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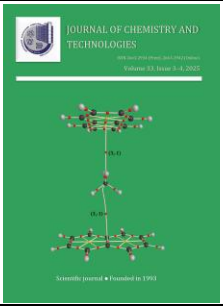

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ESSENTIAL OIL OF LAVENDER FLOWERS (*LAVANDULA ANGUSTIFOLIA* MILL.) FROM LAM DONG, VIETNAM: CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES

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Abstract

The current research examines the sensory qualities, key physicochemical parameters, comprehensive chemical profile, and notable bioactivities—particularly its antibacterial and antioxidant potential—of lavender (*Lavandula angustifolia* Mill.) essential oil. Gas chromatography-mass spectrometry was utilized to evaluate the volatile components of the essential oil extracted from lavender flowers. The results indicated that the primary chemicals responsible for the essential oil's distinctive aroma were linalyl acetate (39.22 %) and β-linalool (24.15 %). Furthermore, the study found that the oil possesses a freezing point below –20 °C. Assessment of the essential oil's biological properties revealed a moderate effectiveness in inhibiting the growth of *Bacillus cereus* and *Escherichia coli*, suggesting possible applications in antibacterial formulations. However, its antioxidant efficacy was significantly lower than that of the reference standard, vitamin C, evidenced by an IC_{50} value of 77.11 mg/mL. By thoroughly examining these characteristics, this study contributes valuable scientific knowledge that can inform quality assessment and explore the broad range of potential applications for lavender essential oil in various industrial sectors.

Keywords: antioxidant capacity; antibacterial activity; *Lavandula angustifolia;* essential oil; gas chromatography–mass spectrometry; paper disk diffusion method.

ЕФІРНА ОЛІЯ КВІТІВ ЛАВАНДИ (*LAVANDULA ANGUSTIFOLIA MILL.*) З ЛАМ ДОНГ, В'ЄТНАМ: ХІМІЧНИЙ СКЛАД ТА БІОЛОГІЧНА АКТИВНІСТЬ

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Анотація

У даному дослідженні вивчаються сенсорні якості, основні фізико-хімічні параметри, комплексний хімічний профіль та значні біологічні властивості, зокрема антибактеріальний та антиоксидантний потенціал ефірної олії лаванди (*Lavandula angustifolia Mill.*). Для оцінки летких компонентів ефірної олії, видобутої з квітів лаванди, було використано газову хроматографію-мас-спектрометрію. Результати показали, що основними хімічними речовинами, відповідальними за характерний аромат ефірної олії, були ліналілацетат (39.22 %) та β-ліналоол (24.15 %). Крім того, дослідження виявило, що олія має температуру замерзання нижче –20 °C. Оцінка біологічних властивостей ефірної олії виявила помірну ефективність у пригніченні росту Bacillus cereus та Escherichia coli, що свідчить про можливе застосування в антибактеріальних препаратах. Однак його антиоксидантна ефективність була значно нижчою, ніж у еталонного стандарту, вітаміну C, про що свідчить значення IC_{50} 77.11 мг/мл. Завдяки ретельному вивченню цих характеристик, дане дослідження робить цінний внесок у наукові знання, які можуть бути використані для оцінки якості та дослідження широкого спектру потенційних застосувань ефірної олії лаванди в різних галузях промисловості.

Ключові слова: антиоксидантна здатність; антибактеріальна активність; *Lavandula angustifolia*; ефірна олія; газова хроматографія-мас-спектрометрія; метод дифузії на паперовому диску.

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Introduction

The flowering plant lavender (*Lavandula angustifolia* Mill.), which belongs to the Lamiaceae family, is well-known for its powerful, calming scent and lovely appearance [1]. The Mediterranean region is the origin of lavender, which is grown extensively worldwide for its high economic worth in the aromatherapy, cosmetic, and pharmaceutical industries as well as for ornamental uses. Essential oil is the most well-known product made from lavender. This valuable liquid has a complicated chemical makeup and a wide range of possible biological activities [2].

The primary method for extracting *Lavandula angustifolia* essential oil (LaEO) from dried or fresh flowers is steam distillation. Through the capture of volatile aromatic molecules, a highly valued product with a distinctive aroma that is described as floral, sweet, and slightly woody is produced [3]. There are hundreds of distinct chemicals that make up the chemical composition of LaEO, with linalool and linalyl acetate often being the two most prevalent ones. The biological activity and aromatic quality of the essential oil are determined by the amount and presence of these components [4].

