EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology, 2026, 27(5), 81–92
https://doi.org/10.12912/27197050/219771
ISSN 2719–7050, License CC-BY 4.0

Received: 2026.02.26
Accepted: 2026.04.15
Published: 2026.05.01

Chemical profiling and comparative antioxidant-antimicrobial activities of *Melissa officinalis* and *Mentha spicata* essential oils from Morocco

Siham El Machrafi[1,2*], Rajaâ Zakhour[3], M'hammed Elkouali[2], Ichraq El Yaakouby[5], Hafsa Mguild[6], Samy Iskandar[4], Meryem El Jemli[4], Ali Zourif[7,8], Mohammed Bouhadi[9], Houda El Hajjouji[1]

[1] Laboratory of Ecology and Environment, Faculty of Sciences Ben M'Sick, Hassan II University of Casablanca, Morocco
[2] Laboratory of Analytical and Molecular Chemistry, Faculty of Sciences Ben M'Sik, Hassan II University of Casablanca, Morocco
[3] Toxicology and Pharmacodynamics of Aromatic and Medicinal Plants Research Team (ERTP.PAM), Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, Mohammed V University, 6203 Rabat, Morocco
[4] Laboratoire Génie des Matériaux pour Environnement et Valorisation (GeMEV), Equipe I3MP, Faculté des Sciences Aïn Chock, B.P., 5366 Maârif, Casablanca, Morocco
[5] Research Laboratory of Microbiology, Infectious Diseases, Allergology and Pathogen Surveillance (LARMIAS), Mohammed VI Faculty of Medicine, Mohammed VI University of Sciences and Health (UM6SS), Casablanca, Morocco
[6] Laboratory for Interdisciplinary Pharmacological Research in Health Sciences, Mohammed VI University of Health Sciences, Casablanca, Morocco
[7] Department of Chemical and Biochemical Sciences, Green Process Engineering (CBS), Mohammed VI Polytechnic University, Morocco
[8] Laboratory of Physical Chemistry of Applied Materials (LCPMA), Faculty of Sciences Ben M'Sick, Hassan II University of Casablanca, Casablanca, Morocco
[9] Laboratory of Biotechnology, Conservation and Valorization of Bioresources, Department of Biology, Faculty of Sciences Dhar Mehraz, Sidi Mohamed Ben Abdellah University, 30000 Fez, Morocco
* Corresponding author's e-mail: siham.elmachrafi-etu@etu.univh2c.ma

ABSTRACT

Melissa officinalis L. and *Mentha spicata* L. are widely used aromatic plants; however, variations in essential oil composition related to geographical origin may significantly influence their biological activities. The present study aimed to characterize the chemical composition and comparatively evaluate the antioxidant and antimicrobial properties of essential oils obtained from plants collected in the Settat region of Morocco. Essential oils were extracted by hydrodistillation and analyzed using GC–MS. Geraniol (30.37%), (Z)-2,6-octadien-1-ol (22.52%), and citronellol (13.54%) were the major constituents of *M. officinalis* oil, whereas (-)-carvone (58.05%) predominated in *M. spicata* oil, followed by trans-carveol (7.29%) and (R)-1-methyl-5-(1-methylethenyl)cyclohexene (5.45%). Antioxidant activity was assessed using DPPH, ABTS, and FRAP assays. *M. spicata* oil demonstrated stronger radical-scavenging activity in DPPH and ABTS assays, while *M. officinalis* showed higher ferric-reducing power. Antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria and one yeast strain. Both oils exhibited higher inhibitory effects against *Staphylococcus aureus* and *Staphylococcus epidermidis* compared with *Pseudomonas aeruginosa.* The obtained results demonstrate distinct compositional profiles of the essential oils, with dominant terpenoid constituents associated with the measured antioxidant and antimicrobial activities. The characterized chemical composition and corresponding bioactivities indicate their suitability for further consideration in pharmaceutical, food, and cosmetic applications.

Keywords: antioxidant activity, antimicrobial activity, GC-MS, *Melissa officinalis*, *Mentha spicata*, essential oils.

INTRODUCTION

Traditional medicine has been practiced for millennia and continues to represent an important source of therapeutic agents. Medicinal plants are particularly valued for their ability to synthesize structurally diverse bioactive secondary metabolites. Among them, species of the Lamiaceae family are recognized for their aromatic properties and broad spectrum of biological activities (Uritu et al., 2018). Ethnobotanical investigations conducted in the Settat region of Morocco have documented the traditional use of *Mentha spicata* L. and *Melissa officinalis* L. in local herbal medicine for the management of various disorders (Bouhadi et al., 2021; Lahyaoui et al., 2025).

Melissa officinalis is traditionally used as an antiseptic and sedative, particularly in the management of digestive disorders (Abdellatif et al., 2023; Pineau et al., 2016). Its biological activity is associated with a complex phytochemical composition that includes phenolic acids such as rosmarinic and caffeic acids, flavonoids including luteolin and quercetin, and volatile constituents such as geranial, neral, and citronellal. Similarly, *Mentha spicata* is characterized by a high content of carvone and other monoterpenoids, which are associated with anti-inflammatory, antioxidant, and antimicrobial effects (Baser et al., 1999). The essential oil of *M. spicat*a is widely used in the food, cosmetic, and pharmaceutical sectors due to its aromatic properties and biological activities (Kiełtyka-Dadasiewicz et al., 2017; Pintilia et al., 2025). In traditional medicine, it is employed to alleviate headaches, nausea, and gastrointestinal disorders (Mahboubi, 2021), effects that have been partly attributed to monoterpenoids such as limonene and 1,8-cineole (Mahendran et al., 2021).

Despite the documented therapeutic relevance of these species, the yield and chemical composition of their essential oils are known to vary significantly depending on environmental conditions, climatic factors, harvesting period, and plant genotype (Shahbazi, 2015). Such variability may directly influence their biological properties, including antioxidant and antimicrobial activities. Although numerous studies have reported the bioactivity of these plants, comparative data integrating chemical profiling and biological evaluation of essential oils from the Settat region of Morocco remain limited. In particular, the relationship between dominant volatile constituents and the observed biological effects has not been sufficiently characterized for locally grown populations.

