

Research Article

Biological Properties and Bioactive Components of *Mentha spicata* L. Essential Oil: Focus on Potential Benefits in the Treatment of Obesity, Alzheimer's Disease, Dermatophytosis, and Drug-Resistant Infections

Mohammed S. Ali-Shtayeh , Rana M. Jamous , Salam Y. Abu-Zaitoun, Ahmad I. Khasati, and Samer R. Kalbouneh

Biodiversity and Environmental Research Center (BERC), Til, Nablus, State of Palestine

Correspondence should be addressed to Mohammed S. Ali-Shtayeh; msshtayeh@yahoo.com

Received 28 May 2019; Accepted 29 August 2019; Published 20 October 2019

Academic Editor: Marisa Colone

Copyright © 2019 Mohammed S. Ali-Shtayeh et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

In the present study, the medicinal aromatic plant *Mentha spicata* has been investigated as a source of essential oil (EO) and pharmaceuticals. The quantity and composition of EO from *M. spicata* cultivated in Palestine were analyzed seasonally over a three-year period. A significantly higher EO content was produced in summer and fall months (2.54–2.79%). Chemical analysis of EO revealed 31 compounds with oxygenated monoterpenes (90%) as the most abundant components followed by sesquiterpene and monoterpene hydrocarbons (6 and 3%, respectively). *M. spicata* can be characterized as a carvone chemotype (65%). EO and carvone have shown strong inhibitory activities against the principal enzymes associated with Alzheimer's disease (AD) and overweight diseases (cholinesterase and porcine pancreatic lipase) and also shown strong antidermatophytic activity against *Microsporum canis*, *Trichophyton rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum*. The pancreatic lipase inhibition and the synergism showed the potential activity of *M. spicata* EO and carvone and that their combinations with standard drugs can be useful for the treatment of obesity and overweight. The results also demonstrated that, in addition to their significant inhibitory activity against biofilm formation of methicillin-resistant *Staphylococcus aureus* (MRSA), *M. spicata* EO and carvone had a strong inhibitory effect on metabolic activity and biomass of the preformed biofilm. The current study supports the utilization of *M. spicata* EO as a traditional medicine and opens perceptions to find more potent substances in the EO for the management of obesity, AD, and dermatophytosis and for combating drug-resistant bacterial infections.

1. Introduction

Spearmint (*Mentha spicata* L.) is a cultivated perennial, rhizomatous, and glabrous plant of the Lamiaceae family and is one of the most common herbs grown commercially worldwide including Palestine. It has been considered one of the most important essential oil crops [1]. Spearmint fresh and dried leaves are used as teas and spice and to flavor foods, dishes, and beverages. Herbage, extracts, and essential oil (EO) of the plant have long been used for combating a number of human diseases or relieving ailments [2].

Spearmint EO is used widely as an aromatic agent in numerous products such as chewing gum, dental cream, and mouth washes, as well as in medications, sweet, fragrance, and ecological pesticides and as antimicrobial agents [3]. The plant is considered a source material for EO that has been found to be a valuable source of natural phenolic antioxidants, cholinesterase inhibitors, pancreatic lipase inhibitors, and biofilm disinfection, antifungal, and antiproliferative agents [4–8].

The most dominant constituent found in spearmint oil worldwide is R(-)-carvone, which offers spearmint its unique smooth characteristic scent [9]. *M. spicata* oil also

contains noteworthy concentrations of limonene, dihydrocarvone, and 1,8-cineole [10–12]. However, carvone is reported to be potential in bacterial growth inhibition, as well as reported as a fungicide, insect repellent, and potato- or flower bulb-sprouting suppressant [13].

In recent years, due to the increasing interest in natural products, plant EOs have attracted more attention in phyto-medicine because of their extensive diversity of bio-activities [14–17]. Furthermore, researches have revealed synergistic effects of standard drugs when applied in combination with specific EOs or some of their main constituents, responsible for exerting such activities [18–25]. Such investigations indicate that the combination of EOs with standard drugs provides significant potential for enhancing the therapeutic effect of existing therapies, developing novel strategies for the management of infectious diseases caused by multidrug-resistant microorganisms, and also reducing any adverse side effects [26].

The effects of seasons on the biochemical features of some EOs of the Lamiaceae family have been described in the literature [16, 27]. However, no information is available on how seasonal variations would affect the EO content, composition, and yields of the major oil constituents of spearmint cultivated in Palestine.

In Traditional Arabic Palestinian Herbal Medicine (TAPHM), the fresh and dried spearmint EOs have been used for the treatment of obesity, dementia, hypertension, abdominal pain, digestive disorders, muscle spasm, flatulence, headache, fever, menstrual pain, asthma, cough, cold, depression, and others [7, 16, 28–31]. In addition, they are commonly used as a memory enhancer and nerve sedative [32]. In animals, spearmint is used as a laxative, diuretic, hypothermia blocker, and flea repellent and for sore throat [33].

The objectives of this study were to (1) study the effect of seasons on the EO content and composition of spearmint cultivated in Palestine; (2) investigate the biological activities of the EOs focusing on their potential benefits in the treatment of obesity, Alzheimer's disease (AD), dermatophytosis, and antibiotic-resistant infections; and (3) verify the use of *M. spicata* EO as a folk medicine in TAPHM and open perspectives to find more effective substances from plant origin for the treatment of important human diseases.

2. Materials and Methods

2.1. Plant Material *M. spicata*. Samples of aerial parts were collected from a spearmint field in the BERC Experimental Station, Til, Nablus, Palestine, during three consecutive years (2015–2017). EOs were separated from the fresh aerial parts by hydrodistillation using a modified Clevenger apparatus following the conditions reported in [16].

2.2. Chemical Analysis of the Essential Oils. Determination of EO composition was performed using gas chromatography-mass spectrometry (GC-MS) following the conditions reported in [16, 27]. The identification of the constituents was based on comparison of their relative retention indices and

spectra with spectra of NIST 98, QuadLib1607 GC-MS, and Adams libraries [34].

2.3. Determination of Antidermatophytic Activity. Essential oil and its main component, carvone, were tested for their antidermatophytic activity against four dermatophyte species: *Microsporum canis*, *Trichophyton mentagrophytes*, *T. rubrum*, and *Epidermophyton floccosum*, using the poisoned food technique as described by Gakuubi et al. [35] and Mohareb et al. [36]. EO and carvone were tested at concentrations ranging from 0.3 to 4 ml/L. Mycelial growth inhibition % (PI) was calculated as follows:

$$\text{mycelial growth inhibition \% (PI)} = \left[\frac{\text{DC} - \text{DT}}{\text{DC}} \right] \times 100, \quad (1)$$

where DC and DT are average diameters of fungal growth in control and treatment groups, respectively. Effective concentration that caused 50% mycelial growth inhibition (EC_{50}) was assessed using Excel.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined following previously reported assays [35, 37].

2.4. Determination of Antibiofilm Activity

2.4.1. Activity against Biofilm Formation. A biofilm-producing bacterial strain, *MRSA-BERC #01*, was used to assess the potential of *M. spicata* EO and carvone to prevent biofilm formation. Aliquots of twofold serial dilutions (100 μL) of EO and carvone were prepared in tryptic soy peptone supplemented with the 0.2% glucose (TSBGlc) medium and added to the 96-well flat-bottom microtiter plate, with final concentrations ranging from 0.078 to 5 $\mu\text{L}/\text{mL}$. Bacterial suspensions (100 μL ; 1×10^6 CFU/mL, final concentration) were then added to the plate. The TSBGlc medium was employed as a negative control. TSBGlc without essential oil was used as a blank [38, 39]. After incubation at 37°C for 24 h, the ability of *MRSA-BERC #01* to form the biofilm in the presence of carvone or EO was then determined using the 2,3,5-triphenyl-tetrazolium chloride (TTC) reduction and crystal violet (CV) assays as mentioned below.

2.4.2. Effects on Established Biofilms. The effect of *M. spicata* EO and carvone on established biofilms was tested following Sabaeifard et al. [40]. In brief, 100 μL of the bacterial suspension (1×10^6 CFU/mL) was added to each well in the microtiter plate and incubated for 24 h at 37°C to allow biofilm formation. After incubation, 100 μL of different concentrations of the EO was added to each well to give final concentrations ranging between 0.078 and 5 $\mu\text{L}/\text{mL}$. TSBGlc was used as the negative control and the blank. The plates were incubated for 24 h after incubation, the planktonic cells were removed by gently aspirating the cell suspension, and the plate was washed twice with PBS. Extra moisture was removed by tapping the plate on a sterile tissue paper. The

microplates were left to dry in a laminar flow desk for 15 min in the upside-down position. The activity of EOs or carvone on the preformed biofilm was subsequently determined using the TTC reduction assay and CV assay [41–45] as mentioned below.

