



Research article

Quality criteria, chemical composition and antimicrobial activity of the essential oil of *Mentha suaveolens* Ehrh

Moha Afrokh^{a,b}, Khadija El Mehrach^b, Khalid Chatoui^a, Mohamed Ait Bihi^b, Hajar Sadki^b, Abdelkader Zarrouk^a, Mohamed Tabyaoui^a, Saida Tahrouch^{b,*}

^a Laboratory of Materials, Nanotechnology and Environment, Faculty of Sciences, Mohammed V University in Rabat, Av. Ibn Battouta, P. O. Box 1014 Agdal-Rabat, Morocco

^b Laboratory of Plant Biotechnology, Faculty of Sciences, Ibn Zohr University, Box.8106, Agadir, Morocco

ARTICLE INFO

Keywords:

Mentha suaveolens Ehrh
Essential oil
Organoleptic and physicochemical characterization
Antimicrobial activity
Oxygenated monoterpenes
GC/MS analysis

ABSTRACT

The aim of the present study is the valorization of the essential oil of *Mentha suaveolens* Ehrh. The research plan and methods included 3-axis: the first axis consists of studying the organoleptic and physicochemical characterization of the essential oil, the second is the chemical analysis carried out by Gas Chromatography/Mass Spectrometry (GC/MS) and the third consists of evaluating its antimicrobial activity against selected microorganisms. The results obtained for the organoleptic and physicochemical properties are as follows: appearance: Liquid, mobile and clear, odor: Strong odor characteristic of *Mentha suaveolens* Ehrh, color: Pale yellow; relative density (0.92), miscibility with ethanol (1V/2V), freezing point ($T_{\text{freezing}} < -10$ °C), refractive index (1.5256), rotating power (+0.825), acid index (1.68), ester index (68.44), saponification index (70.13) and iodine index (12.05). Chemical analysis identified 69 compounds which are mostly oxygenated monoterpenes such as piperitenone oxide (32.55%), pulegone (10.14%), piperitone oxide (8.34%), etc. The microbiological tests were carried out by an agar diffusion test using the essential oil of *Mentha suaveolens* Ehrh. The microbiological tests were carried out by a diffusion test on agar, these tests are carried out on six microbial strains (five bacteria and one yeast). The inhibitory effect of our oil is well marked against bacteria: *Proteus mirabilis* (17.50 ± 0.70 mm at 50 $\mu\text{L/mL}$), *Enterococcus faecalis* (17.00 ± 1.00 mm at 50 $\mu\text{L/mL}$) and *Staphylococcus coagulase negative* (16.33 ± 0.57 mm at 50 $\mu\text{L/mL}$) while it was moderate against *Escherichia coli* (14.33 ± 1.15 mm at 50 $\mu\text{L/mL}$) and *Streptococcus* spp (13.00 ± 0.00 mm at 50 $\mu\text{L/mL}$) as well as against yeast, *Candida albicans* (15.33 ± 1.52 mm at 50 $\mu\text{L/mL}$). It appears from these results that our oil is of high quality and can be used in several areas. The results obtained are therefore promising and thus open the way for manufacturers to use this essential oil of *Mentha suaveolens* Ehrh in the pharmaceutical, cosmetic, agricultural and food industries.

1. Introduction

Aromatic and medicinal plants have been known and used since the dawn of humanity for their cosmetic virtues and their various

* Corresponding author.

E-mail addresses: afrokhmoha@yahoo.fr (M. Afrokh), k.elmehrach@uiz.ac.ma (K. El Mehrach), chatouikhalid@gmail.com (K. Chatoui), hajarsadki38@gmail.com (H. Sadki), azarrouk@gmail.com (A. Zarrouk), tabyaouihamid@gmail.com (M. Tabyaoui), s.tahrouch@uiz.ac.ma (S. Tahrouch).

<https://doi.org/10.1016/j.heliyon.2024.e28125>

Received 29 April 2023; Received in revised form 11 March 2024; Accepted 12 March 2024

Available online 19 March 2024

2405-8440/© 2024 Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

therapeutic properties [1]. The family Lamiaceae (Labiatae) from the Latin labia meaning that the flowers have a characteristic two-lipped shape, it includes nearly 700 species divided into 250 genera. The majority of these plants have medicinal properties and constitute an important source of essential oils (EO) [2]. The essential oils of these plants all have a common characteristic: rich in terpene constituents [3]. These species contain major groups of economically important secondary metabolites and can be used as an ingredient in many formulations [4]. Flavonoids, polyphenols, alkaloids, glycosides, terpenoids, and sesquiterpenes are the primary metabolic chemicals that possess diverse antimicrobial, antifungal, cytotoxic, and antioxidant characteristics [5].

The genus *Mentha* is an important member of this family, and it is represented in Morocco by five main species: *Mentha pulegium* L., *Mentha aquatica* L., *Mentha longifolia* L., *Mentha arvensis* L. and *Mentha suaveolens* Ehrh [6]. *Mentha suaveolens* Ehrh, formerly named *Mentha rotundifolia* (L) Hudson [7] is native to Africa, temperate Asia and Europe [8], it is known in Morocco under the name of "Merssita" or "Timijja" [6]. It is a perennial herb that finds habitats in semi-arid and sub-humid zones along rivers [9]. These plants have several therapeutic benefits, including analgesic, antispasmodic, sedative, appetizing, stimulant, tonic, antiviral, anticonvulsant [10], anti common digestive and respiratory disorders [2]. Furthermore, the pharmacological and biological characteristics of this variety's essential oil include anti-inflammatory, antibacterial, antioxidant, anticancer, and antidiabetic actions [11]. In view of these benefits of this plant, it is necessary to find solutions to the changing environmental conditions that generate abiotic stresses such as exposure to chemicals and biotic stress [12]. Thus, to increase the sources of benefits for human health and obtain significant results [13]. The chemical composition of the essential oil of African MS [14], has revealed its richness in menthone, pulegone, germacrene D, borneol and piperitenone endowed with insecticidal, antibacterial and antifungal properties [15].

The objective of this study is the valorization of this plant, which grows in the region of Er-rich, south eastern of Morocco, by the organoleptic and physicochemical characterization of its essential oil obtained by hydrodistillation. The chemical analysis carried out by Gas Chromatography/Mass Spectrometry and the antimicrobial activity of this essential oil has been investigated against the bacteria *Enterococcus faecalis*, *Proteus mirabilis*, streptococcus spp, *Escherichia coli*, *Staphylococcus coagulase negative* and the fungi: *Candida albicans*.

2. Material and methods

2.1. Plant material

Mentha suaveolens Ehrh (MS) was collected near the town of Er-rich, located 65 km from the province of Errachidia (Latitude: N: 32° 15'33.691", Longitude: O: 4° 29'43.544" and Altitude: 1321 m) in full bloom during September.

2.2. Kinetics study of the extraction

The distillation was carried out with a recycling commonly called cohesion as described in the European Pharmacopoeia. Samples were taken every hour on the aqueous and organic phases and the hydrodistillation kinetics were measured. The hydrodistillation of MS was performed using a Clevenger-type device. The distillation was carried out with a recycling commonly called cohesion as described in the European Pharmacopoeia. This system makes possible to take hourly samples at regular intervals on the aqueous and organic phases, which are intended for measuring hydrodistillation kinetics. The essential oil (EO) obtained was dehydrated using anhydrous sodium sulphate before yield calculating. Afterward, it was stored in brown glass bottles sealed to protect it from air and light at a temperature of 4 °C.

