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Chemical composition and biological activities of essential oils and solvent extracts of *Origanum vulgare* subsp. *glandulosum* Desf. from Tunisia

Afef Béjaoui[1]*, Abdennacer Boulila[2] and Mohamed Boussaid[1]

[1]Laboratory of Plant Biotechnology, Department of Biology, National Institute of Applied Science and Technology, Centre Urbain Nord, B.P. 676, 1080 Tunis Cedex, Tunisia.
[2]Laboratory of Natural Substances, National Institute of Research and Physico-chemical Analysis, Technical Pole of Sidi Thabet, 2020, Ariana, Tunisia.

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This study aimed to quantify the active biological compounds in *Origanum vulgare* subsp. *glandulosum* Desf. Analysis of the chemical composition essential oil from Tunisia was carried out using gas chromatography-mass spectral (GC-MS). The antioxidant capacities of essential oil and extracts were evaluated using free radical scavenging, ferric reducing antioxidant power assay and metal chelating effects. The antidiabetic activity was screened using α-amylase. The oil was dominated by carvacrol (61.20 to 74.03), p-cymene (5.89 to 12.60), and γ-terpinene (1.13 to 6.88). 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power assay (FRAP) assays showed that leaves of *O. vulgare* have a potent antioxidant activities which was comparable to Trolox. The methanol extract have the highest ion chelating ability at around 76.98 mg equivalent ethylenediaminetetraacetic acid (EDTA)/g dry matter (DM). The amounts of chlorophylls A and B were higher than that of lycopene and β-carotene. Essential oils and extracts, screened for α-amylase inhibitory effect, showed a strong capacity to inhibit the degradation of starch by pancreatic and salivary α-amylase. Extracts and essential oils of *O. vulgare* showed substantial antioxidant activity, and detectible inhibitory effect on α-amylase activity, therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Key words: *Origanum vulgare* subsp. *glandulosum*, essential oil, solvent extract, biological activities.

INTRODUCTION

The naturally occurring antioxidants of medicinal plants such as polyphenols and flavonoids exhibit a high ability to donate hydrogen from phenolic hydroxy groups, thereby forming stable free radicals. Thus, the preservative effect of many aromatic plants has increased the interest in finding secondary metabolites with antioxidant properties for use in foods to replace synthetic antioxidants (Amrutha and Bhaskar, 2010).

Oregano is considered one of the most important temperate culinary herbs. The term “oregano” refers to a large number of species that belong to different botanical families and genera. Previous studies in Greece carried out by Kokkini (1989). Two “oregano” plant species, rich in carvacrol were identified, *Origanum onites* L. and *Origanum vulgare* L. Many researchers have shown the great variation in the essential oil yield as well as in carvacrol content in studies which have been carried out in the Mediterranean zone (Kirimer et al., 1995). The variation in oil yield and oil constituents may be attributed to different environmental factors taking into

*Corresponding author. E-mail: afef5@hotmail.com.

account that oil is a metabolic product of plant cells and its quantitative and qualitative composition may be influenced by soil and climatic conditions. The variation of the chemotypes has also been stated by Burkart and Buhler (1997) as the result of interaction between the type of aromatic vegetation and several environmental factors.

The essential oils and extracts of *Origanum* species (Lamiaceae family) native to Mediterranean basin are widely used in pharmaceutical, cosmetic and perfume industry, and for flavouring and preservation of several food products. The essential oils of Oregano species are rich sources of carvacrol, a component of particular biological importance: it is known for its antibacterial and antifungal activities, antispasmodic effects, acetylcholine esterase inhibition, lipid peroxidase inhibition, radical scavenging effect, and cardiac depressant activity (Kirimer et al., 1995). Several studies have been published on biological properties of *Origanum* spp. as antibacterial (Şahin et al., 2004), antioxidant, anti-inflammatory and anticholinesterase (Loizzo et al., 2009), mutagenic and antimutagenic effects (Mezzouga et al., 2007) and antifungal (Khosravi et al., 2011). It has also been used as a traditional remedy to treat various ailments such as a spasmodic, antimicrobial, and aromatic for whooping and convulsive coughs, digestive disorders and menstrual problems (Sokovic et al., 2002; Daferera et al., 2003).

