



- RESEARCH ARTICLE -

## Antibacterial Actions and Potential Phototoxic Effects of Volatile oils of *Foeniculum* sp. (fennel), *Salvia* sp. (sage), *Vitis* sp. (grape), *Lavandula* sp. (lavender)

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### Abstract

In the present study, the volatile compounds of essential oil of *Foeniculum vulgare* (fennel), *Salvia officinalis* (sage), *Vitis vinifera* (grape), *Lavandula angustifolia* (lavender) were analysed by gas chromatography-mass spectrometry (GC-MS) using the Nist and Willey libraries. It was determined that the main components of *Foeniculum* sp. were anethole (41.11%), carvacrol (9.18%). whereas main components of *Salvia* sp were 1.8 cineole (34.09%), caryophyllene (10.95%), camphor (9.44%),  $\alpha$ -pinene (8.42%). *Vitis* sp. contained linoleic acid (36.98%), 2,4-decadienal (30.79%). Finally, volatile component of *Lavandula* sp. was linalool (33.57%), linalyl acetate (30.74%). Phototoxic antibacterial activity of volatile oil of those plants against *Escherichia coli* (ATCC 25293), *Klebsiella pneumoniae* (10031), *Salmonella thymurium*, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925), *Enterococcus faecalis* (ATCC 29212) were examined by using disc diffusion method. We demonstrated that volatile oil effectively can be activated by a standard LED light. *In vitro*, significant phototoxicity was demonstrated by volatile oil of *Foeniculum* sp. and *Vitis* sp. ( $P < 0.05$ ), while minor phototoxicity was induced by *Lavandula* sp. Therefore, volatile oil of plant can be considered as a potential photosensitizer in the photochemical therapy.

### Keywords:

*Foeniculum vulgare*, *Salvia officinalis*, *Vitis vinifera*, *Lavandula angustifolia*, photoactivated volatile oil, antimicrobial action

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## Introduction

*Foeniculum vulgare* (fennel), *Salvia officinalis* (sage), *Vitis vinifera* (grape), *Lavandula angustifolia* (lavender) are natural floristic plants of Turkey and quite important medicinal and aromatic plants due to their strong biological properties (Marotti et al., 1994; Wu et al., 2012; Vislocky & Fernandez, 2010; Hongratanaworakit, 2011). Especially, their essential oils have an important role in traditional usage as antibacterials, antifungals, antivirals, and insecticides (Bakkali et al., 2008). They are terpenes and oxygenated compounds that contribute to pharmacological effects (Prabuseenivasan et al., 2006). *Foeniculum vulgare* (Mill) belongs to family Apiaceae, cultivated in some countries such as India, China and Egypt. In our country, it grows in especially in west and south regions (Ozcan et al., 2007; Diao et al., 2014). The volatile oil of fennel was reported anti-spasmodic, anti-allergic, diuretic, anti-inflammatory, analgesic and antioxidant, antimicrobial properties (Ebeed et al., 2010; Kooti et al., 2015). *Salvia*, a member of Lamiaceae, is widespread in Mediterranean and employed in traditional recipes and comprises more than 1000 species distributed in many regions of the world (Erdemoglu et al., 2006; Kamatou et al., 2008). Many *Salvia* species as *Salvia officinalis* have commercial important and their essential oils were used in traditional medicine and aromatherapy due to many active substances (Walch et al., 2011). Genus *Vitis* were known that have approximately 14,000 cultivars and its different extracts have used in conditions like skin diseases, burning sensations, asthmas hemorrhages, and bronchitis anemia in folk medicine. Anatolia was known as the origin of viticulture, especially for *Vitis vinifera*, in eastern and southeastern regions of Turkey (Parekh & Chanda, 2006; Ergul et al., 2011). Finally, native to a large part of the Mediterranean region, the *Lavandula* genus, belongs to Lamiaceae, is principally cultivated worldwide for its essential oils, especially in aromatherapy products. The genus have approximately 20 species and the most popular are *Lavandula angustifolia*, *Lavandula latifolia* and *Lavandula intermedia*. (Lis-Balchin, 1999, Torras-Claveria et al., 2007).

