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Essential oil composition, total phenolic content, antioxidant and antibiofilm activities of four *Origanum* species from southeastern Turkey

Gürkan Semiz[a], Aslı Semiz[b], and Nazime Mercan-Doğan[a]

[a]Faculty of Arts & Sciences, Department of Biology, Pamukkale University, Denizli, Turkey; [b]Vocational School of Health Services, Medical Laboratory Techniques, Pamukkale University, Denizli, Turkey

ABSTRACT

This study reports a comparative screening of four species of *Origanum* in Turkey, based on their essential oil composition, total phenolic content, antioxidant and antibiofilm activities. The major components of essential oils were *p*-cymene, linalool, and thymol. The total phenolic contents differed from 3.81 to 47.54 mg of GAE/g of extract. The concentrations of flavonoids varied from 12.74 to 58.39 mg of Ru/g of extract. Antioxidant activity was determined *in vitro* using DPPH reagent and expressed as concentration of each extract required to inhibit radical by 50% (IC_{50}) values that ranged from 16.03 to 48.94 µg/ml. Our results indicated that chloroform extracts of species *O. majorana* and *O. onites*, with a total content of polyphenols (47.54 mg of GAE/g and 45.17 mg of GAE/g, respectively) and an IC_{50} of 16.03 µg/ml and 16.89 µg/ml, respectively were more antioxidant. Among the essential oil concentrations tested, maximum antibiofilm activity was found as 92.24% against *M. luteus* NRRL-B 1013 by *O. majorana* essential oil at 50 mg/ml.

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Introduction

Many medicinal plants are known to comprise large amounts of antioxidants which can play an important role in scavenging free radicals and reactive oxygen species. During the time of stress created by drugs, toxic substances, or diseases, the production of active oxygen species increases, which has the potential to cause oxidative damage.[1] Antioxidants have crucial functions in preventing oxidative mechanisms that lead to degenerative diseases.[2] Today, free radicals exhibit important functions in the etiology of cardiovascular diseases, such as cancer, Alzheimer, and Parkinson.[3] Evidence shows that vegetable and fruits consumptions and intakes of certain nonnutrients which can be found in foods decrease the risk of several pathological events, such as cancer [4–7] and cardio- and cerebrovascular diseases.[8] Moreover, plants or their crude extracts have been used in the prevention and/or treatment of some diseases in various different communities around the world.[1]

Origanum is a member of Lamiaceae family and is a herbaceous plant native to the Mediterranean, Euro-Siberian, and Irano-Turanian regions. Many of *Origanum* species are found in the Eastern Mediterranean subregion.[9] As chemicals and aromatic characteristics are variable, *Origanum* plants are extensively used in agriculture and pharmaceutical, and cosmetic industries as a culinary herb, flavouring substances of food products, alcoholic beverages, and perfumery for their spicy fragrance.[10–14] These plants have potential health-promoting benefits and antioxidant properties from phenolic contents.[15] Furthermore, *Origanum* plants have been reported to exhibit multiple biological activities such as antioxidant, antimicrobial, antiviral, antihepatotoxic, and antiulcerogenic activities which are assigned to an array of biologically active plant chemicals

CONTACT Gürkan Semiz gsemiz@pau.edu.tr Faculty of Arts & Sciences, Department of Biology, Pamukkale University, Denizli, Turkey

including triterpenes, proteins and steroids.[16] *O. hypericifolium* and *O. sipyleum* are narrowly distributed endemic species classified as "Least Concern (LC)" category of IUCN (International Union for Conservation of Nature) and taxonomically belongs to section Anatolicon.[17] *O. onites* is a perennial species with woody stem and is commonly known as "Turkish oregano" and *O. majorana* is a culinary herb and is called as "sweet marjoram" because of its citrus flavours. Additionally, Turkey has become a major supplier of *Origanum* herb and its oil to world markets due to its high quality. Turkey dominates the global support of oregano oil, supplying around 70% of global needs.[18]

