Research Article

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Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil

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Abstract

BACKGROUND: There is a growing interest in industry to replace synthetic chemicals by natural products with bioactive properties. Aromatic plants are excellent sources of bioactive compounds that can be extracted using several processes. As far as oregano is concerned, studies are lacking addressing the effect of extraction processes in bioactivity of extracts. This study aimed to characterise the *in vitro* antioxidant and antibacterial properties of oregano (*Origanum vulgare*) essential oil and extracts (in hot and cold water, and ethanol), and the chemical composition of its essential oil.

RESULTS: The major components of oregano essential oil were carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene. Hot water extract had the strongest antioxidant properties and the highest phenolic content. All extracts were ineffective in inhibiting the growth of the seven tested bacteria. In contrast, the essential oil inhibited the growth of all bacteria, causing greater reductions on both *Listeria* strains (*L. monocytogenes* and *L. innocua*).

CONCLUSION: *O. vulgare* extracts and essential oil from Portuguese origin are strong candidates to replace synthetic chemicals used by the industry.

Keywords: oregano; antibacterial activity; antioxidant activity; ethanol extracts; aqueous extracts; essential oil

INTRODUCTION

Synthetic preservatives are widely used for different applications. Yet, since the safety of some synthetic preservatives is questionable, consumers tend to prefer preservative-free products. Therefore, there is a growing interest in assessing the antimicrobial and antioxidant properties of substances from natural sources that can potentially be used by the food and pharmaceutical industries. Essential oils from aromatic and medicinal plants have been known to possess biological activity, such as antimicrobial and antioxidant properties.[1,2]

Oregano (*Origanum vulgare*) is an aromatic herb belonging to the Lamiaceae family, and commonly occurs throughout Asia, Europe, and northern Africa.[3] In folk medicine, *O. vulgare* is used to treat respiratory disorders, dyspepsia, painful menstruation, rheumatoid arthritis, scrofulosis and urinary tract disorders. It is also used as a culinary herb in gastronomy.[3] Previous studies reported the potential of oregano essential oil to preserve food, such as fresh chicken breast meat,[4] swordfish[5] and octopus.[6]

Previous studies on the isolation of *O. vulgare* essential oils from different regions of the world, including Greece,[7] Lithuania,[8] India,[9] Poland[10] and Italy,[11] focused mainly on the chemical composition, although the antioxidant and antimicrobial properties were also explored.[12] Yet, there was no information on the bioactive properties of water and ethanol extracts of *O. vulgare*. Therefore, this study aimed to identify the chemical composition of *O. vulgare* essential oil of Portuguese origin, and to evaluate the total phenolic content, antioxidant activity, and antibacterial properties of the essential oil and water (hot and cold) and ethanolic extracts.

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MATERIAL AND METHODS

Preparation of extracts and essential oil

O. vulgare was collected in Santarém (Ribatejo, Portugal) (39° 21′ 37.44″ N, 8° 45′ 41.04″ W) in summer 2008. Voucher specimens were identified by Dr Carmo Serrano and deposited in the Herbarium of the Portuguese National Institute of Biological Resources, I.P. (n° LISU 191009). The dried aerial parts were used to prepare three extracts (hot water, cold water, and ethanolic) and the essential oil. The extracts were obtained by maceration of dry plant material (100 g) in either boiling water for 3 h (hot water extract); or water for 3 days at room temperature (cold water extract); or ethanol (99%) for 3 days at room temperature (ethanolic extract), without using a successive extraction. The macerates were filtered under vacuum through a Buckner funnel with filter paper (Whatman no. 4), whereas the ethanolic extract was dried in a rotary evaporator under vacuum (40°C, 178 mbar). The extracts were freeze dried at −50°C (Heto-Powerdry, LL3000; Mukarov, Czech Republic). The essential oil was obtained from dry plant material (75 g) by hydrodistillation for 3 h using a modified Clevenger system. During the boiling process, the dried material soaks up water and the essential oil diffuses through the cell walls by means of osmosis, and then it is vapourised and carried away by the stream of steam.[13] Afterwards, the essential oil was dried through anhydrous sodium sulfate. The extracts and essential oil were stored at −20°C. The water extracts concentrations, before the freeze-drying procedure, were 41.7 mg mL^{-1} (hot water extract) and 23.8 mg mL^{-1} (cold water extract), while the essential oil had a density of 896.6 mg mL^{-1}, and the total dry weight of the ethanolic extract was 5.4 g.