2.4.3. TTC Assay. Biofilm metabolic activity was measured using the TTC assay following Sabaeifard et al. [40]. In brief, fifty microliters of TTC (0.2%) mixed with 200 μL of TSBGlc were transferred to each well, and the plates were then kept in the dark at 37°C for 4 h. Following incubation, the TTC solution was aspirated, and the plate was air-dried. Attached red formazan was dissolved in a mixture of acetone (20%) and ethanol (80%). The dissolved red formazan was moved to a new flat-bottom microplate, and absorbance was measured at 500 nm using Epoch Microplate Spectrophotometer (BioTek) [41–45].

2.4.4. Crystal Violet Assay (CV Assay). The effect of EOs on the total biofilm biomass was measured by the CV staining protocol as described by Peeters et al. [45]. In brief, the formed biofilm was washed using PBS, and the remaining biofilm was stained using 0.01% CV. The bound CV was dissolved with 33% acetic acid, and absorbance at 550 nm was measured using Epoch Microplate Spectrophotometer (BioTek).

2.5. Enzyme Inhibitory Activity

2.5.1. Anticholinesterase Assays. Anticholinesterase activities against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were measured spectrophotometrically using the NA-FB method following Ali-Shtayeh et al. [4]. Neostigmine bromide and galanthamine hydrobromide were used as reference standards.

2.5.2. Pancreatic Lipase Inhibition. The ability of the *M. spicata* EO to inhibit PPL was determined based on a spectrophotometric analysis as described previously [16]. Orlistat was used as the reference standard.

The interactions of EO or carvone with orlistat were determined using a checkerboard method as described by Nikkhah et al. [46]. A two-dimensional checkerboard with twofold dilutions of two treatment agents comprising *M. spicata* EO/orlistat and carvone/orlistat was carried out. The evaluated concentrations were in the range of 7 dilutions with final concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 $\mu\text{L}/\text{mL}$ for EO and carvone and 0.063, 0.031, 0.016, 0.008, 0.004, 0.002, and 0.001 $\mu\text{g}/\text{mL}$ for orlistat.

The fractional inhibitory concentration (FIC) was calculated for the first well in each row of the microtiter plate containing the combination with lipase percentage of inhibition more than 50%, given as follows: FIC of compound A (FIC_A) = MIC_{50} (A) in combination/ MIC_{50} (A) alone and FIC of compound B (FIC_B) = MIC_{50} (B) in combination/ MIC_{50} (B) alone, where A is EO or carvone and B is orlistat.

The FIC index (FICI) was calculated as follows:

$$\text{FICI} = (\text{FIC}_A + \text{FIC}_B). \quad (2)$$

The obtained results were inferred as follows: synergistic effect ($\text{FICI} \leq 0.5$), additive effect ($0.5 < \text{FICI} \leq 1$), no interactive effect ($1 < \text{FICI} \leq 4$), and antagonistic effect ($\text{FICI} > 4$) [47, 48]. The results were graphically represented as isobolograms using Excel. The isobolograms were used to define the type of interaction between EO or carvone and orlistat combination. The isobolograms were performed by mixing EO or carvone with orlistat to determine what lipase inhibitory interaction could be observed by different concentrations of EO and orlistat combined. The isobologram curves can be built by plotting the data points of different ratios where each MIC_{50} is defined in relation to the independent MIC_{50} . A concave curve represents synergy, whereas convex curve and straight line indicate antagonistic and additive effects, respectively [49].

2.6. Statistical Analysis. Averages and standard deviations for three simultaneous assays were tested using standard statistical methods. The relationships between the studied phytochemical compositions were expressed by Pearson's correlation coefficient (SPSS software 21).

3. Results and Discussion

3.1. Seasonal Variations in *M. spicata* Essential Oil Content. The EOs obtained from *M. spicata* varied quantitatively according to seasons. *M. spicata* demonstrated a significantly higher ($p \leq 0.05$) EO content in summer and fall months when the plants were in full bloom (2.79% in summer and 2.54% in fall) than that in winter and spring months when the plants have reached the end of their growing cycle (0.87–1.94%) (Table 1). The results demonstrated that EO accumulation in *M. spicata* seems to be metabolically controlled during vegetative and flowering stages of plant growth. Our results are in agreement with those of Hussain et al. [11] who also reported higher EO contents from late summer crops. A similar outcome was also obtained for *Origanum syriacum* and *Clinopodium serpyllifolium* which also showed the maximum EO yield during summer, when the plants were in full bloom [16, 27].

3.2. *Mentha spicata* EO Chemical Composition. The phytochemical analysis results of the EOs are detailed in Table 1. Thirty-one compounds were identified in the *M. spicata* EOs. Eleven of the components (limonene, 1,8-cineole, cis-dihydrocarvone, dihydrocarveol, trans-carveol, cis-carveol, pulegone, carvone, iso-dihydrocarveol acetate, β -bourbonene, and ϵ -caryophyllene) show mean percentages more than 1%, which represent 90% of the total oils (Figure 1). Three of these compounds were the major constituents: carvone (36.9–76.8%), limonene (6.23–9.79%), and dihydrocarveol (2.27–13.76%). Hence, regardless of the season, all *M. spicata* EOs (collected in all seasons) can be considered carvone chemotypes. Our results are in consistent with those of other scientists who also found the EO of *M. spicata* to be of the carvone chemotype [3, 12, 21, 50, 51].

TABLE 1: Constituents (%) of the EOs from cultivated *M. spicata* sampled over a three-year period.

