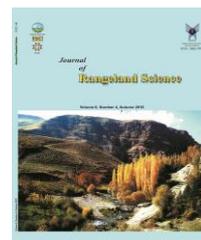


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Research and Full Length Article:

Investigation of Environmental Factors Regarding the Essential Oil and Antibacterial Activity of Lavandin (Case Study: Jowkar, Khoramabad, Alashtar and Aligoodarz from Iran)

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Abstract. Plants produce thousands of primary and secondary metabolites including volatile compounds at various developmental stages throughout their life cycle during flowering, ripening, and maturation. Lavandin known for its volatile compounds is a small aromatic shrub cultivated worldwide for the essential oil. Lavandin essential oil composition is greatly influenced by the environmental and genetic factors. The essential oils from the leaves and flowers of the plant are widely used to enhance the flavor of foods as well as cosmetic and pharmaceutical industries. In order to study the environmental factors regarding the essential oil and antibacterial activity of Lavandin, four populations were collected (Jowkar, Khoramabad, Alashtar and Aligoodarz) in Lorestan province, Iran in May 2014. The hydro-distillated essential oil was analyzed using GC and GC/MS. Results indicated that there were significant differences among the various populations for the main constituents in the essential oils. Analyses revealed 32 compounds constituting 91.63–97.2% of the essential oils. The main constituents were 1, 8-cineole (44.8 to 31.64%), borneol (26.14 to 18.47%), and camphor (14 to 8.41%). According to the results, there was a significant correlation between borneol and altitude ($r=-0.95$). Antibacterial data were analyzed using one-way analysis of variance. The essential oils of Lavandin indicated good inhibitory activities against four bacteria, especially against *Salmonella typhi* with the inhibition zones of 19–23 mm. It was concluded that there was a high variation for the essential oil composition among the populations of Lavandin. In addition, the essential oil of *Lavandula × intermedia* has a good potential source of natural products for being used in food industry.

Key words: Lavandin, Essential oil, Bactria, 1- 8-Cineole

Introduction

Nowadays, there is a renewed or increasing interest in consuming wild food plants (Mohammadi and Azar, 2012). Applications of plants, especially medicinal plants are considered due to human needs for nutrition and treatment during the history (Toupchi, 2011). Rangelands have major roles in the supply of livestock forage, and production of industrial and medicinal plants (Akbarlou and Nodehi, 2016).

Lavenders are widely grown for their essential oils, which have extensive applications in cosmetics, hygiene products and alternative medicine (Zuzarte *et al.*, 2010). Lavender extracts have been traditionally prescribed to treat infertility, infection, anxiety and fever, and have been used as antidepressants, antispasmodics, antifatulent agents, antiemetic remedies and diuretics (Sakamoto *et al.*, 2005). In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods, and chewing gums (Kim and Lee, 2002). Recently, aromatherapy is becoming increasingly popular, and lavender is used in aromatherapy as a relaxant (Farina *et al.*, 2005; Morris, 2002). Other therapeutic effects of lavender such as sedative, carminative spasmolytic, antioxidant, antiviral and antibacterial activities as well as several gastrointestinal nervous and rheumatic disorders have been reported (Torrás-Claveria *et al.*, 2007).

Although over 300 components have been identified in the oils derived from *Lavandula* species, further 26 components are present in the amounts of 0.1 to 1.0%. It follows that the remaining 3% of the oil is comprised of about 250 trace compounds with great structural variations and some important organoleptic properties (Herraiz-Penalver *et al.*, 2013). Due to their extremely low odor threshold, some trace compounds have been found to have a significant contribution to the overall odor profile of

lavender oil. The therapeutic and olfactory properties of lavender essential oils are attributed to monoterpenes, a class of low molecular weight (C_{10}) as isoprenoids (Rabiei *et al.*, 2014). Oil compositions in these plants are primarily determined by plant genotype, but they can be also influenced by developmental and environmental factors (Rabiei *et al.*, 2014).