Based on the known bioactivity of these plants, we hypothesize that the *Melissa officinalis* and *Mentha spicata* essential oils will exhibit significant antioxidant and antimicrobial activities. Additionally, we hypothesize that the chemical profiles of each essential oil will lead to different mechanisms of action and varying efficacy against a range of tested microorganisms.

Therefore, the present study aimed to characterize the chemical composition of essential oils obtained from *Melissa officinalis* L. and *Mentha spicata* L. cultivated in the Settat region of Morocco using GC–MS analysis, and to comparatively evaluate their antioxidant and antimicrobial activities. By integrating phytochemical profiling with multiple in vitro bioassays (FRAP, DPPH, and ABTS), this study sought to determine whether compositional differences are associated with variations in biological efficacy. The generated data provide region-specific insights into the chemical variability and bioactivity of these species and contribute to a more detailed understanding of composition–activity relationships in their essential oils.

MATERIALS AND METHODS

Botanical material and extraction procedure of essential oils

Fresh aerial parts (leaves) of *Melissa officinalis* L. and *Mentha spicata* L. were collected in January and February 2022, respectively, from the Settat region, Morocco (33°06’35.6”N 7°43’01.5”W; altitude around 370 m above sea level). (Figure 1) Botanical identification was confirmed by the Department of Botany at the Scientific Institute of Rabat, Morocco. Voucher specimens were deposited in the institutional herbarium under accession numbers RAB 114745 (*M. officinalis*) and RAB 114746 (*M. spicata*).

The plant materials were air-dried under controlled ambient conditions (20–24 °C; relative humidity 45–55% for 7 to 10 days until a constant weight was achieved. The dried leaves were then separated, crushed using a laboratory grinder, and stored in tightly sealed, dark containers for three weeks to ensure material integrity. For the extraction of volatile compounds, 200 g of the dried

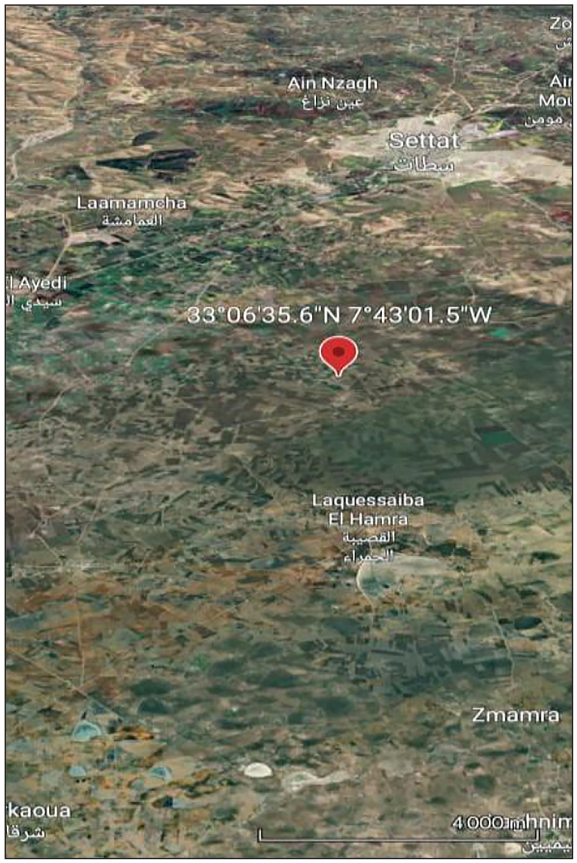

Figure 1. The Settat region (Morocco) and sampling locations for testing: Samples 1 (33°06'35.6"N 7°43'01.5"W; altitude around 370 m above sea level)

material was subjected to hydrodistillation using a Clevenger-type apparatus for 3 h with 1000 ml of distilled water. Extractions were performed in triplicate to ensure reproducibility. Essential oil yield was calculated as a percentage (w/w) relative to the dry plant material. The obtained yields were 1.0% for *M. officinalis* and 1.8% for *M. spicata*. The resulting oils were dried over anhydrous sodium sulfate, filtered, and stored in sealed amber vials at 4 °C until phytochemical and antioxidant analysis (Table 1) (Figure 2).

Gas chromatography-mass spectroscopy analysis

Chemical characterization was performed using a Thermo Trace 1300 gas chromatograph coupled to a TSQ 8000 EVO triple quadrupole mass spectrometer. Chromatographic separation was achieved on a TR-35MS fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature program was as follows: the initial temperature was set at 40 °C and held for 2 min, then increased at a rate of 4 °C/min to 180 °C (held for 0 min), and finally increased at 20 °C/min to a final temperature of 300 °C, which was maintained for 2 min. Helium was used as the carrier gas at a constant flow rate of 1.5 mL/min. Samples were injected in split mode (split ratio 1:33.3; split flow 50 mL/min) with the injector port maintained at 200 °C. Mass spectra were obtained using electron ionization (EI) in full scan mode. The transfer line temperature was maintained at 250 °C, while the ion source was set at 200 °C. Compound identification was based on a comparison of mass spectra with the NIST library and literature data, as well as the calculation of retention indices (RI) relative to a homologous series of n-alkanes (C_8–C_{24}). Relative percentages of compounds were calculated by peak area normalization without correction factors.