RT	RI	Compound	Winter	Spring	Summer	Fall
5.418	939	α -Pinene	0.54 \pm 0.05	0.35 \pm 0.07	0.25 \pm 0.03	0.32 \pm 0.01
5.816	946	Camphene	0.16 \pm 0	0.14 \pm 0.02	0.07 \pm 0.01	0.06 \pm 0
6.393	969	Sabinene	5.51 \pm 1.79	0.69 \pm 0.25	0.14 \pm 0.01	0.22 \pm 0
6.518	974	β -Pinene	0.73 \pm 0.06	0.59 \pm 0.1	0.35 \pm 0.01	0.41 \pm 0
6.846	988	Myrcene	0.51 \pm 0.06	0.36 \pm 0.07	0.17 \pm 0.01	0.23 \pm 0
7.048	988	3-Octanol	0.46 \pm 0.02	0.47 \pm 0.02	0.35 \pm 0.06	0.44 \pm 0.03
7.809	1020	para-Cymene	0.27 \pm 0.04	0.31 \pm 0.01	0.33 \pm 0.01	0.11 \pm 0.02
7.927	1029	Limonene	9.79 \pm 0.55	6.23 \pm 0.1	7.08 \pm 0.65	8.19 \pm 1.13
7.99	1031	1,8-Cineole	1.72 \pm 0.35	2.92 \pm 0.18	3.52 \pm 0.01	2.3 \pm 0.14
8.8	1060	γ -Terpinene	0.25 \pm 0.02	0.42 \pm 0.09	0.05 \pm 0	0.03 \pm 0
9.086	1070	cis-Sabinene hydrate	1.11 \pm 0.2	1.34 \pm 0.37	0.69 \pm 0.23	0.28 \pm 0
12.072	1165	Borneol	0.56 \pm 0.02	1.16 \pm 0.03	0.66 \pm 0.04	0.63 \pm 0.05
12.341	1174	Terpinen-4-ol	1 \pm 0.2	1.13 \pm 0.31	0.47 \pm 0.07	0.18 \pm 0.01
12.53	1186	α -Terpineol	0.12 \pm 0.03	0.19 \pm 0	0.31 \pm 0.01	0.25 \pm 0
12.83	1191	cis-Dihydrocarvone	4.59 \pm 0.39	1.84 \pm 0.01	0.65 \pm 0.18	1.79 \pm 0.56
12.959	1192	Dihydrocarveol	13.76 \pm 0.11	4.82 \pm 0.2	2.27 \pm 0.35	2.49 \pm 1.4
13.511	1215	trans-Carveol	1.23 \pm 0.33	1.13 \pm 0.09	0.52 \pm 0.02	1.28 \pm 0.07
13.904	1226	cis-Carveol	3.57 \pm 0.02	1.71 \pm 0.02	0.73 \pm 0.09	1.07 \pm 0.07
14.12	1233	Pulegone	2.3 \pm 0.16	1.32 \pm 0.08	0.63 \pm 0.06	0.58 \pm 0.05
14.239	1239	Carvone	36.9 \pm 2.67	59.09 \pm 0.74	76.82 \pm 0.87	75.18 \pm 2.54
15.32	1287	Bornyl acetate	0.99 \pm 0.07	0.3 \pm 0.03	0.1 \pm 0.01	0.39 \pm 0.1
16.464	1329	iso-Dihydrocarveol acetate	7.67 \pm 0.76	0.83 \pm 0	0.12 \pm 0.02	0.36 \pm 0.07
16.692	1339	trans-Carvyl acetate	0.87 \pm 0.02	0.35 \pm 0.12	0.06 \pm 0.04	0.31 \pm 0.14
18.123	1387	β -Bourbonene	0.64 \pm 0.12	1.78 \pm 0.08	0.79 \pm 0.02	0.46 \pm 0.03
18.278	1389	β -Elemene	0.96 \pm 0.06	1.48 \pm 0.11	0.38 \pm 0.04	0.3 \pm 0
19.079	1417	ϵ -Caryophyllene	2.27 \pm 0.16	3.87 \pm 0.06	1.38 \pm 0.15	0.81 \pm 0.07
20.2	1430	β -Copaene	0.51 \pm 0.18	0.77 \pm 0.06	0.04 \pm 0	0.23 \pm 0.02
20.7	1484	Germacrene D	0.43 \pm 0.17	1.57 \pm 0.05	0.14 \pm 0	0.15 \pm 0
21.1	1500	Bicylogermacrene	0.15 \pm 0.09	1.03 \pm 0.04	0.06 \pm 0	0.09 \pm 0.02
21.7	1529	trans-Calamenene	0.27 \pm 0.13	0.39 \pm 0.07	0.01 \pm 0	0.11 \pm 0.02
23.2	1582	Caryophyllene oxide	0.18 \pm 0.03	1.45 \pm 0.48	0.84 \pm 0.09	0.73 \pm 0.16
Essential oil yield (%)			0.87 \pm 0.02	1.94 \pm 0.04	2.79 \pm 0.08	2.54 \pm 0.09
Chemical group						
Monoterpene hydrocarbons			8.2 \pm 2.69	2.9 \pm 0.73	1.7 \pm 0.18	1.8 \pm 0.01
Oxygenated monoterpenes			86.4 \pm 4.02	84.8 \pm 0.01	94.7 \pm 0.53	95.3 \pm 0.4
Sesquiterpene hydrocarbons			5.2 \pm 1.29	10.9 \pm 0.04	2.8 \pm 0.23	2.2 \pm 0.17
Oxygenated sesquiterpenes			0.2 \pm 0.05	1.4 \pm 0.68	0.8 \pm 0.12	0.7 \pm 0.22

RT: retention time; RI: retention index.

The percentage of carvone in the EOs of *M. spicata* plants (Table 1 and Figure 1) increased gradually from winter to spring where it reached 59.09%; the percentage reached its maximum level of 76.82 and 75.18% in summer and fall months, respectively. The seasonal variations in phytochemical profiles of the EOs might be due to the impact of the phenological status and environmental conditions which can affect the regulation of the biosynthesis of EOs [11, 16, 52].

The Pearson correlation exhibited significant negative correlation between carvone and other phytochemical components (not presented). Carvone was negatively correlated with cis-carveol ($r = -0.88$), pulegone ($r = -0.83$), and dihydrocarveol ($r = -0.79$), indicating very high reverse correlation between carvone and these components. Lower percentages of carvone in EOs were shown during winter and spring months accompanied by an increased concentration of limonene, dihydrocarveol, pulegone, and cis-carveol.

The data shown in Table 1 demonstrate that the oxygenated monoterpenes (OMs) contained the highest

percentages of all the tested EOs in the range of 84.8–95.3% over the study period, followed by sesquiterpene hydrocarbons in a range of 2.2–10.9% over the study period. Our results are consistent with those of Hussain et al. [11] who also found OMs to be dominant (81.48% and 78.33%) in *M. spicata* oils collected during summer and winter months, respectively, followed by monoterpene hydrocarbons and sesquiterpene hydrocarbons.

Oxygenated monoterpenes generally followed a variation pattern (strong positive correlation between EO yield and OM percentage ($r = 0.696$)) comparable to that of EO yield with the highest OM percentages (94.7–95.3%) concurring with warmer seasons (summer and early fall) characterized by higher plant growth rates when the plants were in full bloom and with the lowest OM percentages (84.8–86.4%) coinciding with cooler weather conditions in winter and early spring characterized by lower plant growth rates when the plants reached the end of their growing season (Figure 2). These results are in agreement with those

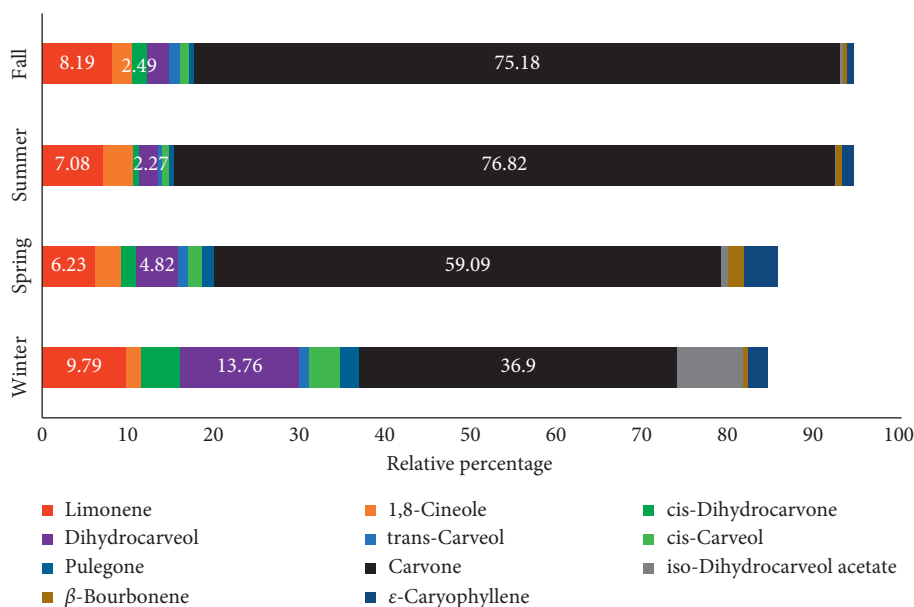


FIGURE 1: Seasonal variation of percentage of *M. spicata* EO major compounds over a 3-year study period.

of Hussain et al. [11] who also described considerable seasonal variations in the content of the EO of *M. spicata*. This result would help scientists and industrialists to select ideal harvest time, allowing producing oil with high concentration of MOs. Since *M. spicata* EOs are rich in MOs, they are predicted to have high antifungal and enzyme inhibitory activities [53, 54].

3.3. *Mentha spicata* Antidermatophytic Activity. Dermatophytes are conceived to affect about 25% the world population [55–57]. The current treatment against dermatophytosis is based on synthetic antimycotic drugs. However, these artificial drugs are somewhat expensive in addition to having adverse reactions and slow action. The use of synthetic antifungal drugs, especially in case the drugs are not well managed, can increase the chances of recurrence and of selecting resistant strains [57, 58]. However, EOs obtained from aromatic plants are very vital natural products which have diverse therapeutic and biological activities, and EOs are known to be inhibitive or lethal to fungi and represent a potential source of new antifungal agents [53]. In view of the growing resistance to the conventional antimycotic agents, the EOs may be beneficial in the clinical management of mycosis, mainly dermatophytosis [59].

In the current study, *M. spicata* EO and carvone exhibited high antidermatophytic activity against *M. canis*, *T. rubrum*, *T. mentagrophytes*, and *E. floccosum* as indicated by PI, MIC, MFC, and EC_{50} (Figures 2 and 3).