The essential oil of *Lavandula* species has been used for centuries as an antibacterial agent (Hanamanthagouda *et al.*, 2010). Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes including food-borne pathogens (Gutierrez *et al.*, 2008). The main constituents of essential oils – mono- and sesquiterpenes including carbohydrates, phenols, alcohols, ethers, aldehydes and ketones – are responsible for the biological activity of aromatic and medicinal plants as well as their fragrance (Djenane *et al.*, 2011). Several recent studies described the antimicrobial properties of essential oil against a number of relevant food-borne microorganisms. These essential oils are suggested as possible natural alternatives to chemical-based antibacterial agents for food safety and preservation (Djenane *et al.*, 2011).

The variation and relationships of chemical composition and oil content seemed to be different in environmental conditions. Rahimmalek *et al.* (2013) showed significant correlations between α -pinene and 1, 8-cineole ($r=0.90$), 1, 8-cineole and limonene ($r=-0.90$), limonene and α -pinene ($r=-0.87$), linalool and linalyl acetate ($r=0.58$), and α -terpineol and limonene ($r=0.50$). Similarly, Messaoud *et al.* (2011) in *Myrtus communis* found significant relationships between the essential oil composition and the eco-geographical origins of the populations. Similar chemotypes in myrtle oils collected from distinct geographic zones in Montenegro also

showed the relationship between environmental factors with chemotypes of myrtle (Mimica-Dukic *et al.*, 2010).

The aim of the present study was to quantify the variations in chemical constituents of the essential oil of populations of *Lavandula × intermedia* collected from four geographical regions of western Iran and relationships between variations of chemical composition contents and the environmental factors involved in different geo-ecological regions.

Materials and Methods

Plant material and site description

The leaves of four Lavandin populations (*Lavandula × intermedia*) were harvested from plant growing sites (Jowkar, Khoramabad, Alashtar and Aligoodarz) in the Zagros regions, western Iran in May 2014 (Fig. 1 and Table 1). All the plants grown in the regions were similar clones of *L. × intermedia*. However, for

the exact identification, three samples were collected from each shrub and then, labeled and the locations were recorded using GPS receiver and a representative voucher specimen (MPH 2208) was placed in the Herbarium of Shahid Beheshti University, Iran; then, they were grinded and mixed for the essential oil extraction.

Soil analyses methods

Soil samples were taken from each region. Soil pH was determined using an automatic probe (Quesada *et al.*, 2010). Nitrogen (N) was analyzed according to Kjeldahl method (Kjeldahl, 1883). Phosphorus (P) and Potassium (K) were determined by flame photometer method (Ellis and MacDonald, 2006). Electrical Conductivity (EC) and soil texture were determined by an electrical conductivity meter (EC meter) and a hydrometer, respectively.

