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Exploring the Chemical Composition and Bioactivity of *Lavandula angustifolia* Essential Oil: A Gas Chromatography Analysis Approach

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ABSTRACT

This research is a comprehensive study of the chemical composition, antimicrobial, and antioxidant properties of the essential oil derived from *Lavandula angustifolia* Moench, commonly known as lavender, sourced from the Hamadan province in Iran. The study utilized Gas Chromatography (GC) analysis to scrutinize the chemical composition of the extracted essential oil. Antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) through a series of microdilutions, while the antioxidant activity was estimated using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay. Variations in the chemical composition of the essential oils from the plant's flowers and leaves were observed, with linalool, linalyl acetate, camphor, and borneol identified as the predominant components in all essential oils. The study revealed significant antimicrobial and antioxidant activities in the essential oil from *L. angustifolia*. Furthermore, the study further investigated the variations in these properties based on the part of the plant used (leaves or flowers) and the plant's conditions (purchased or cultivated). The findings indicate that *L. angustifolia* essential oil holds potential for therapeutic and natural medicine applications.

Keywords: Lavander, Chemical constituents, GC-MS analysis, DPPH assay, Minimum inhibitory concentration

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INTRODUCTION

Lavandula angustifolia (Lamiaceae), a member of the Lamiaceae family, stands out as a widely acclaimed and adaptable herb renowned for its diverse applications across various domains. The plant is characterized by its aromatic grey-green leaves and purple flowers, which contain a complex mixture of volatile compounds that confer distinctive fragrance and biological activities [1-5].

The earliest therapeutic use of *L. angustifolia* can be traced back to Roman and Greek civilizations [6]. *Lavandula* genus is globally cherished for its aromatic and medicinal properties. The name 'Lavender' originates from the Latin term 'lavando', a derivative of the verb 'lavare', translating to 'to wash'. The Romans historically employed lavender to infuse their baths with a delightful fragrance and to reap its health advantages. For centuries, it has been used in various fields, including herbal medicine, cosmetics, perfumes, foods, and aromatherapy [7, 8]. *L. angustifolia*, also commonly known as English or "true" lavender, is one of the 39 species in the *Lavandula* genus, encompassing several hybrid varieties. There are numerous identified cultivars known to this day. As Lis-Balchin pointed out in his book, the global number of lavender cultivars began to rise in the early 17th century [9]. Additionally, he highlighted the challenges in classifying species, hybrids, and cultivars, as the same plant, when cultivated in varying geographical locations and under diverse conditions, can appear completely distinct. As concluded by Handan Giray and colleagues in their paper on the Analysis of World Lavender Oil Markets, the international market for Lavandula oil is evolving dynamically, with growing interest from both established producers and newcomers [10]. The cultivation of lavender for oil extraction offers substantial opportunities for value addition in the agricultural sector. The principal avenues for value addition in lavender oil production encompass essential oils, fresh flowers and plants, dried products, food, and agro-tourism. The production and quality of lavender essential oil are influenced by environmental and developmental conditions, with temperature and flowering stage determining its chemical composition. The essential oil derived from *L. angustifolia* is commonly used in perfumery and cosmetics due to its demonstrated sedative, anxiolytic, and antidepressant

effects on the central nervous system [11-13]. Moreover, essential oils from the *Lavandula* genus are recognized for their antifungal, antibacterial, insecticidal, sedative, spasmolytic, antioxidant, and anticancer properties [14-23]. These oils have the potential to interact with conventional drugs which could be beneficial of reducing lowering the required onset dose of medications and minimizing their associated side effects. The chemical composition of essential oils is complex and influenced by numerous factors. These include the cultivation area, which affects the plant's biochemical synthesis, and environmental parameters that modulate the concentration of specific compounds in the essential oil. The distinctive chemical composition of essential oils is influenced by the plant's morphological characteristics and the methods employed during processing. An in-depth comprehension of these variables is imperative for overseeing the quality of essential oils and for devising tailored cultivation and processing techniques. This knowledge can facilitate the production of oils with specific chemical compositions tailored to particular applications [24, 25].

In general, essential oils derived from plants in *Lavandula* genus exhibit a broad spectrum of biological activities. *L. dentata* essential oil has been found to inhibit a variety of gram-positive and gram-negative bacteria including Salmonella, Enterobacter, Klebsiella, E. coli, and Listeria monocytogenes. Similarly, the essential oil of *L. bipinnata* possesses antibacterial properties against E. coli, P. aeruginosa, S. aureus, B. subtilis, and antifungal properties against A. niger, P. notatum, and C. albicans [26]. *L. angustifolia* species is a flowering plant that is prevalent in Europe and across the Mediterranean. Essential oils derived from *L. angustifolia*, grown in various countries including China, Syria, India, Iran, Romania, Canada, Spain, and France, among others, have been studied in previous research. The predominant compounds found in these essential oils are linalool, limonene, perillyl alcohol, linalyl acetate, cis-jasmone, terpene, coumarin, tannin, caffeic acid, camphor, and α-pinene. [1]. The escalating global health challenges associated with oxidative stress and reactive oxygen species (ROS) have necessitated the search for potent antioxidants. Plants, with their vast array of phytochemicals, have long been a source of potent antioxidant compounds. Also, the increasing prevalence of antibiotic-resistant pathogens has led to a growing interest in the antimicrobial properties of plants. Multiple plants have been used for centuries in traditional medicine systems worldwide, and they continue to be a valuable source of new therapeutic agents. Among these, *L. angustifolia* has attracted significant attention.

