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Chemical Composition, Antimicrobial and Antioxidant Activities of *Lavandula Angustifolia* Essential Oil

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ABSTRACT

Introduction: In response to concerns arising from the application of synthetic preservatives in foodstuffs, several alternative methods have been proposed, such as the application of natural preservatives. This study aimed to evaluate the potential application of natural-based *Lavandula angustifolia* essential oil (LEO) as a food preservative by means of in-vitro antimicrobial and antioxidant assays.

Methods: The main constituents of LEO were determined by GC-MS method. To assess the antibacterial efficacy of the tested LEO, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oil were determined against four important foodborne pathogens by microdilution broth method. Finally, DPPH method was used for the evaluation of the antioxidant properties of the LEO.

Results: According to the chemical analysis, terpenes, like linalool and 1,8- Cineol, constitute the main part of LEO. Antioxidant evaluation revealed that the DPPH radicals inhibitory percentage of LEO in concentrations of 40, 80, 120, 160, 200 and 240 (µg/ml) were 31, 43, 47, 53, 60 and 66 (%), respectively. Regarding antibacterial analysis, the tested LEO could efficiently inhibit and kill the microorganisms including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhimorium* and *Listeria monocytogenes* with the MIC and MBC range of 1.5 to 4 mg/ml.

Conclusion: In conclusion, this work indicates the promising antimicrobial and antioxidant potential of LEO to be considered by food scientists and researchers in future studies.

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Introduction

Microbial contamination and lipid oxidation in food materials are among the most important problems that could threaten public health and/or accelerate the destruction of the nutritional and sensory quality of various food products, especially meat products (1).

Nowadays, there are several methods for increasing shelf life and safety of foodstuffs, such as thermal processing and using synthetic preservatives. Each of these methods has its own limits (2).

For instance, thermal processing can disable many food-borne pathogens, but it can also decrease the nutritional value of food products (3). Moreover, several concerns have been raised over long-term usage of synthetic preservatives which may cause various disorders such as cancer in consumers and increase of microbial resistance (4).

In response to these problems and concerns, several alternative methods have been proposed, such as using natural preservatives. It has been shown that these compounds can improve shelf life and safety of the products with minimal side effects. A very important group of natural preservatives are essential oils (EOs) and plant extracts, which are derived from a wide range of plants (4).

Many of these plants have great economic value, and almost 700,000 plant species with antimicrobial properties are known in tropical regions. These plants mainly have antibacterial, antioxidant, antiviral, antifungal, anticancer and larvicidal properties (5,6). Currently, herbal EOs are used in cosmetics, sanitizers and food packaging materials. The antimicrobial and antioxidant impacts of EOs are well demonstrated in real food models and in-vitro against many foodborne pathogens and spoilage bacteria (7,8).

Among the various plants, *Lavandula angustifolia* is an aromatic medicinal plant that has multiple uses, and its effectiveness has been

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proven against several diseases. The lavender, scientifically known as *Lavandula angustifolia* that belongs to a plant family namely *Lamiaceae* (9).

This plant is grown in the north parts of Iran, the Canary Islands, the Mediterranean region, West Asia, the Arabian Peninsula, and the tropical area of Africa and India. The leaves and flowers of lavender have the highest amount of EO (10). Lavender EO (LEO) has anti-inflammatory, sedative and, most importantly, antibacterial, antifungal and antiseptic properties (9).

The *lamiaceae* family are rich in phenolic compounds and have excellent antioxidant activity (11). It has been proven that the antimicrobial properties of a whole EO are superior to its constituents (12). Due to the antimicrobial properties and monoterpenes compounds of LEO, this EO is applied as a burn treatment and acted successfully against some foodborne pathogens like *Salmonella enterica* and *Listeria monocytogenes* (13,14).

During the last few decades, a huge body of studies was conducted to evaluate the biological properties of natural compounds particularly herbal EOs and extracts (15). Among those active agents, some members of *lamiaceae* family such as thyme, oregano and sage were more studied and examined in different mediums and food models (16,17), while others like lavender, perilla and hyssop were less evaluated. In this concern, although some efforts have been carried out to test the biological properties of LEO (18,19), it seems that more detailed works are still needed to discover its probable unknown biological capacities. Moreover, as far as we know, the antibacterial properties of LEO were mostly evaluated against some special strains of bacteria and comprehensive evaluation has never been carried out yet, particularly regarding foodborne pathogens.

