Diversity and profiles of volatile compounds in twenty-five peppermint genotypes grown in China

Lin Lu, Hua Cao, Han Li, Hao Zhang, Shenchong Li, and Jihua Wang

Flower Research Institute, Yunnan Academy of Agricultural Sciences, Kunming, China

ABSTRACT
Peppermint (Mentha piperita L.) is one of the most cultivated and consumed herbs. The pharmaceutical properties and nutrition values of peppermint oil make it an important commercial product. This study aimed to explore the volatile compounds in the essential oil extracted from different peppermint genotypes commercially grown in China. Oil extraction using a steam distillation followed by gas chromatography-mass spectrometry resulted in the quantification of 53 volatile compounds from five major classes, including esters, alcohols, ketones, terpenes, and volatile compounds. The highest essential oil yields were observed in Golden mint, Silver mint, Scottish mint, and Banana mint. Moreover, three genotypes, viz., Milk fruit mint, Winter Mint, and Pineapple, were screened with the highest diversity of volatile compounds. Major volatiles identified were linalyl acetate, 1-octene-3-acetate, geranyl acetate, neryl acetate, menthol, linalool, menthone, and carvone. The phenotypic characterization coupled with the volatile profiling in this study provides the basis for selecting and improving the investigated peppermint genotypes for specific commercial uses.

Introduction
Peppermint (Mentha piperita L.), an aromatic herb, is a widely used species of the Mentha genus from the Lamiaceae family. Most Mentha spp. have a perennial growth habit with a wide distribution across Europe, Asia, Africa, and America. Since ancient times, various species of the Mentha genus have been consumed as nutrition products and used in cosmetics and traditional medicine. China is among the leading countries producing essential oils from peppermint.

The Mentha species are the most widely used for health and medicinal uses, primarily because of Menthol and Menthone. Besides its culinary use, peppermint is enriched in essential oils, including methanol, menthane, cineol, and polyphenols. The use of Mentha species can be traced back to the herbal pharmacopoeia of ancient Greece. Numerous studies have emphasized the beneficial biological functions associated with peppermint volatiles, including antioxidant, anti-inflammatory, antimicrobial, and antiviral. Peppermint oil is a popular remedy for irritable bowel syndrome, headache, and non-ulcer dyspepsia. The bioactive volatiles can be extracted from M. piperita through steam distillation or hydro-distillation. Furthermore, peppermint oil can be extracted from the stem, leaves, and flower tissues of the plant. However, the distribution pattern of the volatile compounds is an environmentally dependent phenomenon and significantly varies across varieties and different tissues. Therefore, it is pertinent to characterize the differential accumulation of volatiles among different varieties to develop the rationale for the further valorization of peppermint essential oil.
Although extensive research has been carried out to evaluate the accumulation of bioactive compounds in Mentha spp., the diversity of volatiles within species and across varieties is scarce. Therefore, this study aimed to explore the profiles of volatile compounds in 25 peppermint genotypes by utilizing the steam distillation method. The accumulation profile of the volatile compounds will provide a basis for selecting peppermint varieties for corresponding industrial/commercial uses.

Materials and methods

Collection and drying of samples

Twenty-five genotypes classified as Chocolate Mint (Mentha x piperita L. ‘Chocolate’), Corsica Mint (M. requienii Benth), Roman Mint (Clinopodium nepeta L. Kuntze subsp. spruneri (Boiss.) Bartolucci & F.Contia), Milk fruit Mint, Japanese Mint (Mentha arvensis L.), Winter Mint (Mentha spicata L.), Peppermint (Mentha × piperita L. (M. aquatica × M. spicata), Spearmint (M. spicata L.), Pineapple Mint (M. suaveolens Ehrh. ‘Variegata’), Australian Mint (M. austalis R.Br.), Apple Mint (M. suaveolens Ehrh.), Cat Mint (Nepeta cataria L.), Vietnamese Mint (Mentha × gracilis Sole (syn. Mentha × gentilis L.; M. arvensis × M. spicata)), Lemon Mint (Melissa officinalis L.), Silver Mint (M. longifolia L. ‘Silver’), Scottish Mint (Mentha × gracilis Sole (syn. Mentha × gentilis L.; M. arvensis × M. spicata)), Banana Mint (M. arvensis L. ‘Banana’), Milk Mint, Grapefruit Mint (Mentha x piperita L. ‘Grapefruit’), Lavender Mint (Mentha x piperita L. ‘Lavendula’), English Mint (Mentha spicata L.), and Golden Mint (Mentha spicata L. ‘Golden’) were used in this study and grown in the resource nursery of Flower Research Institute, Yunnan Academy of Agricultural Science. Before sampling, Mint plants have been growing for a year. The upper and middle parts of the plants were harvested and taken to the laboratory for distillation and extraction. The sample collection time was from July to August 2020. The samples were collected after 10 o’clock to avoid water droplets. The dried samples (three biological replicates for each variety) were powdered before extraction. The drying was performed in a chamber with the air-flow rate of 1.5 ms⁻¹ at 30°C as previously described. The air-dried peppermint samples were further used for distillation.