The antioxidant and antimicrobial activity of *L. angustifolia* is particularly noteworthy [6, 27], given the crucial role of antioxidants in mitigating the harmful effects of ROS, such as cellular damage, aging, and various chronic diseases. The essential oil of *L. angustifolia* is rich in a variety of bioactive compounds, including linalool, linalyl acetate, camphor, and borneol. These compounds have been shown to inhibit the growth of a wide range of pathogens, making *L. angustifolia* a promising source of new antimicrobial agents [28]. The essential oil of *L. angustifolia* is also characterized by a high content of monoterpenes, including linalool and linalyl acetate, among others. These compounds have been shown to exhibit strong antioxidant activity, thereby contributing to the overall antioxidant capacity of the plant.

However, it is important to note that the chemical composition of *L. angustifolia*, and consequently its antioxidant and antimicrobial potential, can be influenced by several factors. These include the geographical location of cultivation, the stage of plant development at the time of oil extraction, and the extraction method employed.

In this context, the present study aims to investigate the antioxidant, and antimicrobial properties as well as the chemical composition of *L. angustifolia* essential oil extracted from the plant growing in Hamadan province, Iran during the flowering stage. The whole procedure was performed on the leaves and flowers of this plant individually. Furthermore, due to the importance of *L. angustifolia*, two sets of plant material were collected: one purchased from the market in Hamedan province and the other, the cultivated plant in the garden of the pharmacy faculty, Hamadan University of Medical Sciences. We hope that our findings will contribute to the development of new, effective antimicrobial agents and shed light on the potential of *L. angustifolia* as a source of therapeutic compounds.

MATERIALS AND METHODS

Ethical Considerations

The research was approved by The Ethics Committee of Hamadan University of Medical Sciences under the code IR.UMSHA.REC.1400.795.

Preparation of the Plant Material

The aerial parts of *L. angustifolia* (herbarium code: 767) were collected in May 2022 from the medicinal plant garden of the Hamedan Faculty of Pharmacy and the aerial parts of the plant (flower, leaf) were separated from each other and placed away from the sun to dry. Also, dried flowers and leaves of *L. angustifolia* were purchased from the markets of Hamedan and stored at 25 °C, away from sunlight until the day of use. The authentication of plant specimens was done in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

Preparation of the Essential oil

The essential oil was extracted from the leaves and flowers of *L. angustifolia* individually using a hydro-distillation process facilitated by a Clevenger-type apparatus [29, 30]. This involved placing 100 g of the dried components of *L. angustifolia* in a 2-liter flask, to which 1.5 liters of purified water was added. The system was then heated for 3 h for flowers and 5 h for leaves. Following this, the essential oil was separated and dried using sodium thiosulfate anhydrous, then preserved in sealed, dark-colored glass vials at a temperature of 4 °C until use.

GC-MS Analysis

The essential oil derived from leaves and flowers of *L. angustifolia* was subjected to analysis using a ThermoQuest-Finnigan TRACE MS gas chromatograph-mass spectrometer (GC-MS) equipped with a fused methyl silicon DB-5 column (30 m × 0.25 mm × 0.25 mm film thickness). The carrier gas used was helium, flowing at a rate of 1.1 ml/min. The column temperature was initially held at 60 °C for two minutes, then ramped up to 250 °C at a rate of 5 °C/min, and finally maintained at 250 °C for an additional 2 minutes. The injector temperature was set at 250 °C, and the split ratio was adjusted to 1/10. The injection volume was 0.2 ml. The mass spectrometer was operated under the following conditions: an ionization potential of 70 eV and a source temperature of 200 °C. The constituents were identified by comparing their Retention Index with that of C_5-C_{24} *n*-alkanes, as well as by comparing the RI provided in the literature and the mass spectra recorded by the Mainlib and Willey.

DPPH Assay

The study assessed the antioxidant properties of the essential oil obtained from the leaves and flowers of L. *angustifolia* by employing a modified approach based on the method outlined by Motaghed *et al* [31]. This approach involves the discoloration of a purple-colored methanol solution of DPPH, indicating the antioxidative or electron donation potential of the botanical material.

In brief, 750 µl of plant extract and essential oil at various concentrations (ranging from 25000 µg/ml to 50 µg/ml) were combined with 300 µl of 0.3 mM DPPH radicals in methanol, maintaining a ratio of 5:2. The mixture was allowed to stand at room temperature for 30 minutes. The absorbance of the solution was then measured against a blank at 518 nm, with all measurements being performed in triplicate.

The percentage of antioxidant activity was calculated using the formula:

$$AA\% = [(Abs\ Control - Abs\ Sample)/Abs\ Control\ ] \times 100$$

Here, Abs Control represents the absorbance of the control reaction (which contains all reagents except for the test compound), and Abs Sample represents the absorbance of the test compound. BHT was used as a positive standard. The essential oil concentrations that provided 50% inhibition (IC_{50}) were determined from the graph of inhibition percentage plotted against their concentration.

