

ORIGINAL ARTICLE

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**PHYSICOCHEMICAL PROPERTIES,
CHEMICAL COMPONENTS, AND ANTIBACTERIAL
ACTIVITY OF THE ESSENTIAL OIL
FROM *Mentha arvensis* L. LEAVES**

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Abstract

Essential oil (EO) obtained from *Mentha arvensis* L. leaves was separated by the steam distillation method. The following EO physicochemical properties were analyzed: acid value, saponification value, ester value, relative density, absolute density, and freezing point. Using gas chromatography-mass spectrometry, 26 major compounds were detected in the EO, with menthol having the highest level (69.44%). The antioxidant capacity of the EO ($IC_{50} = 330 \text{ mg mL}^{-1}$) was determined with the help of the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The antibacterial activity (against *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) was assessed using the paper disc diffusion method for antibiotic susceptibility testing.

Keywords: antibacterial activity, antioxidant capacity, *Mentha arvensis* L., menthol, herb

Introduction

Mentha arvensis L. is a flowering and strongly aromatic herb species from the family Lamiaceae (commonly known as corn mint, field mint, or wild mint). It has been cultivated widely throughout the world, especially in Europe, western and central Asia, eastern Siberia, etc. [1]. In Vietnam, it occurs naturally in the Mekong River delta, as well as in the provinces of Binh Thuan, Lao Cai, Quang Nam, Lam Dong, Khanh Hoa, etc. [2].

Historically, *M. arvensis* L. has been extensively used as a household remedy and a spice. Nowadays, it is also an important industrial plant grown to produce food (juices, candies, and cakes), cosmetics (perfume), and pharmaceuticals (various drugs) [1, 3]. As far as the latter is concerned, many previous studies have reported that *M. arvensis* L. has an outstanding medicinal potential for treating stomach problems, allergy, liver and spleen diseases, asthma and jaundice, etc. [4, 5].

M. arvensis L. owes its medicinal efficacy to the specific chemical components of the essential oil (EO) extracted from its leaves: this EO contains many bioactive (menthol, isomenthone, *p*-cymene, limonene, caryophyllene, α -pinene, etc. [6]) and phenolic (rosmarinic acid [7] and hesperidin [8]) compounds. However, the quantity of these compounds is significantly influenced by various factors, such as the season, climate, genes, maturity stage, and extraction method.

To recover these precious compounds, the EO can be extracted using various methods, such as supercritical fluid extraction, hydrodistillation, steam distillation, solvent extraction, etc. Among them, steam distillation is applied more widely on an industrial scale [9]. Depending on the extraction method and the type of raw material, the EO obtained will possess different chemical constituents, antioxidant capacity (AC), antibacterial activity (AA), and physicochemical properties. In Khanh Hoa province, *M. arvensis* L. has been especially widely cultivated in recent years with a high output, with local citizens using it as a food seasoning and medicated oil. Nevertheless, until now, to the best of my knowledge, none of the above-mentioned chemical characteristics have been studied for this material from Khanh Hoa province. Therefore, the major aim of this study was to assess the chemical composition and the general quality of the *M. arvensis* L. EO from Khanh Hoa province.

1. Material and Methods

1.1. Plant material. The EO from the leaves of *M. arvensis* L. (Khanh Hoa province, Vietnam) was extracted by steam distillation and stored at 4 °C in a dark glass bottle, without being exposed to direct sunlight, until required for the experiment.

1.2. Bacterial strains. The bacterial strains used in this study include *S. aureus* (ATCC 25923), *S. enteritidis* (ATCC 13076), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853), all provided by the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City (Vietnam).

1.3. Determination of the EO physical properties. The relative density (RD), absolute density (AD), and freezing point (FP) were determined according to ISO 279:1998 and ISO 1041:1973 [10, 11].