Natural products have therapeutic and antimicrobial properties which were published a great amount of data (Lopez et al., 2001). Especially, many studies about antimicrobial action of volatile oil of plants have critical important in pharmacology. Thus, numerous studies were reported to determine antibacterial activities of plant essential oils against pathogen bacteria. However, there is a lack of studies performed to determine antibacterial performance in presence of photo activated oils. Because, the components of essential oil are highly affected by environmental factors (Tip-pyang et al., 2000; Rahimmalek et al., 2009). Thus, our paper have presented a comparison research about volatile oils of fennel, sage, grape, lavender and an antibacterial study in relation to light and dark treatments.

## Material and Method

**Plant material:** Aerial parts of *Foeniculum vulgare* Mill, *Salvia officinalis* L., *Vitis vinifera* L., *Lavandula angustifolia* L. were collected in Mersin-Adana (Turkey), 2015. The botanical identification of the plants were done according to Davis's book (Davis et al., 1988). Dried aerial parts of each plant (100 g) were subjected to hydro distillation for 3 h according to the standard procedure described in the *European Pharmacopoeia*. (Council of Europe, 2004). The oils were solubilized in *n*-hexane, dried over anhydrous sodium sulfate and N<sub>2</sub> to remove *n*-hexane. Samples were stored at +4°C in the dark until tested and analyzed.

**GC/MS analysis:** The GC/MS analysis was performed on an Agilent 7890A; fitted with an apolar HP-5MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.33 µm film thickness);

Column temperature: 60°C, with 20 min initial hold, and then to 240°C at 20°C/min, and then to 300°C (20 min); injection mode Split 1/50. Analysis was coupled to an Agilent Mass Selective Detector MSD 5975; ionization energy voltage 70 eV. Mass spectra were scanned in the range 30–550 amu. Most constituents were identified by gas chromatography-mass spectra by comparison of their Kovats retention indices (Ri) by comparison of the mass spectra on both columns with either those stored in NIST 02 and Wiley 275 libraries or with those from the literature. The Kovats retention indices were determined in relation to a homologous series of *n*-alkanes (C5–C40), under the same operating conditions.

### Antibacterial and Phototoxic Activity screening

The bacteria used for antibacterial and phototoxic activity screening; *Escherichia coli* (ATCC 25293), *Klebsiella pneumoniae* (10031), *Salmonella thyphimurium*, *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25925), *Enterococcus faecalis* (ATCC 29212). The bacteria inoculum was prepared in 4 ml-Tryptic Soy Broth medium and incubated at 37°C, overnight. Then, the culture suspensions were adjusted to 0.5 McFarland Standard Turbidity (~10<sup>4</sup> for bacteria CFU per milliliter) (McFarland, 1987) and prepared daily and stored at +4 °C until use.

The Micro broth dilution technique was employed to compare the antimicrobial activities of the volatile oils. This method was used to determine the minimum inhibitory concentration (MIC; lowest concentration causing complete visible growth inhibition), Minimal Bactericidal Concentration (MBC; lowest concentration killing 99.9% cells) according to guideline of the National Committee for Clinical Laboratory Standards (CLSI, 2014; Dabur et al., 2007). For the antimicrobial activity experiment; volatile oil of each plant was dissolved at 4 mg/ml with dimethyl sulfoxide (DMSO; 15%). A 100 µl portion of the volatile oil samples was poured into the first order wells of a 96-well microplate, which 100 µl portion of the Mueller Hinton Broth (MHB) solution had already been poured. Then, two-fold serial dilutions of volatile oils were performed and final diluted concentration of master was 0.031 mg/ml. Finally, 5 µl of each microorganisms was added into the well. For the negative control, MHB containing DMSO (15 %) and essential oil without microorganisms were used. For the positive control, MHB with 15%DMSO but no oil, was used. All of the plates were incubated at 37 °C for 12 hours, the growth (turbidity) was measured at 600 nm and three replicates were done for each plant oil. Then, 5µl culture of all wells was inoculated on MHB agar plates for 12 hours at 37°C for determine MBC (Fabio et al., 2007; Zora; et al., 2010).

