

Original article

Promising anticancer activity of lavender (*Lavandula angustifolia* Mill.) essential oil through induction of both apoptosis and necrosis

Sevgi Gezici

Department of Molecular Biology and Genetics, University of Kilis 7Aralik, 79000, Kilis, Turkey

Received September 19, 2018; Revised November 12, 2018; Accepted November 18, 2018; Published online December 30, 2018

Abstract

In the current study, potential anticancer and antiproliferative activity of EOs from lavender (*Lavandula angustifolia* Mill.) were determined against A549, H1299, C6 cancer cells and non-tumorous HUVEC cells through induction of both apoptosis and necrosis. Furthermore, the differentiations of the cancer prevention activities of the EOs distilled from first and second year's harvest of the lavender growing under traditional system and plastic mulch were investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypan blue, neutral red uptake assays as well as lactate dehydrogenase (LDH) assay. The EOs exhibited remarkable anticancer and antiproliferative activity against all treated cancer cells, in a dose and time dependent manner. They exerted significantly decreases the cell growth in cancer cells, and also observed higher toxicity towards C6 glioma cells than the A549 and H1299 lung cells. In case of growing conditions of the EOs, higher anticancer and antiproliferative activity were observed under growing plastic mulch as compared to that of traditional system. Furthermore, first year's harvest of the lavender growing under plastic mulch seem to be possessed stronger cytotoxic effect than that of the second year's harvest. Besides, the lysosomal and LDH activity of the EOs were found to be highly in correlation with anticancer activity, and seem to be decreased obviously depending the year. It may be concluded that data obtained from the presented research demonstrates lavender EOs could have possible usage for cancer treatment as an alternative anticancer agent.

Key words: Lavender, anticancer, apoptosis, essential oil, growing condition**1. Introduction**

Lavender (*Lavandula angustifolia* Mill., Lamiaceae) is a plant species that is native to the Mediterranean, Middle Eastern countries, and the Arabian Peninsula. Nowadays, it is cultivated worldwide, which is a result of essential oil (EO) of its flowers has a wide range of pharmacological properties (Basch *et al.*, 2004; Denner, 2009; Hassiotis *et al.*, 2014; Nadalin *et al.*, 2014).

Lavender oil has been used in the field of medicine for herbal treatment purposes since the past. In recent years, it has been widely used in aromatherapy, cosmetics, perfumes, shampoo, detergents, massage oil, food processing, and tea, apart from its uses in medicine as a source of drugs (Lis Balchin and Hart, 1999; Dobetsberger and Buchbauer, 2011; Djilani and Dicko, 2012; Lesage-Meessen *et al.*, 2015).

Lavender oil is suggested to possess a variety of pharmacological activities ranging from antineoplastic to anti-inflammatory, antioxidant, antibacterial, antifungal, antiseptic, antiviral, antidepressive, sedative and immune stimulating effects (Cavanagh and Wilkinson, 2002; Atsumi and Tonosaki, 2007; Yang *et al.*, 2010;

Adaszynska *et al.*, 2013; Nikolic *et al.*, 2014; Carrasco *et al.*, 2016; Shokri *et al.*, 2017). Furthermore, it has potential uses predominantly in the treatment of central nervous system disorders, cardiovascular and respiratory infections, and chronic diseases (Dobetsberger and Buchbauer, 2011; Koulivand *et al.*, 2013; Prusinowska and Emigielski, 2014; Wotman *et al.*, 2017).

Lavender has complex chemical compositions, especially rich in lipophilic components (essential oil) and hydrophilic components (phenolic compounds, anthocyanins, phytosterols, tannins, flavone glycosides, *etc.*) (An *et al.*, 2001; Shellie *et al.*, 2002; Yang *et al.*, 2010; Rajeshwari *et al.*, 2014). It was determined that EO from the flowers of lavender obtained by stem distillation or hydrodistillation, contains more than 300 biologically active chemical compounds and components including linalyl acetate, linalool, lavandulyl acetate, lavandulol, α -terpineol, β -caryophyllene, cis- β -ocimene, camphor, and ketones, of which, linalyl acetate and linalool are identified as the major components of the lavender oil. EOs from lavender are characterized by the presence of terpenes and terpenoids, which are particularly responsible for its biological and pharmacological properties (Umezu *et al.*, 2006; Dupuy *et al.*, 2014; Prusinowska and Emigielski, 2014; Carrasco *et al.*, 2016; Coelho *et al.*, 2017). Additionally, the qualitative and quantitative composition of whole lavender oil can vary significantly depending on genotype, growing location, climatic conditions, and morphological characteristics (Cavanagh and Wilkinson, 2002; Emigielski *et al.*, 2013; Lesage-Meessen *et al.*, 2015; Fahim *et al.*, 2017).

Author for correspondence: Dr. Sevgi Gezici
Assistant Professor, Department of Molecular Biology and Genetics,
University of Kilis 7Aralik, 79000, Kilis, Turkey

E-mail: drsevgigezici@gmail.com, sevgigezici@kilis.edu.tr

Tel.: +90-5376348061

Copyright © 2018 Ukaaz Publications. All rights reserved.

Email: ukaaz@yahoo.com; **Website:** www.ukaazpublications.com

Although, there has been a recent increase in the popularity of plant based natural products as potential therapeutic agents for the modern alternative and complementary medicine, to the best of our knowledge, limited researches have been performed, comprehensive analysis on the cancer prevention activities of the essential oils of lavender (Prashar *et al.*, 2004; Nikolie *et al.*, 2014; Udupa Nayanabhirama, 2016; Majeed, 2017). Further, anticancer and antiproliferative potentials of the lavender EOs in point of growing conditions and harvest year of lavender have not been previously reported elsewhere. As far as our literature survey could ascertain, data presented here could be assumed as the first report for the literature.