1.4. Determination of the acid value (AV) of the EO. According to Quoc [12], 1 g of the EO was dissolved in 5 mL of 96% alcohol, and a few drops of 1% phenolphthalein solution were then added. The mixture obtained was titrated using 0.1 N KOH solution (diluted in 96% ethanol) until a pale pink color appeared for 30 sec:

$$AV = \frac{V_{\text{KOH}} \cdot 0.1 \cdot 56.1}{\text{Mass of essential oil}}.$$

1.5. Determination of the saponification value (SV) of the EO. The SV value was measured as described by Quoc [12] with minor modifications. Initially, 2 g of the EO was dissolved in 25 mL of 0.5 N KOH solution (diluted in 96% ethanol). The mixture was then heated in a water bath for 1 h, and then 25 mL of distilled water and a few drops of 1% phenolphthalein solution were added. The final solution was titrated with 0.5 N HCl solution until the pink color disappeared:

$$SV = \frac{(V_{\text{blank}} - V_{\text{sample}}) \cdot 0.5 \cdot 56.1}{\text{Mass of essential oil}}.$$

The ester value (EV) was determined based on the AV and SV, using the following formula: $ES = SV - AV$.

1.6. Determination of the antioxidant capacity (AC) of the EO. The AC of the EO to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals was determined

according to the method of Kirby and Schmidt [13], with some small changes. The EO was dissolved in ethanol (96%, v/v) to obtain its various concentrations (1024, 512, 256, 128, and 64 mg mL⁻¹). Then, 0.3 mL of the obtained solution was mixed with 2.7 mL of DPPH in ethanol solution (concentration of 40 µg mL⁻¹) and kept in the dark for 30 min at room temperature. The AC was recorded by observing the decrease in absorbance at 517 nm against a control sample (containing only DPPH solution without the tested sample). Ascorbic acid was used as a standard to compare with the AC of the EO. The percent inhibition was plotted against the EO concentrations to estimate the concentration providing 50% inhibition (IC₅₀). The AC was evaluated using the formula below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

1.7. Determination of the antibacterial activity (AA) of the EO. The AA of the EO was determined using the paper disc diffusion method described by Bauer et al. [14]. Briefly, 100 µL of bacterial suspension (0.5 McFarland standard, approximately 1.5·10⁸ CFU mL⁻¹) were spread on MHA media (Mueller-Hinton agar) using a sterile hockey stick. Then, the sterile paper discs (6 mm in diameter) were impregnated with 5 µL of the EO. Gentamicin (10 µg disc⁻¹) and dimethylsulfoxide (DMSO) solution (5%, v/v) were used as positive and negative controls, respectively. Finally, all dishes were incubated for 24 h at 37 °C and the AA was assessed by inhibitory zone with the paper disc of 6 mm in diameter.

1.8. Gas chromatography-mass spectrometry (GC-MS) analysis. The chemical composition of the EO was analyzed using the GC-MS method: 1 µL of the EO was injected into a gas chromatograph (Agilent HP 6890N, USA) with a capillary column (HP-5ms, 30 m × 0.25 mm × 0.45 µm, Agilent Technologies, USA) equipped with a quadrupole mass analyzer (Agilent HP 5972, USA). Helium was used as a carrier gas at a constant flow rate of 0.5 mL min⁻¹ and a split ratio of 10:1. The injection temperature was 250 °C, and the temperature program was set as follows: initial temperature of 50 °C, held for 2 min, increased to 300 °C at a rate of 10 °C min⁻¹, and held for 5 min. Mass spectra were recorded at the ionization energy of 70 eV in the EI mode.

1.9. Statistical data analysis. All experiments were performed in triplicate. The results were expressed as mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) procedure was carried out to test significant differences between the means ($p < 0.05$). The data were analyzed using Statgraphics Centurion XV software (version 15.1.02, Statgraphics Technologies, Inc., USA).

2. Results and Discussion

2.1. Physicochemical properties of the EO from *M. arvensis* L. leaves. Table 1 shows the physicochemical properties of the EO extracted from *M. arvensis* L. leaves. The RD of the obtained EO is similar to that of the *M. arvensis* L. EO from Brazil (0.897) and lower than that of the *M. piperita* EO (0.901) from Brazil [15].

