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The Biological Activities of *Lavandula stoechas* L. against Food Pathogens

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Abstract: Foodborne pathogens are microorganisms as well as a number of parasites, which are capable of infecting humans via contaminated food or water. In recent years, diseases caused by foodborne pathogens have become an important public health problem in the world, producing a significant rate of morbidity and mortality. In traditional medicine, numerous plants and their extracts have used for thousands of years to treat health disorders. Although many studies were made on natural herbs, those involving the antimicrobial, antioxidant and antimutagenic activity of the herb species are rather rare. This study researches the biological activities of ethanol and methanol extracts of *Lavandula stoechas* L., which are prevalent in Turkey. In this study, 8 food pathogens were used for antimicrobial activity studies. Antimicrobial activity studies were done by disk diffusion assay and MIC (minimum inhibitory concentration). DPPH method was used for non-enzymatic antioxidant activity. The *Lavandula* extracts were screened for their antimutagenic activity against sodium azide by Ames test in absence of rat microsomal liver enzyme (-S9). The ethanol and methanol extracts of *Lavandula stoechas* showed antibacterial activity (7 mm) against most of bacteria. The antifungal activity of *L. stoechas* was not determined against *C. albicans* RSKK02029. The lowest MIC value was determined as 3250 µg/mL. The highest radical inhibition was determined as 79 % by *Lavandula stoechas* flower methanol extract. The flower extract of *L. stoechas* (12500 µg/plate) was found to have its highest antimutagenic activity for *Salmonella* Typhimurium TA98. This inhibition value is 42 %. *L. stoechas* leaves extracts (6250 and 3125 µg/plate) showed a moderate positive inhibitory effect for *Salmonella* Typhimurium TA98, and TA100. *L. stoechas* flower extracts (12500 and 6250 µg/plate) showed a moderate positive inhibitory effect (respectively 31 and 30 %) for *Salmonella* Typhimurium TA100. The extracts of *L. stoechas* have antimicrobial, antioxidant and antimutagenic activities.

Keywords: *Lavandula, Antimicrobial Activity, Antioxidant Activity, Antimutagenic Activity*

1. INTRODUCTION

More than 200 known diseases are transmitted through food by a variety of agents that include bacteria, fungi, viruses, and parasites. The Center for Disease Control and Prevention [1,2] estimates that 76 million people get sick, more than 300,000 are hospitalized, and 5,000 Americans die each year from foodborne illness. Food items most likely associated with antibiotic resistant pathogens included dairy products, ground beef, and poultry. Pathogens exhibiting multi-drug resistance to five or more antibiotics were identified in more than half of the outbreaks [3]. Scientists have taken up the issue to solve this problem.

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments [4]. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective,

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fewer side effects and their easy availability [5-7]. Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. Today, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs [8].

Genus *Lavandula* belongs to Lamiaceae family and it includes about 39 species however, the most important species are lavender (*Lavandula angustifolia* Mill.), lavandin (*Lavandula intermedia* Emeric.) and spike lavender (*Lavandula spica* L.) [9]. Lavender is native to the Mediterranean and grows in natural sites of lower parts of mountains. Lavender, small shrubby plant, grows 20–60 cm high with irregular, much branched stems. The leaves are opposite, sessile, lanceolate, linear or lance-shaped and hairy. Flowers are produced in the long spikes on long stems. The spikes consist of rings of 6–10 flowers which are bilabiate, small, 0.8 cm long with blue, tubular and ribbed calyx and violet-blue corolla. The majority of the oil, extracted from the flowers, is contained in the glands on the calyx [10]. According to Nartowska [11] lavender flowers contain essential oil and its components: linalyl acetate (40%), linalool (30%), limonene, β-ocymene, 1,8-cineole, camphor, α-terpineol, borneol, but also phenolic acids, ursolic acid, cumarins flavonoids and sterols. Lavender oil (*Lavandulae aetheroleum*) is known for its antibacterial, antifungal, carminative, antiflatulence, antiholic, sedative and antidepressive activities [12]. Lavender oil is used in phytotherapy to relief cough, neuralgia, insomnia but also for bath and compress [13-15]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [16].

The aim of the investigation presented in this paper is to evaluate the antibacterial, antioxidant and antimutagenic activities of extracts of *Lavandula stoechas* on several food pathogens, as there is a significant lack of information on such activities in literature.