MIC and MBC Evaluation

Determining the minimum inhibitory and lethal concentration by the serial dilution method:

The broth microdilution technique (CLSI 2009) was employed to determine the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of lavender essential oils for the organisms under study [32]. This technique is based on an in vitro antimicrobial susceptibility test using serial concentrations of an antimicrobial agent incorporated into an agar growth medium in separate Petri dishes. These dishes are then inoculated with a bacterial suspension to determine the minimal inhibitory concentration (MIC). To determine the minimum inhibitory and lethal concentration of the essential oil of the flower and leaf from *L. angustifolia* plant, the bacteria were first defrosted and grown on the Soybean Casein Digest Broth (SCDB) culture medium in a 37 °C incubator for 24 h and were transferred to the SCDA (Soybean Casein Digest Agar) culture medium by a sterile swab and placed in an incubator for 24 h. A quantity of grown bacteria was diluted by a loop in a 0.9% sodium chloride solution to obtain 0.5 McFarland turbidity (equivalent to 1.5×10^8 colony-forming unit (CFU)/ml) and then diluted 10 times and 1 ml of it was inoculated into 100 ml of SCDB culture medium. One ml of the inoculated culture medium was entered into 10 separate test tubes and 0.5 ml of essential oil and 0.5 ml of DMSO (Dimethyl sulfoxide) were added. Ten two-fold dilutions from 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.2, 0.1, 0.05 mg/ml of the essential oil was used. To investigate the antimicrobial effects of DMSO instead of essential oil, 0.5 ml of normal saline was added and dilution was performed similarly. The concentrations for which no turbidity was observed after 24 h of incubation are considered MIC. Subsequently, in the last four dilutions without bacterial growth, 10 µl were taken and cultured in the SCDA medium and placed in a 37 °C incubator for 24 h. The first concentration for which no bacterial growth was observed after 24 h of incubation, is considered MBC. The test was repeated three times.

RESULTS

The total essential oil yield from leaves and flowers of *L. angustifolia* purchased from the Hamadan market was 0.39% and 1.52%, respectively.

Besides, the total yield of the essential oil for *L. angustifolia* collected from the university garden was 0.42% and 2.1% for leaves and flowers, respectively. The GC-MS analysis of *L. angustifolia* from Hamadan, Iran, revealed the presence of several compounds (Figs. 1-4). The main constituents were Linalool, 1,8-Cineol, Borneol, and Camphor. Other compounds such as γ-Terpinen, trans-Linalool oxide, cis-Linalool oxide, p-Cymen-7-ol, Linalool acetate, α-Terpineol, 4-Terpineol, Isobornyl formate, Limonene, p-Cymene, Caryophyllene oxide, p-Cumic aldehyde, p-Cymen-8-ol, n-Dodecane, n-Decane, trans-Pinocarveol, Camphene, α-Pinene, β-Pinene, 1-Octen-3-ol, Bornyl acetate, and Ho-trienol were also detected in various amounts (Table 1).

As can be noticed from Table 1, the following components could only be found in the essential oil from flowers of *L. angustifolia* and are absent in the leaves of the plant.

Cryptone: a natural organic compound that is found in various plants. It is a major component of the essential oil of Eucalyptus globulus, a plant known for its medicinal properties. It is also found in Steganotaenia araliacea, a plant used for its medicinal properties and various commodities [33].

α-Cadinol: a cadinane sesquiterpenoid that is found in various plants. It has been identified in Casearia sylvestris, a plant used in folk medicine as an antiseptic and cicatrizing in skin diseases. It has also been found in maize [34].

Myrtenal: a bicyclic monoterpenoid that can be found in numerous plant species including Hyssopus officinalis, Salvia absconditiflora, and Cyperus articulates [34]. It has been shown to inhibit acetylcholinesterase, a common method of treatment for Alzheimer's disease and dementia, in vitro. It also has antioxidant properties.

Trans-p-mentha-2,8-diene-1-ol: a monoterpene that is used in the preparation of THC, a compound found in cannabis. It can be prepared from limonene, a compound found in the peels of citrus fruits [35].

Verbenone: a natural organic compound classified as a terpene that is found naturally in a variety of plants. It is the primary constituent of the oil of Spanish verbena and is also found in the oil of rosemary. Verbenone has a pleasant characteristic odor and is used in perfumery, aromatherapy, herbal teas, spices, and herbal remedies [36]. It also has an important role in the control of bark beetles such as the mountain pine beetle and the Southern pine bark beetle.

Cis-Carveol: a primary terpene found in cannabis, as well as lilacs, nutmeg, cumin, tea tree oil, and apples. It has antioxidative, antibacterial, antifungal, antiseptic, and potential anticancer properties, and is used in food and drink flavorings, air fresheners, and cleaning products [37].

α-Campholene: a major component of the essential oils of many types of plants and flowers. It is produced by an enzyme called Tricyclene synthase [38].

Pinocarvone: a monomer for synthesizing polyketone polymers. It is found in the essential oils of thyme and oregano and in smaller amounts in a wide range of herbs and spices [39].