The present study aimed to demonstrate potential anticancer and antiproliferative activities of EOs, distilled from *L. angustifolia* through induction of both apoptosis and necrosis. For evaluation the anticancer activities of the lavender EOs, different assays were conducted against three cancer cell lines: A549 (human lung carcinoma), H1299 (non-small lung cancer), C6 (glioma), and non-tumorous HUVEC cells; MTT assay for measurement of the cell proliferation, trypan blue exclusion assay to determine the antiproliferative properties, neutral red uptake assay for evaluation lysosomal activities of the cells, and lactate dehydrogenase activity assay to detection of necrosis in the cultured cells. Additionally, the current study was aimed to compare *in vitro* apoptosis and necrosis induction potentials of EOs distilled from first and second year's harvest of the lavender growing under traditional system and plastic mulch.

2. Materials and Methods

2.1 Plant material

Lavender (*Lavandula angustifolia* Mill.), growing under traditional system and plastic mulch, was harvested from first and second year's products during the blooming season from Kilis, Turkey, dried under shade and kept at laboratory conditions at room temperature (25°C) with no direct light until the extraction.

2.2 Extraction of EOs

The dried flowers of *L. angustifolia* were used for extraction of EOs. The flower samples (100 g for each growing condition) were extracted separately by hydrodistillation using a Clevenger Apparatus for 4 h at $100 \pm 5^\circ\text{C}$, and the extracted oils were dried over anhydrous Na_2SO_4 , then the pure essential oils were obtained and stored at 4°C for further analysis.

2.3 Cell lines and culture

A549 (lung carcinoma), H1299 (non-small cell lung cancer), C6 (glioma) and non-tumorous HUVEC (human umbilical vein endothelial cells) cell lines, obtained from the American Type Culture Collection (ATCC, USA) were used for evaluation of the potential anticancer and antiproliferative effects of the lavender EOs. The lung cancer cells (A549 and H1299) were cultured on Roswell Park Memorial Institute Medium (RPMI, Thermo Fisher Scientific), supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco®), 1% antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) in the flasks at 37°C in a humidified CO_2 (5%) incubator. HUVEC and C6 cells were grown in Dulbecco's modified Eagle medium (DMEM): Ham's F12 nutrient medium (1:1) (Thermo Fisher Scientific) with the same additives. All the experiments were run in triplicates using A549, H1299, C6 and HUVEC cells from passage 24 or less than.

2.4 Anticancer activity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed for evaluation cytotoxic properties of the lavender EOs as described by Mosmann (1983) with minor modifications, and all the conditions were same as described in our previous research (Gezici *et al.*, 2017). Briefly, densities of 5×10^3 cells were seeded in 200 µl medium into 96-well plates for 24 h and after seeding, the lavender EOs with concentrations from 6.25 µg/ml to 100 µg/ml were added all wells, and doxorubicin used as a standard cytotoxic agent with different concentrations 0.01-1 µg/ml, and then all the wells incubated at 37°C for 48 h. After incubation, the medium was discarded and 50 µl/well of MTT (Sigma-Aldrich) solution (5 mg/ml) was added into each well and incubated for 4 h at 37°C. The medium-containing MTT was discarded and 200 µl of dimethyl sulfoxide (DMSO, Sigma-Aldrich) was added for both lysis the cells and solubilization formazone. Then, the absorbance was measured at 570 nm with a Thermo Lab systems 408 multiskan multiplate spectrophotometer. The dose response curve was used to generate the IC_{50} (µg/ml) values for each cell line as the concentration of drug needed to produce 50% inhibition of cell growth.

2.5 Antiproliferative activity assay

Antiproliferative activities of the lavender EOs were evaluated against the A549, H1299 and C6, and HUVEC cell lines using trypan blue exclusion method described by Strober (2001) previously. Stock solutions of the EOs were prepared in DMSO and used at 6.25, 12.5, 25, 50 and 100 µg/ml concentrations from each EO, and afterwards incubated at 37°C for 24, 48, and 72 h. At the end of the every 24 h incubation period, 100 µl of cells were collected for counting and added 1ml of 0.05% trypsin-EDTA, and then washed with phosphate buffer saline (PBS, Sigma-Aldrich, USA). Finally, the collected cells were harvested for 10 min at 1000 rpm using a centrifuge (Thermo Fisher Scientific). After removing the supernatant, 20 µl of 0.4% trypan blue solution was added to cell pellet (1:1 dilution of the cell suspension). The cell viability was determined microscopically (Nicon, Japan), and the viable cells were counted by an automated cell counter Cedex XS Analyzer.

2.6 Lysosomal activity assay

In order to determine lysosomal activity of the cells treated with the lavender EOs, neutral red uptake assay (NR) was used as previously reported with minor modifications (Repetto *et al.*, 2008). Briefly, the cells were seeded into 96-well culture plates and incubated for 24 h at 37°C in a humidified CO_2 (5%) incubator. When the cells reached at least 50% confluence, they treated with 0, 5, 10, 50 and 100 µg/ml concentrations of the lavender EOs, before incubation at 37°C for 24, 48, and 72 h. At the end of the incubation periods, they washed with cold PBS at three times, they were then re-incubated with the medium containing 200 µl NR solutions at least 2 h at 25°C. After NR incubation, the cells were subsequently washed with acetic acid and ethanol (1:50) solution, and then fixed with CaCl_2 and formaldehyde (2:1) solution for 2 min to remove the NR dye in each well. Finally, the plates were read at 540 nm using a microplate spectrophotometer system (Thermo Lab systems 408 Multiskan).

2.7 Lactate dehydrogenase (LDH) activity assay

The release of the enzyme lactate dehydrogenase (LDH) activity assay was performed for detection of necrosis in the cultured cells described by Al-Qubaisi *et al.* (2011) previously. The cells (a density of 2×10^4 cells/well) were seeded in 96-well plates with 100 μ l fresh culture medium and incubated at 37°C for 18 h. And then, they were treated with 0, 5, 10, 50 and 100 μ g/ml concentrations of the lavender EOs, as used in the MTT assay and incubated for 24, 48 and 72 h, respectively. For determination of the potential LDH concentration, 40 μ l of culture supernatant was collected from each well and transferred to a new plate, and then 40 μ l of 6% triton X-100 was added to all well. Before measure the absorbance, 100 μ l of buffer I (0.1 M potassium phosphate buffer containing 4.6 mM pyruvic acid, pH 7.5) and 100 μ l of buffer II (0.1 M potassium phosphate buffer containing 0.4 mg/ml reduced β -NADH, pH 7.5) were added to each well containing the culture medium, and mixed by gentle tapping. Additionally, the same protocol was performed for untreated cell lysate as LDH positive control to evaluate total LDH/U well activity. All the plates were incubated at room temperature about 30 min in the dark; afterwards they were read at wavelength 340 nm using an ELISA microplate spectrophotometer system (Thermo Lab systems 408 Multiskan). The percentage of LDH release in medium was calculated and compared to total LDH in cell lysate in the same well, of the followed equation:

$$[(\text{Mean OD from treated LDH activity} - \text{mean OD from untreated LDH activity}) / (\text{mean OD from maximum LDH release activity} - \text{mean OD from untreated LDH activity})] \times 100.$$

The assay was done using three replicates. The dose response curve was used to generate the IC_{50} (μ g/ml) values for each cell line as the concentration of drug needed to produce 50% inhibition of cell growth.