Table 1. Physicochemical properties of the EO from *M. arvensis* L. leaves

No.	Physicochemical properties	Value
1	Absolute density (AD, g mL ⁻¹)	0.8959 ± 0.0001
2	Relative density (RD)	0.8987 ± 0.0003
3	pH	4.670 ± 0.006
4	Freezing point (FP, °C)	-7.33 ± 0.58
5	Acid value (AV, mg KOH g ⁻¹ EO)	1.171 ± 0.009
6	Saponification value (SV, mg KOH g ⁻¹ EO)	25.970 ± 0.007
7	Ester value (EV, mg KOH g ⁻¹ EO)	24.799 ± 0.001

The RD is also higher than that of the EO of *Ageratum conyzoides* L. leaves (0.889) [12]. Essentially, the RD and AD values strongly correlate with each other and depend on the surrounding temperature. In general, ADs of most EOs from plants are lower than that of water: for instance, the *M. arvensis* L. EO in the present study – 0.8959 g mL⁻¹, the *Ceratonia siliqua* pulp and seed EOs – 0.833 and 0.91 g mL⁻¹, respectively [16]. In addition, the pH value of the EO from *M. arvensis* L. leaves is quite low (pH 4.67), which is in agreement with many EOs extracted from various plants, such as *A. conyzoides* leaves (pH 4.46), *M. piperita* (pH 5.2) [12, 15], etc. This can be explained by the strong dependence of the EO physicochemical properties on the natural source of the plant and the EO chemical composition.

Remarkable variations in FP were found between various EOs in previous studies [12, 17]. The FP of the EO in the present study is quite low (-7.33 °C), but this value cannot be compared with any other because there are no published reports on the FP of EOs of this plant, except those by Abdul-Majeed et al. [18] and Quoc [12] for the FP of EOs extracted from *Eucalyptus camaldulensis* Dehnh. leaves (0–1 °C) and *A. conyzoides* leaves (-10.33 °C). In the above cases, the differences in the FP values of EOs from plants were attributed to the influence of major components in these EOs, i.e., menthol, menthone, and isomenthone.

The AV and SV of the *M. arvensis* EO were found to be 1.171 and 25.97 mg KOH g⁻¹ EO, respectively. They were lower than those of basil (AV – 3.95 mg KOH g⁻¹ EO; SV – 198 mg KOH g⁻¹ EO) and lemongrass (AV – 4.09 mg KOH g⁻¹ EO; SV – 143 mg KOH g⁻¹ EO) EOs [19]. The EV obtained from the AV and SV is only 24.799 mg KOH g⁻¹ EO, which is slightly smaller than that of the *C. siliqua* pulp and seeds EO (42.61 and 33.22 mg KOH g⁻¹ EO, respectively) [16]. As yet, there are few published studies on the AV, SV, and EV of the *M. arvensis* L. EO; however, the AV and EV of the EO from *M. arvensis* L. collected from Andhra Pradesh state (India) were 1.33 and 27.64 mg KOH g⁻¹ EO, respectively [20] and are similar to those of our study. According to Quoc [12], distillation techniques, climatic conditions, plant varieties, regions, harvest periods, genotype, type of material, and chemical composition directly affect all these parameters.

2.2. Chemical composition of the EO from *M. arvensis* L. leaves. The chemical composition of the EO from *M. arvensis* L. leaves was determined by GC-MS. A total of 26 compounds were identified and quantified in the EO isolated from the *M. arvensis* L. leaves; they constituted 98% of the oil and were tested by retention times ranging from 6.29 to 21.8 min (Table 2). The EO was found to contain menthol (69.44%), menthone (7.66%), isomenthone (5.65%), and trans-carane (4.34%) as major compounds.

