GRASAS Y ACEITES, 62 (4),
OCTUBRE-DICIEMBRE, 410-417, 2011,
ISSN: 0017-3495
DOI: 10.3989/gya.113610

Antioxidant and antimicrobial activity in the seeds of O*riganum vulgare* L. subsp. *gracile* (C. Koch) Ietswaart and *Origanum acutidens* (Hand.-Mazz.) Ietswaart from Turkey

By **M. Kurşat*[1], İ. Emre[2], Ö.Yılmaz[3]** and **P.Erecevit[3]**

[1]Bitlis Eren University, Faculty of Sciences and Arts, Department of Biology, Bitlis, 13000, Turkey
[2]Firat University, Faculty of Education, Department of Primary Education, Elazig, 23119, Turkey
[3]Firat University, Faculty of Sciences, Department of Biology, Elazig, 23119, Turkey
*Corresponding Author: e-mail: botanikkursat@hotmail.com

RESUMEN

Actividad antioxidante y antimicrobiana en semillas de *Origanum vulgare* L. subsp. *gracile* (C. Koch) Ietswaart y *Origanum acutidens* (Hand.-Mazz.) Ietswaart de Turquía.

En el presente estudio se han determinado algunos compuestos biológicos (ácidos grasos, vitaminas, esteroles y flavonoides), la capacidad de secuestrar radicales libres y la actividad antimicrobiana de dos especies de orégano, O*riganum vulgare* L. subsp. g*racile* (C. Koch) Ietswaart y *Origanum acutidens* (Hand.-Mazz.) Ietswaart. El ácido linolénico ($C_{18:3}$) mostró ser el principal ácido graso en ambas especies, seguido por los ácidos palmítico ($C_{16:0}$), esteárico ($C_{18:0}$), oleico ($C_{18:1}$ n9), linoleico ($C_{18:2}$ n6) y estearidónico ($C_{18:4}$). Además, las dos especies de orégano mostraron contener altos niveles de estigmasterol. También se constató que *Origanum vulgare* L. subsp. gracile (C. Koch) Ietswaart tenía un elevado contenido de β-sitosterol (152,8 ± 2,6 mg / g), gran cantidad de vitaminas D3 (33,1 ± 0,8 mg / g), K1 (29,4 ± 0,8 mg / g), K2 (26,0 ± 0,8 g / g), y bajos contenidos de α-tocoferol (7,8 ± 0,2 mg / g) y vitamina D2 (1,8 ± 0,1 mg / g), mientras que *Origanum acutidens* (Hand.-Mazz) Ietswaart contenía altas concentraciones de vitamina D2 (42,9. ± 1,5 mg / g), y bajas concentraciones de D3 (2,9 ± 0,1 mg / g), α-tocoferol (3,1 ± 0,1 mg / g), γ-tocoferol (3,5 ± 0,2 mg / g), K2 (1.3 ± 0,1 mg / g), acetato de retinol (1,3 ± 0,2 mg / g) y retinol (1,2 ± 0,1 mg / g). El presente estudio también mostró que O*riganum vulgare* L. subsp. gracile (C. Koch) Ietswaart y *Origanum acutidens* (mano.-Mazz.) Ietswaart contenían pequeñas concentraciones de flavonoides. Por otra parte, los extractos de metanol mostraron ser el agente más eficaz contra radicales DPPH en ambas especies de orégano examinados. Los resultados experimentales mostraron que las vitaminas, flavonoides y ácidos grasos de los extractos de ambas especies de orégano fueron eficaces –a diferentes niveles– en la inhibición del crecimiento de los microorganismos ensayados.

PALABRAS CLAVE: Acidos grasos – Capacidad antimicrobiana – DPPH actividad captadora de radicales – Esteroles – Flavonoides – Origanum L. – Vitaminas.

SUMMARY

Antioxidant and antimicrobial activity in the seeds of O*riganum vulgare* L. subsp. *gracile* (C. Koch) Ietswaart and *Origanum acutidens* (Hand.-Mazz.) Ietswaart from Turkey.

The present study determined some biological compounds (fatty acids, vitamins, sterols and flavonoids), radical scavenging capacity and antimicrobial activity of two *Origanum* L. species of O*riganum vulgare* L. subsp. g*racile* (C. Koch) Ietswaart and *Origanum acutidens* (Hand.-Mazz.). Ietswaart. Linolenic acid was found to be the main fatty acid in both species, which was followed by palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n9), linoleic acid (C18:2 n6) and stearidonic acid (C18:4). In addition, the two *Origanum* species were recorded as containing high levels of stigmasterol. It was also found that O*riganum vulgare* L. subsp. g*racile* (C. Koch) Ietswaart had a high β-sitosterol content (152.8±2.6 µg/g); high amounts of D3 (33.1±0.8 µg/g), K1 (29.4±0.8 µg/g), K2 (26.0±0.8 µg/g) vitamins; and low amounts of α-tocopherol (7.8±0.2 µg/g) and D2 (1.8±0.1 µg/g), while *Origanum acutidens* (Hand.-Mazz.) Ietswaart contained high amounts of D2 (42.9±1.5 µg/g) vitamin; and low amounts of D3 (2.9±0.1 µg/g), α-tocopherol (3.1±0.1 µg/g), r-tocopherol (3.5±0.2 µg/g), K2 (1.3±0.1 µg/g), retinol (1.3±0.2 µg/g) and retinol acetate (1.2±0.1 µg/g) vitamins. The present study showed that O*riganum vulgare* L. subsp. g*racile* (C. Koch) Ietswaart and *Origanum acutidens* (Hand.-Mazz.) Ietswaart contained the lowest amount of flavonoid. Furthermore, methanol extracts were recorded to be the most effective agent against the DPPH radical in both *Origanum* L. species examined. Experimental results showed that the vitamins, flavonoids and fatty acid extracts of both *Origanum* L. species were effective in the inhibition of the growth of the tested microorganisms at varying levels.