2.8 Statistical analysis

The data were represented as mean and standard deviation (Mean \pm SD) from at least three independent experiment, and three different microplate wells were used for each concentration. A linear regression analysis was performed to calculate IC_{50} values. Differences between groups were considered as significant when a p -value was set at 0.05, and very significant when a p -value was set at 0.01.

3. Results

3.1 Anticancer activity assay results

In order to determine cytotoxicity and anticancer activities of the EOs of lavender, MTT assay was performed against A549 (lung carcinoma), H1299 (non-small cell lung cancer), C6 (glioma) cancer cells, and non-tumorous HUVEC (human umbilical vein endothelial cells). *In vitro* dosage and time dependent cytotoxic effects of the EOs are presented in Table 1 regarding of IC_{50} values after 48 h treatment period.

Table 1: Cytotoxic effects of lavender EOs against A549, H1299, C6 and HUVEC cell lines for 48 h

Cell lines	Treatment		Concentrations of the Lavender EOs (μ g/ml)				
			6,25 μ g/ml	12,5 μ g/ml	25 μ g/ml	50 μ g/ml	100 μ g/ml
A549	Traditional system	first year	167,516 \pm 0,047**	158,028 \pm 0,031*	137,169 \pm 0,024**	128,3 \pm 0,0582*	114,472 \pm 0,035**
		second year	125,881 \pm 0,012*	130,209 \pm 0,044*	154,904 \pm 0,075**	120,376 \pm 0,031**	121,156 \pm 0,006**
	Plastic mulch	first year	99,839 \pm 0,025**	86,859 \pm 0,012*	80,850 \pm 0,009**	81,736 \pm 0,029**	66,551 \pm 0,027**
		second year	120,214 \pm 0,006*	131,472 \pm 0,049**	149,540 \pm 0,029*	126,276 \pm 0,065**	103,663 \pm 0,042*
H1299	Traditional system	first year	106,396 \pm 0,154*	84,839 \pm 0,028**	78,394 \pm 0,053**	76,174 \pm 0,126*	62,499 \pm 0,034**
		second year	121,042 \pm 0,028*	128,604 \pm 0,001**	120,982 \pm 0,104**	126,013 \pm 0,155**	118,001 \pm 0,016*
	Plastic mulch	first year	106,396 \pm 0,104**	84,839 \pm 0,028*	78,394 \pm 0,053**	76,174 \pm 0,126**	58,499 \pm 0,034**
		second year	125,683 \pm 0,033*	131,185 \pm 0,106*	129,450 \pm 0,026*	104,744 \pm 0,092**	98,024 \pm 0,012**
C6	Traditional system	first year	141,334 \pm 0,006*	101,984 \pm 0,085**	108,785 \pm 0,126**	78,407 \pm 0,064*	55,213 \pm 0,026**
		second year	145,527 \pm 0,012*	134,478 \pm 0,053*	106,590 \pm 0,072*	92,058 \pm 0,068*	69,555 \pm 0,023**
	Plastic mulch	first year	98,918 \pm 0,024**	94,482 \pm 0,038**	85,470 \pm 0,048*	52,594 \pm 0,032**	28,405 \pm 0,001**
		second year	108,619 \pm 0,016*	80,285 \pm 0,001**	76,613 \pm 0,026**	69,295 \pm 0,101**	41,735 \pm 0,041**
HUVEC	Traditional system	first year	22,396 \pm 0,038**	24,102 \pm 0,023**	22,830 \pm 0,107**	20,243 \pm 0,031**	14,942 \pm 0,025**
		second year	38,096 \pm 0,105**	31,986 \pm 0,108**	30,004 \pm 0,053**	19,128 \pm 0,004**	20,306 \pm 0,124**
	Plastic mulch	first year	13,022 \pm 0,124**	19,254 \pm 0,025**	15,940 \pm 0,015**	12,186 \pm 0,042**	8,284 \pm 0,004**
		second year	24,908 \pm 0,029**	24,014 \pm 0,108**	17,685 \pm 0,002**	18,144 \pm 0,104**	12,605 \pm 0,036**
Doxorubicin ^a			5,928 \pm 0,001	5,202 \pm 0,001	4,404 \pm 0,105	4,046 \pm 0,012	3,72 \pm 0,002
DMSO (dimethyl sulfoxide) ^b			0	0	0	0	0

Values are expressed as $IC_{50} \pm$ SD from three independent experiment (n=3).

** p value of <0.01 and * p value of <0.05

^a Doxorubicin, positive control.

^b DMSO; dimethyl sulfoxide, negative control.

Our results revealed from the assay demonstrated that lavender EOs exhibited strong anticancer and cytotoxic activity against all treated cancer cells, and decreased the cell growth by increasing cell death in cancer cells significantly, as figured out in Figure 1. In case of anticancer activities, the EOs of lavender showed the highest growth inhibitory effects towards C6 glioma cell line ($IC_{50} = 28,405 \pm 0,001$ to $69,555 \pm 0,023 \mu\text{g/ml}$), followed by H1299 and A549 lung cancer cell lines (IC_{50} values between $58,499 \pm 0,034$ to $121,156 \pm 0,006 \mu\text{g/ml}$). In addition, the IC_{50} values of lavender EOs obtained from the cancer cells were compared to non-tumorous HUVEC cells in a dose and time dependently (Table 1, Figure 1).

