

Phytochemicals, antioxidant activity and essential oil components of *Mentha longifolia* genotypes

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Article

Keywords: Essential oil, GC-MS, Horse mint, Lamiacea, Pigment

Posted Date: July 11th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3076933/v1>

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Abstract

Mentha longifolia is one of the most important aromatic and native plants of Iran and has a wide range of applications in the pharmaceutical, food and cosmetic industries. In the present study, the phytochemicals and essential oil components, and antioxidant activity of 30 genotypes of *M. longifolia* were studied. The maximum amount of chlorophyll a ($0.40 \text{ mg g}^{-1} \text{ DW}$), chlorophyll b ($0.23 \text{ mg g}^{-1} \text{ DW}$) and total carotenoid content ($18.59 \mu\text{g g}^{-1} \text{ DW}$) were obtained in G26, while beta carotene ($0.026 \mu\text{g g}^{-1} \text{ DW}$) was obtained in G27. Total phenol content, flavonoid content and antioxidant activity by DPPH method ranged between $5.97\text{--}16.06 \text{ mg GAE g}^{-1} \text{ DW}$, $0.59\text{--}3.60 \text{ mg quercetin g}^{-1} \text{ DW}$ and $3.34\text{--}15.72 \text{ mg AAE g}^{-1} \text{ DW}$, respectively. The highest levels of total anthocyanin content ($9.30 \mu\text{mol g}^{-1} \text{ DW}$) and soluble carbohydrate content ($2.01 \text{ mg g}^{-1} \text{ DW}$) were recorded in G10 and G29, respectively. GC-MS analysis revealed that piperitenone oxide (from 1.17 to 69.12%), pulegone (from 2.97 to 67.82%), cis-piperitenone oxide (from 0.78 to 24.89%), thymol (from 0.14 to 22.90%), 1, 8-Cineole (from 0.97 to 22.54%), p-menthone (from 0.17 to 15.42%) were as the main ingredients in 30 studied genotypes. Genotypes were divided into four classes based on Hierarchical cluster heat map analysis. Correlation analysis also showed a good relationship between essential oil constituents and evaluated phytochemical compounds. According to the results of this study, the essential oil of *M. Longifolia* genotypes can be a suitable candidate for use in various pharmaceutical, food and cosmetic industries.

1. Introduction

Mentha longifolia (also known as horse mint or wild mint) belongs to the Lamiaceae family¹. The genus *Mentha* includes approximately 25 to 30 species², which are widely distributed in Europe, Australia Asia Minor as well as Northern parts of Africa, Iran and America³. *Mentha longifolia* is one of the most important medicinal and economic species of *Mentha* genus (*Mentha* spp.)⁴. Natural compounds in *M. longifolia* are essential oils, phenolic compounds, flavonoids and other compounds such as steroids and ceramides^{5,6}. Most of the different parts of horse mint (especially leaves, flowers and stems) are used as flavoring in the food industry and also in herbal tea⁷. The beneficial effects of horse mint in traditional medicine include anti flatulence, antispasmodic, antimicrobial, treatment of digestive diseases and headache⁸. Nowadays, anti-inflammatory, anti-diarrheal, treatment of irritable bowel syndrome (IBS), Liver protection and anti-mutagenic of horse mint have been confirmed as medicinal effects⁹.

Weather conditions, seasonal changes, altitude, lighting, soil type, collection region, genotype and species can be major factors affecting on the quantity and quality of natural products in medicinal plants^{10–12}. Some studies reported that the yield of *Mentha longifolia* essential oil varied between 0.88–1.3%, also detected menthone, pulegone, 1, 8-cineole, piperitenone, carvone, borneol and limonene as the main components^{13–15}. Total phenol content in *Mentha spicata* and *Mentha longifolia* has been registered 50.1 and 67.2 mg tannic acid g^{-1} dry weight, respectively¹⁶. Stanisavljević et al¹⁷ found that the natural dried extract of *Mentha longifolia* has the highest total phenol ($113.8 \text{ mg gallic acid g}^{-1}$) and flavonoid content ($106.7 \text{ mg rutin g}^{-1}$). In another study, the reduction of antioxidant activity by DPPH method in five *Mentha* species was presented in the following order: *Mentha piperita* > *Mentha pulegium* > *Mentha rotundifolia* > *Mentha longifolia* > *Mentha spicata*¹⁸.

Based on the previous studies mentioned above, the horse mint is very rich in terms of natural compounds production. Considering that until now no study has been done on the comparison of natural compounds and chemical profile of essential oil in different genotypes of *M. longifolia*. In this new work, it has been tried to compare 30 different genotypes of *M. longifolia* in terms of the ability to produce important natural compounds (such as total phenol and flavonoid, anthocyanin, carotenoid, beta-carotene and antioxidant capacity) as well as the main components of the essential oil in Iran.