KEY-WORDS: Antimicrobial capacity – DPPH radical scavenging activity – Fatty acids – Flavonoids – Origanum L. – Sterols – Vitamins.

1. INTRODUCTION

Reactive oxygen radicals have been detected to play a significant role in the occurrence and progression of various diseases such as cancer, cardiovascular diseases, atherosclerosis and inflammatory injuries (Verma *et al.,* 2009). Many medicinal plants, especially members of the Lamiaceae-such as oregano, sage and thyme contain large amounts of antioxidants such as polyphenols, ascorbic acid and carotenoids, all of which can play an important role in the absorption and neutralization of the reactive oxygen radicals, quenching singlet and triplet oxygen, or in the decomposition of peroxides (Capecka *et al.,* 2005; Djeridane *et al.,* 2006).

Origanum L. is represented by three groups, 10 sections, 43 species, 6 subspecies and 18 hybrids

(Skoula and Harborne, 2002; Skoula *et al.,* 2008). In addition, *Origanum* is represented in Turkey by 23 species and 32 taxa and *Origanum vulgare* by four subspecies (*Origanum vulgare* L. subsp. *vulgare, Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, *Origanum vulgare* L. subsp. *viride* (Boiss.) Hayek, Origanum *vulgare* L. subsp. *gracile* (C. Koch) (Davis *et al.,* 1982; Ulukapı *et al.,* 2008). *Origanum* species are distributed widely around the Mediterranean Region, more than 80% of which is concentrated exclusively in the East Mediterranean Region (Skoula *et al.,* 2008). Turkey is the world's largest producer of oregano and grows the largest number of oregano varieties (Cetingul *et al.,* 2007). The leaves and dried herbs of oregano have been used for medical purposes for centuries (Chun *et al.,* 2005). Recent research has shown that oregano extracts, among all aromatic herbs, have the most effective antioxidant activity and antimicrobial properties (Milos *et al.,* 2000; Kouri *et al*., 2007) and that oregano is used as an anti-diabetic, carminative, tonic, stimulant, diuretic and to treat asthma (Nakiboglu *et al.,* 2007). Recently, there is an increasing interest in finding natural antimicrobials from different extracts obtained from plant materials. Phenolic compounds, flavonoids or unsaponifiable matter are among the groups of compounds able to inhibit the growth of specific microorganisms (Dib *et al.,* 2010; Mohamed *et al.,* 2010).

In this scope, the aim of the present study was to determine (i) the fatty acid compositions, vitamin and sterol contents; (ii) flavonoid contents and radical scavenging properties; and (iii) antimicrobial activities of vitamin and sterol, fatty acid and flavonoid contents of the seeds of *Origanum vulgare* subsp. *gracile* and *Origanum acutidens.*

2. MATERIALS AND METHODS

2.1. Chemical agents

All chemicals and reagents were purchased from Sigma-Aldrich.

2.2. Materials

Plants

Mature seeds of the *Origanum vulgare* L. subsp. *gracile* (C. Koch) Ietswaart (Elazığ, Baskil-Bolucuk village, 1580-1600 m) and *Origanum acutidens* (Hand.-Mazz.) Ietswaart (Tunceli, 27 km N. of Tunceli, 960 m) were examined in this study. The experiment was repeated three times on each sample.

Microbial strain

A total of 4 bacteria (Escherichia coli ATCC 25922, Klebsiella pneumoniae FMC 5, Staphylococcus aureus COWAN 1, Bacillus megaterium DSM 32), 2 yeasts (Candida albicans FMC 17, Candida glabrata ATCC 66032) and 2 dermatophyte species (Trichophyton sp., Epidermophyton sp.) were used in the present investigation. Microorganisms were provided from the Department of Biology, Firat University, Microbiology Laboratory, Elazig-Turkey.

2.3. Extraction of seeds

Extracts of fatty acid, sterols and lipid soluble vitamins

2 g of seed materials for fatty acid, sterol and vitamin analyses were finely ground in a mill and were then extracted with hexane/isopropanol (3:2 v/v) (Hara and Radin, 1978). The lipid extracts were centrifuged at 10.000 *g* for 5 minutes and filtered, and the solvent was then removed on a rotary evaporator at 40°C. The extracted lipids were stored at −25°C until further analysis. The experiment was repeated three times.