2. MATERIAL and METHODS

21. Plant Material

Lavandula stoechas flowers and leaves were collected in May 2014 form 0- 700 m height above sea level in Izmir. The identity was confirmed by Dr. Olcay Ceylan, Department of Biology, Mugla Sitki Kocman University. The voucher specimens were deposited at the Herbarium of Department of Biology, Mugla Sitki Kocman University. The identification of these specimens was carried out using the Flora of Turkey [17].

2.2. Plant Extraction

The flowers and leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water. Fresh plant materials were air dried, and then the dried materials were powdered in a blender. All samples were stored at ambient temperature until initial sample preparation, after which they were stored at 4 $^{\circ}$C until required for analysis. The air dried and powdered flowers and leaves of the plant samples (40 g) were extracted with ethanol and methanol (250 mg/mL) using the Soxhlet apparatus. All experiments were continued for 4 hours. All of extracts were evaporated and then the extracts were dissolved in their solvent and then kept in small sterile opac bottles under refrigerated conditions until used. All of extracts concentrations were set to 100- 200 mg/mL.

2.3. Microorganisms and Cultivation

The extracts were individually tested against food pathogenic strains such as *Bacillus subtilis* RSKK245, *Staphylococcus aureus* RSKK2392, *Salmonella* Typhimurium RSKK19, *Enterococcus faecalis* ATCC8093, *Escherichia coli* ATCC11229, *Listeria monocytogenes* ATCC7644, *Yersinia enterocolitica* NCTC11174 and *Candida albicans* RSKK02029. The

bacteria were grown for 24 hours at 37 °C in Mueller- Hinton Broth (Merck). *C. albicans* was grown for 24- 48 hour at 30 °C in Sabouraud Dextrose Broth (Merck). These strains of bacteria and *C. albicans* were obtained from ATCC (American Type Culture Collection, USA), RSKK (Refik Saydam National Type Culture Collection, Turkey) or NCTC (National Collection of Type Cultures).

2.4. *In vitro* Antimicrobial Activity

Kirby-Bauer method applied for antimicrobial activity [18]. The extracts of plant were tested by disc diffusion assay. The concentration and quantity of extracts were used as 45 µL of 100-200 mg/mL. In this study, ethanol and methanol were used as organic solvents. The bacteria were maintained on Mueller-Hinton agar plates (MHA, Merck) at 37 °C and yeast was maintained on Sabouraud Dextrose agar plates (SDA, Merck) [18]. Bacteria and *C. albicans* RSKK02029 cultures were adjusted to 0.5 McFarland. The experiments were performed in triplicate. Bacteria were incubated at 37 °C in 24 hours. *C. albicans* RSKK02029 was incubated at 30 °C for 24 hours. After incubation, the inhibition zones formed and then the values of zone were measured. Ethanol and methanol used as negative control. Chloramphenicol (30µg), and nystatin (100µg) antibiotics used as positive control.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The MIC was evaluated on plant extracts as antimicrobial activity. The MIC was taken as the lowest concentration that inhibits growth after incubation. The broth dilution assay was performed as described in the CLSI standards [19,20]. This test was performed at final concentrations of each extract (6500; 3250; 1625; 812.5; and 406.25 µg/mL).

2.6. Determination of non-Enzymatic Antioxidant Activity

The non-enzymatic antioxidant activity was determined using DPPH as a free radical. The stable 2,2-dipheny l-1-picrylhydrazyl- hydrate radical (DPPH) was used for determination of free radical scavenging activities of the flower and leaf extracts. Extract (0.1 mL) was added to 3.9 mL of a 0.1 mM methanol DPPH solution. After incubation for 30 minutes, absorbance of extract was measured at 515 nm using spectrophotometer. Methanol was used as a blank, while methanol with DPPH solution was used as a control [21]. Trolox was used for reference antioxidant. The DPPH scavenging capacity expressed in percentage (%) was calculated using the formula.