Pathogenic bacteria growing in slime-enclosed aggregates known as biofilms are associated with many persistent and chronic bacterial infections such as cystic fibrosis, chronic wounds, otitis media, dental plaque, and infections related to the use of medical devices, such as catheters and implants.[19] These bacteria are especially resistant to antibiotics. Herewith, it is very difficult to combat these diseases. Although most research have focused on antimicrobial effects of essential oils and/or active constituents of plants, information about antibiofilm effects of plants is lacking.[20] This is first study undertook the antibiofilm effects of *O. onites, O. hypericifolium, O. majorana*, and *O. sipyleum*. The present study aimed to examine the distribution of phenolic compounds and antibiofilm acitivity of essential oils in four species of *Origanum* from southeastern Turkey.

Material and methods

Plant materials

Origanum majorana, O. onites, O. hypericifolium, and *O. sipyleum* at the flowering stage were collected from certain localities in southeast Turkey (Table 1). The taxonomic identification of plant materials was confirmed by Dr. Gürkan SEMİZ, in Department of Biology, Pamukkale University, Denizli-Turkey. The voucher specimens have been deposited at the Laboratory of Chemical Ecology of Pamukkale University (Denizli, Turkey).

Preparation of the extracts

Plant samples were dried in a dry and dark place at ambient temperature. The dried flowering shoots and leaves were cut off from the stem and ground in a grinder with a 2 mm diameter mesh. The collected plant material, air-dried and fine powdered (10 g), was extracted with 50 ml of solvent (water, methanol, and chloroform), respectively for 6 h at 50°C in a temperature controlled shaker (Memmert, SV 1422). The resulting extracts were filtered through Whatman No. 1 paper and residues were re-extracted with equal volume of solvents. Methanol and chloroform extracts were evaporated under vacuum at 37°C using rotary evaporator (IKA, RV10). The water extracts were concentrated to dryness under a vacuum on a freeze dry system

Table 1. Collection sites, dates, voucher specimens, and yields of *Origanum* L. species studied.

	Local Name	Locality	Date	Voucher Specimen	Oil yield
O. hypericifolium	Delik Mercan	Denizli, Sandras Mountain, 1509 m, 37°07′01″N 28°50′10″E	16.09.2014	2528	1.54
O. sipyleum	Mor Mercan	Denizli, Servergazi, 884 m, 37°45′42″N 28°58′48″E	18.09.2014	2490	1.10
O. majorana	Beyaz Kekik	Antalya, Akseki, 1240 m, 37°01′58″N 31°49′55″E	14.08.2014	2541	1.86
O. onites	İzmir Kekiği	Denizli, Güzelpınar Village, 1170 m, 37°58′58″N 29°12′24″E	21.06.2014	2603	2.02

(Labconco FreeZone) at −105°C. Same lyophilization procedure was applied to methanol and chloroform extracts to remove moisture from extracts. The obtained extracts were kept in dark and stored at 4°C.

Extraction of the essential oil

Air-dried parts of the *Origanum* species were subjected to steam distillation for 4 h using a Clevenger apparatus to obtain essential oil. The essential oil was dried in anhydrous sodium sulphate and after filtration stored in a sealed dark vial at 4°C until analysis. The yields of the essential oils were calculated by the formula:

$$\text{Yield of essential oil} = \text{volume of essential oil (g)/volume of sample (g) x 100\%}$$

GC/MS analysis

Chemical analyses of the essential oil were performed on gas chromatography-mass spectrometry (Hewlett Packard GC type 7820A, MSD 5975; Hewlett Packard, Wilmington, DE, USA) using a 30-m long HP-5MS (ID 0.25 mm, film thickness 0.25 mm, Hewlett Packard) capillary column. The chromatographic conditions were as follows: helium was used as the carrier gas at 1.2 ml min^{-1}; the temperature program for terpenes ranged from 50°C to 250°C; the heating rate was 5 ° C min^{-1}; SCAN technique (mass numbers from m/z 30 to 350 were recorded; signal ions in monitoring; 93, 133, 136, 161, and 204 m/z) was used; the samples of 1 µl were injected automatically and in the splitless mode. Mass spectra were taken at 70 eV. The individual peaks were identified by comparison of their retention indices (relative to C8-C25 *n*-alkanes for HP-5MS) as well as by comparing their mass spectra with Wiley 7 MS library (Wiley, New York, NY, USA) and NIST02 (Gaithersburg, MD, USA) mass spectral database. A series of *n*-alkanes was also injected under same analytical conditions with that of essential oil for the calculation of Retention Indices (RI). The percentages of the samples were calculated from the GC peak areas with the normalization method. The relative amount of compounds was calculated as mean values from duplicate GC and GC/MS analyses.

