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EXPERIMENTAL PAPER

Composition, antioxidant and antimicrobial activities of cold-pressed and distilled essential oils of *Origanum onites* L. and *Lavandula officinalis* L. relationships evaluated by SEM microscopy

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Summary

Introduction: In order to prevent increased bacterial resistance, it is important to use herbal medicines with less side effects.

Objective: In this study, the chemical composition, antimicrobial, antioxidant activities and SEM images of *Lavandula officinalis* and *Origanum onites* oils obtained in two different ways were determined.

Methods: The antimicrobial activity of the oils was determined against reference and multiple strains of foodborne and pathogenic bacteria. The each essential oil sample were analyzed by GC–MS. Antioxidant activities of the samples were examined through DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging and FRAP assays.

Results: The results indicate that the oils of obtained from *Origanum onites* and *Lavandula officinalis* exhibited relatively strong antibacterial and antifungal activity. In addition, the antioxidant activities detected were remarkable. Morphological changes in bacterial cells treated with essential oil samples were demon-

strated with SEM images. In addition, the chemical components of the oil samples obtained by both water steam and cold press were revealed by GC-MS analysis and compared.

Conclusion: When all the obtained results are evaluated together; these plants could be suitable for using as antimicrobial and antioxidative agents in several industries such as food, cosmetic, etc.

Key words: ***Origanum, Lavandula, essential oil, antioxidant activity, GC-MS analysis, antimicrobial activity***

Słowa kluczowe: ***oregano, lawenda, olejek eteryczny, działanie antyoksydacyjne, analiza GC-MS, działanie przeciwdrobnoustrojowe***

INTRODUCTION

Essential oils (EOs) can be included in the class of secondary components such as terpenoids, phenolics and alkaloids produced in the secondary metabolism of plants. They can be used for a variety of purposes such as drug content, nutritional additives and cosmetic products. The flora of Turkey provides a rich variety of plants, estimated to be around 8,500–9,000 species [1, 2]. Turkey is also is known as the important gene-center of *Lamiaceae* (*Labiatae*) family. There are 45 genera, 546 species and 730 taxa of it in Turkey. The endemism rate of *Lamiaceae* family is 44.2% [3]. *Origanum onites* (*O. onites*) is ranked first among the traded *Origanum* species in Turkey. The chemical composition of its essential oil has already been well documented. Its chemical composition includes monoterpenes, phenolics and sesquiterpenes. Carvacrol was detected as main component (60–82%) of essential oil samples of 13 different *Origanum* obtained from Western Regions of Turkey [4, 5]. The content of essential oils varies depending on various climatic conditions and the method of distillation applied [6].

In Turkey, investigation has been carried out on distinct species of lavender [7]. The most common varieties are *Lavandula officinalis* (*L. officinalis*) and *Lavandula angustifolia* (*L. angustifolia*), growing naturally in Turkey [8].

Antioxidant capacity of essential oils is effective in preventing or eliminating the danger that may arise against the endogenous production of free radicals and other oxidant species. It is also known that EOs exhibit significant *in vitro* antimicrobial properties against disease-causing pathogens and foodborne agents [9]. So, in this study, antimicrobial and antioxidant activity and chemical composition of essential oils extracted from *O. onites* and *L. officinalis* were determined. Furthermore their activity on bacterial cells was also examined using scanning electron microscopy.

MATERIAL AND METHODS

Essential oil material

Lavender (*Lavandula officinalis*) and thyme (*Origanum onites*) essential oils, which are known to be used most frequently for various purposes, were selected as a study material and obtained from the pharmacy commercially. Each sample was obtained from the food additive supplier (Foodstuffs Istanbul, Turkey) and selected among those obtained by both water steam distillation and cold press techniques.

Microorganisms

Both samples were screened for antimicrobial activity against six Gram-positive bacteria (*Listeria monocytogenes* ATCC®7677, *Clostridium perfringens* ATCC 313124, *Bacillus subtilis* B209, *Micrococcus luteus* B1018, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC®10876), six Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC®27853, *Proteus vulgaris* ATCC®7829, *Escherichia coli* ATCC®25922, *Klebsiella pneumoniae* ATCC®13883, *Salmonella enterica* ATCC 14028, *Yersinia enterocolitica* ATCC®27729) and two fungi (*Aspergillus niger* ATCC®9642, and *Candida albicans* ATCC®10231) using disc diffusion and microdilution assays. Mueller Hinton Agar (MHA, Merck) or Mueller Hinton Broth (MHB, Merck) and Sabouraud Dextrose Broth (SDB, Difco) or Sabouraud Dextrose Agar (SDA, Oxoid) were used for growing bacterial and fungal cells, respectively.

Disc diffusion assay

Antimicrobial activity was measured according to Ronald's method [10]. Bacterial strains were grown in MHA for 24 h at 37°C, and fungal strains were

grown in SDA at 27°C for 48 h. Overnight cultures were diluted with 0.9% *w/v* saline solution and turbidities of bacterial and fungal cell solutions were adjusted to 0.5 McFarland. Then, sterile discs with a diameter of 6 mm were placed on agar to load 30 μl of each essential oil extract stock solution prepared at 30, 20 and 10 mg/ml concentrations. As a positive control, nystatin for fungi and amoxicillin and cephazolin for bacteria were used. Alcohol and acetone were also used as a negative control.

Minimum inhibitory concentration (MIC)

The method described by Vanden Berghe and Vietinck was used for determination of minimum inhibitory concentration (MIC) values [11]. For this purpose, each essential oil extract sample was tested at concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. As positive control amoxicillin and cephazolin for bacteria and nystatin for fungi were used. 70% ethanol was also used as negative control. Growth of microorganisms was assessed by a stereo microscope after the incubation period.