O-Cymene: O-Cymene is an organic compound classified as an aromatic hydrocarbon. It is a flammable colorless liquid that is nearly insoluble in water but soluble in organic solvents. It is naturally present in high quantities in the essential oils of thyme and oregano and in smaller amounts in a wide range of herbs and spices [40]. Common plants containing O-Cymene are anise, cannabis, coriander, and cumin. In small quantities, O-Cymene has a pleasant odor described as citrus, woody, sweet, and earthy but can be overwhelmingly harsh and turpentine-like in strong concentrations. For this reason, O-Cymene is used in the perfume industry and added in small amounts to cleaning products. O-Cymene also alters how other aromatics smell and taste, leading to its usage in the food and beverage industry as a flavor additive.

E-β-Ocimene: a monoterpene volatile, is ubiquitously found in plants and is emitted from flowers to attract pollinators and from vegetative tissues as part of inducible defenses against insect herbivores [41]. It is found in plants like *Pyrus betuleafolia* and many angiosperms.

α-Terpinolene: a primary terpene found in cannabis, as well as lilacs, nutmeg, cumin, tea tree oil, and apples. It has antioxidative, antibacterial, antifungal, antiseptic, and potential anticancer properties, and is used in food and drink flavorings, air fresheners, and cleaning products [42].

γ-Cadinene: a group of isomeric hydrocarbons that occur in a wide variety of essential oil-producing plants. The name is derived from the Cade juniper (*Juniperus oxycedrus* L.), the wood that yields an oil from which cadinene isomers were first isolated. It is also found in *Ganoderma lucidum* and Ganoderma sinensis from Basidiomycetes [43].

Nopinone: used in the preparation of a chiral annulated indene derivative, which can be a potentially useful chiral ligand for transition metal complexes in asymmetric transformations. It may also react with secondary amines in cyclohexane to form the corresponding enamines [44).

Tricyclene, also known as 1,7,7-Trimethyltricyclo [2.2.1.0,2,6] heptane, is a major component of the essential oils of many types of plants and flowers. It is produced by an enzyme called Tricyclene synthase [45].

After the removal of free radicals, the absorbance value measured by the spectrophotometric method indicates the amount of DPPH free radicals. The higher this value, the less the ability of the essential oil to remove free radicals.

The IC_{50} values of the essential oils from the leaf and flower of *L. angustifolia* are illustrated in Table 2. The IC_{50} value in BHT is reported as a positive control.

Our findings revealed that an increase in essential oil concentrations will lead to a rise in free radical-scavenging activity. The activity of the essential oil of flowers of *L. angustifolia* collected from the faculty garden was higher than the other samples (P<0.05) but still. it wasn't comparable to BHT (IC_{50}= 11.16 ± 1.05 µg/ml) as the positive control. In our observations, it was discerned that the antioxidant activity exhibited by the essential oils derived from the flowers of the plant was significantly superior compared to the samples containing essential oils extracted from the leaves (P<0.05).

The results of MIC and MBC in terms of the concentration of essential oil in the test tubes on the bacteria are given in Table 3. As the antimicrobial effects of the essential oil from flowers of *L. angustifolia* were not noticeable, the data was not included in the following table.

As the results show (Table 4), DMSO does not have a significant inhibitory effect on 4 strains of bacteria (*Escherichia. coli*, *Staphylococcus. aureus*, *Pseuodomonas aeruginosa*, and MRSA) and lacks the ability to eliminate any bacteria even at its highest concentration, i.e., 25 mg/ml. The MIC values for essential oil flowers from the purchased plant and the one from the faculty garden were similar to each other and equal to 0.2-1.56 mg/ml. The MBC values for the purchased flower and the faculty garden are also 0.39-3.12 mg/mL and 0.78-3.12 mg/ml, respectively.

DISCUSSION

Chemical Composition

The essential oil of *L. angustifolia*, commonly known as lavender, has been extensively studied for its diverse chemical composition and potential applications in various industries. Our recent study on *L. angustifolia* from Hamadan, Iran, has revealed a unique chemical profile that contributes to our understanding of this plant's versatility. In the essential oils derived from the leaves and flowers of *L. angustifolia*, camphor, and borneol were the predominant constituents. The essential oil of the flowers is characterized by a unique presence of linalool as the principal component. The essential oil of the leaves, specifically from the plant harvested in the university garden, exhibited a high concentration of 1,8-cineol, while limonene was present in lower quantities.

Conversely, the essential oil of the leaves from the plant purchased from the Hamadan market demonstrated a low concentration of 1,8-cineol. Interestingly, this sample exhibited the highest concentration of any constituent, with limonene constituting 42.75% of the sample. According the Table 1, the yield of essential oil from flowers is more than leaves, and the plant cultivated in the garden is superior to that of the market. In the case of the difference between flowers and leaves of true lavender, the finding is by those reported by Gonza´lez-Rivera *et al*. [46], Mun˜oz-Bertomeu *et al*. [47], and Guitton *et al*. [48].

