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Antimicrobial Efficacy and Chemical Composition of Essential Oils from Moroccan Medicinal Plants against Multidrug-Resistant *Salmonella* Strains

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ABSTRACT

The rising occurrence of multidrug-resistant bacteria underscores the critical need for alternative therapeutic approaches. This study aimed to investigate the yield, chemical composition, and antimicrobial properties of essential oils extracted from *Thymus satureioides*, *Origanum majorana*, and *Rosmarinus officinalis*, sourced from the Benslimane region in Morocco. Essential oils were extracted using hydrodistillation, their chemical composition was analyzed via gas chromatography-mass spectrometry (GC-MS), and their antimicrobial potential was assessed against 27 multidrug-resistant *Salmonella* strains. The essential oil of *Thymus satureioides* was primarily composed of thymol (28.7%) and borneol (21.2%). *Origanum majorana* contained high levels of terpinen-4-ol (34.2%), while *Rosmarinus officinalis* essential oil was rich in 1,8-cineole (50.3%). *Thymus satureioides* demonstrated the highest efficacy, with the lowest minimum inhibitory concentration (MIC: 0.15 mg/mL) and the most pronounced inhibition zones (30.3 ± 0.02 mm). *Origanum majorana* indicated moderate antibacterial activity. *Rosmarinus officinalis*, on the other hand, showed variable but generally lower efficacy, with inhibition zones reaching up to 16.6 ± 0.1 mm and MIC values ranging from 2.35 to 9.4 mg/mL. These results highlighted the promising potential of Moroccan essential oils, particularly *Thymus satureioides*, as natural antimicrobials to combat multidrug-resistant pathogens in food safety and clinical applications.

Keywords: Essential oil, Food, Multidrug-resistant, *Origanum majorana*, *Rosmarinus officinalis*, *Salmonella*, *Thymus satureioides*

INTRODUCTION

Antibiotic resistance is a global crisis. Pathogenic bacteria, increasingly resilient to existing antibiotics, have led to infections that defy conventional treatments and jeopardize public health (Alamgir, 2018). Compounding this challenge is the emergence of resistant bacterial strains and atypical pathogens, further diminishing the efficacy of synthetic antimicrobial agents. To address these escalating challenges, alternative therapeutic strategies are imperative. Plants, a rich and diverse source of bioactive molecules, offer a promising avenue for the discovery of new antimicrobial compounds and alternative therapeutic agents (Arip et al., 2022). Analyzing the chemical composition of plant-derived active compounds holds the potential to identify substances capable of combating infections without relying on synthetic antibiotics (Vaou et al., 2021). Such natural molecules may provide broad-spectrum activity, targeting resistant bacteria and mitigating the development of further resistance (Iseppi et al., 2021). As current antibacterial agents face mounting limitations, plant-based bioactive compounds, such as phenolics, flavonoids, alkaloids, and terpenoids, emerge as a vital frontier for the development of new, effective therapeutics to control and eliminate infections (Mendelsohn et al., 2023).

Morocco is a global hotspot of plant diversity, home to 4,200 species, including 800 endemics, with 382 species recognized for their medicinal and aromatic properties (Fakchich and Mostafa, 2014; Alistiqsa et al., 2017). In recent years, the Aromatic and Medicinal Plants sector has experienced significant socio-economic growth, contributing to both regional development and international markets. Essential oils extracted from these plants have found applications across diverse industries, including aromatherapy, pharmaceuticals, perfumery, cosmetics, and food preservation. Their widespread use is linked to a combination of broad-spectrum biological activities and highly targeted therapeutic effects, such as antimicrobial, anti-inflammatory, antioxidant, and analgesic properties, positioning plant essential oils as vital resources for innovation in science and industry (Fourmentin and Kfoury, 2024).

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Despite their widespread traditional uses, limited studies have explored the chemical composition of essential oils derived from *Thymus satureioides*, *Rosmarinus officinalis*, and *Origanum majorana*. To the best of available knowledge, no prior study has specifically investigated the antimicrobial activity of essential oils against multidrug-resistant *Salmonella* strains isolated from food products in Casablanca, Morocco. This study aims to address this gap by analyzing the chemical composition of essential oils extracted from Moroccan samples of *Thymus satureioides*, *Rosmarinus officinalis*, and *Origanum majorana*. Furthermore, the antimicrobial efficacy of these essential oils was assessed *in vitro* against 27 multidrug-resistant *Salmonella* strains obtained from various food products.

MATERIALS AND METHODS

Ethical approval

This study was conducted *in vitro* by the guidelines of the Faculty of Sciences Ibn Tofail University Kénitra, Morocco.

Plant material

The leaves of *Thymus satureioides* and *Origanum majorana* were collected in April 2023, and *Rosmarinus officinalis* leaves were gathered in May 2023, all from the Benslimane province in the Casablanca-Settat region of Morocco. The identification of species was primarily performed using macroscopic botanical tests, which involved examining external features such as leaf shape and flower structure (Fennane et al., 1999). Following collection, the leaves were dried under ambient conditions (temperature ranging from 20 to 25°C) in a ventilated room, protected from light, for approximately 30 days. Once dried, the plant material was ground into a coarse powder using a knife mill. The powdered samples were stored in stainless steel containers, shielded from light and moisture, to preserve their integrity for subsequent analysis.

Microorganisms

The 27 microbial strains analyzed in this study consisted of multidrug-resistant *Salmonella* strains, exhibiting high levels of antibiotic resistance (Table 1). These strains were isolated from food products of diverse origins in Casablanca, Morocco, and represent various serovars of significant relevance to food safety and public health concerns in the study region. To ensure viability, the strains were maintained through regular sub-culturing on nutrient agar and preserved by cryopreservation in 30% glycerol at -20°C for future use.

Table 1. Antibiotic resistance profiles of selected strains

Code	Serovar	Antibiotic resistance profile
S60	*S.Kentucky*	CIP ; NAL ; SMX ; TET
S78	*S.Kentucky*	CIP ; NAL ; SMX ; TET
S1	*S. Typhimurium*	CHL ; SMX ; TET ; TMP ; SXT
S7	*S.Schwarzengrund*	CHL ; SMX ; TET ; TMP ; SXT
S19	*S.Typhimurium*	AMP ; SMX ; TET ; TMP ; SXT
S21	*S.Typhimurium*	AMP ; SMX ; TET ; TMP ; SXT
S22	*S.Saintpaul*	NAL ; SMX ; TET ; TMP ; SXT
S23	*S.Chester*	CHL ; SMX ; TET ; TMP ; SXT
S44	*S.Kentucky*	AMP ; CIP ; NAL ; SMX ; TET
S61	*S.Kentucky*	AMP ; CIP ; NAL ; SMX ; TET
S58	*S.New Port*	CIP ; NAL ; SMX ; TET ; TMP
S59	*S.Chester*	CHL ; SMX ; TET ; TMP ; SXT
S62	*S.Kentucky*	AMP ; CIP ; NAL ; TET ; TMP
S67	*S.Saintpaul*	NAL ; SMX ; TET ; TMP ; SXT
S41	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S47	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S51	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S55	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S56	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S57	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S63	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S72	*S.Kentucky*	AMP ; CIP ; GEN ; NAL ; SMX ; TET
S42	*S.Kentucky*	AMP ; CIP ; NAL ; SMX ; TET ; TMP ;
S66	*S.Kentucky*	AMP ; CIP ; NAL ; SMX ; TET ; TMP ; SXT
S70	*S.Hadar*	AMP ; CHL ; CIP ; NAL ; SMX ; TET ; TMP ; SXT
S80	*S.kentucky*	AMP ; CHL ; CIP ; NAL ; SMX ; TET ; TMP ; SXT
S69	*S.kentucky*	AMP ; CTX ; FOX ; CAZ ; CRO ; CHL ; CIP ; ETP ; NAL ; SMX ; TET ; TMP ; SXT

AMP: Ampicillin, CTX: Cefotaxime, FOX: Cefoxitin, CAZ: Ceftazidime, CRO: Ceftriaxone, CHL: Chloramphenicol, CIP: Ciprofloxacin, GEN: Gentamicin, ETP: Ertapenem, NAL: Nalidixic acid, SMX: Sulfonamide, TET: Tetracycline, TMP: Trimethoprim, SXT: Trimethoprim/Sulfamethoxazole.