The EO of *M. spicata* and its main compound (carvone) showed a dose-dependent activity against the test dermatophytes (Figure 3). Generally, as the dose of the EO or carvone increased, the antidermatophytic activity increased, represented by an increase in the mycelial growth inhibition. The results of PI values at different concentrations of oil and carvone are presented in Figure 2. The results indicated that

the radial growth of all tested strains was entirely inhibited by the EO and carvone at 1, 2, and 4 $\mu\text{L}/\text{mL}$ concentrations. However, at lower doses (0.03–0.5 $\mu\text{L}/\text{mL}$), the oil and carvone were generally more active on the mycelia growth of *T. mentagrophytes* than other tested dermatophytes; at 0.5 $\mu\text{L}/\text{mL}$, PI = 94.1% and 100% for oil and carvone, respectively (Figure 2).

The MICs of the EO of *M. spicata* on the test dermatophytes ranged from 0.75 to 2 $\mu\text{L}/\text{mL}$, and the EC_{50} of *M. spicata* EO ranged from 0.25 to 0.46 $\mu\text{L}/\text{mL}$. However, EO showed fungicidal effect on the four studied dermatophytes, and the MFCs ranged from 2 to 4 $\mu\text{L}/\text{mL}$. *T. mentagrophytes* was more susceptible to EO than the other tested fungi with MIC, MFC, and EC_{50} values of 0.44, 1.38, and 0.2 $\mu\text{L}/\text{mL}$, respectively.

The strong antidermatophytic activity may be elucidated by the main component of the EOs, carvone, the oxygenated monoterpene which exhibited strong inhibitory activity against the tested dermatophytes (Figure 2) with PI, MIC, EC_{50} , and MFC values ranging from 80 to 100%, 0.44 to 0.63 $\mu\text{L}/\text{mL}$, 0.2 to 0.35 $\mu\text{L}/\text{mL}$, and 1.38 to 2.25 $\mu\text{L}/\text{mL}$, respectively. The monoterpene alcohols are known to have strong antidermatophytic activity because of their good solubility in water and the presence of the functional alcohol group. Monoterpene alcohols can exert their antidermatophytic effects by increasing the permeability of the plasma membrane, causing the disruption of the cell membrane, and inhibiting the process of respiration on the mitochondrial membrane of fungi leading to death of cells or inhibiting the spore production and germination of dermatophytic fungi [53, 60–63].

3.4. *Mentha spicata* Antibiofilm Activity. MRSA is a common cause of dermal and soft-tissue infections. MRSA has generated increasing concern because of restricted treatment alternatives since its strains are resistant to the whole class of

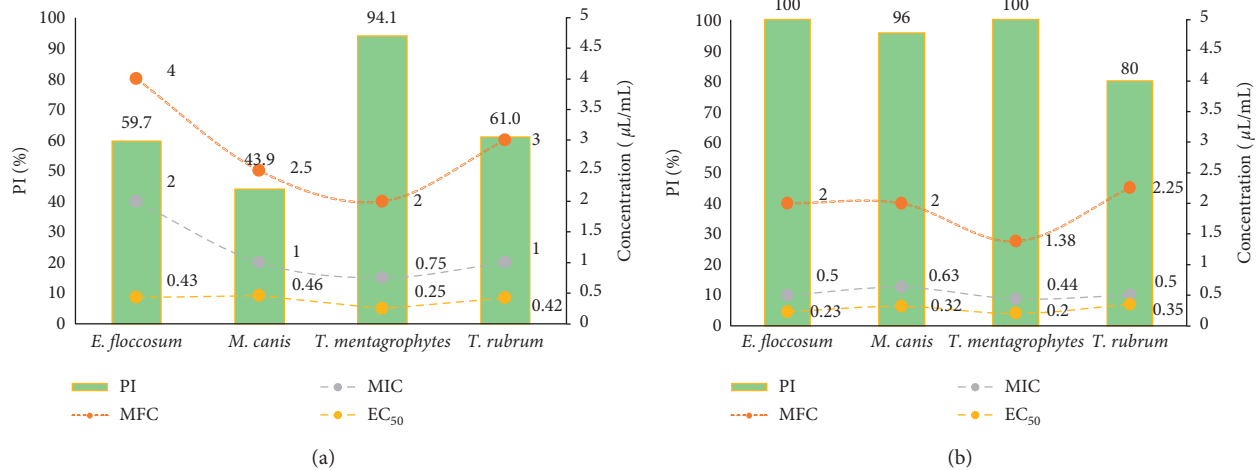


FIGURE 2: Percentage of mycelial growth inhibition (PI) of (a) *M. spicata* EO and (b) carvone against the test dermatophytes with MIC, MFC, and EC₅₀ values.

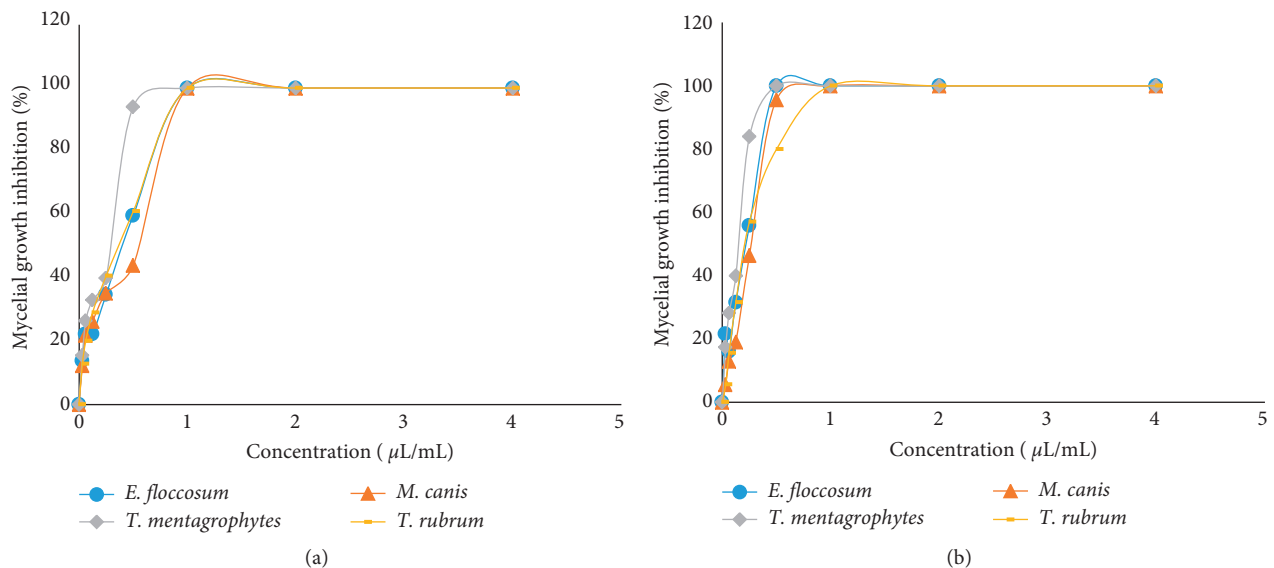


FIGURE 3: Mycelial growth inhibition activity of (a) *M. spicata* EO and (b) carvone against the test dermatophytes.

β -lactam antibiotics [64]. Eradication of MRSA is not always successful because of its ability to form biofilms.

Biofilms are communities of sessile microorganisms that are embedded in an extracellular matrix of proteins, lipids, polysaccharides, and nucleic acids, which confers protection to bacteria against host defenses and inhibits the delivery of antimicrobial agents [65]. Biofilm-associated bacteria are much more resistant to antimicrobial agents. The discovery of anti-infective agents with antibiofilm activity represents an important objective. In fact, inhibition of the biofilm formation effect of *M. spicata* essential oil has been reported in the case of *Vibrio* spp., *Staphylococcus aureus*, and dental biofilm [12, 66, 67].

In the current study, we evaluated the ability of several doses of *M. spicata* EO and its main component (carvone) to inhibit or destroy biofilm on a polystyrene surface formed by MRSA.

3.4.1. Effects on Biofilm Formation (Inhibition of Cell Attachment). Prevention of biofilm formation was examined on MRSA and assessed by crystal violet and TTC assays. The results were expressed as inhibition % of biofilm development. *M. spicata* EO exhibited a significant inhibitory activity on biomass and metabolic activity of MRSA biofilm formation in a dose-dependent manner (Figure 4). The crystal violet assay showed that *M. spicata* EO reduced the number of adherent bacteria (biomass) by 71.3% at 5 $\mu\text{L/mL}$, and the observed biofilm inhibitory concentration (BIC₅₀) was 0.37 $\mu\text{L/mL}$ (Table 2).