The objective of this study was to get knowledge on the chemical composition and biological activities of *O. vulgare* from Tunisia for a possible valorization of essential oil and solvent extracts.

MATERIALS AND METHODS

Plant

The oregano plants used for this study were collected in winter from Sidi Nssir Mountain (35°27'N and 9°33'E) located in North East of Tunisia. The site belongs to the sub-humid bioclimatic zone with a rainfall ranging between 600 and 900 mm/year at altitude of 336 m. Botanical identification of this species was carried out by Prof. M. Boussaid, Biologist (National Institute of Applied Science and Technology (INSAT, Tunisia). Voucher specimens were deposited at the herbarium of INSAT.

Chemicals

Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6 tripyridyl-s-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), $FeCl_3$ $6H_2O$, $FeCl_2$, $4H_2O$, Ferrozine, $FeSO_4$ $7H_2O$, KI, Soluble starch (S-9765), and α-amylase were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used in this study are of higher purity.

Isolation of essential oils

Oils were obtained by hydrodistillation, using a Clevenger-type apparatus, for 3 h of 30 to 60 g of air-dried leaves of each sample. Oil yields were then estimated on the basis of the dry weight of plant material. Oils were recovered directly, from above the distillate, and stored in dark vials at 4°C.

Preparation of the extracts

Phenolic extracts from leaves were obtained by magnetic stirring for 24 h of 50 mg of dry organ powder with 10 ml solvent 100%. Solvents used were pure methanol, ethanol and H_2O.

Essential oils identification

The essential oils were analysed by gas chromatography-mass spectrometry (GC-MS) using a HP 5975C mass spectrometer (Agilent technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30m × 250 µm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 µm film thickness) was used. Oven temperature was programmed to rise from 60 to 220°C at a rate of 4°C min^{-1}; transfer line temperature was 230°C. The carrier gas was He with a flow rate of 0.8 ml/min and a split ratio of 50:1. Scan time and mass range were 1 s and 50 to 550 m/z, respectively (Messaoud et al., 2005).

The identification of oil components was assigned by comparison of their retention indices (RI) relative to C8 to C22 n-alkanes with those of literature or with those of authentic compounds available in the author's laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra (Adams, 2001). The percentage of the components was based on peak area normalization without using correction factors.

Evaluation of antioxidant activity

The antioxidant activities of essential oil and solvent extracts were measured using free radical-scavenging activity (RSA), ferric reducing antioxidant power assay (FRAP) systems and metal chelating activity.

Free radical-scavenging activity

The evaluation of the free radical-scavenging activity was based on the measurement of the reducing ability of antioxidants toward the DPPH radical. Various concentrations of the diluted essential oils were mixed with 0.50 ml DPPH (0.2 mM). The mixture was then shaken and allowed to stand at room temperature in the dark. After 30 min, the decrease in absorbance was measured at 517 nm against a blank (methanol solution) using a UV-vis spectrophotometer. A mixture consisting of 0.50 ml of methanol and 0.25 ml of DPPH solution was used as the control.

The capability to scavenge the DPPH radical was calculated using the following equation:

$$RSA\ (\%) = 100 \times (A\ blank - A\ sample) / A\ blank$$

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound.

Test compound concentration providing 50% inhibition (IC_{50}, expressed in mg/ml) was calculated from the graph plotted inhibition percentage against extract concentrations (2 to 0.25 mg/ml). Synthetic antioxidant Trolox was used as positive control and all tests were carried out in triplicate and the results expressed as means ± standard deviation (SD).