Essential oil extraction

The ground plant material was subjected to hydrodistillation using a Clevenger-type apparatus, following the standardized method outlined in the European Pharmacopoeia. This technique involves boiling the plant material in water, allowing the steam to carry volatile compounds through a condenser, where they are cooled and collected as an essential oil-water mixture. According to the European Pharmacopoeia guidelines, hydrodistillation ensures the efficient extraction of essential oils while preserving their chemical integrity. For each extraction, 100 g of plant material was placed in a 1 L glass flask with 500 mL of distilled water, ensuring a proper ratio to facilitate effective steam distillation without causing overflow. The distillation process was conducted for 3 hours, after which the recovered essential oils were dried using anhydrous sodium sulfate (Na_2SO_4). The purified essential oils were stored in tightly sealed, opaque bottles at a controlled temperature between 4°C and 6°C to preserve their integrity.

Calculation of yield

The essential oil yield, expressed as a percentage, was determined by calculating the ratio of the extracted oil weight to the weight of the dry plant material used, in accordance with the method outlined by Boubrit (2007). The calculation was performed based on the dry matter content. The yield was determined using the following Formula 1.

$$\text{Yield (\%)} = \frac{\text{Mass of essential oil (g)}}{\text{Mass of dry leaves (g)}} \times 100 \qquad \text{(Formula 1)}$$

Chemical composition

The identification of volatile compounds in the essential oils was carried out using gas chromatography coupled with mass spectrometry (GC-MS). The specific operating conditions applied during the analysis are detailed in Table 2.

Table 2. Operational conditions for gas chromatography coupled with mass spectrometry

Parameter	Details
Injected sample volume	0.5 Ml
Split ratio	200:1
Inlet temperature	250°C
Capillary column	HP-5 MS Agilent J & W (50 m x 200 µm x 0.33 µm)
Temperature program	60°C (initial), increase to 220°C at 4°C/min, hold for 10 min, then increase to 240°C at 1°C/min
Carrier gas	Helium, 1.2 mL/min
Scan range	35 to 450 m/z
Ionization energy	70 Ev
Software used	ChemStation (Agilent Technologies)

Evaluation of the antibacterial activity of essential oils

Well diffusion method

The antibacterial activity of the essential oils was evaluated using the well diffusion method, a widely used technique for assessing antimicrobial efficacy. This method measures the ability of essential oils to diffuse through the agar medium and inhibit bacterial growth, as indicated by the formation of inhibition zones. Bacterial strains were first cultured in nutrient broth at 37°C for 18-24 hours, and the culture density was adjusted to a turbidity corresponding to 0.5 on the McFarland scale. Approximately 20 mL of Mueller-Hinton agar was poured into sterile Petri dishes. Once solidified, the agar surface was uniformly inoculated with the bacterial suspension. Sterile punches were used to create wells approximately 6 mm in diameter. Each well was then filled with 50 µL of essential oil, while cefotaxime served as the positive control. The plates were incubated at 37°C for a period ranging from 18-24 hours. After incubation, inhibition zone diameters were measured using a millimeter ruler. The antibacterial activity of the essential oils was determined by comparing these measurements with those of the positive control (Kadiri et al., 2022).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial efficacy of essential oils against multidrug-resistant *Salmonella* strains was assessed by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). These parameters were measured using the broth microdilution method, following the procedure described by Sarker et al. (2007). Essential oils were first prepared as stock solutions and serially diluted (1:2) in Mueller-Hinton broth, ensuring thorough mixing in each dilution. The prepared dilutions were distributed into sterile 96-well microplates, and 0.1 mL of *Salmonella* inoculum (approximately 10^6CFU/mL) was added to each well. To assess bacterial viability, 30 µL of a 15%

resazurin solution was introduced. The microplates were incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was visually identified as the lowest concentration at which bacterial growth inhibition was observed, based on the color change. Wells containing concentrations above the MIC were subsequently used to determine (MBC), defined as the lowest concentration that kills 99.9% of the bacterial population. Each experiment was performed in triplicate to ensure result reliability and robustness.

Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (mean ± SD). Statistical analysis was conducted using GraphPad Prism 10.4.0. A repeated measures ANOVA was applied for the analysis of MIC and MBC, followed by Šídák's post hoc test for multiple comparisons. When the assumption of sphericity was not met, the Geisser-Greenhouse correction was applied. The significance threshold was set at $p < 0.05$.

RESULTS

Essential oil yields

The essential oil extraction process from the three plants collected in the Benslimane region resulted in yields ranging from 1.70 ± 0.025 mL/100 g DM to 2.40 ± 0.025 mL/100 g DM. Table 3 summarizes the specific yields obtained for each plant species. The results revealed variations in essential oil yields among the three studied plants. *Thymus satureioides* exhibited the highest average yield, at 2.37 mL/100 g DM, with a low standard deviation of 0.025, reflecting strong consistency across measurements. *Origanum majorana* produced an average yield of 1.72 mL/100 g DM, accompanied by an exceptionally low standard deviation of 0.025, indicating remarkable homogeneity in essential oil production for this species. Lastly, *Rosmarinus officinalis* yielded an average of 2.10 mL/100 g DM, with a standard deviation of 0.020. While this yield is slightly lower than that of *Thymus satureioides*, it remains relatively high and consistent. Statistical analysis showed significant differences between the yields of the three species ($p < 0.05$).

Chemical composition

The chemical profiles of the essential oils from *Thymus satureioides*, *Rosmarinus officinalis*, and *Origanum majorana*, obtained through gas chromatography-mass spectrometry (GC-MS) analysis, are presented in Table 4. The total identified percentages of volatile compounds in the essential oils were 88.58% for *Thymus satureioides*, 96.47% for *Origanum majorana*, and 98.07% for *Rosmarinus officinalis*, indicating near-complete characterization of their chemical profiles. In *Thymus satureioides*, the predominant compounds were thymol (28.67%), borneol (21.15%), p-cymene (10.66%), and α-terpineol (7.34%). For *Origanum majorana*, the major constituents included terpinen-4-ol (34.17%), α-terpinene (19.4%), γ-terpinene (14.19%), and α-terpineol (7.92%). In *Rosmarinus officinalis*, 1,8-cineole (50.33%), camphor (17.80%), α-pinene (6.40%), and camphene (5.47%) were the dominant components. These findings revealed significant chemical diversity among the three species, each exhibiting a distinct chemical profile that underscores its unique therapeutic potential.

Evaluation of antibacterial activity

Disk diffusion

The evaluation of the antibacterial activity of the essential oils from *Rosmarinus officinalis*, *Origanum majorana*, and *Thymus satureioides* revealed notable differences in their effectiveness. These findings highlighted their promising potential as natural antimicrobial agents (Figure 1). *Thymus satureioides* demonstrated notable antibacterial activity, with inhibition zones reaching remarkably high values of 30.3 ± 0.02 mm. However, variability was observed, with inhibition zones ranging from these high values to more modest measurements around 10.5 ± 0.02 mm (Table 5). These findings highlighted the significant, albeit variable, antibacterial efficacy of *Thymus satureioides* against multidrug-resistant bacterial strains. *Origanum majorana* exhibited variable antibacterial activity, with inhibition zones ranging from modest values, such as 11.1 ± 0.4 mm, to slightly higher measurements of 12 ± 0.2 mm and 15.2 ± 0.3 mm. Notably, for certain strains, a substantial inhibition zone of 30 ± 0.1 mm was observed, indicating stronger antibacterial efficacy in specific cases. Overall, the activity of *Origanum majorana* remains moderate, with significant variability observed between different *Salmonella* serovars ($p < 0.05$), indicating that its antibacterial efficacy is strain-dependent. *Rosmarinus officinalis* exhibited comparatively modest antibacterial activity, with inhibition zones reaching up to 16.6 ± 0.1 mm. Overall, its activity was lower than that of the other essential oils in this study. In contrast, *Origanum majorana* demonstrated notable efficacy, particularly against the growth of *Salmonella enterica* strains, with significantly larger inhibition zones compared to *Rosmarinus officinalis* ($p < 0.05$), highlighting its strong potential as an antimicrobial agent.