Scanning electron microscopy (SEM)

Bacterial cultures were spread on the slide and cultured, then essential oils were released onto these spreads and left to stand for 24 hours. Initially, the preparations were fastened together with 2.5% (*w/v*) glutaraldehyde in a filter-sterilized phosphate buffer (0.1 M, pH 7.4) at room temperature for 1-2 hours and then rinsed three times for 20 minutes in sodium cacodylate buffer (0.1 M). After then as a second decisiveness footstep, it was treated with osmium tetroxide (1% *w/v*) sodium cacodylate buffer (0.1 M) for one hour. As the next step, a quick rinse with distilled water was performed. The fixed sources were then dehydrated for 7 minutes by successively increasing the percentage of ethanol (from 30% to 95%) in the ethanol-water mixtures rapidly, and then two times in pure ethanol for 10-20 minutes. They were placed in an ethanol bath to evaporate.

Dehydrated samples were added to the metal holders with double-sided sticky tape and ultimately coated with gold and palladium in an evaporator. Investigations were completed at 15 kV with a scanning electron microscope (Hitachi SU1510 SEM). From the optic surface of each sample, three fields of view were randomly chosen with magnification from × 1000. Each experiment was repeated three times [12].

DPPH free radical scavenging activity

In order to determine potential of the essential oil samples to destroy DPPH free radical in the reaction environment, the color change of the DPPH solution in methanol was monitored as spectrophotometrically. For this purpose, absorbance of 1 ml of 0.4 mM DPPH solution used as stable radical was firstly measured at 517 nm (A_{blank}). On the other hand, 2% essential oil samples were added to DPPH solution and after incubation for 30 min at room temperature the final absorbance was measured and recorded as A_{sample}. To evaluate the hydrogen atom or electron susceptibility of the essential oil samples, DPPH free radical scavenging activity (SA) was calculated as percentage expression using following equation [13]:

$$\%SA = \frac{A_{blank} - A_{sample}}{A_{blank} \times 100}.$$

ABTS radical scavenging activity

The decoloring of the ABTS radical cation ($ABTS^{\bullet+}$) by the addition of essential oil samples was also monitored spectroscopically to reveal the total antioxidant activity of the samples with a second method [14]. For this purpose, ABTS radical cation was prepared by mixing ABTS solution and potassium persulphate. Sufficient amount of the each essential oil sample was added to $ABTS^{\bullet+}$ solution equilibrated at 30°C after oxidation process for 12–16 h and absorbance of it was adjusted to 0.7 at 734 nm by diluting with ethanol. The mixture is left to wait for the reaction to take place at 30°C fort 30 min. The same was done for various trolox concentrations. Thus, $ABTS^{\bullet+}$ scavenging activity of the samples was calculated as (μmol TX/g sample) utilizing the standard calibration curve.

Ferric-reducing antioxidant power (FRAP) assay

FRAP assay is an inexpensive, repeatable and simple antioxidant activity determination method. To investigate FRAP activities of the oil samples, the method developed by Habib *et al.* was followed [15]. The basis of the FRAP method is that the Fe (III) -TPTZ complex formed in the reaction medium is reduced to Fe (II)-TPTZ in the presence of antioxidants and the resulting blue color intensity is measured at 595 nm [16]. For this purpose 1.2 ml of FRAP reactive freshly prepared just before analysis was added to

appropriate amount of sample to be tested and mixed. Results were calculated as Trolox equivalent (μmol TX/g sample) using the standard calibration graph obtained by treating Trolox, a standard antioxidant, under the same experimental conditions.

GC-MS analysis

GC-MS analysis of the each essential oil samples were performed using GC-MS (Hewlett Packard 5890 Series II GC Plus-Hewlett Packard 5971 Series MS) equipped with a column (Innowax 19091N-136, 60 m×0.250 mm i.d.; film thickness 0.25 μm). GC-MS conditions were adjusted as follows: the oven temperature was 70°C at first and finally increased to 240°C by raising 5°C/min. The carrier gas was helium with a flow rate of 0.77 ml/min. The electron ionization detector's voltage was 70 eV, and the detector temperature was adjusted as 280°C. The compounds absorbed by ethanol were injected into GC-MS in the splitless mode. The compounds were identified by comparing their molecular weights and fragmentations with spectra from the libraries of Wiley and Aromsa.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

The antimicrobial and antifungal activity of essential oils obtained from *O. onites* and *L. officinalis* (distilled water steam method and cold press) were initially evaluated by disc diffusion method using 12 strains of (Gram-positive and Gram-negative) bacteria and 2 fungal strains (*C. albicans* and *A. niger*). Each of EOs exhibited relatively strong antibacterial and antifungal effect. The results are shown in Table 1. The *O. onites* essential oil extracted by water steam method had inhibited growth of Gram-positive and Gram-negative bacteria, as well as fungi and molds [17, 18]. Also, *O. onites* cold press essential oil showed stronger inhibition effect on growth of Gram-positive and Gram-negative bacteria, as well as fungi and molds. The basic ingredients of essential oil of *O. onites* were reported as carvacrol, thymol, and γ-terpinene, borneol, linalool, α-terpinene [19, 20]. The essential oil distilled from *O. onites* contains carvacrol, linalool, *p*-cymene, myrcene, α-terpinene as the basic compounds. Thanks to composition of EOs, they show strong antimicrobial activity [21]. The antibacterial and antifungal activity of EOs has also been reported in different studies [22]. In plenty of statuses the activity was arised from complicated interplay between the distinct compound classes such as phenols, aldehydes, ketones, alcohols, esters, ethers or hydrocarbons found in EOs [21-23]. Although in some cases, the bioactivities of EOs are nearly involved with the efficiency of the major components of the oils [24]. Different studies have revealed that some of these compounds exhibit important antimicrobial properties [21].

