International Journal of Engineering

Journal Homepage: www.ije.ir

Chemical Composition, Antibacterial and Analgesic Activity of *Lavandula stoechas* Flowers from North of Iran

M. Khavarpour*[a], S. M. Vahdat[b], S. Kazemi[c], A. A. Moghadamnia[d], O. Hasanzadeh[a], Z. Salimi[a], N. Rahmanpour[a]

[a] *Department of Chemical Engineering, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*
[b] *Department of Chemistry, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*
[c] *Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran*
[d] *Department of Internal Medicine, Babol University of Medical Sciences, Babol, Iran*

PAPER INFO

Paper history:
Received 08 Mach 2019
Received in revised form 21 July 2019
Accepted 28 July 2019

Keywords:
Antibacterial Activity
Chemical Composition
Essential Oil
Extracts
Lavandula stoechas

ABSTRACT

Aromatic plants have subscribed to the belief that they can be used in food, perfumery, pharmaceutical and cosmetic products due to their nutritional and biological properties. The present study was designed to examine the chemical composition and antibacterial and analgesic properties of *Lavandula stoechas* flowers originating Amol, north of Iran for the first time. The essential oil of *Lavandula stoechas* flowers was obtained by hydrodistillation. Gas chromatography-mass spectrometry examination was used for its analysis. *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis and Bacillus subtilis* were utilized to evaluate the antibacterial activity of essential oil using agar disk diffusion and microdilution assayes. GC-MS analysis revealed Linalol (35.69%) Borneol (14.99%) and 1,8-Cineole (11.45%) as the main compounds of the oil. Antibacterial assayes of various dilutions of essential oil revealed its remarkable antibacterial activity against gram-positive *Staphylococcus aureus.* Furthermore, the water and ethanolic extracts inhibited the pain resulting from hot-plate test. The results proposed *lavandula stoechas* from Amol as an alternative natural bioactive compound which posses antibacterial and analgesic effects to use in pharmaceutical industries.

***doi**: 10.5829/ije.2019.32.08b.02*

1. INTRODUCTION

Lavandula stoechas belongs to the *Lamiaceae* family known as one of the most widely used shrub aromatic plant in traditional medicine, remedy and as preservatives in growth inhibition of food-borne bacteria [1]. It is one of the most cultured and wild species in the Mediterranean region and many regions of Asia [2]. *Lavandula stoechas*, commonly known as "*Ostokhoddous*" in Iran, is a flowering plant with 30 to 100 cm hight, leaves of 1-4cm long and flowers of pink to purple color [3]. Extensive research on *Lavandula* essential oil has shown many its pharmacological activities including anti-inflammatory [4], anti-spasmodic, anticonvulsant, analgesic, sedative, antioxidant [5], antimicrobial and muscle relexant [6]. Literature review demonestrated that the oil contained a complex mixture of bioactive compounds such as linalool, linalyl acetate, 1,8-cineole, fenchone α-pinene and camphor [7]. El Idrissi et al. [8] reported Fenchone (12.55%), α-Campholéne aldehyde (12.08%) and myrtenyl acetate (11.46%) were the major constituents of the essential oil of *Lavandula stoechas* flowers in Morocco. In another study from Morocco, the main components of essential oils were identified as fenchone (31.81%), camphor (29.60%), terpineol (13.14%) and menthone (8.96%) [9]. Zuzarte et al. [10] showed that *Lavandula stoechas* originating from Sardinia Island (Italy) contained high amounts of fenchone and camphor (37.0% and 27.3%, respectivelys).

Several attempts have been also made to describe the antibacterial properties of *Lavandula Stoechas* essential oils from various region. Bouzouita et al. [11] and El Idrissi et al. [8] stated the antibacterial potential of *Lavandula stoechas* oils from Tunisia and Morocco against six bacteria, respectively. The results showed

*Corresponding Author Email: *mkhavarpoor@yahoo.com* (M. Khaarpour)

Please cite this article as: M. Khavarpour, S. M. Vahdat, S. Kazemi, A. A. Moghadamnia, O. Hasanzadeh, Z. Salimi, N. Rahmanpour, Chemical Composition, Antibacterial and Analgesic Activity of *Lavandula stoechas* Flowers from North of Iran, International Journal of Engineering (IJE), IJE TRANSACTIONS B: Applications Vol. 32, No. 8, (August 2019) 1065-1073

that *Staphylococcus aureus* was the more sensitive strain. In spite of several studies undertaken on this herb, there is no information on *Lavandula stoechas* grown in Amol. As far as we know, it is a first report on chemical composition, antibacterial and analgesic activities of *Lavandula stoechas* from Amol to evaluate its potential application as an antibacterial and anti-pain agent in food and pharmaceutical industries.

2. MATERIALS AND METHODS

2. 1. Plant Material The flowers of *Lavandula stoechas* were collected during the flowering period in June 2016 from Amol, Mazandaran province (North of Iran). Samples were air-dried in shadow for 4 days.