FRAP

The FRAP assay was adapted from Gardeli et al. (2008). The FRAP reagent was freshly prepared by mixing TPTZ solution (10 mM TPTZ in 40 mM HCl), ferric solution ($FeCl_3.6H_2O$, 20 mM) and acetate buffer (300 mM, pH 3.6) in proportions of 1:1:10 (v/v). To perform the assay, 900 µl of FRAP working reagent were mixed with 90 µl distilled water. 30 µl of diluted oil were then added and incubated at 37°C in a water bath for 30 min. Absorbance at 593 nm was determined against distilled water blank. Ferrous sulfate heptahydrate solutions (100 to 2000 µM) were used for calibration. All tests were run in triplicate and the results expressed as means ± SD. Mean values were used to calculate the FRAP values. The FRAP values is defined as concentration of an antioxidant having a ferric reducing ability equivalent to that of mM ferrous salt.

Metal chelating activity

The chelating of ferrous ions by *Origanum glandulosum* essential oils and extracts was estimated as described by Dinis et al. (1994). Different concentrations of the extracts were added to a 0.3 ml $FeCl_2$, $4H_2O$ solution (1 mmol/l) and left for incubation for 5 min. The reaction was initiated by adding 0.3 ml of ferrozine (25 mmol/L). Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+}complex formation was calculated using the formula:

Metal chelating effect (%) = (A0 - A1) / A0 × 100

where A0 is the absorbance of the control, and A1 is the absorbance in the presence of the sample or standard. A control containing 300 µl methanol, 300 µl $FeCl_2$ and 300 µl ferrosine was prepared. All tests were run in triplicate and the results were expressed as means ± SD. The determination of the antioxidant power is a standard range of ethylenediaminetetraacetic acid (EDTA) at different concentrations (5 to 40 µg/ml), performed in the same assay conditions. The antioxidant effect of the samples is expressed in mmol EDTA/g of dry plant material.

Chlorophyll analyses

About 50 mg of leaves were air dried and ground into course powder. It was extracted with 20 ml of (n-hexane:acetone, 40:60) for 24 h. After stirring, the mixture was filtered and the supernatant of the sample was transferred in flask and covered with aluminium foil to prevent photodegradation of the pigments. The absorbance of the samples was measured at 453, 505 and 663 nm, respectively using a UV/VIS spectrophotometer. The antioxidant activity was measured in terms of successful using the following equations:

β-carotene = (0,216 × A_{663}) – (1,22 × A_{645}) – (0,304 A_{505}) + (0,452 × A_{453}).
Lycopene = (-0, 0458 × A_{453}) + (0,204 × A_{645}) – (0,304 × A_{505}) + (0,452 × A_{453}).
Chloro A = (0,999 × A_{663}) – (0, 0989 × A_{645}).
Chloro B = (-0,328 × A_{663}) + (1, 77 × A_{645}).

The quantities of chlorophyll a and b, lycopene and ß-carotene are respectively expressed in 0.5 g/20 ml.

Inhibition of α-amylase

The samples were tested for amylase inhibition activity by the agar disc diffusion method, according to Cha et al. (2009). Starch hydrolysis assay was performed on plates that are composed of 1% (w/v) starch dispersed in 1.5% agar. Sterile Whatman No. 1 (ϕ = 8 mm) disc papers were individually placed on agar plates, and then 10 µl of samples (pancreatic α-amylase with or without sample) were applied to the filter paper disc. After incubation at 37°C for 72 h, starch plates were stained by flooding with iodine solution (5 mM I_2 in 3% KI) for 15 min at room temperature. Iodine was removed from the plates by washing with distilled water. Amylase activity was determined by observing the zone diameter of hydrolyzed areas around the wells.
The percentage of inhibition of α-amylase was calculated using the formula:

Amylase activity (%) = (A0 - A1)/A0 × 100

where A0 is the diameter of the negative control, and A1 is the diameter of the sample.