2. Experimental

2.1. Plant material

Fresh aerial parts (including leaves, stems and flowers) of 30 different genotypes of *Mentha longifolia* (Fig. 1) from two provinces in Iran (West Azerbaijan and East Azerbaijan) were collected in August 2022 (Table 1). The plant samples were dried at room temperature (25°C), and were transferred to a dry and cool place until measurement of parameters. The *Mentha longifolia* genotypes identification was done by botanist Dr. Ali Sonboli. The voucher herbarium samples of the collected *Mentha longifolia* genotypes have been deposited at the herbarium of Urmia University (Horticulture department). Vouchers of samples are presented in Table 1. According to "Red data book of Iran" (a preliminary survey of endemic, rare and endangered plant species in Iran/ by Adel Jalili and Ziba Jamzad), *Mentha longifolia* species is not among the threatened species, and according to IUCN statement, this species is among the stable species. Nevertheless, the necessary licenses and permits were obtained from the Natural Resources Organization of Iran to collect this plant. The authors state that the institutional, national, and international guidelines and legislation were followed in the collecting of *Mentha longifolia* genotypes.

Table 1
Geographical information of sampling location of *Mentha longifolia* genotypes

Code	Vouchers of samples	Locality	province	Latitude	Longitude	Height (m)
G1	1506	Miandoab	West Azerbaijan	36° 5' 43.84"	46° 50' 14.31"	2076
G2	1507	Miandoab	West Azerbaijan	37° 1' 26.79"	46° 29' 56.50"	1747
G3	1508	Miandoab	West Azerbaijan	37° 0' 33.23"	46° 28' 21.81"	1700
G4	1509	Naghadeh	West Azerbaijan	36° 55' 40.21"	45° 22' 1.28"	1329
G5	1510	Naghadeh	West Azerbaijan	36° 55' 32.30"	45° 22' 51.95"	1338
G6	1511	Naghadeh	West Azerbaijan	36° 55' 12.54'	45° 22' 32.18"	1370
G7	1512	Urmia	West Azerbaijan	37° 25' 32.54'	45° 7' 39.86"	1321
G8	1513	Urmia	West Azerbaijan	37° 26' 0.43"	45° 8' 39.89"	1320
G9	1514	Urmia	West Azerbaijan	37° 26' 8.88"	45° 6' 33.05"	1334
G10	1515	Boukan	West Azerbaijan	36° 40' 49.55"	46° 6' 14.89"	1332
G11	1516	Boukan	West Azerbaijan	36° 41' 40.10"	46° 2' 44.77"	1469
G12	1517	Boukan	West Azerbaijan	36° 52' 8.69"	46° 1' 34.32"	1356
G13	1518	Shahindj	West Azerbaijan	36° 40' 51.11"	46° 37' 15.51"	1454
G14	1519	Shahindj	West Azerbaijan	36° 44' 6.73"	46° 29' 21.64"	1348
G15	1520	Shahindj	West Azerbaijan	36° 45' 40.13"	46° 28' 0.13"	1339
G16	1521	Bonab	East Azerbaijan	37° 25' 33.48"	46° 5' 35.34"	1396
G17	1522	Bonab	East Azerbaijan	37° 22' 22.23"	46° 6' 35.10"	1330
G18	1523	Bonab	East Azerbaijan	37° 22' 48.18"	46° 4' 10.78"	1287
G19	1524	Ajab Shir	East Azerbaijan	37° 29' 48.28"	45° 58' 24.20"	1359
G20	1525	Ajab Shir	East Azerbaijan	37° 28' 50.59"	45° 55' 35.44"	1311
G21	1526	Ajab Shir	East Azerbaijan	37° 26' 44.49"	45° 52' 58.41"	1280
G22	1527	Malkan	East Azerbaijan	37° 10' 19.11"	46° 15' 12.04"	1596
G23	1528	Malkan	East Azerbaijan	37° 12' 30.31"	46° 17' 54.72"	1829
G24	1529	Malkan	East Azerbaijan	37° 11' 1.46"	46° 19' 18.49"	1679
G25	1530	Maragheh	East Azerbaijan	37° 23' 3.27"	46° 23' 25.64"	1776
G26	1531	Maragheh	East Azerbaijan	37° 24' 0.22"	46° 24' 42.31"	1737
G27	1532	Maragheh	East Azerbaijan	37° 26' 17.65"	46° 25' 20.62"	1810
G28	1533	Tabriz	East Azerbaijan	37° 53' 54.65"	46° 34' 56.87"	2026
G29	1534	Tabriz	East Azerbaijan	37° 50' 24.19"	46° 32' 48.04"	2255
G30	1535	Tabriz	East Azerbaijan	37° 48' 50.47"	46° 32' 4.78"	2380