Extracts of flavonoids

2 g of seed materials were homogenized in 5 ml of 80% methanol. Homogenates were centrifuged at 5000 rpm at 4°C. After centrifugation, the supernatant was concentrated by reduced-pressure rotary evaporation. Each extract was re-suspended in dimethyl sulphoxide (DMSO) to produce a stock solution. The experiment was repeated three times.

2.4. Determination of bioactive properties

Fatty Acids

Fatty acids in the lipid extracts were converted into methyl esters by means of 2% sulphuric acid (v/v) in methanol (Christie, 1990). The fatty acid methyl esters were extracted with n-hexane. The methyl esters were then separated and quantified by gas chromatography and flame-ionization detection (Shimadzu GC 17 Ver.3) coupled to a Glass GC 10 computer software. Chromatography was performed with a capillary column (25 m in length and 0.25 mm in diameter, Permabound 25, Macherey-Nagel, Germany) using nitrogen as carrier gas (flow rate 0.8 ml/min.). The temperatures of the column, detector and injection valve were 130-220, 240, and 280°C, respectively. Identification of the individual methyl esters was performed by frequent comparison with authentic standard mixtures that were analyzed under the same conditions.

Lipid soluble vitamins and sterols

Lipide-soluble vitamins and phytosterols were extracted from the lipid fraction by the method of Sânchez-Machado (2002) with minor modifications. The HPLC analyses were performed using a Shimadzu instrument (Shimadzu, Kyota, Japan) equipped with a UV detector. Seperations were performed on a Supelcosil ™ LC18 (250 × 4.6 mm, 5

μm, Sigma, USA). The mobile phase was acetonitrile/ methanol (75/25 v/v), at a flow rate of 1.0 ml/min. The temperature of the analytical column was kept at 40°C; the injection volume of each sample was 50 μl. Chromatograms were recorded at at 320 nm for retinol (vitamin A) and retinol acetate, and 215 nm for δ-tocopherol, vitamin D, α-tocopherol, α-tocopherol acetate, 202 nm for phytosterols, 265 nm for vitamin K1. Identification of the individual vitamins and phytosterols was performed by frequent comparison with authentic external standard mixtures analyzed under the same conditions (Lôpez-Cervantes *et al.,* 2006). *Class Vp 6.1* software assisted in the interpretation of the data. The results of analyses were expressed as μg/g for each sample.

Flavonoids

A chromatographic analysis was carried out using a PREVAIL C18 reversed-phase column (15×4.6mm, 5μm, USA); the mobile phase was methanol/water/ acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (Zu *et al.,* 2006). This mobile phase was filtered through a 0.45 μm membrane filter (Millipore), then de-aerated ultrasonically prior to use. Catechin (CA), naringin (NA), rutin (RU), resveratrol (RES), myricetin (MYR), morin (MOR), naringenin (NAR), quercetin (QU) and kaempferol (KA) were quantified by DAD separation at 280 nm for CA and NA, 254 nm for RU, MYR, MOR and QU, and 265 nm for KA. Flow rate and injection volume were 1.0 ml/min and l0 μL, respectively. The chromatographic peaks of the extracts were confirmed by comparing their retention times with those of the reference standards. Quantification was carried out by the integration of the peak using the external standard method. All chromatographic operations were carried out at a temperature of 25°C.

2.5. Determination of antioxidant properties

Antioxidant assay by DPPH radical scavenging activity

The free radical scavenging effect of seed extracts was assessed by the decoloration of a methanolic solution of DPPH$^{\bullet}$ according to the method of Liyana-Pathiranan and Shahidi (2005). A solution of 25 mg / L DPPH in methanol was prepared and 4.0 ml of this solution was mixed with 25 and 50 μL of extract in DMSO. The reaction mixture was left in the dark at room temperature for 30 minutes. The absorption of a blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The absorbance of the mixture was measured spectrophotometrically at 517 nm. 1 μM quercetin was used as a reference.

The ability to scavenge DPPH radicals was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control − Abs sample)]/ (Abs control)] × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract /standard.

2.6. Antimicrobial activity

Antimicrobial tests were carried out according to the well agar method using 100 μL of suspension containing 10^6 cells / mL of bacteria, 10^4 cells / mL yeast and cells / mL dermatophyta fungi as per the McFarland standard, inoculated into Mueller Hinton Agar (Difco), Malt Extract Agar (Difco), and Sabouroud Dextrose Agar (Oxoid), respectively. Wells were prepared in the plates with the help of a cork-borer (0. 85 cm). 10 μl of the flavonoid, vitamin and fatty acid extracts were introduced directly into the wells one at a time. The wells were also injected with methanol and hexane. Steril petri dishes (9 cm diameter) were kept at 4°C for 2h. Then, the inoculated plates were incubated at 37±0.1°C for 24 h for bacterial strains and also at 25±0.1°C for 72 h for yeast and dermatophyta fungi. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms (Collins and Lyne, 1987). The standard antibiotics nystatin and streptomysin were used as a positive control (respectively; for yeasts and bacteria) and methanol and hexzane were used as negative control in this study. The experimental studies were repeated three times.