2. 2. Plant Essential Oil and Extract Preparation The dried flowers were ground and 100 g of obtained powders (mesh<25) in 1 L of water was subjected to hydrodistillation for 3 h in Clevenger-type apparatus [12]. The collected essential oil was dried over anhydrous sodium sulphate and after filtration, weighed and stored in a sealed dark vials at 4 ºC until tested and used. The yield was calculated based on dry weight of the sample. For preparation of plant extract, 100 g of powdered dry flowers were extracted with ethanol and water separately by maceration. The extracts were collected and filtered by Whatman filter papers. The yielded extracts were concentrated under vacuum and kept in a dark bottle in a refrigerator at 4 °C until use [13].

2. 3. Gas Chromatography - Mass Spectrophotometry Analysis Separation and identification of essential oil components were performed using gas chromatography-mass spectrophotometry (GC-MS) analysis. The analysis was done on an Agilent 6890A gas chromatograph equipped with a Flame Ionisation Detector (FID), using HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) coupled to a Mass Selective Detector MSD 5975C in EI mode (ionization energy voltage 70 eV). Helium was selected as carrier gas at a flow rate of 1.0 ml/min. Initial column temperature was 60°C with 4 min hold and was raised at the rate of 6 °C/min to 250 °C. Injector and detector temperatures were set at 280 °C. Essential oil samples (1μl) in hexane (HPLC grade) were autoinjected. All components were identified by comparison of their spectra and retention time with those of the Wiley and NIST Libraries in the computer library and literature [14, 15]. Percentage composition was determined using the summation of the peak areas of the entire oil composition.

2. 4. Microbial Strains and Antibacterial Assays *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633 and *Salmonella enteritidis* ATCC 10708 were used in this study. The bacteria species were maintained in Mueller Hinton Agar. The antibacterial activity of the essential oils were assessed by agar disc diffusion and microplate methods.

In agar disc diffusion method, bacteria were cultured overnight at 37°C in Mueller Hinton agar and then adjusted with sterile saline to concentration of 1.5×10^8 CFU/ml. Then, a suspension of the tested bacteria was spread on nutrient agar. Different dilutions of essentiall oils were prepared with DMSO (1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128). Filter paper discs (6 mm in diameter) were individually impregnated with 10 μl of the undiluted and diluted oil aliquots and then placed on the earlier prepared agar plates. The Petri dishes were kept at 4°C for 2 h and then incubated at 37°C for 24 h [9]. Gentamicine and Streptomicin (each of 10 μg) were used as a positive control. Negative controls were prepared using DMSO. The inhibition zone diameters (mm) were measured and considered as antibacterial activity. All tests were carried out in triplicate and reported as means ± standard deviation.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of essential oils against the examined microorganisms were determined by the broth microdilution assay in 96-well microtitre plates [16]. The bacterial suspension were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. A total of 100 μl of essential oil and its serial dilutions were added into sterile round-bottom 96-well plates containing inocula (100 μl) of the tested microorganism. The final volume in each well was 200 μl. Then, microplates were incubated for 24h at 37°C. The MIC was refered to the lowest concentration of essential oil at which no visible growth of the strains was observed. MBC was determined by sampling broth from each well and inoculated in nutrient agar for 24h at 37°C. The lowest concentration of the essential oil with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum.

2. 5. Animals The tests were done on Swiss male mice with mean weight of 18–25 g. Animals were maintained over a 12 h light-dark cycle and humidity of 50–60% at an environment temperature of 23 ± 2°C, in the animal room of the School of Pharmacy, Mazandaran University of Medical Sciences, Iran. They were fed a standard diet and water ad-libitum before use. In each group, six mice were studied. The experiments were performed in a quiet room and in the light period of 8 am to 5 pm [17].

2. 6. Analgesic Properties of the Extract and Hot-plate Test Five groups of six mice were used

for injection as follows: group I – saline solution with 10% Tween 80 (negative control), groups II, III, and IV – different dose of water or ethanolic extract (100, 200 and 400 mg/kg), and group V –10 mg/kg morphine (positive control). Ethanolic extract solutions were prepared in saline solution with 10% Tween 80. In each experiment, mice were injected with 2ml of solution into the subplantar space of the right hind paw. Then, 15, 30, 45, 60, 75 and 90 min after injection, the animals were placed individually on a hot plate (Pars AZMA Co., Isfahan, Iran), maintained at 52 ± 5 ˚C and the time between the placement of the animal on the hot plate and the occurrence of either the licking of the hindpaws, shaking or jumping off from the surface was recorded as the response latency [18]. A cut-off time of 40 s was considered if the animal did not show any reaction to the painful stimulus.

3. RESULT AND DISCUSSION

3. 1. Chemical Composition of Essential Oil

The essential oil of *lavandula stoechas* flowers was in high yield of 1.2%. The GC-MS chromatogram of compounds detected in essential oil is shown in Figure 1. The identified constituents, retention times, chemical structure and their percentage in the oil composition is summarized in Table 1. The GC/MS analysis of oil showed a total of 52 volatile compounds representing 99.40 of the total oil. The most abundant components of flowers oil was Linalol (35.69%) followed by Borneol (14.99%), 1,8-Cineole (11.45%), camphor (4.32%), 4-terpineol (3.72%), α-Bisabolol (3.67%) and cis-Ocimene (3.54%) as the main compounds. The results show that the flowers oil consist of 13 monoterenes, 14 oxygenated monoterpenes, 8 sesquiterpenes and 5 oxygenated sesquiterpenes. Oxygenated monoterpene represented the main fraction of the oil. Esters, alcohols, aldehydes, ketones and diterpenes were other components which exist in the *Lavandula stoechas* oil (Table 1).

