Chemical Composition and Enzymatic Screening of *Micromeria fruticosa* serpyllifolia Volatile Oils Collected from Three Different Regions of West Bank, Palestine

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**Introduction.** Volatile oils (VOs) have been commonly used in cosmetics and food as fragrances, flavoring, and preservative agents or in alternative medicine for their therapeutic effects. This necessitates investigating those plants and their VOs. This study was conducted to investigate the chemical compositions of the VOs of *Micromeria fruticosa* serpyllifolia growing widely in three regions in Palestine (i.e., Hebron, Ramallah, and Nablus districts representing south, middle, and north of West Bank). Afterwards, VOs were subjected to *in vitro* screening and their enzymatic properties were compared. **Methods.** The analysis of chemical components of VOs was performed by gas chromatography coupled with mass spectrometry (GC-MS). The antilipase activity was evaluated using porcine pancreatic lipase and *p*-nitrophenyl butyrate. The antiamylase activity was assessed using porcine pancreatic *\( \alpha \)*-amylase, starch, and 3,5-dinitrosalicylic. **Results.** Plant extracts yield range was 0.67 to 0.99 w/w%. GC-MS analysis showed the high percentages of oxygenated components in the range of 86.1-89.88% and nonoxygenated components in the range of 4.38-4.71%. Seven components were observed, pulegone was the most abundant component in the three samples in the range of 74.43-86.04%, and isomenthone was the second most abundant component with the range of 3.16-14.41%. The sample collected from Nablus showed the most potent antilipase and antiamylase activity with IC50 values of 39.81 \( \mu \)g/mL and 3.31 \( \mu \)g/mL, respectively. **Conclusions.** The study showed that *Micromeria fruticosa* serpyllifolia volatile oils samples from different regions in Palestine contained different proportions of phytochemicals which provided different potential biological activities such as antiobesity and antidiabetes activities that were in line with traditional uses of the plant extracts. The plant extracts showed higher antilipase and antiamylase potency than that of the relative references and there were significant differences in these activities compared to each other.

1. **Introduction**

Dissimilar to conventional single drug, plant extracts or raw plants have a range of phytochemicals and bioactive constituents that provide synergistic effects which allow for multitarget effect in curing of diseases [1]. The medicinal plants and their claimed traditional use are considered one of the major approaches in developing new drug from natural products [2]. Secondary metabolites, including alkaloids, glycosides, flavonoids, phenols, steroids, saponins, tannins, terpenoids, and volatile oils (VOs), are important for healing diseases and are responsible for the therapeutic effect of plants; for example, VOs have anti-inflammatory, anticancer, anthelmintic, antimalarial, antiviral, antibacterial, cholesterol inhibition, and insecticide effects [3, 4]. Volatile oil also called “ethereal oil” or “essential oil” is extracted from different parts of plant (roots, bark, leaves, flowers, fruits, etc.) [5]. The chemical compounds of VOs can be classified into oxygenated (ketones, alcohols, phenols, etc.) and hydrocarbons (limonene, pinene, etc.) [6, 7]. The chemical structures of VOs determine their therapeutic activities [6]. The chemical composition and the aroma of VOs may be different due to growing condition (climate, type of soil and composition, altitude), plant age, geo-climatic location, and environmental conditions of collection time and site [8].
Dangerous health problems causing big load to global health sector such as obesity are a global health danger, which negatively affect personal professional quality of life, morbidity, and mortality [9]. The greatest cost expended for obesity is due to coronary heart disease, diabetes type 2, and hypertension [10]. Orlistat is among the most common drugs used for obesity treatment, but there is still deficiency of safe medicine for treating obesity [9]. Diabetes has caused a major load to the global health sector [11]. Diabetes is a dangerous disease and has serious complications; WHO reported that 8.5% of adults around the world have diabetes, and 1.6 million deaths occurred in 2015 [12]. Therefore, the investigation for antidiabetic agents from plant extract has increased, as discovering new effective drugs is important for controlling the disease [11].