Inhibition of biofilm formation by *M. spicata* EO was confirmed by the TTC assay; in the presence of *M. spicata* EO, the metabolic oxidative activity was noticeably decreased after 24 h of incubation by 89.5% at 5 $\mu\text{L/mL}$ concentration compared to the nontreated biofilm (Figure 5), and the observed BIC₅₀ value was 0.41 $\mu\text{L/mL}$ (Table 2).

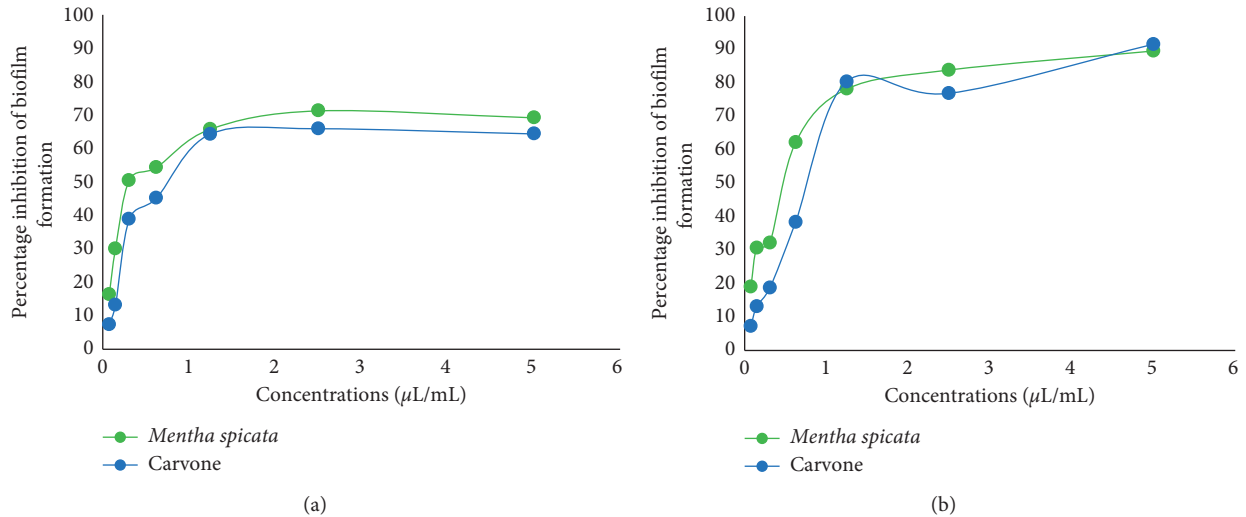


FIGURE 4: Effects of various doses *M. spicata* EO and carvone on biofilm formation indicated as % of inhibition, evaluated by the (a) crystal violet (CV) assay and (b) TTC assay.

TABLE 2: Antibiofilm effect of *Mentha spicata* EO and carvone against the MRSA-positive biofilm strain.

	Effect upon biofilm formation		Effect upon preformed biofilm	
	BIC ₅₀ (µL/mL)			
	CV assay	TTC assay	CV assay	TTC assay
<i>Mentha spicata</i>	0.37 ± 0.05	0.41 ± 0.13	0.39 ± 0.11	0.89 ± 0.05
Carvone	0.53 ± 0.14	0.68 ± 0.1	0.34 ± 0.06	0.66 ± 0.02

These results demonstrated that, in addition to the decreasing number of attached bacteria (biomass) evaluated by the CV assay, *M. spicata* EO has a strong inhibitory effect on metabolic activity of cells embedded in the biofilm.

3.4.2. Effects on Preformed Biofilm. Essential oil from *M. spicata* and carvone showed significant effects on the biomass and oxidative metabolic activity of the preformed biofilm in a dose-dependent manner (Figure 5). *M. spicata* essential oil eradicated more than 75% of preformed biofilm biomass and metabolic activity at 5 µL/mL. The BIC₅₀ value observed by crystal violet and TTC assays was 0.39 and 0.89 µL/mL, respectively.

The results demonstrate that, in addition to its activity against biofilm formation, *M. spicata* EO had a strong inhibitory effect on metabolic activity and biomass of the preformed biofilm. The significant activity of *M. spicata* EO on the MRSA biofilm may be elucidated by the major component of the EOs, carvone (Figures 4 and 5). Carvone has a strong effect on biofilm biomass and metabolic activity with the BIC₅₀ value ranging between 0.34 and 0.68 µL/mL (Table 2).

The success of *M. spicata* EO in inhibiting bacterial adhesion is a promising approach for treating infections of mucosal surfaces by reducing microbial colonization on surfaces and epithelial mucosa which leads to preventing the establishment of bacterial pathogenesis [68, 69].

3.5. Cholinesterase Inhibitory Activity of *M. spicata* Essential Oil. Several plant species from the Lamiaceae family are used in TAPHM to enhance memory [4, 31]. The ability of these plants to boost the cholinergic function by inhibiting cholinesterase is the most acceptable theory for their memory-enhancing activities [4]. In this study, the EO of *M. spicata* showed high levels of inhibitory activity against AChE and BuChE; the EO inhibited cholinesterase enzymes in a concentration-dependent manner, with AChE (IC₅₀ = 23.1 µL/mL) and BuChE (IC₅₀ = 35.0 µL/mL) inhibitory activities (Table 3), matching inhibitory activities of carvone (EO's main component) against AChE (IC₅₀ = 19.01 µL/mL) and BuChE (IC₅₀ = 32.33 µL/mL). It is therefore speculated that the anticholinesterase activity of the major monoterpenoid component (carvone) is accountable for the observed inhibitory effect, of the *M. spicata* aromatic plant [70, 71].

According to the results of the current study, it is highly recommended that the EO extracted from the aerial parts of *M. spicata* be further explored for possible useful effects on neurodegenerative disorders, such as AD, utilizing its cholinesterase inhibition activities.

3.6. Pancreatic Lipase Inhibitory Activity of *M. spicata* Essential Oil. In the current study, *M. spicata* EO and its main component (carvone) were assessed for lipid-lowering activity through inhibition of porcine pancreatic lipase (PPL) (Table 4). *M. spicata* EO and carvone had a lipase inhibitory concentration (MIC₅₀) of 12.5 µL/mL. The fractional inhibitory concentrations (FICs) of the dual combinations of EO or carvone with orlistat are presented in Table 4. Both combinations displayed a synergistic effect against the lipase enzyme. The synergistic effect was presented graphically by using the isobologram method (Figure 6).

In this regard, the PPL inhibitory activities may in part be elucidated by the high content of carvone and other monoterpenes. The results support the view that *M. spicata*

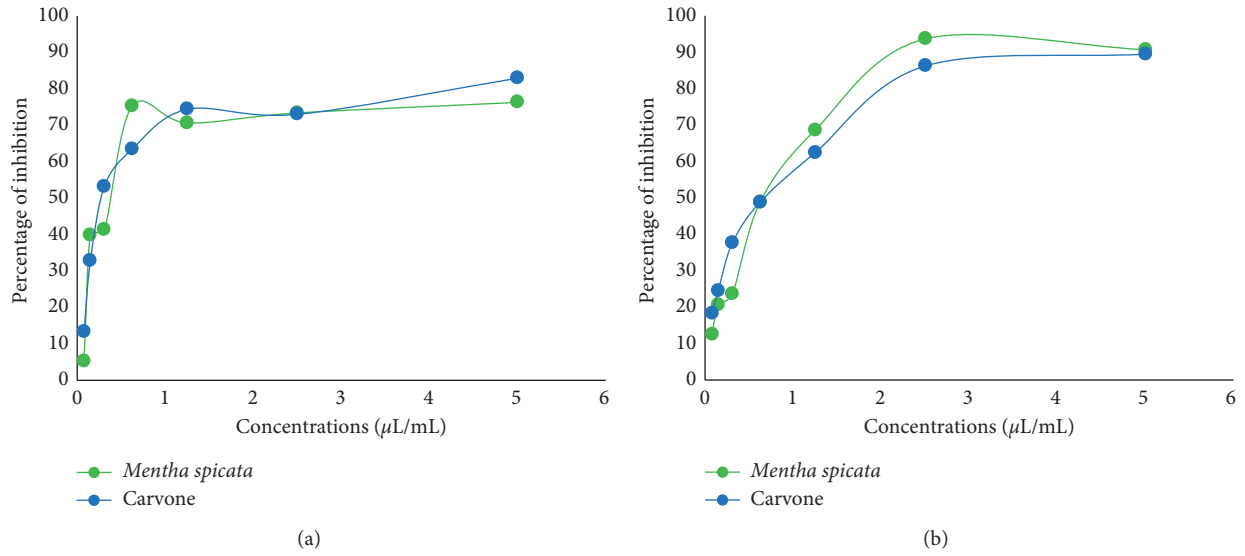


FIGURE 5: Effects of various doses of *M. spicata* EO and carvone on the preformed biofilm presented as % of inhibition, evaluated by the (a) crystal violet (CV) assay and (b) TTC assay.