In vitro antioxidant assays

Evaluation of DPPH radical inhibition capacity

The radical scavenging potential of the essential oils was evaluated according to the method described by Sahin et al. (2004) with slight modifications. A concentration range of [specify range, e.g., 0.05–2 mg/mL] for each essential oil was prepared in methanol. Briefly, an aliquot of 0.1 mL of each preparation was reacted with 3.9 mL of a 60 µM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol. Butylated hydroxytoluene (BHT) was employed as the positive reference antioxidant. The mixtures were thoroughly vortexed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a Multiskan™ Multiplate Photometer UV-Vis spectrophotometer. All tests were performed in triplicate. The radical scavenging activity was expressed as the percentage of DPPH radical inhibition and calculated using Equation 1:

$$Inhibition\ percentage = \\ = \frac{(Abs\ of\ control - Abs\ of\ samples)}{(Abs\ of\ control)} \times 100 \quad (1)$$

The antioxidant activity of each essential oil was expressed as the IC_{50} value, defined as the concentration of the test material required to cause a 50% decrease in the initial DPPH concentration. To determine these values, *Melissa officinalis* was tested at concentrations ranging from 100 to 900 µg/mL, *Mentha spicata* from 50 to 800 µg/mL, and the reference antioxidant (BHT) from 100 to 700 µg/mL. Absorbance was measured at 517 nm using a Multiskan™ Multiplate Photometer UV-Vis spectrophotometer. All

Table 1. Essential oil yield of *Mentha spicata* and *Melissa officinalis* obtained by hydrodistillation

Plant species	Plant material (g, dry)	Water volume (mL)	Extraction method	Duration (h)	Number of replicates	Yield (% w/w, dry basis)
Mentha spicata	200	1000	Hydrodistillation (Clevenger-type apparatus)	3	3	1.8
Melissa officinalis	200	1000	Hydrodistillation (Clevenger-type apparatus)	3	3	1.0

Figure 2. Sample preparation for analysis: (a) plant implantation, (b) dried plant, (c) plant shredder, (d) essential oil extrcation

measurements were performed in triplicate. The IC_{50} values were determined by nonlinear regression analysis (or linear regression, depending on your curve fit) using Origin 2018 software. The final concentration of each oil and the reference standard after the addition of the DPPH reagent was calculated according to Equation 2. Results are expressed as mean ± standard deviation (SD).

$$Final\ concentration = \frac{(Intial\ concentration \times Initial\ volume)}{Final\ volume} \quad (2)$$

ABTS radical scavenging assay

The ABTS•$^{+}$ radical cation was obtained by mixing 7 mM ABTS solution with 2.45 mM potassium persulfateand allowing the mixture to stand in the dark for 16 h at room temperature. Before use, the solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. A volume of 20 µL of essential oil solution (1–900 mg/mL) was added to 2 mL of ABTS•$^{+}$ working solution. After 6 min of incubation in the dark, absorbance was recorded at 734 nm by unsing a MultiskanTM Multiplate Photometer UVVis spectrophotometer. Trolox (100–180 µg/mL) was used as a standard. All experiments were conducted in triplicate. Next, the percentage of radical scavenging activity was worked out from the equation used for the DPPH (Formula 1) test, where Trolox served as the standard reference compound. The real concentration of each oil and reference by adding ABTS reagent solution was calculated by using Formula 2. Results were expressed as mean ± standard deviation.

Ferric reducing antioxidant capacity assay (FRAP)

The ferric reducing antioxidant power method was employed to assess the reducing ability of the essential oils. In brief. An aliquot of 0.2 mL of each tested sample, prepared at different concentrations(100–900 mg/mL), was transferred into a reaction mixture containing 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution [$K_3Fe(CN)_6$]. The resulting mixture was incubated in a thermostatically controlled water bath for 20 minutes at 50 °C to allow the reduction process to occur. Following incubation, the reaction was terminated through the addition of 2.5 mL of 10% trichloroacetic acid, thereby stopping further redox activity. Following vigorous agitation. An aliquot of the resulting supernatant (2.5 mL) was then combined with distilled water (2.5 mL) and 0.5 mL of 0.1% $FeCl_3$ solution. Butylated hydroxytoluene (BHT) was utilized as the standard

antioxidant for comparison. The reducing power was evaluated by measuring absorbance at 700 nmusing a MultiskanTM Multiplate Photometer UVVis spectrophotometer, with increased absorbance indicating stronger reducing potential. Results were expressed as EC_{50} values (µg/mL), calculated from calibration curves R^2 =93. The real concentration of each oil and reference by adding ABTS reagent solution was calculated by using Formula 2. Results were expressed as mean ± standard deviation.

Antimicrobial activity

The antimicrobial activity of *Melissa officinalis* and Mentha spicata essential oils was assessed against five pathogenic microorganisms: *Candida albicans* ATCC 1023, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and the *Escherichia coli* ATCC 8739. The microbial strains were obtained from the Microbiology Department at Cheikh Khalifa International University Hospital, Casablanca, Morocco.

Disc-diffusion technique

The antimicrobial efficacy was evaluated against a panel of five microbial strains: *Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, and *Escherichia coli* ATCC 8739. Bacterial strains were cultured on Mueller–Hinton Agar (MHA), while the yeast strain was cultured on Sabouraud Dextrose Agar (SDA). All procedures were performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI M07-A10 for bacteria and CLSI M27-A3 for yeast).

The susceptibility of the tested microorganisms was assessed using the disk diffusion method (Kalemba and Kunicka, 2003). A microbial suspension (0.5 mL of 10^6 CFU/mL) was uniformly spread onto the surface of the appropriate agar medium. After 15 min of drying, sterile paper disks (Whatman No. 1, 6 mm diameter) were impregnated with 10 µL of essential oil at serial concentrations ranging up to 200 and 400 mg/mL. The oils were diluted using 10% Tween 80 in sterile distilled water. Disks loaded with 10% Tween 80 (without essential oil) served as negative controls. Positive controls included Ceftriaxone (CRO), Teicoplanin (TEC), Ceftazidime (CAZ), and Piperacillin-Tazobactam (TPZ) at a concentration of 30 µg/disk. The plates were incubated at 37 °C for 24 h (bacterial strains) and 48 h (fungal strains). Antimicrobial activity was evaluated by measuring the diameter of the inhibition zones (mm). All assays were conducted in triplicate, and results were expressed as mean ± SD.