While the water steam EO of *O. onites* showed antibacterial activity (3.7-3.4-3.5-3.4 cm/30 μl inhibition zone) against *B. subtillis, S. aureus, P. vulgaris* and *P. aeruginosa* and antifungal activity (3.6–3.5 cm/30 μl inhibition zone) against C. *albicans* and *A. niger*, the cold press EO of *O. onites* showed the higher antibacterial activity (4.5–4.4–4.5–4.2 mm/30 μl inhibition zone) against *B. subtillis, S. aureus, P. vulgaris, P. aeruginosa* and antifungal activity (4.6–3.7 mm/30 μl inhibition zone) against *C. albicans* and *A. niger*.

All identified chemical components of cold press and water steam EOs from *L. officinalis* and *O. onites* are listed in tables 2–3. It was determined that the essential oil obtained from thyme with both hot steam and cold press methods contains these 3 main components in varying proportions as a result of GC-MS analysis (tab. 3) [25]. Oregano is mainly used in food, spices, and pharmaceutical industries. 51–60 components were identified in water steam and cold press EOs obtained from *L. officinalis* Miller, respectively, representing 100%, whereas 58–40 components were identified in the EO obtained from *O. onites* Miller, representing 100%. Cold press *Origanum* oil was characterized by the high content of *p*-cymene (16.16%) and γ-terpinene (14.86%), while water steam *Origanum* oil was characterized by the only main component linalool (17.31%).

In the present study, both oil samples were similar in content, but cold press oil has more variety of compounds. This may be due to the synergistic effect of the ingredients in the oil. A similar result was found in the study of Zengin and Baysal [25].

As can be seen in table 1, the both essential oil of *L. officinalis* exhibited antimicrobial activities, but water steam oil was found to have stronger activity against 14 different pathogenic microorganisms. The cold press essential oil of *L. officinalis* exhibited moderate activity against the microorganisms. In general, weaker activity was exhibited against Gram-negative bacteria, although they were more effective against fungus.

Table 1.

Zone diameters [cm] of inhibition showing the antimicrobial activity of *L. officinalis* and *O. onites* EOs (distilled water steam distillation and cold press) at various concentrations and MIC [mg/ml] values of the samples