Table 3. Essential oil yield of the studied plants

Plant	RHE1 (ml/100g DM)	RHE2 (ml/100g DM)	RHE3 (ml/100g DM)	Average (ml/100g DM)	Standard deviation
Thymus satureioides	2.35	2.40	2.37	2.37	0.025
Origanum majorana	1.70	1.72	1.75	1.72	0.025
Rosmarinus officinalis	2.10	2.08	2.12	2.10	0.020

RHE1, RHE2, and RHE3: Repetitions of essential oil extraction; DM: Dry matter

Table 4. Chemical composition of essential oils from *Thymus satureioide*s, *Origanum majorana* and *Rosmarinus officinalis*

N°	Chemical Compounds	RI	Chemical Formula	Essential Oils		
				T. S	O. M	R. O
1	Tricyclene	928	$C_{10}H_{16}$	0.66	-	-
2	*α*-Thujene	934	$C_{10}H_{16}$	0.19	0.63	-
3	*α*-Pinene	941	$C_{10}H_{16}$	0.71	-	6.40
4	Camphene	952	$C_{10}H_{16}$	0.83	-	5.47
5	Sabinene	975	$C_{10}H_{16}$	1.06	4.81	3.45
6	*β*-Pinene	982	$C_{10}H_{16}$	1.67	0.31	1.31
7	Myrcene	991	$C_{10}H_{16}$	1.36	1.71	-
8	*α*-Phellandrene	1007	$C_{10}H_{16}$	-	0.57	-
9	Delta-3-Carene	1012	$C_{10}H_{16}$	1.18	-	-
10	*α*-Terpinene	1018	$C_{10}H_{16}$	-	19.4	-
11	*O*-Cymene	1024	$C_{10}H_{14}$	0.073	-	-
12	*p*-Cymene	1025	$C_{10}H_{14}$	10.66	2.8	0.805
13	Limonene	1029	$C_{10}H_{16}$	-	0.12	2.02
14	*β*-Phellandrene	1032	$C_{10}H_{16}$	0.047	-	-
15	1,8-Cineole	1035	$C_{10}H_{18}O$	0.62	0.641	50.33
16	*γ*-Terpinene	1058	$C_{10}H_{16}$	1.03	14.191	0.75
17	*cis*-Sabinene Hydrate	1066	$C_{10}H_{18}$	0.498	1.826	-
18	*α*-Terpinolene	1088	$C_{10}H_{16}$	-	1.812	1.50
19	*trans*-Sabinene Hydrate	1098	$C_{10}H_{18}O$	-	-	0.11
20	Camphor	1145	$C_{10}H_{16}O$	0.117	-	17.80
21	*p*-Menth-3-en-8-ol	1149	$C_{10}H_{18}O$	-	1.47	-
22	Borneol	1169	$C_{10}H_{18}O$	21.158	-	4.18
23	Terpinene-4-ol	1178	$C_{10}H_{18}O$	-	34.171	1.88
24	*α*-Terpineol	1193	$C_{10}H_{18}O$	7.34	7.923	0.61
25	Bornyl Acetate	1288	$C_{12}H_{20}O_2$	0.247	-	0.30
26	Thymol	1302	$C_{10}H_{14}O$	28.677	-	-
27	Carvacrol	1310	$C_{10}H_{14}O$	1.889	0.301	-
28	*β*-Caryophyllene	1426	$C_{15}H_{24}$	6.728	3.79	1.16
29	Germacrene-D	1494	$C_{15}H_{24}$	0.012	-	-
30	*γ*-Cadinene	1515	$C_{15}H_{24}$	0.109	-	-
31	*β*-Cadinene	1531	$C_{15}H_{24}$	0.180	-	-
32	OxCaryophyllene Oxide	1575	$C_{15}H_{24}O$	1.538	-	0.41
Total identified (%)				88.583	96.475	98.075

RI: Retention index; T.S: *Thymus satureioides*; R.O: *Rosmarinus officinalis*; O.M: *Origanum majorana*

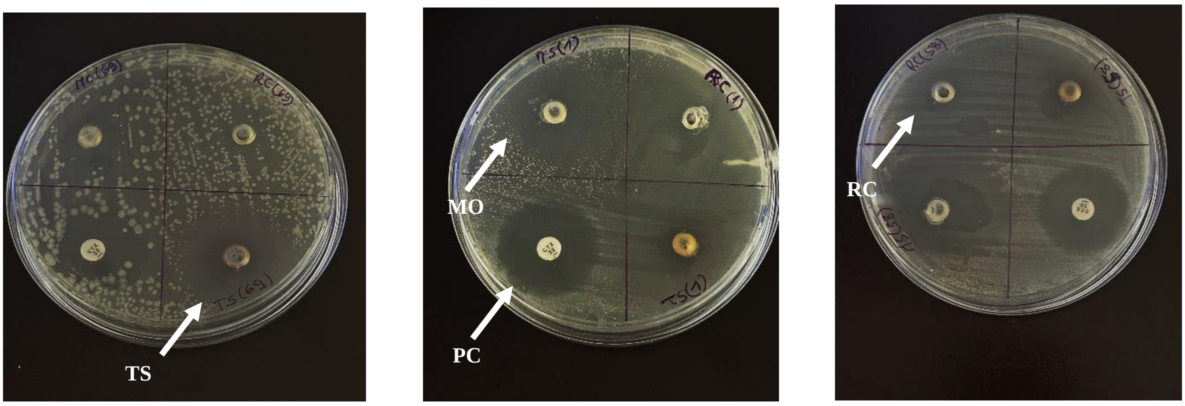

Figure 1. Antibacterial activity (inhibition zone) of essential oils from *Thymus satureioides*, *Origanum majorana*, and *Rosmarinus officinalis* against multidrug-resistant *Salmonella* spp. **TS:** *Thymus satureioides*; **MO:** *Origanum majorana*; **RC:** *Rosmarinus officinalis*; **PC:** Positive control.

