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Inhibition and Antimicrobial Activity of *Lavandula stoechas* Essential Oil on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*

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Abstract **–** In the present study, the volatile compounds of essential oil of *Lavandula stoechas* L. (Lavender) was analysed by gas chromatography-mass spectrometry (GC-MS) using the Nist and Willey libraries. It was determined that the main components of *L. stoechas* were linalyl acetate (36.31%) and linalool (34.18%). Inhibition and antimicrobial activity of volatile oil of the plant against *Escherichia coli* (ATCC 25293), *Staphylococcus aureus* (ATCC25925) and *Candida albicans* were examined by microbroth dilution method. *L. stoechas* EO (Essential Oil) exhibited antimicrobial activity with MIC of between 14.8 and 32.5 µg/mL. The lowest antimicrobial activity were determined against *E. coli* in *L. stoechas* EO (MIC= 32.5 µg/mL), while maximum MIC were noticed as 14.8 µg/mL against *C. albicans.* The most cell inhibition were noted as 91.76% in *C. albicans* at the concentration of 4500 µg/mL of *L. stoechas*. The reductions in living cell number were obtain as 0.54 log in *E. coli*, 1.04 log in *S. aureus* and 1.08 log in *C. albicans* at the same concentration of 4.5 µg/mL. This study showed the strong antimicrobial performance of *L. stoechas* EO in detail. This study showed that *L. stoechas* EO inhibits the growth of microorganisms depending on concentration.

Keywords – *Lavandula stoechas, antimicrobial action, cell inhibition, reduction of living cell*

I. Introduction

Natural products have therapeutic and antimicrobial characteristics and a large quantity of information has been released [1]. In particular, many studies on the antimicrobial action of essential oils have critical significance. Thus, countless studies against pathogenic bacteria have been recorded to determine antimicrobial operations of vital plant oils. Lavandula species are the leading ones of these studies. Lavandula genus includes herbaceous plants, annuals, and small shrubs, having aromatic parts. Among cultivated in the world, the most known species believed to have medicinal and aromatherapic value are *Lavandula angustifolia*, *Lavandula intermedia*, *Lavandula dentata*, *Lavandula officinalis* and *Lavandula stoechas*. Their essential oil have been used for centuries as a therapeutic agent, especially an antimicrobial agent [2]- [4]. Also culinary herbs and food supplements include most Lavender species based on their biological activities. Many studies has performed several investigations on lavender essential oils, mainly on its antifungal, antioxidant, and anti-inflammatory properties [5]-[8].

Lavandula stoechas L. is a member of Lamiaceae family and distributed in all Mediterranean regions. It is rather important in medical treatment as anticonvulsant, sedative, and antispasmodic activities. In addition, *L. stoechas* is used in perfumery and cosmetics due to consist of desired aromatic components [9], [10]. However, no paper was found reporting the reduction of survival and inhibition percentage in pathogen microorganisms on different concentration of *L. stoechas* essential oil. Thus, this study focuses on the key issues such as survival of *E. coli*, *S. aureus* and *C. albicans* in natural antimicrobial agent environments and the rate of inhibition of *L. stoechas* EO.

II. Materials and Method

A. Plant material and Essential oil extraction

L. stoechas. were collected from Fethiye region of Muğla, Turkey in 2018. They were identified and confirmed by comparing it with the specimen located at the Herbarium of Biology, Faculty of Science, Muğla Sıtkı Koçman University, Turkey. The *Hp*EO and *Ls*EO extractions were performed from approximately 100 g of the dried *L. stoechas* via hydrodistillation method for 2 hours from several matrix.

B. Chemical composition

The qualitative and quantitative composition of essential oil analysis were conducted at Giresun University central Research Laboratories Application and Research Center by GC-MS 7890A-(5975C inert MSD) instrument equipped with an Agilent 19091S-433 column (30m x 250 µm film x 0.25 µm thickness). Helium was used as a carrier gas. The temperature was raised from 60°C to 225°C by an increase of 3°C/minutes and then 25 minutes of waiting time were implemented during the analysis. Injection port temperature was 250°C, while detector temperature was 260°C. Characterization of *L. stoechas* EO components was based on the library (Wiley and NIST) comparison with the mass spectra of the injected essential oil samples.