The agar disc diffusion method was employed to compare the phototoxic antimicrobial activities of the volatile oil in dark and light which is a standard white LED source. When experiment performed, a certain amount of bacteria dilution was spread on a solid agar medium in Petri dishes (Nutrient agar). Filter paper discs (6 mm in diameter) were previously soaked in 5 µL of the essential oil and placed on the bacteria plates and then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeters (Tepe et al., 2004, Bouhadjera et al., 2005). For the positive control, Streptomycin (Sigma P, 1 µL) was applied to the discs from stock solution (1 mg/mL). The experiments were performed as triplicate in light and dark treatment. Sterile distilled water was used as negative control. Data on the inhibitory effects of oils on bacteria was analyzed by variance analysis (ANOVA) using Mann-Whitney U. The separation of means was done by using the Least Significant Difference (LSD) test at P<0.05.

## Results

The compositions of volatile oils from *Foeniculum vulgare* (fennel), *Salvia officinalis* (sage), *Vitis vinifera* (grape), *Lavandula angustifolia* (lavender) were determined by comparing the relative retention times and mass spectra from data library (Table 1). In literature, the essential oils were characterized by the dominant presence of one, two or three components in.

**Table 1.** Chemical composition of the volatile oils from *F. vulgare*, *S. officinalis*, *V. vinifera* and *L. angustifolia*. RI: Kovats retention index on HP-5MS column; relative peak area (%): more than 0.05.

Compound	<i>F. vulgare</i>		<i>S. officinalis</i>		<i>V. vinifera</i>		<i>L. angustifolia</i>	
	%	RI	%	RI	%	RI	%	RI
$\gamma$ terminene	-	-	0,25	930	-	-	-	-
$\alpha$ pinene	0,84	975	8,42	937	-	-	0,29	945
Camphene	-	-	3,32	952	-	-	0,31	947
3-octanone	-	-	-	-	-	-	0,90	956
$\beta$ phellandrene	0,11	958	-	-	-	-	-	-
Thujene	0,07	963	-	-	-	-	-	-
$\beta$ -Myrcene	-	-	-	-	-	-	0,72	966
$\beta$ pinene	0,24	995	4,10	971	-	-	-	-
n-Hexyl acetate	-	-	-	-	-	-	1,03	1001
delta-3-carene	0,12	1002	0,08	1002	-	-	1,40	1003
o-cymene	2,36	1003	-	-	-	-	-	-
Limonene	4,97	1004	-	-	-	-	-	-
1,8-Cineole	0,34	1005	34,09	1005	0,78	1004	5,15	1004
$\beta$ -ocimene	-	-	-	-	-	-	1,05	1005
Linalool Oxide	0,21	1008	-	-	-	-	-	-
Trans linalool oxide	0,17	1010	-	-	-	-	0,35	1008
Sabinene hydrate	-	-	0,07	1008	-	-	-	-
Linalool	6,11	1011	0,93	1010	-	-	33,57	1014
$\alpha$ -Pinene oxide	-	-	0,05	1012	-	-	-	-
$\alpha$ -terpinolene	-	-	-	-	-	-	0,24	1013
$\alpha$ -Thujone	-	-	0,48	1013	-	-	-	-
$\beta$ Thujene	-	-	0,89	1014	-	-	-	-
Propanoic acid	-	-	-	-	-	-	0,27	1015
Camphor	0,04	1017	9,44	1017	-	-	6,54	1017
2,8-Menthadien-1-ol	0,20	1015	-	-	-	-	-	-
p-Menthanone	0,26	1018	-	-	-	-	-	-
Borneol	0,15	1019	5,84	1019	-	-	3,70	1019
n-Hexyl butyrate	-	-	-	-	-	-	0,47	1020
$\alpha$ -terpinol	-	-	3,76	1200	-	-	1,77	1200
4-Terpinenol	0,34	1200	-	-	-	-	-	-
B Frenchyl alcohol	0,26	1201	-	-	-	-	-	-
2,4-Decadienal-(E,E)-	-	-	-	-	30,79	1202	-	-
Estragole	3,19	1202	-	-	-	-	-	-
2-Pinen-4-one	-	-	0,09	1203	-	-	-	-