2.7. Determination of Antimutagenic Activity

Antimutagenic activity was evaluated by the *Salmonella*-microsome assay, using the *Salmonella* Typhimurium tester strains TA98 and TA100, kindly provided by Dr. B.N. Ames (Berkeley, CA, USA), without (−S9) metabolisation by the pre-incubation method [22]. The *Salmonella* histidine point mutation assay of Maron and Ames [22] was used to test the antimutagenic activity of extracts without S9 mix. In this study, two different tester strains were employed to measure the antimutagenicities of *Lavandula stoechas* extracts. These strains included *Salmonella* Typhimurium TA98 and TA100. The calculation percentage of inhibition was done according to the formula given by Ong *et al.*, [23]. Sodium azide was used as positive control. Methanol is negative control. Concurrently, a positive control (where mutagen but no extract was added) and a negative control (where no mutagen was added) were also set. The test sample was dissolved in methanol. But mutagen was dissolved in distilled water. In our study, non-toxic concentrations of the test sample used for investigating were 12500, 6250 and 3125 µg/plate. These concentrations were categorized as non-toxic because they showed a well-developed lawn, almost similar size of colonies and no statistical difference in the number of spontaneous revertants in test and control plates.

3. RESULTS and DISCUSSIONS

The antimicrobial activities of ethanol and methanol extracts of *Lavandula stoechas* were evaluated *in vitro* against 8 microorganisms test species, which are known to cause some diseases in foods. Results of antimicrobial activities of used plant extracts against the test bacteria are shown in Table 1. Besides, the inhibition zone diameters of the reference antibiotics to the test microorganisms are shown in Table 2.

The results of antibacterial activities were recorded as zone of inhibition in mm for all the materials used as follows. Results show that the ethanol extracts of *L. stoechas* inhibited the growth of six bacteria and the inhibition zones were 7 mm. Whereas methanol extracts of this plant inhibited the growth of seven bacteria and similarly the inhibition zones were 7 mm. In addition the ethanolic and methanolic extracts of this plant did not determine any anticandidal effects against used yeast. The extracts showed the same effect on *B. subtilis, S. aureus, L. monocytogenes* and *Y. enterocolitica* (Table 1). Chloramphenicol and nystatin antibiotics used as positive control. Chloramphenicol very strongly inhibited the bacterial growths (Table 2).

Table 1. Antimicrobial activities of *Lavandula stoechas* extracts

Microorganisms	Concentration (mg/mL)	Inhibition zone diameters (mm)				Solvents	
		Lsc		Lsy			
		EE	ME	EE	ME	E	M
B. subtilis RSKK245	100	7	7	-	-	-	-
	200	7	7	-	-	-	-
S. aureus RSKK2392	100	7	7	-	-	-	-
	200	7	7	-	-	-	-
S. Typhimurium RSKK19	100	-	-	-	-	-	-
	200	-	-	-	-	-	-
E. faecalis ATCC8093	100	-	-	-	-	-	-
	200	-	-	-	-	-	-
E. coli ATCC11229	100	-	-	-	-	-	-
	200	-	-	-	-	-	-
L. monocytogens ATCC7644	100	-	-	-	-	-	-
	200	-	7	7	-	-	-
Y. enterocolitica NCTC11174	100	7	7	-	-	-	-
	200	7	7	7	7	-	-
C. albicans RSKK02029	100	-	-	-	-	-	-
	200	-	-	-	-	-	-

Lsc: *Lavandula stoechas* (flower) Lsy: *Lavandula stoechas* (leaf) EE: Ethanol extract ME: Methanol extract.
(-): zone did not occur E: Ethanol M: Methanol

Table 2. Antibiotic profils of food pathogens

Microorganisms	Inhibition zone diameter (mm)	
	Chloramphenicol	Nystatin
B. subtilis RSKK245	12	nt
S. aureus RSKK2392	15	nt
S. Typhimurium RSKK19	22	nt
E. faecalis ATCC8093	22	nt
E. coli ATCC11229	21	nt
L. monocytogenes ATCC7644	22	nt
Y. enterocolitica NCTC11174	20	nt
C. albicans RSKK02029	nt	7

NOTE: nt =not tested

Table 3. shows MICs of *Lavandula stoechas* extracts obtained by the broth dilution method. Three bacteria showed the lowest sensitivity to extracts of *Lavandula stoechas* (3250 µg/mL).