*Microcerma fruticosa* subspecies *serpyllifolia* (M. Bieb.) (Lamiaceae), known as White *Microcerma*, is an aromatic herb [13], dominated in the eastern Mediterranean regions including Palestine, has pleasant minty fragrance, and in hot summer summer discovering sensation of coolness [14, 15]. In Palestinian society known as Duqat ‘Adas, ‘Ishbit esh-shai, Qurnya [16, 17], and as Thyme-leaf savory in English, the aerial parts of plant (flower, leaves, and stalk) are used in folk medicine [16]. *M. fruticosa serpyllifolia* is a perennial Mediterranean plant inhabitant in rocky areas that has a height of 20-80 cm. It is short-shrub plant grown in the period of end winter and spring and starts flowering in summer until autumn with white color [15, 18–20]. Stems are straight, whitish, covered with short, dense, and soft hair, thick, and solid. Leaves are greyish-white, thyme-leaved, and covered with very finely hair. Inflorescence is a cluster of cymes with many branching flowers. Corolla is yellow or white, scarcely female, and self-pollinated in unopened flowers [21–23]. *M. fruticosa serpyllifolia* has different uses in traditional medicine such as treatment of hypertension, heart disorders, diarrhea, abdominal pains, colds, headache, wounds, infections such as skin and eye infections and has anti-inflammatory effects [15, 24–28]. In Palestinian society *M. fruticosa serpyllifolia* is considered one of the most wild edible plants in Palestine [14]. The leaves are prepared as tea for colds and relieve intestine and stomach pain in addition to exhaustion and weariness [15]. The extracts of leaves have been used for relief chest, respiratory system, asthma, fever, skin infections, wounds, and eye inflammation [18, 20]; in addition to that, the infusion of *M. fruticosa serpyllifolia* stalks and leaves in Palestinian society is used in treatment of diabetes, cough, headaches, and urinary diseases [16, 20]. The major constituents of *M. fruticosa serpyllifolia* VOs were monoterpenes (pulegone, menthol, isomenthol, limonene, α-pinene, β-pinene, piperitone, and piperitenone oxide), and sesquiterpenes (b-caryophyllene and germacrene) [15, 19, 24]. Studies conducted on the aqueous extract of *M. fruticosa serpyllifolia* showed anti-inflammatory effect and protection against gastric ulcer activities so it can be used as supplement or alternative herbal therapy for NSAIDs which can cause gastric ulcers [18, 25]. *M. fruticosa serpyllifolia* VOs also exhibit antibacterial, antifungal, antioxidants, insecticide, analgesic, anticonvulsants, hepatoprotective, and Central Nervous System (CNS) depressant effects [13, 15, 26, 29].

They can also be used as a natural substance for replacement of synthetic herbicides due to the presence of pulegone which consisted of 70% of oils chemicals [30]. Therefore, the aim of the current study was to identify the chemical composition and the potential enzymatic activities of *M. fruticosa serpyllifolia* growing wildly in three regions in West Bank area in Palestine and to perform a comparative study of the results among three regions in Palestine.

2. Materials and Methods

2.1. Chemical Reagents. Porcine pancreatic lipase, Tris-HCl, PNBP (p-nitrophenyl butyrate), Amylase type VI -B, ≥10 unit/mg, Acarbose, and Calcium Chloride were purchased from Sigma-Aldrich, USA. Orlistat was purchased from Sigma-Aldrich, China, Acetonitrile and dimethyl sulfoxide (DMSO) were purchased from CARLO ERBA, France. 3,5-Dinitrosalysylic acid (DNSA) was purchased from Sigma-Aldrich, India, sodium potassium tartrate tetrahydrate was purchased from Merck, Germany, sodium hydroxide (NaOH) was purchased from Sun Pharm,drug stars, Nablus, Palestine, Disodium hydrophosphate/dihydrosodium phosphate (Na₂HPO₄/NaH₂PO₄) was purchased from Alfa Aesar, USA, Sodium chloride (NaCl) was self-backing (Alshela company, Palestine) as well as Starch (Alzahra company, Nablus, Palestine).