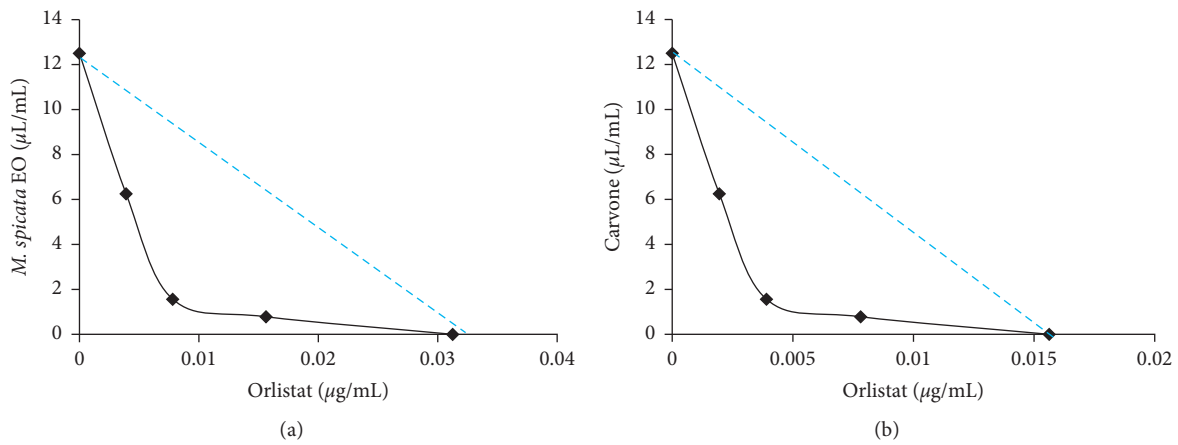


FIGURE 6: Isobologram curve of double combinations of EO or carvone with orlistat against porcine pancreatic lipase.

TABLE 3: Cholinesterase inhibitory activities of essential oil from *M. spicata* growing in Palestine.

	IC ₅₀ (µL/mL)	
	Acetylcholinesterase (AChE)	Butyrylcholinesterase (BuChE)
Essential oil	23.1 ± 0.26	35.0 ± 0.37
Carvone	19.01 ± 0.45	32.33 ± 0.09
Galanthamine	0.0076	0.229
Neostagmine	0.0008	0.104

TABLE 4: FIC and interaction effects of double combinations of EO or carvone with orlistat on pancreatic lipase inhibitory activities.

Combination (A/B)	MIC ₅₀ A (alone) (µL/mL)	MIC ₅₀ B (alone) (µg/mL)	MIC ₅₀ A (in the presence of B) (µL/mL)	MIC ₅₀ B (in the presence of A) (µg/mL)	Checkerboard FIC index
EO/orlistat	12.5	0.032	0.078	0.0039	0.11
Carvone/orlistat	12.5	0.032	0.078	0.0019	0.065

signifies a rich source of antilipase constituents. Jamous et al. [7] have previously reported that the ethanolic extracts of *M. spicata* exhibit a strong PPL inhibitory property with an IC₅₀ of 1.19 mg/ml. However, to the best of our knowledge, EO from this plant or its phytochemicals have not been previously studied for their lipase inhibitory activity.

4. Conclusions

Because of its high biological activities, EO from *Mentha spicata* may be used for the development of new formulations of nutraceutical products for the management of chronic diseases such as Alzheimer's disease and overweight. *M. spicata* EO can fulfill the growing demand of industries for constant and good raw natural sources of antidermatophytic, anticholinesterase, and antiobesity agents that could be safer than synthetic drugs. To attain the optimum oil yield with a high quantity and content of OMs (particularly carvone), *M. spicata* should be extracted when the plants are in full bloom. The strong inhibitory activity of *M. spicata* EOs on AChE and PPL and antidermatophytic properties may be due to their high concentrations of carvone monoterpenes. The results also showed that, in addition to their significant inhibitory activity against biofilm formation of MRSA, *M. spicata* EO and carvone had a strong inhibitory effect on metabolic activity and biomass of the preformed biofilm. Besides the biological activities described here, the results support the use of *M. spicata* EO as a folk medicine. The results also provide further evidence for the utilization of *M. spicata* EO and its principal constituents as an important source of anticholinesterase, antiobesity, antidermatophytic, and anti-MRSA agents.

Abbreviations

AChE:	Acetylcholinesterase
AD:	Alzheimer's disease
BERC:	Biodiversity and Environmental Research Center
BuChE:	Butyrylcholinesterase
EC ₅₀ :	Effective concentration with 50% inhibitory activity
TAPHM:	Traditional Arabic Palestinian Herbal Medicine
IC ₅₀ :	Inhibitory concentration with 50% activity
MFC:	Minimum fungicidal concentration
MIC:	Minimum inhibitory concentration
MRSA:	Methicillin-resistant <i>Staphylococcus aureus</i>
OMs:	Oxygenated monoterpenes
PI:	Percent of inhibition
PPL:	Porcine pancreatic lipase
RI:	Retention index
RT:	Retention time.

Data Availability

The datasets supporting the results of this study will be freely available upon request to the corresponding author (msshtayeh@yahoo.com) for noncommercial use only.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank Prof. N. Dudai for help with GC-MS analysis and Rola Akkawi, Samer Jarrar, and Omar Mallah for technical support. This research was partially funded by the Middle East Regional Cooperation (MERC) Project (Project no. M29-033; Award no. SIS70013GR29033).

References

- [1] B. M. Lawrence, *Mint: The Genus Mentha*, CRC Press, Boca Raton, FL, USA, 2007.
- [2] V. D. Topalov, "Mentha," in *Crop Production*, M. S. Topalov, V. D. Dechev, and I. I. Pehlivanov, Eds., Sofia, Bulgaria: Zemizdat Press, pp. 372–381, 1989.
- [3] V. D. Zheljaskov, C. L. Cantrell, T. Astatkie, and A. Hristov, "Yield, content, and composition of peppermint and spearmints as a function of harvesting time and drying," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 21, pp. 11400–11407, 2010.
- [4] M. S. Ali-Shtayeh, R. M. Jamous, S. Y. Abu-Zaitoun, and I. B. Qasem, "In-vitro screening of acetylcholinesterase inhibitory activity of extracts from Palestinian indigenous flora in relation to the treatment of Alzheimer's disease," *Functional Foods in Health and Disease*, vol. 4, no. 9, pp. 381–400, 2014.
- [5] N. Bernstein, D. Chaimovitch, and N. Dudai, "Effect of irrigation with secondary treated effluent on essential oil, antioxidant activity, and phenolic compounds in oregano and rosemary," *Agronomy Journal*, vol. 101, no. 1, pp. 1–10, 2009.
- [6] H. Özer, M. Sökmen, M. Güllüce et al., "Chemical composition and antimicrobial and antioxidant activities of the essential oil and methanol extract of *Hippomarathrum microcarpum* (bieb.) from Turkey," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 3, pp. 937–942, 2007.
- [7] R. M. Jamous, S. Y. Abu-Zaitoun, R. J. Akkawi, and M. S. Ali-Shtayeh, "Antiobesity and antioxidant potentials of selected palestinian medicinal plants," *Evidence-Based Complementary and Alternative Medicine*, vol. 2018, Article ID 8426752, 21 pages, 2018.
- [8] M. S. Ali-Shtayeh, A. A. Al-Assali, and R. M. Jamous, "Antimicrobial activity of Palestinian medicinal plants against acne-inducing bacteria," *African Journal of Microbiology*, vol. 7, no. 21, pp. 2560–2573, 2013.
- [9] S. Laggoune, M. Öztürk, E. Erol et al., "Chemical composition, antioxidant and antibacterial activities of the essential oil of *Mentha spicata* L. from Algeria," *Journal of Materials and Environmental Science*, vol. 7, no. 11, pp. 4205–4213, 2016.
- [10] L. Jirovetz, G. Buchbauer, M. Shahabi, and M. B. Ngassoum, "Comparative investigations of the essential oil and volatiles of spearmint," *Perfumer and Flavorist*, vol. 27, no. 6, pp. 16–22, 2002.
- [11] A. I. Hussain, F. Anwar, P. S. Nigam, M. Ashraf, and A. H. Gilani, "Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four mentha species," *Journal of the Science of Food and Agriculture*, vol. 90, no. 11, pp. 1827–1836, 2010.
- [12] M. Snoussi, E. Noumi, N. Trabelsi, G. Flamini, A. Papetti, and V. De Feo, "*Mentha spicata* essential oil: chemical composition, antioxidant and antibacterial activities against