Table 3. Minimum inhibitory concentrations of *Lavandula stoechas* extracts

Microorganisms	Lsc (µg/mL)		Lsy (µg/mL)	
	EE	ME	EE	ME
B. subtilis RSKK245	6500	6500	nt	nt
S. aureus RSKK2392	3250	3250	nt	nt
S. Typhimurium RSKK19	nt	nt	nt	nt
E. faecalis ATCC8093	nt	nt	nt	nt
E. coli ATCC11229	nt	nt	nt	nt
L. monocytogenes ATCC7644	nt	3250	3250	nt
Y. enterocolitica NCTC11174	6500	3250	6500	6500
C.albicans RSKK02029	nt	nt	nt	nt

NOTE: Lsc:*Lavandula stoechas* (flower) Lsy:*Lavandula stoechas* (leaf) nt: Not tested EE: Ethanol extract ME: Methanol extract

The non-enzymatic antioxidant activity of plant extract was evaluated by the DPHH radical scavenging capacity. Table 4 shows the percent of DPPH radical scavenging capacity with trolox as reference. The methanol extract showed 79% inhibition at 200 mg/mL concentration. Trolox equivalent value was 2.2 mM/g (Table 4).

Table 4. DPPH radical scavenging capacity of *Lavandula stoechas*(200mg/mL)

Plants	Ethanol extracts		Methanol extracts	
	DPPH İnhibition (%)	TE	DPPH Inhibition (%)	TE
Lsc	40	1,4	79	2,2
Lsy	26	1,1	67	2,0

NOTE: Lsc: *Lavandula stoechas* (flower) Lsy:*Lavandula stoechas* (leaf) TE: Trolox equivalent (mM/g DW); DW: Dry weight

The antimutagenic activities of the extracts were evaluated by the against sodium azide by Ames test in absence of rat microsomal liver enzyme (-S9). Table 5, 6, and 7 shows the percent of inhibition. The flower extract of *L. stoechas* (12500 µg/plate) was found to have its highest antimutagenic activity for *Salmonella* Typhimurium TA98. This inhibition value is 42%. *L. stoechas* flower extracts (12500 and 6250 µg/plate) showed a moderate positive inhibitory effect (respectively 31 and 30%) for *Salmonella* Typhimurium TA100 (Table 5).

Table 5. Antimutagenic activity of *Lavandula stoechas* extract (12500 µg/plak)

Test substances	*Salmonella* Typhimurium TA98		*Salmonella* Typhimurium TA100	
	Revertant	% Inhibition	Revertant	% Inhibition
Control	22		80	
Negative control	24		88	
Positive control	45		131	
Lsc	26	% 42,2	90	% 31,3

NOTE: Lsc: *Lavandula stoechas* (flower)

Table 6. Antimutagenic activity of *Lavandula stoechas* extract (6250 µg/plak)

Test substances	*Salmonella* Typhimurium TA98		*Salmonella* Typhimurium TA100	
	Revertant	% Inhibition	Revertant	% Inhibition
Control	22		80	
Negative control	24		88	
Positive control	45		131	
Lsc	31	% 31,1	92	% 29,8
Lsy	33	% 26,7	91	% 30,5

NOTE: Lsc:*Lavandula stoechas* (flower) Lsy:*Lavandula stoechas* (leaf)

Table 7. Antimutagenic activity of *Lavandula stoechas* extract (3125 µg/plak)

Test substances	*Salmonella* Typhimurium TA98		*Salmonella* Typhimurium TA100	
	Revertant	% Inhibition	Revertant	% Inhibition
Control	22		80	
Negative control	24		88	
Positive control	45		131	
Lsy	35	% 22,2	93	% 29,0

NOTE: Lsy**:** *Lavandula stoechas* (leaf)

4. DISCUSSION

Medicinal plants have proved to be abundant sources of biologically active compounds, many of which have been used as compounds to develop new pharmaceuticals [24]. *L. stoechas* flowers and leaves were selected based on their relevant ethnomedical use. In the present study, extracts of the plant obtained in solvents were tested against eight microorganisms. The antimicrobial activities were compared with the standard antibiotics. Results show that the ethanol extracts of *L. stoechas* inhibited the growth of six bacteria and the inhibition zones were 7 mm. Whereas methanol extracts of this plant inhibited the growth of seven bacteria and similarly the inhibition zones were 7 mm (Table 1). Gören et al. [25] reported that antimicrobial activities of extracts of *L. stoechas* ssp. *stoechas* were found highly effect on bacteria.