2.3. Chromatographic analysis

2.3.1. Gas chromatographic analysis (GC-FID)

The PerkinElmer Autosystem (Whaltham, MA, USA) gas chromatograph (GC) with dual flame ionization (FID) detection was utilized to analyze the samples. It was fitted with fused silica capillary columns (60 m × 0.22 mm internal diameter, 0.25 µm film thickness) and various stationary phases. Rtx-Wax (polyethylene glycol) and Rtx-1 (polydimethylsiloxane). The oven was set to increase the temperature by 2 °C each minute from 60 to 230 °C, and it was then maintained at that temperature isothermally for 35 min. At 280 °C, the temperatures of the injector and detector remained unchanged. Using helium as the carrier gas at a flow rate of 1 mL/min and an injection volume of 0.2 µL of pure oil, samples were injected fractionally (1/50). By utilizing PerkinElmer software, linear interpolation based on the Van den Dool and Kratz equation, and comparing the retention periods with those of a series of linear alkanes (C5–C30) (Restek, Lisses, France), the retention indices (RIs) of the compounds were ascertained.

2.3.2. Gas chromatography-mass spectrometry (GC-MS)

Rtx-1 and Rtx-wax fused silica capillary columns from the PerkinElmer Autosystem XL system were used in conjunction with a PerkinElmer turbo (quadrupole) mass detector to analyze the samples. The carrier gas, helium, was employed at a flow rate of 1 mL/min. The oven temperature was planned to rise from 60 °C to 230 °C at a rate of 2 °C/min and then be isothermally maintained at 230 °C for 35 min, while the ion source temperature was kept at 150 °C. The injector was maintained at 280 °C and 70 eV of ionization energy. Electron ionization mass spectra were obtained using a fractionation of 1/80 and an injection volume of 0.2 µL of pure oil, covering the mass range of 35–350 Da.

2.3.3. Components identification

Two primary approaches were used to identify the components of essential oils: (i) comparing the mass spectra of genuine reference compounds, if feasible, with those of mass spectra libraries like WILEY275, NIST 02, and Adams; and (ii) assessing their retention indices (RIs), which were computed using linear interpolation with respect to the retention times of a series of C-5 to C-30 alkanes, and comparing these data with our own library of genuine compounds or with reference data from literary works [16,17].

2.4. Quality control of the essential oil

2.4.1. Organoleptic properties

In this study the organoleptic characteristics such as color, physical appearance and odor of the essential oil of *Mentha suaveolens* Ehrh (EOMS) were determined and evaluated.

2.4.2. Physical properties

2.4.2.1. Relative density (NFT-75 111, 2000). The determination of the density of extracted oils was carried out using an Eppendorf tube with a capacity of 1 mL. The volume taken was 0.20 mL for the essential oil, as well as for the distilled water. The relative density is given by the following formula(1) [18]:

$$d_{20} = \frac{m_1 - m_0}{m - m_0} \quad (1)$$

With:

m_1 : mass in grams of the Eppendorf containing 0.20 mL of the EO. <https://www.msn.com/fr-xl/feed>

m_0 : mass in grams of the empty Eppendorf.

m : mass in grams of the Eppendorf containing 0.20 mL of distilled water.

2.4.2.2. Miscibility with ethanol (NFT-75 101, 2000). Ethanol is added, in 0.5 mL portionsto 0.5 mL of essential oil. After each addition, the mixture is stirred. When the solution becomes clear, the volume of ethanol is noted [18].The miscibility with ethanol was calculated.

2.4.2.3. Freezing point (NFT-75 102, 2000). The essential oil was placed, in a freezer, with a thermometer, which allowed following slowly and gradually the temperature accompanying the solidification of the oil [18].The freezing point was recorded by the researcher.

2.4.2.4. Rotatory power (NFT-75 113, 2000). Certain chemical compounds exhibit specific rotation $[\alpha]_D^t$, which results in a deviation of the polarization plane of polarized light. This deviation suggests the presence of an asymmetric carbon. The Rotatory power $[\alpha]_D^t$ measured at a temperature t , using the D-line of Sodium (Na) $\lambda = 589 \text{ nm}$ as a light source is expressed by Biot's law(2):

$$[\alpha]_D^t = \frac{\alpha}{L.C} \quad (2)$$

With:

α : Value of the deflection angle of the polarized light read on the polarimeter expressed in degrees.

L : Length of the cell expressed in dm.

C : Concentration of the test solution in g/100 mL.

In order to evaluate the rotation angles a branded polarimeter was used: ATAGO AP-300, equipped with a cell filled with an ethanolic solution of essential oil at a rate of 0.20 g for the sample in 100 mL of solvent.

The instrument measures the observed degree of rotation directly, allowing us to calculate the rotatory power of our essence [18].

2.4.2.5. Refractive index (NFT-75 112, 2000). The refractive index is the ratio between the sine of the angles of incidence and refraction of a light ray of determined wavelength, passing air through the maintained essential oil at a constant temperature. It is measured by means of a refractometer, into which a few drops of distilled water, which is considered as a standard on the prism, are introduced. The device is set at 1.333. These drops are wiped and replaced by a few drops of EO, then read. The refractive index at temperature t is given by the formula(3) [18]:

$$[n]_D^t = n_D^t + 0,00045(t' - t) \quad (3)$$

With:

n_D^t : is the reference refractive index

n_D^t : is the measured refractive index.

t : reference temperature which is at 20 °C.

t' : temperature at the time of measurement.

2.4.3. Chemical properties

2.4.3.1. Acid index (NFT-75 103, 2000). The acid index is defined as the number of milligrams (mg) of potassium hydroxide (KOH) required to neutralize the free acids contained in 1 g of oil.

One gram of essential oil was added in an Erlenmeyer flask with 5 mL of 95% ethanol and about 5 drops of phenolphthalein. The mixture was then titrated with an alcoholic solution of 0.1 N potassium hydroxide (KOH) until the solution turns pink. The acid index (I_a) is determined by the following formula(4) [18]:

$$I_a = V.C. \frac{56,11}{m} \quad (4)$$

With:

56.11: is the molar mass, expressed in grams per mole, of potassium hydroxide.

V: volume in mL of the KOH solution used for the titration.

C: concentration in mol/L of the KOH solution.

m: mass in grams of the test sample.

2.4.3.2. Ester index (NFT-75 104, 2000). The ester index is the number of mg of potassium hydroxide KOH necessary to neutralize the acids released by hydrolysis in a basic medium of the esters contained in 1 g of oil.

One gram of essential oil and 25 mL of an alcoholic solution of 0.5 M KOH potassium hydroxide were introduced into a 100 mL flask using a burette, as well as some pumice stones. The mixture was refluxed for 1 h. After cooling the solution, 20 mL of distilled water and 3 drops of phenolphthalein are added.

Excess KOH was titrated with a solution of 0.5 N hydrochloric acid HCl until the pink color disappears. A blank operation was carried out under the same conditions with the same reagents, replacing the EO with 1 mL of distilled water. The ester index I_e was calculated by the following formula(5) [18]:

$$I_e = \frac{28.05}{m} (V_0 - V_1) - I_a \quad (5)$$

With:

V_0 : volume in mL of the HCl solution (0.5 N), measured for the blank test.

V_1 : volume in mL of the HCl solution (0.5 N), measured for the calculation of I_e .

m: mass in grams of the test sample.

I_a : value of acid index.

2.4.3.3. Saponification index (NFT 60–206, 1984). The saponification index is the number of mg of potassium hydroxide (KOH) needed to neutralize the free acids and saponify the esters contained in 1 g of the substance.

