils were then diluted with 2 mL of ether and filtered through anhydrous sodium sulfate to remove water traces. The resulting essential oils were stored at 4 °C. The oil content was estimated in mL/100 g (dry weight of the plant material).

2.3. Chemical analysis

2.3.1. GC–EIMS analysis

GC–EIMS analysis of the extracts was performed on a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu GCMS-QP 5050 mass-selective detector with the appropriate data system. The GC was equipped with a Grob-type split-splitless injector. The fused silica capillary column (Supelco, SBP-5 with 0.25 μm film thickness, 30 m × 0.25 mm i.d.) was directly coupled to the ion source. Helium was used as a carrier gas with a back pressure of 0.8 Atm. The injector temperature was 250 °C and the oven temperature program started at 50 °C for 5 min and then increased at a rate of 5 °C/min up to 150 °C, retained at this temperature for 10 min and increased again at a rate of 5 °C/min up to 280 °C, where it remained for 20 min.

2.3.2. Identification

The scanning range was 30–700 *m*/*z*. The quantification of the components was based on the total number of fragments (total ion count) of the metabolites, as detected by the mass spectrometer. The identification of the chemical components was carried out based on the retention time of each component (Rt) compared with those of commercially available compounds, by analysis of their mass spectra, by the use of the NIST21, NIST107 and PMW_TOX2 mass spectra libraries (NIST, 2010) as well as by comparison with literature data (Adams, 2007). Calculation of retention indices was performed in accordance to the work of Van den Dool and Kratz (1963), in comparison to the retention times of standard hydrocarbons (C_9–C_{25}). Also, when necessary, co-injection with standard compounds was carried out.

2.4. Antimicrobial screening

The 6 microbial strains used as test organisms were as follows: *E. coli*, *S. cerevisiae*, *Listonella anguillarum* (CECT 522), *Vibrio splendidus* DMC-1 (kindly provided by Prof. T.H. Brikbeck, University of Glaskow), *Vibrio alginolyticus*, isolated from seabream larvae (*Sparus aurata*) (kindly provided by Dr. P. Katharios, Institute of Aquaculture, Hellenic Center for Marine Research) and *Vibrio* sp. isolated from enriched *Artemia metanauplii* homogenate by Dr. P. Makridis at the Institute of Aquaculture, Hellenic Center for Marine Research. *L. anguillarum*, *V. splendidus*, *V. alginolyticus* and *Vibrio* sp. were grown on tryptic soy agar dishes. *S. cerevisiae* were grown on YPD agar dishes and *E. coli* were grown on LB agar dishes.

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil (NCCLS, 1997). For this purpose, Watmann number 1 paper disks with 6 mm diameter soaked with 2 μL of the essential oil were laid on top of the agar culture medium plate previously inoculated (50 μL–10^8 CFU/mL) with the different micro-organisms tested in this work. In addition

Table 1
Origanum samples used in the study.

Sample	Date	Species	Place of collection or purchasing	Geographical coordinates (altitude)
0	Oct/2007	*O. vulgare* subsp. *hirtum*	Pilio, Greece	39°26′N 23°2′E (700 m)
1	Aug/2006	*O. vulgare* subsp. *hirtum*	CC Botanicals Ltd (Warwick, UK)	–
2	Aug/2006	*O. vulgare* subsp. *hirtum*	Agrafa, Greece	39°8′N 21°38′E (700 m)
3	Aug/2006	*O. marjorana*	Pieterpikzonen b.v. (Luinjeberd, NL)	–
4	Aug/2006	*O. vulgare* subsp. *hirtum*	CC Botanicals Ltd (Warwick, UK)	–
5	Oct/2007	*O. vulgare* subsp. *hirtum*	Pilio, Greece	39°26′N 23°2′E (700 m)
6	Oct/2007	*O. vulgare* subsp. *hirtum*	Sisses, Greece	35°22′N 24°28′E (500 m)
7	Aug/2006	*O. onites*	Naxos island, Greece	37°5′N 25°28′E (700 m)
8	Aug/2006	*O. onites*	Naxos island, Greece	37°5′N 25°28′E (700 m)
9	Oct/2007	*O. vulgare* subsp. *hirtum*	Agrafa, Greece	39°8′N 21°38′E (700 m)