Due to its possible therapeutic benefits, LaEO has been utilized in aromatherapy and traditional medicine since ancient times [5]. Numerous actions of LaEO, such as antibacterial, antifungal, anti-inflammatory, antioxidant, sedative, and anxiolytic qualities, have been shown in contemporary scientific investigations [6].

These qualities make LaEO a popular ingredient in cosmetics, personal care products, perfumes, alternative therapies, and natural health care. However, several variables, including as the cultivar, the soil and climate of the producing region, the stage of flower harvest, and the extraction technique, can greatly affect the chemical makeup and biological activity of LaEO. To guarantee its efficient usage and expand its possible uses, it is crucial to research and assess the quality of LaEO from various sources. In particular, lavender grown in Lam Dong (Vietnam) has received little attention and is mainly cultivated as an ornamental plant. This material may exhibit different properties compared to those from other regions.

Materials and methods

Plant material

Lavandula angustifolia flower, which is cultivated and collected in Lam Dong province, Vietnam. Steam distillation is used to extract the flower's essential oil (EO) at 100 °C for three hours. To preserve its quality and long-term efficacy, the extracted essential oil is kept at room temperature in a dark glass bottle.

Bacterial strains

Four bacterial strains were used in this investigation: two Gram-positive bacteria, *Bacillus cereus* (ATCC 11778) and *Staphylococcus aureus* (ATCC 33591), and two Gram-negative bacteria, *Salmonella enteritidis* (ATCC 13076) and *Escherichia coli* (ATCC 25922). The Institute of Biotechnology and Food Technology at the Industrial University of Ho Chi Minh City supplied these bacterial strains.

Chemicals

Dimethyl sulfoxide (DMSO, China), 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, USA) among the compounds utilized in the investigation. Furthermore, the antibacterial and culture testing medium, including nutritional broth (HiMedia, India) and Mueller-Hinton agar (HiMedia, India), as well as other chemicals, were of analytical quality.

Evaluation of the physical properties of LaEO

According to ISO 279 (1998) [7], 1242 (2023) [8], and 7660 (1983) [9], 1041 [10], the following parameters were evaluated relative density (RD), absolute density (AD), acid value (AV), ester value (EV), saponification value (SV), and freezing point (FP), respectively.

Fragrance retention (FR) of LaEO

With a few minor adjustments, the techniques outlined by Quoc [11] were used to estimate the EO's fragrance retention (FR) based on concentration and FR duration. 96 % ethanol was combined with the LaEO to create concentrations of 20, 40, 60, 80, and 100 % (v/v). Two drops of oil were placed on a smell test paper, and the piece was spread uniformly around the paper for a few seconds. The time required for the fragrance of the LaEO to disappear completely under normal conditions was recorded to evaluate fragrance retention.

Gas chromatography–mass spectrometry (GC-MS) analysis

The GC-MS-QP2020 (Shimadzu, Japan) was connected to the gas chromatography GC-2030 (Shimadzu, Japan). For the analysis, a capillary column Rxi-5MS (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm, Shimadzu, Japan) was utilized. Column head temperature: maintained at 50 °C for 4 min, raised at a rate of 2 °C/min to 80 °C, then 5 °C/min to 150 °C, then 10 °C/min to 200 °C, then 20 °C/min to 300 °C, and finally held for 3 min. The temperature of the ion chamber was 230 °C. At a flow rate of 1.69 mL/min, helium gas was employed as the carrier gas. Using a 1:10 split ratio and a head pressure of 100 kPa, divide the flow.

Determination of antioxidant capacity of LaEO

With some adjustments, Quoc [11] described the DPPH technique for measuring the antioxidant capacity LaEO utilizing free radical scavenging activity (RSA). Each of the various EO concentrations was mixed with 96 % ethanol. After mixing 2.7 mL of 0.1 mM DPPH solution with 0.3 mL of EO solution, the mixture was allowed to sit at room temperature for 30 min in the dark. A spectrophotometer was used to measure the DPPH color shift at 517 nm. To create a control sample, vitamin C was utilized. Based on the concentration of LaEO, the percentage inhibition was calculated to find the concentration for 50 % inhibition (IC_{50}). The antioxidant capacity (AC) was calculated using the formula below:

$$\%DPPH_{RSA} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The A_{sample} value is the measured absorbance of the LaEO solution in the presence of DPPH solution, and $A_{control}$ is the measured absorbance of the DPPH solution.