A quantity of 1 g of essential oil was placed in a flask equipped with a condenser, and 25 mL of 0.5 N ethanolic KOH were then added. The mixture was first boiled with stirring for 1 h, then, 3 drops of phenolphthalein were added. The soapy solution was titrated with HCl (0.5 N). At the same time, a blank test was carried out under the same conditions. The saponification index was given by the following formula (6) [19]:

$$I_s = \frac{56.11.N.(V_0 - V)}{m} \text{ (mg KOH / g EO)} \quad (6)$$

With:

56.11: molar mass of KOH.

V_0 : volume in mL of the hydrochloric acid solution for the blank test.

V: volume in mL of the hydrochloric acid solution used for the test portion.

N: exact normality of the hydrochloric solution.

m: mass of the test sample in grams.

2.4.3.4. Iodine index (NFT 60–203, 1984). The iodine index is the mass of iodine in grams likely to be fixed by 100g of the substance.

One gram of essential oil, 20 mL of carbon tetrachloride (CCl_4) and 25 mL of a solution of iodine (1 N) prepared in carbon tetrachloride were put in an Erlenmeyer flask. The mixture was kept in darkness for 2 h, then 20 mL of a solution of potassium iodide (KI) at 50% and 20 mL of distilled water were added. Excess iodine is titrated with 0.1 N sodium thiosulfate pentahydrate solution in the presence of starch paste. Under the same conditions as above, a blank test was performed. The iodine index was calculated according to the following relation (7) [19]:

$$I_i = \frac{12.69.N.(V_0 - V_1)}{m} \quad (7)$$

With:

Table 1
Chemical constituents of *Mentha suaveolens* Ehrh essential oil, in per cent (%).

R T ^o (min)	Calc.	Area	%	Name of compound
10,17		58433904	0,04	ethyl 2-methylbutyrate
13,76	925	59128192	0,04	α -thujene
14,22	932	1166624000	0,82	α -pinene
15,03	944	897449920	0,63	camphene
16,33	966	607782208	0,43	verbenene
16,44	967	142679184	0,10	octen-3-ol
16,62	968	1234428416	0,87	sabinene
17,21	976	839217920	0,59	β -pinene
17,78	999	56475908	0,04	myrcene
18,05	1004	65311732	0,05	α -phellandrene
18,83	1015	54050572	0,04	α -terpinene
19,34	1024	133822360	0,09	<i>p</i> -cymene
19,78	1030	4015885056	2,83	limonene
19,87	1032	267900944	0,19	1,8-cineole (eucalyptol)
20,00	1033	76219888	0,05	(Z)-ocimene
20,49	1042	67859096	0,05	phenyl acetaldehyde
20,66	1044	47633020	0,03	(E)-ocimene
21,45	1056	140807808	0,10	γ -terpinene
22,18	1066	298938816	0,21	<i>p</i> -mentha-1,8-diene
23,39	1084	254868448	0,18	<i>p</i> -mentha-2,4(8)-diene
24,22	1098	2481846016	1,75	linalool
24,34	1101	86026296	0,06	nonanal
26,59	1134	491053504	0,35	dihydrolinalool
27,83	1151	3722602752	2,62	isopulegol
28,10	1155	3512162304	2,48	menthone
28,19	1156	205595632	0,14	isopulegol
28,80	1166	3890161920	2,74	neomenthol
28,94	1168	2587858176	1,82	menthol
29,19	1172	2643717376	1,86	borneol
29,79	1180	5722162176	4,03	terpinen-4-ol + isopulegol
30,04	1184	1007960128	0,71	isomenthol
30,42	1188	1388312064	0,98	<i>cryptone</i>
30,66	1192	966480960	0,68	α -terpineol
32,80	1223	742011328	0,52	coahuilensol, methylether
34,32	1246	1,4383E+10	10,14	pulegone
35,38	1262	1,1826E+10	8,34	<i>piperitone oxide</i>
35,56	1264	472312736	0,33	<i>carveol oxide cis</i>
35,67	1266	227414352	0,16	geranial
36,11	1273	1384287232	0,98	<i>neomenthyl acetate</i>
36,86	1284	1042828800	0,74	isobornyl acetate
37,03	1286	119624336	0,08	N I ^a
37,30	1291	580543104	0,41	bornyl acetate
37,75	1296	426778624	0,30	2,4,6-trimethyl-3-methoxycyclohex2-enone
38,08	1303	361723840	0,26	N I
38,27	1306	920536064	0,65	isomenthyl acetate
38,60	1310	1630003072	1,15	dihydrocarvyl acetate
38,69	1312	444966656	0,31	carvacrol
39,05	1332	260792704	0,18	3-hexenyl <i>butyrate</i>
40,67	1343	4339761664	3,06	piperitenone
43,72	1389	4,6163E+10	32,55	piperitenone oxide
44,04	1394	292077856	0,21	β -elemene
44,72	1404	1559553408	1,10	methyl eugenol
44,80	1406	1006666496	0,71	N I
44,94	1407	2117318912	1,49	N I
45,07	1410	198096160	0,14	α - <i>gurjunene</i>
45,37	1415	1308774272	0,92	N I
45,77	1420	764911040	0,54	(E)-caryophyllene
46,22	1428	121632616	0,09	β -copaene
46,99	1439	152734336	0,11	N I
47,24	1444	266732064	0,19	muurola-3,5-diene <i>cis</i>
47,50	1454	165154592	0,12	spirolechepinene
47,72	1457	119235400	0,08	α -humulene
48,14	1464	100236728	0,07	alloaromadendrene
48,25	1466	185453088	0,13	γ -muurolene
49,45	1484	2147368448	1,51	germacrene D
50,27	1497	475189024	0,34	bicyclogermacrene
51,24	1513	510703200	0,36	γ -cadinene
51,52	1519	158842432	0,11	N I

(continued on next page)

Table 1 (continued)

R T ^a (min)	Calc.	Area	%	Name of compound
51,68	1521	641318272	0,45	δ-cadinene
55,02	1577	397151840	0,28	spathulenol
55,19	1580	383340768	0,27	2-phenethyl tiglate
55,37	1581	213837168	0,15	caryophyllene oxide
57,07	1610	280433024	0,20	α-epi-cadinol
58,60	1636	2045257856	1,44	α-epi-muurolol
58,77	1639	200641952	0,14	α-muurolol (torreyol)
59,28	1658	626716480	0,44	α-cadinol
60,99	1675	98215776	0,07	N I
61,31	1693	115422128	0,08	N I

^a Rt: Retention time, N I: Not Identified.

N: normality of the solution of sodium thiosulfate pentahydrate.

V₀: volume in mL of the sodium thiosulfate pentahydrate solution used for the blank test.

V₁: volume in mL of the sodium thiosulfate solution used for the determination of I_i.

m: mass in grams of the test sample.

2.5. Assessment of antimicrobial activities

Microorganisms used in this study are pathogenic species obtained from Oued Noun Laboratory for Medical Biology Analysis, Guelmim (Morocco) and consisted of five bacteria species namely *Enterococcus faecalis*, *Proteus mirabilis*, *Streptococcus* spp, *Escherichia coli*, *Staphylococcus coagulase negative* and yeast: *Candida albicans*. Purity, viability and identification of the organisms were checked by plating, gram staining, and the Vitek 2 System (Biomérieux vitek).

2.5.1. Antibacterial activity by agar disc diffusion method (DDM)

The antimicrobial activity was performed using the disc diffusion method on Muller-Hinton (MH) and Sabouraud (SB) agar, following the CLSI recommendations [20]. Thus, the saline suspensions of microorganisms (NaCl 0.9%) were first adjusted to match the 0.5 McFarland standard (equivalent to 1×10^8 cfu/mL), and then inoculated onto plate surfaces with a sterile cotton swab. Whatman paper discs (about 5 mm in diameter) sterilized by autoclaving (121 °C for 15 min) were soaked with the test oil at different concentrations (30,40 and 50 µL/mL) and placed on the MH and SB agar surface. After 24 h of incubation at 37 °C and 48 h at 25 °C for bacteria and yeasts respectively, the diameter of inhibition growth zones was measured using a ruler. Each trial was repeated three times.