Dihydrocarvone	0,14	1203	-	-	-	-	-	-
Trans-Carveol	0,34	1204	-	-	-	-	-	-
Fenchyl acetate	0,34	1205	-	-	-	-	-	-
Linalyl acetate	-	-	0,19	1206	-	-	30,74	1207
Lavandulyl acetate	-	-	-	-	-	-	1,95	1210
1- carvone	6,53	1207	-	-	-	-	-	-
Aubepine	5,13	1208	-	-	-	-	-	-
Thyme camphor	-	-	0,13	1211	-	-	-	-
Anethole (anise camphor)	41,11	1220	-	-	-	-	-	-
Carvacrol	9,18	1226	0,09	1213	-	-	-	-
Hexyl tiglate	-	-	-	-	-	-	0,47	1214
Neryl acetate	-	-	-	-	-	-	0,50	1217
$\alpha$ -Terpinene	-	-	0,30	1217	-	-	-	-
$\alpha$ cubebene	-	-	0,04	1218	-	-	-	-
m-Mentha-1,8-diene	0,10	1271	-	-	-	-	-	-
E-ocimenone	0,10	1275	-	-	-	-	-	-
$\alpha$ -Copaene	-	-	0,39	1400	-	-	-	-
2-propanone	0,72	1400	-	-	-	-	-	-
$\alpha$ -Bergamotene	-	-	-	-	-	-	0,19	1400
Eugenol methyl ether	-	-	0,15	1401	-	-	-	-
Zingiberene	-	-	-	-	-	-	0,18	1402
Methyleugenol	0,23	1402	-	-	-	-	-	-
Iso caryophyllene	-	-	0,12	1404	-	-	-	-
(+)-Aromadendrene	0,09	1413	0,08	1405	-	-	-	-
Caryophyllene	0,14	1406	10,95	1406	-	-	1,69	1406
1H-Cycloprop[e]azulene	-	-	0,24	1408	-	-	-	-
$\beta$ caryophyllene	-	-	0,22	1409	-	-	-	-
$\beta$ -Selinene	-	-	2,41	1410	-	-	-	-
Naphthalene	-	-	0,54	1411	-	-	-	-
trans-Caryophyllene	-	-	0,06	1412	-	-	-	-
$\beta$ -Bisabolene	-	-	0,62	1453	-	-	-	-
trans- $\beta$ -Farnesene	-	-	-	-	-	-	0,88	1470
Germacrene B	-	-	0,05	1414	-	-	0,56	1481
delta.-Cadinene	-	-	0,73	1415	-	-	-	-
$\alpha$ -Calacorene	-	-	0,06	1418	-	-	-	-
(-)-Caryophyllene oxide	-	-	0,35	1420	-	-	-	-
Dillapiole	0,25	1600	-	-	-	-	-	-
Caryophyllene oxide	-	-	3,40	1600	-	-	0,43	1603
$\alpha$ -Bisabolol	-	-	-	-	-	-	0,79	1616
(-)-Kaurene	-	-	0,08	1801	-	-	-	-
Methyl palmitate	-	-	-	-	6,90	1806	-	-
Oleic acid, methyl ester	-	-	-	-	5,47	2001	-	-
Ethyl palmitate	0,10	2000	-	-	-	-	-	-
Ethyl linoleate	0,30	2001	-	-	-	-	-	-
Ethyl Oleate	0,17	2002	-	-	-	-	-	-