Determination of the antibacterial activity (AA) of LaEO

The antibacterial capacity (AA) was determined using the paper disk diffusion method based on the method described by Quoc [11], with some modifications. First, 100 µL of bacterial suspension (0.5 McFarland standard concentration, equivalent to approximately 1.5×10^{8} CFU/mL) was evenly spread onto MHA medium. Sterile paper disks (6 mm in diameter) were inoculated with 5 µL of the EO; gentamicin (10 µg/disc) and dimethyl sulfoxide (DMSO) (5 %, v/v) were used as positive and negative controls, respectively. The paper was compressed at 37 °C for 24 h. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the paper disk.

Statistical data analysis

Analysis of variance (ANOVA) and mean comparison were performed using Statistics 20 software. The 95 % confidence level ($p < 0.05$) was calculated using the Tukey HSD method. The results are shown as mean ± standard deviation.

Results and discussion

Determination of physicochemical properties of LaEO

The physical and chemical parameters determined (Table 1) included freezing point, specific gravity, density, acid value (AV), esterification value (EV), saponification value (SV) and aroma retention at different concentrations.

Table 1

Physicochemical properties of LaEO

No.	Physicochemical properties	Value
1	Freezing point (FP, °C)	< −20 °C
2	Relative density (RD)	0.8850 ± 0.0113
3	Absolute density (AD, g/mL)	0.8410 ± 0.0132
4	Acid value (AV, mg KOH/g EO)	1.21 ± 0.16
5	Saponification value (SV, mg KOH/g EO)	95.05 ± 2.32
6	Ester value (EV, mg KOH/g EO)	93.85 ± 2.47
7	Fragrance retention (FR, h):	
	20 % EO	5.75 ± 0.14
	40 % EO	18.66 ± 0.43
	60 % EO	29.76 ± 0.21
	80 % EO	41.12 ± 0.07
	100 % EO	57.21 ± 0.11

The FP of LaEO was recorded to be below -20 °C, indicating its ability to maintain a liquid state at low temperatures. This study's LaEO has a far lower FP than previous research on *Ageratum conyzoides* L. essential oil (−10.33 °C) [12]. Despite being a widely used essential oil, there is little data from earlier research on the FP of LaEO, which makes direct comparisons challenging.

LaEO has RD of 0.8850 and AD of 0.8410 g/mL. These values of LaEO in this study tends to be lower than those of lemongrass EO (0.9015 g/mL) and *Mentha arvensis* L. EO (AD: 0.8959 g/mL, RD: 0.8987) [11; 13].

The quality of essential oils was assessed using chemical indices (AV, EV, and SV). In this study, the AV, EV, and SV of LaEO were 1.21 mg KOH/g, 93.85 KOH/g, and 95.05 mg KOH/g,

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respectively. When compared to Kaffir lime peel EO (EV 10.66 mg KOH/g) and *Cymbopogon citratus* EO (AV 0.55 mg KOH/g, EV 189.21 mg KOH/g, SV 109.76 mg KOH/g), a significant difference was noted [14; 15]. LaEO relatively high EV value indicates possible uses in the medicinal and industrial sectors, while its higher AV value might indicate a greater vulnerability to oxidation [16].

A long-lasting aroma retention was also seen when the LaEO was tested at various concentrations. It ranged from 9.1 hours at 20 % (v/v) concentration to 25.08 h in its pure form. According to Ahmed et al., the essential oil's chemical makeup, which contains aromatic chemicals including limonene, linalool, and citral, may be connected to this smell retention [17]. This finding implies that there is a great chance that LaEO will be used in cosmetics, fragrances, and medical supplies.

Chemical composition of LaEO

The study's total LaEO content consisted of 99.37 % of the 33 useful components found by GCMS analysis (Table 2). This number of compounds differs from earlier research; for instance, Dong et al. [18] found 40 compounds (92.03 % of the total composition) in the essential oil from the flowers and leaves of *L. angustifolia* in China, while Ciocarlan et al. [19] found 41 components (99.79 %) in the LaEO from the Republic of Moldova. Geographical considerations (temperature), agricultural practices, lavender types, and extraction processes may all have an impact on the diversity in the chemical composition of LaEO reported in different research [20].