Table 1 Chemical composition of the essential oil from flowers of *L. angustifolia.*

Components	LFU [a] (%)	LFM [b] (%)	KI
α-Pinene	0.37	0.34	934
Camphene	0.38	0.55	950
1-Octen-3-ol	0.29	ND	975
β-Pinene	0.34	0.32	979
n-Decane	0/63	0.59	998
p-Cymene	0.74	0.71	1025
Limonene	0.84	0.81	1024
1,8-Cineol	17.64	20.22	1026
E-β-Ocimene	0.31	ND	1054
γ-Terpinen	ND	6.59	1067
cis-Linalool oxide	4.98	ND	1072
Linalool	29.50	22.48	1089
trans-Linalool oxide	3.80	4.74	1098
Ho-trienol	ND	0.32	1104
trans-Pinocarveol	0.48	0.52	1142
Camphor	11.41	13.34	1149
Borneol	13.17	15.62	1170
Terpinen-4-ol	1.87	1.32	1181
p-Cymen-8-ol	0.67	0.93	1190
α-Terpineol	2.08	1.86	1193
n-Dodecane	0.63	0.64	1200
Isobornyl formate	1.62	2.09	1232
p-Cumic aldehyde	0.68	0.90	1243
D-Carvone	0.36	0.43	1247
Linalool acetate	2.10	1.45	1257
Bornyl acetate	0.26	0.45	1289
p-Cymen-7-ol	2.29	1.47	1291
Carvacrol	1.06	ND	1301

Lavandulyl isovalerate	0.45	ND	1509
Caryophyllene oxide	0.74	0.86	1588
α-Bisabolol	0.34	0.46	1686
Oxygenated monoterpenes	93.97	88.14	-
Monoterpene hydrocarbons	2.98	9.32	-
Oxygenated sesquiterpenes	1.53	1.32	-
Alkanes	ND	1.23	-
Yield of essential oil	2.1	0.42	-

[a]: *L. angustifolia* Flowers collected from University garden; [b]: *L. angustifolia* Flowers purchased from Hamadan Market

Table 2 Chemical composition of the essential oil from leaves of *L. angustifolia*.

Components	LLU[c] (%)	LLM[d] (%)	KI
Tricyclene	ND	0.13	924
α-Pinene	2.12	0.91	934
Camphene	1.27	0.96	950
β-Pinene	1.36	0.54	979
n-Decane	0.65	0.80	998
p -Cymene	0.58	0.62	1022
O -Cymene	1.99	2.23	1025
Limonene	1.76	42.75	1024
1,8-Cineol	27.29	0.13	1026
E-β-Ocimene	0.46	ND	1054
γ-Terpinen	0.24	ND	1067
Terpinolene	0.32	0.31	1089
Linalool	0.45	0.29	1098
Trans-p-mentha-2,8-diene-1-ol	0.70	0.42	1123
α-Campholene	0.60	0.37	1127
Nopinone	ND	0.27	1140
trans-Pinocarveol	1.52	1.11	1142
Camphor	19.40	21.73	1149
Pinocarvone	0.60	0.47	1166
Borneol	18.32	16.97	1170
Terpinen-4-ol	0.92	0.65	1181
p-Cymen-8-ol	0.54	0.51	1190
Cryptone	2.87	1.10	1191
α-Terpineol	1.03	0.52	1193
Myrtenal	1.46	1.16	1201
Verbenone	0.69	0.43	1213
cis-Carveol	0.66	ND	1221
Isobornyl formate	3.73	1.56	1232
p-Cumic aldehyde	2.22	1.17	1243
D-Carvone	1.15	0.59	1247
Bornyl acetate	0.49	0.64	1289
p-Cymen-7-ol	0.54	0.25	1291
γ-Cadinene	0.31	ND	1518
Caryophyllene oxide	2.20	0.42	1588
α-cadinol	1.54	ND	1652
Oxygenated monoterpenes	85.18	50.34	-
Monoterpene hydrocarbons	10.1	48.45	-
Oxygenated sesquiterpenes	3.74	0.42	-
Sesquiterpene hydrocarbons	0.31	ND	-
Alkanes	0.65	0.8	-
Yield of essential oil	1.52	0.39	-

[c]*: L. angustifoli*a Leaves collected from university garden; [d]: *L. angustifolia* Leaves purchased from Hamadan Market

Table 3 Comparison of the birth activity of free radical (DPPH)

Plant samples	DPPH[a] (IC_{50} µg/ml) (Mean ± SD)
LLM	13574 ± 963.112
LLU	13208 ± 853.304
LFM	11960 ± 894.87
LFU	11708 ± 970.12
BHT	11.16 ± 1.05
Significance level	($p < 0.05$)

[a]: 2,2-Diphenyl-1-picrylhydrazyl

Table 4 Antimicrobial activity of *L. angustifolia* defined as MIC and MBC values

Sample / Essential oil	MRSA[a]		*P. aeruginosa*		*S. aureus*		*E. coli*	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
LFM	1.25	3.12	0.31	0.31	1.25	3.12	0.16	0.63
LFU	0.31	0.63	0.31	0.63	1.25	3.12	0.16	0.63
DMSO	20	>20	10	>20	20	>20	10	>20

[a]: Methicillin-resistant Staphylococcus aureus

Also, Harborne and Williams, reported that the essential oil content of true lavender has large variability not only in different populations but also within the same population [49]. These differences may arise from genotype and climatic conditions, the nature of plant material (fresh or dried), drying method and drying duration, and extraction method also cause variations in the essential oil content and composition [50]. For example, Dusˇkova *et al*. reported that Prolonged storage of dried flowers of lavender causes a reduction in essential oil content obtained by hydrodistillation in the range of 0.007% up to 2.56% per year [51].