Linoleic acid	-	-	-	-	39,43	2004	-	-
Linoleic acid ethyl ester	-	-	-	-	4,88	2007	-	-
<b>Total</b>	<b>84,95</b>		<b>93,01</b>		<b>88,25</b>		<b>96,14</b>	

The results of the chemical analyses of the volatile oils of the different plants are presented in Table 1. It was determined that the main components of *F. vulgare* were anethole (41.11%), carvacrol (9.18%), 1-carvone (6.53%), linalool (6.11%), limonene (4.97%), aubepine (5.13%), estragole (3.19%), *o*-cymene (2.36%) whereas main components of *S. officinalis* were 1,8 cineole (34.09%), caryophyllene (10.95%), camphor (9.44%),  $\alpha$ -pinene (8.42%), borneol (5.84%),  $\beta$ -pinene (4.10%),  $\alpha$ -terpineol (3.76%), caryophyllene oxide (3.40%), camphene (3.32%). *V. vinifera* contained linoleic acid (36.98%), 2,4-decadienal (30.79%), methyl palmitate (6.90%), oleic acid-methyl ester (5.47%). Finally, volatile component of *L. angustifolia* were linalool (33.57%), linalyl acetate (30.74%), camphor (6.54%), 1,8 cineole (5.15%), borneol (3.70).

Terpenes were the most abundant volatiles detected in aromatic plants. In our study, we reported grape oil contain relatively narrow chemical profile while the volatile oils of fennel, sage and lavender were rich in diversity of compound. The common component for volatile oils studied were determined as 1, 8-cineole and its relative percentage was %0.34 for fennel; %34.09 for sage; %0.78 for grape; %5.15 for lavender.

The antibacterial activity of essential oils of *F. vulgare* (fennel), *S. officinalis* (sage), *V. vinifera* (grape), *L. angustifolia* (lavender) were showed on Table 2. The volatile oils investigated showed better activity against Gram-positive than Gram-negative bacteria. The antibacterial activity of oils tested in the study can be presented as: Sage>Lavender>Fennel>Grape. Grape oil showed the lowest antibacterial activity in the microdilution method, MIC at 0, 25–1  $\mu$ g/mL and MBC at 2–4  $\mu$ g/mL. Fennel and Lavender oil showed similar antibacterial activity with MIC of 0,062-1  $\mu$ g/mL and MBC of 0, 5–4  $\mu$ g/mL. Sage oil also exhibited the highest antibacterial activity with MIC of 0,062-0, 5  $\mu$ g/mL and MBC of 0, 5–4  $\mu$ g/mL.

**Table 2.** Antimicrobial activity of *F. vulgare*, *S. officinalis*, *V. vinifera* and *L. angustifolia*. volatile oil indicated as inimal Inhibitory Concentrations (MIC; mg/ml); Minimal Bactericidal Concentration (MBC mg/ml) on the table.

	Fennel		Sage		Grape		Lavender	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Escherichia coli</i> G(-)	1	4	0,5	2	1	>4	1	4
<i>Klebsiella pneumoniae</i> G(-)	1	4	0,25	2	0,5	4	0,5	4
<i>Salmonella typhimurium</i> G(-)	0,5	2	0,5	4	1	2	0,5	2
<i>Bacillus subtilis</i> G(+)	0,125	2	0,5	4	0,5	2	0,25	1
<i>Staphylococcus aureus</i> G(+)	0,062	0,5	0,25	2	0,25	2	0,062	0,5
<i>Enterococcus faecalis</i> G(+)	0,25	1	0,0625	0,5	0,5	2	0,5	2

In part of our containing research into medical plants, we investigated their phototoxic antimicrobial activity as well as volatile oil composition of plants. In this study, the diameter of growth inhibition zone (mm) for incubation with LED light was better than incubation in dark against bacteria against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* (Table 3). The maximal inhibition zone of volatile oils in dark incubation conditions was 2, 64 mm of *L. angustifolia* volatile oil for *E. coli*; the lowest was 0,44 mm of *V. vinifera* against *E. faecalis*.