Steam distillation

Dried samples were weighed into the distillation vessel and connected to the steam generator (VELP Scientific, UDK 142). The distillation was performed according to the method explained by. The condenser of the steam distillation vessel was continuously cooled using a cooler circulator attached to the steam generator. The distillation vessel was subjected to steam at 80% efficiency. About 900 mL of distillate was collected into a 1 L separatory funnel containing about 50 mL of ethyl acetate for 10, 15, and 20 min. The steam distillate was extracted with 3 × 30 mL portions of the same solvent; the combined solvent layer was dried over anhydrous sodium sulfate and evaporated in a rotary evaporator (at 35°C) under vacuum. The concentrated extract was quantitatively transferred into a 10 mL volumetric flask, made up to volume by adding distilled water, and subjected to downstream GC/MS analysis.

GC/MS characterization of volatiles compounds in mint extract

The extracts obtained from the steam distillation process were subjected to gas chromatography (GC) and GC/MS analysis on a Varian Saturn 2000 ion-trap GC/MS for further quantitative analysis of bioactive compounds. The GC apparatus included Agilent technology (HP) 6890 system, a capillary column of HP-5 MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was maintained at 40°C for one minute and raised to 230°C for 10 minutes. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector were maintained at 250°C and 230°C, respectively. Flame ionization detection (FID) was performed at 280°C.
GC/MS analysis was conducted on an HP 6890 GC system with conditions: GC Column: CP-Sil 8 CB (30 m × 0.32 mm i.d., 1 μm film thickness). Injector temperature: 230°C. Column Temp.: 80°C-10 min. −10°C/min. −220°C-6 min. −20°C/min. −300°C-20 min. Ion Trap Temp.: 170°C. The oil compounds were identified by comparing their retention indices (RI) and mass spectra fragmentation with Wiley 5 mass spectra computer.\textsuperscript{[29]} Data obtained were confirmed by comparison of retention indices of authentic compounds.

**Phenotypic characterization**

The phenotypes of the 25 genotypes were visually observed for plant architecture, stem color, stem pubescence, foliage, leaf pubescence, leaf shape, leaf tip, the serrated shape of leaf edge, inflorescence color, and inflorescence shape. Furthermore, the plant height (cm) and leaf size (length × width, cm\(^2\)) were measured for each genotype.

**Results**

**Phenotypic characterization**

This study utilized 25 commercial peppermint genotypes to analyze the general morphological differences, followed by a comprehensive analysis of the volatile compounds. The morphological description of all the genotypes has been presented in Figure 1 and Table 1. Overall, a significant variation in phenotype and aroma could be observed in the genotypes.

**Essential oil yield and characterization of the volatile compounds**

Furthermore, we characterized all 25 genotypes of peppermint for their corresponding volatile profiles. The essential oil was extracted from dried plants. The essential oil percentage extracted from each variety varied from 0.029 to 1.517% (Figure 2 and Table S1). Golden mint, Silver mint, Scottish mint, Banana mint, and Roman mint depicted the highest percentages of essential oil extraction. While Lemon mint, Pineapple mint, Milk mint, and English mint showed the lowest percentages of extracted essential oil. The essential oil from each variety was further characterized by the availability of volatile compounds and their ratios in the essential oils. Overall, 53 volatile compounds were identified in the 25 genotypes, including esters (9), alcohols (10), ketones (8), terpenes (19), and aromatic compounds (7) (Figure 2b). However, the number of volatile compounds in each variety varied significantly, and the occurrence of each volatile compound in the different genotypes was also variable (Figure 2c and Table S2). For instance, \(\beta\)-Caryophyllene, biggerene D, limonene, and 1,8-Cineole from terpenes were present in 21 out of the 25 genotypes with different concentrations.