Total phenolic content

Total phenolic content was estimated spectrophotometrically according to the Folin-Ciocalteu colorimetric method.[21] The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. The samples were incubated at 45°C for 15 min. The absorbance was determined at λ_{max} = 765 nm. It was calibrated against gallic acid standards and expressed the results as mg gallic acid equivalents (GAE)/g extract. Data presented are average values of three measurements for each sample.

Total flavonoid content

Flavonoid content was measured according to aluminum chloride colorimetric method. [22] The sample contained 1 ml of methanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at the room temperature. The absorbance was determined at λ_{max} = 415 nm.[23] A calibration curve was prepared with rutin and the results were expressed in terms of rutin equivalent (mg of Ru/g of extract). Data presented are average values of three measurements for each sample.

Free radical scavenging activity using DPPH

The DPPH free radical scavenging activity of each sample was determined following the method described by Stankovic et al.[23] The stock solution (1 mg/ml) of the plant extract was prepared in methanol. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, and 0.97 µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) methanolic solution (0.2 mM). After 30 min in darkness at room temperature, the absorbance was recorded at 517 nm against a blank (methanol solution). The control samples contained all the reagents except the extract. The DPPH radicals scavenging activity was calculated using the following equation:

$$\%\text{inhibition} = \left[\left(A_{\text{control}} - A_{\text{sample}}\right)/A_{\text{control}}\right] \text{x} 100.$$

IC_{50} was obtained from sigmoidal graph by using non-linear regression analysis. The experiment was performed in triplicate. Synthetic antioxidants [butylated hydroxytoluene (BHT) and quercetin] were used as positive controls.

Microorganisms and medium

Six gram positive (*Staphylococcus aureus* ATCC 33862, *S. aureus* ATCC 29213, *Enterococcus faecalis* M10 (clinic isolate), *E. faecalis* M18 (clinic isolate), *E. faecalis* ATCC 19433, *Micrococcus luteus* NRRL-B 1013) and three gram negative (*Salmonella tphymurium* ATCC 14028, *Enterobacter cloacea* ATCC 28355, *Pseudomonas fluorescens* ATCC 55241) bacteria were used as test microorganisms. The bacterial strains were collected from Bacteriology Laboratory of Pamukkale University. Bacterial cultures were inoculated in growth media Tryptic Soy Broth (TSB) which consisted of peptone from casein (17.0 g/L), peptone from soymeal (3.0 g/L), glucose (2.5 g/L), NaCl (5.0 g/L), and K_2HPO_4 (2.5 g/L). The culture was aerobically incubated and the growth was followed by measuring the optical density (OD) at 600 nm.

Antibiofilm activity

Crystal violet assay was used to test antibiofilm activity of the essential oils against the bacteria on 96-well polystyrene plates.[24] The bacterial cultures were grown in TSB at 37°C and 30°C under aerobic conditions for 24 h. Then, bacterial suspension at 0.5 McFarland turbidity standard was dispensed into each well of 96-well plates in the presence of TSB supplemented with glucose, containing essential oils. The essential oils were prepared at 50 mg/ml, 25 mg/ml, and 12.5 mg/ml ratios. The plates were then incubated for 48 h at 37°C (30°C for *Pseudomonas florescens* ATCC 55241 and *Micrococcus luteus* NRRL-B 1013). Following incubation, crystal violet staining assay was applied. Negative control (cells+TSB) was used as growth control. Each experiment was performed in duplicate. And the biofilm inhibition percentage was calculated by using the following formula:

$$\left[\left(OD_{\text{growth control}} - OD_{\text{sample}}\right)/OD_{\text{growth control}}\right] \text{x} 100$$

