International Food Research Journal 23(4): 1706-1710 (2016)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant and antimicrobial activities of *Origanum vulgare* essential oil

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Article history

Received: 26 June 2015
Received in revised form: 26 December 2015
Accepted: 6 January 2016

Keywords

Antimicrobial activity
Origanum vulgare
Essential oil
Bacteria
Antioxidant activity

Abstract

Oregano*(Origanum vulgare)* essential oil was analyzed by gas chromatography coupled with Mass spectrometry (GC/MS), the major components were Terpinen-4-ol (24.90%), gamma-Terpinen (10.57%), o-Cymene (8.90%), cis-beta-Terpineol (8.73%), alpha-Terpinen (6.67%), beta-Phellandrene (4.84%), alpha-Terpieol (4.18%) and Carvacrol (3.90%) they constituted 72.69% of total oil Oil exhibited antioxidant activity as shown by the consistent values of DPPH free radical-scavenging inhibition (59.09%) at 1000 ppm oil concentration. Antimicrobial activity of essential oil from oregano was also evaluated, among tested microorganisms *P. aeruginosa* demonstrated the highest resistance, while *B. cereus* was the most sensitive to the oregano oil. Minimum inhibitory concentrations (MIC) ranging from 1.56 to 50 µl/ml. Results obtained indicated that oregano essential oil could be used as a potential source of natural antioxidant with possible applications in food systems.

Introduction

Lamiaceae is a common botanical family, members of which are found in the temperate regions worldwide (Cantino, 1992). It includes approximately 220 genera and about 3500 to 4000 species (Almeida and Albuquerque, 2002). Nowadays there is an increasing interest for alternative and efficient compounds for food preservation, having the goal to replace antimicrobial chemical additives. Essential oils of aromatic and medicinal plants present a great potential of application as natural antimicrobial agents. Essential oils have been shown to possess antibacterial, antifungal and antioxidant proprieties (Burt, 2004; Kordali *et al*., 2005). Earlier studies have demonstrated the potent antibacterial properties of oregano (Naim and Tariq, 2006; Lopez *et al*., 2007).

There is a growing interest in studies of natural additives as potential antioxidants. Many sources of antioxidants of plant origin have been studied in recent years. The antioxidant properties of many aromatic plants have shown to be effective in retarding the process of lipid peroxidation in oils and fatty foods, their antioxidant effect was due to the presence of hydroxyl groups in their chemical structure (Shahidi *et al.*, 1992; Vekiari *et al.*, 1993; Shahidi, 2000). A number of studies on antioxidant activities of essential oils from various aromatic plants reported that the oregano essential oil has a considerable antioxidant effect on the process of the fat oxidation (Lagouri *et al*., 1993). Many publications (Cervato *et al*., 2000; Damechki *et al*., 2001; Vichi *et al*., 2001; Bendini *et al.*, 2002) showed antioxidative activities of oregano. Some volatile compounds from essential oils possess the potential as natural agents for food preservation.

The objectives of this study were to determine the chemical composition of oregano hydro-distilled essential oil by GC/MS and to investigate its antimicrobial and antioxidant activities. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were evaluated by broth microdilution method.

Materials and Methods

Plant material

Oregano *(Origanum vulgare)* was collected from Latakia in Syria (during the flowering period). The plant leaves were washed with distilled water and chopped into small pieces shade dried. The dried leaves were ground into powdered form.

Oil extraction

One hundred grams of dried powder were hydro-distilled in Clevenger type apparatus for 3 h, the essential oil was dried over anhydrous sodium sulphate, oil was stored in dark-colored glass bottles and kept at ± 4ºC until analysis.

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Chemical analysis of essential oil

The GC/MS analysis was performed with an Agilent 7890A GC system 5975C inert XL EI/CI MSD with Triple-Axis Detector. The operating conditions were as follows: the GC was equipped with a capillary column DB-HP-5MS (30 m*0.25 mm), 0.25 µm film thickness was used. The carrier gas flow rate was 1ml He/min, Injected volume was 10 µl. Spilt ratio was 1/20. The column temperature was held at 60ºC, programming 4ºC/min to 180ºC, then 10ºC/min to 260ºC. Detector and evaporator temperature was 260ºC.

The components of essential oil were identified by comparing the results of mass-spectra(m/z) of the chemical substances found in the mixtures under study (obtained in the process of chromatography) with the data of mass-spectra library NIST. The range of electronic scanning was 35 to 45 Da and the electronic charge of mass-spectra was 70 eV (Zein *et al*., 2011).