C. Antimicrobial Activity

The antimicrobial activity of essential oil of *L. stoechas* was researched on several pathogens, namely *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* using and modified spectrophotometric microdilution technique. Firstly, the inoculums of microorganisms were prepared in 4 ml Triptic Soy Broth for bacteria, 4 ml Sabouraud Dextrose Broth for yeast and incubated at 37ºC, overnight. After 24 hours, the culture suspensions were adjusted to 0.5 McFarland Standard Turbidity and stored at +4°C until use [11].

D. Spectrophotometric Microdilution Technique

The experiment were performed on 96-well microtiter plates and firstly 50 µL of Mueller Hinton Broth (MHB) medium were added into all wells. Two-fold serial dilutions (50 µL) of *L. stoechas* (4.65 mg/mL) was made on all x-axis along of elisa plate. Columns 11 and 12 were used as negative and positive controls. Finally, 5 µL culture of microorganisms was inoculated on all wells except medium control wells. All plates were incubated for 24 hours at 37°C, turbidity for bacteria was measured at 600 nm, 415 nm for yeast. The optical density was read before, T0 and T24 after 24-hour incubation for MIC assessment. MIC was calculated for each plate using the following formula: The OD was subtracted from theOD for each replicate at T24 for each replicate. The Percent growth = (ODtest /OD control)x100. Percent Inhibition = 100-(OD test well/OD of corresponding control well)x100 for each row of the 96-well plate.We calculated MIC (the lowest concentration of test material which results in 99.9% inhibition of growth) using the R^2 formula on inhibition curve [12], [13].

E. Survival under L. stoechas Essential Oil

Survival of *Escherichia coli, S. aureus* and *C. albicans* were investigated by broth microdilution method in vitro exposure to L. stoechas EO. The percentages of cell viability counts were used to assess logaritmic reduction in cell number compared with negative control (100% survivor). To the formule: The reduction of living cell = The cell number in negative control well (MHB + microorganism) - The cell number in corresponding well (MHB + microorganism + corresponding amount of *L. stoechas* EO).

F. Statistical analysis

Survival All data on antimicrobial activity assay studies were the averages of triplicate analysis. Data were recorded as mean ± SEM (standard error of the mean). Significant differences between means were determined by Tukey HSD (SPSS 25; post hoc-one way ANOVA) test and *p* values <0.05 were regarded as significant

III. Results

The components of EO extracted from *L. stoechas* with their retention time (RT) and area (%) were listed in Table 1. In the present study, linalyl acetate (36.31%), linalool (34.18%), camphor (5.38%), 1,8-cineole (3.98%) and borneol (3.11%) were the major component in the essential oil of *L. stoechas* aerial parts, followed by *lavandulyl acetate* (1.92%), caryophyllene (1.89%), hexyl butyrate (1.45%), *α*-terpineol (1.31%), *β*-farnesene (1.23%), geranyl acetate (1.10%) and less amounts than 1% with camphene, 3-octanone, 3-carene, *cis*-ocimene, *β*-ocimene, neryl acetate, germacrene-D, caryophyllene oxide, *α*-bisabolol, elaidic acid.

Table 1. Chemical composition of *L. stoechas* essential oil. RT: Retention Time. Quantity (%): more than 0.01.