2.2. Chlorophylls, total carotenoid and β -carotene content

Acetone extract was used to measure the amount of chlorophylls, total carotenoid and β -carotene content. For this purpose, 0.5 grams of plant samples were thoroughly mixed with 10 ml of acetone. The obtained mixture was centrifuged (ROTINA 380/380R, Hettich, Germany) at 4000 rpm for 10 minutes. Then, the upper phase of each sample was used for reading at 470, 479, 662 and 645 nm by spectrophotometer (Dynamica Halo XB-10, England). Acetone was used as a control. In the last step, the amount of chlorophyll a and b, total carotenoid content and β -carotene were calculated according to the following formulas¹⁹.

$$\text{Cha} = 11.75A_{662} - 2.350A_{645}$$

$$\text{Chb} = 18.61A_{645} - 3.960A_{662}$$

$$\text{TCC} = 1000A_{470} - 2.270\text{Cha} - 81.4\text{Chb}/227$$

$$\beta\text{-carotene} = 0.854A_{479} - 0.312A_{645} + 0.039A_{663} - 0.005$$

where Cha is the chlorophyll a, Chb is the chlorophyll b, TCC is the total carotenoid content, β -carotene is the beta-carotene and A is the absorbance.

2.3. Preparation of methanolic extracts

0.5 grams of dried samples were homogenized with 10 ml of methanol and water (80:20% ratio) in separate falcons, and finally the samples were extracted by ultrasonic method (Elmasonic, Singen, 120 Hz power, Germany) for 30 min at 30°C.

2.3.1. Total phenol content

Quantitative determination of total phenolic content (TPC) in plant samples was performed using Folin–Ciocalteu reagent²⁰. The reaction components for each sample included 5 µl of methanol extract (80% v/v), 180 µl of distilled water, 1200 µl of Folin (10% v/v) and 960 µl of sodium carbonate (7% w/v). Then the tubes were transferred for 30 minutes in the dark and at room temperature (25°C). In the last step, the absorbance of the samples was read by a spectrophotometer at 760 nm. In this experiment, gallic acid was used as a standard and the TPC was reported in mg gallic acid per gram of dry weight (mg GAE g⁻¹ DW).

2.3.2. Total flavonoid content

Aluminum chloride was used as a reagent to measure total flavonoid content (TFC). 15 µl of methanol extract (80% v/v) from each sample was mixed with 500 µl of aluminum chloride (10% w/v), 500 µl of potassium acetate (1 M) and 1400 µl of distilled water. The tubes were kept for 30 minutes in the dark and at room temperature (25°C). Finally, the absorbance of the samples was read at 415 nm by a spectrophotometer. In this experiment, quercetin was presented as a standard and the TFC was presented in mg of quercetin per gram of dry weight (mg QUE g⁻¹ DW)²¹.

2.3.3. Antioxidant activity

The DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay was used to determine the antioxidant activity of mentha samples. 5 µl of the methanol extract of the samples was added to 2000 µl of prepared DPPH solution. After 5 minutes, the absorbance of completely homogenized samples was recorded at 517 nm in the spectrophotometer. In this experiment, ascorbic acid was used as a standard curve and the antioxidant activity of different mint samples was recorded based on mg of ascorbic acid per gram of dry weight (mg AA g⁻¹ DW). The free radical scavenging activity was obtained according to the following formula²².

$$\text{FRSA (\%)} = (\text{AC} - \text{AS}) / \text{AC} \times 100$$

where FRSA is the free radical scavenging activity, AC is the absorbance of control and AS is the absorbance of sample.

2.4. Total anthocyanin content

Total anthocyanin content (TAC) was measured by Wagner²³ method with some slight modifications. According to this method, first, 0.1 grams of dry plant samples were completely mixed with 10 ml of acidic methanol (Methanol (99): Hydrochloric acid (1) v/v). Then the prepared samples were stored in the dark at 25°C for 24 hours. Finally, after Ben Marin (with 90°C for 10 minutes) and centrifugation (4000 rpm for 10 minutes), the absorbance of the samples was read by a spectrophotometer at 550 nm. The amount of TAC was calculated according to the following formula:

$$A = \epsilon \times B \times C$$

where A is the sample absorption, ϵ is the extinction coefficient (equivalent to 33000 mol cm⁻¹), B is the cuvette width (1 cm) and C is the amount of anthocyanin (mol g⁻¹).

2.5. Soluble carbohydrate content

Soluble carbohydrate content (SCC) was evaluated using anthrone reagent with a spectrophotometer according to Carroll et al (²⁴) method. In the first step, 0.5 grams of the samples were completely homogenized with 5 ml of ethanol (96% v/v). In the second step, after filtering the solution, 5 ml of ethanol (70% v/v) was added to the plant residues again. Then the whole solution was centrifuged (ROTINA 380/380R, Hettich, Germany) at 4000 rpm for 15 minutes. The obtained ethanolic extract was used as the base extract to estimate the SCC. For each sample, 20 µl of prepared extract was added to 3 ml of anthrone solution (150 mg in 100 mL of sulfuric acid). Then the tubes were placed in Ben Marin (100°C) for 10 minutes and after cooling the samples, their absorbance was read at 625 nm. The amount of SCC was calculated using the glucose standard curve.