As shown in Figure 1, growing conditions and harvest year of lavender highly affect the anticancer potentials of the EOs. Regarding of growing conditions of lavender, EOs distilled from growing under plastic mulch showed higher anticancer potential with IC_{50} values in range of $28,405 \pm 0,001$ to $103,663 \pm 0,042 \mu\text{g/ml}$ than the EOs distilled from growing under traditional system with IC_{50} values in range of $55,213 \pm 0,026$ to $121,156 \pm 0,006 \mu\text{g/ml}$ ($100 \mu\text{g/ml}$ concentration, 48 h) towards all the tested cancer cell lines. As for the harvesting year of lavender, first year's harvest of the lavender growing under plastic mulch possessed stronger cytotoxic effect than that of the second year's harvest ranging IC_{50} values between $28,405 \pm 0,001$ to $66,551 \pm 0,027 \mu\text{g/ml}$ at $100 \mu\text{g/ml}$ concentration for 48 h (Table 1, Figure 1).

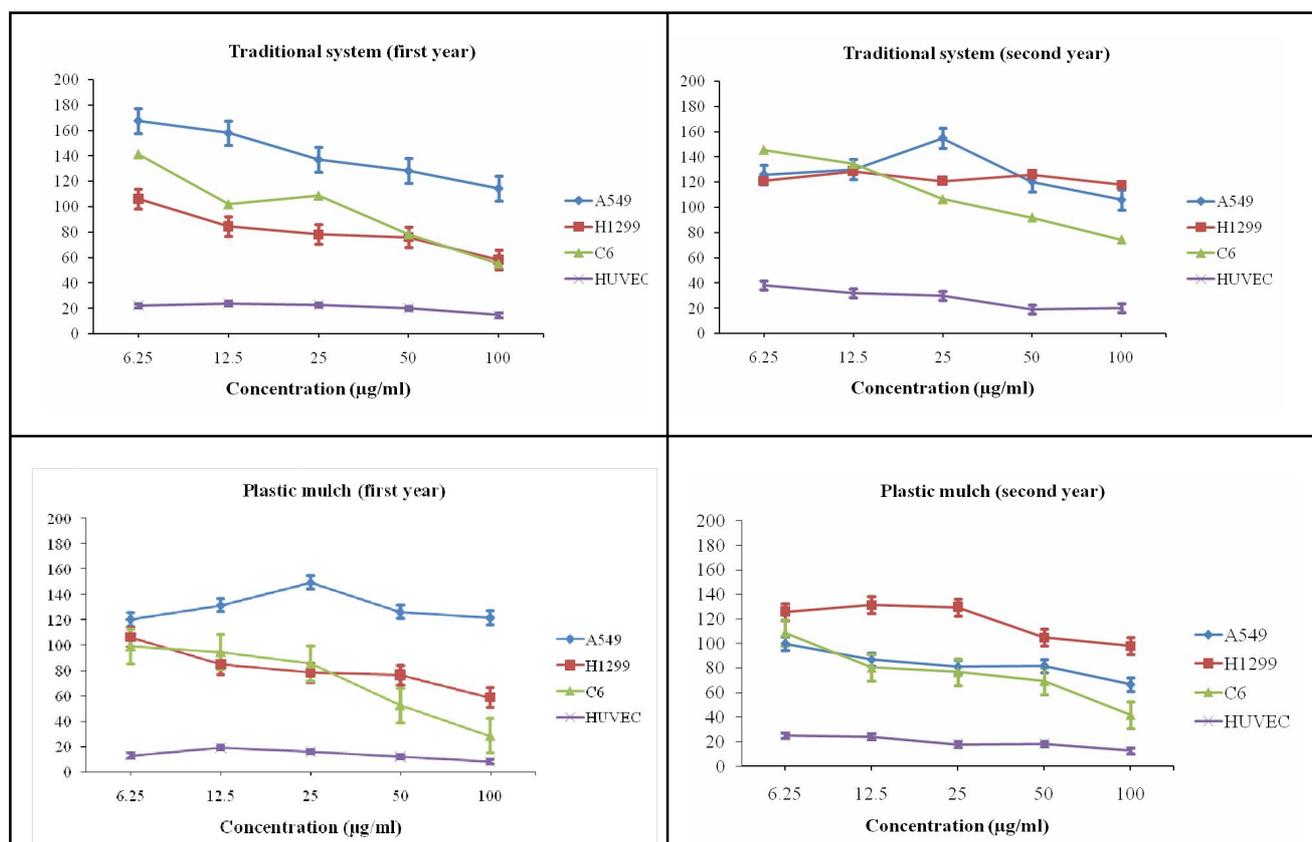


Figure 1: Inhibition effect of lavender EOs under growing traditional system and plastic mulch.

3.2 Antiproliferative activity assay results

Trypan blue assay was assessed to determine antiproliferative effects of the lavender EOs by cell counting after 24, 48 and 72 h treatment with different doses. According to antiproliferative assay, the EOs of lavender exerted remarkable decreasing in the cell viability even at the lowest concentration ($5 \mu\text{g/ml}$). Furthermore, increase in the concentration and exposure time of the EOs resulted in decrease of the cell viability in malignant cells but not in non-malignant HUVEC cells. Results of survival (%) percentage of A549, H1299 and C6 cancer cells, comparing the non-tumorous HUVEC cells after 48 h treatment with the EOs are shown in Figure 2.

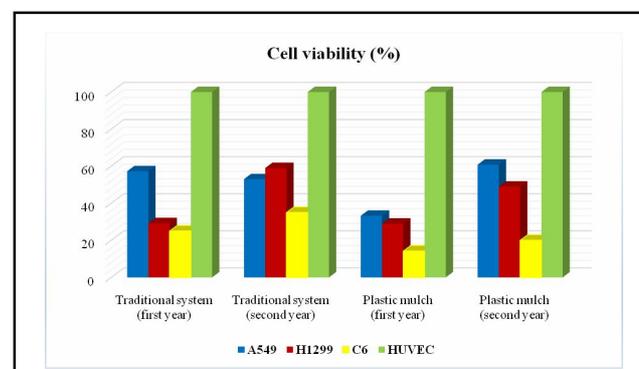


Figure 2: Cell viability (%) of A549, H1299 and C6 cancer cells after 48h treatment with $100 \mu\text{g/ml}$ concentration of the EOs.