RT	Compound	%	RT	Compound	%
9.913	Camphene	0.20	**24.790**	Linalyl acetate	36.31
11.601	3-octanone	0.33	**25.878**	Lavandulyl acetat	1.92
13.512	3-carene	0.85	**28.945**	Neryl acetate	0.61
13.747	1,8-cineole	3.98	**29.786**	Geranyl acetate	1.10
13.947	*cis*-ocimene	0.70	**31.251**	Caryophylle ne	1.89
14.422	*β*-ocimene	0.75	**32.652**	*β*-farnesene	1.23
17.730	Linalool	34.1 8	**33.682**	Germacrene -D	0.31
19.366	Camphor	5.38	**38.031**	Caryophylle ne oxide	0.63
20.333	Borneol	3.11	**42.237**	*α*-bisabolol	0.81
21.323	Hexyl butyrate	1.45	**57.537**	Elaidic	0.33
21.483	(±)-*α*-terpineol	1.31	**Total**		**99.22**

The 24 hour incubation of *L. stoechas* EO with microorganisms was found to be statistically significant in terms of MIC (*p*<0.05) (Table 2). All microorganisms were found to be sensitive to the essential oil. The MIC of *L. stoechas* EO (32.5 µg/mL) against *E. coli* was found to be statistically significant than *L. stoechas* EO (14.8 µg/mL) on *C. albicans*. The moderately antimicrobial activity were determined against *E. coli* and *S. aureus* with MIC= 32.5 µg/mL and MIC= 21.3 µg/mL, respectively, while maximum MIC were noticed as 14.8 µg/mL against *C. albicans* in *L. stoechas*.

Table 2. MIC of *L. stoechas* against tested microbial strains by microdilution method. For positive control: ampicillin (for bacteria) and fluconazole (for yeast) were used as positive control (128 µg/mL). The average MICs were expressed with the standard deviation (±) and significance level ($p \leq 0.05$) and a: same signs differ statistically at the 0.05 level.

Microorganism/MIC	*L. stoechas*	Antibiotics
E. coli (-)	32.5[a]±3.6	64±3.9
S. aureus **(+)**	21.3±0.8	8±2.8
C. albicans	14.8[a]±1.3	128±4.8

As shown in Table 3, *L. stoechas* EO revealed different inhibition activities towards the three microorganism cells investigated. Inactivation of *E. coli*, *S. aureus* and *C.*

albicans by increased doses of the essential oil was similar in that caused an increase cell death. In general, a dose-dependent decrease in the survival of the microorganisms was observed. The applied essential oil at doses of 8.8, 17.6, 35.2, 70.3, 140.6, 281.3, 562.5, 1125, 2250 and 4500 µg/mL led to inhibition between 3.56% and 71.41% for *E. coli*, 1.17% and 90.90% for *S. aureus*, -5.09% and 91.76% for *C. albicans* ($p<0.05$). At a concentration of 4.5 mg/mL, the inhibition by *L. stoechas* were 71.41% for *E. coli*, 90.90% for *S. aureus*, 91.76% for *C. albicans*.

Table 3. The logaritmic reduction in cell population (R) and dose-dependent cell inhibition in *E. coli (E), S. aureus* (S) and *C. albicans (C)* (%) in the presence of *L. stoechas* essential oil during exposure between the concentration of 4500 µg/mL and 8.789 µg/mL for 24 h.

		Concentration									
		8.78	**17.57**	**35.15**	**70.31**	**140.6**	**281.2**	**562.5**	**1125**	**2250**	**4500**
E	**%**	3.56	7.19	37.28	56.34	64.86	67.36	68.41	69.09	70.55	71.41
	R	0.02	0.03	0.20	0.36	0.45	0.49	0.50	0.51	0.53	0.54
S	**%**	1.17	-3.99	-2.26	-2.42	-2.69	-6.08	-4.81	27.71	50.40	90.90
	R	0.01	-0.02	-0.01	-0.01	-0.01	-0.03	-0.02	0.14	0.30	1.04
C	**%**	- 5.09	-3.06	6.83	5.10	5.13	10.48	13.51	28.98	57.98	91.76
	R	- 0.02	-0.01	0.03	0.02	0.02	0.05	0.06	0.15	0.38	1.08