2.2. Instrumentation. Grinder (Moulinex model, Uno. China) was used to fracture the dried herbs. Balance max 220 g (Radway, Poland) was used to weigh the plant material, microwave-ultrasonic cooperative extractor/reactor (CW-2000, China) was utilized for chemical screening of VOs, water bath was from Memert, Germany, Ultraviolet-Visible (UV-Vis) Spectrophotometer (Jen WAT 7315, UK) was utilized for assessment the enzymatic activities of VOs, water bath was from Memert, Germany, water bath sonicator was from MRC, Haifa, and heater was from Lab-Tech, Korea. pH meter was used to adjust pH of disodium hydrophosphate/dihydrosodium phosphate (Na₂HPO₄/NaH₂PO₄). Micropipettes were from Microliter, BRAND, Germany.

2.3. Plant Material Collection and Treatment. The aerial parts of *M. fruticosa serpyllifolia* were collected in April of 2017 from three cities in the West Bank (WB) in Palestine: Nablus, Ramallah, and Hebron which represented north, middle, and south regions of the WB in Palestine, respectively (three samples were collected from each city). The samples were botanically identified and coded by Dr. Nidal Jaradat the Pharmacognosist at An-Najah National University (ANNU) under the voucher specimen code: Pharm-PCT-1575. The extraction of VOs followed the procedure in [31]. The fresh aerial parts of *M. fruticosa serpyllifolia* were separated carefully, washed two times with distilled water, and dried for two weeks in the shade at room temperature. The dried specimens were fractured and stored in well closed plastic bags for future use in the Laboratory of Pharmacognosy at An-Najah National University Faculty of Medicine and Health Sciences.
2.4. Extraction of Volatile Oils. The VO samples of the three samples of *M. fruticosa serpyllifolia* plant were extracted by microwave-ultrasonic method which was examined by Jaradat et al. [31], by which the suspension of plant fru-2.5. GC-MS Analysis. The chemical composition of the three samples of *M. fruticosa serpyllifolia* VO samples was separated and quantified using Shimadzu QP-5000 GC-MS. The method-2.6. ComponentsIdentification. The mass spectrometry data center of the national institute of standards and technology (NIST) was used as a reference to identify the chemical components of the VO samples by comparing their MS spectra with data of NIST in addition to using Kovats index in the literature to compare their retention times. The quantitative data were obtained electronically from integrated peaks, area percentages without the use of correction factor [32, 33].-2.7. Pancreatic Lipase (PL) Inhibition. The porcine pancreatic lipase (PPL) inhibitory assay was conducted using the methods from Jaradat et al. [34], Bustanji et al. [35], and Siew-Ling et al. [36] with some modifications. VO samples were prepared by dissolving 12.5 mg of amylase enzyme in buffer ([Na4HPO4/NaH2PO4 (0.02 M)], NaCl (0.006 M) at pH 6.9). Working solution of concentrations 10, 50, 100, 500, and 1000 μg/mL was prepared using buffer (Na4HPO4/NaH2PO4 (0.02 M)). NaCl (0.006 M) at pH 6.9). Acarbose was used as a reference. The stock and working solutions of Acarbose were prepared using the same procedure of *M. fruticosa serpyllifolia* VO samples. α-Amylase activity was determined by measuring the hydrolysis of p-nitrophenol to p-nitrophenolate at 405 nm using UV-Vis spectrophotometer. The lipase inhibition activity of *M. fruticosa serpyllifolia* VO or Orlistat as a reference was identified by measuring the effect on the enzyme reaction rate after adding extracts, compared with the control. % was calculated by using the following equation [37].

\[
\text{%I} = \frac{[\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Test}}]}{\text{Absorbance}_{\text{Control}}} \times 100 \%
\]

where %I is the percentage inhibition of pancreatic lipase.

