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RESEARCH ARTICLE (Open Access)

Antimicrobial Activity and Chemical Composition of Albanian Oregano

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Abstract

Antimicrobial activity of methanolic and aqueous methanolic extracts of *oregano* was tested against: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas* spp, *Candida albicans* ATCC 10231, *Listeria monocytogenes* ATCC 19111 and *Salmonella typhimurium* ATCC 14028. Antimicrobial activity of oregano essential oil was also tested against: *E. coli, P. aeruginosa, S. aureus and C. albicans*. Only oregano essential oil was active against microorganisms selected. Essential oil of oregano was analysed by GC-MS. Eighteen components were identified representing 99.48 % of the oil. Monoterpenes phenols and derivatives (borneol, 4-terpineol, carvacrol methyl ether, thymoquinone, thymol, carvacrol) represented 74.66 % of essential oil. Carvacrol, *p*-cymene, thymol and γ-terpinene were the main components. Sesquiterpenes such as *trans*-caryophyllene, α-humulene, β-bisabolene, δ-Cadinene, caryophyllene oxide were also found.

Keywords: Oregano; oil, antimicrobial; microorganism, GC⁄MS.

1. Introduction

Origanum vulgare L., (Lamiaceae) is a medicinal and aromatic plant widely distributed in mediterrean area. Oregano is the world commercially most valued spice known and used for centuries. *O. vulgare* volatile oil is known to possess antioxidant [30;26;14] antimicrobial [24;12;13], antifungal [28], antihelmentic [22], antiinflamatory properties [25;19]. Studies on antimicrobial activity of *O. vulgare* essential oil showed that is particularly active versus Gram-positive pathogens and particularly *Bacillus cereus* and *B. subtilis* [3;9], *Burkholderia cepacia* [15], *Staphylococcus aureus* [1;10], *Salmonella* [21] but is also active against gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli* , *Klebsiella pneumoniae* [20], *Aeromonas hydrophila* [2]. Oregano had shown antifungal activity against pathogenic fungi: anti-*Aspergillus* activity [6] and specially against *Aspergillus flavus* and *Aspergillus niger* [11;4], *A. fumigatus* [7], *Microsporum canis* [16], *Trichophyton mentagrophytes* [17] *Candida* spp. [8].

Higher inhibitory capacity was observed in the oils with a higher percentage of phenolic components (carvacrol and thymol) [21]. Studies showed that carvacrol has diverse biological activities such as: antioxidant, antibacterial, antifungal [28], anticancer, anti-inflammatory, hepatoprotective, spasmolytic, and vasorelaxant [27;18;29]. The survey in the literature showed that antimicrobial activity of the volatile part of oregano was most widely studied. Limited data on antimicrobial activity polyphenols of oregano were found. No data were found about *O. vulgare (*of Albanian origin) phenolics compounds and their biological activity.

2. Material and Methods

2.1. Reagents and standards

Nutrient agar (NA, Biolife), Patato dextrose agar (PDA, Liofilchem), methanol (Analar Normapur), DMSO (Sigma-Aldrich), cefazolin (Sefazol, Mn pharmaceuticals), hexane (Sigma-Aldrich).

2.2. Isolation of essential oil

Aerial parts of wild growing *O. vulgare* were collected in the region of Shkodra in Albania. Essential oil was prepared by hydrodistillation in a Clevenger-type apparatus, using hexane as a recipient solvent.

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2.3. Preparation of oregano methanolic and aqueous-methanolic extracts

4.99 g of dried plant materials *O.vulgare* was extracted with 100 ml aqueous methanol 80 % for two hours with constant shaking at 135 rpm and temperature 30˚C. The extracts were filtered using a filter paper and concentrated under reduce pressure at 45 ˚C using rotatory evaporator (Laborota 4000, Heidolph) at 90 rpm and t=45˚C. The water phase was removed in thermostat (t=45˚C). The green-brown residue of *O. vulgare* were diluted in 10 ml DMSO. Methanolic extracts were prepared in the same way. The yield of methanolic extract was determined to be 800.7 mg or 16.05 % for *O. vulgare*. The residue was diluted in methanol.

2.4. Antimicrobial activity of oregano methanolic and aqueous-methanolic extracts

The antimicrobial activity was tested by the disc-diffusion method as already described by Bauer *et al.* (1966) against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Pseudomonas* spp, *Candida albicans* ATCC 10231, *Listeria monocytogenes* ATCC 19111 and *Salmonella typhimurium* ATCC 14028 (obtained from Profarma sh.a. Pharmaceutical company and Faculty of Biotechnology and Food, Agricultural University of Tirana). The selected microorganisms were taken from stock culture and grown in NA (*E. coli*, *S. aureus*, *Pseudomonas* spp. *L. monocytogenes* and *S. thyphimurium*) and modified PDA (*C. albicans*). The plates were incubated at 37 ˚C for 24h. The bacterial suspensions was adjusted to a density of bacterial cells of 1.0×10^8 CFU/mL. A sterile cotton-tipped swab immersed in this bacterial suspension was used to inoculate the entire surface of a NA and PDA. Four sterile paper disc 8mm impregnated with 10 µL diluted solution of *O. vulgare* extracts (1mg/mL), positive control cefazolin (3mg/mL) and negative control (DMSO or methanol) were aseptically placed on inoculated agar surface. The plates were incubated at 37˚C for 24h. After 24h the diameter of inhibition zones was measured. The experiments were conducted in triplicate.