Therefore, this study was aimed to examine the in-vitro antioxidant and antibacterial properties of LEO (against a range of important Gram-negative and Gram-positive foodborne pathogens) and evaluate its chemical composition.

Materials and Method

Materials

Lavender EO was obtained from Johare Taam Co. (Mashhad, Iran) and kept in refrigeration condition at 4 °C until used in the dark. Dimethyl sulfoxide (DMSO) and Brain Heart Infusion (BHI) agar and broth were purchased from Merck (Germany). All of the solvents and reagents like 2,2-diphenyl-1-picrylhydrazyl (DPPH) were of the analytical grade.

Methods

Chemical analysis of LEO

Chemical analysis of the LEO was performed by means of Gas Chromatography-Mass Spectrometry System (GC-MS, Agilent 7890A/5975C), equipped with a capillary column with 0.25 mm diameter and 30 m length and film thickness of 0.25 µm. The temperature of oven was programmed from 50 °C to 240 held for 3 and 1 min respectively at a rate of 5 °C/min. The temperature of injector port was 250 °C and the flow rate of helium (as carrier gas) was set at 1 mL/min. The identification of resulting GC-MS peaks was carried out by comparing with data from National Institute of Standards and Technology (NIST 08) commercial libraries, the Wiley (2001 data software) and the literature.

Evaluation of the antibacterial activity of LEO

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LEO were determined by microdilution broth assay according to the methods of the CLSI laboratory standard and Merrikhi Ardebili et al (2019) with some modifications(20). Six strains of Gram-positive and Gram-negative foodborne pathogens including *Staphylococcus aureus* (25923 ATCC), *Escherichia coli* (25922 ATCC), *Bacillus cereus* (10876 ATCC), *Salmonella typhimorium* (14028 ATCC), *Escherichia coli* (12900 NTCC) and *Listeria monocytogenes* (7644 ATCC) were used in this study for MIC and MBC evaluation. (21,22).

To prepare the bacterial suspension, the inoculum of bacterial strains was obtained from an overnight BHI culture and transferred to tubes containing 4 or 5 mL of 0.9% saline to achieve 0.5 McFarland standard turbidity with the final density of 1×10^8 CFU/mL.

To prepare LEO dilutions, the studied LEO was dissolved in 1 mL DMSO in a sterile tube to obtain a mixture of 4% wt (or 40 mg/mL) of the LEO.

Eight sterile microtubes were selected to dilute the essential oil. Twofold serial dilution was made by serially transferring 500 µL of the LEO

dilutions to next microtubes containing 500 µl of BHI. Finally, a concentration range of 40 to 0.31 mg/mL of LEO in 500 µl sterile test tubes containing BHI broth were obtained.

In the next step, 20 µL of each inoculum was added to the different rows of a 96-well microplate containing 160 µL of BHI broth. Subsequently, 20 µL of the resulting LEO dilutions were transferred into the relevant wells. Consequently, the final concentrations of the LEO in each well was 4 to 0.31 mg/mL. Wells without LEO (20 µL of the inoculums and 180 µL of BHI broth) and wells without the tested bacteria (20 µL of LEO and 180 µL of BHI broth) were considered as positive and negative controls, respectively. The tested microplates were then placed on a plate shaker at 100 rpm for 60 s, and finally incubated for 24 hours at 37 °C. The MICs of LEO, was defined as the lowest concentrations of the tested LEO which did not allow cell growth (no turbidity in the well) after 24 h at 37 °C. For determination of MBC values, non-turbid wells were inoculated on BHI agar and incubated at 37 °C for 24 h the lowest concentrations with no obvious bacterial growth on the agar (lowest concentration that can cause the death of 99.9% of bacteria) were considered as MBC values of the LEO against the tested bacteria.