2.5.2. Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentrations (MIC) of tested oils were determined using the 96-well plate microdilution method [21]. Two-fold serial dilutions of the essential oil were made with BHI sterile broth. 25 µL of diluted oil were dispensed in each well of columns 1 to 10, while columns 11 and 12 were reserved respectively for microbial inoculums and BHI medium broth (as a control to monitor sterility). 5 µL of microbial suspension previously adjusted to 0.5 Mac Farland (10^8 CFU/mL) was then added to all wells except the column 12 which serves as a negative control. BHI medium of 70 µL was distributed in the 96 wells and the microplate was incubated at 37 °C for 24 h. After incubation, the visual turbidity was observed and recorded. The MIC was determined visually as the lowest concentration that led to growth inhibition.

2.5.3. Determination of minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by plating directly 100 µL of the content of wells with concentrations higher than the MIC value. The MBC was determined after incubation for 24 h at 37 °C and it was considered as the lowest concentration that totally inhibits growth. The antibacterial effect was judged to be bactericidal or bacteriostatic depending on the ratio: MBC/MIC. Indeed, if $MBC/MIC \leq 4$, the effect is bactericidal and if $MBC/MIC > 4$, the effect is bacteriostatic [22].

3. Results and discussion

3.1. Determination of the essential oil yield

According to the results, the evolution of the EO yield has a progressive growth. Thus, from 5 h of extraction; the curve is bent and marks a plateau, which corresponds, the maximum yield that can be achieved, under the conditions considered. It corresponds to a plateau that marks the end of the extraction process. The essential oil obtained gives a yield ranging from 0.53 to 1.93% during one to 6 h.

The output and content of the essential oil are influenced by several factors. Seasonal changes, the plant portion utilized, its maturity level, its place of origin, and its genetic traits are a few of these.

3.2. Chromatographic analysis

The essential oil of *Mentha suaveolens* Ehrh is characterized by the presence of piperitenone oxide as the main constituent with a content of 32.55%. The latter is followed by pulegone (10.14%), piperitone oxide (8.34%), terpinen-4-ol + isopulegol (4.03%), piperitenone (3.06%), limonene (2.83%), neo menthol (2.74%), isopulegol (2.62%), etc. Other compounds are identified but at relatively low percentages such as borneol (1.86%), menthol (1.82%), linalool (1.75%), etc. (Table 1).

Numerous studies have been devoted to the chemical composition of the essential oils of MS. For example, a study carried out on the essential oil of the fresh aerial parts of MS collected in the Al Hoceima National Park region (North of Morocco) confirmed the presence of high quantities of Piperitenone oxide (44.3%), followed by Z-piperitone oxide (19.1%), terpinen-4-ol (3.8%), 1,2-epoxymethyl acetate (3.5%) and *trans*-hydrate Sabinene (3.1%) [23]. In addition, a study carried out on the EO of this species collected in the North-West, confirmed the presence of the piperitenone oxide (56.28%), piperitenone (11.64%) and pulegone (6.16%) [24]. While a study recently carried out by Ref. [25], shows that Piperitenone oxide was the major constituent of the Azrou EO (74.69%), followed by low percentages of γ -muurolene (5.53%), pulegone (2.34%), limonene (1.85%), etc. In general, previous research on the chemical composition of the essential oil of *Mentha suaveolens* collected in various regions in Morocco has shown in the majority of cases, high percentages for constituents belonging to the class of oxygenated monoterpenes, such as pulegone 85.47% in Beni-Mellal [26]; menthol 40.50% in Boulemane [27]; piperitenone oxide 81.69% in M'irt [28]; piperitone 33.03% in Oulmes [29].

3.3. Quality control of the essential oil of *Mentha suaveolens* Ehrh

3.3.1. Determination of organoleptic properties

Organoleptic and physico-chemical properties are a means of verifying and controlling the quality of essential oil. Thus, the results of the determination of organoleptic characteristics (appearance, color and odor) of the EOMS, grouped in Table 2, are close to those of *Mentha piperita* L [18].

3.3.2. Determination of physical properties

The quality control verification results of the *Mentha suaveolens* Ehrh performed by the determination of the physical properties are grouped in Table 3.

The miscibility with ethanol of the EOMS is a volume of essential oil for every two volumes of ethanol (1V/2V), we found that the miscibility with ethanol of our oil is in agreement with those of Benbouali [30]. This does not allow us to conclude that our essential oil has a good miscibility with ethanol.

The freezing temperature of the EOMS is below $-10\text{ }^{\circ}\text{C}$ ($T_{\text{freezing}} < -10\text{ }^{\circ}\text{C}$).

Comparing the value of the relative density mentioned previously, we found that this value is close to that of the EO of *Mentha piperita* L which varies between 0.820 and 0.9900, according to AFNOR [18].

According to Ref. [31], the refractive index gives information on the quality of the EO; however, it varies with the content of monoterpenes and oxygenated derivatives, a high content of monoterpenes would give a high index. For some authors, the low refractive index of EO indicates its low refraction of light, which could favor its use in cosmetics [18]. Based on the value of the refractive index of 1.5256, our EO has good quality and contains a large amount of monoterpenes and oxygenated derivatives. Thus, the value found for our study is close to that of the oil of *Mentha piperita* L which varies between 1.4600 and 1.4670 according to AFNOR [18] and in good agreement with those of Benbouali [30].

The rotary power is an important criterion of purity of the EO, which makes it possible to determine if it is optically active [32]. Thus, if the direction of rotation of the analyzer is in the clockwise direction, the oil studied is dextrorotatory (+). If the rotation of the analyzer is in the counterclockwise direction, the oil studied is levorotatory (−) [33].

According to the angle of rotation obtained $\alpha = +0.33^{\circ}$, the rotational power value of the essential oil of *Mentha suaveolens* Ehrh is +0.825. This oil is therefore dextrorotatory (+).

3.3.3. Determination of chemical properties

The chemical properties of the essential oil of *Mentha suaveolens* Ehrh are shown in Table 4.

The amount of free acids present is indicated by the acidity index. Our study's index was less than 2, indicating that the essence was well preserved and that there were few free acids present [18]. In addition, an acid index of less than 2 serves as an indicator of oil quality. Our obtained results for the acid index agree with those given by Ref. [18] for the species *Mentha piperita* L. and are in agreement with those of Benbouali [30].

In general, the higher the ester index, the better the quality of the EO [34]. From the chemical properties obtained, it appeared that

Table 2
Organoleptic properties of the essential oil of *Mentha suaveolens* Ehrh.

Organoleptic properties	<i>Mentha suaveolens</i> (our results)	<i>Mentha suaveolens</i> [18]	<i>Mentha Piperita</i> [18]
Aspect	Liquid, mobile and crystal clear	Liquid, mobile and crystal clear	Liquid
Odor	Strong characteristic smell at <i>Mentha suaveolens</i> Ehrh	Spicy. Penetrating	The strong smell, mint very refreshing.
Color	Pale yellow	Pale yellow	Transparent to pale yellow

Table 3
Physical properties of the essential oil of *Mentha suaveolens* Ehrh.