Chemicals

Phosphate buffer, trichloroacetic acid, ferric chloride, ascorbic acid, and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were purchased from Fluka (Buchs, Germany); Folin–Ciocalteu reagent, gallic acid, α,α-diphenyl-β-picrylhydrazyl (DPPH), potassium hexacyanoferrate(III), and dimethylsulfoxide from Sigma–Aldrich (Steinheim, Germany); ferrous sulfate, sodium acetate, tryptic soy agar, and plate count agar from Merck (Darmstadt, Germany); sodium carbonate and butylated hydroxytoluene (BHT) from BDH (Poole, UK); sodium sulfate anhydrous from Panreac Química S.A.U. (Barcelona, Spain); brain heart infusion broth from Oxoid (Basingstoke, UK); ethanol had a purity grade of 99% and the water used was distilled and Milli-Q purified.

Gas chromatography–mass spectrometry analysis

The *O. vulgare* essential oil was analysed on an Agilent 6890 gas chromatograph interfaced to an Agilent 5973N mass selective detector (Agilent Technologies, Palo Alto). A vapourisation injector operating in the split mode (1:50) at 250°C was used, into which a fused silica capillary column (30 m length × 0.32 mm internal diameter × 0.25 μm film thickness; HP-5MS; 5% diphenyl–95% dimethyl polydimethylsiloxane; Agilent Technologies) was installed. The oven temperature was programmed at 45°C for 1 min, raised to 250°C at 5°C min^{-1}, and maintained at 250°C for 5 min. Helium was used as carrier gas at 30 cm s^{-1} and the injection volume was 1 μL. The transfer line, ion source, and quadrupole analyser temperatures were maintained at 280°C, 230°C and 150°C, respectively, and a turbo molecular pump (10^{-5} Torr) was used. In the full-scan mode, electron ionisation mass spectra in the range 40–400 Da were recorded at 70 eV electron energy. A solvent delay of 3 min was selected. The acquisition data and instrument control were performed by the MSD ChemStation software (G1701CA, version C.00.00; Agilent Technologies, Santa Clara, CA, USA). The identity of each compound was assigned by comparison of their retention index relative to a standard mixture of *n*-alkanes,[14] as well as by comparison with the mass spectra characteristic features obtained with the Wiley's library spectral data bank (G1035B, Rev D.02.00; Agilent Technologies, Santa Clara). For semi-quantification purposes the normalised peak area abundances without correction factors were used.

Antioxidant activity assays

Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) assay was based on the methodology of Benzie and Strain[15] modified by Deighton *et al*.[16] Briefly, FRAP reagent was prepared by combining TPTZ (1 mmol L^{-1}) and ferric chloride (2 mmol L^{-1}) in sodium acetate (0.25 mol L^{-1}; pH 3.6). The sample (0.2 mL) was mixed with FRAP reagent (1.8 mL), allowed to stand for 4 min at room temperature and the absorbance was determined at 593 nm (ATI-UNICAN-UV2; Unicam, Cambridge, UK). *O. vulgare* extracts were diluted in water and the essential oil was diluted in ethanol 50%. Negative controls with water (aqueous and ethanolic extracts) or ethanol 50% (essential oil) were also included. All determinations were performed in triplicate.

The difference between the absorbance of sample and the negative control was calculated and the reducing capacity of samples was compared with that of a reaction with a ferrous ion standard solution. The standard was prepared from ferrous sulfate that reacted with the TPTZ reagent, following the same procedure as with samples. The absorbance was plotted against ferrous ion concentration in the range 0.125–3.500 μmol L^{-1} Fe^{2+}, and FRAP values were expressed as μmol Fe^{2+} g^{-1} sample.