Similarly, menthol, menthone, myrcene, carvone, linalyl acetate, and linalool were identified in 14, 13,13, 12, 11, and 11 genotypes, respectively. The remaining volatiles occurred in less than 10 genotypes. The differential occurrence pattern of the volatile compounds suggested a significant genotypic variation in the accumulation and availability of volatile compounds in the essential oils extracted from the 25 peppermint genotypes.

The volatile compounds in the essential oil extracted from the peppermint genotypes were quantified using GC/MS chromatograms. The accumulation profiles of the volatile compounds in the essential oils have been presented in Figure 3. The total ion chromatograms indicating the retention time for the quantified volatiles in each variety showed reliability and repeatability of the quantification results (Figure S1).

Linalyl Acetate, menthyl acetate, 1-octene-3-acetate, geranyl acetate, neryl acetate, dihydrocarvyl acetate, neomenthol acetate, nepetalactone, and carvyl acetate were among the esters identified in the essential oils. Linalyl acetate was identified in 11 genotypes with the highest relative concentration in
A7 (Japanese mint, 48.6%) followed by A22 (Grapefruit mint, 42.9%), A17 (Lemon mint, 37.6%), and A4 (Orange mint, 30.7%). Menthol, linalool, elemenol, chloranthol, neomenthol, alpha-terpineol, dihydrocarvol, terpineol-4-ol, 3-octanol, and citronellol belonging to the alcohol group were also identified. Menthol, linalool, and elemenol showed the highest occurrence in 14, 13, and 9 genotypes, respectively. Peppermint genotypes A31 (Golden Mint), A5 (Candy Mint), and A9 (Peppermint) had the highest relative contents of methanol (76.5, 31.8, and 25.2%, respectively).

Furthermore, eight compounds from Ketones, including menthone, longleaf menthone, carvone, piperonone oxide, dihydrocarvone, piperonone, and isomenthone were identified. Menthone and carvone were among the most occurring volatiles and were identified in 13 and 12 genotypes, respectively. A3, A11, A12, A19, A31 depicted the highest concentrations of ketones. Interestingly, terpenes were most the abundant volatiles (including β-caryophyllene, biggerene D, limonene, 1,8-cineole, myrcene, trans-β-farnesene, β-pinene, α-pinene, cis-ocilene, β-bourbonene, γ-terpinene, trans-hinoene, bicyclic myrene, cypresene, α-terpinene, a-thujene, trans-ocimene, biggerene D isomer, and caryophyllene oxide) in the genotypes. β-caryophyllene, biggerene D, limonene, and
Table 1. Morphological description of 25 genotypes of peppermint.