Physical properties	<i>Mentha suaveolens</i> (our results)	<i>Mentha suaveolens</i> [30]	<i>Mentha piperita</i> [18]
Density at 20 °C	0.920	0.930	0.820–0.9900
Miscibility with ethanol	1V/2V	90% 2V	–
Freezing point	T _{freezing} < –10 °C	–	–
Refractive index	1.5256	1.5416	1.4600–1.4670
Rotary power	+0.825	–	–

Table 4
Chemical properties of the essential oil of *Mentha suaveolens* Ehrh.

Chemical properties	<i>Mentha suaveolens</i> (our results)	<i>Mentha suaveolens</i> [30]	<i>Mentha piperita</i> [18]
Acid index	1.68	1.78	<5
Ester index	68.44	71.12	53–76
Saponification index	70.13	–	–
Iodine index	12.05	–	–

our essential oil is of good quality.

According to the results obtained, the ester index is in good agreement with that given by AFNOR [18] for the species *Mentha piperita* L. which varies between 53 and 76 and in good agreement with that of Benbouali [30].

The saponification index is related to the length of the constituent fatty acids of the oil. Indeed, a low saponification index therefore corresponds to fatty acids having a longer carbon chain. A high saponification index results in a high level of short chain fatty acids and a higher glycerol content [35].

From the saponification index value obtained, it appears that our EO does not contain free fatty acids having a high molecular weight.

The iodine index is the generally accepted parameter expressing the degree of unsaturation, ie the number of carbon-carbon double bonds in fats or oils [36] also reported that the higher the amount of unsaturation, the greater the uptake of iodine; therefore, the higher the iodine index, the higher the degree of unsaturation. In this regard, examination of Table 4 shows that the iodine index value of the oil is low, that shows that our essential oil is less saturated. While a study recently carried out by Ref. [25], shows that the iodine values (gram of iodine absorbed/100g of fat) obtained for *Mentha suaveolens* oil varied from 107.354 ± 0.018 to 106.61 ± 0.019 g/100 g from the Azrou and Ifrane regions respectively [25]. The production, concentration, physical-chemical characteristics, and composition of essential oils can all be influenced by a variety of circumstances. These include the plant's age, its genotype, its parts utilized, its surroundings, its drying process, its extraction technique, its harvesting period, its farming practices, and its drying methods and length [37].

3.3.4. Antimicrobial activity

The results of the antimicrobial activity of the EOMS are summarized in Table 5.

The EO of round-leaved mint, evaluated in this study, is characterized by the variability of its activity with respect to the microorganisms tested Round-leaf mint EO, evaluated in this study, is characterized by the variability of its activity with respect to the microorganisms tested. Its strong inhibitory effect is marked against bacteria: *Proteus mirabilis* (17.50 ± 0.70 mm at 50 µL/mL), *Enterococcus faecalis* (17.00 ± 1.00 mm at 50 µL/mL) and *Staphylococcus coagulase negative* (16.33 ± 0.57 mm at 50 µL/mL) while it was moderate against *Escherichia coli* (14.33 ± 1.15 mm at 50 µL/mL) and *Streptococcus* spp (13 ± 0.00 mm at 50 µL/mL) and our results are closer to those obtained by Ref. [39] on *Escherichiacoli* (wild) (14.00 mm) and *Proteus mirabilis* (9.00 mm). Recently, the results of the diffusion test conducted by Ref. [40] showed an inhibition of bacterial growth proportional to the diameter zone. In addition, the diameters of inhibition produced by the EO vary according to the bacterial strain. The highest inhibition zone was observed in *Staphylococcus aureus* (21.00 ± 1.00 mm), followed by *Escherichia coli* (17.00 ± 0.50 mm), *Klebsiella pneumoniae* (16.00 ± 0.50 mm), *Streptococcus faecalis* (14.00 ± 0.50 mm), *Salmonella* spp. (14.00 ± 0.40 mm) and *Pseudomonas aeruginosa* (13.00 ± 0.30

Table 5
Sensitivity of the studied strains to the essential oil of *Mentha suaveolens* Ehrh.

Strains	Essential oil of <i>Mentha suaveolens</i>			Nitrofurantoin (cystitis) [38]		
		Inhibition zones (mm)			Critical diameter (mm)	
Bacteria	Concentration (µL/mL)	30	40	50	S ≥	R <
	<i>Staphylococcus coagulase négative</i>	11.66 ± 0.57	13 ± 1.00	16.33 ± 0.57	13	13
	<i>Streptococcus</i> spp	10.50 ± 0.70	12.00 ± 0.00	13.00 ± 0.00	15	15
	<i>Enterococcus faecalis</i>	12.00 ± 0.57	14.66 ± 0.57	17.00 ± 1.00	15	15
	<i>Escherichia coli</i>	11.50 ± 1.41	13.33 ± 0.57	14.33 ± 1.15	11	11
	<i>Proteus mirabilis</i>	11.50 ± 0.70	14.50 ± 0.70	17.50 ± 0.70	11	11
Yeasts	<i>Candida albicans</i>	10.66 ± 1.15	12.00 ± 1.15	15.33 ± 1.52	–	–

mm).

Using the solid-phase and microtiter analysis method, essential oil extracts from plants grown in different parts of Morocco were analyzed and their effectiveness against 19 bacterial strains, both Gram-positive and Gram-negative, as well as three species of fungi, was assessed [41]. The essential oil containing a high concentration of pulegone showed significant inhibition of all bacterial strains, while that containing a high proportion of piperitone oxide (PO) showed weaker activity. It appears that the activity of oils rich in PO and piperitone oxide is less pronounced. These results suggest that the efficacy of essential oils is closely linked to their specific chemical composition, an observation corroborated by another analysis in which the main aromatic components of these oils were tested against the same organisms. It seems that oxidized piperitone has around half the potency of piperitone oxide against a variety of microorganisms, including yeasts. These findings imply that the most fragrant component of wild mint essential oil extract (EOMS) that may be the most active is pulegone [41]. The presence of significant concentrations of oxygenated monoterpenes with possible antibacterial and antifungal characteristics, pulegone and menthone, may account for our essential oil's antimicrobial action [42,43].

The measured MIC values are consistent with the zones of inhibition achieved, since the MIC is inversely proportional to the diameter of the inhibition zones (Table 6). The highest MBC/MIC value was observed in *Enterococcus faecalis*. On the other hand, the lowest values were observed in *Escherichia coli* and *Proteus mirabilis*. The minimum inhibitory concentration (MIC) for *Staphylococcus aureus* was found to be 0.48 mg/mL, whereas *Salmonella* spp. and *Pseudomonas aeruginosa* showed a MIC of 7.81 mg/mL, as reported by Ref. [40]. Moreover, the minimum bactericidal concentration (MBC) varied from 15.62 mg/mL in *Pseudomonas aeruginosa* to 0.48 mg/ml in *Staphylococcus aureus*.

Additionally, reference data [44] indicates that when *M. suaveolens* is compared to the reference antibiotics utilized, its MIC (minimum inhibitory concentration), MBC (minimum bactericidal concentration), and MFC (minimum fungicidal concentration) values are extremely low. This implies that, subject to thorough in vitro and in vivo toxicological and pharmacological evaluations, this essential oil (EO) is a very strong antibacterial agent that may find use in the food industry and pharmaceutical formulations. According to reference data [44], the EOs examined had bactericidal properties since their BMC/MIC and MFC/MIC ratios were less than or equal to four. Chemicals are classified as bacteriostatic or fungistatic if their CMB/CMI and CMF/CMI ratios are larger than four, and as bactericidal or fungicidal if their ratios are less than or equal to four [45].

The precise mechanism of action of several EO constituents is still not known, although pioneering studies in the past have provided some insights [46]. However, before investigating the effect of EO on microbes, we should have a closer look at the cell-wall structure of Gram-negative and Gram-positive bacteria (Fig. 1(a,b)).