2.6. Essential oil extraction

Essential oil (EO) extraction from different genotypes was performed by water distillation using the Clevenger system for 3 hours. For this purpose, 20 grams of dry material from each sample was poured into a 500 ml flask with 100 ml of water. After dehydrating with sodium sulfate (Na₂SO₄), the essential oils were stored in a dark vial at 4°C until analysis by GC-MS. Essential oil yields were calculated according to the following formula²⁵:

$$\text{EOYS} = \text{VEO} / \text{DWPM} \times 100$$

where EOYS is the essential oil yields, VEO is the volume of essential oil and DWPM is the dry weight of plant material.

2.6.1. Gas Chromatography-Mass Spectrometry analysis

Essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS). For GC–MS analysis was used an Agilent 7890 A gas chromatograph coupled to a 5975A mass spectrometer using a HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed as follows: 3 min at 80°C, subsequently 8°C min⁻¹ to 180°C, held for 10 min at 180°C. Helium was used as carrier gas at a flow rate of 1 ml min⁻¹ and the Electron impact (EI) was 70 eV. The injector was set in a split mode (split ratio of 1:500) and mass range acquisition was from 40 to 500 m/z. Essential oil constituents were identified by using the calculated linear retention indices (Wiley 2007; NIST 2005) and mass spectra with those reported in the NIST 05 and Wiley 07.

2.7. Statistical analysis

Quantitative data was analyzed by SPSS software. A completely randomized design (CRD) with three replications was used for all the experiments performed in the present study. Means were compared using the least significant difference test (LSD). Visualization (i.e. Hierarchical cluster heat map analysis) and correlation between data was analyzed by R software.

3. Results and Discussion

3.1. Chlorophylls, total carotenoid and β -carotene content

The analysis results demonstrated a significant difference in pigments such as Cha, Chb, TCC and β -carotene content among 30 *Mentha* genotypes ($P < 0.01$). According to Table 2, the amounts of Cha, Chb, TCC and β -carotene content varied between 0.07–0.40 mg g⁻¹ DW, 0.02–0.23 mg g⁻¹ DW, 3.17–18.59 μ g g⁻¹ DW and 0.004–0.026 μ g g⁻¹ DW, respectively. Their highest amount was detected in G26, G27, G26 and G25 genotypes, respectively. Carotenoids and chlorophylls are natural compounds that have the ability to reduce oxidative stress and protect against light^{26,27}. Other applications of carotenoids can be mentioned to create a molecular signal, a precursor for the production of phytohormones (such as abscisic acid) and strigolacton²⁸. Beta-carotene (a type of carotenoid) is also important precursor for the production of vitamin A²⁹. Therefore, accurate determination of the quantity of pigments can play an effective role in maintaining the health of the plant³⁰. The biosynthesis of plant pigments is greatly affected by environmental factors such as high light, salinity and drought³¹. Previous studies have documented that altitude and light quality can have a positive effect on the production and accumulation of plant pigments^{32–33}. In the present study, the highest amount of pigments among the 30 studied genotypes was obtained almost at high altitudes (above 1500 meters). According to previous reports, blue light has a positive effect on the amount of pigments compared to red light³³. Therefore, to some extent, the obtained results can be related to the high altitudes and light quality.

Table 2
Content of pigment and phytochemical compounds in *Mentha longifolia* genotypes