In addition, the maximal inhibition zone of photo activated volatile oils was 4,75 mm of *S. officinalis* against *E. coli*; the lowest was 1,18 mm of *V. vinifera* against *S. aureus*. Strong antibacterial activity in dark treatment was noticed for *F. vulgare* (0,9–1,9 mm), *Salvia officinalis* (1,2–1,9 mm), *V. vinifera* (0,44–2,00 mm) and especially for *L. angustifolia* (2,23–2,64 mm) against bacteria. More strong antibacterial activity was shown for *Foeniculum* sp. (2,1–3,1 mm), *S. officinalis* (2,76–4,75 mm), *V. vinifera* (1,18–3,01 mm) and for *Lavandula* sp. (2,6–4,7 mm) against bacteria in the presence of photoactivated volatile oil. The phototoxic antibacterial activity of volatile oils tested in disc diffusion method can be presented as: *F. vulgare* (dark) < *F. vulgare* (light); *S. officinalis* (dark) < *S. officinalis* (light); *V. vinifera* (dark) < *V. vinifera* (light); *L. angustifolia* (dark) < *L. angustifolia* (light).

**Table 3.** Antibacterial activity of *F. vulgare*, *S. officinalis*, *V. vinifera* and *L. angustifolia* volatile oils (5  $\mu$ L) in disc-diffusion method, inhibition zones in dark or light treatment (mm). Abbreviations: strep: Streptomycin. The same letter means that differences of inhibition zone were statistically significant.

	<i>Fennel</i>		<i>Sage</i>		<i>Grape</i>		<i>Lavender</i>		<i>Strep.</i>	
	dark	light	dark	light	dark	light	dark	light	dark	light
<i>Escherichia coli</i> G(-)	1,9	2,9	1,74 <sup>c</sup>	4,75 <sup>c</sup>	2	3,01	2,64	3,95	2,53	2,86
<i>Klebsiella pneumoniae</i> G(-)	1,2 <sup>a</sup>	3,1 <sup>a</sup>	1,9	3,8	0,76	1,21	2,35	4,7	2,33	2,03
<i>Salmonella typhimurium</i> G(-)	0,9 <sup>b</sup>	2,3 <sup>b</sup>	1,9	3,8	0,5 <sup>e</sup>	1,8 <sup>e</sup>	2,3	4,7	3,69	3,55
<i>Bacillus subtilis</i> G(+)	1,27	2,1	1,2	2,76	0,5 <sup>f</sup>	1,35 <sup>f</sup>	2,38	2,6	2,86	2,66
<i>Staphylococcus aureus</i> G(+)	1,52	2,1	1,9	3,15	0,59	1,18	2,47	2,73	3,88	3,65
<i>Enterococcus faecalis</i> G(+)	1,16	2,49	1,22 <sup>d</sup>	3,77 <sup>d</sup>	0,44 <sup>g</sup>	2,64 <sup>g</sup>	2,23	3,78	2,55	2,99