In the previous report by El Idrissi et al. [8] indicated that only 18 components were present in arial parts of *Lavandula stoechas* (flowers), correspond to 80.68% of the total oil. Many authors also reported different components in *Lavandula stoechas* essential oil from Turkey [19], Australia [20], Algeria [21] and southern Sardinia (Italy) [22]. These qualitative and quantitative differences between the present results and other studies could be attributed to geographical origins, harvest time and environmental factors [23]. Differences between constituents of the essential oil will also lead to differences in nutritional and medicinal uses. However, literture review shows that Linalool, 1,8-cineole, linalyl acetate, camphor and fenchone are the main oxygenated monoterpenes compounds in most *Lavandula* species [24], which are in accordance with our study (Table 1).

3.2. Antimicrobial Potential of *Lavandula stoechas* Essential Oil

In vitro antimicrobial activities of *Lavandula stoechas* essential oils were assessed using disk diffusion and microdilution techniques. Table 2 represents the inhibition zone of undiluted and diluted essential oils determined against tested microorganisms. The result showed that the essential oil exerted different inhibiting activity. The gram-positive *Staphylococcus aureus* was the most susceptible strain examined to the undiluted oil of *Lavandula stoechas* flowers with the strongest inhibition zone (32±1.29 mm). On the contrary, the antimicrobial activity of the essential oils against the gram-negative *Salmonella enteritidis* was the lowest for *Lavandula stoechas* flowers. The essential oil of *Lavandula stoechas* flowers with dilution of 1/32 showed a good activity for *Staphylococcus aureus* compared to gentamicin and streptomicin antibiotics (10 µg). Furthermore, all undiluted essential oils depicted considerable antibacterial activity compared with that of standard antiniotics. Our results were in accordance with result previously reported by El Idrissi et al. [8] which in *Staphylococcus aureus* showed the highest sensitivity to essential oils. Additionally, some oxygenated monoterpenes, such as linalool, borneol, camphor and 1,8-cineol were corroborated to have different amounts of antibacterial potential against various bacteria [25]. Therefore, the significant antibacterial properties of the essential oil of *lavandula stoechas* is correlated to the compounds present in this essential oil.

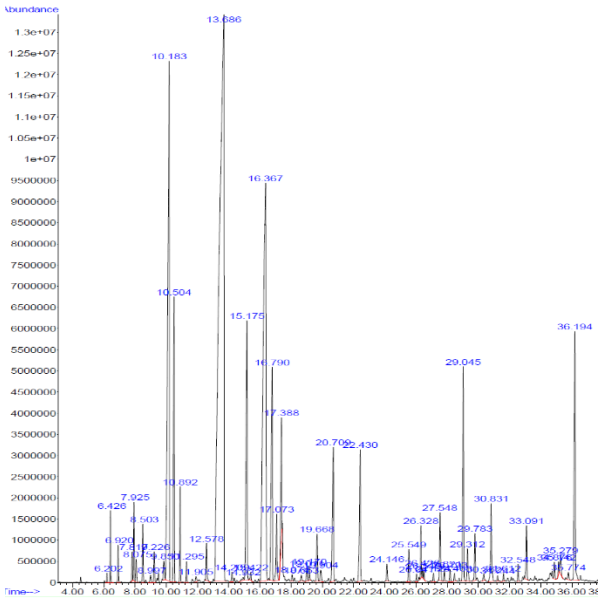
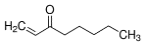
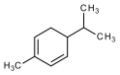
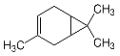
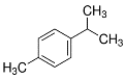
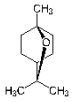
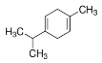
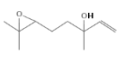
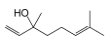
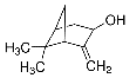
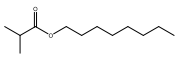
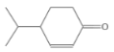
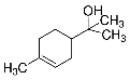
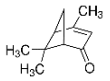
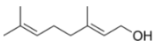
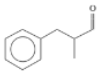
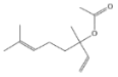
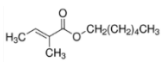
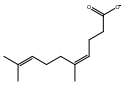
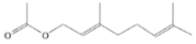
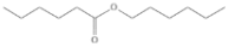
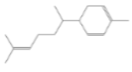


Figure 1. GC-MS Chromatogram of the Essential Oils of *Lavandula stoechas* Flowers

TABLE 1. Chemical composition of the essential oil from *Lavandula stoechas* flowers identified by GC/MS analysis

