Chemical Constituents and in Vitro/In Vivo Pharmacological Effects of *Mentha piperita* L. Essential oil in Different Regions of Algeria

Latifa Halli [1,2], Lilya Harchaoui [1], Mohammed Messaoudi [3*], Thoraya Dahmane [4], Fatiha Aid [1], Barbara Sawicka[5], Wafa Zahnit [6]

[1] Faculty of Biological Sciences, University of Science and Technology Houari Boumediene, Algiers, Algeria.
[2] Research and Development Center, SAIDAL GROUP, Algiers, Algeria.
[3] Nuclear Research Centre of Birine, Djelfa, Algeria, Ain Oussera, Djelfa, Algeria.
[4] Department of Agronomic Sciences, University Djillali Bounaama Khemis Miliana, Faculty of Natural and Life Sciences and Earth Sciences, Algeria.
[5] Department of Plant Production Technology and Commoditties Science, University of Life Sciences in Lublin, Lublin, Akademicka 15 str., Poland.
[6] Department of Chemistry, Faculty of Mathematics and material sciences, Kasdi Merbah University, Laboratory of Valorization and Promotion of Saharan Resources (VPRS), Ouargla, Algeria.

ABSTRACT

This study focuses on *Mentha piperita* L. cultivated in two regions with distinct bioclimatic levels. The aim is to assess and compare the yield, chemical composition, and pharmacological activities of the plant in these diverse environmental conditions. The phytochemical profile of *Mentha piperita* L essential oils was established using gas chromatographic analysis. The pharmacological activities were performed in vivo and in vitro using analgesic, anti-inflammatory, antibacterial and antioxidant assays. The optimal yield of essential oil is obtained from the region of Oued Souf with 1.02% and that of the region of Algiers is equal to 0.86%. The GC/MS analysis revealed a richness of the essential oil in Linalool as well as its derivatives linalyl acetate for Oued Souf and linalyl butyrate for Algiers. In all pharmacological activities, the essential oil of *Mentha piperita* L from Oued Souf region was significantly more potent than essential oil from Algiers. This study can contribute to the application of *Mentha piperita* L essential oil in the pharmaceutical industry as a promising natural reservoir of volatile compounds with noteworthy therapeutic properties.

Keywords: Anti-inflammatory, GC/MS, Lamiaceae, *Mentha piperita* ,phytochemical profile, linalool.

1. INTRODUCTION

In recent years, the exploration of natural remedies for various health conditions has gained considerable attention, prompting extensive research into traditional medicinal plants .These plants have been widely employed for addressing diverse ailments, with certain ones remaining integral to customary therapeutic practices for various health conditions [1]. Pain, inflammation, and bacterial infections remain pervasive challenges in healthcare, necessitating the quest for novel therapeutic agents with efficacy and without side effects [2]. Nowadays, advances in plant biochemistry have shown that plant species can synthesize thousands of different chemical constituents (phenolic compounds, alkaloids and terpenoids) [3, 4]. Essential oils possess a complex chemical composition; constituting a blend of diverse molecules, notably terpenes (non-aromatic hydrocarbons) and oxygenated compounds (alcohol, aldehyde, ketone, and ester)[5, 6].Various factors such as the harvesting time,

[*]***Corresponding author: Mohammed Messaoudi***
messaoudi2006@yahoo.fr
Received: 30/7/2024 Accepted: 21/9/2024.
DOI: https://doi.org/10.35516/jjps.v18i1.3069

cultivation location, plant part utilized, and production method are among the numerous variables influencing the chemical composition and quality of essential oils derived from distinct plant species [7]. Their pharmacological properties encompass stimulative, diuretic, analgesic, antiseptic, and antimicrobial properties [8, 9]. Lamiaceae family plants are abundant in essential oils, hold considerable importance in natural medicine, pharmacology, cosmetology, and aromatherapy [3]. Among these plants, *Mentha piperita* L that occupies a privileged place in digestive phytotherapy and used in traditional medicine for its antispasmodic, antimicrobial and antioxidant properties [3, 10]. Peppermint is perennial plant that grows to be 50-80 cm tall and the rhizomes are wide spreading, fleshy, and bear fibrous roots, whereas the leaves are dark green, and flowers are purple, it encompasses a persistent chalice surrounds the fruit divided into four parts, this type of mint would arise from a crossing between Mentha aquatic and *Mentha spicata*.

Currently, several varieties, descendants of this hybridization, are cultivated in the world. They are particularly prevalent in temperate and Mediterranean regions, in cool zones and in neutral environments [11, 12]. Although the genus is native of the Mediterranean region. It is cultivated all over the world for its flavor, its scent and as well as for the different medicinal and pharmaceutical applications. Peppermint oil is one of the most produced and consumed essential oils. The chemical composition of *Mentha piperita* varies greatly according to the soil and the harvesting time. Peppermint essential oil is mainly constituted of menthol (30% to 40%), menthone (20% to 65%), esters, coumarins and sulfur compounds [13, 14]. However, other authors have reported the existence of particular chemo-types like limonene (33.37%), and 1,8-Cineole (30.75%) chemo-types in Spain [15], and Terpinene chemo-type (19.7%) in Iran [16].

To the best of our knowledge, there is no scientific research that includes a phytochemical and pharmacological comparison of peppermint from various bioclimatic levels. Therefore, this work is interested to *Mentha piperita* L cultived in two regions located in different bio climatic levels in order to compare the yield, the chemical composition and pharmacological activities.

2. MATERIALS AND METHODS

2.1. Plant material

Mentha piperita L was collected in two different regions, a region with subhumid climate (Algiers), and in arid climate for the other (Oued Souf). The plants were collected in March 2022 from the two regions. The botanical identification of the plants was carried out at the National Institute of Agronomy, Algeria (INA) [17]. A standard voucher specimen is conserved in the herbarium of university of Florida (U.S.A) (voucher N◦:241569). The freshly collected plant at flowering stage, was dried in a dry and ventilated place, shielded from the light for 15 days, and then kept in bags. The aerial part (leaves, flowers and stems) was used for the extraction of the essential oil.