Table 5. Inhibition diameters (mm) of tested essential oils against *Salmonella*

Codes	Essential oils			Antibiotic
	Rosmarinus officinalis	***Thymus saturéoides***	***Origanum majorana***	**CTX**
S70	6 ± 0.1	10.5 ± 0.1	6 ± 0.1	30
S80	6 ± 0.1	10.5 ± 0.1	6 ± 0.1	30
S23	6 ± 0.1	11 ± 0.5	8 ± 0.1	30
S21	11 ± 0.4	15.3 ± 0.1	8 ± 0.1	35
S19	11 ± 0.4	15.3 ± 0.1	6 ± 0.1	35
S62	11.3 ± 0.1	15.7 ± 0.4	8 ± 0.1	25
S61	11.3 ± 0.1	15.7 ± 0.4	6 ± 0.1	25
S69	15 ± 0.2	20.5 ± 0.1	30 ± 0.1	20
S22	16.6 ± 0.1	15.7 ± 0.1	11 ± 0.1	30
S1	10 ± 0.1	30.3 ± 0.1	15.2 ± 0.3	30
S7	10 ± 0.1	30.3 ± 0.1	15.2 ± 0.3	30
S67	6 ± 0.1	11 ± 0.1	8 ± 0.1	30
S41	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S42	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S47	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S51	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S55	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S56	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S57	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S63	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S72	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S66	14.2 ± 0.1	12.2 ± 0.3	11.1 ± 0.4	30
S58	8 ± 0.1	20.3 ± 0.4	11.5 ± 0.1	20
S59	8 ± 0.1	15 ± 0.1	12 ± 0.2	28
S60	7 ± 0.1	9 ± 0.1	7 ± 0.1	12
S78	8 ± 0.1	9 ± 0.1	7 ± 0.1	12
S44	12 ± 0.5	15.1 ± 0.2	12 ± 0.4	30

CTX: Cefotaxime

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The essential oils of *Rosmarinus officinalis*, *Origanum majorana*, and *Thymus satureioides* demonstrated variable activity against multidrug-resistant *Salmonella* strains. Among these, *Thymus satureioides* demonstrated the highest antibacterial potency, with a minimum inhibitory concentration as low as 0.15 mg/mL and a correspondingly low minimum bactericidal concentration for most of the tested strains. This combination of low MIC and MBC underscores its considerable potential as a promising antimicrobial agent against multidrug-resistant bacteria. In contrast, *Rosmarinus officinalis* and *Origanum majorana* showed more variable effects. For certain strains, *Rosmarinus officinalis* exhibited moderate MIC values ranging from 2.35 to 9.4 mg/mL, while its MBC values occasionally reached much higher concentrations, up to 75.2 mg/mL. This finding suggests that while *Rosmarinus officinalis* displays a notable inhibitory effect, its bactericidal activity often requires substantially higher concentrations.

Origanum majorana exhibited a minimum inhibitory concentration (MIC) ranging from 0.17 mg/mL to 0.9 mg/mL, demonstrating its antibacterial potential. However, its effectiveness varied across different *Salmonella* strains, with some requiring higher MIC values, up to 0.9 mg/mL. The minimum bactericidal concentration (MBC) also displayed significant variability, ranging from 2.73 mg/mL to 43.75 mg/mL. While this essential oil exhibited inhibitory effects at MIC values as low as 0.17 mg/mL, its bactericidal activity was limited against certain highly resistant *Salmonella* strains, as indicated by the highest MBC value of 43.75 mg/mL (Figure 2).

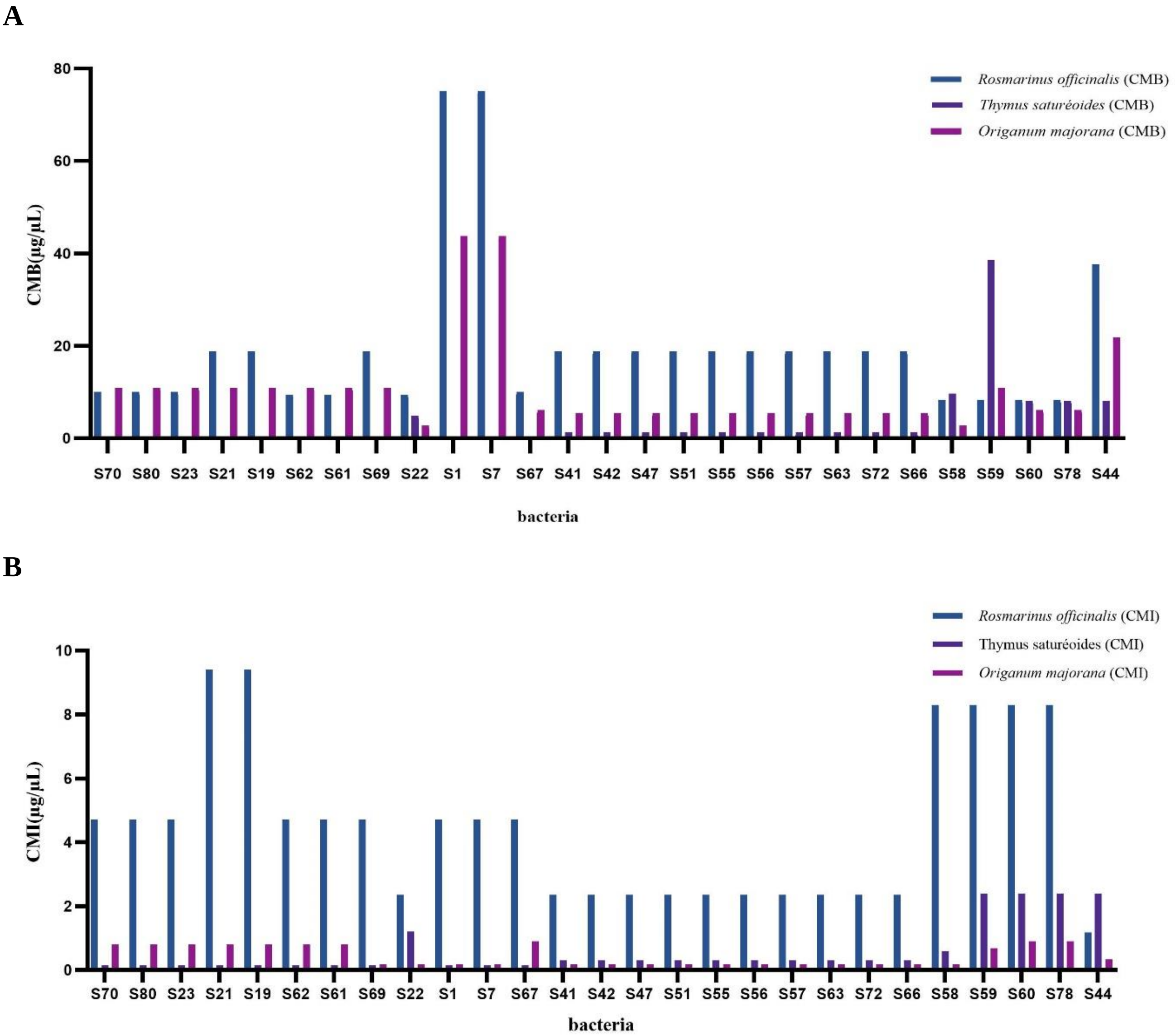

Figure 2. Comparison of minimum inhibitory and minimum bactericidal concentrations of essential oils from *Thymus satureioides*, *Origanum majorana*, and *Rosmarinus officinalis* against multidrug-resistant *Salmonella* spp., **A**: Comparison of MICs for each essential oil. **B**: Comparison of MBCs for each essential oil

DISCUSSION

Essential oils are complex mixtures derived from various plant parts and are rich in aromatic components, such as terpenes. These oils are widely utilized across multiple fields, including medicine, cosmetics, and the food industry (Burt, 2004; Bakkali et al., 2008). The existing literature highlights their diverse biological activities, including significant antiseptic, antibacterial, antiviral, antioxidant, antiparasitic, antifungal, and insecticidal properties (Kalemba and Kunicka, 2003). Morocco is recognized as one of the leading global suppliers and producers of aromatic plants, including *Artemisia herba-alba Asso*, *Mentha pulegium* L., *Lavandula stoechas* L., and *Rosmarinus officinalis* L. These plants yield high-value-added products, playing a significant role in Morocco's economic development (Ait Bouzid et al., 2024). In line with previous studies on the antimicrobial efficacy of essential oils, the present study confirms the potent antibacterial activity of *Thymus satureioides*, *Origanum majorana*, and *Rosmarinus officinalis* against multidrug-resistant *Salmonella* strains. Similar findings have been reported by Hezil et al. (2022), who demonstrated that oregano essential oil is among the most effective against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, a study by Soković et al. (2010) indicated that *Rosmarinus officinalis* exhibits antimicrobial activity against several Gram-positive and Gram-negative bacteria, supporting the results obtained in this study.