Essential oils (EOs)		Microorganisms													
		LM Mean ±SE	*EC* Mean ±SE	*CP* Mean ±SE	*PA* Mean ±SE	*PV* Mean ±SE	*KP* Mean ±SE	*SA* Mean ±SE	*AN* Mean ±SE	*CA* Mean ±SE	*BS* Mean ±SE	*SE* Mean ±SE	*ML* Mean ±SE	*YE* Mean ±SE	*BC* Mean ±SE
Steam distillation *O. onites*															
(1)	10 mg/ml	2.526±0.046	2.120±0.026	2.020±0.026	2.220±0.026	1.950±0.051	2.030±0.026	2.320±0.026	2.430±0.026	2.250±0.026	2.350±0.026	1.850±0.026	1.786±0.005	1.983±0.011	2.230±0.026
	15 mg/ml	2.620±0.026	2.320±0.026	2.220±0.026	2.760±0.026	2.310±0.026	2.400±0.026	2.540±0.026	2.520±0.026	2.520±0.026	2.620±0.026	2.120±0.026	1.960±0.051	2.320±0.026	2.400±0.026
	20 mg/ml	2.920±0.026	2.620±0.026	2.820±0.026	3.020±0.026	2.820±0.026	2.520±0.026	2.920±0.026	3.320±0.026	3.320±0.026	3.520±0.026	3.260±0.026	2.220±0.026	2.650±0.026	2.520±0.026
	30 mg/ml	3.020±0.026	3.220±0.026	3.390±0.026	3.420±0.026	3.500±0.026	3.000±0.026	3.410±0.026	3.670±0.026	3.570±0.026	3.770±0.026	3.470±0.026	2.760±0.026	3.260±0.026	3.000±0.026
O. onites		≤12.5	≤12.5	≤12.5.	≤25.0	≤6.25	≤6.25	≤12.5	≤25.0	≤12.5	≤12.5	≤12.5	≤12.5	≤12.5	≤6.25
Steam distillation *L. officinalis*															
(2)	10 mg/ml	1.600±0.000	2.220±0.026	1.856±0.051	1.986±0.005	1.876±0.051	1.883±0.011	2.420±0.026	2.450±0.026	2.450±0.026	2.250±0.026	1.750±0.026	1.853±0.005	2.050±0.026	1.986±0.005
	15 mg/ml	2.020±0.026	2.330±0.026	2.320±0.026	2.413±0.023	2.230±0.026	2.000±0.026	2.760±0.026	2.620±0.026	2.520±0.026	2.620±0.026	2.220±0.026	2.220±0.026	2.520±0.026	2.413±0.023
	20 mg/ml	2.320±0.026	3.110±0.026	2.720±0.026	2..813±0.023	2.640±0.026	2.520±0.026	3.120±0.026	3.420±0.026	3.320±0.026	3.770±0.026	3.020±0.026	2.450±0.026	3.020±0.026	2.813±0.023
	30 mg/ml	2.720±0.026	3.220±0.026	3.320±0.026	3.313±0.023	3.220±0.026	3.000±0.026	3.560±0.026	3.670±0.026	3.470±0.026	3.840±0.026	3.370±0.026	2.520±0.026	3.370±0.026	3.213±0.023
L. officinalis		≤12.5	≤25.0	≤12.5	≤12.5	≤6.25	≤12.5	≤6.25	≤6.25	≤6.25	≤6.25	≤12.5	≤6.25	≤12.5	≤12.5
Cold press *O. onites*															
	10 mg/ml	2.726±0.044	2.720±0.024	3.020±0.053	2.520±0.063	2.950±0.051	3.330±0.026	2.520±0.021	2.230±0.024	2.750±0.027	2.550±0.025	2.050±0.005	2.286±0.005	2.483±0.011	3.230±0.037
	15 mg/ml	2.920±0.026	2.790±0.023	3.220±0.028	2.960±0.005	3.010±0.025	3.800±0.025	2.940±0.027	2.620±0.016	2.920±0.028	2.820±0.022	2.120±0.023	2.560±0.051	2.820±0.026	3.400±0.022
	20 mg/ml	3.420±0.026	3.720±0.026	3.920±0.022	3.620±0.026	3.920±0.022	4.220±0.026	3.900±0.025	3.120±0.042	3.720±0.021	3.920±0.024	3.460±0.022	3.220±0.026	3.650±0.026	3.820±0.002
	30 mg/ml	3.720±0.023	3.820±0.012	4.390±0.024	4.220±0.025	4.550±0.024	4.700±0.025	4.410±0.032	3.670±0.005	4.570±0.014	4.570±0.025	3.870±0.027	3.760±0.026	4.160±0.026	3.900±0.010
O. onites		≤12.5	≤12.50	≤12.50	≤12.50	≤6.25	≤6.25	≤12.5	≤12.5	≤6.25	≤6.25	≤12.5	≤3.125	≤12.5	≤6.25
Cold press *L. officinalis*															
	10 mg/ml	1.300±0.000	1.220±0.005	1.286±0.051	1.386±0.005	1.176±0.050	1.883±0.011	1.400±0.026	1.430±0.022	1.850±0.020	1.200±0.025	1.250±0.056	1.153±0.005	1.050±0.028	1.186±0.005
	15 mg/ml	1.420±0.002	1.330±0.024	1.420±0.026	1.400±0.023	1.230±0.025	2.000±0.028	1.730±0.026	1.620±0.027	1.920±0.026	1.420±0.004	1.420±0.046	1.210±0.026	1.220±0.026	1.413±0.022
	20 mg/ml	2.020±0.041	1.510±0.026	1.700±0.026	1.600±0.025	1.340±0.026	1.720±0.025	2.100±0.026	2.420±0.026	2.310±0.026	2.070±0.025	2.000±0.026	1.450±0.030	1.520±0.026	1.713±0.023
	30 mg/ml	2.120±0.022	1.720±0.026	1.780±0.026	1.713±0.023	1.420±0.020	2.000±0.023	2.570±0.026	2.670±0.034	2.400±0.026	2.240±0.005	2.170±0.026	1.520±0.000	1.770±0.026	1.913±0.024
L. officinalis		≤12.5	≤50.0	≤50.0	≤25.0	≤12.5	≤25.0	≤12.5	≤12.5	≤12.5	≤12.5	≤12.5	≤6.25	≤25.0	≤12.5
Ampicillin		2.800±0.00	1.900±0.00	4.316±0.028	3.226±0.046	2.900±0.00	1.52±0.010	1.00±00	NT	NT	3.56±0.00	3.540±0.034	0.600±0.00	2.666±0.57	2.650±0.026
Cephazolin		3.313±0.023	1.900±0.00	4.316±0.028	2.833±0.028	0.600±0.00	1.72±0.010	0.600±0.00	NT	NT	3.826±0.109	3.516±0.040	3.573±0.023	3.433c±0.57	2.820±0.026
Nystatin		NT	NT	NT	NT	NT	NT	NT	1.700±0.00	1.700±0.00	NT	NT	NT	NT	NT
Ampicillin		≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	NT	NT	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125
Cephazolin		≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125	NT	NT	≤3.125	≤3.125	≤3.125	≤3.125	≤3.125
Nystatin		NT	NT	NT	NT	NT	NT	NT	≤25.0	≤12.5	NT	NT	NT	NT	NT
Solvents		–	–	–	–	–	–	–	–		–	–	–	–	–

Average values are expressed as mean of triplicates ±SD (standart deviation). NT, not tested – no, PA: *Pseudomonas aeruginosa*; PV: *Proteus vulgaris*; EC: *Escherichia coli,* KP: *Klebsiella pneumoniae,* LM: *Listeria monocytogenes;* CP: *Clostridium perfringens*, SE: *Salmonella enterica*, BS: *Bacillus subtilis;* ML: *Micrococcus luteus*; SA: *Staphylococcus aureus*; YE: *Yersinia enterocolitica*; BC: *Bacillus cereus*; CA: *Candida albicans;* AN: *Aspergillus niger*

Table 2.