3. RESULTS AND DISCUSSION

3.1. Seed composition

Fatty acid compositions in the seeds of Origanum vulgare subsp. *gracile* and *Origanum acutidens*

The main fatty acids found in the seeds of *O. vulgare* subsp. *gracile* and *O. acutidens* were identified as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n9), linoleic acid (C18:2 n6), linolenic acid (C18:3 n3) and stearidonic acid (C18:4) (Table 1). Both species were determined to

Table 1
Fatty acid compositions in the seeds of O. vulgare subsp. gracile and O. acutiden

Fatty acids (%)	*O. vulgare* subsp. *gracile*	O.acutidens
C16:0	4.4±0.1	4.2±0.4
C18:0	1.5±0.1	1.9±0.1
ΣSFA	**5.9±0.1**	**6.1±0.3**
C16:1 n 7	0.4±0.1	–
C18:1 n9	5.7±0.3	7.5±0.5
ΣMUFA	**6.1±0.2**	**7.5±0.5**
C18:2 n6	15.4±0.4	13.5±0.2
C18:3 n3	67.8±0.6	67.6±0.6
C18:3 n6	1.2±0.1	0.9±0.1
C18:4	4.2±0.3	4.7±0.2
ΣPUFA	**88.6±0.4**	**86.7±0.3**

contain similar fatty acids. α-linolenic acid was found to be the major fatty acid (C18:3 n3; 67.8±0.6% in *O. vulgare* subsp. *gracile*; 67.6±0.6% in *O. acutidens*) in both species examined. Low amounts of γ- linolenic acid (C18:3 n 6) were found in both species (0.9±0.1-1.2±0.1%). The second major fatty acid was found to be linoleic acid (C18:2 n 6) (15.4±0.4% in *O. vulgare* subsp. *gracile*; 13.5±0.2 % in *O. acutidens*) in both species.

It was found that *O. acutidens* (7.5±0.5 %) includes more oleic acid constituents than *O. vulgare* subsp. *gracile* (5.7±0.3%). The results of the present study support the findings of the previous literature studies (Marin *et al.,* 1991; Azcan *et al*., 2004). Azcan *et al.* (2004) determined that *O. vulgare* seeds had fatty acid compositions similar to those of *O. vulgare* examined in the present study. Azcan *et al*. (2004) indicated in the scope of the same study that *O. vulgare* contained palmitic acid (5.5%), stearic acid (2.1%), oleic acid (5.9%) linoleic acid (18.8%) and α-linolenic acid (61.8%) (Azcan *et al.,* 2004). Moreover, Marin *et al.* (1991) recorded that the seed oil of *O. vulgare* was composed of palmitic acid (4.2%), stearic acid (1.3%), oleic acid (6.1%), and linolenic acid (64.6%). When compared to the present study, Marin *et al.* (1991) found higher amounts of linoleic acid in the *O. vulgare* species they studied (23.8%).

Lipide-soluble vitamin and sterol contents in the seeds of Origanum vulgare subsp. *gracile* and *Origanum acutidens*

Conforti *et al.* (2009) revealed the antioxidant capacity of phytosterols. They also reported that plant sterols had a considerable reducing effect on the plasma total cholesterol and LDL cholesterol (Conforti *et al.,* 2009). Several studies demonstrated that phytosterols (stigmasterols, β-sitosterols) have protective effects against reactive oxygen radicals (Yoshida and Niki, 2003; Vivacons and Moreno, 2005). Regarding the sterol contents of the seeds of *Origanum* species examined in the present study, two *Origanum* species were found to contain high amounts of stigmasterol (*O. vulgare* subsp. *gracile*, 2262.2±3.3 μg/g; *O. acutidens,* 1097.9±4.6 μg/g). In addition, *O. vulgare* subsp. *gracile* (56.3±2 μg/g) was recorded to have a higher ergosterol content than *O. acutidens* (5.5±0.3 μg/g). *While* β-sitosterol was found in *O. vulgare* subsp. *gracile* (152.8±2.6 μg/g), it was not recorded in *O. acutidens*.

Furthermore, *O. vulgare* subsp. *gracile* was found to include D3 (33.1±0.8 μg/g), K1 (29.4±0.8 μg/g), K2 (26.0±0.8 μg/g), α-tocopherol (7.8±0.2 μg/g), D2 (1.8±0.1 μg/g) and retinol acetate (0.3±0.1 μg/g) (Table 2) while *O. acutidens* was found to include D2 (42.9±1.5 μg/g), D3 (2.9±0.1 μg/g), α-tocopherol (3.1±0.1 μg/g), r-tocopherol (3.5±0.2 μg/g), K2 (1.3±0.1 μg/g), retinol (1.3±0.2 μg/g) and retinol acetate (1.2±0.2 μg/g) (Table 2). Lagouri and Boskou (1996) suggested that oregano contained all tocopherol homologues, with γ-tocopherol as the major component (Logouri and Boskou, 1996), which conflicts with the findings of the present study. However, Demo *et al*. (1998) determined the presence of only α-tocopherol in Lamiaceae species.