Genotypes	Cha (mg g ⁻¹ DW)	Chb (mg g ⁻¹ DW)	TCC (μg g ⁻¹ DW)	β-carotene (μg g ⁻¹ DW)	TPC (mg GAE g ⁻¹ DW)	TFC (mg QUE g ⁻¹ DW)	DPPH (mg AAE g ⁻¹ l)	TAC (μmol g ⁻¹ DW)	SCC (mg g ⁻¹ DW)
G1	0.28 ^{defg} ± 0.00	0.11 ^{fg} ± 0.00	11.61 ^j ± 0.05	0.017 ^j ± 0.00	11.82 ^{ef} ± 0.62	3.36 ^b ± 0.16	11.65 ^{de} ± 0.20	1.04 ^l ± 0.02	1.06 ⁿ ± 0.01
G2	0.26 ^{efghi} ± 0.00	0.10 ^{ghi} ± 0.00	11.21 ^k ± 0.09	0.016 ^{jk} ± 0.00	8.74 ^{lmn} ± 0.24	2.22 ^f ± 0.18	15.18 ^a ± 0.12	1.41 ^{kl} ± 0.05	1.27 ^l ± 0.00
G3	0.15 ^{kl} ± 0.00	0.05 ^{mno} ± 0.00	6.53 ^t ± 0.04	0.009 ^s ± 0.00	11.18 ^{fgh} ± 0.45	2.22 ^f ± 0.01	15.50 ^a ± 0.21	1.03 ^l ± 0.01	1.78 ^{cd} ± 0.04
G4	0.07 ^m ± 0.00	0.02 ^p ± 0.00	3.17 ^v ± 0.01	0.004 ^u ± 0.00	7.36 ^{op} ± 0.31	0.76 ^l ± 0.01	6.10 ⁱ ± 0.10	3.32 ^j ± 0.15	1.52 ^{hijk} ± 0.05
G5	0.18 ^{jk} ± 0.00	0.07 ^{klm} ± 0.00	11 ^{fg} ± 0.04	0.010 ^f ± 0.00	6.13 ^u ± 0.05	0.63 ^l ± 0.07	3.34 ^k ± 0.47	8.12 ^b ± 0.89	1.30 ^l ± 0.06
G6	0.31 ^{cde} ± 0.00	0.14 ^{de} ± 0.00	13.10 ^h ± 0.08	0.019 ^h ± 0.00	7.34 ^{op} ± 0.45	0.59 ^l ± 0.13	6.52 ^{hi} ± 0.62	5.05 ^h ± 0.33	1.60 ^{fghi} ± 0.02
G7	0.10 ^{lm} ± 0.00	0.03 ^{op} ± 0.00	5.37 ^u ± 0.03	0.008 ^t ± 0.00	13.90 ^b ± 0.22	3.31 ^b ± 0.06	15.72 ^a ± 0.68	3.45 ⁱ ± 0.19	1.93 ^{ab} ± 0.01
G8	0.23 ^{fghi} ± 0.00	0.10 ^{ghi} ± 0.00	12.86 ^{hi} ± 0.07	0.019 ^g ± 0.00	12.66 ^{cd} ± 0.67	1.15 ^k ± 0.08	15.00 ^a ± 0.25	6.73 ^f ± 0.22	1.62 ^{efgh} ± 0.02
G9	0.14 ^{kl} ± 0.00	0.05 ^{no} ± 0.00	7.69 ^s ± 0.04	0.011 ^q ± 0.00	13.16 ^c ± 0.32	1.59 ^{ij} ± 0.10	12.17 ^{cde} ± 0.72	7.53 ^{cd} ± 0.62	1.48 ^{jk} ± 0.06
G10	0.29 ^{def} ± 0.00	0.13 ^{de} ± 0.00	12.65 ⁱ ± 0.03	0.018 ⁱ ± 0.00	6.46 ^u ± 0.36	0.61 ^l ± 0.05	6.56 ^{hi} ± 0.64	9.30 ^a ± 0.24	1.24 ^{lm} ± 0.02
G11	0.18 ^{jk} ± 0.00	0.07 ^{klm} ± 0.00	8.05 ^r ± 0.02	0.011 ^q ± 0.00	15.13 ^a ± 0.10	2.43 ^e ± 0.14	15.50 ^a ± 0.48	7.05 ^{def} ± 0.26	0.78 ^o ± 0.06
G12	0.20 ^{hijk} ± 0.00	0.08 ^{ijkl} ± 0.00	9.13 ^{op} ± 0.02	0.013 ^m ± 0.00	8.60 ^{lmn} ± 0.80	1.72 ^{hi} ± 0.07	12.68 ^{bc} ± 0.71	1.03 ^l ± 0.03	1.25 ^{lm} ± 0.08
G13	0.37 ^{abc} ± 0.00	0.17 ^c ± 0.00	17.05 ^c ± 1.47	0.025 ^b ± 0.01	8.29 ^{lmn} ± 0.35	1.57 ^{ij} ± 0.13	13.21 ^b ± 0.60	5.82 ^j ± 0.56	1.63 ^{efgh} ± 0.05
G14	0.20 ^{ijk} ± 0.00	0.07 ^{klm} ± 0.00	8.91 ^p ± 0.04	0.013 ^o ± 0.00	5.97 ^u ± 0.27	1.09 ^k ± 0.08	4.33 ^j ± 0.87	5.75 ^g ± 0.23	1.14 ^{mn} ± 0.00
G15	0.28 ^{defg} ± 0.12	0.19 ^b ± 0.03	16.71 ^d ± 0.09	0.024 ^{cd} ± 0.00	6.19 ^u ± 0.25	0.97 ^k ± 0.08	7.40 ^h ± 0.85	9.07 ^a ± 0.15	1.73 ^{de} ± 0.06
G16	0.34 ^{bcd} ± 0.00	0.15 ^{cd} ± 0.00	15.45 ^f ± 0.28	0.022 ^e ± 0.00	8.06 ^{no} ± 0.25	1.81 ^h ± 0.10	12.12 ^{cde} ± 0.08	6.84 ^{ef} ± 0.24	1.31 ^l ± 0.00
G17	0.22 ^{ghij} ± 0.00	0.09 ^{hijk} ± 0.00	10.40 ^m ± 0.10	0.015 ⁱ ± 0.00	8.99 ^{lm} ± 0.46	2.16 ^f ± 0.05	10.34 ^f ± 0.25	7.70 ^{bc} ± 0.35	1.54 ^{ghijk} ± 0.10
G18	0.32 ^{cde} ± 0.00	0.15 ^d ± 0.00	15.44 ^f ± 0.14	0.022 ^e ± 0.00	10.88 ^{ghi} ± 0.47	2.03 ^{fg} ± 0.11	7.01 ^{hi} ± 0.08	7.43 ^c ± 0.28	1.47 ^{jk} ± 0.06
G19	0.29 ^{def} ± 0.00	0.12 ^{ef} ± 0.00	14.63 ^g ± 0.17	0.021 ^f ± 0.00	6.70 ^{pu} ± 0.15	0.78 ^l ± 0.02	3.46 ^k ± 0.81	1.08 ^l ± 0.03	1.68 ^{def} ± 0.00
G20	0.19 ^{ijk} ± 0.00	0.07 ^{klm} ± 0.00	9.48 ⁿ ± 0.03	0.014 ^m ± 0.00	11.41 ^{fg} ± 0.27	2.82 ^c ± 0.02	15.32 ^a ± 0.51	1.15 ^l ± 0.03	1.77 ^{cd} ± 0.06
G21	0.34 ^{bcd} ± 0.00	0.15 ^d ± 0.00	16.12 ^e ± 3.50	0.024 ^{bc} ± 0.01	11.29 ^{fgh} ± 0.74	2.56 ^{de} ± 0.16	9.63 ^f ± 0.21	7.36 ^{cde} ± 0.43	1.57 ^{fghij} ± 0.07
G22	0.10 ^{lm} ± 0.00	0.04 ^{op} ± 0.00	5.20 ^u ± 0.30	0.007 ^t ± 0.00	11.03 ^{fgh} ± 0.10	2.58 ^{de} ± 0.18	8.73 ^g ± 0.48	4.05 ⁱ ± 0.29	1.63 ^{efgh} ± 0.03
G23	0.18 ^{jk} ± 0.00	0.06 ^{lmn} ± 0.00	8.49 ^q ± 0.09	0.012 ^p ± 0.00	12.18 ^{de} ± 0.32	2.64 ^{cd} ± 0.18	8.69 ^g ± 0.57	8.15 ^b ± 0.20	1.11 ⁿ ± 0.11