Result of this study showed that tested plant flower extracts were found to same effective against *S. aureus* RSKK2392 and *Bacillus subtilis* RSKK245 (7 mm) (Table 1). Oskay et al. [26] showed that the most susceptible organism was methicillin resistant *S. aureus* (MRSA) which was sensitive to methanol and ethanol extracts. Khosravi and Malecan [27] reported that alcoholic extract of *L. stoechas* has significantly inhibitory effect on the growth of *Staphylococcus aureus*. These results are like with our study.

In this study, the extracts were not inhibited the growths of *S.* Typhimurium and *E. coli* (Table1). Cherrat et al. [28] reported that *Lavandula stoechas* is effective against Gram (+) bacteria and Gram (-) bacteria is weakly effective. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria [29,30]. These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure [31].

In addition, the ethanolic and methanolic extracts of this plant did not determine any anticandidal effects against used yeast (Table 1). Zuzarte et al. [32] reported that the oil of *Lavandula stoechas* had low antifungal activity in their study. According to Adam et al. [33], *Lavandula angustifolia* showed moderate to low antifungal activities against *Malassezia furfur*,

Trichophyton rubrum, and *Trichosporon beigelii*. Uzun et al. [34] reported that *L. stoechas* extract was not inhibited growth of *Candida albicans*. These results are like with our study.

In this study, three bacteria showed the lowest sensitivity to extracts of *Lavandula stoechas* (3250 µg/mL). Ünsal et al. [35] reported that MIC value of *Lavandula* subsp. *stoechas cariensis* was 19.52 µg/mL. Results of our study are higher than their results. In our results, MIC values are 6500 µg/mL for *B. subtilis* and 3250 µg/mL for *S. aureus* (Table 3). Nunes et al. [36] determined that MIC values are 9830 µg/mL for *B. cereus* and 9830 µg/mL for *S. aureus*. Results of our study are better than their results.

In this study, the extracts of *Lavandula stoechas* have different free radical inhibition. The flower methanol extract showed 79% free radical inhibition at 200 mg/mL concentration (Table 4). Researchers found several compounds to be present in *L. stoechas* essential oils are known to possess antioxidant activities. These include eugenol, carvacrol, thymol, terpinolene, α- terpinene, γ-terpinene [37], and terpinen-4-ol [38]. Matos et al. [39] reported that chemical composition of *L. stoechas* were fenchone (42%), camphor (35%) and oxygen-containing monoterpenes (87%). These chemical composition differences might be caused by geographic origins, climatic and seasonal conditions, the time of collection, the stage of development, the method of extraction and even might be correlated to the existence of new chemotypes [40]. Some authors reported the antioxidant activity of *L. angustifolia* and *L. luisieri* extracts [41,42]. Cherrat et al. [28] reported that *Lavandula stoechas* has high antioxidant properties in their study, which supports our work.

The flower extract of *L. stoechas* (12500 µg/plate) was found to have its highest antimutagenic activity for *Salmonella* Typhimurium TA98. This inhibition value is 42 % (Table 5). In determining the antimutagenic potential of a sample, a value smaller than 25% inhibition of the mutagen activity indicates a weak or non-antimutagenic effect, a moderate effect when the value is between 25 and 40% and strong antimutagenicity when the value is greater than 40% [43].

5. CONCLUSION

It is inferred that extracts of *L. stoechas* is moderately effective against food pathogens and can be utilized as sources of natural antimicrobial agents. Our results support the use of this plant in traditional medicine and suggest that some of the plant extracts possess compounds with good antibacterial properties that can be used as antibacterial agents in the search for new drugs.

The results obtained in this report clearly demonstrate that greater part of tested extracts exhibited strong antioxidant activities, particularly, to scavenge free radicals generated from DPPH reagent, especially *Lavandula* flower extract. The methanolic extract of *L. stoechas* flowers, should be beneficial as an antioxidant protection system for the human body against oxidative damage. This plant with high antioxidant ability can be used on development research and may be a source of natural antioxidants for potential exploit in food, cosmetic and pharmaceutical industries. However, further researches are needed to explore the bioactive compounds in this selected medicinal plant. Undoubtedly, the antimicrobial and antioxidant effects of *L. stoechas* should be investigated further. Fractionation and characterization of the active compounds should be do further works to investigate.

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Conflict of Interests

Authors declare that there is no conflict of interests.

6. REFERENCES

[1] CDC (Centers for Disease Control and Prevention). (2003). *Multistate outbreak of Salmonella Serotype Typhimurium infections associated with drinking unpasteurized milk*. MMWR, 52:613. Illinois, Indiana, Ohio, and Tennessee, ABD.