The applied *L. stoechas* essential oil at doses of 8.8, 17.6, 35.2, 70.3, 140.6, 281.3, 562.5, 1125, 2250 and 4500 µg/mL led to reduction in live cell count between 0.02 and 0.54 log cfu/mL for *E. coli*, 0.01 and 1.04 cfu/mL for *S. aureus*, -0.02 and 1.08 cfu/mL for *C. albicans* ($p<0.05$). The high reduction of living cell were obtain as 1.08 log in *C. albicans* at the concentration of 4.5 mg/mL of *L. stoechas*. The others also: It was 1.04 log in *S. aureus* and 0.54 log in *E. coli* at the same concentration. *E. coli* cell population during exposure in the essential oil (2.25 mg/mL) poured medium decreased 0.53 log according to the control experiments which is without addition of essential oil. *S. aureus* and *C. albicans* cell population decreased 0.30 log and 0.38 log, respectively. As for inactivation of *E. coli*, *S. aureus* and *C. albicans* under the same doses of *L. stoechas* EO, the 1125 mg/mL EO decreased the initial count of 0.51, 0.14, 0.15 log cfu/mL in the control samples (2 log cfu/mL), respectively ($p<0.05$). It was 0.50, -0.02, 0.06 log cfu/mL for *E. coli*, *S. aureus* and *C. albicans* in the 562.5 mg/mL EO, respectively (Figure 1).

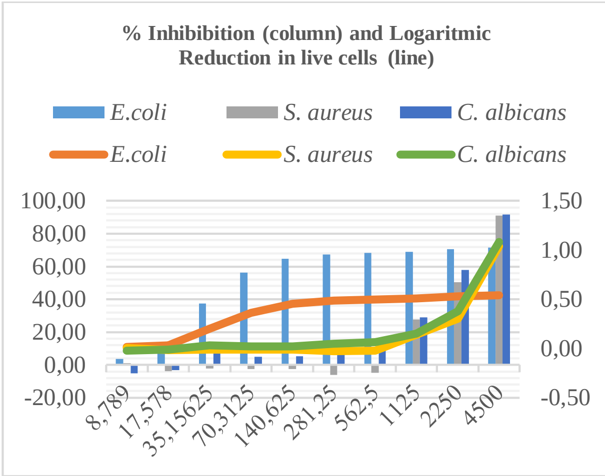

Figure 1. The logaritmic reduction in cell population (line) and dose-dependent cell inhibition in *E. coli. S. aureus* and *C. albicans* in the presence of *L. stoechas* essential oil during exposure between the concentration of 4500 µg/mL and 8.789 µg/mL for 24 h. The cell number in control samples: 2 log cfu/mL

IV. DISCUSSION

Although the most widely common chemotype of *L. stoechas* is the camphor-fenchone, we noted the linalyl acetate, linalool, camphor which have strong pharmacological activities such as antimicrobial, anti-inflammatory [14]. Many authors have attributed this chemical difference to differences in habitat, season and plant origin [15], [16]. In existing literature, the oil of *L. stoechas* were noted mostly consists of linalyl acetate, 1,8-cineole camphene and fenchone [17]. Soylu et al. (2006) obtained that the main components of *L. stoechas* were 1,8-Cineole (35.5%), sabinene (15.0%), α-terpineyl acetate (14.2%), a-pinene (7.5%) [18]. In another study, the major compounds in *L. stoechas* EO were reported to be fenchone (11.27-37.48%), camphor (1.94-21.8%), 1,8-cineole (0.16-8.71%), and viridiflorol (2.89-7.38%) [19]. The GC-MS profile depicted that fenchone (55.79%), camphor (18.18%), 1,8-cineole (8.03%), and myrtenyl acetate (6.25%) were the major components in *L. stoechas* EO [20]. Similarly, the featured constituents of the essential oils from various populations of *L. stoechas* L. ssp. *stoechas* in Greece were α-pinene, 1,8-cineole, fenchone, camphor and myrtenyl acetate, all components of different amounts due to different habitat [21]. The component of fenchone from the stems/leaves and flowers of *L. stoechas* L. ssp. *stoechas* mostly was extracted on varying proportions such as 13.13%, 27.08%, 52.60% [16].