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil identified several compounds. According to Table 1., oxygenated monoterpenes are significantly higher than other types of compounds in the flowers and leaves of *L.angustifolia*. The finding is in align with the review presented by Boukhatem *et al*. [17]. As observed in Table 1., the concentration of oxygenated monoterpenes is higher than other compounds in the flowers and leaves of *L.angustifolia*. Although limonene (a hydrocarbon monoterpene) is one of the major components of the essential oil obtained from the leaves of specimens purchased from the market. This high content of limonene may be explained by the duration time of the isolation of essential oil because the extended distillation causes the breakdown of esters to monoterpenes such as limonene [52].

These compounds are often associated with the characteristic aroma of lavender and have been reported in other studies [24, 53]

In addition to these, we detected other compounds such as γ-Terpinen, trans-Linalool oxide, cis-Linalool oxide, p-Cymen-7-ol, α-Terpineol, 4-Terpineol, Isobornyl formate, Limonene, p-Cymene, Caryophyllene oxide, p-Cumic aldehyde, p-Cymen-8-ol, n-Dodecane, n-Decane, trans-Pinocarveol, Camphene, α-Pinene, β-Pinene, 1-Octen-3-ol, Bornyl acetate, and Ho-trienol in varying amounts.

However, the detection of certain compounds such as p-Cymen-7-ol, p-Cumic aldehyde, and p-Cymen-8-ol in our sample indicates a unique chemical profile that warrants further investigation.

The collective presence of these components plays a crucial role in defining the biological properties of the essential oil. This observation aligns with numerous studies that have investigated the distinct chemical compositions of the flowers and leaves of this plant. These findings underscore the importance of considering the source of the essential oil in *L. angustifolia* when examining its potential applications. In line with our study, research conducted by Smigielski *et al*. examined the chemical composition of essential oils derived from both fresh and dried flowers, as well as the aerial parts of *L. angustifolia* [54]. They observed that the process of drying led to a decrease in the concentrations of key components such as linalyl acetate (from 34.4% to 19.7%), 1,8-cineole (from 1.5% to 0.5%), β-ocimene (from 8.2% to 2.9%), and caryophyllene (from 4.0% to 1.0%). However, the concentrations of other components remained unchanged. Interestingly, the essential oil derived from dried flowers introduced new compounds, including lavandulyl acetate (4.5%), linalool oxide (1.9%), and cryptone (0.9%). When compared to the essential oil from fresh aerial parts of lavender, the essential oil from dried aerial parts had lower amounts of linalool (from 31.2% to 26.5%) and α-limonene (from 3.8% to 1.2%). however, new compounds such as β-myrcene (1.1%) and lavandulol (0.7%) were introduced.

The delightful aroma of the plants is primarily due to the presence of monoterpenoids that are synthesized and accumulate in the aerial parts, mainly in flowers [55]. The most appreciated lavender oils for the perfume and cosmetic industries are those that are highly valued in linalyl acetate and linalool content and low content in camphor, while those richer in camphor are predominantly utilized in aromatherapy and herbal medicine [24]. A research paper published in Natural Product Communications identified 78 compounds in the essential oil of *L. angustifolia* [56]. The major constituents of the oil were linalool (30.6%), linalyl acetate (14.2%), geraniol (5.3%), β-caryophyllene (4.7%), and lavandulyl acetate (4.4%). Another study published in Molecules analyzed *L. angustifolia* essential oil using GC-MS and identified a total of 40 compounds, accounting for 92.03% of the total essential oil compositions [54]. A research article published in MDPI states that the main components of the essential oil are typically linalool (20–45%) and its acetate (25–46%). The percentage of other ingredients falls usually within the following ranges: limonene (<1.0%), eucalyptol

(<2.5%), camphor (<1.2%), terpinen-4-ol (0.1–6.0%), lavandulol (>0.1%), lavandulyl acetate (>0.2%), and α-terpineol (<2.0%). Al-Younis et al. examined the chemical profile of essential oils from *L. angustifolia* obtained from the aerial parts of plants grown in Syria, and found a similar composition with borneol (16.25%) and linalool (35.12%) as the primary components, respectively [57]. Meanwhile, the essential oils from L. angustifolia collected in Xinjiang and the Himalayan region showed linalyl acetate (28.89%) and (47.56%) respectively, as the principal molecule [58]. Luu Thai Danh *et al*. examined the impact of three distinct extraction techniques - hydrodistillation, supercritical CO2 extraction (SCE), and hexane extraction - on the yield, chemical composition, antimicrobial and antioxidant properties of *L. angustifolia* essential oil [59]. Even though the extraction methods resulted in essential oils with different chemical compositions, four key compounds - linalool, linalyl acetate, camphor, and borneol - constituted approximately 80% of the identified compounds in all extracts. Linalool was the most prevalent compound, making up about 53%, 43%, and 33% of the hydrodistilled, SCE, and hexane extracts, respectively.

The characteristic scent of lavender primarily originates from compounds such as linalool, linalyl acetate, 1,8-cineole, o-cymene, borneol, and camphor [60].