2.8. α-Amylase Inhibition. The α-amylase inhibition assay was done according to procedure conducted by N. Nirmal-i et al. [11] with some modifications. The assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method. *M. frut-icosa serpyllifolia* VO stock solution (S.S) of 1 mg/mL was prepared in a minimum amount of DMSO 10% and was further dissolved in buffer (Na4HPO4/NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9). Working solution of concentrations 10, 50, 100, 500, and 1000 μg/mL was prepared using buffer (Na4HPO4/NaH2PO4 (0.02 M)). NaCl (0.006 M) at pH 6.9). Acarbose was used as a reference. The stock and working solutions of Acarbose were prepared using the same procedure of *M. fruticosa serpyllifolia* VO samples. α-Amylase solution (2 unit/mL) was prepared by dissolving 12.5 mg of amylase enzyme in buffer ([Na4HPO4/NaH2PO4 (0.02 M)], NaCl (0.006 M) at pH 6.9). A volume of 200 μL of α-amylase solution (2 unit/mL) was mixed with 200 μL of each VO samples working solution and incubated for 10 min at 37°C. Then 200 μL of the starch solution was added and incubated for 3 min. The reaction was stopped by the addition of 200 μL DNSA reagent and boiled for 10 min in a water bath at 85—90°C. The mixture was cooled to ambient temperature and diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-Vis. spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 μL of buffer. Acarbose was used as a positive control sample. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using (2). The % α-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph [11].

\[
\% \alpha –\text{amylase inhibition} = \frac{[\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Test}}]}{\text{Abs}_{\text{Control}}} \times 100 \%
\]
Table 1: The total % of yields, chemical compounds, total identified compounds, and chemical groups of the three samples of *M* *fruticosa* *serpyllifolia* VOs.

|                  | % total VO | % total VO | % total VO |
|------------------|------------|------------|------------|
|                  | Nablus     | Ramallah   | Hebron     |
| (w/w) % yield    |            |            |            |
| α-Pinene         | 0.91       | 0.71       | 0.83       |
| β-Pinene         | 1.48       | 0.94       | 1.08       |
| β-Myrcene        | < 0.04     | 0.26       | 0.35       |
| D-Limonene       | 1.73       | 1.65       | 1.26       |
| Isocaryophyllene | 0.26       | 1          | 1.19       |
| Isomenthone      | 3.16       | 3.84       | 14.41      |
| Pulegone         | 82.94      | 86.04      | 74.43      |
| Total non-oxygenated constituents | 4.38 | 4.56 | 4.71 |
| Total oxygenated constituents | 86.1 | 89.88 | 88.84 |
| Total identified components % | 90.48 | 94.44 | 93.55 |

where % α-amylase inhibition is the percentage inhibition of amylase.

2.9. Statistical Analysis. Statistical analysis was conducted using one-way ANOVA with post hoc Tukey-Kramer HSD multiple comparison calculation; p values less than 0.05 or 0.01 were considered statistically significant [38].

3. Results

3.1. Chemical Composition. Volatile oils of the three samples of *M* *fruticosa* *serpyllifolia* were extracted using Microwave-Ultrasonic Apparatus, and the produced oils were viscous, colorless, and with a nice peppermint smell. The average percentage of VOs yield (w/w%) of the three samples was Nablus (0.67% ± 0.29), Ramallah (0.99% ± 0.55), and Hebron (0.70% ± 0.17) (Table 1). The data were expressed as mean ± STDV (n=3).

The chemical analysis conducted using GC-MS characterized the VOs with seven components classified into oxygenated ingredients mainly ketones and non-oxygenated ingredients mainly hydrocarbons in all three samples with different proportions (Table 1). The most abundant components in all three samples were pulegone and isomenthone. The total identified components in the three samples were almost consistent in which 90.48, 94.44, and 93.55% of the constituents were identified in Nablus, Ramallah, and Hebron districts, respectively. Detailed results are represented in Table 1. Other five common compounds identified in all three samples with total percentage less than 2% were D-Limonene, β-Pinene, Isocaryophyllene, α-Pinene, and β-Myrcene (Table 1).