Radical scavenging activity using DPPH assay

Antioxidant activity was determined by DPPH assay. A solution of DPPH in methanol (60 µM) was prepared and different concentrations of essential oil were prepared in methanol (100, 250, 500, 750 and 1000 ppm). 1ml of DPPH solution was added to 1ml of the different concentrations of oil in methanol and shaken vigorously, the mixtures were allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm in a spectrophotometer, and the DPPH radical concentration was calculated using the following formula:

$$\text{Scavenging effect \%} = [(A_0 - A_1)/A_0]^* 100$$

Where A_0 was the absorbance of the control sample (without essential oil) and A_1 was the absorbance in the presence of the sample (Badee *et al*., 2013).

Antimicrobial activity

The antimicrobial activity of the essential oil was tested against five strains of bacteria and fungi: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Candida albicans*. The strains were provided by National Commission for Biotechnology, Damascus, Syria. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the essential oil were determined using the broth microdilution method (Manhor *et al*., 2001).

A stock solution was prepared by diluting peppermint oil in Tween 80%, various concentrations of the essential oil were obtained in the following scheme: 400 µl of the essential oil were placed in a sterile assay tube and 400 µl of Tween 80% and 200 µl of broth were added, following a serial dilution to reach final oil concentrations ranging from 200 to 0.0487µl/ml (Manohar *et al*., 2001). The dilutions were inoculated with 10µl of the tested bacteria/fungi at account of approximately 105cells/ml for bacteria and fungi with a negative control. Bacteria were incubated at 37ºC for 24 h, while fungi were incubated at at 28ºC for 48 h.

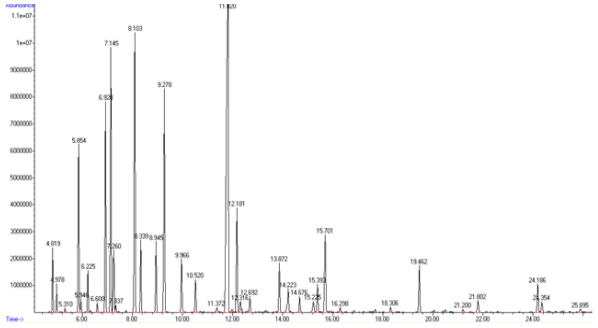

Figure 1.Typical (GC-MS) chromatogram of oregano essential oil

The MIC was defined as the lowest concentration of the essential oil that inhibited the visible growth (no turbidity) (Niculae *et al*.,2009(. Afterwards 10 µl of each tube were transferred to agar plates and incubated for 24 h for bacteria and 48 h for fungi. MBC and MFC were the lowest concentration associated with no visible growth of bacteria or fungi, respectively, on the agar plates (Fu *et al*., 2007).

Result and Discussion

Chemical constituents of oregano oil

The essential oil of oregano were analyzed by GC/MS system (Figure 1). The components and their relative proportions (Area%) are listed in Table 1. Thirty five components were identified, the major components of oil were: Terpinen-4-ol (24.90%), gamma-Terpinen (10.57%), o-Cymene (8.90%), cis-beta-Terpineol (8.73%), alpha-Terpinen (6.67%), beta-Phellandrene (4.84%), alpha-Terpieol (4.18%) and Carvacrol (3.90%), they constituted 72.69% of total oil, indicating that oregano oil belonged to Terpinen-4-ol chemotype. GC analysis also revealed that some compounds are presented in essential oil in appreciable amounts such as beta-Thujene (2.69%), Terpinolene (2.43%), p-Menth-8-en-1-ol (2.42%), (-)-Carvone (1.96%), beta-Myrcene (1.19%) and Thymol (1.09%). Other constituents were less than 1% such as alpha-Pinene, beta-Pinene, Alpha-Phellandrene , Camphene, Borneol, trans-Piperitol,