Minimum inhibitory concentration assay

The minimum inhibitory concentration (MIC) of the essential oils was determined using the broth microdilution method in 96-well microplates, following the procedures described by Chen et al. (2024) and Tina et al. (2025) in accordance with CLSI standards. Briefly, serial two-fold dilutions of the essential oils were prepared in [specify broth, e.g., Mueller-Hinton broth for bacteria / sabouraud broth for yeast] supplemented with 10% Tween 80. A volume of 100 µL of each oil concentration (ranging from 0.097 to 100 mg/mL) was added to wells containing 100 µL of microbial inoculum (10^6 CFU/mL).The microplates were incubated at 37 °C for 24 h for bacterial strains and 48 h for the yeast strain. Following incubation, microbial growth was assessed visually by the presence or absence of turbidity. MIC was defined as the lowest concentration of the essential oil that showed no visible growth. All assays were executed in triplicate to ensure reproducibility.

Statistical analysis

All experimental measurements were conducted in three replicates, with All experimental findings were analyzed using a one-way ANOVA, with statistical computations carried out in IBM SPSS (version 25), where a confidence level of 95% ($p < 0.05$) was used to identify significant variations between treatment groups. To further delineate these differences, multiple comparisons of average valueswere executed through Tukey's post hoc test. All experiments were performed in triplicate and the results were expressed as mean ± standard deviation (SD). Data normality was evaluated using the Shapiro–Wilk test. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test with IBM SPSS Statistics version 25. Differences were considered statistically significant at $p< 0.05$.

RESULTS

Phytochemical composition

The chemical constituents of the essential oils from *Melissa officinalis* and *Mentha spicata* were characterized via GC–MS (Figure 3). The original chromatograms, showing all detected peaks and integration data, are provided in the supplementary materials (Figures S1 and S2). The identified components are presented with their corresponding retention times and percent compositions, expressed as peak area percentages (Table S1 and S2). As shown in Table S1 (Supplementary Files), geraniol was identified as the predominant component of *M. officinalis*, accounting for 30.37% of the total composition, followed by (Z)-2,6-octadien-1-ol, 3,7-dimethyl- (21.7%), and citronellol (13.54%). Conversely, the principal components identified in *M. spicata* were carvone (58.5%), trans-carveol (7.29%), and 1-methyl-5-(1-methylethenyl)-(R)-Eucalyptol cyclohexene (5.45%) (Table S2, Supplementary File).

Antioxidant activity

The antioxidant potential was examined using three distinct assays: DPPH, ABTS, and FRAP. As summarized in Table 2, *M. spicata* essential oil exhibited higher antioxidant activity than *M. officinalis* in both DPPH (926.60 ± 5.25 µg/mL) and ABTS (1772.60 ± 217.10 µg/mL) tests. However, this trend was reversed in the FRAP assay, where *M. officinalis* oil displayed a superior reduction capacity (2702.99 ± 33.50 µg/mL) (Tables 3–5).

Antimicrobial activity

Table 6 presented the qualitative and quantitative antibacterial activity results for essential oils from *Melissa officinalis* and *Mentha spicata* plants. Against *Pseudomonas aeruginosa*, both extracts exhibited very limited activity, with inhibition zones not ex-ceeding 8 mm and relatively high MIC values (3.12 mg/mL). This weak effectiveness contrasts with reference antibiotics, particularly piperacillin/tazobactam (27 mm) and ceftriaxone

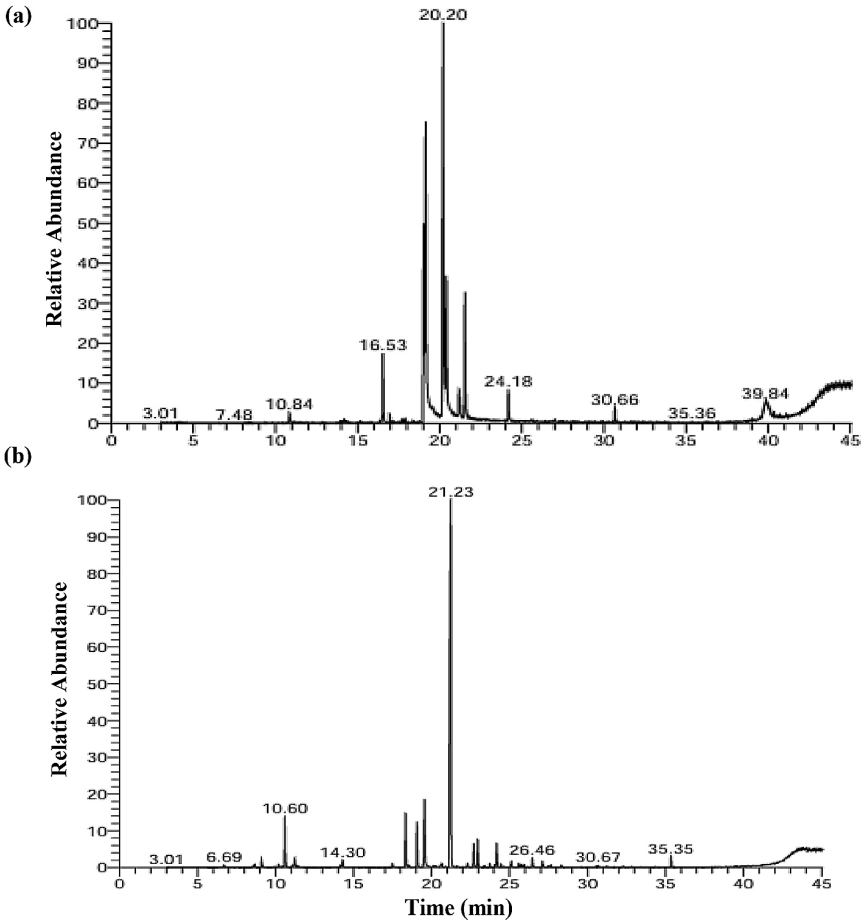

Figure 3. The phytochemical constituents of the essential oil from (a) *Melissa officinalis* plant, (b) *Mentha spicata* plant

Table 2. IC_{50} of essential oils from *Melissa officinalis* and *Mentha spicata*