Statistical analysis

All measurements were performed in triplicate and results were expressed as mean ± standard deviation (SD) of each triplicate test. The data were subjected to analysis of variance, and appreciate mean separation was conducted using Tukey's multiple range test in SPSS 15.0 software.[25]

Results and discussion

Chemical composition of the essential oils

The composition of essential oils obtained from *Origanum* species is shown in Table 2. Overall, 34 compounds representing >97% of the oils were identified by GC and GC/MS. The essential oil yield for the collected samples, ranging from 1.10% (v/w) in *O. sipyleum* to 2.02% (v/w) in *O. onites* based on the dry weight of the samples. Although the total contents of terpene profile are quite similar, there are differences in the content of individual components. *p*-Cymene, linalool, and thymol are the most abundant terpenoids in *Origanum* species sampled. Earlier studies similarly reported the essential oil compositions of the same *Origanum* species from different regions and found that carvacrol, *p*-cymene, terpinen-4-ol, and thymol were the major components but their percentages varied from the results of the current study.[26–30] The differences in the chemical composition of *Origanum* essential oils among studies may be related to different environmental and climatic conditions, sampling time, genetic origins of plants, vegetative plant phases, and extraction and quantification methods. Carvacrol, thymol, and linalool are known to possess strong antioxidant properties [31–33] and carvacrol and thymol also exhibit antibacterial activity against several bacteria.[10,34]

Table 2. Chemical composition (%) of essential oils of *Origanum* species growing in southeastern Turkey.

No	Compounds[a]	RI (min)[b]	*O. hypericifolium*	*O. sipyleum*	*O. majorana*	*O. onites*
1	α-thujene	930	1.68	-	1.57	1.22
2	α-pinene	937	1.46	11.03	0.81	1.09
3	camphene	952	1.12	-	0.32	0.83
4	sabinene	975	0.44	0.66	0.21	-
5	β-pinene	979	0.58	0.54	0.32	0.21
6	1-octen-3-ol	984	1.15	-	0.24	0.28
7	β-myrcene	992	1.47	1.60	2.22	2.00
8	α-phellandrene	1004	0.49	-	0.41	0.45
9	3-carene	1011	-	-	0.12	0.12
10	α-terpinene	1018	1.46	1.04	2.85	2.17
11	p-cymene	1025	**33.08**	**13.21**	6.38	5.57
12	β-phellandrene	1030	-	-	0.82	0.87
13	1,8-cineole	1035	-	-	0.53	-
14	trans-β-ocimene	1039	-	0.43	0.52	0.30
15	γ-terpinene	1060	14.92	9.61	11.63	6.43
16	α-terpinolene	1088	0.59	-	0.30	0.44
17	linalool	1099	0.35	-	**38.38**	14.35
18	camphor	1144	-	3.72	-	-
19	borneol	1166	4.06	-	0.88	2.87
20	terpinen-4-ol	1177	1.91	-	2.98	5.66
21	α-terpineol	1189	0.12		-	0.48
22	carvacrol methyl ether	1240	-	5.45	-	-
23	carvone	1245	-	-	-	-
24	thymol	1291	22.90	5.69	26.11	**42.15**
25	carvacrol	1299	5.28	13.14		8.47
26	trans-β-caryophyllene	1405	1.99	7.35	0.84	0.68
27	aromadendrene	1439	-	4.24	-	0.46
28	α-humulene	1454	0.18	-	-	-
28	germacrene D	1480	-	5.70	-	-
30	bicyclogermacrene	1495	0.20	5.90	0.31	-
31	β-bisabolene	1509	-	-	-	1.04
32	δ-cadiene	1513	0.16	-	-	-
33	caryophyllene oxide	1581	1.54	-	-	0.22
34	α-cadinol	1652	-	8.01	-	-

[a]Compounds listed in order their elution.
[b]RI: Retention index measured relative to *n*-alkanes on HP-5MS column. (-) = not detected.
Bold values indicate compounds present in highest amounts.