| # | Var. | Ph (cm) | Shape                      | Stem hair   | Leaf dia (length*width)/ cm | Foliage       | Leaf pubescence | Leaf shape | Leaf tip | Serrated shape of leaf edge | Color | Inflorescence shape | aroma                                      |
|---|------|---------|----------------------------|-------------|----------------------------|---------------|-----------------|-------------|----------|---------------------------|-------|-------------------------|-------------------------------------------|
| 1 | A1   | 20–35   | Half upright and half creeping | Absent      | 3.0*2.3 Folds Sparse         | oval          | Blunt tip       | Jagged     | Lavender | Cyme                      | Strong fragrance, cool and slightly sweet |
| 2 | A2   | 15–30   | Creeping                   | Absent      | 1.6*1.8 Flatten Absent       | Heart-shaped  | Blunt tip       | Whole      | White    | Spikes                    | Glandular odor                            |
| 3 | A3   | 28–35   | upright                    | Absent      | 1.4*1.3 Flatten Absent       | Broad oval    | Blunt tip       | Sharp      | Shallow seration Jagged   | Light pink      | Cyme                      | Fragrant                                  |
| 4 | A4   | 35–45   | Upright and less            | Absent      | 4.5*3.4 Folds Absent         | Broad oval    | Blunt tip       | Fine-pointed seration | Lavender | Cyme                      | Strong mint flavor                       |
| 5 | A5   | 20–30   | upright and half            | Present     | 3.9*2.6 Folds Absent         | Oval          | Apex            | No blossoms | No blossoms | Light savory mint     | A typical candy flavor with a refreshing taste |
| 6 | A6   | 35–45   | upright                    | Present     | 3.6*2.7 Folds Dense          | Broad oval    | Blunt tip       | Shallow seration | Lavender | Spikes                    | Light savory mint                         |
| 7 | A7   | 50–72   | upright                    | Absent      | 4.2*2.2 Folds Absent         | Wedge-lanceolate | Apex            | Jagged     | White    | Cyme                      | Strong mint flavor                       |
| 8 | A8   | 20–40   | upright                    | Absent      | 1.3*0.5 Flatten Absent       | Lanceolate    | Apex            | Whole, no jagged | No blossoms | Light incense            | Peppery with a refreshing taste           |
| 9 | A9   | 15–25   | upright                    | Sparse      | 4.5*2.6 Folds Absent         | Oblong        | Apex            | Fine-pointed seration | No blossoms | White        | Spikes                    | Strong mint flavor                       |
| 10| A10  | 40–50   | upright                    | Sparse      | 4.6*2.9 Folds Absent         | Oval          | Blunt tip       | Serrations are deep and blunt | Lavender | Spikes                    | Strong mint flavor                       |
| 11| A11  | 25–35   | upright                    | Present     | 3.5*2.7 Folds Absent         | Broad oval    | Blunt tip       | Blunt      | No blossoms | Light savory mint     | Light savory mint                         |
| 12| A12  | 58–72   | upright                    | Absent      | 5.0*3.5 Folds Absent         | Oblong-lanceolate | Blunt tip       | Neatly serrated jagged | White    | Spikes                    | Light fragrance                           |
| 13| A14  | 35–42   | upright                    | Present     | 4.7*3.6 Folds Absent         | Oval          | Blunt tip       | Jagged     | Lavender | Cyme                      | Apple scent                              |
| 14| A15  | 30–40   | upright                    | Have        | 2.1*1.2 Folds Absent         | Oblong-lanceolate | Blunt tip       | Neatly serrated jagged | Lavender | Cyme                      | Light incense                            |
| 15| A16  | 35–50   | upright                    | Have        | 3.2*2.0 Folds Absent         | Oval          | Apex            | Fine-pointed seration | pink      | Spikes                    | Strong mint flavor                       |
| 16| A17  | 20–30   | upright                    | without     | 5.4*5.1 Folds Absent         | Broad oval    | Blunt tip       | Serrations are deep and blunt | No blossoms | Cyme                      | Lemon scent                             |(Continued)
| #  | Var. | Ph (cm) | Shape     | Leaf dia (length*width)/cm | Foliage     | Leaf pubescence | Leaf shape      | Leaf tip            | Serrated shape of leaf edge | Color  | Inflorescence shape | Aroma                                           |
|----|------|---------|-----------|-----------------------------|-------------|-----------------|-----------------|--------------------|--------------------------|--------|---------------------|--------|
| 17 | A18  | 40–50   | upright   | Have                        | 4.2*2.2     | Folds           | Leaf dorsal is densely fluffy | Ovoid-lanceolate | Sharp                  | Neatly serrated Jagged     | Lavender | Spikes              | Strong aroma and refreshing taste Light savory mint |
| 18 | A19  | 30–45   | upright   | Have                        | 3.7*2.0     | Folds           | Leaf dorsal with sparse villi | Wedge             | Apex                   | Neatly serrated            | Lavender | Cyme                | Light mint                           |
| 19 | A20  | 30–40   | upright   | Have                        | 4.9*2.5     | Folds           | Leaf dorsal dense villi       | Ovoid-lanceolate | Sharp                  | Neatly serrated Blunt teeth | Lavender | Spikes              | Strong aroma and refreshing taste Light mint                           |
| 20 | A21  | 30–45   | upright   | Have                        | 5.0*4.2     | Folds           | Densely short villi           | Broad oval        | Blunt tip              | Sharp Neatly serrated Blunt teeth | No blossoms | No blossoms | Fragrant                                      |
| 21 | A22  | 50–60   | upright   | Have                        | 3.7*2.3     | Folds           | Leaf dorsal is densely fluffy | Oblong-lanceolate | Sharp                  | Neatly serrated Blunt teeth | No blossoms | No blossoms | Strong aroma and refreshing taste Light savory mint |
| 22 | A23  | 25–35   | upright   | without                     | 4.8*3.6     | Flatten         | No leaf hairs               | Ovate-lanceolate | Taper                  | Neatly serrated            | No blossoms | No blossoms | Strong fragrance                                      |
| 23 | A24  | 30–40   | upright   | Have                        | 3.1*2       | Folds           | Sparse leaf hairs on the back | Ovate-lanceolate | Sharp                  | Neatly serrated Blunt teeth | No blossoms | No blossoms | Strong aroma and refreshing taste Light savory mint |
| 24 | A25  | 20–35   | Adult plants stand upright  | without      | 1.3*0.9      | Flatten         | Leaves are sparsely fluffy on both sides | Oblong-lanceolate | Blunt tip              | Whole Neatly serrated Whole | Lavender | Cyme                | Strong aroma and refreshing taste Light savory mint |
| 25 | A31  | 65–75   | upright   | Have                        | 4.9*2.5     | Folds           | Leaf dorsal with sparse villi | Oblong-lanceolate | Sharp                  | Neatly serrated            | White   | Cyme                | Strong mint flavor                           |
1,8-cineole were quantified in the essential oils from the different genotypes. Limonene showed the highest relative contents in A1 (Chocolate Mint, 39.7%), A19 (Scottish Mint, 26.2%), A10 (Spearmint, 15.4%), and A20 (Banana Mint, 13.0%). In comparison, A17 (Vietnamese Mint, 14.6%) had the highest relative concentration of β-caryophyllene.