Cha, Chlorophyll a; Chb, Chlorophyll b; TCC, Total carotenoid content; β-carotene, beta-carotene; TPC, Total phenol content; TFC, Total Flavonoid content; DPPH, Antioxidant activity by DPPH assay; TAC, Total anthocyanin content; SCC, Soluble carbohydrate content; **P < 0.01

Genotypes	Cha (mg g ⁻¹ DW)	Chb (mg g ⁻¹ DW)	TCC (μg g ⁻¹ DW)	β-carotene (μg g ⁻¹ DW)	TPC (mg GAE g ⁻¹ DW)	TFC (mg QUE g ⁻¹ DW)	DPPH (mg AAE g ⁻¹ DW)	TAC (μmol g ⁻¹ DW)	SCC (mg g ⁻¹ DW)
G24	0.23 ^{fghij} ± 0.00	0.08 ^{hijkl} ± 0.00	10.81 ^l ± 0.19	0.016 ^k ± 0.00	16.06 ^a ± 0.72	3.60 ^a ± 0.15	11.97 ^{cde} ± 0.28	6.70 ^f ± 0.09	1.64 ^{efg} ± 0.00
G25	0.40 ^{ab} ± 0.00	0.21 ^a ± 0.00	18.31 ^a ± 0.14	0.026 ^a ± 0.01	10.97 ^{gh} ± 0.24	2.67 ^{cd} ± 0.02	15.14 ^a ± 0.17	1.71 ^k ± 0.06	1.42 ^k ± 0.03
G26	0.40 ^a ± 0.00	0.22 ^a ± 0.00	18.59 ^a ± 0.13	0.026 ^a ± 0.00	9.84 ^{jk} ± 0.10	2.16 ^f ± 0.10	11.70 ^{cde} ± 0.68	4.95 ^h ± 0.26	1.85 ^{bc} ± 0.00
G27	0.31 ^{cde} ± 0.12	0.23 ^a ± 0.00	17.42 ^b ± 0.45	0.024 ^c ± 0.00	9.22 ^{kl} ± 0.57	1.92 ^{gh} ± 0.11	12.28 ^{cd} ± 0.64	7.48 ^{cd} ± 0.33	1.73 ^{de} ± 0.04
G28	0.19 ^{ijk} ± 0.00	0.08 ^{ijkl} ± 0.00	9.37 ^{no} ± 0.06	0.014 ^{mn} ± 0.00	10.16 ^{ij} ± 0.19	2.51 ^{de} ± 0.03	11.80 ^{cde} ± 0.05	1.04 ^l ± 0.04	1.48 ^{ijk} ± 0.13
G29	0.27 ^{efgh} ± 0.00	0.10 ^{fgh} ± 0.00	10.55 ^{hij} ± 0.28	0.019 ^g ± 0.00	1.86 ^{gh} ± 0.52	1.86 ^{gh} ± 0.08	11.26 ^e ± 0.47	3.33 ^l ± 0.22	2.01 ^a ± 0.01
G30	0.23 ^{fghij} ± 0.00	0.09 ^{hij} ± 0.00	10.26 ^m ± 0.31	0.015 ⁱ ± 0.00	8.66 ^{lmn} ± 0.44	1.42 ^g ± 0.08	8.77 ^g ± 0.34	8.13 ^b ± 0.32	1.44 ^k ± 0.01
Significance	**	**	**	**	**	**	**	**	**
Cha, Chlorophyll a; Chb, Chlorophyll b; TCC, Total carotenoid content; β-carotene, beta-carotene; TPC, Total phenol content; TFC, Total Flavonoid content; DPPH, Antioxidant activity by DPPH assay; TAC, Total anthocyanin content; SCC, Soluble carbohydrate content; ** <i>P</i> < 0.01									