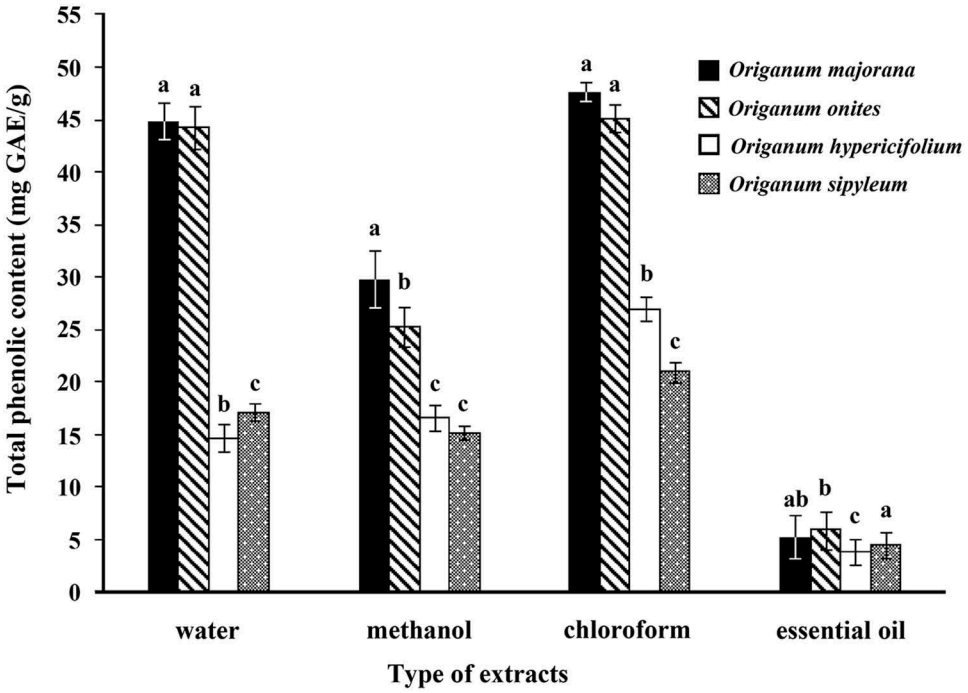

Figure 1. Total phenolic contents in the extracts of *O. majorana, O. onites, O. hypericifolium*, and *O. sipyleum* expressed in terms of gallic acid equivalent (mg of GAE/g of extract). results are presented as the mean from three independent experiments and expressed as relative mean ± standard deviation. Those with the same letter are not significantly different at the $P < 0.05$ level.

Total phenolic and flavonoid content

The total phenolic contents of the extracts and essential oil ranged between 3.81 to 47.54 mg of GAE/g of extract (Fig. 1). *O. majorana* and *O. onites* chloroform extracts had the highest total phenolic content (47.54 mg GAE/g and 45.17 mg GAE/g, respectively). Water extracts of *O. majorana* and *O. onites* also contain high levels of phenolics (44.81 mg GAE/g and 44.26 mg GAE/g, respectively), while phenolic contents in methanol and essential oil extracts were relatively lower. Varying solubility of the phenolic compounds due to different solvents could be explained by the solvent polarity.[35] Moreover, phenolics may also be associated with other plant metabolites (e.g. carbohydrates and proteins). These results suggest that methanol and ethanol seem to be more efficient in extracting lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone. Additionally, ethanol is also safe for human consumption.[36]

The concentrations of flavonoids varied from 12.74 to 58.39 mg of Ru/g of extract (Fig. 2). Chloroform extracts of *O. majorana* and *O. onites* have been found to be rich in flavonoids with a value of 57.50 mg of Ru/g and 58.39 mg of Ru/g, respectively, than other extracts examined and essential oils. Similar to the phenolic contents, the water extracts of *O. majorana* and *O. onites* also contain high levels of flavonoid (50.24 mg of Ru/g and 49.35 mg of Ru/g, respectively). Based on these results, chloroform seem to be the best solvent for extracting flavonoids and phenolics from *Origanum* species.