## Discussion

The components present in the volatile oil obtained from fennel are similar to those reported but the relative percentage of their as anethole and limonene differed (Chowdhury et al., 2009, Raal et al., 2012). While we showed that anethole and carvacrol were the main component of fennel oil, some papers reported that the main components of plant were methyl chavicol and anethole (Marotti et al., 1994) estragole and fenchone (Ozcan et al., 2006), anethole and estragole (Khalid et al., 2015). The chemical nature of volatile oils from *Salvia* species have mostly similar. However, diverse factors such as ecological, physiological conditions and genetic diversity have affected the composition and yield of essential oil of plant (Li et al., 2015; Figueiredo et al., 2008). In our study, *S. officinalis* mainly consisted of 1,8 cineole, caryophyllene, camphor,  $\alpha$ -pinene and borneol. However, the major component of essential oils of some *Salvia* genus were beta-caryophyllene and caryophyllene oxide for *S. hydrangea* and *S. ballotiflora* (Sonboli et al., 2006; Cardenas-Ortega et al., 2015); aromadendrene oxide-(1), cadinol and germacrene D for *S. miltiorrhiza*; Limonene, terpineol and eudesmol for *S. przewalskii* and thujene for *S. officinalis* (Li et al., 2015). *Vitis* species have active components for human diet because of their nutritional value and vital compounds to be protected from disease. Several study have reported focused on the biological properties and identification of aroma chemistry attributable to the cultivar (Lüker et al. 2004; Sanchez-Plomo et al., 2010). We reported the results of volatile oil of grape profile which have 2,4-decadienal, linoleic acid, and kaurene as the major component while grape produce numerous sesquiterpenoid volatiles such as (E)- $\beta$ -caryophyllene, (+)-valencene,  $\alpha$ -humulene, (-)-7-epi- $\alpha$ -selinene and (E,E)- $\alpha$ -farnesene (Buchbauer et al., 1995, Martin et al., 2009). Barbagallo et al. also reported more than 50 components and focused that limonene and cymene were the main component of *Vitis*

*vinifera* (Barbagallo et al., 2014). Lavenders are the most valuable essential-oil bearing crops and widely used for both traditional medicine and aromatic purposes in cosmetics and pharmacological area. All of the lavender species have different percentages of terpenic alcohols (linalool, lavandulol, terpinen-4-ol), camphor, 1,8-cineole, b-caryophyllene (Lis-Balchin, 2002; Harborne & Willams, 2002; Peter, 2004) while we reported that linalool, linalyl acetate, camphor, 1,8-cineole are the main component of *L.angustifolia*.

The volatile oils of *Foeniculum* sp. (fennel), *Salvia* sp. (sage), *Vitis* sp. (grape), *Lavandula* sp. (lavender) are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Their antimicrobial actions is assigned to a number of small oil compounds, which also in pure form demonstrate high biological activity. When volatile oils and their compounds absorbed into the cells, producing many toxic radicals, have damaged the membranes or protein structures in the cell (Bakkali et al., 2008). We reported *F.vulgare* volatile oil exhibit more strong antimicrobial activity against Gram-positive bacteria than Gram negative. Many study supported our study, indicating that gram-positive strains of bacteria, especially *B. subtilis*, are less resistant than gram-negative bacteria, especially *E.coli* to fennel oils (Ruberto et al., 2000, Ozcan et al., 2006, La Cantore et al., 2004). The MIC and MCC values indicate that the volatile oil of *Salvia officinalis* was more efficient on all of the microorganisms. This result demonstrated that the antimicrobial action of *S. officinalis* can be attributed to the presence of different concentrations of thujone, 1,8-cineole, camphor and other minor components (Dorman & Deans, 2000; Sur et al., 1991). *Salvia* species have strong antimicrobial effective even in very low concentrations (Tepe et al., 2005, topçu & Goren, 2007, Martins et al., 2015) as our study.

Our MIC values about the antimicrobial action of *Vitis vinifera* showed that the oil were mostly more effective against the Gram-positive bacteria (*B. Cereus*, *E. faecalis* and *S. aureus*), compared to the data for the Gram-negative ones. The same results was observed by Boussaada et al., (2008) and Michielin et al., (2009), Oliveira et al., 2013 studying extracts from other methods.