Main chemical constituents of the essential oils (distilled water steam method and cold press) of the *L. officinalis* identified by GC/MS analysis and their retention indices

Lavandula officinalis (water steam method)					*Lavandula officinalis* (cold press)				
No	Ret. Time	Area [%]	Height [%]	Name	No	Ret. Time	Area [%]	Height [%]	Name
1	5.474	0.11	0.26	pentanol <1-methyl->	1	1.291	0.13	0.20	amyl methyl ketone
2	7.110	0.08	0.23	hexanol <n->	2	1.408	0.15	0.36	piruvate <ethyl->
3	9.167	0.19	0.38	pinene <α->	3	1.684	0.27	0.28	isoamyl alcohol
4	9.314	0.19	0.50	pinene <α->	4	1.782	0.15	0.32	adipic ketone
5	9.714	0.23	0.43	camphene	5	1.838	0.26	0.40	acetoin
6	9.849	0.22	0.55	camphene	6	9.111	0.06	0.07	thujene <β->
7	10.827	0.14	0.24	pinene <β->	7	9.344	3.15	3.14	pinene <α->
8	10.931	0.10	0.27	pinene <β->	8	9.875	0.09	0.09	camphene
9	11.201	0.08	0.23	vinyl amyl carbinol	9	10.863	0.66	0.83	sabinene
10	11.295	0.69	1.20	amyl ethyl ketone	10	10.953	0.65	0.70	pinene <β->
11	11.435	1.00	1.89	amyl ethyl ketone	11	11.449	3.95	4.56	hept-5-en-2-one <6-methyl->
12	11.491	0.77	1.66	**myrcene**	12	11.607	2.52	2.37	myrcene
13	11.614	1.79	3.48	**myrcene**	13	12.053	0.11	0.14	phellandrene <α->
14	11.710	0.18	0.25	butanoate <butyl->	14	12.277	0.08	0.09	carene <δ-3->
15	11.810	0.14	0.30	hexanol <ethyl->	15	12.543	0.13	0.16	terpinene <α->
16	12.412	0.89	1.34	ethanoate <hexyl->	16	12.872	2.82	2.90	cymene <p->
17	12.531	1.17	2.49	ethanoate <hexyl->	17	13.083	8.64	7.95	**limonene**
18	12.781	1.02	1.62	cymene <p->	18	13.143	5.88	9.18	**eucalyptol**
19	12.898	2.58	3.57	cymene <p->	19	13.431	0.39	0.63	pinene <α->
20	12.955	0.45	1.91	**limonene**	20	13.834	0.86	1.08	ocimene <(E)-, β->
21	13.048	3.54	5.25	**eucalyptol**	21	14.214	0.37	0.40	terpinene <γ->
22	13.140	2.34	4.83	**eucalyptol**	22	14.783	0.06	0.05	linalool oxide <*cis*->
23	13.361	0.89	1.81	pinene <α->	23	15.383	0.41	0.38	terpinolene
24	13.432	0.86	2.20	pinene <α->	24	16.004	18.50	12.09	**linalool**
25	13.785	1.91	3.30	ocimene <(E)-, β->	25	16.094	0.11	0.17	furan <2-acetyl-, 5-methyl->
26	13.848	1.21	3.39	ocimene <(E)-, β->	26	17.025	0.05	0.06	terpinolene
27	14.140	0.26	0.69	terpinene <γ->	27	17.189	0.26	0.44	dihydrolinalool
28	14.210	0.84	1.88	terpinene <γ->	28	17.594	6.59	7.49	**camphor**
29	15.345	0.44	0.68	terpinolene	29	18.572	0.04	0.06	benzoate <ethyl->
30	16.160	18.12	7.39	**linalool**	30	18.843	1.56	2.46	terpinen-4-ol
31	16.268	6.58	7.70	**linalool**	31	18.977	0.05	0.04	azanaphthalene <1->
32	16.426	0.40	1.04	octene <3-acetoxy->	32	19.344	0.06	0.08	terpineol <α->
33	16.861	0.31	0.61	isopulegyl acetate	33	19.449	0.06	0.09	**camphor**
34	17.024	0.34	0.53	hydrocinnamaldehyde	34	20.450	0.10	0.15	fenchyl acetate <endo->
35	17.656	4.86	4.99	**camphor**	35	20.779	0.04	0.05	geranyl formate
36	17.740	0.33	0.70	isobutyrate <hexyl->	36	21.049	0.14	0.23	isopulegyl acetate
37	17.846	1.05	2.21	**linalool**	37	21.486	0.08	0.12	geranyl formate
38	18.445	2.35	3.43	isoborneol	38	21.899	22.01	15.34	**linalyl acetate**
39	18.832	0.35	0.76	terpinen-4-ol	39	22.412	0.50	0.84	dihydrolinalool
40	19.133	0.10	0.16	furan <2,5-dimethyl->	40	22.711	1.02	1.70	dihydrocarvyl acetate

Table 2. (*continued*)

Lavandula officinalis (water steam method)					*Lavandula officinalis* (cold press)				
No	Ret. Time	Area [%]	Height [%]	Name	No	Ret. Time	Area [%]	Height [%]	Name
41	19.406	2.26	3.38	butyrate <hexyl->	41	22.870	1.43	2.07	terpinyl acetate <α->
42	19.560	0.13	0.17	furan <2,5-dimethyl->	42	23.365	0.20	0.30	terpineol <γ->
43	19.792	0.10	0.17	ısoborneol	43	23.964	0.06	0.10	terpinyl acetate <α->
44	20.118	0.16	0.33	terpinen-4-ol	44	24.517	0.46	0.73	dihydrocarvyl acetate
45	20.698	0.09	0.19	bornyl acetate	45	25.166	8.57	8.61	terpinyl acetate <α->
46	21.045	0.26	0.36	butanoate <hexyl-, 3-methyl->	46	25.224	2.16	4.89	terpineol <γ->
47	21.235	0.17	0.18	butanoate <hexyl-, 3-methyl->	47	25.575	0.37	0.63	neryl acetate
48	22.004	16.75	7.53	**linalyl acetate**	48	26.230	0.81	1.33	geranyl acetate
49	22.440	0.15	0.30	**linalyl acetate**	49	27.542	0.22	0.33	himachalene <α->
50	23.018	1.57	1.37	lavandulyl acetate	50	27.989	0.32	0.35	coumarin
51	23.908	14.63	3.38	**carvacrol**	51	33.069	2.44	2.97	phthalate <diethyl->
52	26.241	0.58	0.96	geranyl acetate					
53	27.559	1.31	2.43	himachalene <α->					
54	27.790	0.12	0.20	aromadendrene					
55	28.165	0.08	0.10	aromadendrene					
56	28.665	0.36	0.76	farnesene <(e)-, β->					
57	30.377	0.65	1.02	bisabolene <β->					
58	32.440	0.65	0.25	phthalate <diethyl->					
59	32.770	0.45	0.29	phthalate <diethyl->					
60	52.407	0.43	0.28	farnesol <*cis, cis*->					

Table 3.