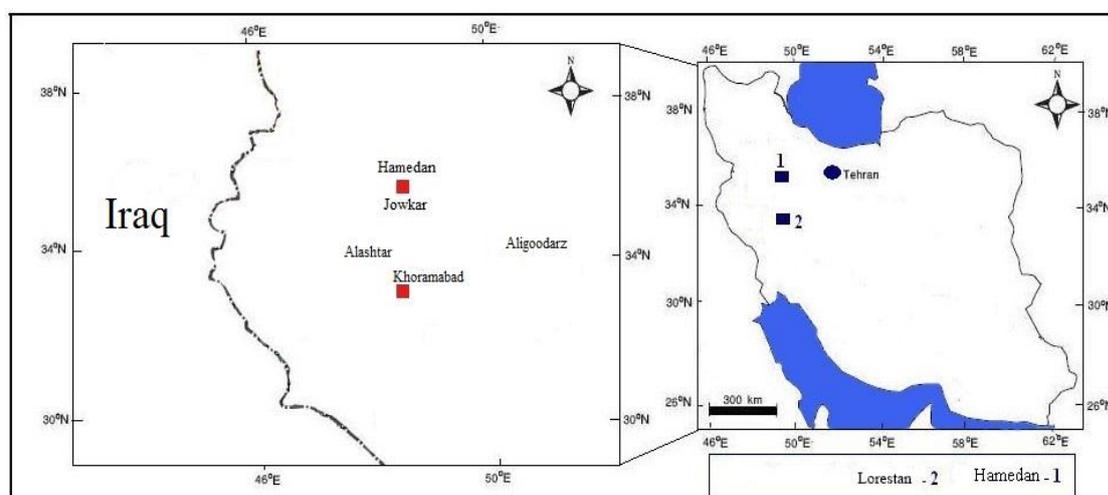


Fig. 1. Collection sites of different Lavandin populations

Essential oil extraction

In all regions (Jowkar, Khoramabad, Alashtar and Aligoodarz), the leaves were dried in the shade at room temperature (25 °C) for ten days. The essential oil was extracted from 100 g of plant tissues in water contained in a 2 L flask and heated by the heating Jacket at 100 °C for 3 h in a Clevenger-type apparatus. Afterward, the essential oils

were dried over anhydrous sodium sulfate and then, stored in vials at 4 °C prior to be analyzed. The yield based on dry weight of the sample was calculated (ml/100g) (Bajalan and Ghasemi Pirbalouti, 2014).

Essential oil compound identification

Composition of essential oils was determined by GC and GC-MS. GC

analysis was done on an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses). The carrier gas was helium at a flow of 0.8 ml/min. Initial column temperature was 60 °C and was programmed to increase from 4 °C/min to 280 °C. The split ratio was 40:1. The injector temperature was set at 300°C. The purity of helium gas was 99.99% and 0.1 µl samples were injected manually in the split mode. GC-MS analysis was done on the mentioned Agilent Technologies 5975 Mass system. Mass spectra were recorded at 70eV. Mass range was from m/z 50-550. Constituents were identified by comparison of their KI (Kovats index) relative to C₅-C₂₄ n-alkanes obtained on a nonpolar HP-5MS column by comparison of the KI provided in the literature and mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology) and Willey (Chem Station data system). The individual constituents were identified by retention indices and compared with constituents known from the literature (Adams, 2007). The percentage of composition was computed from the GC peak areas without using any correction factors.

Antibacterial test

The antibacterial activities of the essential oils against *Salmonella typhi* (PTCC 1609), *Enterococcus faecalis* (PTCC 1237), *Staphylococcus epidermis* (PTCC 1435), and *Pseudomonas aeruginosa* (PTCC 1310) bacteria were determined with the disc diffusion method (NCCLS, 2002) in Boroujerd University. Briefly, bacterial suspensions were adjusted to 1×10⁷ CFU/ml and spread in TSA or PCA using sterile cotton swabs. Subsequently, filter paper discs (6 mm Ø; Whatman #1) were

placed on the surface of Petri dishes and impregnated with 20 µl of essential oil. Control was prepared with DMSO. After staying at 4 °C (2 h), all Petri dishes were incubated at 30 °C (24 h). All the determinations were performed in triplicate. Antibacterial activity was evaluated by measuring the radius of the inhibition zones to the nearest millimeter (Teixeira *et al.*, 2013). The antibacterial data were analyzed using one-way analysis of variance.

Statistical analysis

The antibacterial data were analyzed using one-way analysis of variance using the SAS statistical software. The significance of differences among treatment means was tested using Duncan's multiple range tests at $p \leq 0.05$ levels. Pearson correlation among the compounds and some environment properties was estimated.

Results

Site geographical features and soil characteristics

The Geographical features and soil characteristics of different Lavandin populations are presented in Table 1. The highest altitudes were in Aligoodarz and Jowkar, respectively and Alashtar and Khoramabad had lower altitudes. It can be clearly seen that Khoramabad and Alashtar were rich in Nitrogen (N) and Phosphorus (P). Furthermore, Potassium (K) in Aligoodarz (509 ppm) and Khoramabad (448 ppm) was higher than that in Alashtar (418 ppm) and Jowkar (385 ppm). The amounts of EC were between 0.58 d sm⁻¹ in Alashtar and 0.82 d sm⁻¹ in Khoramabad (Table 1). In addition, Khoramabad, Alashtar and Aligoodarz had clay loam soil and the soil texture in Jowkar was sandy loam.