Table 2. Chemical composition of the EO from *M. arvensis* L. leaves

No.	Compound	RT (min)	(%)
1	Diacetone alcohol	6.29	1.94
2	α -pinene	8.57	0.52
3	Sabinene	9.56	0.23
4	L- β -pinene	9.68	0.59
5	β -myrcene	9.92	0.32
6	3-octanol	10	0.22
7	<i>o</i> -xylene, 4-ethyl-	10.84	0.06
8	D-limonene	10.96	2.35
9	1,8-cineole	11.06	0.13
10	Isopulegol	13.97	0.43
11	Menthone	14.19	7.66
12	Isomenthone	14.45	5.65
13	Menthol	14.77	69.44
14	Neomenthol	14.96	1.28
15	Levomenthol	15.11	1.87
16	<i>n</i> -valeric acid cis-3-hexenyl ester	16.03	0.48
17	Pulegone	16.27	0.65
18	Piperitone	16.63	0.58
19	(+)-3-menthene	17.04	0.08
20	trans-carane	17.49	4.34
21	3-menthene	17.87	0.11
22	(-)- β -bourbonene	19.73	0.12
23	Decyl acetate	19.94	0.07
24	Caryophyllene	20.51	0.46
25	3,3,6-trimethyl-1,4-heptadien-6-ol	21.48	0.17
26	D-germacrene	21.8	0.23

In addition, the obtained results revealed that the terpene group also appears in the EO (in small amount, including α -pinene, limonene, 3-menthene, sabinene, etc. Significant variations were observed in the chemical profile of the *M. arvensis* L. EO: for instance, menthol, *p*-menthone, isomenthone, and neo-menthol of the EO of *M. arvensis* L. aerial parts from India account for 71.40%, 8.04%, 5.42%, and 3.18%, respectively [21], while the EO of *M. arvensis* L. leaves from the southeastern region of Macedonia contained menthol (32.47%), isomenthone (15.97%), 1,8-cineol (5.4%), and neomenthol (5.24%) [6].

Principally, menthol is a major component of the EO, accounting for a high proportion in the flower, leaves, and whole plant of *M. arvensis* L. [6]. According to Ribeiro-Santos et al. [22], the chemical composition of EOs is significantly influenced by different factors, such as the development stage, variety, geographical origin, part of the plant used, age, season and condition of the plant when harvested, as well as the extraction method, analysis conditions, and the solvent used.

The findings of this study show that menthol is present in the EO extracted from *M. arvensis* L. leaves in Vietnam in an amount similar to or higher than that of EOs from elsewhere, confirming that this compound is precious and can be widely used in the food and pharmaceutical industries.

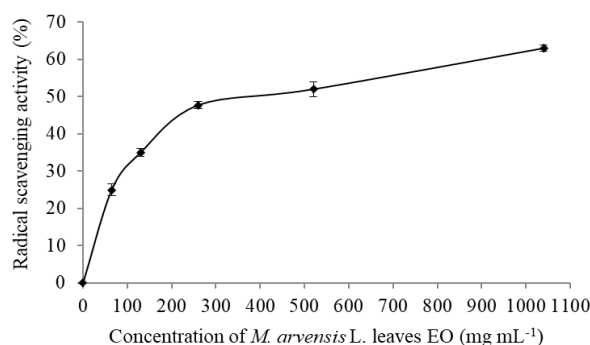


Fig. 1. Radical scavenging activity of the EO from *M. arvensis* L. leaves

2.3. Antioxidant capacity (AC) of the EO from *M. arvensis* L. leaves. As seen in Fig. 1, the AC value of the EO significantly increased with a rise in the EO concentration (up to 1024 mg mL⁻¹) (Fig. 1). The IC₅₀ of the EO was 330 mg mL⁻¹, while that of the control sample (Vitamin C) was only 15.25 µg mL⁻¹. These estimates prove that the AC of the EO is significantly lower than that of the control sample and the EO of *M. arvensis* L. from Brazil (IC₅₀ = 26.72 mg mL⁻¹) [8]. Compared to other EOs isolated from various materials, the AC in this study is also lower than that of the EO of *A. conyzoides* leaves (IC₅₀ = 8 mg mL⁻¹) [12], *C. siliqua* pulp (IC₅₀ = 7.8 µg mL⁻¹), and *C. siliqua* seeds (IC₅₀ = 31.25 µg mL⁻¹) [16]. Almost all plant EOs possess AC; however, it depends strongly on the chemical composition of the EO or the synergistic effect of its compounds. In this study, most major and minor compounds in the EO possessed AC, such as menthol [23], menthone [24], α-pinene, and 1,8-cineole [25], which is advantageous for using the EO from *M. arvensis* L. in food production.