*Values are expressed as Mean \pm SD from three independent experiment (n = 3). HUVEC cells were used as control and set as 100%.

As supported by cytotoxicity assay, results of trypan blue assay showed that cell growth and viability in cancer cell lines were inhibited by the EOs depending on growing conditions and harvest year of lavender. On the other hand, EOs distilled from growing under plastic mulch indicated significant reduction in cell growth of all the cancer cell lines even at the lowest concentration (5 $\mu\text{g/ml}$), as compared to that of growing under traditional system. As can be seen from Figure 2, it was observed that first year's harvest of the

lavender growing under plastic mulch or traditional system caused much more inhibitory effects in the cell viability of the tested cancer cells than the second year's harvest of the lavender (Figure 2).

3.3 Lysosomal activity assay results

Lysosomal activities of the lavender EOs were observed in a time (for 24 h, 48 h, and 72 h) and dose dependent manner (0, 5, 10, 50 and 100 $\mu\text{g/ml}$) against the A549, H1299 and C6 cancer cells, and the results for 48 h treatment were shown in Figure 3 for each treated cell line.

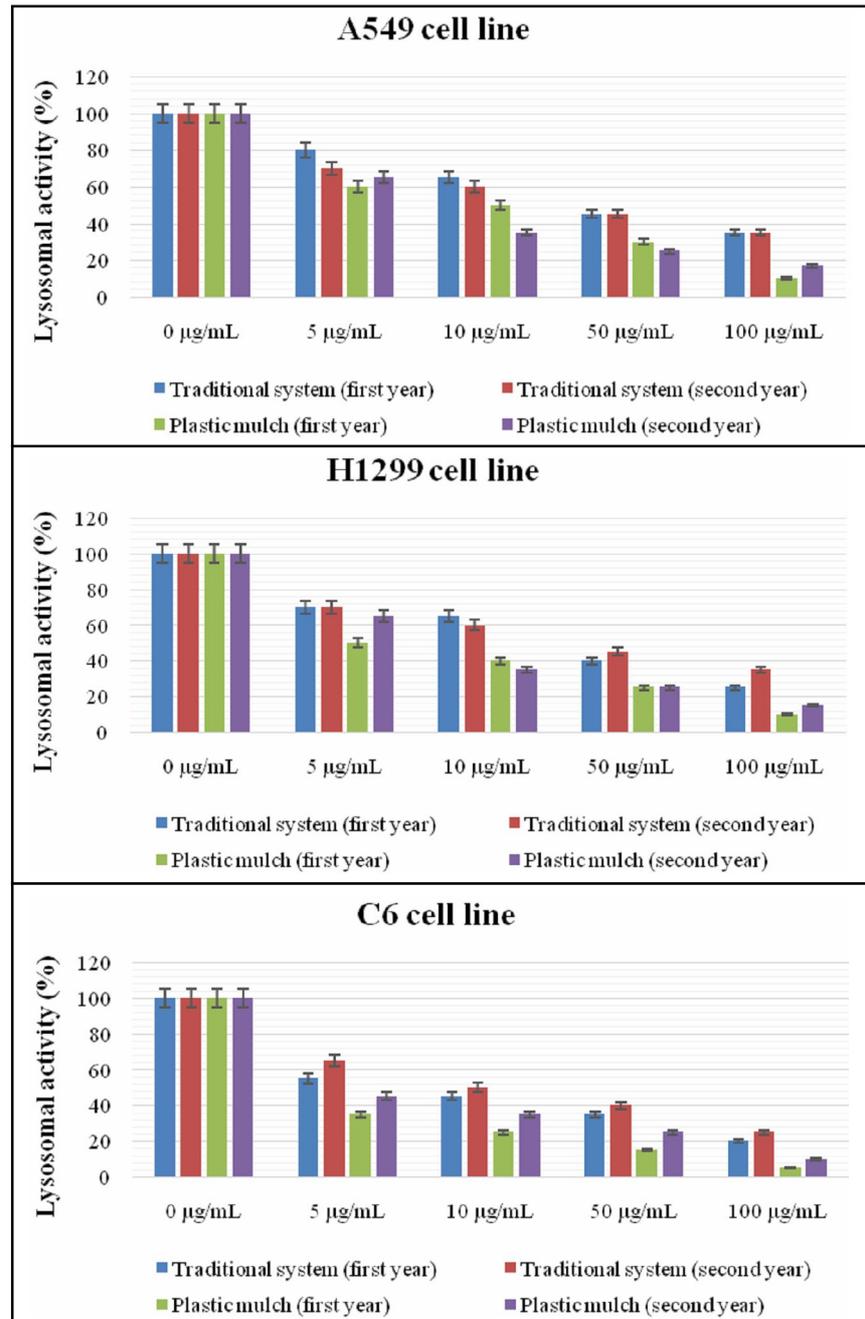


Figure 3: Lysosomal activity (%) of A549, H1299 and C6 cancer cells after 48 h treatment.

*Values are expressed Mean \pm SD from three independent experiment (n = 3). The dose of 0 $\mu\text{g/ml}$ was used as control and set as 100%.

According to results of lysosomal activity assay, first year's harvest of the lavender growing under plastic mulch had stronger effects on lysosomal activity than the growing under traditional system, which is consistent with anticancer and antiproliferative assay results. Additionally, increasing EOs exposure time and dose resulted in increasing the lysosomal functions and membrane permeability in the cancer cells, which is again compliant with anticancer and antiproliferative assay results.

3.4 Lactate dehydrogenase (LDH) activity assay results

The release of LDH from the A549, H1299 and C6 cancer cells was measured after treatment with 0, 5, 10, 50 and 100 $\mu\text{g/ml}$ concentrations of lavender EOs. The leakage of LDH was shown in Table 2 regarding of IC_{50} values in a time dependent manner for 24, 48, and 72 h treatment period. Non-tumorous HUVEC cells were used as control and doxorubicin used as standard cytotoxic agent (Table 2).