Aromatic compounds identified from the essential oils of the genotypes were among the least abundant classes. P-cymene, carvacrol, menthol furan, 1,3-dimethoxy-2-methylphenol, geranial neral and 2-methoxy-4-ethyl-6-methylphenol were identified in 4,3,3,1,1,1, and 1 genotypes, respectively. P-cymene was quantified in A6, A8, A21, and A24, while Carvacrol was quantified only in A4, A6, and A8.

Overall, there are three genotypes viz., A6 (Milk fruit Mint), A8 (Winter Mint), and A11 (Pineapple Mint), containing more than 15 volatile compounds. Also, there are eight genotypes, including A3 (Roman Mint), A7 (Japanese Mint), A18 (Silver Mint), A19 (Scottish Mint), A20 (Banana Mint), A22 (Grapefruit Mint), A23 (Lavender Mint), and A31 (Golden Mint) with the relative content of volatile compounds in their essential oils above 90%.
Discussion

This study utilized 25 peppermint genotypes commercially produced in China and evaluated their essential oil based on gas chromatography-mass spectrometry (GC/MS). GC/MS is a known method used for metabolic profiling of essential oils and has been used in many studies to evaluate volatile profiles.\[30–33\] Compared to GC, GC/MS has proven to be an efficient method for volatile quantification in medicinal herbs.\[34\]

Morphological descriptions of the 25 peppermint genotypes presented distinctive characteristics for each variety. Moreover, descriptive markers and their association with volatile compounds can result in rapid screening for future breeding programs. Aside from the observed prevalent phenotypic differences, peppermint genotypes depicted a significant variation in essential oil yield. Golden mint, Silver mint, Scottish mint, and Banana mint showed the highest essential oil yields. Previous reports also suggested a genotypic variation concerning essential oil yield among different peppermint genotypes.\[35,36\] Several studies concerning *Daucus carota* L.,\[37,38\] *Prunus persica* L.,\[39\] apple,\[40\] and tomato\[41\] have reported genome-wide association studies (GWAS) using genotype-trait association to decipher molecular mechanism underlying variation of volatile composition. Furthermore,
developing a bi-parental population with contrasting phenotypes regarding oil yield in peppermint, followed by quantitative trait loci mapping, could yield significant insights into the molecular control of oil yield and composition in peppermint. A similar approach has been achieved in many crop plants.\textsuperscript{[42–46]}