Main chemical constituents of the essential oils (distilled water steam method and cold press) of the *O. onites* identified by GC/MS analysis and their retention indices

Origanum onites (water steam method)					*Origanum onites* (cold press)				
No	Ret. Time	Area [%]	Height [%]	Name	No	Ret. Time	Area [%]	Height [%]	Name
1	8.970	0.26	0.68	thujene <α->	1	1.364	0.12	0.47	piruvate <ethyl->
2	9.099	0.31	0.94	thujene <α->	2	1.414	0.22	0.65	piruvate <ethyl->
3	9.221	1.25	3.08	pinene <α->	3	1.439	0.12	0.41	propylene glycol
4	9.348	1.64	4.54	pinene <α->	4	1.750	0.16	0.26	acetoin
5	9.752	0.36	1.02	camphene	5	1.820	0.25	0.37	acetoin
6	9.865	0.54	1.67	camphene	6	1.889	0.11	0.33	pyrazole <1H, 3-methyl->
7	10.845	0.15	0.38	pinene <β->	7	2.288	0.05	0.12	nona-2(E),6(E)-dienal
8	10.951	0.21	0.65	pinene <β->	8	2.869	0.06	0.17	sorbic aldehyde
9	11.060	0.27	0.44	vinyl amyl carbinol	9	2.918	0.21	0.41	sorbic aldehyde
10	11.279	0.44	1.22	vinyl amyl carbinol	10	4.402	0.08	0.09	ısovalerate <ethyl->
11	11.608	**4.15**	6.53	**myrcene**	11	5.906	0.11	0.12	furfural
12	11.724	**3.63**	7.04	**myrcene**	12	6.532	0.25	0.34	hex-2(E)-enal
13	12.009	0.36	0.97	phellandrene <α->	13	6.692	0.05	0.05	hex-3(Z)-enyl formate>
14	12.109	0.69	1.94	phellandrene < α->	14	8.894	0.06	0.09	tricyclene
15	12.215	0.15	0.34	carene <δ-3->	15	9.123	2.10	3.33	thujene <α->
16	12.326	0.24	0.73	carene <δ-3->	16	9.358	2.91	4.68	pinene <α->

Table 3. (*continued*)

Origanum onites (water steam method)					*Origanum onites* (cold press)				
No	Ret. Time	Area [%]	Height [%]	Name	No	Ret. Time	Area [%]	Height [%]	Name
17	12.535	**2.45**	4.29	**terpinene < α ->**	17	9.887	1.25	2.00	camphene
18	12.693	**3.42**	5.59	**terpinene <α->**	18	10.335	0.08	0.09	benzaldehyde
19	13.120	**10.92**	5.93	**cymene <*p*->**	19	10.972	0.63	1.04	pinene <β->
20	13.203	1.83	6.36	pseudolimonene	20	11.167	0.23	0.31	vinyl amyl carbinol
21	13.469	0.42	1.06	pinene < α ->	21	11.641	**6.09**	8.70	**myrcene**
22	13.900	0.66	1.03	ocimene <(E)-, β->	22	12.077	0.77	1.28	phellandrene <α->
23	14.449	**9.86**	7.55	**terpinene <γ->**	23	12.304	0.37	0.58	carene <δ-3->
24	15.406	1.27	3.02	terpinolene	24	12.583	**4.59**	6.68	**terpinene <α->**
25	16.248	**17.31**	8.85	**linalool**	25	12.970	**16.16**	13.29	**cymene <*p*->**
26	18.418	0.97	1.34	ısoborneol	26	13.078	1.91	3.72	ocimene <(Z)-, β->
27	18.880	2.23	2.85	terpinen-4-ol	27	13.155	0.18	0.28	eucalyptol
28	19.460	0.46	0.42	salicylate <methyl->	28	13.255	0.07	0.06	benzyl alcohol
29	22.735	0.27	0.29	carvone	29	13.450	0.30	0.40	pinene <α->
30	23.189	1.76	1.18	**carvacrol**	30	13.856	0.37	0.46	ocimene <(E)-, β->
31	24.218	**24.66**	6.07	**carvacrol**	31	14.330	**14.86**	14.49	**terpinene <γ->**
32	26.003	0.36	0.57	**carvacrol**	32	14.559	0.08	0.15	sabinene hydrate <*trans*->
33	27.655	2.00	4.44	himachalene <α->	33	14.792	0.05	0.08	linalool oxide <*cis*->
34	28.085	0.17	0.40	bergamotene <α-*trans*->	34	15.396	0.93	1.31	terpinolene
35	30.496	2.02	4.14	bisabolene <β->	35	15.982	**13.70**	11.41	**linalool**
36	30.685	0.16	0.39	cadinene <γ->	36	16.073	0.05	0.07	hex-3(Z)-enyl butyrate
37	32.466	0.87	0.70	phthalate <diethyl->	37	18.384	0.65	0.78	ısoborneol
38	32.660	0.41	0.49	phthalate <diethyl->	38	18.830	0.51	0.64	terpinen-4-ol
39	32.935	0.50	0.42	phthalate <diethyl->	39	19.341	0.07	0.09	**terpineol <α->**
40	52.464	0.37	0.45	farnesol <*cis, cis*->	40	19.454	0.14	0.17	**terpineol <α->**
					41	21.320	0.19	0.34	ısoeugenyl phenylacetate
					42	21.501	0.08	0.11	carvone
					43	21.883	0.09	0.11	nerol
					44	22.858	0.06	0.07	**carvacrol**
					45	23.076	0.58	0.63	**carvacrol**
					46	23.632	23.82	11.99	**carvacrol**
					47	24.154	0.05	0.06	eugenol
					48	25.882	0.07	0.09	**carvacrol**
					49	26.063	0.05	0.08	copaene <α->
					50	26.383	0.06	0.09	bourbonene <β->
					51	27.579	2.41	3.81	himachalene <α->
					52	28.051	0.07	0.11	bergamotene <α-*trans*->
					53	28.201	0.26	0.39	aromadendrene
					54	28.680	0.11	0.18	humulene <α->
					55	30.032	0.16	0.24	viridiflorene
					56	30.407	0.89	1.49	bisabolene <β->
					57	30.619	0.07	0.11	cadinene <γ->
					58	30.895	0.08	0.13	cadinene <δ->