- planktonic and biofilm cultures of vibrio spp. strains," *Molecules*, vol. 20, no. 8, pp. 14402–14424, 2015.
- [13] K. Oosterhaven, B. Poolman, and E. J. Smid, "S-carvone as a natural potato sprout inhibiting, fungistatic and bacteristatic compound," *Industrial Crops and Products*, vol. 4, no. 1, pp. 23–31, 1995.
- [14] Y. Zu, H. Yu, L. Liang et al., "Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells," *Molecules*, vol. 15, no. 5, pp. 3200–3210, 2010.
- [15] M. Sylvestre, A. Pichette, A. Longtin, F. Nagau, and J. Legault, "Essential oil analysis and anticancer activity of leaf essential oil of croton flavens L. from Guadeloupe," *Journal of Ethnopharmacology*, vol. 103, no. 1, pp. 99–102, 2006.
- [16] M. S. Ali-Shtayeh, R. M. Jamous, S. Y. Abu-Zaitoun et al., "Secondary treated effluent irrigation did not impact chemical composition, and enzyme inhibition activities of essential oils from *Origanum syriacum* var. *syriacum*," *Industrial Crops and Products*, vol. 111, pp. 775–786, 2018.
- [17] E. Pinto, K. Hrimpeng, G. Lopes et al., "Antifungal activity of ferulago capillaris essential oil against candida, cryptococcus, aspergillus and dermatophyte species," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 32, no. 10, pp. 1311–1320, 2013.
- [18] R. Ravizza, M. B. Gariboldi, R. Molteni, and E. Monti, "Linalool, a plant-derived monoterpene alcohol, reverses doxorubicin resistance in human breast adenocarcinoma cells," *Oncology Reports*, vol. 20, no. 3, pp. 625–630, 2008.
- [19] D. Dangkong and W. Limpanasithikul, "Effect of citral on the cytotoxicity of doxorubicin in human B-lymphoma cells," *Pharmaceutical Biology*, vol. 53, no. 2, pp. 262–268, 2015.
- [20] S. Carnesecchi, K. Langley, F. Exinger, F. Gosse, and F. Raul, "Geraniol, a component of plant essential oils, sensitizes human colonic cancer cells to 5-fluorouracil treatment," *Journal of Pharmacology and Experimental Therapeutics*, vol. 301, no. 2, pp. 625–630, 2002.
- [21] E. Fitsiou, G. Mitropoulou, K. Spyridopoulou et al., "Phytochemical profile and evaluation of the biological activities of essential oils derived from the Greek aromatic plant species *Ocimum basilicum*, *Mentha spicata*, *Pimpinella anisum* and *Fortunella margarita*," *Molecules*, vol. 21, no. 8, pp. 1–15, 2016.
- [22] N. N. R. Cardoso, C. S. Alviano, A. F. Blank et al., "Synergism effect of the essential oil from *Ocimum basilicum* var. *Maria bonita* and its major components with fluconazole and its influence on ergosterol biosynthesis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2016, Article ID 5647182, 12 pages, 2016.
- [23] P. S. X. Yap, B. C. Yiap, H. C. Ping, and S. H. E. Lim, "Essential oils, a new horizon in combating bacterial antibiotic resistance," *The Open Microbiology Journal*, vol. 8, no. 1, pp. 6–14, 2014.
- [24] S. Shin and S. Lim, "Antifungal effects of herbal essential oils alone and in combination with ketoconazole against *Trichophyton* spp.," *Journal of Applied Microbiology*, vol. 97, no. 6, pp. 1289–1296, 2004.
- [25] M. S. A. Khan and I. Ahmad, "Antifungal activity of essential oils and their synergy with fluconazole against drug-resistant strains of *Aspergillus fumigatus* and *Trichophyton rubrum*," *Applied Microbiology and Biotechnology*, vol. 90, no. 3, pp. 1083–1094, 2011.
- [26] K. Sak, "Chemotherapy and dietary phytochemical agents," *Chemotherapy Research and Practice*, vol. 2012, Article ID 282570, 11 pages, 2012.
- [27] M. S. Ali-Shtayeh, R. M. Jamous, S. Y. Abu-Zaitoun et al., "Chemical profile and bioactive properties of the essential oil isolated from *Clinopodium serpyllifolium* (M. Bieb.) Kuntze growing in Palestine," *Industrial Crops and Products*, vol. 124, pp. 617–625, 2018.
- [28] M. S. Ali-Shtayeh, R. M. Jamous, R. M. Jamous, and N. M. Y. Salameh, "Complementary and alternative medicine (CAM) use among hypertensive patients in Palestine," *Complementary Therapies in Clinical Practice*, vol. 19, no. 4, pp. 256–263, 2013.
- [29] M. S. Ali-Shtayeh, R. M. Jamous, and R. M. Jamous, "Plants used during pregnancy, childbirth, postpartum and infant healthcare in Palestine," *Complementary Therapies in Clinical Practice*, vol. 21, no. 2, pp. 84–93, 2015.
- [30] M. S. Ali-Shtayeh, Z. Yaniv, and J. Mahajna, "Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants," *Journal of Ethnopharmacology*, vol. 73, no. 1-2, pp. 221–232, 2000.
- [31] M. S. Ali-Shtayeh, R. M. Jamous, J. H. Al-Shafe' et al., "Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): a comparative study," *Journal of Ethnobiology and Ethnomedicine*, vol. 4, no. 1, p. 13, 2008.
- [32] M. S. Ali Shtayeh and R. M. Jamous, *Traditional Arabic Palestinian Herbal Medicine*, TAPHM. Til, Nablus, Biodiversity and Environmental Research Center, Til, Nablus, Palestine, 2008.
- [33] M. S. Ali-Shtayeh, R. M. Jamous, and R. M. Jamous, "Traditional Arabic Palestinian ethnoveterinary practices in animal health care: a field survey in the West Bank (Palestine)," *Journal of Ethnopharmacology*, vol. 182, pp. 35–49, 2016.
- [34] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation, Carol Stream, IL, USA, 2007.
- [35] M. M. Gakuubi, A. W. Maina, and J. M. Wagacha, "Antifungal activity of essential oil of *Eucalyptus camaldulensis* dehn. against selected *Fusarium* spp.," *International Journal of Microbiology*, vol. 2017, Article ID 8761610, 7 pages, 2017.
- [36] A. S. O. Mohareb, M. E. I. Badawy, and S. A. M. Abdelgaleil, "Antifungal activity of essential oils isolated from Egyptian plants against wood decay fungi," *Journal of Wood Science*, vol. 59, no. 6, pp. 499–505, 2013.
- [37] S. A. Euloge, K. Sandrine, D.-A. Edwige, S. Dominique, and S. Mohamed, "Antifungal activity of *Ocimum canum* essential oil against toxinogenic fungi isolated from peanut seeds in post-harvest in Benin," *International Research Journal of Biological Sciences*, vol. 1, no. 7, pp. 20–26, 2012.
- [38] S. Chusri, K. Sompetch, S. Mukdee et al., "Inhibition of staphylococcus epidermidis biofilm formation by traditional thai herbal recipes used for wound treatment," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 159797, 8 pages, 2012.
- [39] M.-H. Lin, F.-R. Chang, M.-Y. Hua, Y.-C. Wu, and S.-T. Liu, "Inhibitory effects of 1,2,3,4,6-penta-O-galloyl- β -D-glucopyranose on biofilm formation by *Staphylococcus aureus*," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 3, pp. 1021–1027, 2011.
- [40] P. Sabaeifard, A. Abdi-Ali, M. R. Soudi, and R. Dinarvand, "Optimization of tetrazolium salt assay for *Pseudomonas aeruginosa* biofilm using microtiter plate method," *Journal of Microbiological Methods*, vol. 105, pp. 134–140, 2014.
- [41] J. Chojniak, I. Biedroń, and G. Plaza, "TTC- based test as an efficient method to determine antibiofilm activity of silver nanoparticles," *E3S Web of Conferences*, vol. 17, Article ID 00015, 2017.
- [42] P. Knezevic and O. Petrovic, "A colorimetric microtiter plate method for assessment of phage effect on *Pseudomonas*