Antioxidant activity

DPPH (radical scavenging ability assay)

The hydrogen atoms or electrons donation capacity of the LEO was measured from the bleaching of purple coloured methanol solution of DPPH (23).

Five mL of a 0.004% methanol solution of DPPH was added to 50 µl of various concentrations of LEO (40, 80, 120, 160, 200, and 240 µg/ mL) and the mixture was vigorously stirred. The test tubes were kept in a dark place at room temperature for 30 minutes. Afterward, the absorbance of the solutions was assessed at 570 nm. It should be noted that in the control sample, LEO was replaced by 50 µl of methanol. The radical scavenging activity of LEO was calculated by using the following equation:

$$\text{Radical scavenging (\%)} = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

Where A_{blank} and A_{sample} are the absorbances of the control and the sample, respectively. EC50 value (µg/ml DPPH), demonstrating the effective concentration of LEO at which 50% of the DPPH radicals are scavenged, was calculated by interpolation from the linear regression analysis. Butylated hydroxytoluene (BHT, 1.0 mg/mL) was used as positive control.

Statistical analysis

The data obtained in this study were statistically analyzed using SPSS software (version 16) and the data were compared with one-way ANOVA with Tukey test. Statistically, the mean difference at the 5% probability level was significant.

Results and discussion

Chemical analysis

The main chemical constituents of LEO with the corresponding retention time and concentrations are presented in Table 1. As can be seen, six major compounds of the LEO were linalool, 1,8- Cineol, roesfuran epoxide, camphor, isomenthol and menthon in order of abundance.

Base on the studies that assessed the chemical composition of lavender plant by GC/MS method (24–28), linalool, linalyl acetate, 1,8-cineole, camphor, caryophyllene, borneol, beta-pinene and carvacrol were identified as the main constituents of LEO. In a study, different samples of lavender obtained from Italy, Greece, Bosnia-Herzegovina, France and Spain were evaluated (24). The results showed that the main compounds in LEO obtained from different geographical areas were linalool (61.98-0.94), linalyl acetate (0.28-2.89), 1,8 cineole (1.51-8.11), Carvacrol (29.78-44), caryophyllene (0.22-2.89), borneol (2.5-7.6) and camphor (1.5-5.3). In fact, linalool and camphor were the only constituents that similarly presented in the tested LEO in the present study with almost similar concentrations.

Hanamanthagouda et al. (2010) investigated the antimicrobial properties of lavender essential oil and evaluated its constituents by GC/MS method (29). During this analysis, the main constituents of lavender essential oil, trans-carveol (18.93%), pollen (8.45%), camphor (7.09%) and menthol (5.89%) were identified. Trans-carveol and pollen are used as aromatic substances in cosmetics and food industries. Camphor and menthol are two compounds with antibacterial properties that are easily absorbed through the skin and create a cool feeling on the skin (30).

Among the above-mentioned compounds in the tested LEO, linalool and 1,8-Cineol were repeatedly reported as good antimicrobial and antioxidant agents (31,32)

Table 1. Chemical analysis of *Lavandula angustifolia* Essential Oil by GC-MS

Compounds	Retention time (min)	GC area (%)
p-cymene	10.02	0.9
Limonene	10.08	0.8
1,8- Cineol	10.11	21.5
Linalool	12.85	44.94
Camphor	14.11	5.6
Menthone	14.86	4.2
Roesfuran epoxide	16.06	9.3
Isomenthol	16.39	4.8
Dihydrocarvone	17.04	3.8
Total		95.84

Antioxidant properties

One of the most common methods for evaluating the antioxidant activity of plant EOs is DPPH method. This method is based on trapping the number of free radicals of a substance called 2,2- diphenyl-1-picrylhydrazyl using antioxidants which reduce the absorption at 520 nm. Calculating 50% inhibition concentration (IC50) is a good way to evaluate and compare the antioxidant activity of different substances. IC50 expresses the amount of antioxidant required to reduce free radicals by 50%. Figure 1 shows the results obtained during the measurement of the percentage of DPPH radical inhibited by different concentrations of LEO and BHT. As shown, as the concentration of BHT or LEO increased, the percentage of free radical scavenging elevated. It was also observed that the percentage of free radical scavenging for all concentrations of LEO was lower than BHT.