2.2. Microbial strains

The strains of the collections American Type Culture Collection (ATCC) were used to evaluate the antimicrobial potential of *Mentha piperita* L. essential oils, four bacterial strains (Staphylococcus aureus, (ATCC 6538), Pseudomonas aeruginosa, (ATCC 9027), Escherichia coli, (ATCC 4157), Bacillus subtilis, (ATCC 9372) and a yeast strain Candida albicans, (ATCC 24443) were tested. The strains were activated at 37°C for 24 hoursand at 25°C for 48 hours for the yeast [18].

2.3. Experimental animals

Adult male albino mice, Mus musculus, (average weight = 20±5 g) were obtained from the Pasteur Institute of Algeria. The animals were housed in standard laboratory conditions with a temperature of 24±2˚C and a 12-hour light/dark cycle. Animals received food and water ad libitum.

2.4. Essential oil extraction

The essential oil extraction from peppermint was carried out by Clevenger hydrodistillation [19]. A quantity of

100 grams of dried plant material from each plant was placed in a flask with 1 liter of water, equipped with a Clevenger apparatus and a condenser. The heating mantle was adjusted to a temperature of 90 °C. Distillation continued for 3 hours after the recovery of the first distillate drop. The obtained oils were preserved in an airtight brown glass bottle at 4 °C until utilization. The yield (R %) is defined as the ratio of the mass of essential oil (m HE) obtained to the mass of dry vegetable matter (m MVS) used, according to the following formula (1).

$$R\ (\%) = \frac{\text{m HE}}{\text{m MVS}} \times 100 \qquad (1)$$

2.5. Phytochemical analysis

A gas chromatographic analysis (HP Agilent 6890 Network GC System technology) coupled to a mass spectrometer (HP Agilent 5973 Network technology mass selective detector) was performed, in order to determine the different constituents of *Mentha piperita* L essential oils. Samples were separated on a capillary column HP-5MS (5% diphenyle and 95% dimethylpolysiloxane) 30m long and 0.32 mm diameter. The carrier gas is helium with a flow of 1 $mL.min^{-1}$. A volume of 0.5 µL of sample was injected in split mode with a ratio of 70/30. The oven temperature was programmed as follows: 60 °C for 8 minutes followed by an increase in temperature at a rate of 2 °C/min until 250 °C for 10 minutes. The temperature of the interface (GC-MS) was 280 °C and the temperature of the injector was 250 °C. The identification of the different constituents of *Mentha piperita L*. essential oil of was carried out based on mass spectra and retention indices (IR). They are calculated using a linear interpretation of the retention time of an alkane's series ranging from C8 to C22 [20].

2.6. Pharmacological activities

2.6.1 Antimicrobial activity

The qualitative evaluation of the antimicrobial activity was determined using disk diffusion method [21, 22]. Muller-Hinton's medium was used for bacteria and Sabouraud's for yeast. Sterile discs (MN 640w filter paper, Macherey-Nagel Gmbh and co, KG Germany), 9 mm in diameter were impregnated with 20µL of essential oil and then put down delicately on the agar seeded previously with a bacterial suspension of the five strains ATCC references adjusted to the 0.5 McFarland. Petri dishes were incubated at 37 °C for 24 hours for the bacteria and at 25 °C for 48 hours for the yeast [23]. The antimicrobial activity of the essential oil was determined by measuring the diameter of the inhibition zone (in mm) produced around the disks after incubation [24]. To determine the Minimum Inhibitory Concentration (MIC), cultures of bacteria or yeast (107-108CFU/mL) were carried out in the presence of decreasing concentrations (from 2% to 0.03%) of peppermint essential oil [25]. Dilutions of the essential oil were prepared in DMSO. The MIC was determined from Petri dishes where there is no visible culture [26].

2.6.2. Antioxidant activity

To measure the antioxidant activity of *Mentha piperita*, the DPPH test (diphenylpicrylhydrayl) was used according to the protocol described by Singh et al. (2015) [27]. In brief, 0.5 mL volumes of *Mentha piperita* L. at concentrations ranging from 10 to 50 µg/mL were combined with 1.5 mL of DPPH (0.004 g/L in methanol). Subsequently, the mixture underwent a 30-minute incubation in darkness at room temperature, following which absorbance was gauged at 517 nm against a methanol control using a Cecil CE2041 spectrophotometer from England. This process was iterated thrice for each concentration and sample. The Percentage of Inhibition (PI %) is determined using formula 2.

$$PI\ \% = (1 - \text{Sample})/(\text{Blank}) \times 100 \qquad (2)$$

A positive control is prepared using a solution of Butylated hydroxytoluene (BHT) as standard antioxidant. The variation of the reducing power according to the concentration of HE and BHT allows us to calculate the Inhibitory Concentration IC_{50} [28]. The extract concentration required to scavenge 50% of the DPPH radical is called IC_{50} and lower IC_{50} values indicate higher antiradical activity.

2.6.3. In-Vivo pharmacological activities

Analgesic activity

The analgesic potential of *Mentha piperita* L. essential oils was evaluated using acetic acid induced writhing test described by [29]. 6 groups each comprised of 6 mice (n=6) were used in this investigation. 4 groups received an oral dose of 0.5 mL of essential oils respectively at doses of 100 and 500 mg/kg per body weight. 2 other groups used as positive and negative controls, were treated respectively with paracetamol® at 500 mg/Kg b.w and saline solution. 30 minutes after treatment, animals were intraperitoneal injected with 0.5 mL of 0.6% acid acetic to induce writhes, then the count of abdominal contractions during 15 min. The protection percentage against abdominal writhes was used to assess the analgesic effect and was calculated as in formula 3.

Protection (%) = [(Wt -We/Wt)] 100 (3)

In which, We represents the mean value of writhes in treated groups and Wt represents the mean value of writhes in control groups.

- *Anti-inflammatory activity*

Carrageenan induced paw edema model was used to assessed the anti-inflammatory activity of *Mentha piperita* L. essential oils according to [30]. Mice were divided into 6 groups of six animals. One group served as negative control received orally 0.5 mL of saline solution, 4 groups were treated with *Mentha piperita* L. essential oils at doses of 100 and 500 mg/kg b.w. While, the reference group received 500 mg/kg b.w of Diclofenac. A volume of 0.05 mL of 1% carrageenan freshly prepared in saline solution was injected intradermal into the plantar side of the right hind paw of the mice one hour after the oral route of treatments. 4 hours after carrageenan injection, animals were sacrificed by cervical dislocation and the two posterior legs were cut at tarsal joint, then immediately weighed.