No	Component	RT(min)	(%)	Exact mass (g/mol)	Chemical structure	MS Fragment-ions	Formula
1	α-Thujene	6.204	0.07	136.234		57, 65, 77, 93, 105, 121, 136	$C_{10}H_{16}$
2	α-Pinene	6.425	0.58	136.24		55, 67, 77, 90, 93, 105, 113, 121, 128, 136	$C_{10}H_{16}$
3	Camphene	6.918	0.32	136.24		67, 93, 121	$C_{10}H_{16}$
4	Sabinene	7.816	0.25	136.23		57, 63, 69, 77, 87, 93, 105, 115, 121, 128, 136	$C_{10}H_{16}$
5	2-β-Pinene	7.924	0.69	136.23		69, 93, 121	$C_{10}H_{16}$
6	1-Octen-3-ol	8.073	0.27	128.21		57, 66, 72, 79, 85, 91, 99, 110, 127	$C_8H_{16}O$
7	β-Myrcene	8.505	0.56	136.23		55, 69, 79, 93, 107, 121, 136	$C_{10}H_{16}$
8	1-Phellandrene	8.998	0.09	136.24		55, 65, 71, 77, 84, 93, 105, 115, 121, 136	$C_{10}H_{16}$
9	3-Carene	9.229	0.32	136.24		58, 67, 77, 93, 105, 121, 129, 136, 152	$C_{10}H_{16}$
10	Benzene, 1-methyl-4-(1-methylethyl)-	9.85	0.27	134.21		65, 91, 119, 136	$C_{10}H_{14}$
11	1,8-Cineole	10.184	11.45	154.249		55, 63, 71, 81, 93, 100, 108, 115, 125, 139, 154	$C_{10}H_{18}O$
12	cis-Ocimene	10.502	3.54	136.24		67, 93, 121, 354, 446	$C_{10}H_{16}$
13	B-trans-Ocimenen	10.892	0.91	136.24		55, 67, 79, 86, 93, 105, 115, 121, 128, 136	$C_{10}H_{16}$
14	γ-Terpinene	11.293	0.21	136.23		65, 93, 115, 136	$C_{10}H_{16}$
15	Cis- Linaloloxide	11.904	0.08	170.252		59, 68, 83, 94, 101, 125, 137, 155	$C_{10}H_{18}O_2$
16	α-Terpinolene	12.577	0.42	136.24		67, 93, 121, 155	$C_{10}H_{16}$
17	Linalool L	13.686	35.69	154.25		71, 93, 121, 154	$C_{10}H_{18}O$
18	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	14.210	0.13	154.2493		58, 71, 79, 86, 93, 103, 111, 121, 139, 154	$C_{10}H_{18}O$

19	trans-Pinocarveol	14.934	0.08	152.23		56, 70, 83, 92, 109, 119, 134	$C_{10}H_{16}O$
20	Camphor	15.175	4.32	152.23		55, 69, 81, 95, 108, 123, 137, 152	$C_{10}H_{16}O$
21	Isobutyric acid, octyl ester	15.422	0.08	200.3178		71, 89, 109, 138	$C_{12}H_{24}O_2$
22	Borneol L	16.367	14.99	154.25		55, 62, 69, 81, 95, 103, 110, 121, 128, 139, 154	$C_{10}H_{18}O$
23	4-Terpineol	16.788	3.72	154.249		57, 64, 71, 79, 86, 93, 103, 111, 118, 125, 136, 154	$C_{10}H_{18}O$
24	2-Cyclohexen-1-one, 4-(1-methylethyl)-	17.075	0.87	138.207		56, 67, 74, 81, 89, 96, 103, 110, 123, 131, 138, 150	$C_9H_{14}O$
25	α-Terpineol	17.379	1.67	154.25		59, 67, 74, 81, 93, 100, 107, 114, 121, 129, 136, 154	$C_{10}H_{18}O$
26	Verbenone	18.072	0.10	150.22		56, 67, 77, 84, 91, 99, 107, 115, 122, 135, 150	$C_{10}H_{14}O$
27	Trans -(+)-Carveol	18.652	0.09	152.24		55, 84, 109, 137, 354, 446	$C_6H_{10}O$
28	Bornyl formate	19.011	0.16	182.263		67, 95, 121, 137, 167	$C_{11}H_{18}O_2$
29	trans-Geraniol	19.171	0.17	154.25		57, 69, 84, 93, 103, 111, 121, 139, 154, 166	$C_{10}H_{18}O$
30	Propanal, 2-methyl-3-phenyl-	19.669	0.64	148.2017		57, 69, 77, 85, 92, 103, 111, 119, 133, 148, 158, 171	$C_{10}H_{12}O$
31	Linalyl acetate	20.711	2.44	196.29		55, 69, 80, 93, 107, 121, 136, 154, 169, 196	$C_{12}H_{20}O_2$
32	Lavandulyl acetate	22.431	1.95	196.29		55, 69, 80, 93, 107, 121, 136, 150, 164, 181	$C_{12}H_{20}O_2$
33	Hexyl Tiglate	24.147	0.21	184.2753		55, 83, 101, 124, 151, 169, 353	$C_{11}H_{20}O_2$
34	Neryl acetat	25.549	0.35	196.29		56, 69, 80, 93, 107, 121, 136, 154, 166, 196	$C_{12}H_{19}O_2$
35	Geranyl acetate	26.329	0.55	196.29		69, 93, 121, 154, 196, 446	$C_{12}H_{20}O_2$
36	Hexanoic acid, hexyl ester	26.427	0.08	200.3178		56, 69, 84, 99, 107, 117, 129, 144, 157, 168	$C_{12}H_{24}O_2$
37	Zingiberene	26.819	0.18	204.1878		69, 93, 119, 161, 189, 446	$C_{15}H_{24}$