In this study, the calculated IC50s for LEO and BHT were 133±0.37 and 98±0.46 µg/ml, respectively. In general, the lower the IC50 index value, the greater the antioxidant activity. In the present study, the DPPH radicals inhibitory percentage of LEO in concentrations of 40, 80, 120, 160, 200 and 240 (µg/ml) were 31, 43, 47, 53, 60 and 66 (%), respectively.

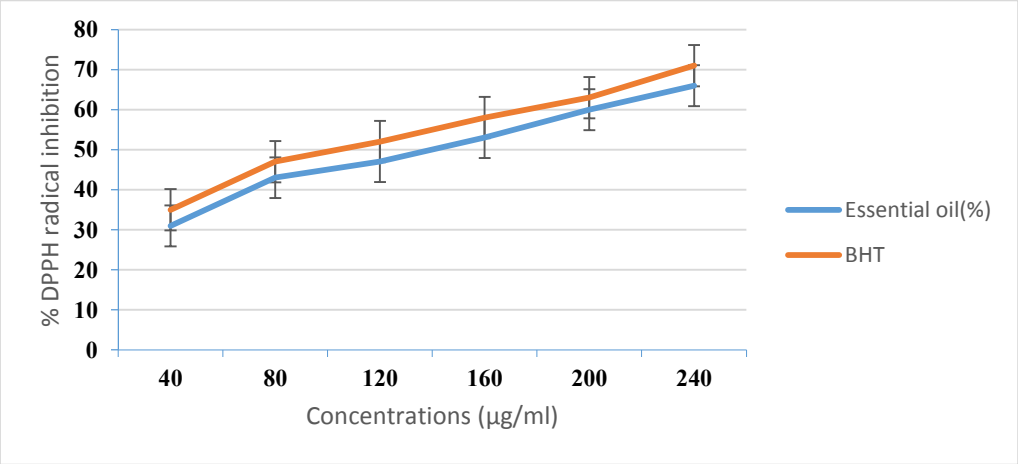

Figure 1. DPPH scavenging activity of *Lavandula angustifolia* essential oil. Date are expressed as mean ± SD (n = 3).

It has been well demonstrated that the antioxidant activity of EOs depends on the mobility of hydrogen atoms in the hydroxyl group of phenolic compounds (33). Moreover, some compounds like linalool showed significant antioxidant effects.

In a study by Yadikar et al. (2018), the antioxidant properties of seven phenolic compounds from *Lavandula angustifolia* were compared with vitamin C (11). IC50 levels for the studied compounds such as lavandufurandiol and lavandunat were reported as 3.63 and 0.04 µg/ml, respectively, and in fact, their antioxidant effects were comparable with vitamin C (with IC50 of 5.34 µg/mL) as an ideal antioxidant agent. In general, the closer the

value of IC50 of a compound to the IC50 of vitamin C, the stronger the antioxidant properties of the substance.

In another study, Barkat et al. (2012) examined the antioxidant activity of *Lavandula stoechas* EO and vitamin E. The results of this work showed that the percentage of inhibition of DPPH radical by the tested essential oil at concentrations of 600 and 500 µg/mL were 50% and 42%, respectively, while the level of antioxidant activity for vitamin E in these concentrations were 60% and 53%, respectively. They also reported the antioxidant activity of the tested EO was increased in a dose-dependent manner.

The findings of the aforementioned studies beside the present work revealed that the antioxidant impacts of *Lavandula* genus EO are comparable with the well-known commercial antioxidants such as ascorbic acid and BHT.

Antimicrobial properties

Table 2 shows the MICs and MBCs of the LEO against six food-borne pathogens based on the broth microdilution method. The results revealed that the LEO had different degrees of growth inhibition activity against all of the tested microorganisms.

Regarding MIC values, the highest and the lowest antibacterial activity of the LEO were recorded against *B. cereus* and *L. monocytogenes* respectively. On the other hand, the MBCs showed that with the exception of *Escherichia coli*, the same values as MICs were recorded for the other bacterial pathogens. In fact, in this study *L. monocytogenes* was the least sensitive strains in comparison with the others ($P < 0.05$).