Table 1. Geographical and soil characteristics of different Lavandin populations

Collection site	Location UTM	Altitude	EC (dsm ⁻¹)	N (%)	P (ppm)	K (ppm)	pH	Soil texture
Jowkar	28°71'36" E 38°11'449" N	1702	0.76	0.186	10.8	385	7.6	Sandy loam
Khoramabad	25°40'29" E 37°11'858" N	1218	0.82	0.203	11.5	448	7.8	Clay Loam
Alashtar	24°61'99" E 37°50'496" N	1605	0.58	0.235	12.5	418	7.9	Clay Loam
Aligoodarz	37°87'85" E 36°97'711" N	2040	0.71	0.183	8.5	509	7.8	Clay Loam

Essential oil composition

Essential oil components of four Lavandin accessions were determined. All the compounds except those with trace amounts are shown in Table 2. Results showed a high chemical polymorphism among Lavandin populations. The main constituents of the essential oil were 1, 8-cineole, borneol and camphor (Table 2). 1, 8-cineole content was ranged from 44.8% in Jowkar to 31.64% in Aligoodarz.

For borneol, the highest and lowest values were belonged to Alashtar

(26.14%) and Khoramabad populations (18.47%), respectively. Camphor showed a good chemical variation among the populations. It was varying from 8.41% in Khoramabad to 14% in Jowkar population.

As illustrated in Table 2, the samples belonging to Jowkar populations revealed the richest amount of 1, 8-cineole. The environmental conditions of this population consisted of low Potassium (K) and a relatively different soil texture (Table 2).

Table 2. Chemical composition of essential oils of four Lavandin populations

Compounds	Retention index	Jowkar	Khoramabad	Alashtar	Aligoodarz
α -Thujene	935	tr ^a	0.30	tr	0.18
α -Pinene	935	1.2	2.76	0.99	1.72
Camphene	950	0.85	0.89	0.59	0.77
Verbenene	955	tr	0.03	tr	tr
Sabinene	974	0.3	1	0.3	0.61
β -Pinene	978	0.89	2.08	0.84	1.31
Myrcene	991	tr	1.11	tr	0.5
α -Phellandrene	1008	tr	0.11	tr	0.07
Delta-3-Carene	1008	tr	2.11	0.44	1.18
<i>p</i> -Cymene	1025	1.52	1.47	1.24	1.35
Limonene	1030	2.05	tr	1.75	tr
β -Phellandrene	1030	tr	6	tr	5.05
1,8-Cineole	1032	44.8	33.26	40.43	31.64
γ -Terpinene	1061	tr	tr	tr	0.11
<i>trans</i> -sabinene hydrate	1039	tr	0.4	0.29	0.23
α -Terpinolene	1089	tr	0.16	tr	0.08
Linalool	1090	tr	0.44	tr	0.32
<i>cis</i> -Pinocarveol	1140	tr	0.68	0.85	0.77
Camphor	1146	14.4	8.41	11.21	11.8
Borneol	1167	20.53	18.47	26.14	20.01
Terpinen-4-ol	1179	0.8	0.89	0.92	0.97
Crypton	1179	1.7	2.52	2.01	3.08
α -Terpinol	1191	0.84	1.56	1.18	1.41
Myrtenol	1198	tr	0.57	0.7	tr
Bornyl acetate	1286	1.41	0.91	1.33	1.13
Cuminyl aldehyde	1286	0.95	1	1.07	1.33
(-)-Carvone	1286	tr	0.53	0.67	0.7
Geranyl acetate	1386	0.94	1.09	0.65	1.64
β -Caryophyllene	1422	1.19	0.33	tr	0.36
Caryophyllene oxide	1586	tr	1.04	1.79	1.61
(+)-Epi-bicyclosesquiphellandrene	1608	tr	1.51	1.81	tr
α -Cadinol	1652	tr	tr	tr	2.14
Total (%)		94.37	91.63	97.2	92.07

^a tr = traces

Correlation between traits

Another assumption in this research was the probable correlation of environmental factors with essential oil components. In this regard, the correlation of the content of major compounds with environmental factors such as soil and climatic conditions in collection regions was determined (Table 3). Results showed that the highest negative correlation was between borneol and altitude (r=-0.95)

indicating the upper altitude that had lower borneol. Other significant correlations were between 1, 8-cineole and potassium (r=-0.93) indicating that higher values of 1, 8-cineole were obtained in soil with lower available K and between borneol and pH (r=-0.93) indicating that higher values of borneol were obtained in alkalid soil with higher CaO. There was also a high correlation between CaO and pH (r=0.96) (Table 3).