Table 2: Comparison of IC_{50} ($\mu\text{g/ml}$) values obtained from LDH release assay for A549, H1299, and C6 cancer cell lines

Lavender EOs	Hours	Cancer Cells ^a		
		A549	H1299	C6
	24 h	27,04 \pm 1,13**	28,02 \pm 0,02*	24,04 \pm 0,18**
Traditional system (first year)	48 h	45,02 \pm 0,05**	41,34 \pm 0,22**	25,94 \pm 0,04**
	72 h	64,07 \pm 0,08*	48,12 \pm 1,04*	34,08 \pm 1,05**
	24 h	30,06 \pm 0,15**	31,83 \pm 0,21*	27,12 \pm 0,02**
Traditional system (second year)	48 h	38,85 \pm 0,03*	46,95 \pm 0,03**	35,06 \pm 0,03**
	72 h	67,02 \pm 0,12**	55,46 \pm 1,01**	45,16 \pm 0,12**
	24 h	29,06 \pm 0,64*	21,09 \pm 0,08**	14,10 \pm 1,02**
Plastic mulch (first year)	48 h	38,07 \pm 1,64**	30,25 \pm 0,12**	18,96 \pm 1,09**
	72 h	43,01 \pm 1,04**	35,09 \pm 0,06**	26,06 \pm 1,04**
	24 h	40,11 \pm 1,02**	38,75 \pm 1,14*	21,96 \pm 1,18**
Plastic mulch (second year)	48 h	40,85 \pm 1,03**	43,69 \pm 0,16**	23,88 \pm 1,06**
	72 h	54,14 \pm 1,08**	48,03 \pm 1,03**	42,08 \pm 1,02**
Doxorubicin ^b		5,48 \pm 0,01	3,64 \pm 0,02	3,12 \pm 0,01

^aThe cells (a density of 2×10^4 cells/well) were seeded for each treatment, and values are expressed as Mean \pm SD from three independent experiments. ^b Doxorubicin, positive control. ** p value of <0.01 and * p value of <0.05 .

As summarized in the Table 2, the IC_{50} values for treated cancer cells were calculated as compared to non-tumor HUVEC cells in a time dependent manner. In this assay, a significant increasing was observed in the IC_{50} value from LDH by depending the time for all the EOs, as supported by IC_{50} values determined from cytotoxicity assay. The EOs distilled from growing under plastic mulch were found to have high LDH activity with IC_{50} values in range of $14,10 \pm 1,02$ to $54,14 \pm 1,08$ $\mu\text{g/ml}$, whereas, the EOs distilled from growing under traditional system found moderate LDH activity with IC_{50} values in range of $24,04 \pm 0,18$ to $67,02 \pm 0,12$ $\mu\text{g/ml}$ (Table 2).

In addition to LDH release activity differences as depending on the growing conditions, the differences of LDH release activity of the EOs were presented depending on types of cancer cell line in a time dependent manner. As presented in Table 2, the EOs distilled from lavender growing under plastic mulch showed the highest LDH release effects against C6 glioma cell line ($\text{IC}_{50} = 14,10 \pm 1,02$ $\mu\text{g/ml}$, for 24 h), whilst the lowest LDH release effects were observed against A549 adenocarcinoma cell line ($\text{IC}_{50} = 54,14 \pm 1,08$ $\mu\text{g/ml}$, for 72 h). Moreover, it was determined that first year's harvest of the lavender growing under plastic mulch lead to higher LDH leakage activity on cancer cells as compared to that of second year's harvest of the lavender growing under traditional system (Table 2).

4. Discussion

Cancer has become a growing health problem around the world with the high numbers of cases and deaths. Meanwhile, it is predicted that it will be more critical health problem in both developed and developing countries for the next years (Siegel *et al.*, 2018). More recently, the researchers have mainly focused on examining the use of plant-derived natural products with less or no side effects for prevention and treatment in the cancer cases, since an increase in the incidences of drug resistance and side effects of drugs used for cancer therapy. A recent increase in the popularity of plant-derived natural products has increased interest in their essential oils as a potential herbal therapy (Cragg and Newman, 2005; Jinukuti and Giri, 2015; Manoharachary and Nagaraju, 2016; Belkhodja *et al.*, 2017; Gezici and Sekeroglu, 2017; Karik *et al.*, 2018).

Our results indicated that lavender EOs distilled from growing under traditional system and plastic mulch had significant anticancer and antiproliferative effects even at the lowest concentration and minimum exposure time. It was observed that the cell growth and viability in cancer cell lines were inhibited by the EOs depending on growing conditions and harvest year of lavender. On the other hand, first year's harvest of the lavender growing under plastic mulch or traditional system caused much more inhibitory effects in

the cell viability of the A549, H1299 and C6 cancer cells than the second year's harvest of the lavender. Our findings were consistent with previously studies, which were performed to reveal the variations in constituents of lavender EOs derived from growing location, different cultivars, climatic conditions, and other characteristics. The cell proliferation and growth inhibitory effects of the EOs may be due to the fact that rich constituent of terpenes and terpenoids such as linalool, linalyl acetate, cineole, α -ocimene, lavandulol, terpinen-4-ol and camphor (Cavanagh and Wilkinson, 2002; Koulivand *et al.*, 2013; Coelho *et al.*, 2017; Shokri *et al.*, 2017).

In the light of antiproliferative assay results, when the cells were exposed to lavender significantly, inhibition was observed in the cell growth in a time and dose dependently. Whilst, the data produced from this assay points to the cell growth inhibitory effects of the EOs on exposed compared to cells that were not exposed to the EOs. On this basis, that might be a positive factor in order to suggest lavender EOs could offer additional benefit for its use in the management of decreasing the amount of cancer cells.