RESULTS

Yields and chemical composition of essential oils

The essential oils isolated from *O. vulgare* were obtained in different yields, according to individuals ranging from 1.87 to 3.42%. Forty of their components were identified, amounting for 90.31 to 98.14% of the total oils from the samples isolated by hydrodistillation. The identified components in oil, and their percentages, are listed in Table 1.

The oxygenated monoterpene fraction was the most representative one in all the oils, ranging from 66.18 to 78.17%. This fraction was dominated by the oxygen-containing compounds, carvacrol being clearly the main component of all oils (74.03%), p-cymene (12.60%) and γ-Terpinene (6.88%) were the second and third main components of all oils. Borneol was present also in a relatively high amount in all oils (3.19%).

Antioxidant and metal chelating activities

The essential oils and solvent extracts showed a substantial antioxidant activity (Table 2), although the two essays (DPPH and FRAP) yielded different values of antioxidant activity. It exerted an antioxidant activity which was comparable to Trolox as shown by DPPH assay. However, the DPPH assays show that Trolox exhibited similar antioxidant activity (IC_{50} = 0.5 mg/ml) than the essential oils and MeOH extract (IC_{50} = 0.625 and 0.6 mg/ml, respectively) (Table 2). The plant extracts of *Origanum* gave ferrous equivalent values at the order of essential oils (0.89 mg/ml), MeOH extract (0.43 mg/ml) > EtOH extract (0.24 mg/ml) > aqueous extract (0.18 mg/ml).

A substantial metal chelating capacity was observed in the MeOH and EtOH extracts of *O. vulgare.* The metal scavenging capacities observed in MeOH extract was found to be 76.98 mg equivalent EDTA/g dry matter (DM). However, relatively high metal scavenging activities

Table 1. Mean percentage of volatiles of *Origanum vulgare* isolated from the aerial parts collected during the vegetative phase.