Reducing power

The capacity of *O. vulgare* extracts and its essential oil to reduce iron(III) to iron(II) was determined according to the modified method of Oyaizu.[17] Briefly, the sample (1 mL) was mixed with phosphate buffer (2.5 mL, 0.2 mol L^{-1}, pH 6.6) and potassium hexacyanoferrate(III) (2.5 mL, 10 mg mL^{-1}). After 30 min of incubation at 50°C in the dark, trichloroacetic acid (2.5 mL, 0.1 g mL^{-1}) was added and the mixture kept at room temperature for 10 min. Afterwards, this mixture (2.5 mL) was added to water (2.5 mL) and ferric chloride (0.5 mL, 1 mg mL^{-1}), vigorously mixed, and the absorbance measured at 700 nm in a spectrophotometer (ATI-UNICAN-UV2). Extracts and essential oil were diluted in ethanol. All determinations were performed in triplicate. Negative (ethanol) and a positive (ascorbic acid in the 10–40 μg mL^{-1} range) control reactions were performed. The results were expressed as μmol ascorbic acid g^{-1} sample.

Free radical scavenging

The scavenging effect of DPPH free radical was assessed by the modified method of Kondo *et al*.[18] Briefly, each extract or essential oil (0.1 mL) at different concentrations (in 95% ethanol) was added to DPPH (2 mL, 0.21 mmol L^{-1} in 95% ethanol). The mixture was shaken, left for 60 min at room temperature in the dark, and the absorbance was measured at 517 nm in a spectrophotometer (ATI-UNICAN-UV2). The percentage DPPH inhibition was calculated using the equation:

$$\text{inhibition}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction (blank with 0.1 mL ethanol and DPPH) and A_{sample} is the absorbance of the sample reaction (0.1 mL sample diluted in ethanol and DPPH). The sample concentration (in 1 mL reaction mixture) providing 50% inhibition (EC_{50}) was estimated by plotting percentages of inhibition against concentrations of sample. All determinations were performed in triplicate. EC_{50} (μg mL^{-1}) was also estimated for the synthetic antioxidant reagent BHT.

To standardise DPPH results, the antioxidant activity index (AAI), proposed by Scherer and Godoy,[19] was calculated as follows:

$$AAI = \frac{C_{DPPH}}{EC_{50}}$$

where C_{DPPH} is the DPPH concentration (μg mL^{-1}) in the reaction mixture. Samples were classified as showing poor antioxidant activity when $AAI < 0.5$, moderate antioxidant activity when $0.5 < AAI < 1.0$, strong antioxidant activity when $1.0 < AAI < 2.0$, and very strong when $AAI > 2.0$.[19]

Phenol content

Total phenol content was measured using a modified Folin–Ciocalteu assay.[20] Briefly, water (5 mL), sample (1–3 mL) and Folin–Ciocalteu reagent (0.5 mL) were mixed, allowed to stand for 5–8 min at room temperature, followed by the addition of sodium carbonate (1.5 mL, 0.2 g mL^{-1}) together with water to obtain a final volume of 10 mL. The solution was mixed, allowed to stand for 2 h and filtered (0.45 μm polytetrafluoroethylene filter, Whatman) prior to absorbance reading at 750 nm in a spectrophotometer (ATI-UNICAN-UV2). Aqueous and ethanolic extracts were diluted in water, whereas the essential oil was diluted in ethanol 50%. All determinations were performed in triplicate. Negative controls were performed with water (aqueous and ethanolic extracts) or ethanol 50% (essential oil). Total phenol content was quantified by comparison of samples absorbance values with those of gallic acid reaction. The calibration curve of gallic acid was prepared in the 5–25 mg L^{-1} range, and results were expressed as mg gallic acid g^{-1} sample.