A study comparing hydrodistillation and hexane extraction of Australian true lavender flowers found that hydrodistilled oil had a higher linalool content (52.59% vs. 33.35%) and a lower linalyl acetate level (9.27% vs. 25.73%) compared to oil extracted using solvents [59]. As it can be noticed from Table 1., Linalyl acetate content is lower in the essential oil from flowers and it is absent in the essential oil of leaves which can be justified in this manner. However, our study also detected several other compounds in varying amounts, indicating a complex chemical profile. The composition and quality of lavender essential oils are influenced by a variety of factors. These include the plant's genotype, the specific plant part from which they are derived, the stage of the plant's growth, environmental conditions like soil and climate, the harvest timing, the methods used for drying, and the techniques employed for extraction [24]. Therefore, while our results may differ from those of other studies, they still contribute valuable information to the overall understanding of the chemical composition of *L. angustifolia* essential oil.

In conclusion, our study contributes to the growing body of research on *L. angustifolia* by providing new insights into its chemical composition. The unique profile of the essential oil from the Hamadan region opens up potential avenues for its use in pharmaceutical, food, and flavor industries, cosmetics, perfumery, and aromatherapy. Future work should focus on understanding the biosynthesis of these compounds and their physiological effects. This could pave the way for the development of new lavender-based products and applications.

Antioxidant

Garzoli *et al*. reported that In their study, the essential oil from *L. angustifolia*, both in liquid and vapor phases, was found to have linalool (49.9%) as its main component [61]. The authors also indicated that the IC50 value for *L. angustifolia* Essential Oil was 7.75 ± 0.10 µg/ml.

Numerous factors contribute to the chemical and biological diversity observed in medicinal and aromatic plants. These include the area of cultivation, farming practices, microclimate conditions, the plant's life stage (whether vegetative or reproductive), and genetic differences [62].

The research conducted by Nikšić *et al*. focused on the analysis of the chemical makeup and the antimicrobial, antioxidant, and antiproliferative properties of essential oils derived from *L. angustifolia* flowers cultivated in the southern region of Bosnia and Herzegovina [63]. The essential oil of *L. angustifolia* was found to be rich in monoterpene alcohols, accounting for 51.8% of its composition, with linalool, lavandulol, terpinen-4-ol, and a-terpineol being the primary constituents. This was followed by monoterpene esters, which made up 22.6% of the oil. The essential oil demonstrated significant antibacterial activity, particularly against Gram-negative strains. The oil also exhibited the ability to neutralize DPPH radicals into a harmless form, DPPH-H, with an inhibitory concentration of 50% (IC50) of 0.421 mg/ml, and this activity was observed to be dose-dependent.

In a study conducted by Smigielski *et al*., they analyzed the chemical composition and evaluated the antioxidant and antimicrobial properties of essential oils derived from both fresh and dried flowers and aerial parts of lavender (*L. angustifolia*) [54]. The primary volatile constituents identified were linalool (26.5-34.7%), linalyl acetate (19.7-23.4%), β-ocimene (2.9-10.7%), and α-terpineol (2.8-5.1%). The essential oils of lavender demonstrated significant activity against bacteria such as B. subtilis, S. aureus, E. coli, and P. aeruginosa, as well as yeast and filamentous fungi including Candida sp., A. niger, and P. expansum. These organisms' growth was inhibited at concentrations ranging from 0.4 to 4.5 µg/ml. The essential oil from dried flowers exhibited the highest antioxidant activity (IC50= 22.1 mg/ml), while the oil from fresh aerial parts showed the least activity (IC50= 77.11 mg/ml).

The antioxidant activity of *L. angustifolia* has been extensively studied. A study by Dobros *et al*. investigated the effect of several extraction procedures applied to three cultivars of *L. angustifolia*on the yield of the polyphenolic compounds and antioxidant activity [53]. The study found that the antioxidant activity was determined by DPPH assay for antiradical properties

(104.58–206.77 µmol Trolox/g) and FRAP assay for reducing properties (79.21–203.06 µmol Trolox/g).

Antimicrobial

Our study's results on the antimicrobial properties of *L. angustifolia* essential oil show some differences when compared to other research findings.

In our study, the lowest MIC and MBC for the essential oil of purchased flower of *L. angustifolia* were observed for the E. Coli bacteria at a concentration of 0.16 mg/ml and *Pseudomonas aeruginosa* at a concentration of 0.31 mg/ml, respectively. The lowest MIC and MBC were observed on Staphylococcus aureus and MRSA which were determined at concentrations of 1.25 mg/ml and 2.5 mg/ml, respectively. For the essential oil of *L. angustifolia* collected from the university garden, the highest and lowest MIC were for *S. Aureus and E. Coli* at concentrations of 1.25 mg/ml and 0.16 mg/ml, respectively. The lowest MBC was for *E. coli*, *Pseudomonas aeruginosa*, and MRSA at a concentration of 0.63 mg/ml.

As demonstrated in Table 3, the antimicrobial properties of the essential oil derived from the flowers of *L. angustifolia* exhibit greater potency against gram-negative bacteria such as *E. coli* and *P. aeruginosa*, with their Minimum Inhibitory Concentration (MIC) ranging from 0.16 to 0.31 mg/ml. This is in contrast to gram-positive bacteria like *S. aureus* and MRSA.