The results revealed variable essential oil yields among the three studied species, with average values of 2.37 mL/100 g DM for *Thymus satureioides*, 1.72 mL/100 g DM for *Origanum majorana*, and 2.10 mL/100 g DM for

Rosmarinus officinalis. These findings align with previous studies conducted in various regions of Morocco, where similar yields were reported under comparable climatic and soil conditions. For instance, Amarti et al. (2010) observed essential oil yields in the Middle Atlas under a semi-arid Mediterranean climate characterized by hot summers and mild, wet winters, along with well-drained, calcareous soils. These conditions are similar to those in the Benslimane region, which may explain the comparable yields. Similarly, several studies have demonstrated that environmental factors such as soil composition, temperature variations, and altitude significantly influence essential oil production. For instance, a study on *Thymus fedtschenkoi* in Iran highlighted that increasing altitude alters plant growth and essential oil composition, indicating a direct impact on yield (Ghelichnia, 2018). Likewise, studies on *Salvia officinalis* have shown that soil type and seasonal effects modify essential oil ratios, emphasizing the role of soil composition in oil production (Stojanovic et al., 2013). The notably high yield of *Thymus satureioides* supported the findings of Azzouzi et al. (2024), who underscored the significant potential of this species in semi-arid environments. For *Rosmarinus officinalis*, studies carried out in Spain and Greece under similar Mediterranean climates reported yields ranging between 1.5 and 2.4 mL/100 g DM, confirming the competitiveness of the yield observed in the Benslimane region (Serralutzu et al., 2020; Kokkini et al., 2021). In contrast, the relatively lower yield observed for *Origanum majorana* may be attributed to its greater sensitivity to the specific environmental conditions in Benslimane. This trend is consistent with findings from Boulila (2022), who reported similar sensitivities in rosemary cultivated in Mediterranean regions, and from Baser et al. (2011), who observed comparable results in studies conducted in Turkey. These variations in yield, observed both nationally and internationally, underscore the combined influence of ecophysiological factors and distillation techniques. They highlight the importance of adapting agricultural practices and optimizing harvesting methods to specific environmental conditions to maximize essential oil extraction efficiency.

The chemical composition analysis of the essential oils, performed using gas chromatography-mass spectrometry (GC-MS), revealed the dominance of thymol (28.67%) and borneol (21.15%) in *Thymus satureioides*, terpinen-4-ol (34.17%) and α-terpinene (19.4%) in *Origanum majorana*, and 1,8-cineole (50.33%) and camphor (17.80%) in *Rosmarinus officinalis*.

Thymol, the predominant compound in the essential oil of *Thymus satureioides*, is widely recognized for its potent antimicrobial and antifungal properties (Marchese et al., 2016). Previous studies have demonstrated that thymol possesses potent antimicrobial properties, effectively targeting a wide range of pathogens, including multidrug-resistant bacteria and fungi (Hammer et al., 1999; Nostro et al., 2004). In Morocco, similar studies on *Thymus satureioides* reported thymol concentrations ranging from 25% to 30%, consistent with the results of the present study, Ziyyat and Boussouf (2012). In contrast, studies on related *Thymus* species in Spain and Turkey have documented lower thymol concentrations, typically around 20% (Rota and Herrera, 2008; Altundag and Ozturk, 2011). This comparatively high thymol content observed in Moroccan *Thymus satureioides* underscores its potential as a superior source for therapeutic applications, particularly in combating infections.

Borneol contributes significantly to the antimicrobial effects of the essential oil. Moreover, studies have highlighted its analgesic and anti-inflammatory properties, which further enhance the oil's therapeutic potential (El Idrissi and Aboudaoud, 2010). Comparatively, a study conducted in the Atlas region of Morocco reported borneol concentrations ranging from 18% to 20%, which are slightly lower than those observed in the present study Chaieb and Hajlaoui (2007). Similar values have been documented in France, where borneol constitutes approximately 20% of the components identified in *Thymus* essential oils (Gheldof and Engeseth, 2002).

The dominance of terpinen-4-ol in the essential oil of *Origanum majorana* aligns with previous studies conducted in Morocco. For instance, Benjilali et al. (1982) reported a similar predominance of terpinen-4-ol, with comparable values, in samples collected from various Moroccan regions. Additionally, international studies have also reported this compound as a predominant constituent. *Origanum majorana* essential oils from Mediterranean regions exhibited terpinen-4-ol concentrations ranging between 30% and 35%, as observed by Skocibusic et al. (2006). These findings confirm that the high concentration of terpinen-4-ol is a consistent characteristic across diverse geographical regions, reinforcing its potential as a potent antimicrobial agent. However, regional variability has been noted for other constituents. For example, Kamari et al. (2023) reported α-terpinene as a major component but observed slightly lower values compared to those of the current study. Similarly, studies conducted in Turkey and Spain found that α-terpinene content in *Origanum* samples typically ranged between 15% and 20% (Lawrence, 2001).

1,8-Cineole, also known as eucalyptol, was identified as the major compound in the essential oil of *Rosmarinus officinalis*, with a significant concentration of 50.33%. Similar findings have been reported in Morocco; for instance, Bendaoud (2009) observed 1,8-Cineole content ranging from 40% to 55% in samples collected from northern Morocco, values closely aligning with those of the present study. Further supporting this trend, reported 1,8-Cineole concentrations of approximately 48% in *Rosmarinus officinalis* essential oils from Iran (Moghtader, 2012). These findings highlight the relative uniformity of 1,8-Cineole composition across geographically distinct regions, reinforcing its prominence as a key component in rosemary essential oils.

Camphor, another key compound in the essential oil of *Rosmarinus officinalis*, is well known for its analgesic, antimicrobial, and antispasmodic properties (Zuccarini and Soldani, 2009). In this study, camphor was present at a concentration of 17.80%, which aligns closely with findings from other Moroccan studies. For instance, Elamrani (2000) reported camphor content ranging between 15% and 20% in samples collected across Morocco, reflecting the consistency in climatic conditions and distillation methods. Similarly, Viuda-Martos (2007) quantified camphor concentrations at approximately 16% in *Rosmarinus officinalis* essential oils from Spain, further corroborating the results of the present study. These observations suggest that camphor is a reliable chemical marker of *Rosmarinus officinalis*, regardless of geographical origin.

Essential oils, volatile compounds produced through the secondary metabolism of plants, play a critical role in defense against pathogens. Renowned for their antioxidant and antimicrobial properties, these compounds are invaluable for both plant protection and human applications (Ben Miri, 2025). Multiple studies have confirmed their effectiveness against a wide range of microorganisms, including both Gram-negative and Gram-positive bacteria, as well as various molds and yeasts (Kalemba and Kunicka, 2003; Nazzaro et al., 2013; Bouyahya et al., 2019). For example, *Thymus vulgaris* essential oil has been shown to be highly effective against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, with minimum inhibitory concentrations (MIC) as low as 0.34 mg/mL (Nazzaro et al., 2013). Similarly, *Zingiber cassumunar* essential oil exhibits strong antimicrobial properties against bacterial and fungal pathogens (Chaieb and Hajlaoui, 2007). The antimicrobial mechanism of essential oils is primarily attributed to their ability to disrupt microbial cell membranes, leading to cell lysis and death (Dorman and Deans, 2000).

The antibacterial activity of essential oils is largely affected by the structural composition of the target microorganism's cell wall. Gram-positive bacteria tend to be more susceptible, as their membranes facilitate direct interaction with the hydrophobic constituents of essential oils (Soković et al., 2010; Zengin and Baysal, 2014). In contrast, the outer lipopolysaccharide membrane of Gram-negative bacteria acts as a barrier, restricting the entry of hydrophobic compounds and thereby reducing their susceptibility (Zengin and Baysal, 2014).