Antibacterial activity

Strains and growth conditions

The antibacterial activity tests included foodborne spoilage and pathogenic bacteria purchased from American Type Culture Collection (ATCC) or Spanish Type Culture Collection (CECT): *Brochothrix thermosphacta* (CECT 847; Gram positive), *Escherichia coli* (ATCC 25922; Gram negative), *Listeria innocua* (CECT 910; Gram positive), *Listeria monocytogenes* (CECT 5873; Gram positive), *Pseudomonas putida* (CECT 7005; Gram negative), *Salmonella typhimurium* (ATCC 14028; Gram negative), and *Shewanella putrefaciens* (CECT 5346; Gram negative). These strains were kept at −70°C in a cryopreservative solution (Microbank; Pro-lab Diagnostics, Richmond Hill, ON, Canada) and were inoculated in tryptic soy agar (TSA) and incubated overnight at 30°C, except *L. monocytogenes*, which was inoculated in plate count agar (PCA). Subsequently, one colony from these cultures was inoculated on brain heart infusion broth and incubated at 30°C for 18–24 h with shaking (75 rpm), in order to obtain freshly cultured microbial suspensions (10^8–10^9 cells mL^{-1}) for tests.

Paper disc diffusion method

The antibacterial activity of *O. vulgare* extracts and essential oil was determined using the paper disc diffusion method.[21] Briefly, the bacterial suspensions were adjusted to 1×10^7 CFU mL^{-1} and spread in TSA or PCA (10–15 mL per 90-mm diameter Petri dish) using a sterile cotton swab. Subsequently, filter paper discs (6 mm diameter; Whatman No. 1) were placed on the surface of Petri dishes and impregnated with 20 μL of the extracts or essential oil at different concentrations. Essential oils were diluted in dimethylsulfoxide, while ethanolic and aqueous extracts were diluted in ethanol and water, respectively. Negative controls were prepared using the same solvents employed to dissolve samples. After remaining at 4°C for 2 h, Petri dishes were incubated at 30°C for 24 h, except *L. monocytogenes*, which was incubated for 48 h. All determinations were performed in triplicate. Antibacterial activity was evaluated by measuring the radius of inhibition zones to the nearest millimetre, and the minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited growth of bacteria.

Effect of minimum inhibitory concentration on the liquid medium

The MIC of extracts and essential oil was tested for antibacterial activity using liquid media. The bacterial suspensions were adjusted to 1×10^7 CFU mL^{-1} in brain heart infusion broth, and 180 μL were added to each well of 96-well plates. Subsequently, 20 μL of extract or essential oil were added, in order to obtain the MIC determined with the paper disc diffusion method. Negative controls were prepared with the solvents used to dissolve extracts and essential oil, as well as positive controls with solvents and bacteria, but without the extract or essential oil. Plates were incubated at 4°C (2 h) and 30°C (24 h). The absorbance at 595 nm was measured using a micro plate reader (Bio-Rad model 680; Bio-Rad, Hercules, CA, USA) before and after incubation period, and the microbial concentration was determined by viable counts on TSA or PCA. All determinations were performed in triplicate.

Statistical analysis

Differences between extracts and essential oil were tested with analysis of variance (ANOVA). In order to satisfy ANOVA assumptions data were transformed, followed by multiple comparisons tests (Tukey HSD) to identify differences between groups. If transformed data could not met ANOVA assumptions and non-parametric analysis of variance (Kruskall–Wallis) was performed, followed by non-parametric multiple comparisons test (Dunn). Statistical analyses were tested at a 0.05 level of probability with the software STATISTICA™ 6.1 (Statsoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Chemical composition of the essential oil

Sixty-four compounds were identified in *O. vulgare* essential oil, accounting for 92.3% of the whole composition (Table 1). The essential oil was mainly composed of oxygenated monoterpenes (53.8%) and monoterpene hydrocarbons (26.4%). Within oxygenated monoterpenes, carvacrol (14.5%), thymol (12.6%), β-fenchyl alcohol (12.8%), and δ-terpineol (7.5%) were the major compounds detected, while γ-terpinene (11.6%) and α-terpinene (3.7%) were the most abundant monoterpene hydrocarbon detected. Additionally, 1-methyl-3-(1-methylethyl)-benzene (6.8%) also represented a substantial fraction of *O. vulgare* essential oil. Carvacrol, thymol, γ-terpinene, and linalool are known to possess strong antioxidant properties,[22–24] and carvacrol and thymol also exhibit antibacterial activity against several bacteria.[12,25,26]