However, these findings are not consistently replicated across different cultivars as observed in other studies evaluating the essential oil from this plant. For instance, research conducted by Smigielski *et al*. demonstrated that the essential oil of *L. angustifolia* cultivated in Poland is more effective against gram-positive bacteria (B. Subtilis and S. Aureus) with a MIC of 0.9 µg/ml, as opposed to gram-negative bacteria *(E. coli* and *P. aeruginosa*) with a MIC of 1.8 µg/ml [54].

In alignment with this research, Inouye *et al.* found that the antimicrobial effects of the essential oil of Lavender are more pronounced against gram-positive bacteria *S. aureus* (MIC: 100 µg/ml) compared to gram-negative bacteria *E. coli* (MIC>1600 µg/ml) [64]. These variations underscore the need for further investigation into the antimicrobial properties of *L. angustifolia* across different cultivars and preparation methods.

Comparatively, a study by Leong *et al*. found that *L. angustifolia* essential oil showed potent antibacterial activity against *Klebsiella pneumoniae*, MRSA, and *S. aureus* with MIC values of 32, 16, and 16 µg/ml, respectively [65]. Another study found that the lavender essential oil showed good antibacterial activity against *Bacillus subtilis, Pseudomonas fluorescens, Xanthomonas campestris, Erwinia carotovora* at 300 µg/ml concentration, and *Erwinia amylovora, Candida utilis* at 150 µg/ml, respectively.

Research by Luu Thai Danh *et al*. analyzed the antimicrobial effects of *L. angustifolia* essential oils extracted by supercritical CO2, Hexane, and hydrodistillation [59]. They reported that the antimicrobial effectiveness of the essential oils varied significantly. None of the oils were as effective as the antibiotic gentamicin, and none were effective against P. aeruginosa. However, gentamicin inhibited P. aeruginosa with a zone of 36.3 mm. The oil from hydrodistillation had the highest antimicrobial activity against all tested microbes (except *P. aeruginosa*), especially against *C. albicans* and *S. aureus*, with inhibition zones of 28.2 and 28.1 mm, respectively. The hexane extract had the least activity, while the solvent extract was only effective against *S. aureus* and *C. albicans* with small inhibition diameters of 7.6 and 8.2 mm, respectively. The SCE oil's antimicrobial activities were higher than the solvent extract but lower than the hydrodistilled oil.

A comparative analysis of the current study with previous research on the essential oil of *L. angustifolia* reveals that the antimicrobial properties of the variant cultivated in Hamadan province are marginally less potent than those of the variants found in Europe.

In the spirit of the combination of herbal extracts and essential oils with the antibiotic drugs, Tamri *et al*. compared the synergistic antibacterial effect of combined *Scrophularia striata* extract and antibiotics against Pseudomonas aeruginosa and methicillin-resistant *S. aureus* (MRSA) [66]. they noted that the plant extract augments the antibacterial efficacy of antibiotics. A combination of *Scrophularia striata* extract (SSE) and Vancomycin demonstrated a range of synergistic to additive effects in combating MRSA. Similarly, when SSE was combined with Gentamicin, it exhibited synergistic to additive effects against *P. aeruginosa*. The interaction of Ceftazidime with SSE resulted in an additive effect against *P. aeruginosa*. The most notable outcome was the synergistic effect observed between SSE and Piperacillin-Tazobactam against *P. aeruginosa*. In summary, their study suggested that S. striata holds promise in enhancing the antibacterial properties of antibiotics and could potentially contribute to the development of new compounds that exhibit synergistic effects when combined with standard antibiotics.

The extraction methods used to obtain the essential oil can also impact the results. Different extraction methods can yield essential oils with different chemical compositions, even from the same plant material. This is because different extraction methods may be more or less efficient at extracting different compounds.

The specific cultivar of *L. angustifolia* used can also influence the results. Different cultivars of the same plant species can have different chemical compositions due to genetic differences and differences in their growing conditions.

Therefore, while our results may differ from those of other studies, they still contribute valuable information to the overall understanding of the antimicrobial properties of *L. angustifolia* essential oil. Our study underscores the need for further research to fully understand the factors influencing the antimicrobial activity of *L. angustifolia* essential oil and to explore its potential therapeutic applications.

CONCLUSION

This research demonstrated the variations in the chemical composition, antimicrobial, and antioxidant properties of the essential oil derived from the leaves and flowers of *L. angustifolia* concerning its cultivation habitat. The findings indicate that the essential oils of *L. angustifolia* from Hamadan province, Iran possess greater efficacy against gram-negative bacteria. Moreover, the antioxidant activity of the essential oil from the flowers of the plant is more pronounced than that from the one derived from leaves. This was also observed that certain components are exclusively present in the essential oil from the flowers or leaves and vice versa. This could potentially contribute to the observed variations in the biological properties of the different essential oils derived from this plant. Given the widespread use of *L. angustifolia* in natural medicine, aromatherapy, perfume, and the cosmetic industry, it would be beneficial to conduct more comprehensive and extensive research on each chemical constituent and its relative abundance in the essential oil. This could further enhance our understanding of the plant's therapeutic potential and applications in various industries.

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