3.2. Lipase Inhibition Activity. The hydrolysis of p-nitrophenyl butyrate to p-nitrophenol was used to measure the influence of *M* *fruticosa* *serpyllifolia* VOs of three samples on pancreatic lipase enzyme. The assay was detected by comparing to Orlistat lipase inhibitory agent; the three VOs samples showed varied antilipase activity while Nablus VO sample showed the highest potency with IC₅₀ value of 39.81 μg/mL but with a maximum inhibition % of 65.40%. However, Orlistat owned potency at IC₅₀ value of 43.64 μg/mL with antilipase inhibition of 99.13%. The results of IC₅₀ values and the antilipase activity of the three samples and Orlistat are shown in Table 2 and Figure 1. Comparative statistical analysis of the findings of the three samples of VOs showed that there were significant differences in antilipase potency and efficacy of VOs compared to Orlistat (p < 0.01). In addition, there were significant differences in antilipase potency and efficacy of VOs compared to each other (p < 0.01).

3.3. α-Amylase Inhibition Assay. *In vitro* assay of alpha amylase inhibitory activities by using starch as a substrate and Acarbose as a positive control was conducted on *M* *fruticosa* *serpyllifolia* VOs of the three samples. Our findings revealed that the three samples of VOs showed different...
The VOs samples from Ramallah and Hebron showed little potency and efficacy of the antiamylase inhibition compared to Acarbose. There were significant differences in antiamylase potency and efficacy of VOs compared to Acarbose and compared to each other ($p < 0.01$).

### 4. Discussion

The qualitative and quantitative differences in the chemical composition of VOs might be attributed to several factors such as geographical factors (location), climatic effects of the plants, harvest season, nature of the soil, age of the plant parts (young or adult), and time of collection. In addition, the effect of the environment on the secondary metabolic profile of *Micromeria fruticosa* is a model for environmental metabolomics of plants. The north region represented by Nablus city showed the highest percent in $\alpha$-Pinene, $\beta$-Pinene, and D-Limonene. The middle region represented by Ramallah city owned the highest percent in Pulegone, and the south region represented by Hebron city showed the highest percent in $\beta$-Myrcene, Isocaryophyllene, and Isomenthone and in total nonoxygenated constituents. These cities are calcified into different biogeographical zones in West Bank in Palestine, such as the central highlands, and the eastern slope which has influence on climate, elevation, the average rainfall, and temperature that affect the rate of biochemical reaction and the weathering process of soil.

The yields of *M. fruticosa* serpyllifolia VOs in the current study were lower than that the findings of a study conducted in Palestine studied by Shehab et al., [24] which reported a yield of VOs of 2.2%. Also our data were lower in yield than that of *M. fruticosa serpyllifolia* growing in Turkey examined by Gulluce et al., [28] who reported a yield of 1.85% of VOs of the plant collected in the flowering period.

The GC-MS analysis identified seven compounds listed in Table 1. Studies were conducted previously on *M. fruticosa serpyllifolia* VOs. Table 4 summarizes different relevant literature with the most important finding. In Palestine Shehab et al. (2012) reported that pulegone, neo-Menthol, and Isomenthone were the dominant compounds [24], while in Lebanon Pulegone and D-limonene were the prevalent components (Al-Hamawi et al., 2011) [13] and for that growing in Turkey piperitenone, pulegone, and Isomenthone were the most abundant components (Gulluce et al., 2004) [28]. Isa Telci and Mustafa Ceylan (2007) [26] reported that the VOs of subspecies of *Micromeria fruticosa* belong to different chemotypes, (a) pulegone, linalool, and p-menthene and (b) piperitenone and linalool type, and revealed that pulegone was the most prominent compound in *Micromeria* species mainly in *M. fruticosa*. In the current study Isocaryophyllene was lower than that identified in VO sample of Palestine (Table 4) [24]. The rest of the components in recent study such as D-limonene, $\beta$-pinene, and $\alpha$-pinene were presented in higher levels than that of the samples of the Palestinian