Table 4.
Antioxidant activities of essential oil extracts at 2% concentrations

Essential oil samples	DPPH [% Inhibition]		FRAP [μmol TX/g oil]		ABTS [μmol TX/g oil]	
	cold	steam	cold	steam	cold	steam
Origanum onites	89.23	73.37	4947.27	177.67	2912.4	119.74
Lavandula officinalis	2.48	–	15.12	–	22.62	5.302

While the water steam EO of *L. officinalis* strong antibacterial activity (3.8–3.5–3.2–3.3cm/30 μl inhibition zone) against *B. subtillis, S. aureus, P. vulgaris,* and *P. aeruginosa*, and antifungal activity (3.6-3.4 cm/ 30 μl inhibition zone) against *C. albicans* and *A. niger*, the cold press EO of *L. officinalis* showed the moderate antibacterial activity (2.2–2.5–1.4–1.7 cm/30 μl inhibition zone) against *B. subtillis, S. aureus, P. vulgaris, P. aeruginosa* and antifungal activity (2.6–2.4 mm/ 30 μl inhibition zone) against *C. albicans* and *A. niger.* Distinct terpenoid components of EOs are able to interact to either decrease or increase of antimicrobial influences [26]. Since there is a big gap in the literature concerning this subject, the relationship between the antimicrobial activities of these EOs on bacterial and fungal species and the structure was evaluated by SEM technique.

Evaluation results of MIC of the essential oils obtained from *O. onites* by means of the agar dilution method are also reported in table 1. While the MIC values of cold press EO from *O. onites* on the tested microorganisms vary between ≤3.125 and ≤12.5 mg/ml, these values for water steam EO from *O. onites* vary between ≤6.25 and ≤25 mg/ml. In other words, the antimicrobial efficacy of cold press essential oil sample obtained from *O. onites* is much more striking. On the contrary, it was determined that the amount of essential oil sample obtained from *L. officinalis* with the cold press technique, varying from ≤ 6.25mg/ml to ≤50 mg/ml, can only inhibit the microorganisms tested, while the oil obtained by the water steam technique from the same plant were sufficient to inhibit the organisms at smaller amounts (≤6.25 – ≤12.5 mg/ml).

As can be seen from table 2, the main components in water steam EO from *L. officinalis* oil were eucalyptol (5.86%), linalool (25.75%) camphor (4.86%) linalyl acetate (16.90%) and carvacrol (14.63%).

Although *L. officinalis*' cold press EO has almost the same basic components, their ratios vary: limonene (8.64%), eucalyptol (5.88%), linalool (18.50%), camphor (6.65%), linalyl acetate (22.01%) and terpinyl acetate <α> (10.06). The lower antimicrobial activity of the cold press EO from *L. officinalis* compared to the water steam EO can be attributed to the absence of carvacrol.

According to all three methods, the calculated antioxidant activity values of thyme were higher than those of lavender. On the other hand, in case of the essential oil sample obtained from *O. onites* by using cold press method, observed antioxidative activity values were higher than those obtained by the water steam method. This difference as more than 20 times is particularly striking in FRAP and ABTS tests. It has already known that thymol is a main constituent of thyme having a great impact on the ABTS method [27]. On the other hand, thymol, carvacrol and terpinene are known as prominent components in the DPPH test [27]. Interestingly, all of these mentioned substances were detected in samples by GC-MS analysis. Therefore, it is not really entirely true to attribute the antioxidant activity to one or a few active compounds. All minor and major constituents should be considered together. The antioxidant activity of the oil sample obtained by the cold press method is significantly higher in the case of FRAP and ABTS tests than those obtained by the steam method [28].

For SEM analyzes, essential oil samples were treated with *S. aureus* representing Gram(+) and *E. coli* representing Gram(–) bacteria among microorganisms for 24 hours. At the end of this period, the resulting morphological changes in the outlook of the cells were observed by SEM. SEM images revealed distinctions between essential oil-treated and untreated bacterial cell structures. Non-treated cells were intact in regular rod or coccal shaped and had smooth surfaces as can be seen in figures 1A-2A, while bacterial cells treated with essential oil underwent significant structural changes that were clearly noticeable in the figures 1B–C and 2B–C. In other words, the morphological changes observed with SEM studies revealed that essential oil samples can damage the structural integrity of both Gram(+) and Gram(–) cells.