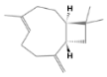
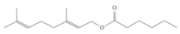
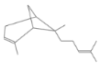
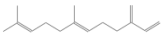
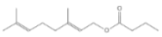
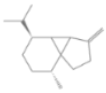
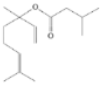
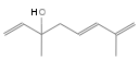
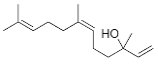
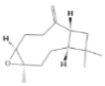
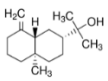
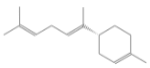
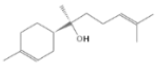
38	trans-Caryophyllene	27.549	0.87	204.36		69, 93, 133, 161, 189	$C_{15}H_{24}$
39	geranyl hexanoate	29.818	0.13	252.398		57, 69, 80, 93, 107, 121, 136, 155, 177, 189, 203, 224	$C_{10}H_{28}O_2$
40	α-Bergamotene	28.193	0.13	204.357		55, 69, 79, 93, 107, 119, 133, 149, 161, 175, 189, 204	$C_{15}H_{24}$
41	β-Funebrene	28.471	0.10	204.3511		69, 93, 112, 133, 161, 179, 204	$C_{15}H_{24}$
42	Trans-β-Farnesene	29.046	2.64	204.357		69, 93, 113, 133, 161, 204, 446	$C_{15}H_{24}$
43	Geranyl butyrate	29.313	0.40	224.344		57, 69, 80, 93, 107, 121, 136, 155, 168, 181, 204, 224	$C_{14}H_{24}O$
44	β-Cubebene	29.785	0.52	204.357		55, 81, 105, 133, 161, 204	$C_{15}H_{24}$
45	bicyclogermacrene	30.36	0.09	204.357		55, 68, 79, 93, 107, 121,134, 147, 161, 175, 189, 204, 220	$C_{15}H_{24}$
46	Linalyl isovalerate	30.833	1.07	238.371		57, 69, 80, 93, 107, 121, 136, 156, 169, 204, 222, 238	$C_{15}H_{26}O_2$
47	Hotrienol	31.613	0.08	152.237		57, 71, 82, 93, 107, 119, 133, 145, 160, 173, 187, 202, 218	$C_{10}H_{16}O$
48	Nerolidol	32.548	0.18	222.37		55, 69, 81, 93, 107, 123, 136, 147, 161, 177, 189, 204, 220	$C_{15}H_{26}O$
49	Caryophyllene oxide	33.092	0.61	220.356		55, 69, 79, 93, 109, 121, 135, 149, 161, 177, 187, 205, 220	$C_{15}H_{24}O$
50	β-Eudesmol	35.141	0.10	222.372		58, 81, 105, 134, 161, 204, 446	$C_{15}H_{26}O$
51	Trans-α-Bisabolene	35.28	0.31	204.357		59, 69, 81, 93, 105, 121, 132, 143, 161, 179, 189, 204, 220, 238	$C_{15}H_{24}$
52	α-Bisabolol	36.194	3.67	222.3663		69, 109, 134, 161, 183, 204, 354, 446	$C_{15}H_{26}O$
Monoterpene Hydrocarbos			8.23				
Oxygenated monoterpenes			73.25				
Sesquiterpene hydrocarbons			4.84				
Oxygenated sesquiterpens			5.63				
Other			7.45				
Total identified			99.40				

TABLE 2. Diameter of Microbial Inhibition Zones (mm) Determined by Disk Diffusion Assay

Plant name and standard	Oil dilutions and antibiotics weight	Gram positive bacteria		Gram negative bacteria	
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Salmonella enteritidis*
Lavandula stoechas flowers	1	32±1.29	28±1.50	29±2.10	20±1.10
	1/2	29±1.05	26±1.30	27±1.40	16±0.90
	1/4	28±0.99	25±0.90	23±1.90	13±0.20
	1/8	28±0.92	20±0.89	22±0.90	10±0.10
	1/16	23±0.40	17±0.78	17±0.90	10±0.09
	1/32	20±0.75	14±0.60	15±0.40	10±0.02
	1/64	20±0.45	12±0.30	11±0.30	-
	1/128	11±0.60	11±1.10	11±0.10	-
Gentamicin	10µg	24±1.20	19±0.10	19±1.10	18±0.90
Streptomicin	10µg	20±0.21	14±0.09	16±1.00	15±0.10

All tests were performed in triplicate. Values are given as mean ± SD (n=3)