Table 2
Lipide-soluble vitamin and sterol contents in the seeds of *O. vulgare* subsp. *gracile* and *O. acutidens*

Vitamins (μg/g)	***O. vulgare* subsp. *gracile***	***O.acutidens***
K1	29.4±0.8	–
K2	26.0±0.8	1.3±0.1
γ-tocopherol	–	3.5±0.2
D2	1.8±0.1	42.9±1.5
D3	33.1±0.8	2.9±0.1
α-tocopherol	7.8±0.2	3.1±0.1
Retinol	–	1.3±0.2
Retinol acetate	0.3±0.1	1.2±0.2
Sterols (μg/g)		
Ergosterol	56.3±2	5.5±0.3
Stigmasterol	2262.2±3.3	1097.9±4.6
ß-sitosterol	152.8±2.6	–

Flavonoid contents

Due to their naturally high phenolic antioxidant contents, the use of Lamiaceae extracts (which includes oregano, rosemary, thyme and spearmint) can be important in the scope of the antioxidant applications (Chun *et al.,* 2005). Many previous studies have demonstrated the antioxidant and free radical scavenging activities of various polyphenols (Kukiç *et al.,* 2006; Nakiboglu *et al.,* 2007).

The seeds of *O. vulgare* subsp. *gracile* were found to include little or no flavonoids, except for morin (42.8±1.3 μg/g). Naringenin (10.3±0.4 μg/g) and morin (4.5±0.1μg/g) were determined as the main flavonoid contents of *O. acutidens* (Table 3). *O. acutidens* was detected to include little or no myricetin (1.2±0.2 μg/g), kaempferol (0.5±0.2 μg/g), rutin, catechine or naringin (Table 3). However, *Origanum vulgare* was suggested by Wojdylo *et al.* (2007) to not contain any quercetin, kaempherol, luteolin, apigenin, or myricetin but to contain high amounts of caffeic acid and neochlorogenic acid (Wojdylo *et al.,* 2007).

Ivanova *et al*. (2005) determined the total phenolic contents of *O. vulgare* water extracts as 1653.61μM while Hernandez-Hernandez *et al.* (2009) indicated that ethanol extracts of oregano contain high amounts of phenols and that this high phenol concentration is not correlated with their high antioxidant activity.

Oregano species (except the two *Origanum* species examined in this study) were reported by

the previous literature studies to contain quercetin, apigenin, myricetin, naringenin, kaempferol, rutin, luteolin and coumarin (Skerget *et al.,* 2005; Proestos *et al.,* 2006; Proestos and Komaitis 2008; Ünver *et al.,* 2009; Sellami *et al.,* 2009; Chatzopoulou *et al.,* 2010).

3.2. Antioxidant Activity

Radical scavenging activity by DPPH method

The methanol extracts of the two *Origanum* species examined in this study were found to be the most effective extracts against the DPPH radical (Table 3, Figure 1). While the radical scavenging activity of the methanol extracts in the seeds of *O. vulgare* varied in the range of 87.4±1.2% (for 25 µl) and 89.3±0.4% (for 50 µl) , in the seeds of *O. acutidens* it varied from 89.6±0.6% (for 25 µl) to 90.5±0.5% (for 50 µl).

Table 3
Flavonoid contents and radical scavenging capacities in the seeds of *O. vulgare* subsp. *gracile* and *O. acutidens*

Flavonoids (µg/g)	*O. vulgare* subsp. *gracile*	O.acutidens
Myricetin	0.2±0.8	1.2±0.2
Morin	42.8±1.3	4.5±0.1
Quercetin	0.3±0.1	–
Kaempferol	0.1±0.1	0.5±0.2
Catechin	–	–
Naringin	–	–
Naringenin	–	10.3±0.4
Rutin	6.4±0.5	–
Inhibition %		
25 µl	87.4±1.2	89.6±0.6
50 µl	89.3±0.4	90.5±0.5

Several previous literature studies have reported findings similar to those of the present study (Kulisica *et al.,* 2004; Ivanova *et al.,* 2005; Kouri *et al.,* 2007). *O. vulgare* was reported to have lower ·OH scavenging activity and DPPH radical scavenging capacity when compared to *Origanum sipyleum,* and *Sideritis sipylea (*Nakiboglu *et al.,* 2007). Ünver *et al.* (2009) found that the water extracts of *O. vulgare* had a free radical scavenging activity higher than that of *Salvia officinalis* but lower than that of *Mentha piperita*. In contrast, an oregano study by Capecka *et al*. (2005) recorded a lower rate of DPPH scavenging accompanied by a significantly high inhibition of linoleic acid peroxidation.

3.3. Antimicrobial activity

Table 4 lists the antimicrobial activities of vitamins, flavonoids and fatty acids in two *Origanum* species, negative control groups and standard antibiotics. No previous literature study reported the antimicrobial activity of the extracts of vitamins, flavonoids and fatty acids of the two species examined in the present study. Therefore, this is the first study which demonstrates the antimicrobial activity of biological compounds contained in the seeds of these plants. Results of the present study show that the vitamin, flavonoid and fatty acid extracts of the two species inhibited the growth of microorganisms used in the test at varying levels. However, some extracts were observed to have no effect on the tested microorganisms and the flavonoids and vitamin extracts to have a generally reduced effect compared to the antibiotic group.