S/N	Compound	RI[a]	Sample								Average[b]
			1	2	3	4	5	6	7	8	
1	α-thujene	924	0.85	1.18	1.20	1.04	0.80	1.24	0.66	0.92	0.99 (0,07)
2	α-pinene	940	0.52	-	0.48	0.65	0.39	0.68	0.41	0.42	0.44 (0,07)
3	Camphene	953	0.47	0	0.39	0.44	0.33	0.66	0.29	0.37	0.37 (0,07)
4	sabinene	979	-	0.04	0.06	0.04	0.08	0.08	-	0.04	0.04 (0,01)
5	β-pinene	983	-	-	-	0.31	-	0	0.25	-	0.07 (0,05)
6	B-myrcene	992	1.24	0.42	0.77	1.42	0.67	0.89	0.93	1.23	0.95 (0,12)
7	3-octanol	995	0.13	0.14	0.17	0.12	0.09	0.28	-	0.19	0.14 (0,03)
8	α-phellandrene	1006	0.23	0.29	0.21	0.26	0.17	0.21	-	0.23	0.20 (0,03)
9	Delta-car-3-ene	1009	0.08	0.10	0.11	0.09	0.11	0.10	0.08	0.08	0.09 (0,00)
10	α-Terpinene	1017	1.24	1.60	1.07	1.37	0.84	1.55	1.20	1.15	1.25 (0,09)
11	p-Cymene	1026	7.90	8.99	10.76	9.87	5.89	12.60	7.56	8.39	9.00 (0,73)
12	Limonene	1030	0.53	0.68	-	-	0.36	-	0.41	0.50	0.31 (0,10)
13	Trans-alpha-ocimene	1035	0.11	0.18	0.17	1.39	0.03	0.89	0.92	1.33	0.63 (0,20)
14	β-ocimene	1040	-	0.07	-	0.07	-	0.04	0.06	0.08	0.04 (0,01)
15	γ-terpinene	1059	5.75	6.88	1.13	6.34	2.06	2.58	4.49	4.75	4.25 (0,75)
16	Cis-Sabinene hydrate	1072	0.64	0.68	1.36	0.81	0.76	1.30	0.63	0.67	0.86 (0,11)
17	α-terpinolene	1088	0.19	0.26	0.12	0.19	0.10	0.17	0.12	0.18	0.17 (0,02)
18	Trans-Sabinene hydrate	1101	0.37	0.39	0.57	-	0.35	0.57	0.34	0.44	0.38 (0.06)
19	α-Campholene aldehyde	1125	-	-	-	0.08	0.08	0.18	0.04	0.15	0.07 (0.02)
20	Camphor	1141	-	-	0.09	-	-	0.09	-	0.06	0.03 (0.02)
21	Borneol	1164	3.72	4.25	3.26	2.38	2.24	4.87	1.80	2.96	3.19 (0.37)
22	Terpinen-4-ol	1181	1.03	1.17	0.82	0.95	-	0.77	0.67	0.99	0.80 (0.13)
23	Carvone	1240	-	-	-	0.09	-	0.12	0.09	0.11	0.05 (0.02)
24	Carvacrol methyl ether	1242	0.03	-	0.12	0.14	-	0.07	0.08	0.08	0.07 (0.02)
25	Thymol	1293	0.39	0.29	0.24	0.21	0.07	0.34	0.29	0.27	0.26 (0.03)
26	Carvacrol	1302	68.45	62.09	67.64	61.08	74.03	61.20	63.50	62.08	65.01 (1.6)
27	Eugenol	1353	-	-	-	0.04	0.06	0.07	-	-	0.02 (0.01)
28	Carvacryl acetate	1367	0.73	0.74	0.78	0.52	0.72	0.10	0.46	1.61	0.71 (0.15)
29	β-caryophyllene	1421	1.15	1.25	3.04	1.62	1.13	2.35	1.47	2.30	1.79 (0.25)
30	Aromadendrene	1437	0.15	0.23	0.11	0.17	0.06	0.12	0.16	0.08	0.14 (0.02)
31	α- Humulene	1445	-	-	-	-	-	0.12	0.09	0.11	0.04 (0.02)
32	Germacrene D	1477	0.13	0.20	0.12	0.17	0.16	0.13	0.15	0.46	0.19 (0.04)
33	α-amorphene	1487	0.15	0.20	0.15	0.22	0.20	0.27	0.20	0.31	0.21 (0.02)
34	Bicyclogermacrene	1491	0.34	0.42	0.44	0.38	0.36	0.76	0.35	0.29	0.42 (0.05)

Table 1. Contd.

35	β-bisabolene	1510	0.84	1.17	0.70	1.64	0.56	1.24	1.49	2.87	1.31 (0.26)
36	Spathulenol	1581	0.25	0.25	0.23	0.36	0.12	0.41	0.33	0.17	0.27 (0.03)
37	Caryophyllene oxide	1585	0.38	0.43	0.80	0.82	0.39	0.50	0.74	-	0.51 (0.10)
38	β-Eudesmol	1643	-	-	-	0.06	-	-	0.05	-	0.01 (0.01)
39	δ-Muurolene	1685	-	-	0.07	0.06	-	-	-	-	0.02 (0.01)
40	Lanceol	1770	0.15	0.17	0.08	0.33	-	-	0	-	0.09 (0.04)
Total identified		-	98.14	94.76	97.26	95.73	93.21	97.55	90.31	95.87	95.35
Chemical classes		-	-	-	-	-	-	-	-	-	-
Monoterpene hydrocarbons		-	19.11	20.69	16.47	23.48	11.83	21.69	17.38	19.67	18.79
Oxygenated monoterpenes		-	75.36	69.61	74.88	66.18	78.17	69.43	67.86	69.27	64.35
Sesquiterpene hydrocarbons		-	2.76	3.47	4.63	4.26	2.47	4.99	3.91	6.42	12.72
Oxygenated sesquiterpenes		-	0.78	0.85	1.11	1.57	0.51	0.91	1.12	0.17	1.31
Others		-	0.13	0.14	0.17	0.24	0.23	0.53	0.04	0.34	0.35

[a]Retention indices (HP-5MS); -: not detected; (): standard deviation.