Five major classes of volatiles, viz., esters, alcohols, ketones, terpenes, and aromatic compounds, were identified in this study, with terpenes as the most occurring class (with 19 volatiles compounds). However, their composition in the different genotypes varied significantly. Linalyl Acetate, identified in 11 peppermint genotypes, is known for its anti-inflammatory effects.\textsuperscript{[47,48]} Japanese mint, Grapefruit Mint, Lemon mint, and Orange mint essential oils had the highest relative contents (\%) of linalyl Acetate. Several studies have demonstrated the antioxidant and therapeutic effects of methyl acetate,\textsuperscript{[11]} 1-octene-3-acetate,\textsuperscript{[49]} geranyl acetate,\textsuperscript{[50]} neryl acetate,\textsuperscript{[51]} dihydrocarvyl acetate,\textsuperscript{[52]} nepetalactone,\textsuperscript{[53,54]} carvyl acetate.\textsuperscript{[55]} Interestingly linalyl acetate, 1-octene-3-acetate, geranyl acetate, and neryl acetate were present in two peppermint genotypes: Grapefruit and Orange mint.

Alcohols are the most prominent volatiles in mint species.\textsuperscript{[56]} Menthol, a characteristic compound of Mentha spp., was quantified in 14 genotypes, with Golden Mint having the highest relative contents (up to 74\%). Linalool was identified in 11 peppermint genotypes with relative contents ranging from 1.55\% to 21.41\%. Linalool is considered an effective remedy for neuropathic pain.\textsuperscript{[57]} The alcohol compounds, including elemenol, chloranthol, neomenthol, alpha-terpineol, terpineol-4-ol, 3-octanol, and citronellol, were relatively less abundant in the mint samples. These volatiles have been previously reported for pharmacological uses.\textsuperscript{[58–62]}

The presence of ketones in peppermint essential oil has been reported earlier.\textsuperscript{[63,64]} This study also identified eight volatiles belonging to the Ketones group. Menthone and carvone were among the most abundant volatiles in the peppermint genotypes. Many studies have reported the allelopathic effects of menthone, emphasizing its importance in pharmaeutics.\textsuperscript{[11,65,66]} The second most abundant ketone, carvone, was quantified in 11 peppermint genotypes. Spearmint, Silver Mint, and Banana Mint depicted the highest relative contents for carvone. Carvone is mainly used as a reversible suppressant of sprouting in stored potatoes or flower bulbs.\textsuperscript{[67]} However, it is also used commercially as an inhibitor of bacteria, fungi, and insect repellent.\textsuperscript{[68–70]}

Furthermore, we identified 19 compounds belonging to terpenes, the most abundant volatile class in our study. Interestingly, \(\beta\)-caryophyllene, biggerene D, limonene, and 1,8-cineole were present in most of the genotypes with variable relative concentrations. \(\beta\)-caryophyllene has been reported to fight against oral plaque\textsuperscript{[71]} and has anti-cancer properties.\textsuperscript{[72]} Vuuren et al.\textsuperscript{[73]} demonstrated the antimicrobial activities with additive, synergistic or antagonistic interaction between limonene, and 1,8-cineole, depending on their relative concentration levels.

In brief, we extracted the essential oil from 25 genotypes and characterized their volatile compounds using GC/MS. We identified three genotypes, viz., Milk fruit mint, Winter Mint, and Pineapple mint, with more than 15 volatile compounds. Our data provide a basis for further exploitation of peppermint genotypes depending on their corresponding volatile profiles. Integration with genetic and genomic studies could clarify the molecular mechanisms of the presence/absence or abundance of key volatile compounds in peppermint. For instance, a study on population genetics and genotype-trait association of peppermint gene pools could provide potential insights into the variation of peppermint volatile composition. Furthermore, we screened out eight genotypes, including Roman Mint, Japanese Mint, Silver Mint, Scottish Mint, Banana Mint, Grapefruit Mint, Lavender Mint, and Golden Mint, with significant contents of the volatile compounds. These genotypes can be further selected for various commercial uses.

**Availability of data and material**

The data used in this study can be obtained in the manuscript and its supplementary files.
Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was funded by National Key R&D Program of China [No. 2020YFD1000400], Key R&D Program of Yunnan Provincial Science and Technology Department of China [No. 2018BB014; No. 2016BC008; No. 2016BB009].

Authors’ contributions

JHW and HZ were the leading investigators of this research program. LL planned and designed the research; LL and HC performed the majority of the experiments with the help of HL; LL and SCL contributed reagents, materials, and analysis tools; LL analyzed the data; LL wrote the paper with suggestions from JHW, HZ and HC. All authors commented on the article before submission. The author(s) read and approved the final manuscript.