Free radical scavenging activity using DPPH

DPPH free radical scavenging activities of species *O. majorana, O. onites, O. hypericifolium*, and *O. sipyleum* extracts and essential oil are shown in Table 3. Antioxidant activity values that are determined *in vitro* using DPPH reagent and expressed as concentration of each extract and essential

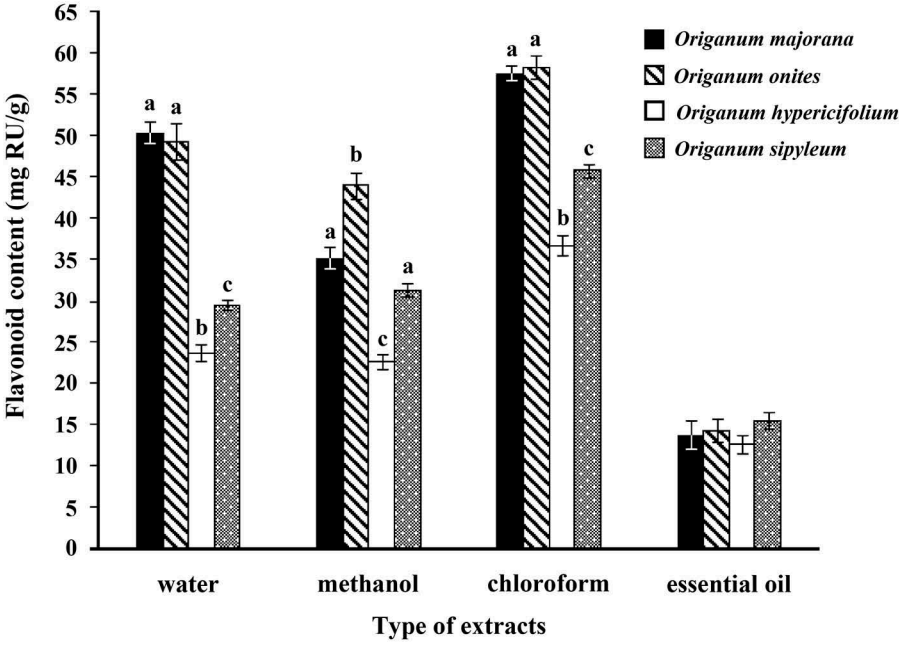

Figure 2. Flavonoid contents in the extracts of *O. majorana, O. onites, O. hypericifolium*, and *O. sipyleum* expressed in terms of rutin equivalent (mg of Ru/g of extract). results are presented as the mean from three independent experiments and expressed as relative mean ± standard deviation. Those with the same letter are not significantly different at the $P < 0.05$ level.

Table 3. DPPH scavenging activity of extracts from *O. majorana, O. onites, O. hypericifolium*, and *O. sipyleum* presented as IC_{50} values.

Type of extract	Water	Methanol	Chloroform	Essential oil
Origanum majorana	18.02 ± 0.36	23.35 ± 0.25	16.03 ± 0.12	46.78 ± 0.33
Origanum onites	18.32 ± 0.22	24.12 ± 0.21	16.89 ± 0.15	46.94 ± 0.45
Origanum hypericifolium	44.89 ± 0.42	42.64 ± 0.35	24.40 ± 0.22	48.94 ± 0.51
Origanum sipyleum	41.88 ± 0.47	46.35 ± 0.48	36.85 ± 0.35	46.65 ± 0.49

Each value represents the mean ± SD ($n = 3$). IC_{50} values were expressed as µg/ml.