Tests	*M. officinalis* (µg/mL)	*M. spicata* (µg/mL)	BHT (µg/mL)	Trolox (µg/mL)
DPPH	1509.15 ± 167.40[a]	926.60 ± 5.25[b]	27.91 ± 2.03[c]	-
ABTS	2055.10 ± 600.60[a]	1772.60 ± 217.10[b]	-	2.07 ± 0.03
FRAP	2702.99 ± 33.50[a]	5474.72 ± 517.56[b]	16.87 ± 0.10[c]	-

Table 3. Experimental results of the DPPH radical scavenging activity of *Mentha spicata* and *Melissa officinalis*

R	DPPH of *Mentha spicata*							
	Concentration (mg/ml)	Real concentration (mg/ml)	DO	A%	Negative test	IC50 (µg/mL)	Moyenne	Ecart type
R1	10	0.243902439	0.7459	13.6589883	0.8639	922.90143	926.616667	5.25413894
	20	0.487804878	0.6988	19.1110082				
	40	0.975609756	0.6373	26.2298877				
	50	1.219512195	0.6251	27.6420882				
R2	10	0.243902439	0,7281	15.719412		930.331904		
	20	0.487804878	0,6787	21.4376664				
	40	0.975609756	0,6373	26.2298877				
	50	1.219512195	0,6251	27.6420882				
R3	10	0.243902439	0,7370	14.6892002		926.616665		
	20	0.487804878	0,6888	20.2743373				
	40	0.975609756	0,6373	26.2298877				
	50	1.219512195	0,6251	27.6420882				
	DPPH of *Melissa officinalis*							
R1	10	0.243902439	0,771	7.35	0.5883	1390.73316	1509.13192	167.44
	20	0.487804878	0,4818	44.2296562				
	30	0.731707317	0,4353	49.6122236				
	40	0.975609756	0,3667	57.5529575				
	50	1.219512195	0,3431	60,2847552				
R2	10	0.243902439	0,59	5.85		1627.53067		
	20	0.487804878	0,5096	41.0116912				
	30	0.731707317	0,4798	44.4611645				
	40	0.975609756	0,4234	50.9896979				
	50	1.219512195	0,3631	57.9696724				
R3	10	0.243902439	0,6805	6,6		1509.13192		
	20	0.487804878	0,4957	42,6206737				
	30	0.731707317	0,4575	47,0366941				
	40	0.975609756	0,3950	54.271326				
	50	1.219512195	0,3531	57.627214				

(20 mm), confirming that the tested extracts are not a promising alternative against this highly resistant bacterium. Regarding *Escherichia coli*, both extracts showed moderate activity, with inhibition zones around 12 mm at the highest concentration and an MIC of 1.56 mg/mL. Although this activity was similar between *M. officinalis* and *M. spicata*, it remained clearly lower than that of standard antibiotics such as ceftriaxone (30 mm), teicoplanin (23 mm), and piperacillin/tazobactam (30 mm). The most significant activity was observed against Gram-positive bacteria. Indeed, for *Staphylococcus aureus*, both extracts demonstrated strong inhibition, but with different profiles. *Melissa officinalis* showed greater potency, reflected by a particularly low MIC (0.19 mg/mL), whereas *Mentha spicata* displayed larger inhibition zones (up to 21.5 mm) but with a slightly higher MIC (0.78 mg/mL). These results bring both ex-tracts close to the effectiveness of reference antibiotics (CRO = 22 mm,

Table 4. Experimental results of the ABTS radical scavenging activity of *Mentha spicata* and *Melissa officinalis*

R	ABTS of*Mentha spicata*							
	Concentration (mg/ml)	Real Concentration (mg/ml)	DO	A%	Negative test	IC50 (µg/mL)	Moyenne	Ecart type
R1	10	0.476190476	0.4953	20.17728	0.6205	1926.241	1772.664	217.1905
	20	0.952380952	0.4107	33.81144				
	30	1.428571429	0.3626	41.56326				
	50	2.380952381	0.2744	55.7776				
	60	2.857142857	0.1908	69.2506				
R2	10	0.476190476	0.5272	15.03626		1619.087		
	20	0.952380952	0.0821	33.81144				
	40	1.428571429	0.2727	56.05157				
	50	2.380952381	0.2385	61.56326				
	60	2.857142857	0.1908	69.2506				
R3	10	0.476190476	0.51125	17.60677		1772.664		
	20	0.952380952	0.24640	33.81144				
	40	1.428571429	0.31765	48.807415				
	50	2.380952381	0.25645	58.67043				
	60	2.857142857	0.19080	69.2506				
	ABTS Melissa officinalis							
R1	10	0.476190476	0.6204	0.128783	0.6212	1625.927	2055.101	606.9431
	20	0.952380952	0.5348	13.90856				
	30	1.428571429	0.5054	18.64134				
	40	1.904761905	0.4128	33.54797				
	60	2.857142857	0.2801	54.90985				
R2	10	0.476190476	0.6102	1.770766		2484.274		
	20	0.952380952	0.5348	13.0856				
	30	1.428571429	0.4878	21.47457				
	40	1.904761905	0.4746	23.59948				
	60	2.857142857	0.3287	47.08628				
R3	10	0.476190476	0.6153	0.949774		2055.100		
	20	0.952380952	0.5348	13.49708				
	30	1.428571429	0.4966	20.057955				
	40	1.904761905	0.3087	28.573725				
	60	2.857142857	0,3044	57.627214				

TEC = 20 mm, CAZ = 27 mm), highlighting their promising potential. A similar trend was observed for *Staphylococcus epidermidis*, where *Melissa officinalis* proved clearly more effective than *Mentha spicata*. With inhibition zones ranging from 15 to 18 mm and an MIC of 1.56 mg/mL, it approached the activity of reference antibiotics (CRO = 25 mm, CAZ = 20 mm). In contrast, *M. spicata* showed weaker inhibition (8–11.5 mm) and a higher MIC (6.25 mg/mL), indicating more modest activity. Finally, against *Candida albicans*, both extracts exhibited weak inhibition, with inhibition zones of only 6–7 mm. However, *M. spicata* demonstrated greater potency in terms of MIC (0.39 mg/mL vs. 2.34 mg/mL for *M. officinalis*), although these results remain far below those expected for significant antifungal activity (Table 7).