Table 2. DPPH radical scavenging activity (IC_{50}), reducing power, metal chelating, and α-amylase inhibition activities of *O. vulgare* essential oils and solvent extracts.

Parameter	DPPH radical-scavenging IC_{50}[b] (mg/ml)	FRAP (mg/ml)	Metal chelating[c] (mg/g)	α-Amylase inhibition (%)		
				1/10	1/20	1/40
Essential oil	0.625±0.09	0.89±0.03	38.11±0.8	90	80	75
MeOH	0.6±0.04	0.43±0.05	76.98±0.39	97.5	95	90
EtOH	0.7±0.05	0.24±0.02	48.95±0.33	97.5	95	90
H_2O	0.7±0.05	0.18±0.02	31.68±0.86	95	90	80
Trolox	0.5±0.02	-	-	-	-	-

[a]Results are reported as mean ± standard deviation of three replicates (95% confidence)); [b]mg trolox equivalents/ml; [c]mg equivalent EDTA/g DM.

were detected in EtOH 48.95% as well as the essential oils (38.11 mg equivalent EDTA/g DM) (Table 2).

Chlorophyll analyses

Pigments content (chlorophylls a, b, β-carotene, and lycopene) in *O. vulgare* were detected in varying concentrations (β-carotene = 1.86 ± 0.14; lycopene = 4.5 ± 0.16; Chloro A = 11.15 ± 0.16, and Chloro B = 18.09 ± 0.31). The highest percentage of chlorophylls in extract was obtained and it reached 23 and 36.18%.

Chlorophyll extracts are used extensively as a dye in in coloring resins, edible fats, cosmetics, lotios, perfume, and leather. Some experimental data suggest that chlorophyll may have some anti-mutagenic and anti-carcinogenic potential, and may help protect against some toxins, and it may ameliorate some drug side effects (Guil-Guerrero et al., 2003).

Inhibition of α-amylase

The inhibition of α-amylase activity, is considered to be an effective strategy for the control of diabetes by diminishing the absorption of glucose (Hara and Honda, 1990). The degradation of starch by pancreatic α-amylase was inhibited by oils of *O. vulgare*. However, the dilutions (1/10, 1/20, and 1/40) displayed high inhibition activity of α-amylase (90, 80 and 75%, respectively) (Table 2). This study also tested the inhibitory activity of methanolic, ethanolic and aqueous extracts against porcine pancreatic α-amylase. These extract exhibited stronger inhibitory activities against pancreatic α-amylase (97.5, 97.5 and 95%, respectively) at dilution (1/10) (Table 2).

DISCUSSION

Origanum is one of the economically important plants of the Lamiaceae. This genus is characterised by extensive morphological and chemical diversity. GC–MS analysis of the essential oil indicated the main components to be carvacrol, p-cymene, and γ-terpinene. The composition of the essential oil from *O. vulgare*, which was previously analyzed by other research groups, was shown to depend on the species, climate, altitude, time of collection and growth stage.

The essential oil composition of *Origanum syriacum* var. *bevanii* growing in Turkey contained carvacrol (64.1%) and p-cymene (12.3%) as major components (Başer et al., 2003). Previous studies reported that the essential oil from the same plant from Egypt contained only carvacrol (76.7%) as the main constituent (Fleisher and Fleisher, 1991). *O. vulgare* from Italy was described by Giamperi et al. (2002). Our results differed from those previously reported in Algerian *O. glandulosum* essential oil (Ruberto et al., 2002), *O. vulgare* from Italy and *Origanum calcaratum* from Greece (Giamperi and Fraternale, 2002) and France (Figuérédo et al., 2006). This may be attributable that the amounts of the essential oil differ by geographic region, collect time, altitude and climate.