The anti-inflammatory activity was calculated as percentage inhibition of edema following the 4.

Inhibition% = [(PEC - PET)/PEC] 100 (4)

where, % PEC is the percentage edema of the control (negative control) and % PET percentage edema of the test (diclofenac and *Mentha piperita* L. essential oils). The percentage of edema is calculated using the formula 5.

PE (%) = [M(RPW)-M(LPW)]/M(LPW) 100 (5)

In which, M(RPW) is the mean weight of the right paw per group, and M(LPW) is the mean weight of the left paw per group.

2.7. Statistical Analysis

The results were presented as the mean ± standard deviation (SD). To assess significant differences among the studied samples, an analysis of variance (ANOVA) test followed by Tukey's multiple comparison test was conducted using Statistica version 6.0 statistical software. The values $P<0.05$ were considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1. Yields of essential oils

The results obtained showed that the yields of essential oil extracted by hydrodistillation average is 1.02±0.01% for mint extracts from Oued Souf region and 0.82±0.01% for those of Algiers region (Table 1).

Table 1. Yields percentage (%) of *Mentha piperita* L. essential oils from Algiers and Oued Souf regions.

Oued Souf region	Algiers region	AFNOR standars
1.02±0.01	0.82±0.01	0.38 - 1.20

All values are represented as mean ± SD of three measurements

As shown in Table 1, the extraction yield of *Mentha piperita* L. essential oil from Oued Souf region is higher than those from Algiers region. These yields are within the range allowed by the AFNOR standards. The current investigation centered on exploring the phytochemical diversity and biological properties of *Mentha piperita* L.

essential oils cultivated in Algeria. The yield of *Mentha piperita* L. essential oil is different depending on the harvest region, that of Oued Souf has a higher yield than that of Algiers. Generally, the essential oil yield depends usually on the soil properties, the harvest period and finally the extraction techniques [31]. Our yields are higher than those obtained by *Mentha piperita* L. essential oils of Benin, estimated at 0.45±0.02% [32] and in Russia with a yield of 0.34% [33].

3.2. Phytochemical analysis

Chemical composition of peppermint essential oil from Algiers and Oued Souf regions were established using gas chromatography coupled to mass spectrometry allowed us to identify 99.10% of the constituents for mint essential oil grown in Algiers and 98.46% of the constituents of the essential oil of that grown at Oued Souf (Table 2). A high proportion of linalool and its acetate and butyrate derivatives (Table 2; Figure 1) characterized both essential oils. Linalool is the major constituent of peppermint from both sources with respectively 34.66 and 25.36% for Algiers and Oued Souf. On the other hand, linalool derivatives are characteristic of the study regions, linalyl butyrate (26.87%) is characteristic of the essential oil of the Algiers region, whereas Oued Souf's one is characterized by linalyl acetate (22.04%).

The chromatograms of peppermint essential oil from Algiers and Oued Souf regions are respresented in Figure 1.

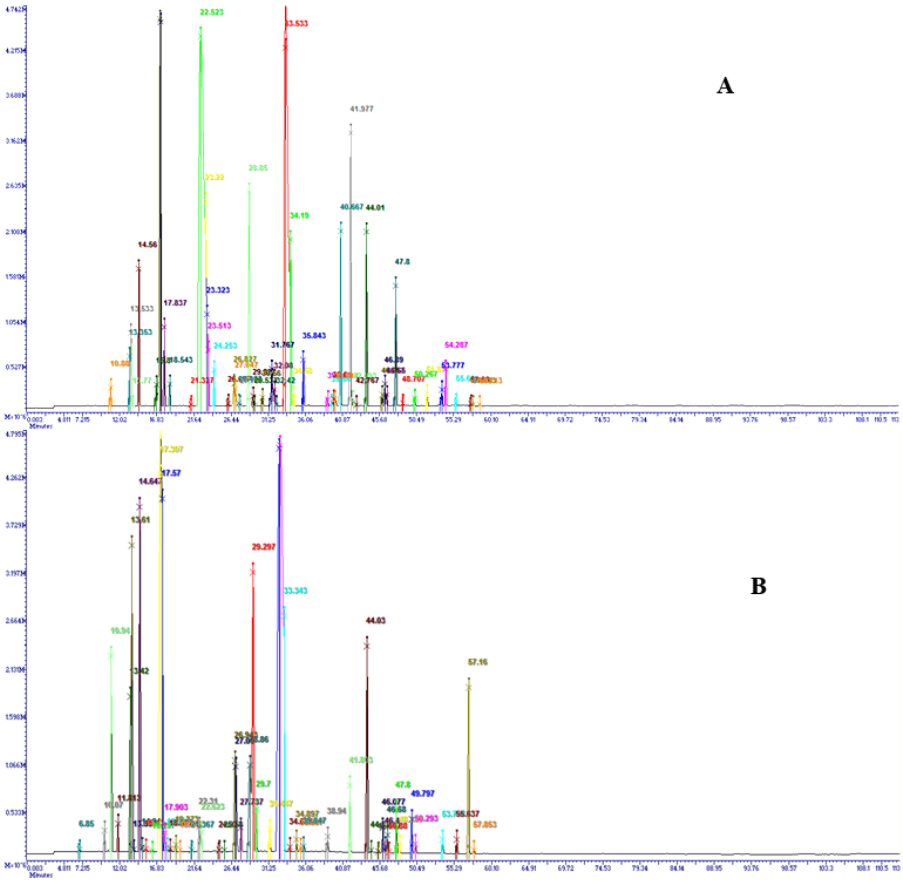

Figure 1. GC-MS chromatograms of *Mentha piperita* L. essential oils (A- Algeriers region, B- Oued Souf region).

Table 2. Chemical composition of the essential oil of peppermint from two different regions.