oil required to inhibit radical by 50% (IC_{50}) ranged from 16.03 to 48.94 µg/ml. A higher DPPH radical scavenging activity is associated with a lower IC_{50} values. When compared to the activity of reference antioxidant rutin (IC_{50} = 9.28 µg/ml) [23], DPPH scavenging activity of chloroform extract of species *O. majorana* and *O. onites* is high (IC_{50} = 16.03 µg/ml and 16.89 µg/ml, respectively). Water extract of species *O. majorana* and *O. onites* also showed considerable DPPH scavenging activity with IC_{50} value of 18.02 µg/ml and 18.32 µg/ml, respectively). These results show that extracts from *O. majorana* and *O. onites* have antioxidant power and ability to scavenge free radicals. There is enough evidence to support that the antioxidant activity of plant materials is well correlated with the content of phenolic compounds.[23,37,38] In the last decade, polyphenol-rich foods and herbs have received specific focus because of their biological effects including antioxidant activity.[39] Various researches on the antioxidant activity of plant extracts have shown a high linear correlation between the values of phenolic content and antioxidant activity.[40–44] It is well known that phenolic substances such as flavonoids, phenolic acids, and tannins contribute directly to the antioxidant capacity of plants.[45,46] Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [47] implicated in several diseases. Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress.[3] Generally, plant materials rich in

phenolics are increasingly being used in food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food.[41,48]

Our results show that *O. majorana* and *O. onites* can be a source of polyphenols and flavonoids and can provide considerable benefits when used as food ingredients and for human consumption. Earlier studies reported that *Origanum* has important biological activities and acts against different types of diseases.-[20,49,50] However, safety and toxicity issues of these extracts and essential oil should be evaluated beforehand.

Microbial tests

Biofilm is more resistant to antimicrobials compared to planktonic cells. Biofilm-related infections threaten the human life and cause recurrent infections in hospitals. Therefore, discovery of alternative drugs for curing biofilm infections has become one of the attractive areas for researchers. *Origanum* is well known for its antimicrobial activity against such bacteria like *Escherichia coli, Klebsiella pneumonia, Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa, Vibrio splendidus, Sarcina lutea*, and *Salmonella typhimurium*.[51–55] However, there is no adequate information regarding the antibiofilm properties of *Origanum* species. In this study, the effects of four essential oils of *Origanum* species on biofilm formation of six gram positive and three gram negative bacteria was determined (Table 4). Essential oils consistently showed an inhibitory effect on biofilm formation of pathogens with a dose dependent manner and as the essential oil concentrations increased the biofilm inhibition percentage also increased. The maximum biofilm inhibition activity was observed at 50 mg/ml. Maximum biofilm inhibition rates for *O. onites* was 58.73% against *S. aureus* ATCC 29213, *O. hypericifolium* and *O. majorana* was 87.40% and 92.24% against *M. luteus* NRRL-B 1013, respectively. Also, maximum biofilm inhibition rate of *O. sipyleum* was 85.71% against *S. tphymurium* ATCC 14028. In addition, the biofilm biomass of *P. fluorescens* ATCC 55241 and *S. tphymurium* ATCC 14028 were inhibited at 56.58% and 56.69% by the *O. hypericifolium and O. majorana* essential oil at 25 mg/ml concentration, in consecutive order.

On the other hand, 12.5 mg/ml concentration of essential oils exhibited lower antibiofilm activity against bacteria species tested. Biofilm inhibition percentage of *O. onites* ranged from 13.58% to 27.43%, *O. hypericifolium* ranged from 10.00% to 44.28%, *O. majorana* ranged from 16.02% to 48.25%, *O. sipyleum* ranged from 12.55% to 32.45% at 12.5 mg/ml of essential oils (Table 4). Essential oil of *O. onites* prevented the biofilm formation of *S. aureus* ATCC 29213, *E. faecalis* ATCC 19433 and *P. fluorescens* ATCC 55241 over 50% concentration of 50 mg/ml. *O. hypericifolium* exhibited higher inhibition activity against bacteria. Over the 60% biofilm inhibition percentages were determined on *S. aureus* ATCC 29213, *M. luteus* NRRL-B 1013, *E. faecalis* ATCC 19433, and *P. fluorescens* ATCC 55241 by *O. hypericifolium. O. majorana* had the biofilm inhibition percentage of 92.24% against the *M. luteus* NRRL-B 1013 at 50 mg/ml oil concentration.