Table 1. Chemical constituents of oregano essential oil by GC/MS

No	compound	RT min	Area (%)
1	1-Isopropyl-4-methylbicyclo[3.1.0]hex-2-ene	4.82	1.63
2	alpha-Pinene	4.98	0.75
3	Camphene	5.31	0.15
4	beta-Phellandrene	5.85	4.84
5	beta-Pinene	5.95	0.44
6	beta-Myrcene	6.23	1.19
7	Alpha-Phellandrene	6.60	0.32
8	alpha-Terpinen	6.93	6.67
9	o-Cymene	7.15	8.90
10	beta-Thujene	7.26	2.69
11	Cineole	7.34	0.23
12	gamma-Terpinen	8.10	10.57
13	p-Menth-8-en-1-ol	8.34	2.42
14	Terpinolene	8.95	2.43
15	cis-beta-Terpineol	9.28	8.73
16	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	9.97	1.96
17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	10.52	1.24
18	Borneol	11.37	0.23
19	Terpinen-4-ol	11.82	24.90
20	alpha-Terpieol	12.18	4.18
21	trans-Piperitol	12.32	0.44
22	2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, trans-	12.69	0.61
23	(-)-Carvone	13.87	1.96
24	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate	14.22	1.22
25	4-Isopropyl-5-methylhex-2-yne-1,4-diol	14.68	0.65
26	Bornyl acetate	15.23	0.58
27	Thymol	15.39	1.09
28	Carvacrol	15.70	3.90
29	1,3-Dioxolane, 2,2-dimethyl-4,5-di-1-propenyl-	16.30	0.21
30	Geranyl acetate	18.31	0.23
31	beta-Caryophyllene	19.46	1.94
32	Gamma-Elemene	21.80	0.59
33	Ent-Spathulenol	24.19	1.29
34	Caryophyllene oxide	24.35	0.51
35	(-)-Spathulenol	25.90	0.20

Bornyl acetate, Geranyl acetate, Caryophyllene oxide and (-)-Spathulenol. Similar chemical composition with little variations in component concentration were observed for oregano essential oil by other researchers (Badee *et al.*, 2013).

Antioxidant activity of oregano oil

Results indicate that the antioxidant capacity of oregano oil was dependent on the concentration tested. Considerable DPPH radical scavenging activity was evident at all tested concentrations of oil. The antioxidant activity was 59.09% at 1000 ppm of oil, while it was 54.8% at 100ppm, the antioxidant activity ranged from 54.8% to 59.09% from the lowest to the highest concentration, respectively.

These results agree with the results obtained by Kulisic *et al.* (2003) who found that the oregano essential oil possesses remarkable antioxidant properties. These results indicate that oregano essential oil could be used as a potential resource of natural antioxidants for food industry that makes it interesting to try its application as natural antioxidant additive in some final food products.

Antimicrobial activity

This study have demonstrated that the oregano oil has shown a strong antibacterial activity against the tested bacteria strains. The MIC, MBC and MFC values for the tested essential oil concentrations are presented in Table 2, bacterial growth inhibition was influenced by the essential oil concentration. After incubation, it was observed that bacterial growth was considerably reduced with increasing concentration of essential oil. Badee *et al*. (2013) reported an inhibitory activity of oregano oil against C. albicans, which was similar to that obtained in this study where the MIC of C. albicans was 25µl/ml and the MFC was 50 µl/ml. while P. aeruginosa was the most resistance strain with a MIC of 50 µl/ml and a MBC of 100 µl/ml, Niculae *et al.* (2009) reported that oregano oil was found effective against pathogenic strains of *P. aeruginosa* and *E. coli*. In another study it was also shown that oregano oil exhibited antibacterial activity against *P. aeruginosa* and *E. coli* (Dikbas *et al*., 2009). Among the tested strains, *B. cereus* was the most sensitive to oregano oil with a MIC of 1.56 µl/ml and a MBC of 3.125 µl/ml, Costa *et al.* (2009) have reported an antibacterial activity of oregano oil against *S. aureus*, another study also reported the antibacterial potential of oregano oil against *S. aureus* and B. cereus (Cosentino *et al*.,1999). The antimicrobial activities have been explained through

Table 2. Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) or minimal fungicidal concentration(MFC) of oregano essential oil

Microorganisms	Oregano essential oil concentrations (µl/ml)													MIC (µl/ml)	MBC/ MFC (µl/ml)
	200	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.195	0.098	0.0487		
C. albicans	-	-	-	+	+	+	+	+	+	+	+	+	+	25	50
P. aeruginosa	-	-	+	+	+	+	+	+	+	+	+	+	+	50	100
E. coli	-	-	-	+	+	+	+	+	+	+	+	+	+	25	50
S. aureus	-	-	-	-	-	-	+	+	+	+	+	+	+	3.125	6.25
B. cereus	-	-	-	-	-	-	-	+	+	+	+	+	+	1.56	3.125

- Absence of growth, + Presence of growth

terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes can also contribute to the overall antimicrobial effects (Burt, 2004).

Conclusion

Our study demonstrated that oregano oil have both antioxidant and antibacterial properties, the essential oil of oregano may be suggested as a new potential source of a natural antimicrobial for the food industry after testing the toxic and irritating effects on human, therefore further studies are necessary to estimate the potential for utilizing oregano essential oil as additives for extending safety and shelf-life of food products.

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