- aeruginosa* biofilm,” *Journal of Microbiological Methods*, vol. 74, no. 2-3, pp. 114–118, 2008.
- [43] S. Shakeri, R. K. Kermanshahi, M. M. Moghaddam, and G. Emtiazi, “Assessment of biofilm cell removal and killing and biocide efficacy using the microtiter plate test,” *Biofouling*, vol. 23, no. 2, pp. 79–86, 2007.
- [44] H. L. Brown, A. H. M. van Vliet, R. P. Betts, and M. Reuter, “Tetrazolium reduction allows assessment of biofilm formation by *Campylobacter jejuni* in a food matrix model,” *Journal of Applied Microbiology*, vol. 115, no. 5, pp. 1212–1221, 2013.
- [45] E. Peeters, H. J. Nelis, and T. Coenye, “Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates,” *Journal of Microbiological Methods*, vol. 72, no. 2, pp. 157–165, 2008.
- [46] M. Nikkiah, M. Hashemi, M. B. Habibi Najafi, and R. Farhoosh, “Synergistic effects of some essential oils against fungal spoilage on pear fruit,” *International Journal of Food Microbiology*, vol. 257, pp. 285–294, 2017.
- [47] J. Gutierrez, C. Barry-Ryan, and P. Bourke, “The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients,” *International Journal of Food Microbiology*, vol. 124, no. 1, pp. 91–97, 2008.
- [48] J. Krisch, R. Tserennadmid, and C. Vágvölgyi, “Essential oils against yeasts and moulds causing food spoilage,” in *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances*, A. Méndez-Vilas, Ed., pp. 1135–1142, Formatex Research Center, Badajoz, Spain, 2011.
- [49] S. F. van Vuuren, S. Suliman, and A. M. Viljoen, “The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobials,” *Letters in Applied Microbiology*, vol. 48, no. 4, pp. 440–446, 2009.
- [50] A. E. Edris, A. S. Shalaby, H. M. Fadel, and M. A. Abdel-Wahab, “Evaluation of a chemotype of spearmint (*Mentha spicata* L.) grown in Siwa Oasis, Egypt,” *European Food Research and Technology*, vol. 218, no. 1, pp. 74–78, 2003.
- [51] M. D. Soković, J. Vukojević, P. D. Marin et al., “Chemical composition of essential oils of *Thymus* and *mentha* species and their antifungal activities,” *Molecules*, vol. 14, no. 1, pp. 238–249, 2009.
- [52] V. Masotti, F. Juteau, J. M. Bessière, and J. Viano, “Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisiamolineri* and its biological activities,” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 24, pp. 7115–7121, 2003.
- [53] P. Baidara and S. Korpole, *Recent Trends in Antifungal Agents and Antifungal Therapy*, Springer, Berlin, Germany, 2016.
- [54] A. N. E. Gendy, M. Leonardi, L. Mugnaini et al., “Chemical composition and antimicrobial activity of essential oil of wild and cultivated *Origanum syriacum* plants grown in Sinai, Egypt,” *Industrial Crops and Products*, vol. 67, pp. 201–207, 2015.
- [55] B. Havlickova, V. A. Czaika, and M. Friedrich, “Epidemiological trends in skin mycoses worldwide,” *Mycoses*, vol. 51, pp. 2–15, 2008.
- [56] M. S. Ali-Shtayeh, S. Yaish, R. M. Jamous, H. Arda, and E. I. Husein, “Updating the epidemiology of dermatophyte infections in Palestine with special reference to concomitant dermatophytosis,” *Journal de Mycologie Médicale*, vol. 25, no. 2, pp. 116–122, 2015.
- [57] D. Zimmermam-Franco, E. Bolutari, H. Polonini et al., “Antifungal activity of *Copaifera langsdorffii* desf oleoresin against dermatophytes,” *Molecules*, vol. 18, no. 10, pp. 12561–12570, 2013.
- [58] A. Espinel-ingroff, “Novel antifungal agents, targets or therapeutic strategies for the treatment of invasive fungal diseases: a review of the literature (2005–2009),” *Revista Iberoamericana de Micología*, vol. 26, no. 1, pp. 15–22, 2009.
- [59] M. Zuzarte, M. J. Gonçalves, C. Cavaleiro, A. M. Dinis, J. M. Canhoto, and L. R. Salgueiro, “Chemical composition and antifungal activity of the essential oils of *Lavandula pedunculata* (miller) cav,” *Chemistry & Biodiversity*, vol. 6, no. 8, pp. 1283–1292, 2009.
- [60] O. Toncer, S. Karaman, and E. Diraz, “An annual variation in essential oil composition of *Origanum syriacum* from southeast anatolia of Turkey,” *Journal of Medicinal Plants Research*, vol. 4, no. 11, pp. 1059–1064, 2010.
- [61] S. D. Cox, C. M. Mann, J. L. Markham et al., “The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil),” *Journal of Applied Microbiology*, vol. 88, no. 1, pp. 170–175, 2000.
- [62] F. Deba, T. D. Xuan, M. Yasuda, and S. Tawata, “Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *Radiata*,” *Food Control*, vol. 19, no. 4, pp. 346–352, 2008.
- [63] F. Nazzaro, F. Fratianni, R. Coppola, and V. De Feo, “Essential oils and antifungal activity,” *Pharmaceuticals*, vol. 10, no. 4, p. 86, 2017.
- [64] N. E. Holmes and B. P. Howden, “What’s new in the treatment of serious MRSA infection?,” *Current Opinion in Infectious Diseases*, vol. 27, no. 6, pp. 471–478, 2014.
- [65] C. L. Quave, L. R. W. Plano, T. Pantuso, and B. C. Bennett, “Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*,” *Journal of Ethnopharmacology*, vol. 118, no. 3, pp. 418–428, 2008.
- [66] D. Vetas, E. Dimitropoulou, G. Mitropoulou, Y. Kourkoutas, and E. Giaouris, “Disinfection efficiencies of sage and spearmint essential oils against planktonic and biofilm *Staphylococcus aureus* cells in comparison with sodium hypochlorite,” *International Journal of Food Microbiology*, vol. 257, pp. 19–25, 2017.
- [67] I. Rasooli and S. Shayegh, “The effect of *Mentha spicata* and *Eucalyptus camaldulensis* essential oils on dental biofilm,” *International Journal of Dental Hygiene*, vol. 7, no. 3, pp. 196–203, 2009.
- [68] C. Bavington and C. Page, “Stopping bacterial adhesion: a novel approach to treating infections,” *Respiration*, vol. 72, no. 4, pp. 335–344, 2005.
- [69] M. Sandasi, C. M. Leonard, and A. M. Viljoen, “The in vitro antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*,” *Letters in Applied Microbiology*, vol. 50, no. 1, pp. 30–35, 2010.
- [70] M. Miyazawa, H. Watanabe, K. Umemoto, and H. Kameoka, “Inhibition of acetylcholinesterase activity by essential oils of *Mentha* species,” *Journal of Agricultural and Food Chemistry*, vol. 46, no. 9, pp. 3431–3434, 1998.
- [71] S. K. Bardaweel, M. M. Hudaib, K. A. Tawaha, and R. M. Bashatwah, “Studies on the in vitro antiproliferative, antimicrobial, antioxidant, and acetylcholinesterase inhibition activities associated with chrysanthemum coronarium essential oil,” *Evidence-Based Complementary and Alternative Medicine*, vol. 2015, Article ID 790838, 6 pages, 2015.