In figure 1, it is seen that the essential oil treatment creates pores in the outer membrane of *E. coli* ATCC®25922 and *S. aureus* ATCC®6538 cells, and

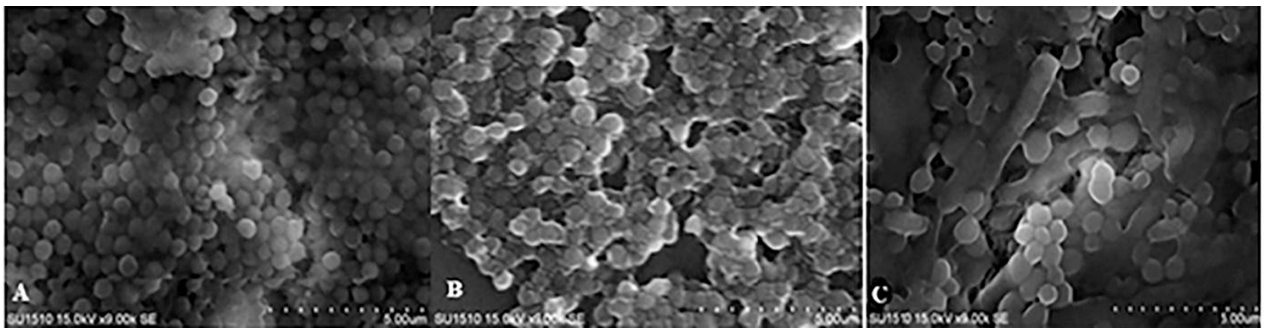

Figure 1.

SEM images of untreated *S. aureus* ATCC 6538 cells (**A**) treated bacterial cells with *O. onites* (**B**) and *L. officinalis* (**C**)

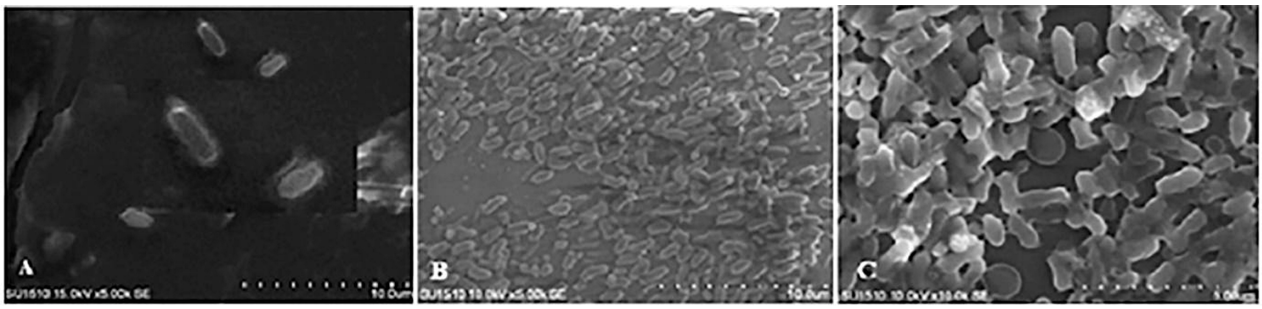

Figure 2.

SEM images of untreated *E. coli* ATCC®25922 cells (**A**) treated bacterial cells with *O. onites* (**B**) and *L. officinalis* (**C**)

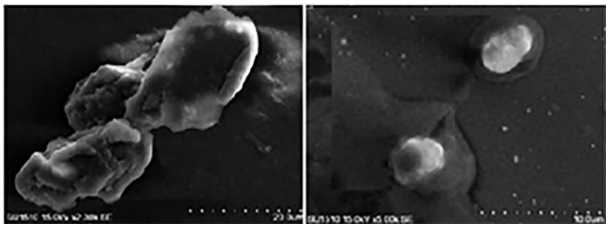

Figure 3.

SEM images of bacterial cells surrounded by essential oil**:** *E. coli* ATCC®25922 with *O. onites* (**A**); *S. aureus* ATCC®6538 with *L. officinalis* (**B)**

cellular components can be easily expelled through these pores, and also wrinkling occurs in the cells. It is thought that the outer membrane or cell wall of the bacteria could most likely be a cellular target for essential oil-containing substances. Moreover, the difference in cell wall composition of different bacterial species is partly responsible for their varying sensitivity to essential oils. Combinations of essential oil such as eucalyptol, carvacrol and linalool terpene components caused permeability change of the outer membrane, alteration of cell membrane function, and seepage of intracellular materials. This was also supported by our conclusions from the cell constituent affranchise tests. There are plenty of probable clarifications of the observation. Some scientists have proposed that the injury to the cell wall and cell membrane was the bereavement of structure entirety [29]. Bacteria cells treated with components at oil densities revealed severe counterproductive impact on the cell morphology of the tested pathogens, showing large surface decadence and aberrant cell breakage, as well as complete lysis of dead cell formation (fig. 1-3). Combined essential oil treatments changed outer membrane and the construction of the cells and made them more pervious.

When all data are evaluated, it is revealed that essential oils, which are the main components of plant-derived compounds, can interact with each other and with bacterial cells to increase or decrease each other's antimicrobial activity. Therefore, interactions between essential components lead to contribution or synergistic influences. Thus, it is significant to research both single constituents and combinations in studies of the antibacterial effect of plant-derived compounds. In addition, depending on the presence

of these components and their possible synergistic effects, the antioxidant activities of the essential oil samples tested also differ. In both plant cases, although the antioxidant activity of the oil sample obtained by cold press is significantly higher, *O. onites* essential oils are much richer in terms of antioxidant activity.

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