### Table 2: Lipase inhibition assay of the three samples of *M. fruticosa serpyllifolia* VOs and Orlistat.

|                  | Orlistat | Nablus | Ramallah | Hebron |
|------------------|----------|--------|----------|--------|
| $IC_{50}$ µg/mL  | 43.64    | 39.81  | 43.73    | 51.21  |
| Antilipase activity | 99.13%  | 65.41% | 54.94%   | 36.92% |

$a p < 0.01$ compared to Orlistat, $b p < 0.01$ compared to Nablus sample, and $c p < 0.01$ compared to Ramallah.

### Table 3: $\alpha$-Amylase inhibition assay of the three samples of *M. fruticosa serpyllifolia* VOs and Acarbose.

|                  | Acarbose | Nablus | Ramallah | Hebron |
|------------------|----------|--------|----------|--------|
| $IC_{50}$ µg/mL  | 21.38    | 3.31$^a$ | 3.40$^{ab}$ | 3.35$^{abc}$ |
| antiamylase activity | 91.39%  | 64.34%$^a$ | 23.77%$^{ab}$ | 25.00%$^{abc}$ |

$a p < 0.01$ compared to Acarbose, $b p < 0.01$ compared to Nablus, and $c p < 0.01$ compared to Ramallah.

![Figure 2](image-url): $\alpha$-Amylase inhibition assay of *M. fruticosa serpyllifolia* VOs from different regions of Palestine.
samples from different regions in Palestine were investigated by the inhibition of α-amylase activity. According to our knowledge, there were no previous studies conducted for the purpose of assessing the activity of *M. fruticosa serpyllifolia* VOs against α-amylase enzyme. The inhibition of α-amylase activity of *Sideritis galactica* Bornm VOs sample growing in Turkey studied by Zengin et al. (2016) [45] was related to abundance of monoterpene hydrocarbons ingredients mainly α-pinene and β-pinene. In screening the α-amylase inhibitory activity of *J. phoenicea* volatile oil growing in Tunisia, the results showed powerful α-amylase inhibition properties due to presence of terpenes like α-pinene [37]. The *M. fruticosa serpyllifolia* VOs sample from Nablus owned the highest amount of α-pinene and β-pinene components (0.91 and 1.48%, respectively) compared with samples of Ramallah and Hebron which may explain its highest potency against α-amylase enzyme.

Since the volatile oils of *Micromeria fruticosa serpyllifolia* have a potential inhibitory activity against pancreatic lipase and α-amylase enzymes, it is of high importance to take into consideration pulegone toxicity. The European Medicines Agency (EMA), committee on herbal medicinal products (HMPC), concluded that pulegone is considered a hepatotoxin; depending on that, the recommended daily dose of pulegone for 60 kg person by EMA would correspond to 2.3 mg/kg body weight (bw) [46] taking into account the density of pulegone (0.9346 g/mL) [47], and the daily recommended dose as mentioned above could be recommended by specialists as the safe volume of volatile oil ingestion [48].

### 5. Conclusions

*M. fruticosa serpyllifolia* VOs from different regions in Palestine represented by three cities showed variable antilipase and antiamylase activities depending on the phytochemical constituents of the volatile oils. *M. fruticosa serpyllifolia* VOs of three regions owned the same chemical components but in difference proportions. The sample from north Palestine (Nablus) exhibited highest antilipase and antiamylase activity due to higher amount of α-pinene and β-pinene. Further in
vivo studies are needed to evaluate the potential pharmacological activities and to assess the safety and toxicity of plant extract. Also further studies are required to isolate the basic components responsible for potential pharmacological activities.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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**Supplementary Materials**

Chemical structure of the main components of *M. fruticosa serpyllifolia* VOs. GC-MS chromatograms and analysis. (Supplementary Materials)

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