In light of our results obtained for the essential oil on the microbial strains tested, we note that Gram-positive bacteria are more sensitive to antimicrobial action than Gram-negative ones. The structural distinctions between Gram-positive and Gram-negative bacteria explain this greater reactivity. In addition, antibacterial action against Gram-negative bacteria, namely *Escherichia coli*, is relatively weak. The lipopolysaccharide-based outer membrane of this bacterium, which envelops the cell wall in peptidoglycan and limits access to antimicrobial substances, could explain this discovery [48]. There is no discernible difference in minimum inhibitory concentrations (MICs) between Gram-positive and Gram-negative bacteria, according to other researchers who have studied the antibacterial action of essential oils [49–53]. The phospholipid bilayer or outer membrane of bacteria, changes in fatty acid composition, increased membrane flexibility leading to leakage of potassium ions and protons, disruption of glucose uptake, inhibition of enzymes or disruption of cellular processes are some of the possible mechanisms for the antimicrobial action of essential oils [54]. Studies have shown that interactions between terpenes and membrane proteins and phospholipids result in metabolite deficiency, disruption of oxidative phosphorylation, suppression of nucleic acid synthesis and inhibition of the cellular respiratory chain. According to other studies, bioactive substances can significantly target the cell membrane, rendering microbial cells inactive [55]. In fact, the thickening of the cell walls of Gram-positive strains due to interactions between bioactive chemicals and bacteria leads to cell lysis. The negatively charged outer layer of Gram-negative bacteria can interact with other substances, making it easier for the molecule to enter the intracellular space and causing disruption [56]. The chemical composition of essential oils, the surrounding environment and the structures of the targeted bacteria influence their effectiveness as antimicrobials (Gram-positive or Gram-negative) [57].

4. Conclusions and future prospects

The present study focused on the valorization of the essential oil of *Mentha suaveolens* Ehrh for its use in the industrial and medicinal fields (pharmaceutical, cosmetic, agri-food, etc.). It turns out from the results obtained for the organoleptic properties (appearance:

Table 6
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the essential oil of *Mentha suaveolens* Ehrh.

Strains	Essential oil of <i>Mentha suaveolens</i>			Nitrofurantoïne (cystitis) [38]	
	MIC (μL/mL)	MBC (μL/mL)	MBC/CMI	S≤	R>
Bacteria					
<i>Escherichia coli</i>	20	20	1	64	64
<i>Staphylococcus coagulase négative</i>	20	40	2	64	64
<i>Enterococcus faecalis</i>	2,5	10	4	64	64
<i>Proteus mirabilis</i>	10	10	1	64	64
<i>Streptococcus</i> spp	10	20	2	64	64
Yeasts					
<i>Candida albicans</i>	10	–	–	–	–

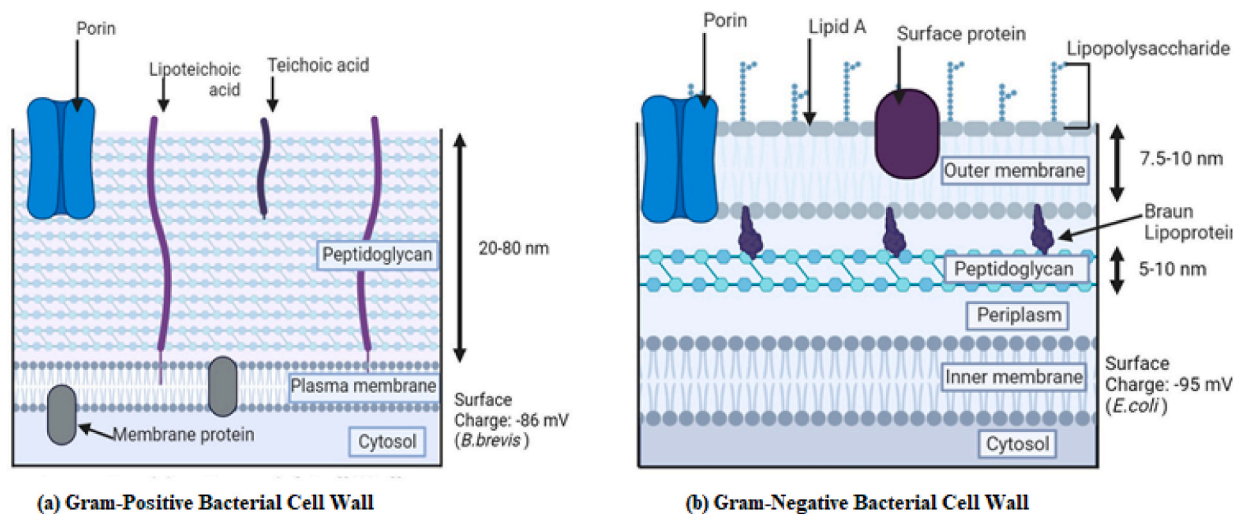


Fig. 1. (a,b): The bacterial cell wall: (a) The Gram-positive envelope and (b) The Gram-negative envelope [47].

Liquid, mobile and clear, odor: Strong odor characteristic of *Mentha suaveolens* Ehrh and color: Pale yellow) and physicochemical (relative density (0.92), miscibility to ethanol (1V/2V), freezing point ($T_{\text{freezing}} < -10$ °C), refractive index (1.5256), turning power (+0.825), acid index (1.68), d index ester (68.44), saponification index (70.13) and iodine index (12.05)) that our essential oil turns out to be of good quality. Thus, chemical analysis shows that our oil is made up of several oxygenated monoterpene compounds such as piperitone oxide with a high percentage (32.55%), pulegone (10.14%), piperitone oxide (8.34%), etc. In addition, microbiological tests carried out on six microbial strains (five bacteria and one yeast) show a well-marked inhibitory effect of our oil against bacteria: *Proteus mirabilis* (17.50 ± 0.70 mm at 50 $\mu\text{L}/\text{mL}$), *Enterococcus faecalis* (17 ± 1.00 mm at 50 $\mu\text{L}/\text{mL}$) and *Staphylococcus coagulase negative* (16.33 ± 0.57 mm at 50 $\mu\text{L}/\text{mL}$) while it was moderate against *Escherichia coli* (14.33 ± 1.15 mm at 50 $\mu\text{L}/\text{mL}$) and *Streptococcus* spp (13 ± 0.00 mm at 50 $\mu\text{L}/\text{mL}$) as well as against a yeast, *Candida albicans* (15.33 ± 1.52 mm at 50 $\mu\text{L}/\text{mL}$). The results obtained are therefore promising and thus open the way for manufacturers to use the essential oil of *Mentha suaveolens* in the pharmaceutical, cosmetic, agricultural and particularly food industries. In addition, these properties could make them viable alternatives to conventional preservatives, thus increasing the shelf life of various food products. However, its application on an industrial scale as an ingredient in perfumes, cosmetics and aromatic scents must be linked to its chemical composition. Indeed, dose management is necessary and is defined as a series of interventions aimed at optimizing good antimicrobial activity in foods. In addition to playing an antimicrobial and preservative role in food preservation, it also helps protect or improve certain physicochemical properties of foods in order to maintain overall food quality.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

the authors confirm that data supporting the findings of this study are available within the article.

CRediT authorship contribution statement

Moha Afrokh: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Khadija El Mehrach:** Writing – review & editing, Writing – original draft, Supervision. **Khalid Chatoui:** Methodology, Formal analysis. **Mohamed Ait Bihi:** Methodology, Conceptualization. **Hajar Sadki:** Formal analysis. **Mohamed Tabyaoui:** Writing – original draft, Supervision, Conceptualization. **Saida Tahrouch:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to warmly thank Professor Jean-Marie BESSIÈRE (Montpellier, France) for his important and valuable help.