The antibacterial activities of *Origanum majorana*, *Thymus satureioides*, and *Rosmarinus officinalis* have been extensively examined in both national and international studies (Bouazza et al., 2014; Lagha et al., 2019; Zuhairi et al., 2020). The findings of this study align with the broader body of study while offering some unique insights. The results of the present study demonstrated a particularly strong inhibitory effect of *Thymus satureioides* essential oil, with inhibition zones ranging from 10.5 mm to 30.3 mm, indicating notable efficacy against multidrug-resistant bacterial strains. The MIC and MBC tests further confirmed this observation, with remarkably low MIC values (0.15 µg/µL), signifying the ability of the oil to inhibit bacterial growth at minimal concentrations. Similarly, the low MBC values (0.15 µg/µL) highlight the bactericidal potential of the oil at these same concentrations. Comparable results were observed in a study conducted in Spain, where MIC values ranged from 0.12 to 0.25 µg/µL against *Salmonella Typhimurium* (Ruberto et al., 2000). The significant antibacterial efficacy of *Thymus satureioides* is largely due to its elevated concentrations of carvacrol and thymol, two compounds widely recognized for their potent antimicrobial effects. Moroccan studies have further corroborated these findings, demonstrating the significant antibacterial effects of Thymus essential oil against foodborne pathogens such as *Salmonella* spp. (Zantar et al., 2011; Bouyahya et al., 2019). These studies reported MIC values consistent with those in our work, along with similarly notable inhibition zones ranging between 10.5 mm and 30.3 mm. Collectively, these results reinforce the potential of *Thymus satureioides* essential oil as a natural alternative to conventional antibiotics, offering promising applications for combating multidrug-resistant bacterial infections in both food safety and clinical settings.

The essential oil of *Origanum majorana* demonstrated significant antibacterial activity, although it was slightly less effective than that of *Thymus satureioides*. Diffusion tests revealed variable antibacterial efficacy depending on the bacterial strains, which may be attributed to the moderate ability of the oil to diffuse through the culture medium. This variability likely stems from the chemical nature of the oil's components, which interact differently with bacterial cell membranes. These findings are consistent with a previous study conducted in Morocco. For example, a study in the Rif region reported similar MIC values for *Origanum majorana* essential oil, ranging from 0.2 to 0.5 µg/µL against *Salmonella* strains (Boutabia et al., 2016). Similarly, a study conducted in Italy by Soković et al. (2010) reported MIC and MBC values within a similar range to those observed in our study, further confirming the antibacterial potential of the tested essential oils. Their findings demonstrated that the essential oils exhibited strong inhibitory and bactericidal effects against a variety of bacterial strains, reinforcing the validity of the results of the present study. Although *Origanum majorana* essential oil exhibits inhibitory effects, it sometimes requires higher concentrations to achieve complete bacterial elimination. The presence of β-caryophyllene is likely a major contributor to its antibacterial activity, while other compounds, such as terpenes, may play a role in the oil's overall synergistic effects. This combination of components may explain the observed variability in efficacy across the tested bacterial strains.

The essential oil of *Rosmarinus officinalis* exerts its antibacterial effects primarily through the disruption of the bacterial cell membrane. Active compounds, such as cineole and camphor, interact with membrane lipids, altering their permeability and causing leakage of intracellular content, ultimately leading to bacterial death. Additionally, *Rosmarinus officinalis* can inhibit bacterial respiration by blocking key enzymes involved in energy production, thereby interfering with bacterial metabolism. Although less pronounced, this essential oil can also impair protein and DNA synthesis, disrupting bacterial replication and cell division. Despite these mechanisms, the antibacterial efficacy of *Rosmarinus officinalis* remains more moderate compared to phenol-rich essential oils, such as those of *Origanum majorana* and *Thymus satureioides* (Olivas-Méndez et al., 2022). This observation is consistent with findings from Moroccan studies, which reported that *Rosmarinus officinalis* exhibits antimicrobial activity but often requires higher MIC values relative to phenol-rich essential oils (Celikel and Kavas, 2008). While *Rosmarinus officinalis* has demonstrated efficacy against certain pathogens, its overall activity is lower than that of *Origanum majorana* and *Thymus* essential oils (Celikel and Kavas, 2008), a trend that aligns with the results of this study. Here, *Rosmarinus officinalis* required higher concentrations to inhibit and eliminate bacteria. When comparing the antimicrobial activities of *Origanum majorana*, *Thymus satureioide*s, and *Rosmarinus officinalis*, it becomes clear that differences in their chemical compositions play a crucial role in determining their varying levels of efficacy against microbial strains.

CONCLUSION

This study evaluated the yield, chemical composition, and antibacterial properties of essential oils extracted from *Thymus satureioides*, *Origanum majorana*, and *Rosmarinus officinalis* collected in the study area. The highest essential oil yield was obtained from *Thymus satureioides*, averaging 2.40 mL/100 g of dry matter (DM). Chemical analyses performed using gas chromatography coupled with mass spectrometry (GC-MS) revealed several bioactive components, with carvacrol and thymol being the most dominant. The evaluation of antibacterial activity against multidrug-resistant *Salmonella* strains demonstrated significant efficacy, particularly for *Thymus satureioides*, which exhibited the strongest bacterial growth inhibition. These findings emphasize the potential of essential oils as natural alternatives to conventional antimicrobials in combating multidrug-resistant infections. Future studies should focus on identifying the precise mechanisms of action of these essential oils, conducting *in vivo* assessments to validate their therapeutic potential, and exploring synergistic effects when combined with existing antibiotics.

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Author's contributions

Chaima Sabri conducted conceptualization, data collection, analysis, and initial drafting of the manuscript. Fatima Zahra Kadiri assisted with data collection, validation of results. Safaa Sabri supervised the analysis and contributed to scientific interpretations. Sara Razzak contributed to the result analysis. Mohamed Rida Salam coordinated in experimental work and technical review. Youness Taboz conducted the general supervision, scientific guidance, and final approval of the manuscript. All authors checked and approved the findings of this study and the last edition of the submitted manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and submission, and redundancy, have been checked by all authors.

REFERENCES

Ait Bouzid H, Ibourki M, Hamdouch A, Oubannin S, Asbbane A, Hallouch O, Bijla L, Jamal K, Majourhat K, and Gharby S (2024). Moroccan aromatic and medicinal plants: A review of economy, Ethnobotany, chemical composition, and biological activities of commonly used plants. Food and Humanity, 2: 100259. DOI: https://www.doi.org/10.1016/j.foohum.2024.10025

Alamgir ANM (2018). Phytoconstituents-active and inert constituents, metabolic pathways, chemistry and application of phytoconstituents, primary metabolic products, and bioactive compounds of primary metabolic origin. Springer International Publishing, pp. 25-164. DOI: https://www.doi.org/10.1007/978-3-319-92387-1_2

Alistiqsa F, Bouassab A, and Allouch M (2017). Etude ethnobotanique des plantes medicinales de la région de Tanger cas de Hjar Nhal et Melloussa [Ethnobotanical study of medicinal plants in the Tangier region: Case of Hjar Nhal and Melloussa]. International Journal of Advanced Research, 5(11): 310-322. DOI: https://www.doi.org/10.21474/IJAR01/5772

Altundag E and Ozturk M (2011). Ethnomedicinal studies on the plant resources of East Anatolia, Turkey. Procedia - Social and Behavioral Sciences, 19(5): 756-777. DOI: https://www.doi.org/10.1016/j.sbspro.2011.05.195

Amarti F, Satrani B, Ghanmi M, Farah A, Aafi A, Aarab L, Ajjouri M, and Chaouch A (2010). Chemical composition and antimicrobial activity of the *Thymus algeriensis* Boiss. & Reut. and *Thymus ciliatus* (Desf.) Benth. essential oils of Morocco. Biotechnology, Agronomy and Society and Environment, 14(1): 141-148. Available at: https://www.cabidigitallibrary.org/doi/full/10.5555/20103134466