DISCUSSION

The chemical profiles of essential oils from *M. officinalis* and *M. spicata* are known to exhibit considerable variation, largely affected by environmental factors including light intensity, cultivation conditions, geographic origin, nutrient

Table 5. Results of the experimental FRAP assay using *Mentha spicata* and *Melissa officinalis*

R	FRAP of *Mentha spicata*					
	Concentration (mg/ml)	Real concentration (mg/ml)	DO	IC50 (µg/mL)	Moyenne	Ecart type
R1	10	0.534759358	0.0999	5108.81393	5474.78483	517.561015
	20	1.069518717	0.118			
	30	1.604278075	0.1652			
	60	3.20855615	0.2542			
	70	3.743315508	0.4379			
R2	10	0.534759358	0.0906	5840.75574		
	20	1.069518717	0.118			
	30	1.604278075	0.1316			
	60	3.20855615	0.269			
	70	3.743315508	0.4379			
R3	10	0.534759358	0.09525	5474.78485		
	20	1.069518717				
	30	1.604278075				
	60	3.20855615				
	70	3.743315508				
R	FRAP of *Melissa officinalis*					
R1	10	0,534759358	0.0762	2679.31034	2702.99749	33.4986826
	20	1,069518717	0.1881			
	30	1,604278075	0.2589			
	60	2,673796791	0.5105			
	70	0,534759358	0.0762			
R2	10	0,534759358	0.0957	2726.68464		
	20	1,069518717	0.1881			
	30	1,604278075	0.2941			
	60	2,673796791	0.5105			
R3	10	0,534759358	0.08595	2702.99749		
	20	1,069518717	0.1881			
	30	1,604278075	0.2765			
	60	2,673796791	0.5105			

Note: DO – optical density, SD – standard deviation.

availability, temperature, harvest stage, and genetic diversity (El Machrafi et al., 2025). Such variability has been well-documented in the scientific literature (Othman and Kamel, 2021; Singh and Agarwal, 2013). The GC-MS analysis of the essential oils in this research demonstrated a complex mixture of bioactive constituents, confirming a distinct profile for each plant. Our finding that geraniol is the dominant compound in *M. officinalis*. Carvone is the major component in *M. spicata* aligns with previous research with previous research on these species and confirms the chemotype of the plants from the Settat region (Hajlaoui et al., 2021; Hosnaroodi and Ghavam, 2025). The presence of these oxygenated monoterpenes is directly linked to the observed biological activities. In addition to these major constituents, various studies have identified other key compounds in *Melissa officinalis* essential oil, including neral, citral, β-caryophyllene, and caryophyllene oxide (Abdellatif et al., 2023; Alizadeh and Shahidi, 2019), while *Mentha spicata* is known to contain significant amounts of limonene, piperitone, and ter-pinen-4-ol (Şahin et al., 2004). Antioxidant activity can be assessed using several analytical methods, each based on different mechanisms of action (Argyropoulos and Müller, 2014; Saeb and Gholamrezaee, 2012). In this study, the antioxidant potential of the essential oils was investigated using three analytical approaches, including

Table 6. Antimicrobial susceptibility of essential oils from *Melissa officinalis* and *Mentha spicata*

Parameter	DIZ (mm)		MIC (mg/mL)	DIZ (mm)		MIC (mg/mL)	CRO	TEC	CAZ	TPZ
	200 mg/mL	400 mg/mL		200 mg/mL	400 mg/mL					
Escherichia coli	6 ± 0.0	12.33 ± 1.33	1.56	6 ± 0.0	12.66 ± 0.48	1.56	30	23	NT	30
Pseudomonas aeruginosa	8 ± 0.0	6 ± 0.0	3.12	7 ± 0.11	6 ± 0.0	3.12	20	NT	NT	27
Candida albicans	7 ± 0.0	7 ± 0	2.34	6 ± 0.0	6 ± 0.0	0.39	NT	NT	NT	NT
Staphylococcus epidermidis	15 ± 2	18 ± 2	1.56	8 ± 0.0	11.5 ± 0.0	6.25	25	NT	20	NT
Staphylococcus aureus	7 ± 1	12 ± 0.0	0.19	10 ± 1.16	21.5 ± 0.5	0.78	22	20	27	NT

Note: NT – no test.

Table 7. In vitro assessment of the antimicrobial activity of essential oils derived from *Mentha spicata* and *Melissa officinalis*

	Mentha Spicata								*Melisse Officinalis*							
Parameter	R1(200 mg/ml)	R2(200 mg/ml)	R3(200 mg/ml)	Mean ±SD	R1(400 mg/ml)	R2(400 mg/ml)	R3(400 mg/ml)	Mean ±SD	R1(200 mg/ml)	R2(200 mg/ml)	R3(200 mg/ml)	Mean ±SD	R1 (400 mg/ml)	R2 (400 mg/ml)	R3 (400 mg/ml)	Mean± SD
E coli	6	6	6	6±0.0	12	13	13	12.67 ±0.58	6	6	6	6±0.0	11	12	14	12.33 ±1.33
S aureus	9	10	11	10±1.0	21	22	22	21.67 ±0.58	6	7	8	7±0.0	12	12	12	12± 0.0
P. aeruginosa	7	7	7	7±0.0	5	5	5	5±0.0	8	8	8	8±0.0	5	5	5	5±0.0
S. epidermidis	11	12	11	11.33 ±0.58	8	8	8	8±0.0	13	15	17	15±2.0	16	18	20	18±2
Candida	6	6	6	6±0.0	6	6	6	6±0.0	7	7	7	7±0.0	7	7	7	7±0.0

the DPPH and ABTS assays, which are based on a hydrogen atom transfer mechanism, and the FRAP assay, which relies on an electron transfer mechanism. The results indicate that both essential oils possess notable antioxidant potential, although with different strengths. The essential oil of *M. spicata* showed stronger activity in the DPPH and ABTS assays, suggesting its components are highly effective at donating a hydrogen atom to neutralize free radicals (Medjeldi et al., 2022; Nguyen et al., 2026). In contrast, the essential oil of *Melissa officinalis* demonstrated a greater tendency for electron transfer, as evidenced by its stronger performance in the FRAP assay. This distinction highlights the value of using multiple assays to fully characterize a plant's antioxidant potential, as a single method may not capture the full spectrum of activity.