It can be seen in Table 4 that the antimicrobial activities of the vitamin extracts of *O. vulgare* subsp. *gracile,* except for *C. glabrata*, had an inhibition zone in the 8.3±0.2-23.6±0.3 mm range. However, the flavonoid extracts of *O. vulgare* subsp. *gracile* did not have any activity against gram-negative bacteria but had an antimicrobial effect on the

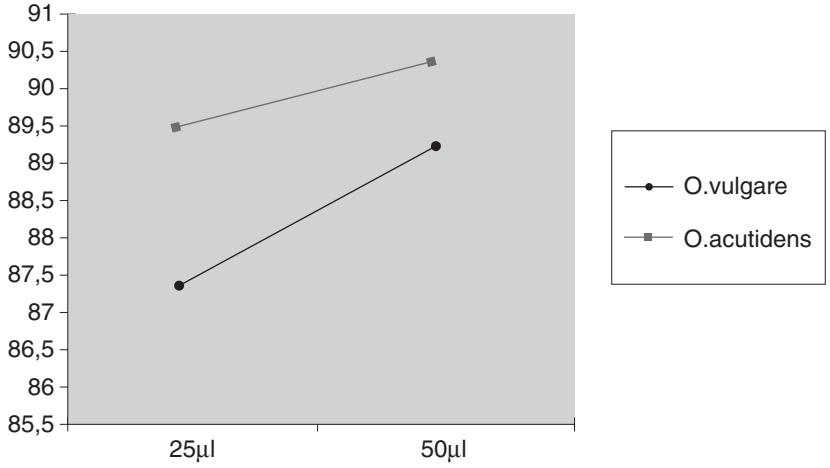

Figure 1
Radical inhibition activities of different volumes of methanol extracts of *O. vulgare* subsp. *gracile* and *O. acutidens* seeds by DPPH radicals

Table 4
Antimicrobial activities of seed extracts containing vitamins, flavonoids and fatty acids. O. v.: *O vulgare* subsp. *gracile*, O*. a.: O acutidens* Standart: *:Nystatin (antifungal, 30 µg/disc), **: Streptomysin sulphate (antibacterial, 10 µg/disc), Control: methanol and hexane (10 µl) NT: not tested

Microorganisms	Inhibition zone (mm)								
	Vitamins		Flavonoids		Fatty acids		Negative Control		Positive Control
	O. v	O. a	O. v	O. a	O. v	O. a	Methanol	Hexane	Standart antibiotics
E. coli	8.3±0.2	11.2±0.2	–	10.4±0.3	8.2±0.3	–	–	15.4±0.2	10.3±0.3**
K.pneumoniae	9.1±0.2	10.5±0.1	–	–	8.5±0.6	8.7±0.2	–	14.5±0.3	9.5±0.3**
S. aureus	9.5±0.3	8.3±0.4	8.2±0.3	8.3±0.2	8.7±0.2	–	–	13.3±0.4	13.4±0.1**
B. megaterium	8.5±0.4	10.3±0.2	8.4±0.2	9.6±0.2	–	–	–	12.4±0.1	9.4±0.3**
C. albicans	11.1±0.1	13.6±0.3	9.2±0.3	8.7±0.2	–	8.6±0.2	–	17.2±0.1	18.2±0.2*
C. glabrata	–	8.5±0.4	9.1±0.1	10.3±0.3	–	8.4±0.3	–	11.1±0.2	12.6±0.4*
Trichophyton sp.	23.6±0.3	19.1±0.3	15.4±0.2	13.4±0.2	–	–	–	17.4±0.4	NT
Epidermophyton sp.	8.4±0.3	8.4±0.3	8.3±0.1	11.3±0.2	8.4±0.1	13.2±0.3	–	9.3±0.3	NT

*:Nystatin (Antifungal, 30 µg/disc), **: Streptomysin sülfat (antibacterial,10 µg/disc), Control (methanol and hexane):10 µL, NT: not tested,

following tested microorganisms: *S. aureus* (8.2±0.3 mm), *B. megaterium* (8.4±0.2 mm), C*. albicans* (9.2±0.3 mm)*, C. glabrata* (9.1±0.1mm)*, Epidermophyton* sp. (8.3±0.1) and *Trichophyton* sp. (15.4±0.2 mm). But, the fatty acid extracts of *O. vulgare* subsp. *gracile* showed very little antimicrobial activity against *E. coli* (8.2±0.3 mm), *K. pneumoniae* (8.5±0.6 mm), *S. aureus* (8.7±0.2 mm), *Epidermophyton* spp. (8.4±0.1mm), except for *B. megaterium,* C*. albicans, C. glabrata* and *Trichophyton* sp. (Table 4).