The results of the MIC and MBC indicated that the oil exhibited significant, but various levels of antibacterial activity against all tested strains (Table 3). The strong antibacterial activity of the essential oil of *Lavandula stoechas* flowers can be ascribed to the presence of high concentration of oxygenated monoterpenes (72.48 %) [26]. The flowers essential oil depicted the highest activity against gram-positive *Staphylococcus aureus* with the MIC and MBC values of 1/64 and 1/32 (stated as the dilution of essential oils), respectively. However, *Salmonella enteritidis* showed less susceptibility. Our results demonstrated that gram-positive bacteria were more sensitive to the essential oil than gram-negative bacteria, which is in accord to some previous reports [9, 11]. The higher susceptibility of gram-positive bacteria may be attributed to their cell membrane structure which restricts diffusion of hydrophobic compounds through its outer wall due to lipopolysaccharide layers [1]. The ability of *Lavandula* essential oils to inhibit gram-positive and gram-negative bacteria growth suggests that these essential oils can have different application in the food and pharmaceutical industries.

TABLE 3. MIC and MBC of *Lavandula stoechas* Essential Oil against the Test Bacteria

Microoganism	*Lavandula stoechas* flowers	
	MIC	MBC
Staphylococcus aureus	1/64	1/32
Bacillus subtilis	1/32	1/16
Escherichia coli	1/32	1/32
Salmonella enteritidis	1/8	1/4

MIC: Minimum inhibitory concentration (as oil dilution)
MBC: Minimum bactericidal concentration (as oil dilution)

3.3. Analgesic Potential of *Lavandula stoechas* Extract The mean response latency to pain stimulus in hot plate test is shown in Figure 2. The results revealed that the effect of both extracts was dose dependent. It is proposed that different doses of extracts may exhibit different effects on the pain system. The mean response latency reached to a maximum of 31.66 s for dose of 400 mg/kg water extract in the case of placement of the mice on the hot plate at 60 min after injection. Furthermore, the pain latency time of 400 mg/kg water extract was more than morphine for times ranging 60 to 90 min.

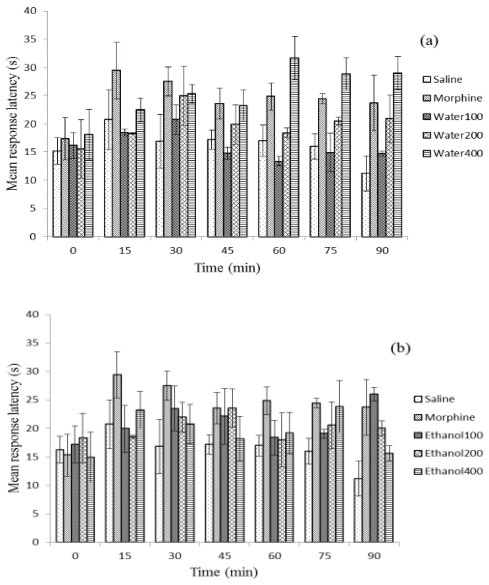


Figure 2. The effect of *Lavandula stoechas* (a) water and (b) ethanol extract on the pain induced by hot-plate test. The results were shown by (mean ± SEM) the time of pain in 6 month

However, results showed that morphine (10 mg/kg) was more effective than ethanol extract. In total, the injection of *Lavandula stoechas* extract to mice inhibited pain in the hot-plate test. Since, the analgesic effect of 1,8-cineole and Linalool was confirmed in some study [27, 28], the extracts may reach in 1,8-cineole and Linalool which prevent the pain resulting from hot-plate test.

4. CONCLUSION

The essential oil of *Lavandula stoechas* flowers from Amol was examined for the first time. Fifty-two constituents were identified in the flowers essential oil. The main components of the oils were mostly monoterpene hydrocarbons and oxygenated monoterpenes. Antibacterial assays highlighted that the essential oil at various dilutions ranged from 1/2 to 1/128 were active against examined bacteria especially gram-positive strains. *Staphylococcus aureus* was the more sensitive strain noted by large growth inhibition halos. The injection of water and ethanolic extracts to mice depicted inhibition effect on pain induced by hot-plate test. Thanks to high amounts of oxygenated monoterpenes and monoterpenes compounds of oil which have therapeutic and nutritional properties, the studied *Lavandula stoechas* has potential applications as possible natural alternatives to chemical-based antibacterial and anti-pain agents in food, pharmaceutical and cosmetic industries. For further studies, it is proposed to assay other pharmacological activities of the mentioned *lavandula stoechas* such as antioxidant and anti-inflammatory effects.

5. ACKNOWLEDGMENTS

The authors are thankful for the facilities provided to carry out this research in laboratory of Ayatollah Amoli Branch, Islamic Azad University.