Table 1. Chemical composition of volatiles in the *Origanum vulgare* essential oil

Compound	RI	%[a]
Monoterpene hydrocarbons	—	**26.4**
α-Thujene	853	2.2
α-Pinene	861	0.7
Camphene	877	0.1
Sabinene	909	1.0
β-Pinene	912	0.4
β-Myrcene	929	1.3
α Phellandrene	945	0.4
Δ^3-Carene	952	0.1
α-Terpinene	961	3.7
β-Phellandrene	978	0.9
cis-β-Ocimene	990	1.6
trans-β-Ocimene	1002	1.5
γ-Terpinene	1016	11.6
α-Terpinolene	1053	0.9
neo-Allo-ocimene	1488	tr
Sesquiterpene hydrocarbons	—	**3.6**
α-Cubebene	1416	tr
β -Elemene	1435	0.1
trans-Caryophyllene	1467	tr
α-Bergamotene	1484	0.1
Allo-aromadendrene	1489	0.1
Germacrene D	1537	0.3
β-Selinene	1543	tr
Ledene	1552	tr
Bicyclogermacrene	1555	0.3
α-Muurolene	1558	tr
β-Bisabolene	1565	2.1
Selina-3,7(11)-diene	1577	0.2
β-Cadinene	1583	0.2
cis-α-Bisabolene	1602	0.1
γ-Cadinene	1709	0.1
Copaene	1713	tr
Oxygenated monoterpenes	—	**53.8**
Eucalyptol	988	0.3
Linalool	1076	2.6
trans-1-Methyl-4-(1-methylethyl)-2-cyclohexen-1-ol	1097	0.3
α-Terpineol	1118	0.2
Menthone	1138	0.7
Borneol	1154	0.4
δ-Terpineol	1171	7.5
trans-Piperitol	1193	0.1
β-Fenchyl alcohol	1197	12.8
cis-*p*-Menth-1-en-3-ol	1199	0.1
cis-Piperitol	1207	0.1
Pulegone	1262	1.0
Piperitone	1271	tr
Carvacrol	1330	14.5
Oxygenated sesquiterpenes	—	**1.4**
(+)-Spathulenol	1644	0.5
Caryophyllene oxide	1649	0.6
Veridiflorol	1658	tr
Isospathulenol	1704	0.1
Cadinol	1709	0.1
α-Cadinol	1723	0.1
Oxygenated diterpenes	—	tr
Epimanoyl oxide	2040	tr
Others	—	**7.1**
1-Octen-3-ol	921	0.2
1-Methyl-3-(1-methylethyl)-benzene	982	6.8
p-Cymen-7-ol	1181	0.1
Thymyl methyl ether	1241	0.1
Carvacryl methyl ether	1253	0.4
Thymol	1316	12.6
Methyleugenol	1452	tr
Hexadecanoic acid	2005	tr
2,3,5,6-Tetramethylphenol	2067	0.1
Total identified		**92.3**

[a] Normalised peak areas without correction factors.
tr, traces (< 0.05%); RI, retention index.

The composition of *O. vulgare* essential oil from different geographical origins has been characterised by several authors, with carvacrol and thymol as the major components, though the proportions vary widely (Table 2). Other chemotypes have also been reported as important essential oil components, such as *p*-cymene, γ-terpinene, caryophyllene, spathulenol, and germacrene-D (Table 2). The differences in the chemical composition of *O. vulgare* essential oil may be related to distinct environmental and climatic conditions, seasonal sampling periods, geographic origins, plant populations, vegetative plant phases, and extraction and quantification methods.[9,11]