Table 3. Correlation of major components and some environmental factors

	1,8-Cineole	Camphor	Borneol	Altitude	CaO	N	P	K	pH
Camphor	0.696								
Borneol	0.453	0.191							
Altitude	-0.256	0.207	-0.953*						
CaO	0.208	-0.366	0.804	-0.672					
N	-0.314	-0.267	0.539	-0.278	0.836				
P	0.492	-0.404	-0.332	0.061	0.319	-0.709			
K	-0.927*	-0.661	-0.503	-0.577	0.708	0.269	0.442		
pH	-0.500	0.110	-0.925*	0.802	-0.964*	-0.791	0.418	-0.597	
EC	-0.393	0.647	0.136	-0.375	-0.432	-0.739	0.404	-0.124	0.244

*: Significant at the 5% probability level

Antibacterial activity

The antibacterial activity of the essential oils was evaluated using the disc diffusion method (Fig. 2). The results of mean comparisons between antibacterial activities in each region indicated significant antibacterial activity effects of the essential oils in the investigated populations (p ≤ 0.05) for antibacterial activity against the tested bacteria. In general, a total of Lavandin essential oil showed relatively high inhibitory activities against four tested pathogenic bacteria. The highest antibacterial activity

was observed against *S. typhi* with the strongest inhibition zones (23 mm) for the essential oil from Aligoodarz population; however, the essential oil from Jowkar population exhibited weak inhibition zones against *S. typhi* (19 mm). After *S. typhi*, *E. faecalis* had the highest antibacterial activity (ranged from 19 mm in Alashtar to 16 mm in Jowkar and Khoramabad). In contrast, *Pseudomonas aeruginosa* had the lowest antibacterial activity with the average values of 9 mm in Khoramabad and 8 mm in other areas (Fig. 2).

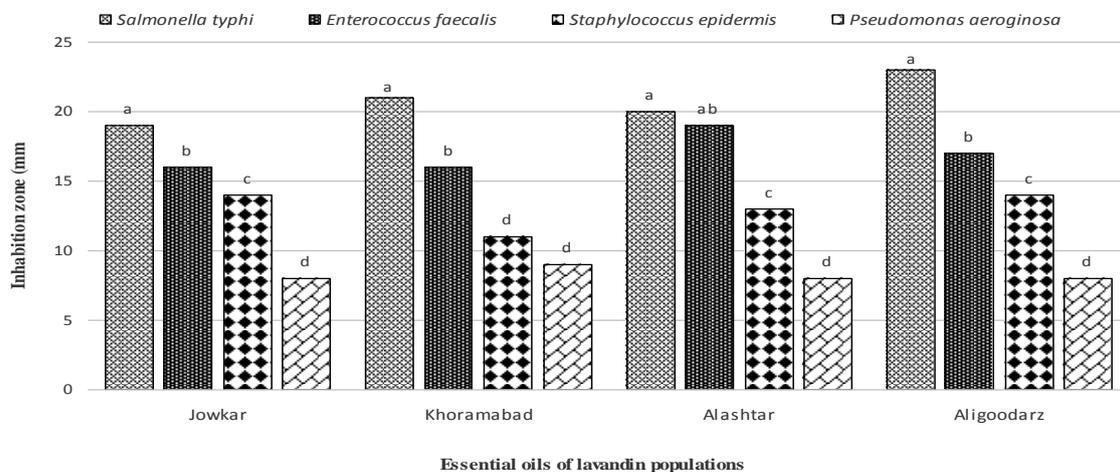


Fig. 2. Antibacterial activity of the essential oils from lavandin leaves collected from different habitats (Means with different letters that are statistically significant at 5% probability level)