6. REFERENCES

1. Burt, S., "Essential oils: their antibacterial properties and potential applications in foods-a review", ***International Journal of Food Microbiology***, Vol. 94, No.3, (2004), 223-253.
2. Tuttolomondo, T., Dugo, G., Ruberto, G., Leto, C., Napoli, E.M., Potorti, A.G., Fede, M.R., Virga, G., Leone, R. and Anna, E.D., "Agronomical evaluation of Sicilian biotypes of *Lavandula stoechas L. spp. stoechas* and analysis of the essential oils", ***Journal of Essential Oil Research***, Vol. 27, No. 2, (2015), 115-124.
3. El Hamdaoui, A., Masanda, F., Boubaker, H., Leach, D., Bombarda, I., Vanloot, P., El Aouad, N., Abbad, A., Boudyach, E.H. and Achemchem, F., "Essential oil composition, antioxidant and antibacterial activities of wild and cultivated *Lavandula mairei Humbert*", ***Biochemical Systematics and Ecology***, Vol. 76, (2018), 1-7.
4. El Hachimi, F., Alfaiz, C., bendriss, A., Cherrah, Y. and Alaoui, K., "Anti-inflammatory activity of the seed oil of *Zizyphus lotus (L.) Desf.*", ***Phytothérapi***, Vol. 15, No. 3, (2017),147-154.
5. Tuttolomondo, T., La Bella, S., Licata, M., Virga, G., Leto, C., Saija, A., Trombetta, D., Tomaino, A., Speciale, A. and Napoli, E.M., "Biomolecular characterization of wild Sicilian oregano: phytochemical screening of essential oils and extracts, and evaluation of their antioxidant activities", ***Chemistry & Biodiversity***, Vol. 10, No. 3, (2013), 411-33.
6. Tuttolomondo, T., Leto, C., Leone, R., Licata, M., Virga, G., Ruberto, G., Napoli, E.M. and La Bella, S., "Essential oil characteristics of wild Sicilian oregano populations in relation to environmental conditions", ***Journal of Essential Oil Research***, Vol. 26, No. 3, (2014), 210-20.
7. Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M., "Biological effects of essential oils–a review", ***Food and Chemical Toxicology***, Vol. 46, No. 2, (2008), 446-475.
8. El Idrissi, M., El Amrani, K., Choukrad, M. and Lhoussain, L., "Chemical composition and antimicrobial activity of essential oils of *Lavandula Stoechas L.* from Morocco", ***World Journal of Pharmacy and Pharmaceutical Sciences***, Vol. 5, No. 9, (2016), 316-324.
9. Bouyahya, A., Et-Touys, A., Abrini, J., Talbaoui, A., Fellah, H., Bakri, Y. and Dakka, N., "*Lavandula stoechas* essential oil from Morocco as novel source of antileishmanial, antibacterial and antioxidant activities", ***Biocatalysis and Agricultural Biotechnology***, Vol. 12, (2017), 179-184.
10. Zuzarte, M., Goncalves, M.J., Cavaleiro, C., Benzarti, A., Marongiu, B., Maxia, A., Piras, A. and Salgueiro, L., "Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus* herba-barona essential oils", ***Industrial Crops and Products***, Vol. 44, (2013), 97-103.
11. Bouzouita, N., Kachouri, F., Hamdi, M., Chaabouni, M.M., Aissa, R.B., Zgoulli, S., Thonart, P., Carlier, A., Marlier, M. and Lognay, G.C., "Volatile constituents and antimicrobial activity of *Lavandula stoechas L.* oil from Tunisia", ***Journal of Essential Oil Research***, Vol. 17. No. 5, (2005), 584-586.
12. Majdi, M., Dastan, D. and Maroofi, H., "Chemical Composition and Antimicrobial Activity of Essential Oils of *Ballota nigra Subsp. kurdica* From Iran", ***Jundishapur Journal of Natural Pharmaceutical Products***, Vol. 12, No. 3, (2017), e36314.
13. Husseini, H., Sahraei, H., Meftahi, G.H., Dargahian, M., Mohammadi, A., Hatef, B., Zardooz, H., Ranjbaran, M., Hosseini, S.B., Alibeig, H., Behzadnia, M., Majd, A., Bahari, Z., Ghoshooni, H., Jalili, C., Golmanesh, L., "Analgesic and anti-inflammatory activities of hydro alcoholic extract of Lavandula officinalis in mice: possible involvement of the cyclooxygenase type 1 and 2 enzymes", ***Brazilian Journal of Pharmacognosy***, Vol. 26, (2016), 102-108.
14. McLafferty, F.W. and Stauffer, D.B., The Wiley/NBS registry of mass spectral data, J Wiley and Sons: New York, 1989.
15. Adams, R.P., Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed. Allured Publishing. Carol Stream, Illinois, 2007.
16. Kazemi, M., "Chemical composition and antimicrobial activity of essential oil of *Matricaria recutita"*, ***International Journal of Food Properties***, Vol. 18, No. 8, (2015), 1784-1792.
17. Zimmermann, M., "Ethical guidelines for investigations of experimental pain in conscious animals", ***Pain***, Vol. 16, No. 2, (1983), 109-110.
18. King, M.A., Bradshaw, S., Chang, A.H. and Pintar, G.W., "Potentiation of Opioid Analgesia in Dopamine 2 Receptor Knock-Out Mice: Evidence for a Tonically Active Anti-Opioid

System", ***Journal of Neuroscience***, Vol. 21, No. 19, (2001), 7788-7792.