References

[1] Salehi, B.; Stojanović-Radić, Z.; Matejić, J.; Sharopov, F.; Antolak, H.; Kręgiel, D.; Sen, S.; Sharifi-Rad, M.; Acharya, K.; Sharifi-Rad, R. Plants of Genus Mentha: From Farm to Food Factory. Plants 2018, 7(3), 70. DOI: 10.3390/plants7030070.
[2] Lawrence, B. M.; Mint: The Genus Mentha; Boca Raton: CRC Press, 2006.
[3] Mamadalieva, N. Z.; Akramov, D. K.; Ovdì, E.; Tiezzi, A.; Nahar, L.; Azimova, S. S.; Sarker, S. D. Aromatic Medicinal Plants of the Lamiaceae Family from Uzbekistan: Ethnopharmacology, Essential Oils Composition, and Biological Activities. Medicines 2017, 4(1), 8. DOI: 10.3390/medicines4010008.
[4] Mahendran, G.; Rahman, L. U. Ethnomedicinal, Phytochemical and Pharmacological Updates on Peppermint (Mentha x Piperita L.)—A Review. Phytotherapy Res. 2020, 34(9), 2088–2139. DOI: 10.1002/ptr.6664.
[5] Desam, N. R.; Al-Rajab, A. J.; Sharma, M.; Mylabathula, M. M.; Gokulanpalli, R. R.; Albratty, M. Chemical Constituents, in Vitro Antibacterial and Antifungal Activity of Mentha × Piperita L.(peppermint) Essential Oils. J. King Saud Univ. Sci. 2019, 31(4), 528–533. DOI: 10.1016/j.jksus.2017.07.013.
[6] Kligler, B.; Chaudary, S. Peppermint Oil. Am. Family Phys. 2007, 75(7), 1027–1030.
[7] Herro, E.; Jacob, S. E. Mentha Piperita (Peppermint). Dermatitis. 2010, 21(6), 327–329. DOI: 10.2310/6620.2011.10080.
[8] Nair, B. Final Report on the Safety Assessment of Mentha Piperita (Peppermint) Oil, Mentha Piperita (Peppermint) Leaf Extract, Mentha Piperita (Peppermint) Leaf, and Mentha Piperita (Peppermint) Leaf Water. Int. J. Toxicol. 2001, 20, 61–73.
[9] McKay, D. L.; Blumberg, J. B. A Review of the Bioactivity and Potential Health Benefits of Peppermint Tea (Mentha Piperita L.). Phytother. Res. 2006, 20(8), 619–633. DOI: 10.1002/ptr.1936.
[10] Loolaei, M.; Moasefi, N.; Rasouli, H.; Adibi, H. Peppermint and Its Functionality: A Review. Arch. Clin. Microbiol. 2017, 8, 54.
[11] Balakrishnan, A. Therapeutic Uses of peppermint-a Review. J.Pharm.Sci. Res. 2015, 7, 474.
[12] Riachi, L. G.; De Maria, C. A. Peppermint Antioxidants Revisited. Food Chemistry. 2015, 176, 72–81.
[13] Dundar, E.; Olgun, E. G.; Isiksoy, S.; Kurkcuoglu, M.; Baser, K. H. C.; Bal, C. The Effects of intra-rectal and intra-peritoneal Application of Origanum Onites L. Essential Oil on 2, 4, 6-trinitrobenzensulfonic acid-induced Colitis in the Rat. Exp. Toxicol. Pathol. 2008, 59(6), 399–408. DOI: 10.1016/j.etp.2007.11.009.
[14] Liu, Y.; Song, M.; Che, T.; Bravo, D.; Pettigrew, J. Anti-inflammatory Effects of Several Plant Extracts on Porcine Alveolar Macrophages in Vitro. J. Anim. Sci. 2012, 90(8), 2774–2783. DOI: 10.2527/jas.2011-4304.
[15] Ruberto, G.; Baratta, M. T. Antioxidant Activity of Selected Essential Oil Components in Two Lipid Model Systems. Food Chem. 2000, 69(2), 167–174. DOI: 10.1016/S0308-8146(99)00247-2.
[16] Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. Food Chem. 2006, 97(4), 654–660. DOI: 10.1016/j.foodchem.2005.04.028.
[17] Lawson, M.; Knight, R.; Tran, K.; Walker, G.; Roberts-Thomson, I. Failure of Enteric-coated Peppermint Oil in the Irritable Bowel Syndrome: A Randomized, Double-blind Crossover Study. J. Gastroenterol. Hepatol. 1988, 3 (3), 235–238. DOI: 10.1111/j.1440-1746.1988.tb00244.x.
[18] Spanier, J. A.; Howden, C. W.; Jones, M. P. A Systematic Review of Alternative Therapies in the Irritable Bowel Syndrome. Arch. Internal Med. 2003, 163(3), 265–274. DOI: 10.1001/archinte.163.3.265.
[64] Maffei, M.; Codignola, A. Photosynthesis, Photorespiration and Herbicide Effect on Terpene Production in Peppermint (Mentha Piperita L.). *J. Essent. Oil Res.* 1990, 2(6), 275–286. DOI: 10.1080/10412905.1990.9697886.