Antioxidant activity of the extracts and essential oil

FRAP analysis revealed essential oil and cold water extract as showing the strongest antioxidant activity, followed by hot water extract and ethanolic extract (Table 3). The reducing power analysis showed statistically the highest antioxidant activity for hot water extract, followed by ethanolic extract, cold water extract, and essential oil (Table 3). The DPPH free radical scavenging activity followed the same trend of reducing power analysis. *O. vulgare* essential oil (AAI = 0.05) revealed poor antioxidant activity, while cold water extract (AAI = 0.55), ethanolic extract (AAI = 1.23), and hot water extract (AAI = 3.16) were classified as moderate, strong and very strong antioxidants, respectively, accordingly to the AAI index and antioxidant categories defined by Scherer and Godoy.[19] The AAI values obtained for the essential oil were within those reported by Şahin *et al*.[27] for the same plant species. The AAI index of the positive control BHT was within the values obtained in previous studies.[28,29]

Total phenolic content revealed statistically higher values in hot water extract, followed by essential oil, ethanolic extract, and cold water extract (Table 3). The phenol values obtained for hot water and ethanolic extracts were lower than those reported by Chun *et al*.[30] for oregano. Such differences might be attributed to distinct plant origins, seasonal sampling periods (not specified), part of plant used (dried powder of the whole plant), and extraction methods (longer in hot water and several ethanol concentrations were used). Since phenols are able to prevent oxidation[31] the greater antioxidant activity of hot water extract could be attributed to the higher content of phenols in this extract, compared with the remaining extracts. Although high total phenolic content was detected in hot water extract and essential oil, the latter showed

Table 2. Major constituents of *Origanum vulgare* essential oils reported in different studies and corresponding origin, sample seasonal period and extraction method employed

Origin	Sampling period	Extraction method details	Compounds detection method	Compounds and their concentrations in the essential oil	Reference
Greece	ND	4 h hydrodistillation (Clevenger apparatus)	Fourier transform Raman spectroscopy	Carvacrol (8.6–80.8%); thymol (1.8–63.7%); *p*-cymene (5.2–13.0%); γ-terpinene (5.8–7.5%)	7
Vilnius district (Lithuania)	1995, 1997–1999	Hydrodistillation	GC-MS and GC-FID	β-Ocimene (10.8–21.6%); germacrene D (7.5–29.4%); β-caryophyllene (2.3–16.6%); sabinene (2.1–15.0%)	8
Kumaon Himalaya (India)	August 2008	2 h steam distillation	GC-MS and GC-FID	Thymol (0–82.0%); carvacrol (0–27.4%); germacrene D (trace–13.3%); germacrene D-4-ol (0–9.5%); β-caryophyllene (0.4–8.8%)	9
Poland	ND	Hydrodistillation (Deryng)	GC-MS	Carvacrol (3.6–9.1 g kg^{-1}); thymol (2.14–8.44 g kg^{-1}); γ-terpinene (1.5–4.9 g kg^{-1})	10
Calabria (southern Italy)	June–July	2 h hydrodistillation (Clevenger apparatus)	GC-MS and GC-FID	Thymol (7.9–55.5%); carvacrol (0.3–56.6%); γ-terpinene (12.6–32.6%); *p*-cymene (3.6–9.7%); methyl carvacryl ether (1.9–4.7%)	11
Unknown origin (Portuguese market)	ND	4 h hydrodistillation (Clevenger apparatus)	GC-MS and GC-FID	Thymol (32.6%); γ-terpinene (25.9%); *p*-cymene (10.7%); β-caryophyllene (4.5%)	12
Iti montain (central Greece)	ND	2 h hydrodistillation	GC-MS and GC-FID	Thymol (45.2%); carvacrol (33.1%); *p*-cymene (7.4%); γ-cymene (5.5%)	38
Kozani (Greece)	July 2002	3 h steam distillation (Clevenger apparatus)	GC-MS and GC-FID	Carvacrol (88.7%); *p*-cymene (3.4%); γ-terpinene (3.2%); β-caryophyllene (1.1%)	26
Vojvodina province (Serbia and Montenegro)	July 2000	Hydrodistillation	GC-MS and GC-FID	Carvacrol (61.3%); thymol (13.9%); γ-terpinene (3.1%)	34
Crete (Greece)	ND	2 h using a microstream distillation–extraction apparatus	GC-MS	Thymol (63.3%); γ-terpinene (12.7); *p*-cymene (9.9%); carvacrol (7.8%)	39
Argentina	November 2007 and 2008	1 h hydrodistillation (Clevenger apparatus)	GC-MS and GC-FID	Thymol (20.5–26.1%); *trans*-sabinene hydrate (27.8–32.5%); γ-terpinene (5.4–15.5%); α-terpinene (4.2–4.6%); terpinen-4-ol (3.5–5.0%)	32
Mountain Taygetos (Peloponnese, Greece)	ND	2 h hydrodistillation (Clevenger apparatus)	GC-MS and GC-FID	Carvacrol (74.6%); *p*-cymene (9.7%); γ-terpinene (5.9%)	40
Dalmatia (Croatia)	October 1998	3 h hydrodistillation	GC-MS	Thymol (40.4%); carvacrol (24.8%); *p*-cymene (16.8%)	41
Market in Pisa (Italy)	January 2004	2 h hydrodistillation (Clevenger apparatus)	GC-MS	Carvacrol (54.7%); thymol (22.1%); γ-terpinene (6.0%); *p*-cymene (5.5%)	42
Oltu valley, Erzorum (Turkey)	ND	3 h hydrodistillation (Clevenger apparatus)	GC-MS	Caryophyllene (14.4%); spathulenol (11.6%); germacrene-D (8.1%); α-terpineol (7.5%); caryophyllene oxide (5.8%)	27
Island of Euboea (Greece)	ND	2 h hydrodistillation (Clevenger apparatus)	GC-MS and GC-FID	Carvacrol (79.6%); *p*-cymene (8.8%); thymol (2.5%); γ-terpinene (2.1%)	33