3.2. Total phenol content

The quantitative amount of TPC is reported in Table 2. The TPC in 30 genotypes of Horse mint was different between 5.97 and 16.06 mg GAE g⁻¹ DW. The TPC was found to be highest in G24 and the lowest in G14. Phenols are natural products that are synthesized from shikimic acid or malonate/ acetate pathway³⁴. The presence of phenolic compounds has caused higher plants resistant to the challenge of changes in environmental conditions³⁵. Harvesting time, altitude, high temperature, sunlight, genotype and plant species are considered to be effective factors in determining the quantitative and qualitative characteristics of phenolic compounds^{10,36,37}. Nikavar and Kamalnejad⁽¹⁸⁾ reported the TPC in five *Mentha* species including *M. piperita* (433.60 μg mg⁻¹ DW), *M. pulegium* (358.10 μg mg⁻¹ DW), *M. rotundifolia* (331.31 μg mg⁻¹ DW), *M. longifolia* (288.82 μg mg⁻¹ DW) and *M. spicata* (150.91 μg mg⁻¹ DW). Elmastaş et al.⁽³⁸⁾ found TPC between 9.39–28.27 μg GAE g⁻¹ DW for *M. spicata*, between 21.56–23.99 μg GAE g⁻¹ DW for *M. piperita* and between 9.92–21.17 μg GAE g⁻¹ DW for *M. villosa nervata*. Other researchers showed the level of TPC varied among species, i.e. 14.66 mg GAE g⁻¹ DW for *M. villosa*, 15.10 mg GAE g⁻¹ DW for *M. rotundifolia*, 17.00 mg GAE g⁻¹ DW for *M. pulegium*, 31.40 mg g⁻¹ mg GAE g⁻¹ DW for *M. piperita*, 32.90 mg g⁻¹ mg GAE g⁻¹ DW for *M. arvensis* and 43.21 mg g⁻¹ mg GAE g⁻¹ DW for *M. aquatica*³⁹. In previous studies, TPC was recorded from 14.81 mg GAE g⁻¹ (for *M. cervina*) to 58.93 mg GAE g⁻¹ (for *M. suaveolens*) in twelve *Mentha* species⁴⁰. In another study, the TPC in four types of aqueous, methanolic, ethanolic and acetic extracts of *M. aquatica* was obtained, respectively 20.12, 45.30, 44.95 and 45.15 mg GAE g⁻¹⁴¹. According to the mentioned articles, the results of this work also showed that the TPC was probably affected by different genotypes.

3.3. Total flavonoid content

The level of TFC is exhibited in Table 2. The TFC of the *mentha* Genotypes varied from 0.59 to 3.60 mg QUE g⁻¹ DW. G24 had the highest TFC while G6 had the lowest TFC. Flavonoids in higher plants have special functions such as increasing the tolerance of plants against biotic and abiotic stresses, molecular signaling as well as antioxidant and allelopathic effects⁴². Flavonoids as the most important bioactive compounds play a role in plant-environment interaction⁴³. Fatiha et al.⁽⁴⁴⁾ exhibited TFC between 1.98 (methanolic extract) to 7.98 (acetic extract) mg QE g⁻¹ DW for *M. spicata*. In another research, the TFC of *M. pulegium*, *M. spicata* and *M. rotundifolia* was reported 0.85, 2.45 and 3.30 mg QE g⁻¹ DW, respectively⁴⁵. Bahadori et al.⁽⁴⁶⁾ obtained the TFC of *M. longifolia* in ethanolic extract and infusion 23.68 mg RE g⁻¹ DW and 46.18 mg RE g⁻¹ extract, respectively. In general, the production and accumulation of phytochemical compounds in plants may be influenced by various factors, including genotype and geographical location⁴⁷. Therefore, according to the results of the present study, G24 can be introduced as a rich source of TPC and TFC.