[2] CDC (Centers for Disease Control and Prevention). (2004) *Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food*. MMWR, 53:338. United States.

[3] www.cspinet.org. (2013). DeWaal, C.S., J.D., and Grooters, S.V., M.P.H. *Antibiotic resistance in foodborne pathogens*. Center for Science in the Public Interest, pp22. Washington, DC.

[4] Grabley, S., & Thiericke, R. (1999). The impact of natural products on drug discovery. *Drug Discovery from Nature*, Vol. 3. Springer: Berlin, pp. 51-55.

[5] Ahmed, M., Ahamed, R. N., Aladakatti, R. H., & Ghosesawar, M. G. (2002). Reversible anti-fertility effect of benzene extract of *Ocimum sanctum* leaves on sperm parameters and fructose content in rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 13(1), 51-60.

[6] Singab, A.N.B., El-Beshbishy, H.A., Yonekawa, M., Nomura, T., & Fukai, T. (2005). Hypoglycemic effect of Egyptian *Morus alba* root bark extract: effect on diabetes and lipid peroxidation of streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 100(3), 333-338.

[7] Bhandari, M.R., Jong-Anurakkun, N., Hong, G., & Kawabata, J. (2008). α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata, Haw.). *Food Chemistry*, 106(1), 247-252.

[8] Ogbera, A.O., Dada, O., Adeleye, F., & Jewo, P. I. (2010). Complementary and alternative medicine use in diabetes mellitus. *West African Journal of Medicine*, 29(3), 158-162.

[9] Kara, N., & Baydar, H. (2013). Determination of lavender and lavandin cultivars (*Lavandula* sp.) containing high quality essential oil in Isparta, Turkey. *Turk J Field Crops*, 18, 58-65.

[10] Raev, R.T., Jordanov, R., & Zheljazkov, V. (1996). Induced polyploidy in lavender. *Acta Hort. (ISHS)*, 426, 561–572.

[11] Nartowska, J. (2012). Rośliny lecznicze – lawenda. *Panacea*, 3 (40), 5–7.

[12] Sabara, D., & Kunicka-Styczyńska, A. (2009). Lavender oil–flavouring or active cosmetic ingredient. *Scientific Bulletin of the Technical University of Lodz*, 78, 33-41.

[13] Ghelardini, C., Galeotti, N., Salvatore, G., & Mazzanti, G. (1999). Local anaesthetic activity of the essential oil of *Lavandula angustifolia*. *Planta Medica*, 65(08), 700-703.

[14] Cavanagh, H.M.A., & Wilkinson, J.M. (2002). Biological activities of lavender essential oil. *Phytotherapy Research*, 16(4), 301-308.

[15] Hritcu, L., Cioanca, O., & Hancianu, M. (2012). Effects of lavender oil inhalation on improving scopolamine-induced spatial memory impairment in laboratory rats. *Phytomedicine*, 19(6), 529-534.

[16] Nascimento, G.G.F., Locatelli, J., Freitas, P.C., & Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247-256.

[17] Davis, P.H. (1988). *Flora of Turkey and East Aegean Islands*. Edinburgh: University Press.

[18] Bauer, A.W., Kirby, W.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493.

[19] CLSI (Clinical and Laboratory Standarts Institute). (2003). *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically- 6th Edition*. Approved Standard M7-A, CLSI, Wayne, Philadelphia, USA.

[20] CLSI (Clinical and Laboratory Standarts Institute). (2006). *Performance Standards for Antimicrobial Susceptibility Testing -16th Edition*. Informational supplement, M100-S16, CLSI, Wayne, Philadelphia, USA.

[21] Brand-Williams, W., Cuvelier, M.E., & Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30.

[22] Maron, D.M., & Ames, B.N. (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutation Research*, 113(3-4), 173-215.

[23] Ong, T.M., Whong, W.Z., Stewart, J., & Brockman, H.E. (1986). Chlorophyllin: a potent antimutagen against environmental and dietary complex mixtures. *Mutation Research*, 173(2), 111-115.

[24] Palombo, E.A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. *Evidence-Based Complementary and Alternative Medicine*, 2011. doi: http://dx.doi.org/10.1093/ecam/nep067.