Arip M, Selvaraja M, Tan LF, Leong MY, Tan PL, Yap VL, Chinnapan S, Tat NC, Abdullah M, K D et al. (2022). Review on plant-based management in combating antimicrobial resistance—mechanistic perspective. Frontiers in Pharmacology, 13: 879495. DOI: https://www.doi.org/10.3389/fphar.2022.879495

Azzouzi FE, Chaouqi S, Makkaoui M, Briguiche H, and Zidane L (2024). Ethnopharmacological study of medicinal plants used in the treatment of skin diseases in the Western Middle Atlas region (Morocco). Journal of Experimental Biology and Agricultural Sciences, 12(1): 93-105. DOI: https://www.doi.org/10.18006/2024.12(1).93.105

Bakkali F, Averbeck S, Averbeck D, and Idaomar M (2008). Biological effects of essential oils—A review. Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 46(2): 446-475. DOI: https://www.doi.org/10.1016/j.fct.2007.09.106

Baser KHC, Ozek T, Tümen G, and Sezik E (2011). Composition of the essential oils of Turkish *Origanum* species with commercial importance. Journal of Essential Oil Research, 5(6): 619-623. DOI: https://www.doi.org/10.1080/10412905.1993.9698294

Ben Miri Y (2025). Essential oils chemical composition and diverse biological activities : A comprehensive review. Natural Product Communications, 20(1): 1934578X241311790. DOI: https://www.doi.org/10.1177/1934578X241311790

Bendaoud H (2009). GC/MS analysis and antimicrobial and antioxidant activities of essential oil of *Eucalyptus radiata*. Journal of the Science of Food and Agriculture, 23(1): 72-82. DOI: https://www.doi.org/10.1002/jsfa.3585

Benjilali B, Tantaoui-Elaraki A, Ayadi A, and Ihlal M (1982). Method to Study antimicrobial effects of essential oils: application to the antifungal activity of six Moroccan essences. Journal of Essential Oil Research, 47(10): 748-752. DOI: https://www.doi.org/10.4315/0362-028X-47.10.748

Bouazza F, Hassikou R, Satrani B, Ghanmi M, Ennadir J, El-Hani Y, and Khedid K (2014). *In vitro* antibacterial activity of *Thymus satureioides*, *Mentha pulegium*, and *Origanum vulgare* essential oils against *Escherichia coli* isolated from raw sheep milk. International Journal of Agriculture Innovations and Research, 2(6): 1011-1016. Available at: https://www.semanticscholar.org/paper/In-vitro-antibacterial-activity-of-Thymus-Mentha-Bouazza-Hassikou/6158ff48c42bae02abc1ed146e8701d3c2a8f304

Boulila A (2022). The essential oil compositions of *Rosmarinus officinalis* L. leaves growing in Mersin, Turkey. European Journal of Chemistry, 47(5): 102-112. DOI: https://www.doi.org/10.5155/eurjchem.11.4.370-376.2048

Boutabia L, Telailia S, Bouguetof I, and Guenadil F (2016). Composition chimique et activité antibactérienne des huiles essentielles de *Rosmarinus officinalis* L. de la région de Hammamet (Tébessa-Algérie) [Chemical composition and antibacterial activity of essential oils of *Rosmarinus officinalis* L. from the Hammamet region (Tébessa-Algeria)]. Bulletin de la Société Royale des Sciences de Liège [Bulletin of the royal society of sciences of Liège], pp. 1-85. Available at: https://popups.uliege.be/0037-9565/index.php?id=6050

Bouyahya A, Abrini J, Dakka N, and Bakri Y (2019). Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. Journal of Pharmaceutical Analysis, 5(9): 301-311. DOI: https://www.doi.org/10.1016/j.jpha.2019.03.001

Burt S (2004). Essential oils : Their antibacterial properties and potential applications in foods--A review. International Journal of Food Microbiology, 94(3): 223-253. DOI: https://www.doi.org/10.1016/j.ijfoodmicro.2004.03.022

Celikel N and Kavas G (2008). Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech Journal of Food Sciences, 26(3): 174-181. DOI: https://www.doi.org/10.17221/1603-CJFS

Chaieb K and Hajlaoui H (2007). The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata (Syzigium aromaticum* L. Myrtaceae): A short review. Phytotherapy Research, 21(6): 501-506. DOI: https://www. doi.org/10.1002/ptr.2124

Dorman HJD and Deans SG (2000). Antimicrobial agents from plants : antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2): 308-316. DOI: https://www.doi.org/10.1046/j.1365-2672.2000.00969.x

El Idrissi M and Aboudaoud M (2010). Morphological and genetic diversity assessment of cultivated *Thymus satureioides* (endemic species) of Morocco. Journal de Génie Génétique et de Biotechnologie, 23(1): 100467. DOI: https://www.doi.org/10.1016/j.jgeb.2025.100467

Elamrani A, Derwich E, Benziane Z, and Chabir R (2000). Aromatic and medicinal plants of Morocco: chemical composition of essential oils of *Rosmarinus officinalis* and *Juniperus phoenicea.* International Journal of Applied Biology and Pharmaceutical Technology, 12(2): 160-165. Available at: https://www.fortunejournals.com/ijabpt/pdf/21025-II-Derwich%20et%20al%5B1%5D.pdf

Fakchich J and Mostafa E (2014). Ethnobotanical survey of medicinal plants used by people in oriental Morocco to manage various ailments. Journal of Ethnopharmacology, 154(1): 76-87. DOI: https://www.doi.org/10.1016/j.jep.2014.03.016

Fennane M, Oualidi J, Faris F, Ibn Tattou M, Mathez J, Ouchbani S, Ouyahya A, Raymaud C, Salvo Tierra Á, and Abidine AZ (1999). Flore pratique du Maroc. Manuel de détermination des plantes vasculaires [Practical flora of Morocco. Identification manual of vascular plants]. Travaux de l'Institut Scientifique, Série Botanique, Tome 1, Institut Scientifique, Rabat, Maroc, pp. 1-36. DOI: https://www.doi.org/10.13140/RG.2.1.3227.8889

Fourmentin S and Kfoury M (2024). Essential oils: The renaissance of natural and sustainable ingredients. Technologie et Innovation, 24(9): 1-21. DOI: https://www.doi.org/10.21494/ISTE.OP.2024.1059

Gheldof N and Engeseth N (2002). Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricultural and Food Chemistry, 50(19): 6215-6222. DOI: https://www.doi.org/10.1021/jf0256135

Ghelichnia H (2018). Essential oil composition of *Thymus fedtschenkoi* ronniger at different growing altitudes in Mazandaran, Iran. Cercetari Agronomice in Moldova, 2(174): 75-83. DOI: https://www.doi.org/10.2478/cerce-2018-0018

Hammer KA, Carson CF, and Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 86(6): 985-990. DOI: https://www.doi.org/10.1046/j.1365-2672.1999.00780.x

Hezil D, Benseghir H, Benamrouche S, Zaidi S, Zineddine R, Bessas A, and Msela A (2022). Chemical composition and antibacterial activity of *Rosmarinus officinalis* essential oil from Algeria. Agricultura, 120: 24-34. Available at: https://journals.usamvcluj.ro/index.php/agricultura/article/view/14332

Iseppi R, Mariani M, Condò C, Sabia C, and Messi P (2021). Essential oils : A natural weapon against antibiotic-resistant Bacteria responsible for nosocomial infections. Antibiotics, 10(4): 417. DOI: https://www.doi.org/10.3390/antibiotics10040417