References

- [1] M. Afrokh, S. Tahrouch, K. Elmehrach, et al., Ethnobotanical, phytochemical and antioxidant study of fifty aromatic and medicinal plants, *Chemical Data Collections* 43 (2023) 100984, <https://doi.org/10.1016/j.cdc.2022.100984>.
- [2] J. Fakchich, M. Elachouri, An overview on ethnobotanical-pharmacological studies carried out in Morocco, from 1991 to 2015: systematic review (part 1), *J. Ethnopharmacol.* 267 (2021) 113200, <https://doi.org/10.1016/j.jep.2020.113200>.
- [3] D. Cox-Georgian, N. Ramadoss, C. Dona, et al., Therapeutic and medicinal uses of terpenes, *Med. Plants: from farm to pharmacy* (2019) 333–359.
- [4] M. Afrokh, K. Boumhara, K. Chatoui, et al., Phytochemical screening and in vitro antioxidant activities of *Mentha suaveolens* Ehrh. extract, *International Journal of Secondary Metabolite* 10 (3) (2023) 332–344, <https://doi.org/10.21448/ijsm.1148664>.
- [5] W. Luo, Z. Du, Y. Zheng, et al., Phytochemical composition and bioactivities of essential oils from six Lamiaceae species, *Ind. Crop. Prod.* 133 (2019) 357–364, <https://doi.org/10.1016/j.indcrop.2019.03.025>.
- [6] A. El Fadl, N. Chtaina, Etude de base sur la culture de la menthe du Maroc, Programme Régional de lutte intégrée contre les organismes nuisibles (Integrated Pest Management) au Proche Orient. Report, Office National de sécurité sanitaire des produits alimentaires (ONSSA), 2010.
- [7] R. Harley, C. Brighton, Chromosome numbers in the genus *Mentha* L., *Bot. J. Linn. Soc.* 74 (1) (1977) 71–96, <https://doi.org/10.1111/j.1095-8339.1977.tb01168.x>.
- [8] B. Abbaszadeh, S.A. Valadabadi, H.A. Farahani, et al., Studying of essential oil variations in leaves of *Mentha* species, *Afr. J. Plant Sci.* 3 (10) (2009) 217–221.
- [9] M. Fennane, M. Ibn-Tattou. Flore pratique du Maroc. Série Botanique, N° 36, Edit. institutScientifique, Rabat, 1999.
- [10] L. Civitelli, S. Panella, M.E. Marocci, et al., In vitro inhibition of herpes simplex virus type 1 replication by *Mentha suaveolens* essential oil and its main component piperitenone oxide, *Phytotherapie* 21 (6) (2014) 857–865, <https://doi.org/10.1016/j.phymed.2014.01.013>.
- [11] W. Dhifi, S. Bellili, S. Jazi, et al., Essential oils' chemical characterization and investigation of some biological activities: a critical review, *Medicines* 3 (4) (2016) 25, <https://doi.org/10.3390/medicines3040025>.
- [12] P. Tunsagool, P. Kruawuangmol, A. Sunpapao, et al., Global metabolic changes by *Bacillus* cyclic lipopeptide extracts on stress responses of para rubber leaf (2022), <https://doi.org/10.21203/rs.3.rs-1957161/v3>.
- [13] E. Hendarto, A. Setyaningrum, Production and king grass nutritional quality number of sources of nitrogen fertilizer, *HighTech and Innovation Journal* 3 (3) (2022) 252–266, <https://doi.org/10.28991/HIJ-2022-03-03-02>.
- [14] A. Kasrati, C.A. Jamali, K. Bekkouche, et al., Essential oil composition and antimicrobial activity of wild and cultivated mint timija (*Mentha suaveolens* subsp. timija (Briq.) Harley), an endemic and threatened medicinal species in Morocco, *Nat. Prod. Res.* 27 (12) (2013) 1119–1122, <https://doi.org/10.1080/14786419.2012.708661>.
- [15] H. Oumzil, S. Ghoulami, M. Rhajaoui, et al., Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*, *Phytother Res.: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 16 (8) (2002) 727–731, <https://doi.org/10.1002/ptr.1045>.
- [16] R. Adams, Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectrometry, Allured Publishing Corporation, Carol Stream, 2007.
- [17] W. Koenig, D. Hochmuth, D. Joulain, Mass Finder 2.1 (Including the Library) of Terpenoids and Related Constituents of Essential Oils, E-B Verlag, Hamburg, 1998.
- [18] Afnor, Huiles essentielles. Monographies Relatives Aux Huiles Essentielles, Tome2. 6ème ed. Paris, 2000.
- [19] AFNOR, *Recueil de normes françaises des corps gras*, graines oléagineuse, produits dérivés, 1984.
- [20] P. Wayne, Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests 19th ed. approved standard, CLSI document M100-S19 29 (2011) (2009). M100-S21.
- [21] P. Wayne, NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Disc Susceptibility Test," Approved Standard: M2-A6, 1997.
- [22] P. Berche, J. Gaillard, M. Simonet, in: Flammarion (Ed.), Les bactéries des infections humaines vol. 53, Médecine-Sciences, Paris, 1988, pp. 506–513.
- [23] A. Salhi, A. Bouyanzer, A. Chetouani, et al., Chemical composition, antioxidant and anticorrosion activities of *Mentha Suaveolens*, *Journal of Materials and Environmental Sciences*, JMES 8 (5) (2017) 1718–1728.
- [24] A. Bouyahya, O. Belmehti, J. Abrini, et al., Chemical composition of *Mentha suaveolens* and *Pinus halepensis* essential oils and their antibacterial and antioxidant activities, *Asian Pac. J. Tropical Med.* 12 (3) (2019) 117–122.
- [25] N. Zekri, H. Elazzouzi, A. Ailli, et al., Physicochemical characterization and antioxidant properties of essential oils of *M. Pulegium* (L.), *M. Suaveolens* (Ehrh.) and *M. spicata* (L.) from Moroccan middle-atlas, *Foods* 12 (4) (2023) 760, <https://doi.org/10.3390/foods12040760>.
- [26] M. El Arch, B. Satrani, A. Farah, et al., Composition chimique et activités antimicrobienne et insecticide de l'huile essentielle de *Mentha rotundifolia* du Maroc, *Acta Bot. Gall.* 150 (3) (2003) 267–274, <https://doi.org/10.1080/12538078.2003.10515996>.
- [27] E. Derwiche, Z. Benziane, R. Taouil, et al., Comparative essential oil composition of leaves of *Mentha rotundifolia* and *Mentha pulegium* a traditional herbal medicine in Morocco, *Am.-Eurasian J. Sustain. Agric. (AEJSA)* 4 (1) (2010) 47–54.
- [28] N. Zekri, H. Sabri, S. Khannouchi, et al., Phytochemical study and fumigant toxicity of *Mentha suaveolens* Ehrh essential oil from Morocco against adults of *S. oryzae* (L.), *Australian Journal of Basic and Applied Sciences* 7 (14) (2013) 599–606.
- [29] N. Benayad, Les Huiles Essentielles Extraites des Plantes Médicinales Marocaines: Moyen Efficace de Lutte Contre les Ravageurs des Denrées Alimentaires Stockées, University of Sciences, Rabat, Morocco, 2008. Ph.D. Thesis.
- [30] M. Benbouali, in: Mémoire de magister, U.H.B. Bouali (Eds.), Valorisation des extraits de plantes aromatique et médicinales de: «*Mentha rotundifolia* et *Thymus Vulgaris*», 2006.
- [31] K. Koba, K. Sanda, C. Raynaud, et al., Activités antimicrobiennes d'huiles essentielles de trois *Cymbopogon* sp. africains vis-à-vis de germes pathogènes d'animaux de compagnie, *Ann. Med. Vet.* 148 (2004) 202–206.
- [32] P. Franchomme, R. Jollois, *L'aromathérapie Exactement*, Limoges: Editions Roger Jollois, 1990.
- [33] H.R. Juliani, J.A. Zygadlo, R. Scrivanti, et al., The essential oil of *Anemia tomentosa* (Savigny) Sw. var. *anthriscifolia* (Schrad.) Mickel, *Flavour Fragrance J.* 19 (6) (2004) 541–543, <https://doi.org/10.1002/ffj.1341>.
- [34] M. Fauconnier, "Ylang-ylang essential oil: its quality record and its distillation follow-up, in: H.o. Comoros (Ed.), Presentation for the GIE Spices, 2006.
- [35] R. Ferhat, S. Laroui, M. Abdeddaim, Huile et profil en acides gras des amandes du *Crateagus azarolus* L, *Leban. Sci. J.* 15 (2) (2014) 73–79.
- [36] Y. Pomeranz, *Food Analysis: Theory and Practice*, Springer Science & Business Media, 2013.
- [37] L.P.T. Quoc, Physicochemical properties components, and antibacterial activity *Mentha arvensis* L. leaves 1 (2022) 36–45.
- [38] R. Bonnet, F. Caron, J. Cavallo, et al., Comité de l'Antibiogramme de la Société Française de Microbiologie, Recommandations 2012 (2012).
- [39] N. Zekri, Thèse de Doctorat, N° d'ordre: 3049, 197 P. Étude Phytochimique et Activités Biologiques des Huiles Essentielles et des Extraits des *M. pulegium* (L.), *M. suaveolens* (Ehrh.), et *M. spicata* (L.) du Moyen-Atlas Marocain, Faculté des Sciences, Université Mohammed V, Rabat, 2017.
- [40] A. Ed-Dra, F.R. Filai, M. Bou-Idra, et al., Application of *Mentha suaveolens* essential oil as an antimicrobial agent in fresh Turkey sausages, *J. Appl. Biol. Biotechnol.* 6 (1) (2018) 7–12.
- [41] H. Oumzil, S. Ghoulami, M. Rhajaoui, et al., Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*, *Phytother Res.* 16 (8) (2002) 727–731, <https://doi.org/10.1002/ptr.1045>.