Table 2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Lavender essential oil against some food-borne pathogens by microdilution broth method*.

Pathogens		MIC (mg/mL)	MBC (mg/mL
Gram (+)	*Staphylococcus aureus*	2 ± 0[b]	2 ± 0[b]
	Listeria monocytogenes	4 ± 0[a]	4 ± 0[a]
	Bacillus cereus	1.5 ± 0.7[b]	1.5 ± 0.7[b]
Gram (-)	*Salmonella typhi*	2 ± 0[b]	2 ± 0[b]
	Escherichia coli O157:H7	2 ± 0[b]	2 ± 0[b]
	Escherichia coli (ATCC 25922)	2 ± 0[b]	4 ± 0[a]

* Data are expressed as mean ± standard deviation (n=3)

[a–b] Different lowercase letters within a column indicate significant differences ($P < 0.05$).

Herbal EOs have great potential to be used against many food-borne pathogens. In this work, the antibacterial effects of LEO were evaluated on six different and important pathogens. The standard microdilution broth method was chosen to assess the in-vitro antibacterial strength of LEO via determining MIC and MBC values. Our findings showed that the tested LEO could efficiently inhibit and kill the microorganisms with the MIC and MBC range of 1.5 to 4 mg/ml.

Concerning the antimicrobial properties of LEO, some efforts have been made over the years, and different results were reported. In a study by Predoi et al. (2018) the in vitro antimicrobial activity of basil and lavender EOs against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were investigated(7). They reported a very good inhibitory activity of the LEO with a MIC range of <0.1% (or <1 mg/ml) for *E. coli* reference strain to 0.78% (or 7.8 mg/ml) for *S. aureus* strains. In fact, their findings were comparable to the results obtained in the present study. Moreover, in another work, the MIC of a LEO against *S.aureus* and *E.coli* were 1.6 and 1.2 mg/ml respectively (18), which were also in accordance with our findings.

On the other hand, higher antibacterial effects of LEO were reported by some authors. For instance, Tardugno et al. (2018) evaluated the MIC of four cultivars of Lavandula EOs against *Salmonella enterica* (10 food strains) and *Listeria monocytogenes* (24 strains) (26). The reported MICs for the examined LEOs were ≥ 10.0 µL/mL (against *Salmonella*) and 0.3 µL/mL versus *L. monocytogenes*, revealing evident and greater activity in comparison with the MICs recorded in the present work.

In fact, the abovementioned variation in the MIC values and antibacterial impacts of an EO is due to several reasons including the herb intrinsic factors, testing method and the strain of tested microorganisms. Between these parameters,

intrinsic factors of EOs including pre-harvest (such as growth condition, genotype, irrigation, etc.) and post-harvest factors (such as storage condition, processing, etc.) were stated as the main factors affecting the biological properties of the herbal extracts and oils (34).

It is also worthy to compare the antimicrobial strength of other herbal oils with LEO. Among different herbs, thyme, oregano, clove and rosemary are well known for their strong antibacterial properties (15). In this concern, in a study by Puškárová et al. (2017), MIC values of thyme, oregano and clove against *Escherichia coli* were 1.2, 0.2 and 1.2 mg/ml respectively (35). These findings indicate satisfactory antibacterial properties of lavender oil between important plant EOs.

Various antibacterial mechanisms were mentioned for EOs in the literature. Among those, the cellular damages caused by lipophilic compounds of EOs and subsequent increase of permeability and release of cellular contents of bacteria were indicated as the main antibacterial mechanism of EOs (36).

Conclusion

In the present work, the antioxidant and antibacterial activity of LEO was examined in order to evaluate its potential for food application. In this regard, both antioxidant and antibacterial analysis indicate satisfactory biological effects of the oil especially comparing to the well-known food preservatives and herbal EOs. Getting together, this work revealed the promising food preservative potential of LEO to be considered by food scientists and researchers in future studies.

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Conflicts of interest

The authors notify that they have no conflicts of interest

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