Table 2

Chemical composition of LaEO

No.	RT. (min)	Compounds	Content (%)
1	7.621	α-Pinene	4.72
2	8.343	Camphene	0.11
3	9.737	β-Pinene	6.14
4	10.086	1-Octen-3-ol	0.09
5	11.225	Pseudolimonene	0.05
6	12.449	o-Cymene	0.44
7	12.760	**D-Limonene**	8.50
8	12.867	Eucalyptol	7.91
9	13.350	cis- β-Ocimene	0.07
10	13.980	trans- β-Ocimene	0.13
11	16.456	cis - Linalool oxide	0.03
12	17.575	**β-Linalool**	24.15
13	19.782	6,7-Dihydrolinalool	0.29
14	20.083	Camphor	0.69
15	21.425	Lavandulol	0.05
16	21.588	Borneol	0.31
17	22.079	Terpinen-4-ol	0.36
18	22.849	α-Terpineol	0.10
19	24.293	cis - Geraniol	1.06
20	24.469	β-Citronellol	0.81
21	24.590	α-Terpiny acetate	0.24
22	24.822	cis – Citral	0.07
23	25.469	**Linalyl acetate**	39.22
24	26.025	trans-Citral	0.08
25	26.705	Lavandulyl acetate	0.26
26	28.279	β-Terpinnyl acetate	0.13
27	28.773	α-Terpinyl acetate	2.68
28	29.213	Neryl acetate	0.13
29	29.832	Geranyl acetate	0.23
30	31.005	β-Caryophyllene	0.18
31	32.045	trans- β-Farnesene	0.08
32	25.172	Caryophyllene oxide	0.04
33	36.995	α-Bisabolol	0.02
Total:			99.37 %
Monoterpene hydrocarbons			20.16 %
Oxygenated monoterpenes			78.89 %
Sesquiterpene hydrocarbons			0.26 %
Oxygenated sesquiterpenes			0.06 %

Similar to the primary components detected in LaEO from China and the Republic of Moldova, the primary chemicals identified in LaEO in this study were linalyl acetate (39.22 %), β-linalool

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(24.15 %), and D-limonene (8.50 %). However, there were notable differences in the amounts of these compounds. For instance, in China, the amounts of D-limonene, β-linalool, and linalyl acetate were 0.58 %, 19.71 %, and 26.61 %, respectively [19]. Although their contents may differ depending on the variety, provenance, and extraction technique, this study is also in line with the findings of Kozuharova et al. [21], who identified linalyl acetate and linalool as the two primary components that influence the quality of LaEO. Both linalool and linalyl acetate are well-known for their pharmacological uses, especially their calming and anti-inflammatory properties. Inhaling linalool has also been demonstrated to produce good psychedelic effects [22].

In addition to the most significant compounds, a number of noteworthy bioactive compounds with low abundance were also found, such as terpinen-4-ol, α-pinene, and β-pinene. These compounds have demonstrated strong antifungal activity against both Gram-positive and Gram-negative bacteria [23]. Major antiparasitic chemicals found in many essential oils, such as eucalyptol and borneol [24], have demonstrated promise for usage as preservatives. This implies that LaEO is useful for applications in food and medicine in addition to the cosmetics and fragrance sectors.

Monoterpenes (99.05 % with 28 compounds, of which monoterpene hydrocarbons accounted for 20.16 % and oxygenated monoterpenes for 78.89 %) made up the majority of LaEO in this study, according to chemical composition analysis. Sesquiterpenes (0.32 % with four compounds) made up a minor portion. This demonstrates that monoterpenes, which are also prevalent in many other essential oils, are the most valuable and distinctive class of chemicals found in LaEO.

Determination of the antioxidant capacity of LaEO

Table 3 shows that both the control and test samples showed a favorable connection between antioxidant content and DPPH inhibition. The AC of the LaEO was much lower than that of vitamin C (IC_{50} = 3.56 μg/mL), as seen by the LaEO's IC_{50} value of 250 mg/mL, which was significantly higher than that of the powerful antioxidant (vitamin C).