IR	Compounds	Relative percentage (%)	
		Algiers region	**Oued Souf region**
1004	Alpha pinene	-	*0,30*
1008	alpha- Thujene	0,19	-
1014	Sabinene	-	0,13
1015	Beta pinene	-	0,15
1018	alpha-phellandrene	0,57	-
1019	beta- Thujene	0,857	-
1019	Beta- Myrcene	-	0,97
1023	alpha –Myrcene	1,64	-
1029	Para –Cymene	-	0,22
1032	o-cymene	0,3	-
1034	Eucalyptol	9,89	3,1
1037	trans -alpha-ocimene	0,88	3.47
1038	Gamma terpinene	-	0.27
1040	Beta-cis-ocimene	0,22	1.27
1047	Alpha-terpinolene	-	0.16
1056	Linalool	34,66	25.36
1059	n-Amylisovalerate	0,828	-
1060	1-Octen-1-ol, acetate	0,52	0.35
1063	3-Octanol, acetate	0.375	-
1073	Isomenthone	0,31	-
1074	4-Octene-2,7-diol, 2,7-dimethyl-, Z-	0.267	-
1082	alpha-Terpineol	5.903	8.05
1084	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	0.148	-
1094	cis-geraniol	0.708	-
1095	cis-isopulegone	0.338	-
1099	Linalyl acetate	-	22.04
1099	Geraniol	-	6.54
1101	linalyl butyrate	26.873	-
1102	linalyl formate	0,63	-
1197	Nerylacetate	-	2.4
1223	2,6-Octadien-1-ol, 3,7-dimethyl-, acetat	2.93	0.33
1259	p-Mentha-1,45 ,8-triene	-	0.98
1269	Geranylacetate	4.605	4.46
1282	alpha.-Gurjunene	-	0.34
1500	Trans-caryophyllene	2.595	2.2
1514	trans-.beta.-Farnesene	-	0.35
1519	Alpha-Humulene	0.125	0.19
1522	Germacrene D	0.235	1.84
1535	cis-muurola-4(14),5-diene	1,74	-
1549	Elemol	-	5.62
1561	Viridiflorol	0.43	1.50
1573	Alpha- selinene	0.131	1.57
1581	Alpha –eudesmol	-	3.16
1588	Valencene	-	0.86
1590	Trans cirtal	0.207	0.28

The phytochemical analysis revelead that the composition of monoterpenes and sesquiterpenes of peppermint grown in Algeria from Algiers and oued souf regions differs from that grown in France, in India and in Italy by the presence of linalool and its derivatives and the absence of menthol and its derivatives [34, 35].This characteristic composition could allow us to consider that the peppermint cultivated in the two regions (North and South) of Algeria and Morocco [36], would be a new chemotype for Algeria with linalool and its derivatives as the majority compound. The studies carried out on the essential oil of *Mentha piperita* L. showed a great diversity in its chemical composition. Indeed, the existence of specific chemotypes has been demonstrated in Spain, chemotype of limonene (33.37%), and 1.8-Cineole (30.75%) [19], and in Iran, chemotype of Terpinene (19.7%) and Pipertitinone oxide (19.3%) [10]. The essential oil of peppermint grown in Morocco, Iran**,** ~~in~~ Russia and in the Himalayas is consisting essentially of menthol and its derivatives, menthyl acetate, menthone, and menthofurane [34, 37, 38]. Thus, these different chemotypes of the essential oil of *Mentha piperita* L may be due to ecological factors and the difference in geographical positions [35, 39].

3.3. Antimicrobial activity

The antimicrobial activity of the essential oils was determined by measuring the inhibition zone diameters surrounding the absorbent discs after incubation under suitable conditions for the tested germ development (Table 3).

Table 3. Antimicrobial activity of peppermint essential oil on a variety of microbial strains.

			Inhibition diameter (mm)	
		Strains	**Algiers region**	**Oued Souf region**
Bacteria	Gram +	*Bacillus subtilis*	20.33±0.,57	22.00±0.50
		Staphyloccocus aureus	18.66±0.57	20.83±1.75
	Gram -	*Escherichia coli*	13.83±0.28	15.83±0.76
		Pseudomonas aeruginosa	9.16±0.50	9.50±0.28
Yeast		*Candida albicans*	48.16±0,28	50.33±1.15

All values are represented as mean ± SD of three measurements.

The findings obtained indicated that the essential oil of *Mentha piperita* L. from the Oued Souf region is more effective than that from the Algiers region against all strains tested.

As per the Chifundera *et al.* (1990) [40], the essential oil of *Mentha piperita* L. exhibits Strong effect against Gram+ bacteria (*Bacillus subtilis* and *Staphyloccocus aureus*) whose inhibition zones ranged from 18.66±0.57 to 22.00±0.50 and a moderate inhibitory activity against Gram- bacteria : *Escherichia-coli* with inhibition zones of about 13.83±0.28 and 15.83±0.76 ,respectively. For Oued Souf and Algiers regions, while showing no inhibitory effect against *Pseudomonas aeruginosa* (Table 3). *Candida albicans* strain showed high sensitivity to *Mentha piperita* L. essential oil for both samples with inhibition zones of 48.16±0.28 mm (Algiers region) and 50.33±1.15 mm (Oued Souf region). Inhibition zone diameter of yeast is much larger than that observed for bacteria. Therefore, the antifungal activity of *Mentha piperita* L. essential oil is more important than the antibacterial activity.

3.3.1. Quantitative determination (MIC)

The MIC results show that the five strains are sensitive to different concentrations of *Mentha piperita* essential oil (Table 4), with the exception of Pseudomonas aeruginosa from Algiers region, which shows resistance. The MIC values obtained are quite low; they range within 0.125 and 0.03%.We found that the essential oil of *Mentha piperita* from Oued Souf region has a MIC of 0.25% for Gram + bacteria (Bacillus subtilis and Staphylococcus aureus), and a MIC of 0.5% for Escherichia coli, 0.125% for Pseudomonas aeruginosa and a MIC < 0.03% for Candida albicans.

Table 4. MIC values of Peppermint essential oil from two regions (+: Growth (resistant strain).-: Inhibition (sensitive strain).