2.5 Antimicrobial activity of oregano essential oil

Sterile paper discs were soaked with 5 µL of undiluted essential oil and placed on the inoculated surface of Petri dishes containing solidified Tryptic Soy Agar. As microorganism we used: *Staphylococcus aureus* ATCC (American Type Culture Collection) 6538 (1.0×10^5 U.F.C./ml), *Pseudomonas aeruginosa* ATCC 9027 (1.1×10^5 U.F.C./ml) and *Escherichia coli* ATCC 8739 (7.1×10^4 U.F.C./ml). Petri dishes were incubated at 37^{o}C for 24h. The antimicrobial activity was evaluated by the inhibition zone observed. Antifungal activity was tested on C.albicans ATCC 1231.

2.6 GC−MS analysis of oregano essential oil

Qualitative and semi-quantitative chemical characterization of essential oil was performed by GC-MS technique, using Agilent Technologies 6890N gas chromatograph coupled with Agilent Technologies 5975B electron ionization mass-selective detector. A 1 µL aliquot of essential oil hexane solution, diluted 100-fold with hexane, was injected into a split/splitless inlet at 250 °C, with a split ratio 1:10. Helium (purity 5.0) was used as a carrier, with a constant flow of 1 mL/min. Components were separated on a non-polar Agilent Technologies HP-5 ms column (30 m × 0.25 mm, 0.25 µm), using the temperature program starting at 50 °C, increasing 8 °C/min to 120 °C, then 15 °C/min to 230 °C, and finally 20 °C/min to 270 °C, and holding at 270 °C for 16.9 min (total run time 35 min). Effluent was delivered to the mass spectrometer via a transfer line held at 280 °C. Ion source temperature was 230 °C, electron energy 70 eV, and quadrupole temperature 150 °C. To achieve better correlation between experimental and library spectra, standard spectra tune was used. Data were acquired in scan mode (*m/z* range 35–400), with solvent delay of 2.30 min. Data were processed using Agilent Technologies MSD ChemStation software (revision E01.01.335) combined with AMDIS (ver. 2.64) and NIST MS Search (ver. 2.0d). AMDIS was used for deconvolution, i.e. co-eluting compounds peak area determination and pure spectra extraction, and NIST MS Search provided search algorithm complementary to PBM algorithm of ChemStation. The compounds were identified by comparison of mass spectra with data libraries (Wiley Registry of Mass Spectral Data, 7th ed. and NIST/EPA/NIH Mass Spectral Library 05) and confirmed by comparison of linear retention indices with literature data (Adams, 2001). Diesel oil, containing C_8–C_{28} *n*-alkanes, was used as a standard for determination of linear retention indices (LRI). Relative amounts of components, expressed in

percentages, were calculated by normalization measurement according to peak area in total ion chromatogram.

3. Results and Discussion

3.1. Antimicrobial activity of oregano methanolic and aqueous−methanolic extracts

Methanolic and 80% aqueous-methanolic extracts of *O.vulgare* failed to inhibit growth of *S. aureus, E. coli, P. aeruginosa, C. albicans, L. monocytogenes and S. typhimurium*. The results are shown in Table 1 and represents the net zone of inhibition after subtracting of the diameter (8mm) of paper discs. As a reference standard cefazolin was used. The standard was active against *S. aureus* (32mm), *E. coli* (20 mm) and *S. typhimurium* (17mm). Sahin *et al.* (2004) reported that methanolic extracts from aerial parts of *O. vulgare* plants showed no antimicrobial activity (against 10 bacteria and 15 fungi and yeast species).

Table 1. Antimicrobial activity of oregano extracts

Microorganisms	*Diameter of inhibition (mm)*		
	O. vulgare M	*O. vulgare AM*	*Cefazolin*
S. aureus	-	-	32
E. coli	-	-	20
P. aeruginosa	-	-	-
S. typhimurium	-	-	17
L. monocytogenes	-	-	-
C. albicans	-	-	*

(-)means no zone of inhibition, *not tested; M−methanolic, AM−aqueous methanolic.

Dulger and Gonuz (2004) showed that *O. vulgare* methanolic extracts were moderately active against: *S. aureus, P. vulgare* and *Mycobacterium smegmatus* (among 12 different bacteria yeast and fungi) at the concentration 200 mg/ml^{-1}.