Thus, in addition to anticancer and antiproliferative assays, lysosomal activity assay was performed to analyze lysosomes activities of the cells that the dye can diffuse cellular membrane and accumulate in the lysosomes. When the cells were treated with NR, the dye penetrates the cell membrane and accumulates intracellularly in the lysosomes that leads to change in sensitivity of lysosomal membrane irreversibly. Therefore, it is possible to evaluate the number of viable cells response to the EOs, which is the basis of NR assay. Based on our results, it can be clearly concluded that the EOs significantly accelerate reducing uptake of NR into the cell membrane, due to presence of rich bioactive compounds, particularly linalool, linalyl acetate, and limonene as reported previously (Dupuy *et al.*, 2014; Prusinowska and Ćemigielski, 2014; Lesage-Meessen *et al.*, 2015; Carrasco *et al.*, 2016).

It is noteworthy that combination of anticancer, antiproliferative and LDH activities of the EOs are known to be associated with fighting cancer efficiently. On this basis, the EOs were also tested whether possess lactate dehydrogenase activity or not. LDH is a cytoplasmic enzyme that plays role in the conversion of pyruvate to lactate, this enzyme releases from the necrotic cell membranes when the cell membrane is damaged. Although, apoptosis and necrosis are two major metabolic processes observed in disease pathologies; necrosis is considered as a passive and accidental form of cell death causes the release of intracellular contents into extracellular milieu (Chan *et al.*, 2013). Therefore, measuring the activity of LDH enzyme gives information about the percentage of dead and necrotic cells, as observed in our study.

5. Conclusion

Overall, these results demonstrated that lavender EOs have significantly anticancer and antiproliferative activities against the cancer cell lines through induction of both apoptosis and necrosis in a time and dose dependent manner, even at lower concentration and minimum exposure time. Although, there is considerable evidence and information about pharmacological properties of lavender EOs; there has been no study so far comparing anticancer potentials of EOs distilled from first and year's harvest of the lavender growing under traditional system and plastic mulch. As far as our literature survey, the results presented in this research

could be the first report, which was conducted for investigation of *in vitro* anticancer and antiproliferative activities of the lavender EOs along with potential lysosomal and lactate dehydrogenase releasing. However, these results obtained from the presented research could be useful to support the possible usage of the EOs distilled from lavender as a promising anticancer agent in cancer treatment, further *in vivo* studies should be performed to ascertain of the mechanisms underlying the remarkable anticancer effects of lavender EOs.

Acknowledgements

The author would like to thank Gaziantep University, Medical Biology Department, Genetics Laboratory for their technical support. In addition, the author would like to thank Professor Nazim Sekeroglu from Kilis 7 Aralık University, Turkey for his kind contribution of obtaining the plant materials.

References

- Adaszyńska, M.; Swarczewicz, M.; Dzięcio³, M. and Dobrowolska, A. (2013). Comparison of chemical composition and antibacterial activity of lavender varieties from Poland. *Nat. Prod. Res.*, **27**(16): 1497-1501.
- Al-Qubaisi, M.; Rozita, R.; Yeap, S.K.; Omar, A.R.; Ali, A.M. and Alitheen, N.B. (2011). Selective cytotoxicity of goniothalamin against hepatoblastoma HepG2 cells. *Molecules*, **16**(4): 2944-2959.
- An, M.; Haig, T. and Hatfield, P. (2001). On-site field sampling and analysis of fragrance from living lavender (*Lavandula angustifolia* L.) flowers by solid-phase microextraction coupled to gas chromatography and ion-trap mass spectrometry. *J. Chromatogr. A.*, **917**(1-2): 245-250.
- Atsumi, T. and Tonosaki, K. (2007). Smelling lavender and rosemary increases free radical scavenging activity and decreases cortisol level in saliva. *Psychiat. Res.*, **150**(1): 89-96.
- Basch, E.; Foppa, I.; Liebowitz, R.; Nelson, J.; Smith, M.; Sollars, D. and Ulbricht, C. (2004). Lavender (*Lavandula angustifolia* Miller). *J. Herb. Pharmacother.*, **4**(2): 63-78.
- Belkhodja, H.; Meddah, B. and Gezici, S. (2017). Anti-inflammatory effects of essential oils from *Rosmarinus officinalis* and *Populus alba* on experimental models of acute and chronic inflammation in rats. *Ind. J. Pharm. Educ. Res.*, **51**(3):185-189.
- Carrasco, A.; Tomas, V.; Tudela, J and Miguel, M.G. (2016). Comparative study of GC MS characterization, antioxidant activity and hyaluronidase inhibition of different species of Lavandula and Thymus essential oils. *Flavour Fragr. J.*, **31**(1):57-69.
- Cavanagh, H. and Wilkinson, J. (2002). Biological activities of lavender essential oil. *Phytother. Res.*, **16**(4):301-308.
- Chan, F.K.M.; Moriwaki, K and De Rosa, M.J. (2013). Detection of necrosis by release of lactate dehydrogenase activity. In: *Immune Homeostasis Humana Press*, Totowa, NJ, pp: 65-70.
- Coelho, L. S.; Correa-Netto, N.F.; Masukawa, M.Y.; Lima, A.C.; Maluf, S.; Linardi, A. and Santos-Junior, J.G. (2017). Inhaled *Lavandula angustifolia* essential oil inhibits consolidation of contextual-but not tone-fear conditioning in rats. *J. Ethnopharmacol.*, **215**: 34-41.
- Cragg, G.M. and Newman, D.J. (2005). Plants as a source of anti-cancer agents. *J. Ethnopharmacol.*, **100**(1): 72-79.
- Denner, S.S. (2009). *Lavandula angustifolia* Miller: English lavender. *Holist. Nurs. Pract.*, **23**(1): 57-64.
- Djilani, A. and Dicko, A. (2012). The therapeutic benefits of essential oils. In: *Nutrition, Well-Being and Health*: In Tech. Ed. Jaouad Bouayed, pp:155-178.