Concentrations			Regions	2.00%	1.0%	0.5%	0.2%	0.12%	0.06%	0.03%
Bacteria	Gram+	*Bacillus subtilis*	Algiers	-	-	-	-	-	-	+
			Oued Souf	-	-	-	-	+	+	+
		Staphylococus aureus	Algiers	-	-	-	-	-	-	+
			Oued Souf	-	-	-	-	+	+	+
	Gram-	*Escherichia coli*	Algiers	-	-	-	-	+	+	+
			Oued Souf	-	-	-	+	+	+	+
		Pseudomonas aeruginosa	Algiers	+	+	+	+	+	+	+
			Oued Souf	-	-	-	-	-	+	+
Yeast		*Candida albicans*	Algiers	-	-	-	-	-	-	+
			Oued Souf	-	-	-	-	-	-	(≤0.03)

The antimicrobial activity was assessed using disk diffusion method which revealed the efficacies of *Mentha piperita* L. essential oil from Oued Souf region is more effective than that from the Algiers region against all strains tested. The antimicrobial effect of medicinal plants is mainly associated to the presence of secondary metabolites [41]. The action mode of the essential oils depends on the active components type and characteristics, especially their hydrophobic property which allows them to penetrate the double layer of phospholipids of the bacterial cell membrane. This can induce a change in the fluidity of the membrane, a chemo-osmotic disturbance and a leakage of ions (potassium ion) [42]. The obtained findings of this activity can be attributed to its richness in oxygenated monoterpenes (linalool, alpha-terpineol, eucalyptol), which has shown its power to fight against many bacterial strains tested, despite the presence of a low concentration of alpha-terpineol and eucalyptol. these compounds being known for their antibacterial activity [43]. Indeed, these compounds lead to lesions in the microorganism's cell membrane, increasing cell permeability and consequently a loss of cellular constituents and thus the death of the bacteria [44]. Phenols (thymol, carvacrol and eugenol), alcohols, (α-terpineol, terpinen-4-ol, linalool), aldehydes, ketones and more rarely carbides are chemical compounds known for their antimicrobial efficacy and broad spectrum [45]. Alcohols are generally better known for both their bacteriostatic action on vegetative cells, through protein denaturation. Aldehydes, which are strongly electronegative, are powerful antimicrobial agents when reacting with the vital nitro compounds (proteins and nucleic acids) of bacteria [46].

3.4. Antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl DPPH method was used to assess the antioxidant capacity of peppermint essential oils. The averages of percentage inhibition (Pi) and IC_{50} values for peppermint essential oils and BHT as a function of concentration are listed in Table 5.

Table 5. Inhibition percentages and IC_{50} values for essential oils and BHT. All values are represented as mean ± SEM of three measurements; [a] $P < 0.05$ compared with reference.

Concentration µg/mL	10	20	40	60	100	150	IC_{50}
Pi%(HE) Oued Souf	46.51±0.51	50.66±0.57	54.25±1.09	57.50±0.50	67.52±0.50	74.73±0.46	23.33±1.20[a]
Pi%(HE) Algiers	18.64±1.19	23.46±1.34	38.49±1.47	65.72±2.57	86.52±2.52	89.55± 0.7	38.37±0.88[a]
Pi%(BHT)	36.09±5.24	47.21±4.41	62.10±6.47	72.33±8.45	85.13±3.42	92.08±2.86	21.79±2.62

The essential oil from Oued Souf region has a strong significantly ($P<0.05$) antioxidant activity at low concentration (46.51%) than the one from Algiers region and particularly compared to BHT; an increase inantioxidant activity is observed according to the concentration. However, the antioxidant activity of the Oued Souf sample is significantly ($P<0.05$) higher than that of Algiers. The antiradical activities obtained show that essential oils have a moderate antioxidant activity with IC_{50} values of 23.33±1.02, and 38.37±0. 88µg/mL for the peppermint essential oils of Oued Souf and Algiers respectively, while the IC_{50} value of the standard antioxidant BHT was 21.79±2.62 mg/mL close to that of IC_{50} of Oued Souf (23.33±1.02 µg/mL) (Table 5).

The antioxidant potential of *Mentha piperita* L. essential oils is probably related to the presence of alcohol, linalool in both samples, and to other oxygenated substances such as alpha terpineol and eucalyptol. Monoterpenes and oxygenated sesquiterpenes contribute redox characteristics to essential oils, leading to their antioxidant potential [47]. The essential oils of peppermint from Turkey (menthol (38.06%)), Morocco (menthone (29.01%)), and Egypt (Menthol (40.47%)) have an anti-free radical capacity much less important than that of our extracts composed mainly of linolool and its derivatives [7, 48].

3.5. In-vivo pharmacological activities

➢ *Analgesic activity*

The acetic acid induced writhing test was used to evaluate the analgesic potential of *Mentha piperita* L. essential oils. The obtained findings on the acetic acid induced abdominal constrictions tested in mice is showed in Figure 2.

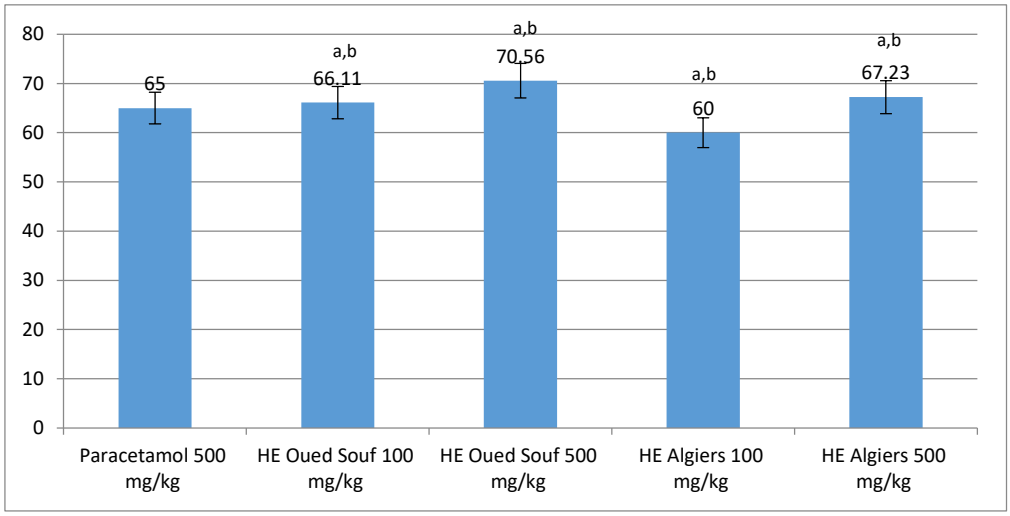

Figure 2. Analgesic effect of *Mentha piperita* L. essential oils and Paracetamol® using acetic acid induced writhing test.