ND, not described; GC-MS, gas chromatography/mass spectrometry; GC-FID, gas chromatography/flame ionisation detector.

Table 3. Antioxidant activity (FRAP, reducing power and DPPH) and phenol content of *Origanum vulgare* extracts and essential oil

Assay	Hot water extract	Cold water extract	Ethanolic extract	Essential oil
FRAP (μmol Fe^{2+} g^{-1} of sample)	27.9 ± 1.6^{b}	37.7 ± 1.3^{a}	12.6 ± 0.1^{c}	38.5 ± 2.3^{a}
Reducing power (μmol ascorbic acid g^{-1} of sample)	621.7 ± 24.0^{a}	203.3 ± 18.0^{b}	232.7 ± 58.7^{b}	74.5 ± 0.5^{c}
DPPH – EC_{50} (μg mL^{-1})	25.1 ± 2.2^{d}	144.3 ± 4.0^{b}	64.1 ± 1.2^{c}	1509.1 ± 119.0^{a}
Total phenols (mg gallic acid g^{-1} of sample)	17.8 ± 0.1^{a}	6.4 ± 0.1^{d}	13.5 ± 0.3^{c}	16.3 ± 0.3^{b}

Values are presented as average ± standard deviation.
Different letters in the same row denote significant differences ($P < 0.05$).
FRAP, ferric reducing antioxidant power; DPPH, α,α-diphenyl-β-picrylhydrazyl; EC_{50}, sample providing 50% inhibition.

Table 4. Antibacterial activity of *Origanum vulgare* extracts and essential oil against foodborne spoilage and pathogenic bacteria

Method and sample	Salmonella typhimurium	Escherichia coli	Listeria innocua	Listeria monocytogenes	Shewanella putrefaciens	Brochothrix thermosphacta	Pseudomonas putida
Disc diffusion method							
Inhibition radius (mm)							
Hot water extract (41.7 mg mL^{-1})	ND	ND	ND	ND	ND	ND	ND
Cold water extract (23.8 mg mL^{-1})	ND	ND	ND	ND	ND	ND	ND
Ethanolic extract (27.7 mg mL^{-1})	2	1	2	2	ND	ND	ND
Essential oil (896.6 mg mL^{-1})	32	29	17	23	32	18	9
MIC (mg mL^{-1})							
Hot water extract	ND	ND	ND	ND	417	417	ND
Cold water extract	ND	ND	ND	ND	238	ND	ND
Ethanolic extract	13.9	6.9	13.9	6.9	277	277	ND
Essential oil	2.2	1.1	2.2	2.2	4.5	4.5	0.4
Effect of MIC on liquid medium							
Logarithmic reductions							
Ethanolic extract	4.7	0	5.2	4.5	—	—	—
Essential oil	7.2	6.9	8.0	8.0	—	4.5	1.2
Absorbance changes							
Ethanolic extract	+0.028	−0.083	−0.084	−0.307	—	—	—
Essential oil	−0.162	−0.317	−0.732	−0.550	+0.047	−0.096	−0.141