- Dobetsberger, C. and Buchbauer, G. (2011). Actions of essential oils on the central nervous system: An updated review. *Flavour Fragr. J.*, **26**(5): 300-316.
- Dupuy, N.; Gaydou, V. and Kister, J. (2014). Quantitative analysis of lavender (*Lavandula angustifolia*) essential oil using multiblock data from infrared spectroscopy. *Am. J. Analyt. Chem.*, **5**(10):633.
- Emigielski, K.B.; Prusinowska, R.; Krosowiak, K. and Sikora, M. (2013). Comparison of qualitative and quantitative chemical composition of hydrolate and essential oils of lavender (*Lavandula angustifolia*). *J. Essent. Oil Res.*, **25**(4):291-299.
- Fahim, M.; Shrivastava, B.; Shrivastava, A.K.; Ibrahim, M.; Parveen, R and Ahmad, S. (2017). Review on extraction methods, antioxidant and antimicrobial properties of volatile oils. *Ann. Phytomed.*, **6**(2): 5-46.
- Gezici S.; Sekeroglu, N. and Kijjoa A. (2017). *In vitro* anticancer activity and antioxidant properties of EOs from *Populus alba* and *Rosmarinus officinalis* growing in south Eastern Anatolia of Turkey. *Ind. J. Pharm. Educ. Res.*, **51**(3):498-503.
- Gezici, S. and Sekeroglu, N. (2017). Regulation of micro RNAs by natural products and bioactive compounds obtained from common medicinal plants: Novel strategy in cancer therapy. *Ind. J. Pharm. Educ. Res.*, **51**(3):483-488.
- Hassiotis, C.N.; Ntana, F.; Lazari, D.M.; Poullos, S. and Vlachonassios, K.E. (2014). Environmental and developmental factors affect essential oil production and quality of *Lavandula angustifolia* during flowering period. *Ind. Crop. Prod.*, **62**:359-366.
- Jinukuti, M.G. and Giri, A. (2015). Anticancer activity of acetone and methanol extracts of *Terminalia chebula* Retz and *Withania somnifera* (Linn.) Dunal on HeLa cell line. *Ann. Phytomed.*, **4**(2): 88-92.
- Karik, U.; Cinar, O.; Tunçtürk, M.; Sekeroglu, N. and Gezici, S. (2018). Essential Oil composition of some sage (*Salvia* spp.) species cultivated in İzmir (Turkey) ecological conditions. *Ind. J. Pharm. Educ. Res.*, **52**(4):102-107.
- Koulivand, P.H.; Khaleghi Ghadiri, M. and Gorji, A. (2013). Lavender and the nervous system. *J. Evid. Based Complementary Altern. Med.*, pp: 1-10.
- Lesage-Meessen, L.; Bou, M.; Sigoillot, J.C.; Faulds, C.B. and Lomascolo, A. (2015). Essential oils and distilled straws of lavender and lavandin: A review of current use and potential application in white biotechnology. *Appl. Microbiol. Biotechnol.*, **99**(8):3375-3385.
- Lis Balchin, M. and Hart, S. (1999). Studies on the mode of action of the essential oil of lavender, *Lavandula angustifolia* P. Miller. *Phytother. Res.*, **13**(6):540-542.
- Manoharachary, C. and Nagaraju, D. (2016). Medicinal plants for human health and welfare. *Ann. Phytomed.*, **5**(1):24-34.
- Majeed, M. (2017). Evidence-based medicinal plant products for the healthcare of world population. *Ann. Phytomed.*, **6**(1):1-4.
- Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**(1-2): 55-63.
- Nađalin, V.; Lepojevia, Ž.; Ristia, M.; Vladia, J.; Nikolovski, B. and Adamovia, D. (2014). Investigation of cultivated lavender (*Lavandula officinalis* L.) extraction and its extracts. *Chem. Ind. Chem. Eng. Q.*, **20**(1):71-86.
- Nikolia, M.; Jovanovia, K.K.; Markovia, T.; Markovia, D.; Gligorijevia, N.; Radulovia, S. and Sokovia, M. (2014). Chemical composition, antimicrobial, and cytotoxic properties of five Lamiaceae essential oils. *Ind. Crop. Prod.*, **61**: 225-232.
- Prashar, A.; Locke, I.C. and Evans, C.S. (2004). Cytotoxicity of lavender oil and its major components to human skin cells. *Cell Prolif.*, **37**(3): 221-229.
- Prusinowska, R. and Emigielski, K.B. (2014). Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. *Herba Pol.*, **60**(2):56-66.
- Rajeshwari, C.U.; Shobha, R.I. and Andallu, B. (2014). Phytochemicals in diet and human health with special reference to polyphenols. *Ann. Phytomed.*, **3**(2):4-25.
- Repetto, G.; Del Peso, A. and Zurita, J.L. (2008). Neutral red uptake assay for the estimation of cell viability/cytotoxicity. *Nat. Protoc.*, **3**(7): 1125.
- Shellie, R.; Mondello, L.; Marriott, P. and Dugo, G. (2002). Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography. *J. Chromatogr. A*, **970**(1-2):225-234.
- Shokri, A.; Saedi, M.; Fakhar, M.; Morteza-Semnani, K.; Keyghobadi, M.; Teshnizi, S.H.; Kelidari, H.R. and Sadjadi, S. (2017). Antileishmanial Activity of *Lavandula angustifolia* and *Rosmarinus officinalis* essential oils and Nano-emulsions on *Leishmania major* (MRHO/IR/75/ER). *Iran. J. Parasitol.*, **12**(4): 622.
- Siegel, R.L.; Miller, K.D. and Jemal, A. (2018). Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians*, **68**(1):7-30.
- Strober, W. (2001). Trypan blue exclusion test of cell viability. *Curr. Protoc. Immunol.*, **A3. B. 1-A3. B. 3**.
- Udupa, Nayanabhirama (2016). Status on herbal drugs and their future properties. *Ann. Phytomed.*, **5**(1):1-3.
- Umez, T.; Nagano, K.; Ito, H.; Kosakai, K.; Sakaniwa, M. and Morita, M. (2006). Anticonflict effects of lavender oil and identification of its active constituents. *Pharmacol. Biochem. Behav.*, **85**(4):713-721.
- Wotman, M.; Levinger, J.; Leung, L.; Kallush, A.; Mauer, E. and Kacker, A. (2017). The efficacy of lavender aromatherapy in reducing preoperative anxiety in ambulatory surgery patients undergoing procedures in general otolaryngology. *Laryngoscope Investig. Otolaryngol.*, **2**(6):437-441.
- Yang, S.A.; Jeon, S.K.; Lee, E.J.; Shim, C.H. and Lee, I.S. (2010). Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. *Nat. Prod. Res.*, **24**(2):140-151.