The results revealed that the oral intake of *Mentha piperita* L. essential oil from Algiers and Oued Souf regions led to a significant ($P < 0.05$) reduction in the number of writhing in a dose-dependent manner (Figure 2). *Mentha piperita* L. essential oil obtained from the Oued Souf region presented the highest analgesic potential with significantly ($P<0.05$) inhibition percentages of 66.11%±1.50 and 70.56%±2.08 respectively at 100 and 500 mg/kg doses. This analgesic effect is greater than that of paracetamol 500 mg/kg (65.00%±2.00). The essential oil of *Mentha piperita* L. from Algiers at dose of 500 mg/kg exhibited analgesic action superior than paracetamol with 67.23%±1.52 inhibition.

➢ *Anti-inflammatory activity*

The results demonstrating the anti-inflammatory activity of *Mentha piperita* L. essential oils, as assessed through the carrageenan-induced hind paw edema test, are presented in Table 6.

Table 6. Anti-inflammatory activity of *Mentha piperita* L. essential oils on carrageenan induced paw oedema in mice.

Groups	Dose (mg/kg b.w)	Oedema percentage (%)	Inhibition p ercentage (%)
Control (saline)	---	46.08±1.93	---
Diclofenac®	100	12.98±1.72[a]	71.83±1.16[a]
***Mentha piperita* L Algiers**	100	18.07±0.97[a,b]	60.78±0.81 [a,b]
	500	15.51±1.23 [a,b]	66.34±1.19 [a,b]
***Mentha piperita* L Oued Souf**	100	16.03±1.13 [a,b]	65.21±0.74 [a,b]
	500	13.17±0.21 [a,b]	71.41±1.05 [a,b]

All values are represented as mean ± SEM of six measurements; Tukey test:a $P < 0.05$ compared with control; b $P < 0.05$ compared with Diclofenac®.

As depicted in Table 6, the essential oils of *Mentha piperita* L., along with Diclofenac®, demonstrated a significant reduction ($P<0.05$) in paw edema inflammation compared to the control. The *Mentha piperita* L. essential oil from the Oued Souf region exhibited the highest dose-dependent anti-inflammatory potential, ranging from 65.21% at 100 mg/kg to 71.41% at a 500 mg/kg dose. This anti-inflammatory efficacy was equal to the reference drug Diclofenac® (71.83%). The administration of *Mentha piperita* L. essential oil from Algiers region at doses of 100 and 500 mg/kg, exhibited a decreased inhibition percentage of paw edema compared to *Mentha piperita* L. from the Oued Souf region and the reference drug (with percentages of 60.78% and 66.34%, respectively).

Similar results were observed in analgesic and anti-inflammatory activities, the essential oil of *Mentha piperita* L. from the Oued Souf region was the most active. These results suggest the existence of active substances on the mediators common to inflammation and the painful process, such as histamine and prostaglandins. Terpenoids are recognized for their inflammatory effects [49, 50]. These molecules probably act as non-steroidal anti-inflammatories by inhibiting the enzymatic activity of cyclooxygenase and thus limiting the quantity of pro-inflammatory mediators produced during this process [41, 51, 52]. The exclusive presence of monoterpenes as Beta pinene, sabinene, Beta- Myrcene and sesquiterpenes such as Elemol and Alpha–eudesmol in the essential oil of *Mentha piperita* L. from Oued Souf region can explain its analgesic and anti-inflammatory potentialities exceeding that of Algiers region [53-55].

4. CONCLUSION

This is the first work about Phytochemical composition and pharmacological activities of *Mentha piperita* L essential oil cultivated in two different regions in Algeria. It has been demonstrated that ecological factors and geographical positions can affect the yeilds, phytochemical content and pharmacological properties. The essential oil of *Mentha piperita* L. growing in Oued Souf region is more efficacy in pharmacological activities. Thus, this study may contribute to the potential use of *Mentha piperita* L. essential oil in the pharmaceutical industry as a promising natural reservoir of bioactive compounds possessing noteworthy therapeutic properties.

Ethical approval

The experimental procedures were approved by the Ethical Committee of Animal Experimentation (CEEA) of University of Sciences and Technology Houari Boumediene (USTHB) with approved Ref N°: CEEA-USTHB-08-2023/11118, Algeria.

Conflict of interest: The authors declare no conflicts of interest.

REFERENCES

1. El-Seedi H. R., Burman R., Mansour A., Turki Z., Boulos L., Gullbo J., Göransson U. The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from *Urginea maritima*. *J Ethnopharmacol.* 2013; 145(3):746-757.
2. Boucher H. W., Talbot G. H., Bradley J. S., Edwards J. E., Gilbert D., Rice L. B., Scheld M., Spellberg B., Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis.* 2009; 48(1):1-12.
3. Hubert J., Berger M., Daydé J. Validation of a High-performance Liquid Chromatography-Ultraviolet Method to Quantify Soy Sapogenols A and B in Soy Germs from Different Cultivars and in Soy Isoflavone-Enriched Supplements. *J Food Sci.* 2005; 70(8):c471-c477.
4. Zahnit W., Smara O., Bechki L., Bensouici C., Messaoudi M., Benchikha N., Larkem I., Awuchi C. G., Sawicka B., Simal-Gandara J. Phytochemical profiling, mineral elements, and biological activities of *Artemisia campestris* L. grown in Algeria. *Horticulturae.* 2022; 8(10):914.
5. Sun J., Sun P., Kang C., Zhang L., Guo L., Kou Y. Chemical composition and biological activities of essential oils from six Lamiaceae folk medicinal plants. *Front Plant Sci.* 2022; 13:919294.
6. Shrestha M., Sindhu K., Sah B. S., Jha P. K., Khaitu S., Pandey B., Yadav R. K., Gautam A., Yadav B. Hydroethanolic Leaf Extract of *Murraya koenigii*: Phytochemical Constituents and Biological Evaluation of its Toxicity and Antipyretic Activity in Wistar Albino Rats. *Jordan Journal of Pharmaceutical Sciences.* 2024; 17(4):811-817.
7. Derwich E., Benziane Z., Boukir A. Chemical composition and in vitro antibacterial activity of the essential oil of *Cedrus atlantica*. *Int J Agric Sci.* 2010.
8. Guzmán E., Lucia A. Essential oils and their individual components in cosmetic products. *Cosmetics.* 2021; 8(4):114.
9. Godfrey E. O., Ejike O. O., Ojoniko A. W., Esther I. I., Faith O., Ugbedeojo A. A., Abdullahi B. I. Evaluating the Nutritional and Chemical Composition of *Treculia Africana* and *Vigna Subterranea* L. Seeds Collected from Kogi State, Nigeria. *Jordan Journal of Pharmaceutical Sciences.* 2024; 17(4):767-782.
10. Yadegarinia D., Gachkar L., Rezaei M. B., Taghizadeh M., Astaneh S. A., Rasooli I. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. *Phytochemistry.* 2006; 67(12):1249-1255.
11. Tafrihi M., Imran M., Tufail T., Gondal T. A., Caruso G., Sharma S., Sharma R., Atanassova M., Atanassov L., Tsouh Fokou P. V. The wonderful activities of the genus *Mentha*: Not only antioxidant properties. *Molecules.* 2021; 26(4):1118.
12. Abu-Darwish D., Shibli R., Al-Abdallat A. M. Phenolic Compounds and Antioxidant Activity of *Chiliadenus montanus* (Vhal.) Brullo. grown in vitro. *Jordan Journal of Pharmaceutical Sciences.* 2024; 17(3):611-628.
13. Jean B. *Pharmacognosie, phytochimie, plantes médicinales* (4e éd.). Lavoisier. 2009.
14. Kalemba D., Synowiec A. Agrobiological interactions of essential oils of two menthol mints: *Mentha piperita* and *Mentha arvensis*. *Molecules.* 2019; 25(1):59.
15. Kizil S., Hasimi N., Tolan V., Kilinc E., Yuksel U. Mineral content, essential oil components and biological activity of two *Mentha* species (*M. piperita* L., *M. spicata* L.). *Turk J Field Crops.* 2010; 15(2):148-153.
16. Quézel P., Santa S. *Nouvelle flore de l'Algérie et des régions désertiques méridionales.* 1962.
17. Zerrouk S., Seijo M. C., Boughediri L., Escuredo O., Rodríguez-Flores M. S. Palynological characterisation of Algerian honeys according to their geographical and botanical origin. *Grana.* 2014; 53(2):147-158.
18. Bauer A., Kirby W., Sherris J. C., Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966; 45(4_ts):493-496.