Table 4 shows that the vitamin extracts obtained from the seeds of *O. acutidens* have higher antibacterial and antifungal activity than all the tested microorganisms: *Trichophyton spp.* (19.1±0.3 mm), *C. albicans* (13.6±0.3 mm), *E. coli* (11.2±0.2 mm), *K. pneumoniae* (10.5±0.1 mm), *B. megaterium* (10.3±0.2 mm)*, S. aureus* (8.3±0.4 mm*), C. glabrata* (8.5±0.4 mm), E*pidermophyton* spp. (8.4±0.3 mm). The flavonoid extracts obtained from *O. acutidens* showed inhibitory effects against all the tested microorganisms: *Trichophyton sp.* (13.4±0.2 mm), *Epidermophyton* sp. (11.3±0.2 mm), *E. coli* (10.4±0.3 mm), *C. glabrata* (10.3±0.3mm), *B. megaterium* (9.6±0.2 mm), *C. albicans* (8.7±0.2 mm), *S. aureus* (8.3±0.2 mm) except for *K. pneumoniae.* In contrast, the fatty acids in *O. acutidens* had no antimicrobial activity against *E. coli*, *S. aureus*, *B. megaterium* or *Trichophyton* spp.and very little activity against the tested microorganisms (8.4±0.3-13.2±0.3 mm zone of inhibition). Some literature studies showed that the extracts of *O. vulgare* inhibited the growth of bacteria and fungi and the synthesis of microbial metabolites (Oliveira *et al.,* 2009). According to Kursat and Erecevit (2009), the plant extracts of *O. vulgare* had less antimicrobial activity against *S. aureus* (9 mm) and *B. megaterium* (9 mm) but more activity against *K. pneumoniae* (17 mm) and *Epidermophyton spp.*(11 mm). Dikbaş *et al.* (2009) showed that the essential oils of *O. acutidens* had a strong antibacterial effect against both gram-positive and gram-negative bacteria. Yiğit and Kandemir (2002) recorded antimicrobial activity of the ethanol extracts of *O. acutidens* against gram-positive bacteria but no such activity against gram-negative bacteria or the yeast species *Candida albicans.* A previous study by Kordalı *et al.* (2008) reported the inhibitory effects of the essential oils isolated from *O. acutidens* on the mycelial growth of 17 phytopathogenic fungal species (Kordalı *et al.,* 2008).

REFERENCES

Azcan N, Kara M, Demirci B, Başer KHC. 2004. Fatty acids of the seeds of *O. onites* L. and *O. vulgare* L. *Lipids* **39** (5), 487-489.

Capecka E, Mareczek A, Lejas M. 2005. Antioxidant activity of fresh and dry herbs of some Lamiaceae species. *Food Chemistry* **93,** 223-226.

Cetingul IS, Bayram I, Akkaya AB, Uyarlar C, Yardımcı M, Şahin EH, Şengör E. 2007. Utilisation of oregano (*Origanum vulgaris*) in laying quails (*Coturnix coturnix japonica*) (2): The effects of oregano on performance, carcass yield, liver and someblood parameters. *Archiva Zootechnica* **10,** 57-65.

Chatzopoulou A, Karioti A, Chrysoula G, Vivancos VL, Kyriazopoulos P, Golegou S, Skaltsa H. 2010. Depsides and other polar constituents from *Origanum dictamnus* L. and their in vitro antimicrobial activity in clinical strains. *J. Agric. Food Chem.* **58,** 6064–6068

Chun SS, Vattem DA, Lin YT, Shetty K. 2005. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori. Process Biochemistry* 40, 809–816.

Christie WW. 1990. Gas Chromatography and lipids. The oily press: Glaskow: UK 573-577.

Collins CH, Lyne PM. 1987. Microbiological Methods Butter Morths & Co (Publishers) Ltd. London, pp. 450.

Conforti, F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, Tubaro A, Menichini F. 2009. The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol content. *Food Chemistry* **112,** 587-594.

Davis PH, Mill RR, Tan K. 1982. Flora of Turkey and the East Aegean Islands, Edinburgh University Press, vol 7, Edinburgh, 297-313.

Demo A, Petrakis C, Kefales P, Boskou D. 1998. Nutrient antioxidants in some herbs and Mediterrranean plant leaves. *Food Research International* **31** (5), 351-354.

Dib MA, Bendahou M, Bandiabdellah A, Djabou N, Allali A, Tabti B, Paolini J, Costa J. 2010. Partial chemical composition and antimicrobial activty of *Daucus crinitus* Desf. extracts. *Grasas y Aceites* **61,** 271-278.

Dikbas N, Kotan R, Dadasoglu F, Karagöz K, Çakmakçı R. 2009. Correlation between major constitiuents and antibacterial activities of some plant essential oils against some pathogenic bacteria. *Turkish Journal of Science* & *Technology* **4** (1), 57-64.

Djeridane A, Yous M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. 2006. Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry* **97,** 654–660.

Hara A, Radin NS. 1978. Lipid extraction of tissues with a low-toxicity solvent. *Anal. Biochem.* **90** (1), 420-426.

Hernández-Hernández E, Ponce-Alquicira E, Jaramillo-Flores ME, Legarreta IG. 2009. Antioxidant effect rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.) extracts on TBARS and colour of model raw pork batters. *Meat Science* **81,** 410–417.

Ivanova D, Gerova D, Chervenkov T, Yankova T. 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* **96,** 145–150.

Kordalı S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E. 2008. Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and p-cymene. *Bioresource Technology* **99**, 8788–8795.

Kouri G, Tsimogiannis D, Bardouki H, Oreopoulou V. 2007. Extraction and analysis of antioxidant components from *Origanum dictamnus. Innovative Food Science* and *Emerging Technologies* **8,** 155-162.