[25] Gören, A.C., Topçu, G., Bilsel, G., Bilsel, M., Aydoğmuş, Z., & Pezzuto, J.M. (2002). The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. stoechas. *Zeitschrift für Naturforschung C*, 57(9-10), 797-800.

[26] Oskay, M., Oskay, D., & Kalyoncu, F. (2009). Activity of some plant extracts against multi-drug resistant human pathogens. *Iranian Journal of Pharmaceutical Research*, 8(4), 293-300.

[27] Khosravi, A., & Malecan, M. (2004). Effects of *Lavandula stoechas* extracts on *Staphylococcus aureus* and other Gram negative bacteria. *J Qazvin Univ Med Sci* , 7(5), 3-9.

[28] Cherrat, L., Espina, L., Bakkali, M., Pagán, R., & Laglaoui, A. (2014). Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium*, *Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes. *Innovative Food Science & Emerging Technologies*, 22, 221-229.

[29] Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jäger, A.K., & Van Staden, J. (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology*, 68(1), 267-274.

[30] Parekh, J., & Chanda, S. (2006). *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L.(Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *African Journal of Biomedical Research*, 9, 89-93.

[31] Yao, J.D., Moellering R.C. (1995). Antibacterial agents. In: Manual of Clinical Microbiology, (Eds. Murray, P., Baron, E., Pfaller, M., Tenover, F., Yolken, R.). *American Society of Microbiology*, Washington DC, 1281-1290.

[32] Zuzarte, M., Gonçalves, M.J., Cavaleiro, C., Cruz, M.T., Benzarti, A., Marongiu, B., & Salgueiro, L. (2013). Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. *Industrial Crops and Products*, 44, 97-103.

[33] Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., & Arsenakis, M. (1998). Antifungal activities of *Origanum vulgare* subsp. hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *Journal of Agricultural and Food Chemistry*, 46(5), 1739-1745.

[34] Uzun, E., Sariyar, G., Adsersen, A., Karakoc, B., Ötük, G., Oktayoglu, E., & Pirildar, S. (2004). Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. *Journal of Ethnopharmacology*, 95(2), 287-296.
[35] Ünsal, Ç., Vural, H., Sariyar, G., Özbek, B., & Ötük, G. (2010). Traditional medicine in Bilecik province (TURKEY) and antimicrobial activities of selected species. *Turkish Journal of Pharmaceutical Sciences*, 7(2), 139-150.
[36] Nunes, R., Pasko, P., Tyszka-Czochara, M., Szewczyk, A., Szlosarczyk, M., & Carvalho, I.S. (2017). Antibacterial, antioxidant and anti-proliferative properties and zinc content of five south Portugal herbs. *Pharmaceutical Biology*, 55(1), 114-123.
[37] Ruberto, G., & Baratta, M.T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*, 69(2), 167-174.
[38] Lertsatitthanakorn, P., Taweechaisupapong, S., Aromdee, C., & Khunkitti, W. (2006). *In vitro* bioactivities of essential oils used for acne control. *International Journal of Aromatherapy*, 16(1), 43-49.
[39] Matos, F., Miguel, M.G., Duarte, J., Venâncio, F., Moiteiro, C., Correia, A.I., & Pedro, L.G. (2009). Antioxidant capacity of the essential oils from *Lavandula luisieri, L. stoechas* subsp. Lusitanica, *L. stoechas* subsp. Lusitanica x *L. luisieri* and *L. viridis* grown in Algarve (Portugal). *Journal of Essential Oil Research*, 21(4), 327-336.
[40] Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223-253.
[41] Hohmann, J., Zupkó, I., Rédei, D., Csányi, M., Falkay, G., Máthé, I., & Janicsák, G. (1999). Protective effects of the aerial parts of *Salvia officinalis*, *Melissa officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation. *Planta Medica*, 65(06), 576-578.
[42] Beirao, A.R.B., & Bernardo-Gil, M.G. (2005). Antioxidant from *Lavandula luisieri*. *Proceeding of 2nd Mercosur Congress on Chemical Engineering and 4th Mercosur Congress on Process Systems Engineering*. Costa Verde, Rio de Janeiro, Brazil.
[43] Negi, P. S., Jayaprakasha, G. K., & Jena, B.S. (2003). Antioxidant and antimutagenic activities of pomegranate peel extracts. *Food Chemistry*, 80(3), 393-397.