Discussion

Lavender essential oil is a complex mixture of monoterpenes and sesquiterpenes. The most abundant monoterpenes found in Lavandin include linalool, linalool acetate, borneol, camphor, and 1, 8-cineole. Among these, camphor, linalool, and linalool acetate are key determinants of the Lavandin essential oil quality (Lis-Balchin, 2002; Upson and Andrews, 2004). Essential oils with a high linalool and linalool acetate to camphor ratio are considered to be of “high quality”, and thus, are used in cosmetic products and aromatherapy (Lane *et al.*, 2010). Essential oils added to alternative medicines are typically rich in camphor and 1, 8-cineole. Lavender essential oil also contains sesquiterpenes such as caryophyllene, bergamotene and nerolidol with trace amounts of other terpenoid compounds such as perillyl alcohol. *L. × intermedia* contain large quantities of camphor while producing a small amount of linalool and linalool acetate, making it more useful for the alternative medicine industry (Cavanagh and Wilkinson, 2002). In present study, there was a good diversity in the amounts and kinds of components (Table 1). There are many reports regarding the variation of Lavandin essential oils in different countries. Nevertheless, it has a very different amount of chemical composition in comparison with other studies (Stanojevic *et al.*, 2011; Zheljazkov *et al.*, 2012). The changes in the essential oil composition in their study might be caused by several environmental (climatic, seasonal, geographical) and genetic differences (Stanojevic *et al.*, 2011).

The assumption in present research was the probable correlation of environmental factors with essential oil components. Soil pH had a high correlation with borneol, 1, 8-cineole, CaO, N, P, K and Altitude. Soil pH affects the availability of several mineral nutrients such as nitrogen, phosphorus,

potassium, sulfur, calcium, magnesium, iron, manganese, boron, copper, zinc and molybdenum, and all of these nutrients are available in the pH range of 5.5 to 6.5 (Taiz and Zeiger, 1998). Loss of plant available phosphate in soils occurs by phosphate fixation, which is strong in acid mineral soils. Such losses can be dramatically reduced by liming soils to a pH of 6–7 (Konrad, 1997). Different levels of soil pH influenced the plant fresh and dry weight and their chemical composition (Shukla *et al.* 1998).

In present study, there was a significant correlation between altitude and Borneol. Altitude has a strong effect on oil essential compounds. It is known that an increase in altitude of 100 m entails a fall in mean temperature of 0.6°C to 0.7°C. Temperature is considered as an important physical factor that may change plant secondary metabolites (Rathcke and Lacey, 1985).

Differences among antibacterial activities between populations are because of different amounts of chemical composition in their essential oils harvested in different regions. The majority of researches on the antibacterial activity of lavender essential oil has been based on the oil derived from *L. angustifolia* with the therapeutic potential of essential oils produced from other varieties such as *L. intermedia* (lavandin), *L. stoechas* (French lavender) and *L. allardii* which might be largely ignored (Haig *et al.*, 2009). In some studies, essential oil components were recognized for their antibacterial efficacy: caryophyllene (Ozturk *et al.*, 2009), terpinen-4-ol (Kotan *et al.*, 2007), borneol, α -pinene, and terpineol (Dorman and Deans, 2000). Camphor as the major component of the Lavandin essential oil has antibacterial properties itself (Sokovic *et al.*, 2007; Mahboubi and Kazempour, 2009). Some studies also suggest the synergistic effect of minor components on the chemical composition of the essential oils in relation to its

antimicrobial activity (Gill *et al.*, 2002; Mourey and Canillac, 2002). The interaction between essential oil compounds can produce four possible types of effects: indifferent, additive, antagonistic, or synergistic effects. It has been reported that essential oils containing aldehydes or phenols such as cinnamaldehyde, citral, eugenol, carvacrol or thymol as major components showed the highest antibacterial activity, followed by essential oils containing terpene alcohols (Sachetti *et al.*, 2005; Bassole and Juliani, 2012; Bajalan and Ghasemi Pirbalouti, 2014).