These results highlight the great variability of chemical composition of oregano oil through their components. These differences could be due either to ecological and genetic factors. The oil of Tunisian samples collected from the North of Tunisia at Nefza, Krib and Bargou regions at the flowering stage was shown to be rich in thymol (18 to 31%), γ-terpinene (11 to 24%) and p-cymene (35 to 46%) (Mechergui et al., 2010). The aromatic monoterpenes mainly presented in the genus *Origanum* are p-cymene, thymol, carvacrol and their precursor, γ-terpinene. Carvacrol is a common component of the oils from oregano. It is frequently used in several products as a flavoring and/or as an antimicrobial agent, showing a broad-spectrum of activity against bacteria, yeasts and fungi (Knowles et al., 2005).

The *O. vulgare* essential oil and MeOH extract analyzed in this research showed potent antioxidant activities. According to GC-MS analysis, the species contains high levels of phenolic compounds (carvacrol). The antioxidant activity of *O. vulgare* essential oil is thus likely thus related to carvacrol (Zheng and Wang, 2001). In several study, carvacrol was found to be main antioxidant constituents of the oils isolated from several *Origanum* spp. (Puertes-Mejia et al., 2002).

In the antioxidant activities, inferiority of the ethanol and aqueous extract could be attributed to the synergistic effects of phenolic acids e.g. rosmarinic acid and polyphenols as well as other chemicals such as flavonoids. This potentiality is mostly correlated to the type of chemical and phenolic compounds according to the essential oils and solvent extracts. The antioxidant capacity variation in our study might be attributed to chemical composition such as carvacrol, and thymol known for their antioxidant potential (Mastelić et al., 2008).

Many medicinal plants have shown antidiabetic activity (Latha et al., 2004). Rosmarinic acid-containing oregano extracts yielded higher than expected amylase inhibition, suggesting that other phenolic compounds such as carvacrol may contribute to additional amylase inhibitory activity (McCue et al., 2004). These results agree with other reports, demonstrating the ability of phenolic substances to interact with and/or inhibit proteins/enzymes (Rohn et al., 2002). Various phenolic compounds have been considered as antioxidant agents, it has been shown that the activity of α-amylase is effectively inhibited by flavonoids, such as naringenin, kaempferol, luteolin, apigenin, (+)/catechin/(-)/epicatechin, diadzein and epigallocatechin gallate (Tadera et al., 2006), indicating that polyphenolic compounds are able to inhibit the activities of carbohydrate-hydrolysing enzymes, due to their ability to bind with proteins (Shobana et al., 2009).

Origanum contains various phenolic compounds which fall under two major categories, phenolic acids such us gallic acid, coumaric acid, vanillic acid and flavonoids (rutin and naringenin) (Oukil et al., 2011). It has been shown that MeOH, EtOH and aqueous extract from *Origanum* contains a larger amount of phenolic compound. However, these findings indicate that the differences in the inhibitory effects of *Origanum* activity of α-amylase could be due to the levels of phenolic compounds in this extracts.

Conclusions

This study reported an estimation of chemical composition and biological activities of *O. vulgare* essential oils and solvent extracts. Available information indicates that analysis of α-amylase activity has not previously been reported. A total of 40 essential compo-

nents were identified. Essential oils and MeOH extract exhibited the highest antioxidant capacity and metal chelating activity. They also showed the highest capacity degradation of starch by pancreatic α-amylase as well as the EtOH and aqueous extracts. The results show that the type of extraction solvent strongly influenced the antioxidant properties of the obtained extracts. Further studies are required to determine the types of bioactive compounds present in the plant extract as well as those responsible for antioxidant activity.

There is a great interest in natural sources of antioxidants to prevent oxidative degradation of foods and to minimize oxidative damage to living cells.

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