19. Del Castillo M. R., Blanch G. P., Herraiz M. Natural variability of the enantiomeric composition of bioactive chiral terpenes in *Mentha piperita*. *J Chromatogr A.* 2004; 1054(1-2):87-93.
20. Staniszewska M., Kula J., Wieczorkiewicz M., Kusewicz D. Essential oils of wild and cultivated carrots—the chemical composition and antimicrobial activity. *J Essent Oil Res.* 2005; 17(5):579-583.
21. Boudjema K., Guerdouba A., Hali L. Composition, physicochemical analysis, antimicrobial and anti-inflammatory activities of the essential oils obtained from *Ruta chalepensis* L. growing wild in northern Algeria. *J Chem Soc Pak.* 2018; 40(6):1054-.
22. Sacchetti G., Maietti S., Muzzoli M., Scaglianti M., Manfredini S., Radice M., Bruni R. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem.* 2005; 91(4):621-632.
23. Franzin L., Pennazio M., Cabodi D., Rossini F. P., Gioannini P. Clarithromycin and amoxicillin susceptibility of *Helicobacter pylori* strains isolated from adult patients with gastric or duodenal ulcer in Italy. *Curr Microbiol.* 2000; 40:96-100.
24. Ponce A., Fritz R., Del Valle C., Roura S. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT - Food Sci Technol.* 2003; 36(7):679-684.
25. Lambert R., Skandamis P. N., Coote P. J., Nychas G. J. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J Appl Microbiol.* 2001; 91(3):453-462.
26. European-Council. *European Pharmacopoeia* 2007.
27. Singh R., Shushni M. A., Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arab J Chem.* 2015; 8(3):322-328.
28. Benmouloud A., Zatra Y., Belkadi A., Halli L., Kacimi L., Souames S., Aknoun-Sail N. Evaluation of gastroprotective effect of jujube honey in ethanol-induced stomach ulcer in mice. *Indian J Tradit Knowl.* 2023; 22(1):150-159.
29. Vogel H. G. *Drug discovery and evaluation: pharmacological assays.* Springer Science & Business Media. 2002.
30. Levy L. Carrageenan paw edema in the mouse. *Lif Sci.* 1969; 8(11):601-606.
31. Bruneton J. *Pharmacognosie, phytochimie, plantes médicinales* (Lavoisier ed.). Tec et Doc édition. 1999.
32. Degnon G., Adjou E., Metome G., Dahouenon-Ahoussi E. Efficacy of essential oils of *Cymbopogon citratus* and *Mentha piperita* in stabilizing the fresh cow's milk in southern Benin. 2016.
33. Myadelets M., Domrachev D., Cheremushkina V. A study of the chemical composition of essential oils of some species from the Lamiaceae L. family cultivated in the Western Siberian Region. *RUSS J BIOORG.* 2013; 39:733-738.
34. Dwivedi S., Khan M., Srivastava S. K., Syamasunnder K., Srivastava A. Essential oil composition of different accessions of *Mentha* × *piperita* L. grown on the northern plains of India. *Flavour Fragr J.* 2004; 19(5):437-440.
35. Maffei M., Mucciarelli M. Essential oil yield in peppermint/soybean strip intercropping. *Field Crops Res.* 2003; 84(3):229-240.
36. Debbab A., Mosaddak B., Aly A., Hakiki A., Mosaddak M. Chemical characterization and toxicological evaluation of the essential oil of *Mentha piperita* L. growing in Morocco. *Sci Stu Res.* 2007; 8(3):281-288.
37. Saharkhiz M. J., Motamedi M., Zomorodian K., Pakshir K., Miri R., Hemyari K. Chemical composition, antifungal and antibiofilm activities of the essential oil of *Mentha piperita* L. *Int Sch Res Notices.* 2012; 2012.
38. Verma R., Rahman L., Verma R., Chauhan A., Yadav A., Singh A. Essential oil composition of menthol mint (*Mentha arvensis*) and peppermint (*Mentha piperita*) cultivars at different stages of plant growth from Kumaon region of Western Himalaya. *Open Access J Med Aromat Plants.* 2010; 1(1):13-18.
39. Liu W., Yin D., Li N., Hou X., Wang D., Li D., Liu J. Influence of environmental factors on the active substance production and antioxidant activity in *Potentilla fruticosa* L. and its quality assessment. *Sci Rep.* 2016; 6(1):28591.