3.2. Antimicrobial activity of oregano essential oil

Oregano essential oil showed interesting antimicrobial activity against all selected microorganisms. The results are shown in Table 2. Oregano essential oil was active against gram positive bacteria *S. aureus*. Alexopoulos *et al.* 2011; De Souza *et al*. 2009 showed antibacterial activity of oregano essential oil against *S. aureus* (34mm).

Table 2. Antimicrobial activity of oregano essential oil

Microorganisms	*Diameter of inhibition (mm)*
	Oregano essential oil
S. aureus	34
E. coli	25
P. aeruginosa	12
C. albicans	24

Oregano essential oil was active against gram negative bacteria *P. aureuginosa* (12mm) and *E. coli* (25mm). The results are in accordance with research done by Bozin *et al.* 2006. Oregano showed antifungal activity against *C. albicans* (24mm). Cleff *et al.* 2010 showed antifungal activity of oregano essential oil against *C. albicans*.

3.3. GC analysis of oregano essential oil

Monoterpenes phenols and derivatives (borneol, 4-terpineol, carvacrol methyl ether, thymoquinone, thymol, carvacrol) represented 74.66 % of essential oil. Carvacrol (monoterpenic phenol isomeric with thymol) was the main component. The second major component was *p*-Cymene and the third one was thymol. Oregano was also rich in γ-terpinene. Sesquiterpenes found were: *trans*-Caryophyllene, α-Humulene, β-Bisabolene, δ-Cadinene, caryophyllene oxide. Eighteen components identified in oregano essential oil are shown in Table 3. Chromatogram of oregano essential oil is represented in Figure 1.

Carvacrol as the main component found in oregano essential oil possess a variety of biological and pharmacological properties including antioxidant, antibacterial, antifungal and antiviral [28].

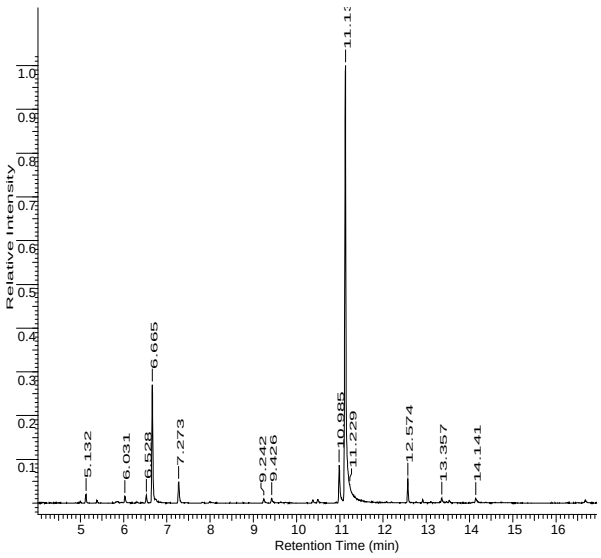

Figure 1. GC chromatogram of oregano essential oil.

Table 3. Qualitative and quantitative chemical characterization of essential oil performed by GC-MS.

t_r [min]	LRI*	ID	area%
5.129	937	α-Pinene	0.89
5.387	952	Camphene	0.26
6.034	992	β-Myrcene	0.66
6.527	1020	α-Terpinene	0.88
6.666	1028	*p*-Cymene	13.37
6.738	1032	Limonene	0.46
7.277	1062	γ-Terpinene	2.62
9.244	1174	Borneol	0.57
9.428	1185	4-Terpineol	0.65
10.380	1315	Carvacrol methyl ether	0.38
10.492	1320	Thymoquinone	0.46
10.987	1343	Thymol	5.31
11.129	1350	Carvacrol	68.50
12.574	1437	*trans*-Caryophyllene	2.25
12.920	1472	α-Humulene	0.31
13.359	1519	β-Bisabolene	0.74
13.534	1539	δ-Cadinene	0.40
14.144	1609	Caryophyllene oxide	0.76

Essential oils sometimes cause skin irritation or allergic reaction. Scientific Committee on Consumer Safety clasiffied a total of 54 individual fragrance substances and 28 natural extracts (essential oils) as 'established contact allergens in humans'. The major components of oregano essential oil (carvacrol, thymol and p-cimene) do not belong to this list of allergens. Only α-pinene is a known allergen in humans however its concentration is in *O. vulgare* essential oil was low (0.89%). *O. vulgare* essential oil can be used in cosmetic products to help for inhibition of growth of microorganisms.

4. Conclusions

Among the oregano extracts, essential oil showed interesting antimicrobial activity against human pathogens *S. aureus, E. coli, P. aureuginosa and C. albicans*. Oregano essential oil was rich in carvacrol, p-cymene and thymol and this conponents do not belong to the list of human allergens. *O. vulgare* essential oil can be used in cosmetic products for the inhibition of growth of microorganisms.

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