Previous studies showed the essential oils of Lavender species is widely effective against the growth of a wide range of pathogens, particularly, *E. coli*, *S. aureus* and *C. albicans* [22], [23]. These effect comes from the components such as linalyl acetate, linalool, camphor, fenchone,caryophyllene, hexyl butyrate, α-terpineol, β-farnesene that it possesses. The MIC values of L. *angustifolia* essential oil extracted by hydrodestilation and having high linalool ratio (52.59%) were found to be over 1.25 mg/mL. against *E. coli*, *S. aureus* and *C. albicans* [24]. In another study, the essential oils of *L. angustifolia* and *Lavandula intermedia* which include great amount of caryophyllene and camphor, respectively, effectively inhibited against *Staphylococcus aureus*, *E.coli* [4]. Also, Soylu et al. (2006) showed that Lavender EO were strong fungisidal at relatively above concentrations of 12.8 µg/mL [18].

In our study, linalyl acetate (36.31%), linalool (34.18%), camphor (5.38%) were be found high level and they were effective to all pathogens. Similarly, Arıdogan et al (2002) demonstrated that *L. hybrida* contain linalool (32.8%), linalyacetate (29.9%) and had anti-staphylococcus activity (8 mm) and had no anti- *E. coli* activity (0 mm) by disc diffusion method [25]. In another study, the *L. stoechas* EO exhibited good antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans* at the concentrations (MICs) ranging from 0.16 to 11.90 mg/mL [19]. In addition, it was reported that linalyl acetate showed low antibacterial activity with MIC at 7.0-10.0 μg/mL in the microdilution method, while linalool showed bacteriostatic activity at 4.0-7.0 μg/mL and bactericidal effect at 4.0-9.0 μg/mL [26].

L. stoechas EO showed above 70% inhibitory effect at concentration of 4.5 mg/mL on the pathogens. They were 91.76 for *C. albicans*, 90.90% for *S. aureus* and 71.41% for *E. coli*. Normally, the inhibition activity was decreased in the lower concentration of *L. stoechas* EO. The inhibition ratio were 57.98% for *C. albicans*, 50.40% for *S. aureus* and 70.55% for *E. coli* in 2250 mg/mL L. stoechas EO. At the concentration of 1125 mg/mL *L. stoechas* EO, it was 28.98% for *C. albicans*, 27.71% for *S. aureus* and 69.09% for *E. coli*. Remarkably, inhibition activity of *L. stoechas* EO (562.5 mg/mL) were not reported on S. aureus. However, there were significantly activity were noted 68.41% for *E. coli* and 13.51% for *C. albicans* at the same concentration. Few studies have reported the inhibition rate on pathogens of essential oil of *L. stoechas*. *Staphylococcus aureus* was reported to be rather sensitive to *Lavandula stoechas* EO which its main component were fenchone (68.2%) and camphor (11.2%) [17]. Linalool and linalyl acetat caused more than 50% inhibition on *C. albicans* [27].

As can be seen, the logarithmic reduction in the number of cells with percent inhibition is shown in the same graph. The count of viable cells decreased as the concentration of the essential oil increased. For inactivation of microorganisms, the degree of inhibitory effects of *L. stoechas* EO was *E. coli*>*C. albicans*>*S. aureus* at mostly high concentration, for example, 4.5 mg/mL (p<0.05). There is a study about this. Dadalioğlu and Evrendilek (2004) reported that Spanish *L. stoechas* essential oil with the concentration of 5 and 80 µL/mL reduced the live cell count around 4 log cfu/mL. As for inactivation of *S. aureus* under the 5 and 10 µL/mL doses of the lavender decreased the initial count of about 3 log cfu/mL [20].

V. CONCLUSION

In conclusion, the inhibition and antimicrobial potential of *L.* stoechas against *E. coli, S. aureus* and *C. albicans* were demonstrated. All microorganisms were sensitive to the oil and the inhibition ratio and also the reduction in cell count were reported for different concentrations of the oil. The results in our study will shed light on future research.

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