40. Chifundera K. B., Kizungub M. Phytochemical screening and antibacterial testing of *Ficus sycomorus* extracts. Communication shorts. *Fitoterapia.* 2016; 535-539.
41. Harchaoui L., Ouafi S., Chabane D. UPLC-MS profiling, antimicrobial and antipyretic activities of *Deverra scoparia* Coss. & Dur. extracts. *Indian J Tradit Knowl.* 2022; 21(1):40-47.
42. Carson C. F., Mee B. J., Riley T. V. Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. *ANTIMICROB AGENTS CH.* 2002; 46(6):1914-1920.
43. Jusoh S., Sirat H. M., Ahmad F., Basar N., Bakar M. B., Jamil S., Haron S. Essential oils of leaves and pseudo stems *Alpinia malaccensis* and antimicrobial activities. *J Phys Conf Ser.* 2020; 1529(4):042050.
44. Meincken M., Holroyd D., Rautenbach M. Atomic force microscopy study of the effect of antimicrobial peptides on the cell envelope of *Escherichia coli*. *Antimicrob Agents Chemother.* 2005; 49(10):4085-4092.
45. Dorman H. D., Deans S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol.* 2000; 88(2):308-316.
46. Desam N. R., Al-Rajab A. J., Sharma M., Mylabathula M. M., Gowkanapalli R. R., Albratty M. Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha* × *Piperita* L. (peppermint) essential oils. *J King Saud Univ Sci.* 2019; 31(4):528-533.
47. Ruberto G., Baratta M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 2000; 69(2):167-174.
48. Gharib F., da Silva J. T. Composition, total phenolic content and antioxidant activity of the essential oil of four Lamiaceae herbs. *Med Aromat Plant Sci Biotechnol.* 2013; 7(1):19-27.
49. Jusoh S., Sirat H. M., Ahmad F., Basar N., Bakar M. B., Jamil S., Haron S. Essential oils of leaves and pseudo stems *Alpinia malaccensis* and antimicrobial activities. *J Phys Conf Ser.* 2020; 1529(4):042050.
50. Meincken M., Holroyd D., Rautenbach M. Atomic force microscopy study of the effect of antimicrobial peptides on the cell envelope of *Escherichia coli*. *Antimicrob Agents Chemother.* 2005; 49(10):4085-4092.
51. Dorman H. D., Deans S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J Appl Microbiol.* 2000; 88(2):308-316.
52. Desam N. R., Al-Rajab A. J., Sharma M., Mylabathula M. M., Gowkanapalli R. R., Albratty M. Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha* × *Piperita* L. (peppermint) essential oils. *J King Saud Univ Sci.* 2019; 31(4):528-533.
53. Ruberto G., Baratta M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* 2000; 69(2):167-174.
54. Gharib F., da Silva J. T. Composition, total phenolic content and antioxidant activity of the essential oil of four Lamiaceae herbs. *Med Aromat Plant Sci Biotechnol.* 2013; 7(1):19-27.

المكونات الكيميائية والتأثيرات الدوائية في المختبر/في الجسم الحي لزيت النعناع الفلفلي من نوع *Mentha piperita* L. في مناطق مختلفة من الجزائر

لطيفة حالي[1,2]، ليليا حرشاوي[1]، محمد مسعودى[*3]، ثريا دحمان[4]، فتيحة عيد[1]، باربارا سويكا[5]، وفاء زحنيت[6]

[1] كلية العلوم البيولوجية، جامعة العلوم والتكنولوجيا هواري بومدين، الجزائر العاصمة، الجزائر.

[2] مركز البحث والتطوير، مجمع صيدال ، الجزائر العاصمة، الجزائر.

[3] مركز البحث النووي ببيرين، عين وسارة الجلفة ، الجزائر.

[4] قسم العلوم الفلاحية، جامعة جيلالي بونعامة خميس مليانة، كلية العلوم الطبيعية والحياة وعلوم الأرض، الجزائر.

[5] قسم تكنولوجيا إنتاج النباتات وعلوم السلع، جامعة العلوم الحياتية ، لوبلين، شارع أكاديميكا 15، بولندا.

[6] قسم الكيمياء، كلية الرياضيات وعلوم المواد، جامعة قاصدي مرباح، مختبر تثمين وترقية الموارد الصحراوية، ورقلة، الجزائر.

ملخص

تتناول هذه الدراسة النعناع الفلفلي *Mentha piperita L.* المزروعة في منطقتين تتمتعان بمستويات بيئية مناخية مختلفة. الهدف هو تقييم ومقارنة الإنتاج والتركيب الكيميائي والأنشطة الدوائية للنبات في هذه الظروف البيئية المتنوعة . تم تحديد الملف الكيميائي النباتي لزيوت النعناع الفلفلي باستخدام تحليل الكروماتوغرافيا الغازية. تم إجراء الأنشطة الدوائية في المختبر وفي الجسم الحي باستخدام اختبارات مسكنة، ومضادة للالتهابات، ومضادة للبكتيريا، ومضادة للأكسدة. تم الحصول على أعلى إنتاجية من الزيت العطري من منطقة وادي سوف بنسبة 1.02%، بينما كان إنتاج منطقة الجزائر 0.86%. كشفت تحليلات GC/MS عن غنى الزيت العطري بالينالول وكذلك مشتقاته مثل أسيتات الينالیل في وادي سوف وبوتيرات الينالیل في الجزائر. في جميع الأنشطة الدوائية، كان زيت النعناع الفلفلي من منطقة وادي سوف أكثر فعالية بشكل ملحوظ من الزيت العطري من الجزائر. يمكن أن تساهم هذه الدراسة في تطبيق زيت النعناع الفلفلي في صناعة الأدوية كمصدر طبيعي واعد للمركبات المتطايرة ذات الخصائص العلاجية البارزة.

الكلمات الدالة: مضاد للالتهابات، كروماتوغرافيا الغاز/مطياف الكتلة، الشفوية (أو الفصيلة الشفوية)، النعناع الفلفلي، الملف الكيميائي النباتي، الينالول.

[*] ***المؤلف المراسل: محمد مسعودى***

messaoudi2006@yahoo.fr

تاريخ استلام البحث 2024/7/30 وتاريخ قبوله للنشر 2024/9/21.