Kadiri F, Zahid B, Thoume A, Nait Irahal I, Bennis F, Chegdani F, and Blaghen M (2022). Characterization and valorization of a biosurfactant produced by *Aeromonas salmonicida* isolated from the Marchika lagoon of Nador. Biocatalysis and Agricultural Biotechnology, 45: 102523. DOI: https://www.doi.org/10.1016/j.bcab.2022.102523

Kalemba D and Kunicka A (2003). Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, 10(10): 813-829. DOI: https://www.doi.org/10.2174/0929867033457719

Kamari FE, Chlouchi A, Hamzaoui NE, Harmouzi A, Lhilali I, Amrani J, ElMouhdi K, Omari HE, and Abdellaoui A (2023). Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Origanum majorana* growing in Middle Atlas of Morocco. Tropical Journal of Natural Product Research, 7(10): 422-4237. DOI: https://www.doi.org/10.26538/tjnpr/v7i10.16

Kokkini S, Karousou R, and Lanaras T (2021). Biological and pharmaceutical properties of essential oils of *Rosmarinus officinalis* L. and *Lavandula officinalis* L. Natural Product Communications, 16(8): 205-210. DOI: https://www.doi.org/10.1016/j.matpr.2021.03.495

Lagha R, Ben Abdallah F, AL-Sarhan BO, and Al-Sodany Y (2019). Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against *Escherichia coli* Isolated from UTI patients. Molecules, 24(6): 1161. DOI: https://www.doi.org/10.3390/molecules24061161

Lawrence BM (2001). Progress in essential oils. Perfumer and Flavorist, 26: 37-45. Available at: https://img.perfumerflavorist.com/files/base/allured/all/document/2016/02/pf.PF_26_06_044_13.pdf

Marchese A, Orhan I, Daglia M, Barbieri R, Di Lorenzo A, Nabavi Sa, Gortzi O, Izadi M, and Nabavi S (2016). Antibacterial and antifungal activities of thymol : A brief review of the literature. Food Chemistry, 210: 402-414. DOI: https://www.doi.org/10.1016/j.foodchem.2016.04.111

Mendelsohn E, Ross N, Zambrana-Torrelio C, Van Boeckel TP, Laxminarayan R, and Daszak P (2023). Global patterns and correlates in the emergence of antimicrobial resistance in humans. Proceedings of the Royal Society B: Biological Sciences, 290(2007): 20231085. DOI: https://www.doi.org/10.1098/rspb.2023.1085

Moghtader M (2012). Comparative study of the essential oil composition of *Rosmarinus officinalis* L. grown in Iran and other countries. International Journal of Biological Sciences, 4(2): 112-115. DOI: https://www.doi.org/10.1007/s10600-012-0132-5

Nazzaro F, Fratianni F, De Martino L, Coppola R, and De Feo V (2013). Effect of essential oils on pathogenic Bacteria. Pharmaceuticals, 6(12): 1451-1474. DOI: https://www.doi.org/10.3390/ph6121451

Nostro A, Blanco AR, Cannatelli MA, Enea V, Flamini G, Morelli I, Sudano Roccaro A, and Alonzo V (2004). Susceptibility of methicillin-resistant *staphylococci* to oregano essential oil, carvacrol and thymol. Microbiology Letters, 230(2): 191-195. DOI: https://www.doi.org/10.1016/S0378-1097(03)00890-5

Olivas-Méndez P, Chávez-Martínez A, Santellano-Estrada E, Guerrero Asorey L, Sánchez-Vega R, Rentería-Monterrubio AL, Chávez-Flores D, Tirado-Gallegos JM, and Méndez-Zamora G (2022). Antioxidant and antimicrobial activity of Rosemary (*Rosmarinus officinalis*) and Garlic (*Allium sativum*) essential oils and chipotle pepper oleoresin (*Capsicum annum*) on beef hamburgers. Foods, 11(14): 2018. DOI: https://www.doi.org/10.3390/foods11142018

Rota M, and Herrera A (2008). Antimicrobial activity and chemical composition of *Thymus* species essential oils. Journal of Food Protection, 71(1): 119-125. DOI: https://www.doi.org/10.1016/j.foodcont.2007.07.007

Ruberto G, Baratta MT, Deans SG, and Dorman HJD (2000). Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* Essential Oils. Planta Medica, 66(8): 687-693. DOI: https://www.doi.org/10.1055/s-2000-9773

Sarker S, Nahar P, and Kumarasamy Y (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods, 42(4): 321-324. DOI: https://www.doi.org/10.1016/j.ymeth.2007.01.006

Serralutzu F, Stangoni A, Amadou B, Tijan D, Re G, Marceddu S, Dore A, and Bullitta S (2020). Essential oil composition and yield of a *Rosmarinus officinalis* L. natural population with an extended flowering season in a coastal Mediterranean environment and perspectives for exploitations. Genetic Resources and Crop Evolution, 67(7): 1777-1793. DOI: https://www.doi.org/10.1007/s10722-020-00939-y

Skocibusic M, Bezic N, Dunkic V, and Radonic A (2006). Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, Agaricus bisporus. Phytotherapy Research, 20(4): 274-278. Available at: https://research.wur.nl/en/publications/antimicrobial-activity-of-essential-oils-and-their-components-aga

Soković M, Glamočlija J, Marin PD, Brkić D, and van Griensven LJLD (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an *in vitro* model. European Journal of Plant Pathology, 116(3): 211-224. DOI: https://www.doi.org/10.1007/s10658-006-9053-0

Stojanovic D, Lakušić B, Ristić M, Slavkovska V, and Lakusic D (2013). Variations in essential oil yields and compositions of *Salvia officinalis* (*Lamiaceae*) at different developmental stages. Botanica Serbica, 37(2): 127-139. Available at: https://www.semanticscholar.org/paper/Variations-in-essential-oil-yields-and-compositions-Stojanovic-Lakušić/758a311584b5d52b756461285ce1fc2aabc60ab1

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, and Bezirtzoglou E (2021). Towards advances in medicinal plant antimicrobial activity : a review study on challenges and future perspectives. Microorganisms, 9(10): 2041. DOI: https://www.doi.org/10.3390/microorganisms9102041

Viuda-Martos M (2007). Antifungal activities of thyme, clove and oregano essential oils in Spain. Journal of Food Safety, 55(19): 8178-8184. DOI: https://www.doi.org/10.1111/j.1745-4565.2007.00063.x

Zantar S, Laglaoui A, Farah A, and Bakkali M (2011). Effect of harvest time on yield, chemical composition, antimicrobial and antioxidant activities of *Thymus vulgaris* and *Mentha pulegium* essential oils. European Journal of Medicinal Plants, 148(1): 66-71. DOI: https://www.doi.org/10.9734/EJMP/2015/17513

Zengin H and Baysal AH (2014). antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming *bacteria* and cell structure-activity relationships evaluated by SEM microscopy. Molecules, 19(11): 17773. DOI: https://www.doi.org/10.3390/molecules191117773

Ziyyat A and Boussouf A (2012). Effet relaxant de l'huile essentielle d' *Artemisia herba-alba* Asso. sur les contractions du jéjunum des rongeurs [Relaxing effect of *Artemisia herba-alba* Asso. essential oil on rodent jejunum contractions]. Journal Marocain de Chimie, 80(2): 457-468. https://www.doi.org/10.3797/scipharm.1106-13

Zuccarini P and Soldani G (2009). Camphor : Benefits and risks of a widely used natural product. Acta Biologica Szegediensis, 53: 77-82. Available at: https://www2.sci.u-szeged.hu/ABS/2009/Acta%20HPc/5377.pdf

Zuhairi J, Jookar F, Rahimi-Moghaddam A, and Yazdani M (2020). Antioxidant, cytotoxic_and antibacterial activity of *Rosmarinus officinalis* l. Essential oil against bacteria isolated from urinary tract infection. European Journal of Integrative Medicine, 38(58): 101192. DOI: https://www.doi.org/10.1016/j.eujim.2020.101192