In our experiment, we reported *Lavandula angustifolia* volatile oil contained many antimicrobial compounds, including terpenic alcohols (linalool, lavandulol, terpinen-4-ol), camphor, 1,8-cineole, b-caryophyllene. It was known that especially linalool, among them, was demonstrated to be the strongest active against a wide range of pathogen microorganisms (Cavanagh & Wilkinson, 2002). According to Table 2, The Volatile oil of Lavender were rather active on microorganisms with even low concentrations. This results was consistent with many studies (Kunicka-Styczynska et al., 2009; Hanamanthagouda et al., 2010). In addition, Danh and coworkers showed volatile oil obtained with either hydrodistilled and solvent-extracted from lavender have similarly efficiency and MIC values on microorganisms (Danh et al., 2013). When essential oils and their compartmentation with light exposure absorbed into the cells, producing some toxic radicals, have damaged the membranes or protein structures in the cell (Bakkali et al., 2008). In our study, we compared with photoactivated (light) and non-photoactivated (dark) volatile oil against pathogen microorganisms. Differences of inhibition zone in dark and light treatment were statistically significant for some plants and microorganisms. They were inhibition zone of *F.vulgare* against *K.pneumoniae* (Gram-): 1,2 mm (dark) and 3,1 mm (light); *F.vulgare* against *S. typhimurium* (Gram-): 0,9 mm (dark) and 2,3 mm (light); *S. officinalis* against *E. coli* (Gram-): 1,74 mm (dark) and 4,75 (light); *S. officinalis* against *E. faecalis* (Gram+): 1,22 mm (dark) and 3,77 mm (light); *V.vinifera* against *S. typhimurium* (Gram-): 0,5 mm (dark) and 1,8 mm (light); *V.vinifera* against *B. subtilis* (Gram+): 0,5 mm (dark) and 1,35 mm (light); *V.vinifera* against *E. faecalis*: (Gram+) 0,44 mm (dark) and 2,64 mm (light).



It was previously shown that some components in volatile oils studied have phototoxic effect in the cells. Photoactivation of *Citrus* sp. essential oil with ICG and IR diode laser exhibited remarkable lethal effect on *Candida* cells (Fekrazad et al., 2015). Phototoxic activity of *Citrus* is primarily attributed to the limonene, telinene, pinene, sabinene and myrcene abundantly present in the plant (Razzaghi-Abyaneh et al., 2009). Ethanol extracts of *Eugenia uniflora* and *Momordica charantia* showed antibacterial activity against an *E. coli* strain when exposed to UV-A light. *E. uniflora* and *M. charantia* have rich in flavonoids and terpenes (Coutinho et al., 2010). *Citrus bergamia* and *Citrus medica* exhibited a selective cytotoxic activity against the A375 cell line (malignant melanoma). While the volatile oil of *C. bergamia* was characterized by limonene, linalyl acetate,  $\gamma$ -terpinene, linalool and  $\beta$ -pinene as major components, the main compounds of *C. medica* oil were limonene,  $\gamma$ -terpinene, citral, geranial,  $\beta$ -pinene and  $\alpha$ -pinene (Menichini et al., 2010). An interesting study was introduced about cow urine which have certain nonvolatile and volatile components which have high antimicrobial activity (Shaw et al., 2007). It was observed that photo-activated cow urine with combined Bavchi and Neem oil had shown synergistic antimicrobial effect on some *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Lactobacillus acidophilus*, *Micrococcus luteus* and *Klebsiella pneumoniae* (Upadhyay et al., 2010).

According to the researches of photodynamic therapy (PDT), toxic concentration of some substances named photosensitizers (PS) with light in presence of oxygen induce cell destruction. This principle have been used to kill both Gram (+) and Gram (-) bacteria in the antimicrobial photodynamic therapy. Especially, the studies done with synthetic photosensitizers have shown that Gram (-) bacteria were more resistance than Gram (+) because of differences in their cell (Usacheva et al., 2001; Demidova et al., 2004). In our study, volatile oils known natural substances, showed similar effects against both Gram (+) and Gram (-) bacteria with regard to antimicrobial activity.

Plant oils have all known pharmacological agent for ages and their constituents are very efficient in treating diseases as antimicrobial agents. However, studies about their phototoxic antimicrobial activity were inadequate. In this study, we compared with photoactivated and non-photoactivated volatile oil against some pathogen bacteria. Differences of inhibition zone were statistically significant for some plants. Photoactivation of volatile oil studied with light exhibited remarkable lethal effect on bacteria. Thus, when studying an essential oil, it could be of interest to reported phototoxic possible cytotoxic performance its as well as its content.

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