2.4. Antibacterial activity (AA) of the EO from *M. arvensis* L. leaves. The EO from *M. arvensis* L. leaves shows AA against four bacterial strains (Table 3). The inhibition zones of the positive control, listed in susceptible order, are *S. aureus* > *S. enteritidis* > *P. aeruginosa* > *E. coli*, while those of the EO, arranged in susceptible order, are *S. enteritidis* > *S. aureus* > *P. aeruginosa* > *E. coli*. The obtained results demonstrate that the AA of the EO is stronger than that of the positive control for *S. enteritidis*.

Table 3. Antibacterial zones of the EO from *M. arvensis* leaves

No.	Bacterial strains	Inhibition zones of gentamycin, mm	Inhibition zones of EO, mm
1	<i>S. enteritidis</i>	18.0 ± 0.71 ^{Ab}	22.8 ± 0.85 ^{Bc}
2	<i>S. aureus</i>	25.3 ± 0.65 ^{Bc}	21.2 ± 0.95 ^{Ab}
3	<i>E. coli</i>	15.3 ± 0.65 ^{Ba}	11.9 ± 0.88 ^{Aa}
4	<i>P. aeruginosa</i>	16.3 ± 0.6 ^{Ba}	12.0 ± 0.40 ^{Aa}

Different lowercase letters in the same column denote significant differences ($p < 0.05$) with respect to the types of bacterial strains.

Different capital letters in the same row denote significant differences ($p < 0.05$) with respect to the antibacterial agents.

According to Nazim et al. [26], the EO from *M. arvensis* L. leaves also inhibited the growth of both Gram-positive (*S. aureus*, *B. subtilis*, and *Streptococcus pyogenes*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria, and these results are

in agreement with those of our study. In addition, Pandey et al. [21] used the disc diffusion method to demonstrate that the EO extracted from *M. arvensis* L. leaves exhibited *in vitro* efficacy against human pathogenic fungi, such as *Fusarium oxysporum* and *Trichophyton mentagrophytes*. Many previous studies have reported that the AA of EOs depends on the chemical constituents and their concentration. According to Mihajlov et al. [6], some compounds contained in EOs such as menthol, menthone, 1,8-cineole, limonene, and caryophyllene, can effectively act against Gram-positive (*S. aureus* and *Enterococcus faecalis*) and Gram-negative (*E. coli*, *P. aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumonia*, and *Salmonella* sp.) bacteria. Therefore, the results discussed in this article are in good correlation with those published in the literature.

Conclusions

Our observations suggest that Khanh Hoa province has great potential for the commercial production of the high-quality EO from *M. arvensis* L. leaves rich in menthol, menthone, and isomenthone. Notably, the EO from the *Mentha* plant native to the region under study is characterized by high AA and AC. The physicochemical properties of this EO are also beneficial. The data obtained could be helpful in further research on using EOs, in particular those from *M. arvensis* L. of Khanh Hoa province, for medicinal or pharmaceutical purposes.

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Физико-химические свойства, химические компоненты и антибактериальная активность эфирного масла из листьев *Mentha arvensis* L.

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Аннотация

Эфирное масло (ЭМ) из листьев *Mentha arvensis* L. получено методом паровой дистилляции и проанализировано по следующим физико-химическим свойствам: кислотное число, число омыления, эфирное число, относительная и абсолютная плотность и температура замерзания. С помощью газовой хроматографии-масс-спектрометрии в ЭМ было обнаружено 26 основных соединений, при этом содержание ментола было самым высоким (69.44%). Показано, что выделенное ЭМ обладает антиоксидантной емкостью ($IC_{50} = 330$ мг/мл) по реакции с ДФПГ (2,2-дифенил-1-пикрилгидразилом) и антибактериальной активностью (относительно *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* и *Escherichia coli*) по методу диффузии на бумажном диске для определения чувствительности к антибиотикам.

Ключевые слова: антибактериальная активность, антиоксидантная способность, *Mentha arvensis* L., ментол, растительное сырье

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