Table 4. The biofilm inhibition effect of essential oils of *Origanum* species, expressed as percentage inhibition.

Essential oils	Concentration (mg/ml)	Bacteria								
		S.a.1	S.a.2	E.f.1	M.l.	E.f.2	E.f.3	E.c.	S.t.	P.f.
O. onites	50	48.46	58.73	47.44	43.62	34.83	51.40	34.55	38.74	53.32
	25	38.97	37.39	33.58	32.14	22.90	29.25	27.54	32.90	31.16
	12.5	13.58	27.43	24.94	20.77	16.77	14.05	24.20	22.93	21.87
O. hypericifolium	50	37.69	62.29	41.57	87.40	32.41	68.19	41.40	49.00	68.68
	25	32.30	41.26	41.57	49.69	20.16	40.72	28.34	38.31	56.58
	12.5	19.23	40.14	20.25	38.28	10.00	26.22	17.35	32.05	44.28
O. majorana	50	32.69	62.19	40.93	92.24	67.11	75.37	37.11	68.34	66.25
	25	23.97	52.33	36.24	50.30	34.19	49.14	25.60	56.69	51.02
	12.5	16.02	26.93	22.17	31.68	24.74	32.19	21.00	47.47	48.25
O. sipyleum	50	30.89	57.31	48.82	52.45	41.55	56.50	66.13	85.71	74.89
	25	20.89	39.73	25.47	25.67	23.01	49.14	39.24	45.64	46.11
	12.5	16.53	23.47	15.45	12.55	18.09	19.93	28.16	25.58	32.45

S.a.1: *S. aureus* ATCC 33862, S.a.2: *S. aureus* ATCC 29213, E.f.1: *E. faecalis* M10, M.l.: *M. luteus* NRRL-B 1013, E.f.2: *E. faecalis* M18, E. f.3: *E. faecalis* ATCC 19433, E.c.: *E. cloacea* ATCC 28355, S.t.: *S. tphymurium* ATCC 14028, P.f.: *P. fluorescens* ATCC 55241.

Also, *O. majorana* showed good antibiofilm activity at 25 mg/ml and 12.5 mg/ml essential oil concentrations. Additionally, *S. tphymurium* ATCC 14028 and *P. fluorescens* ATCC 55241 were more susceptible to *O. sipyleum* essential oil than other strains. In an earlier study, essential oils from six different populations of *Origanum vulgare* subsp. *hirtum* were investigated for their antibiofilm properties and it was found that one sample showed the highest effect against all bacterial strains tested at 50 mg/ml and its inhibition percentages rangedfrom 30% to 52%.[56] Similarly, the essential oils of *O. vulgare* subsp. *viride* have different rates of antibiofilm activity against *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, P. fluorescens*, and the yeast *Candida albicans*.[57]

Conclusion

In this study, free radical scavenging and antibiofilm activity, total phenolic and flavonoid content of three extracts and essential oils of four *Origanum* species, were determined. Extracts of both *O. majorana* and *O. onites* had a certain level of radical scavenging effect, depending on proportion of their total phenolic content. In addition, the chloroform extracts had a stronger radical scavenging effect and the water extracts had a higher total antioxidant capacity. As a result, *O. majorana* and *O. onites* can be used in pharmaceutical products as a source of natural antioxidants. In present study, the extracts of *Origanum* were highly efficient antibiofilm agent. These data have verified our hypothesis that these essential oils have variable antibiofilm effects on pathogens tested. The results suggest that essential oils of *Origanum* species may be of great value to industries that experience problems related to biofilms.

Our study should be broadened for future phytochemical and pharmacological studies for other species of *Origanum*. This may have the potential to be of a vital importance and to pave the way for new therapeutic products. What is more, as the extracts of the genus *Origanum* and its essential oils can function as dietary supplements or for medicinal purposes, it is important to monitor them in order to secure authenticity and product quality as toxic adulterants may be life threatening. Nevertheless, further studies are necessary to evaluate the cost and efficacy of these extracts on industrial applications.

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