In terms of antimicrobial activity, both essential oils showed selective efficacy, with a greater inhibitory effect against Gram-positive bacteria. This is a commonly reported finding and and can be attributed to the intrinsic structural differences between Gram-positive and Gram-negative bacteria. The lipophilic structure of essential oil components allows them easily to permeate and disrupt the simple peptidoglycan layer of Gram-positive bacterial cell walls, resulting inleakage of cellular contents and subsequent cell death (Nourbakhsh et al., 2022). Conversely, Gram-negative bacteria possess a structurally complex, multilayered outer membrane enriched with lipopolysaccharides, which functions as an effective permeability barrier and consequently reduces their susceptibility to essential oils (Gulluce et al., 2007).

While our study found limited activity against *P. aeruginosa*, a Gram-negative bacterium, it is important to acknowledge that some literature reports successful inhibition of Gram-negative bacteria by *Mentha spicata* essential oil, suggesting that efficacy can be influenced by specific oil composition and the microbial strain (Shahbazi, 2015). The antibacteri-al properties of essential oils are generally attributed to their content of phenols, terpenes, and aldoketones (Guimarães et al., 2019). The enhanced antimicrobial activity observed at higher concentrations can be attributed to increased levels of monoterpenes like carvone, limonene, and geraniol, which have well-established antibacterial properties. This observation opens a crucial avenue for future research: the investigation of synergistic effects. Combining these essential oils

with other natural extracts or even conventional antibiotics may enhance their efficacy and help combat the rise of antibiotic resistance by targeting multiple sites on the bacterial cell (Gulluce et al., 2007). The observed compositional variations and mechanisms of action underscore the potential for creating powerful new formulations by blending these essential oils. This study, therefore, provides a foundational characterization of these locally sourced plants, validating their traditional use and paving the way for more detailed investigations into their therapeutic applications.

CONCLUSIONS

The present study established the chemical composition and *in vitro* biological activities of essential oils obtained from *Melissa officinalis* and *Mentha spicata* cultivated in the Settat region of Morocco. GC–MS analysis demonstrated distinct compositional profiles, with geraniol predominating in *M. officinalis* oil and carvone in *M. spicata* oil. Comparative antioxidant assessment revealed differential activity patterns depending on the assay mechanism. *M. spicata* essential oil exhibited stronger radical-scavenging activity in DPPH and ABTS assays, whereas *M. officinalis* demonstrated higher ferric-reducing capacity in the FRAP assay. These findings indicate that variations in dominant oxygenated monoterpenes are associated with differences in antioxidant performance. Both essential oils showed measurable antimicrobial activity, with greater inhibitory effects observed against Gram-positive bacteria than Gram-negative strains and yeast. This variation in susceptibility is consistent with structural differences in microbial cell envelopes. The results support the hypothesis that compositional differences between the two essential oils are associated with variations in antioxidant and antimicrobial efficacy. The study provides region-specific data contributing to the understanding of composition-activity relationships in essential oils of these species.

REFERENCES

1. Abdellatif, F., Begaa, S., Messaoudi, M., Benarfa, A., Ouakouak, H., Hassani, A., Sawicka, B., Simal Gandara, J., (2023). HPLC–DAD analysis, antimicrobial and antioxidant properties of aromatic herb Melissa officinalis L., aerial parts extracts. *Food Analytical Methods 16*, 45–54. https://doi.org/10.1007/s12161-022-02385-1
2. Bouhadi, M., El-Atmani, Z., Talbi, M., Elkouali, M. H., Ainane, T. (2019). Evaluation of the toxicity of chromium VI contaminated irrigation water on the mint (*Mentha spicata*) crop. *Evaluation 3*, 421–427.
3. Alizadeh, B.B., Shahidi, F., (2019). *Melissa officinalis* essential oil: Chemical compositions, antioxidant potential, total phenolic content and antimicrobial activity. *Nutrition and Food Sciences Research 6*(1), 17–25. https://doi.org/10.29252/nfsr.6.1.17
4. Argyropoulos, D., Müller, J., (2014). Changes of essential oil content and composition during convective drying of lemon balm (*Melissa officinalis* L.). *Industrial Crops and Products 52*, 118–124. https://doi.org/10.1016/j.indcrop.2013.10.020
5. Baser, K.H.C., Kürkçüoglu, M., Tarimcilar, G., Kaynak, G., (1999). Essential oils of *Mentha species* from Northern Turkey. *Journal of Essential Oil Research 11*, 579–588. https://doi.org/10.1080/10412905.2012.676775
6. Chen, S., Li, Z., Chitrakar, B., Gu, Z., Ban, X., Hong, Y., Cheng, L., Li, C., (2024). Minimum inhibitory concentration (MIC) of essential oils against microorganisms: Methods, function, accuracy factors and application. *Food Reviews International 40*, 3445–3470. https://doi.org/10.1080/87559129.2024.2357612
7. El Machrafi, S., Elkouali, M., Sadia, T., Iskandar, S., Rahhal, R., Bouhadi, M., El Jemli, M., El Hajjouji, H., (2025). Phytochemical composition and antioxidant activities of *Mentha spicata* L. and *Melissa officinalis* L. from the Settat region of Morocco: A comparative study of extraction methods. *Journal of Ecological Engineering 26*, 164–177. https://doi.org/10.12911/22998993/208642
8. Guimarães, A.C., Meireles, L.M., Lemos, M.F., Guimarães, M.C.C., Endringer, D.C., Fronza, M., Scherer, R., (2019). Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules 24*, 2471. https://doi.org/10.3390/molecules24132471
9. Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., Polissiou, M., Adiguzel, A., Ozkan, H., (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. longifolia. *Food chemistry 103*, 1449–1456. https://doi.org/10.1016/j.foodchem.2006.10.061
10. Hajlaoui, H., Arraouadi, S., Noumi, E., Snoussi, M., Kadri, A., (2021). *Melissa officinalis* L. essential oil: Chemical composition, antioxidant, antibacterial and antifungal activities-in vitro study. *Journal of Pharmaceutical Research International 33*, 1529–1537. https://doi.org/10.9734/jpri/2021/v33i60b34775
11. Hosnaroodi, V.G., Ghavam, M., (2025). Evaluation of the effect of soil and irrigation water characteristics on the chemical composition and antimicrobial activity of *Mentha spicata* L. essential oil: A plant