Kukiç J, Petrovic S, Niketic M. 2006. Antioxidant activity of four endemic *Stachys* taxa. *Biol. Pharm. Bull.* **29** (4), 725-729.

Kulisica T, Radonic A, Katalinic V, Milos M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry* **85**, 633–640.

Kurşat M, Ercevit P. 2009. The Antimicrobial activites of methanolic extracts of some Lamiaceae members collected from Turkey. *Turkish Journal of Science* & *Technology* **4**, 81-85.

López-Cervantes J, Sánchez-Machado DI, Ríos-Vázquez NJ. 2006. High-performance liquid chromatography method for the simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp waste hydrolysate. *Journal of Chromatography A* **1105**, 135–139.

Liyana-Pathiranan CM, Shahidi F. 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. Agric. Food Chem.* **53**, 3–2440.

Logouri V, Boskou D. 1996. Nutrient antioxidants in oregano. *International J. Food Science* **47,** 439-497.

Marin PD, Sajdl V, Kapor S, Tatic B, Petkovic B. 1991. Fatty acids of Saturejoideae, Ajugodeae and Scutellariodeae (Lamiaceae). *Phytochemistry* **30,** 2979-2982.

Milos M, Mastelic J, Jerkovic I. 2000. Chemical composition and antioxidant e.ect of glycosidically bound volatile compounds from oregano (Origanum vulgare L. ssp. hirtum). *Food Chem.* **71,** 79-83.

Mohamed AA, Khali AA, El-Beltagi HES. 2010. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). *Grasas y Aceites* **61,** 65-70.

Nakiboglu M, Urek RO, Kayali HA, Tarhan L. 2007. Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. *Food Chemistry* **104,** 630-635.

Oliveira JLTM, Diniz MFM, Lima EO, Souza EL, Trajano VN, Santos BHC. 2009. Effectiveness of *Origanum vulgare* L. and *Origanum majorana* L. Essential oils in inhibiting the growth of bacterial strains isolated from the patients with conjunctivitis. *Brazilian Archives of Biology* and *Technology* **52**, 45-50.

Proestos C, Boziaris IS, Nychas G-JE, Komaitis M. 2006. Analysis of flavonoids and phenolic acids in Grek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chemistry* **95,** 664-671.

Proestos C, Komaitis M. 2008. Application of microwave-assisted extraction to the fast extraction of plant phenolic compounds. *LWT* **41**, 652–659.

Sánchez-Machado DI, López-Hernandez J, Paseiro-Losado P. 2002. High-performance liquid chromatographic determination of α-tocopherol in macroalgae. *J. Chromatography* A **976**, 277–284.

Sellami IH, Maamouri E, Chahed T, Aidi-Wannes W, Kchouk ME, Marzouk B. 2009. Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (*Origanum majorana* L.) *Industrial Crops and Products* **30**, 395-402.

Skerget M, Kotnik P, Hadolin M, Hras AR, Simonic M, Knez Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* **89,** 191-198.

Skoula M, Harborne JB. 2002. The taxonomy and chemistry of *Origanum*. In: Kintzios (Eed.), S.E. (Ed.), Oregano, the genera *Origanum* and Lippia –Medicinal and Aromatic Plants Series – Industrial Profiles, vol. 25. Taylor & Francis, London, pp. 67–108.

Skoula M, Grayer RJ, Kite GC, Veitch NC. 2008. Exudate flavones and flavanones in *Origanum* species and their interspecific variation. *Biochemical Systematics and Ecology* **36,** 646–654.

Ulukapı K, Demiral S, Onus AN, Ülger S. 2008. Bazı *Origanum* türlerinde dışarıdan GA3 uygulamalarının *in vivo* ve *in vitro* koşullarda çimlenme üzerine etkilerinin araştırılması. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi* **21** (1), 123-129.

Ünver A, Arslan D, Özcan MM, Akbulut M. 2009. Phenolic content and Antioxidant activity of some spices. *World Applied Sciences Journal* **6** (3), 373-377.

Verma AR, Vijayakumar M, Mathela CS, Rao CV. 2009. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food and Chemical Toxicology* **47,** 2196–2201.

Vivacons M, Moreno JJ. 2005. Beta-sitosterol modulates antioxidant enzyme response in RAW 264.7 macrophages. *Free Radical Biology* & *Medicine* **39,** 91-97.

Wojdylo A, Oszmianski J, Czemerys R. 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem.* **105,** 940-949.

Yiğit D, Kandemir A. 2002. Antimicrobial activity of some endemic plants (*Salvia cryptantha*, *Origanum acutidens*, *Thymus sipyleus* ssp. *sipyleus)*. *Erzincan Eğitim Fakültesi Dergisi,* 4-2.

Yoshida Y, Niki E. 2003. Antioxidant effects of phytosterol and its components. *Journal of Nutritional Science* and *Vitaminology* **49,** 277-280.

Zu YG, Li CY, Fu YJ, Zhao CJ. 2006. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. *J. Pharmaceutical and Biomedical Analysis* **41,** 714–719.

Recibido: 8/11/10
Aceptado: 28/3/11