19. Giray, E.S., Kirici, S., Kaya, D.A., Turk, M., Sonmez, O. and Inan, M., "Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of *Lavandula stoechas"*, ***Talanta***, Vol. 74, No. 4, (2008), 930-935.
20. Moon, T., Wilkinson, J. and Cavanagh, H., "Antibacterial activity of essential oils, hydrosols and plant extracts from Australian grown *Lavandula spp"*, ***International Journal of Aromatherapy***, Vol. 16, No. 1, (2006), 9-14.
21. Bekka-Hadji, F., Bombarda, I. and Touati, A., "Antibacterial activity against methicillin-resistant *Staphylococcus aureus* of five essential oils from Algerian medicinal plants *(Lamiaceae)"*, ***Journal of Essential Oil Research***, Vol. 28, No. 6, (2016), 518-527.
22. Angioni, A., Barra, A., Coroneo, V., Dessi, S. and Cabras, P., "Chemical composition, seasonal variability, and antifungal activity of *Lavandula stoechas L. ssp. stoechas* essential oils from stem/leaves and flowers", ***Journal of Agricultural and Food Chemistry***, Vol. 54, No. 12, (2006), 4364-4370.
23. Saeidnia, S., Gohari, A.R., Mokhaber-Dezfuli, N. an Kiuchi, F., "A review on phytochemistry and medicinal properties of the genus *Achillea"*, ***Daru***, Vol. 19, No. 3, (2011), 173-186.
24. Alizadeh, A. and Aghaee, Z., "Essential oil constituents, phenolic content and antioxidant activity of *Lavandula stricta Delile* growing wild in southern Iran. ***Natural Products Research***, Vol. 30, No. 19, (2016), 2253-2257.
25. Mahdian, F., Mahboubi, M., Rahimi, E. and Moslehi Shad, M., "Chemical Composition and Antimicrobial Activity of the Essential Oil of T*anacetum persicum*", ***Jundishapur Journal of Natural Pharmaceutical Products***, Vol. 12, No. 3, (2016).
26. Tajkarimi, M., Ibrahim, S.A. and Cliver, D., "Antimicrobial herb and spice compounds in food", ***Food Control***, Vol. 21, No. 9, (2010), 1199-1218.

27 . Hajhashemi, V., Ghannadi, A. and Sharif, B., "Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill", ***Journal of Ethnopharmacology***, Vol. 89, No. 1, (2003), 67-71.

28. Santos, F. and Rao, V., "Antiinflammatory and antinociceptive effects of 1, 8-cineole a terpenoid oxide present in many plant essential oils", ***Phytotherapy Research***, Vol. 14, No. 4, (2000), 240-244.

Chemical Composition, Antibacterial and Analgesic Activity of *Lavandula stoechas* Flowers from North of Iran

M. Khavarpour[a], S. M. Vahdat[b], S. Kazemi[c], A. A. Moghadamnia[d], O. Hasanzadeh[a], Z. Salimi[a], N. Rahmanpour[a]

[a] *Department of Chemical Engineering, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*
[b] *Department of Chemistry, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran*
[c] *Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran*
[d] *Department of Internal Medicine, Babol University of Medical Sciences, Babol, Iran*

P A P E R I N F O

Paper history:
Received 08 Mach 2019
Received in revised form 21 July 2019
Accepted 28 July 2019

Keywords:
Antibacterial Activity
Chemical Composition
Essential Oil
Extracts
Lavandula stoechas

چکیده

این باور وجود دارد که گیاهان معطر می‌توانند در محصولات غذایی، عطر، دارویی و آرایشی به دلیل خواص تغذیه‌ای و بیولوژیکی آن‌ها مورد استفاده قرار گیرند. مطالعه حاضر به منظور بررسی ترکیبات شیمیایی و خواص ضد باکتریایی و ضد درد گل *اسطوخودوس* در آمل، شمال ایران برای اولین بار طراحی شده است. اسانس گل *اسطوخودوس* توسط تقطیر با آب تهیه شد. برای تجزیه و تحلیل آن از آزمون کروماتوگرافی گازی- طیف سنجی جرمی استفاده شد. *استفیلوکوکوس اورئوس*، *اشرشیا کلی*، *سالمونلا انتریتیدیس* و *باسیلوس سابتیلیس* برای ارزیابی فعالیت ضد باکتریایی روغن اسانس با استفاده از روش‌های انتشار دیسک آگار و میکرودایلوشن استفاده شد. تجزیه و تحلیل GC-MS نشان داد لینالول (۳۵/۶۹٪) بورنول (۱۴/۹۹٪) و ۱،۸ سینئول (۱۱/۴۵٪) به عنوان ترکیبات اصلی روغن هستند. آزمایشات ضد باکتری برای رقت‌های مختلف روغن اسانس، عملکرد ضد باکتریایی قابل توجهی را نسبت به *استفیلوکوکوس اورئوس* گرم مثبت نشان داد. علاوه بر این، عصاره‌های آب و اتانولی باعث کاهش درد ناشی از آزمون صفحه داغ شد. نتایج، *اسطوخودوس* آمل را به عنوان ترکیب فعال زیستی طبیعی جایگزین پیشنهاد می‌کند که دارای اثر ضد باکتری و ضد درد برای استفاده در صنایع دارویی است.

***doi**: 10.5829/ije.2019.32.08b.02*