[65] Ligor, M.; Buszewski, B. Determination of Menthol and Menthone in Food and Pharmaceutical Products by solid-phase microextraction–gas Chromatography. *J. Chromatogr. A.* 1999, 847(1–2), 161–169. DOI: 10.1016/S0021-9673(99)00139-9.

[66] Sarheed, M. M.; Rajabi, F.; Kunert, M.; Boland, W.; Wetters, S.; Miadowitz, K.; Kaźmierczak, A.; Sahi, V. P.; Nick, P. Cellular Base of Mint Allelopathy: Menthone Affects Plant Microtubules. *Front. Plant Sci.* 2020, 11, 1320. DOI: 10.3389/fpls.2020.546345.

[67] Elmastaş, M.; Dermirtas, I.; Isildak, O.; Aboul-Enein, H. Y. Antioxidant Activity of S-Carvone Isolated from Spearmint (*Mentha Spicata* L. Fam Lamiaceae). *J. Liq. Chromatogr. Relat. Technol.* 2006, 29(10), 1465–1475. DOI: 10.1080/10826070600674893.

[68] Helander, I. M.; Alakomi, H.-L.; Latva-Kala, K.; Mattila-Sandholm, T.; Pol, I.; Smid, E. J.; Gorris, L. G.; von Wright, A. Characterization of the Action of Selected Essential Oil Components on Gram-negative Bacteria. *J. Agric. Food Chem.* 1998, 46(9), 3590–3595. DOI: 10.1021/jf980154m.

[69] Smid, E. J.; de Witte, Y.; Gorris, L. G. Secondary Plant Metabolites as Control Agents of Postharvest Penicillium Rot on Tulip Bulbs. *Postharvest. Biol. Technol.* 1995, 6(3–4), 303–312. DOI: 10.1016/0925-5214(95)00010-4.

[70] Lee, S.; Tsao, R.; Peterson, C.; Coats, J. R. Insecticidal Activity of Monoterpenoids to Western Corn Rootworm (Coleoptera: Chrysomelidae), Twospotted Spider Mite (Acari: Tetranychidae), and House Fly (Diptera: Muscidae). *J. Econ. Entomol.* 1997, 90(4), 883–892. DOI: 10.1093/jee/90.4.883.

[71] Pieri, F. A.; de Castro Souza, M. C.; Vermelho, L. L. R.; Vermelho, M. L. R.; Perciano, P. G.; Vargas, F. S.; Borges, A. P. B.; da Veiga-Junior, V. F.; Moreira, M. A. S. Use of β-caryophyllene to Combat Bacterial Dental Plaque Formation in Dogs. *BMC Vet. Res.* 2016, 12(1), 1–8. DOI: 10.1186/s12917-016-0842-1.

[72] Fidyt, K.; Fiedorowicz, A.; Strządała, L.; Szumnny, A. β -caryophyllene and β -caryophyllene oxide-natural Compounds of Anticancer and Analgesic Properties. *Cancer Med.* 2016, 5(10), 3007–3017. DOI: 10.1002/cam4.816.

[73] Vuuren, S. V.; Viljoen, A. M. Antimicrobial Activity of Limonene Enantiomers and 1, 8-cineole Alone and in Combination. *Flavour Fragr. J.* 2007, 22(6), 540–544. DOI: 10.1002/ffj.1843.