Radius of the paper disc is not included. The values of absorbance change with '+' and '−' indicate an increase and decrease in the absorbance values, respectively.
MIC, minimum inhibitory concentration; ND, inhibition not detected.

the weakest antioxidant activity with the reducing power and DPPH methods, but not with the FRAP assay. Dambolena *et al.*[32] reported that other compounds beside phenols may also be responsible for the antioxidant activity.

Antibacterial activity of the extracts and essential oil

Almost all bacteria tested showed resistance to hot and cold water extracts, except *S. putrefaciens* and *B. thermosphacta* (Table 4). In contrast, the ethanolic extract was able to inhibit the growth of most bacteria, except *P. putida*, being *S. putrefaciens* and *B. thermosphacta* the most resistant and *E. coli* and *L. monocytogenes* the most sensitive bacteria (Table 4). A microdilution method (liquid medium) was used to quantify the bacterial reduction caused by the MIC of extracts, and bacterial concentration was determined by counts of viable cells. In liquid medium, the MIC of ethanolic extract was still able to decrease *S. typhimurium, L. innocua*, and *L. monocytogenes* concentrations (Table 4).

O. vulgare essential oil was very effective in inhibitinig the growth of all bacterial strains tested, with MIC values below 5 mg mL^{-1} (Table 4). Taking into account the inhibition zone using undiluted essential oil, *P. putida* was the most resistant strain, despite revealing the lowest MIC value. The values obtained with *E. coli*, *S. typhimurium*, and *L. monocytogenes* are in accordance with those obtained by Sivropoulou *et al.*[33] and Faleiro *et al.*[12] while other studies reported higher antibacterial activity.[26,34] The results for the MIC of *O. vulgare* essential oil showed a strong reduction of most bacteria tested in liquid medium, particularly with both *Listeria* strains (Table 4). The antibacterial activity observed with the essential oil could be related to the presence of carvacrol and thymol, since these compounds are able to inhibit the growth of *E. coli* and *L. monocytogenes*.[12,25,26] Oregano essential oil affects the murein composition, influencing the number of muropeptides of *L. monocytogenes* and *E. coli* cell walls,[35,36] and a similar inhibition mechanism is expected to occur with the remaining bacterial strains.

Antibiotics are active at concentrations of 10 μg mL^{-1}, and plant extracts active at concentrations of 100 μg mL^{-1} have a good potency level.[37] The extracts tested here are active at higher concentration levels, as the results for the disc diffusion method showed, but results obtained with the liquid medium assay suggest that the active concentration could be lower if this method was used for determination of the MIC.

The absorbance of the bacterial suspensions with the MIC of extracts was measured before and after the incubation period. Results (Table 4) revealed a reduction for all bacteria, which is in accordance with the bacterial counts. However, the results did not follow the same trend in ethanolic extract, and therefore, future studies using microdilution methods for MIC evaluation should focus on bacterial counts instead of absorbance or visual inspection.

CONCLUSIONS

This study suggests that the hot water extract of *O. vulgare* of Portuguese origin has strong antioxidant capacity. Additionally, the *O. vulgare* ethanolic extract and essential oil revealed antibacterial properties, though being stronger with the essential oil. In this context, *O. vulgare* extracts and essential oil have strong potential to be used as alternatives to synthetic chemicals in industries whereas oxidation and microbial contamination are problems. Nonetheless, safety and toxicity issues of these extracts and essential oil still need to be evaluated beforehand.

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