- [42] E. El-Kashoury, H.I. El-Askary, Z. Kandi, et al., Chemical and biological study of *Mentha suaveolens* Ehrh. cultivated in Egypt, *J. Med. Plants Res.* 8 (2014) 747–755, <https://doi.org/10.5897/JMPR2014.5324>.
- [43] S. Sutour, P. Bradesi, J. Casanova, et al., Composition and Chemical Variability of *Mentha suaveolens* ssp. *suaveolens* and *M. suaveolens* ssp. *insularis* from Corsica, *Chem. Biodivers.* 7 (4) (2010) 1002–1008, <https://doi.org/10.1002/cbdv.200900365>.
- [44] N. El Hachlafi, N. Benkhaira, S.H. Al-Mijalli, et al., Phytochemical analysis and evaluation of antimicrobial, antioxidant, and antidiabetic activities of essential oils from Moroccan medicinal plants: *Mentha suaveolens*, *Lavandula stoechas*, and *Ammi visnaga*, *Biomed. Pharmacother.* 164 (2023) 114937, <https://doi.org/10.1016/j.biopha.2023.114937>.
- [45] E.M. Abdallah, Antibacterial activity of *Hibiscus sabdariffa* L. calyces against hospital isolates of multidrug resistant *Acinetobacter baumannii*, *Journal of Acute Disease* 5 (6) (2016) 512–516, <https://doi.org/10.1016/j.joad.2016.08.024>.
- [46] H. Hajian-Maleki, M. Shams-bakhsh, Identification of the chemical profile and evaluation of the antimicrobial effect of *Eryngium billardieri* Delar essential oil component against bacterial species of agricultural and food interest, *Front. Microbiol.* 14 (2023), <https://doi.org/10.3389/fmicb.2023.1249780>.
- [47] K.G. Kaiser, V. Delattre, V.J. Frost, et al., Nanosilver: an old antibacterial agent with great promise in the fight against antibiotic resistance, *Antibiotics* 12 (8) (2023) 1264, <https://doi.org/10.3390/antibiotics12081264>.
- [48] Leila Riahia, Myriam Elferchichi, Hanene Ghazghazic Jed Jebali, Sana Ziadi, Chedia Aouadhi, Hnia Chograni Yosr Zaouali Nejia Zoghلامي, Mliki Ahmed, Phytochemistry, antioxidant and antimicrobial activities of the essential oils of *Mentha rotundifolia* L. in Tunisia, *Ind. Crop. Prod.* 49 (2013) 883–889, <https://doi.org/10.1016/j.indcrop.2013.06.032>.
- [49] M. Saleem, M.T. Saeed, Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent, *J. King Saud Univ. Sci.* 32 (1) (2020) 805–810, <https://doi.org/10.1016/j.jksus.2019.02.013>.
- [50] M.-C. Ou, Y.-H. Liu, Y.-W. Sun, et al., The composition, antioxidant and antibacterial activities of cold-pressed and distilled essential oils of *Citrus paradisi* and *Citrus grandis* (L.) Osbeck, *Evid. base Compl. Alternative Med.* 2015 (2015), <https://doi.org/10.1155/2015/804091>.
- [51] T.T. Diep, M.J.Y. Yoo, C. Pook, et al., Volatile components and preliminary antibacterial activity of tamarillo (*solanum betaceum* cav.), *Foods* 10 (9) (2021) 2212, <https://doi.org/10.3390/foods10092212>.
- [52] G. Mandalari, R. Bennett, G. Bisignano, et al., Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry, *J. Appl. Microbiol.* 103 (6) (2007) 2056–2064, <https://doi.org/10.1111/j.1365-2672.2007.03456.x>.
- [53] M.A. Al-Saman, A. Abdella, K.E. Mazrou, et al., Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (*Citrus japonica* Thunb), *J. Food Meas. Char* 13 (2019) 3221–3229, <https://doi.org/10.1007/s11694-019-00244-y>.
- [54] M. Angane, S. Swift, K. Huang, et al., Essential oils and their major components: an updated review on antimicrobial activities, mechanism of action and their potential application in the food industry, *Foods* 11 (3) (2022) 464, <https://doi.org/10.3390/foods11030464>.
- [55] G. Zengin, C. Sarikürkçü, A. Aktümsek, et al., Antioxidant potential and inhibition of key enzymes linked to Alzheimer's diseases and diabetes mellitus by monoterpen-rich essential oil from *Sideritis galatica* Bornm. Endemic to Turkey, *Record Nat. Prod.* 10 (2) (2016) 195.
- [56] Y. Ceylan, K. Usta, A. Usta, et al., Evaluation of antioxidant activity, phytochemicals and ESR analysis of *Lavandula stoechas*, *Acta Phys. Pol., A* 128 (2B) (2015), <https://doi.org/10.3390/foods11030464>.
- [57] M.K.A. Swamy, M.S. Sinniah, U. R., *Antimicrobial properties of plant essential oils against human pathogens and their mode of action: , n* updated review. *Evid.-Based Complement, Altern. Med.* (2016), <https://doi.org/10.1155/2016/3012462>.