Conclusion

In conclusion, a high level of chemical polymorphism was observed among Lavandin populations in Iran. The main constituents were 1, 8-cineole, borneol and camphor. 1, 8-Cineole content was ranged from 44.8% in Jowkar to 31.64% in Aligoodarz. The correlation of soil conditions in collection regions with the major essential oil components was also evaluated. The highest correlation coefficient was between borneol with the altitude ($r=-0.953$) and pH ($r=-0.925$), and between 1, 8-cineole and potassium ($r=-0.927$). The essential oil of Lavandin is effective for the inhibition or control of bacteria strains, especially *Salmonella typhi* and so, it could be used as a natural antimicrobial agent.

Acknowledgments

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اثر برخی عوامل محیطی بر ترکیبات اسانس و فعالیت آنتی باکتریایی اسطوخودوس (مطالعه موردی: جوکار، خرم آباد، الشتر و الیگودرز، ایران)

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چکیده. گیاهان هزاران نوع از متابولیت‌های اولیه و ثانویه در مراحل مختلف رشدی خود و در سراسر چرخه زندگی همانند گلدهی، رسیدگی و بلوغ تولید می‌کنند. انواع مختلف اسطوخودوس *Lavandula sp.* در اغلب نقاط دنیا به دلیل وجود ترکیبات فرار در اندام هوایی مخصوصا در گل‌ها و برگ‌ها به صورت گسترده‌ای کشت می‌شوند. از مهمترین کاربردهای اسانس این گیاه می‌توان به استفاده از آن در ساخت داروهایی با منشاء گیاهی، محصولات آرایشی و بهداشتی و صنعت عطر سازی اشاره کرد. هدف از مطالعه حاضر بررسی تنوع در ترکیبات اسانس جمعیت‌های مختلف اسطوخودوس در غرب ایران می‌باشد. با توجه به اهمیت مطالعه پیرامون متابولیت‌های ثانویه و عوامل محیطی موثر بر آنها، این مطالعه به تحقیق در رابطه با ارتباط بین شرایط محیطی و متابولیت‌های ثانویه برگ اسطوخودوس ($Lavandula \times intermedia$) در چهار منطقه در غرب ایران (جوکار، خرم آباد، الشتر و الیگودرز) در خرداد ماه سال ۱۳۹۳ پرداخته است. بدین منظور پس از جمع‌آوری و خشک شدن نمونه‌ها، عمل اسانس‌گیری برای برگ‌های اسطوخودوس به روش تقطیر با آب توسط دستگاه کلونجر انجام گردید. سپس با استفاده از دستگاه GC/MS نوع و درصد ترکیبات تشکیل دهنده اسانس در نمونه‌ها مشخص گردید. بر اساس نتایج به دست آمده مهمترین ترکیبات تشکیل دهنده اسانس اسطوخودوس شامل ۸۱- سینئول (۳۱/۶۴-۴۴/۸٪)، بورنئول (۱۸/۴۷-۲۶/۱۴٪) و کامفور (۸/۴۱-۱۴٪) بودند. نتایج نشان داد که بیشترین همبستگی بین بورنئول و ارتفاع از سطح دریا ($r=-0.95$) مشاهده شد. داده‌های مربوط به فعالیت آنتی باکتریایی بصورت تجزیه واریانس یک طرفه آنالیز آماری و مقایسه شدند. بررسی فعالیت آنتی باکتریایی اسانس اسطوخودوس به روش انتشار دیسک نشان داد که این گیاه دارای فعالیت آنتی باکتریایی خوبی بر علیه باکتری‌های مورد استفاده در این تحقیق بخصوص *Salmonella typhi* (با قطر هاله عدم رشد بین ۱۹-۲۳ میلی متر) بود. نتایج این تحقیق نشان دهنده تنوع بالایی در جمعیت‌های مختلف اسطوخودوس می‌باشد. همچنین اسانس استخراج شده از گیاه اسطوخودوس می‌تواند به عنوان یک منبع طبیعی در صنایع غذایی مورد استفاده قرار گیرد.

کلمات کلیدی: اسطوخودوس، اسانس، باکتری، 1,8-Cineole