used in traditional medicine of Kashan people. *Agricultural Water Management 309*, 109331. https://doi.org/10.1016/j.agwat.2025.109331

12. Kalemba, D., Kunicka, A., (2003). Antibacterial and antifungal properties of essential oils. *Current medicinal chemistry 10*, 813–829.
13. Kiełtyka-Dadasiewicz, A., Okoń, S., Ociepa, T., Król, B., (2017). Morphological and genetic diversity among peppermint (*Mentha* × *piperita* L.) cultivars. *Acta Scientiarum Polonorum Hortorum Cultus 16*, 151–161. https://doi.org/10.24326/asphc.2017.3.15
14. Lahyaoui, M., Dakka, N., El Menyiy, N., Sobeh, M., Razi, P., Goh, K.W., Al-Worafi, Y.M., Farrukh, M.J., Bouyahya, A., (2025). Ethnobotanical survey of medicinal and aromatic plants used by the population of Settat Province, Morocco. *Heliyon 11*. https://doi.org/10.1016/j.heliyon.2025.e42323
15. Mahboubi, M., (2021). *Mentha spicata* L. essential oil, phytochemistry and its effectiveness in flatulence. *Journal of Traditional and Complementary Medicine 11*, 75–81. https://doi.org/10.1016/j.jtcme.2017.08.011
16. Mahendran, G., Verma, S.K., Rahman, L.-U., (2021). The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L.): A review. *Journal of Ethnopharmacology 278*, 114266. https://doi.org/10.1016/j.jep.2021.114266
17. Medjeldi, S., Benrachou, N., Bouchelaghem, S., Jallouli, S., Farès, N., Elkahoui, S., (2022). Essential oils and alcohol extracts of northwest Tunisia *M. communis* and *M. spicata*: phytocompounds, antioxidant and antibacterial activities. *Current Bioactive Compounds 18*, 44–52. https://doi.org/10.2174/1573407217666211008124528
18. Nguyen, V.K., Tran, T.H., Tran, Q.T., Le Thi, H.N., Hoang, B.N., (2026). Applying response surface methodology to optimize microwave-assisted extraction process from *Mentha Crispa* in Vietnam. *Natural Product Communications 21*. https://doi.org/10.1177/1934578X251382334
19. Nourbakhsh, F., Lotfalizadeh, M., Badpeyma, M., Shakeri, A., Soheili, V., (2022). From plants to antimicrobials: Natural products against bacterial membranes. *Phytotherapy Research 36*, 33–52. https://doi.org/10.1002/ptr.7275
20. Othman, S.I., Kamel, F.H., (2021). In vitro antibacterial activity of *Mentha spicata* essential oil against some pathogenic bacteria. *Polytechnic Journal 11*, 13–15. https://doi.org/10.25156/ptj.v11n1y2021.pp13-15
21. Pineau, S., Legros, C., Mattei, C., (2016). The medical use of lemon balm (*Melissa officinalis*) and Valerian (*Valeriana officinalis*) as natural sedatives: Insight into their interactions with GABA transmission. *International Journal of Clinical Pharmacology & Pharmacotherapy 1*, 1–8. https://doi.org/10.15344/2456-3501/2016/112
22. Pintilia, S., Petcu, E., Ionescu, N., Ion, V.A., (2025). A comprehensive review of mint essential oils – composition, quality and applications. *Published in Scientific Papers. Series B, Horticulture, LXIX*(2), 881–891.
23. Saeb, K., Gholamrezaee, S., (2012). Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth. *Asian Pacific Journal of Tropical Biomedicine 2*, S547–S549. https://doi.org/10.1016/S2221-1691(12)60271-8
24. Şahin, F., Güllüce, M., Daferera, D., Sökmen, A., Sökmen, M., Polissiou, M., Agar, G., Özer, H., (2004). Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. *Food control 15*, 549–557. https://doi.org/10.1016/j.foodcont.2003.08.009
25. Shahbazi, Y., (2015). Chemical composition and in vitro antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. *Journal of pathogens 2015*, 916305. https://doi.org/10.15171/PS.2015.10
26. Singh, C.S., Agarwal, R., (2013). Evaluation of antibacterial activity of volatile oil from *Mentha spicata* L. *Journal of drug delivery and therapeutics 3*, 120–121. https://doi.org/10.22270/jddt.v3i4.580
27. Tina, S., Khibech, O., Zourif, A., Iskandar, S., Mohamed, K.H., Ndayambaje, M., Lhousaine, B., El Jemli, M., 2025. Phytochemical characteristics, antioxidant, and antimicrobial activities and in silico prediction of bioactive compounds from *Cedrus atlantica* Wood Tar. *Biophysica 6*, 3. https://doi.org/10.3390/biophysica6010003
28. Uritu, C.M., Mihai, C.T., Stanciu, G.-D., Dodi, G., Alexa-Stratulat, T., Luca, A., Leon-Constantin, M.-M., Stefanescu, R., Bild, V., Melnic, S., (2018). Medicinal plants of the family Lamiaceae in pain therapy: A review. *Pain Research and Management 2018*, 7801543. https://doi.org/10.1155/2018/7801543