Table 3

Antioxidant capacity of LaEO

Test sample	IC_{50}
Vitamin C (μg/mL)	$3.56^{a} \pm 0.84$
LaEO (mg/mL)	$250^{b} \pm 3.62$

Note : $^{a, b}$ represent statistically significant differences across column ($p < 0.05$).

Numerous studies have documented variations in the AC of LaEO derived from various sources. According to Smigielski et al. [25], the essential oils from dried flowers had a stronger IC_{50} of 22.1 mg/mL than the essential oils from fresh stems and leaves, which had an IC_{50} of 77.11 mg/mL. The essential oil from *L. angustifolia* in Italy has an IC_{50} of 26.26 mg/mL [26], but the study conducted in Croatia by Blažeković et al. [27] revealed that IC_{50} values varied from 15.06 μg/mL to 45.25 μg/mL. Thus, the IC_{50} of the essential oils in this study was higher than those reported above, indicating weaker AC.

The chemical makeup of LaEO may be connected to its antioxidant properties. One important ingredient, linalool, has been demonstrated to have high antioxidant qualities and the ability to treat a variety of illnesses [21]. Despite making up only 4.72 percent, α-pinene has antioxidant qualities and improves the genetic activity of human blood cells [28]. The combination of these substances demonstrates how LaEO may be used in medicine.

Determination of the antibacterial activity of LaEO

All investigated bacterial strains were significantly inhibited by gentamicin, according to the data shown in Table 4.

Table 4

Antibacterial zones of LaEO

Test strains	Diameter of the inhibitory zones of LaEO (mm)	Diameter of the inhibitory zones of gentamicin (mm)
S. aureus	$7.47^{Aa} \pm 0.55$	$20.47^{Ab} \pm 1.48$
S. enteritidis	$8.43^{Ba} \pm 0.58$	$26.53^{Cb} \pm 2.01$
E. coli	$11.23^{Da} \pm 0.25$	$21.50^{Abb} \pm 1.22$
B. cereus	$9.87^{Ca} \pm 0.38$	$24.53^{BCb} \pm 1.63$

Note : $^{a-b}$ represent statistically significant differences across rows and $^{A-D}$ represent statistically significant differences across columns ($p < 0.05$).

The study's findings demonstrated that gentamicin and LaEO differed significantly in their antibacterial activity. The antibacterial effects of LaEO were significantly weaker (7.47–11.23 mm), but gentamicin shown robust activity with considerable inhibition zone widths on all bacterial strains (20.47–26.53 mm). In particular, LaEO was most effective against *E. coli* (11.23 mm), but gentamicin was most inhibitory against *S. enteritidis* (26.53 mm) and *B. cereus* (24.53 mm). Interestingly, both compounds had the lowest inhibitory zone widths on *S. aureus*, measuring only 20.47 mm for gentamicin and 7.47 mm for LaEO. This difference may be due to the special cell wall structure of *S. aureus*, which reduces the effectiveness of both antibiotics and essential oils.

Essential oils' capacity to alter the structure of cell walls and membranes, particularly membrane proteins, is frequently the source of their antibacterial action [29]. Terpenoid chemicals are primarily responsible for this action. According to Julaeha et al. [29], terpenoids have the ability to intercalate into the lipid bilayer of cell membranes, producing enlargement, enhanced mobility, and permeability. This can impair protein function, decrease respiration, and change ion transport. Additionally, some research has demonstrated that essential oils high in α-terpinyl acetate exhibit antibacterial activity against harmful bacteria like *E. coli* and *S. aureus*, albeit a lesser one [30].

Conclusion

The distinctive physical and chemical characteristics, varied chemical makeup, and noteworthy biological activities of LaEO were identified in this study. Although the ratio may vary, the essential oil's chemical makeup primarily consists of linalyl acetate and β-linalool, which is in line with earlier observations. Despite being less effective than vitamin C, the essential oil's antioxidant action nevertheless exhibits some promise. It is noteworthy that LaEO can inhibit the growth of certain bacterial particles, indicating its potential use in antibacterial drugs. The study's findings enhance the scientific framework surrounding LaEO and offer a valuable database for assessing its quality and exploring its possible uses across a range of sectors.

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