3.4. Antioxidant activity

Determining antioxidant activity in plants using the DPPH method is one of the unique features. The level of antioxidant activity in *Mentha* genotypes ranged between 3.34–15.72 mg AA g⁻¹ DW (Table 2). Antioxidant activity was significantly different among genotypes (*P* < 0.01). Among the 30 studied genotypes, the highest and lowest amount of antioxidant activity was obtained in G7 and G5, respectively. DPPH is a stable free radical that is used nowadays to evaluate the antioxidant activity due to its easiness, rapidity and low cost^{48,49}. The single electron in the DPPH free radical alone causes a purple color, but it changes to yellow in the presence of an antioxidant compound⁵⁰. Previous studies introduced *M. piperita* as the best species with antioxidant activity among other *Mentha* species⁵¹. The antioxidant activity of nine varieties of *Mentha* species was investigated at three times 10, 20, and 30 minutes. Based on the obtained results, the levels of their antioxidant were from the highest to lowest in the following order: *M. suaveolens* > *M. longifolia* > *M. officinalis* > *M. piperita* > *M. pulegium* > *M. royleana* > *M. spicata* > *M. arvensis* > *M. citrate*⁵². In a paper, Abootalebian et al.⁽¹⁶⁾ found the maximum antioxidant activity in Mzin6 of *M.*

longifolia (49.3% in), comparing of different *M. spicata* and *M. longifolia* accessions. In another research, the free radical scavenging capacity in *M. longifolia* was detected 23.8%⁵³. In general, the antioxidant capacity of medicinal plants is related to the presence of phytochemical compounds⁵⁴. Antioxidants are essential elements that protect most organisms against free radicals⁵⁵. Based on the results of the present study, the introduction of G7 as the best genotype with maximum antioxidant activity is probably due to the presence of large amounts of natural ingredients such as phenolic and flavonoid compounds.

3.5. Total anthocyanin content

The results of comparing the averages showed that all the studied genotypes had significant differences in TAC ($P < 0.01$). TAC value among *Mentha* genotypes detected between 1.03 and 9.30 $\mu\text{mol g}^{-1}$ DW (Table 2). The minimum of TAC was observed in G3 and G12, as well as its maximum in G10. Anthocyanins as color compounds are responsible for creating red, blue and purple color⁵⁶. These compounds are soluble in water and are considered a subgroup of flavonoids⁵⁷. The beneficial effects of anthocyanins in higher plants include adsorption of pollinators, prevention of DNA damage, filtration against ultraviolet (UV) radiation, flower color and protection against oxidative stress⁵⁸. Previous studies exhibited the TAC of *M. piperita* in three methanolic, ethanolic and methanolic/ethanolic extracts to be 0.1, 0.86 and 1.74 $\text{g } 100 \text{ g}^{-1}$, respectively⁵⁹. Some studies have reported that the level of anthocyanins in three cultivars of *Ocimum basilicum* was affected by plant maturity and cultivar⁶⁰. The data obtained from our study show that the amount of TAC was variable among the 30 *Mentha* genotypes. Based on the reviewed studies, the reason for this difference can be related to the maturity stage of the plant and the harvest time.

3.6. Soluble carbohydrate content

The results listed in Table 2 show a significant difference between genotypes in terms of SCC ($P < 0.01$). The highest amount of SCC was obtained in G29 (2.01 mg g^{-1} DW), while the lowest amount was obtained in G11 (0.78 mg g^{-1} DW). Soluble sugars as a source of energy for plant cells play an important role in the plant tolerance mechanism under stress conditions⁶¹. In addition, soluble sugars are involved in growth and development processes⁶². The content of total sugar in *M. piperita* collected from three different regions was reported between 1.78 and 1.94%⁶³. The results of our study show that the SCC is completely diverse in different altitudes and genotypes. Therefore, it can be said that despite a series of common reactions, the amount of SCC production may be affected by genotype and environmental conditions.

3.7. GC-MS analysis

The essential oil yield among different *Mentha* genotypes was recorded between 0.12 to 0.56% (Table 3). In an article, the essential oil yield has been differently mentioned in various *Mentha longifolia* parts, i.e. 1.34 ml 100 g^{-1} in leaf, 0.76 ml 100 g^{-1} in stem and 0.97 ml 100 g^{-1} in flower⁶⁴. Figure 2 shows the chromatograms of two *Mentha* genotypes (G3 and G21). The major compounds identified in the essential oil are presented in Table 3. The main essential oil ingredients of the studied genotypes included piperitenone oxide (1.17–69.12%), pulegone (2.97–67.82%), cis-piperitenone oxide (0.78–24.89%), thymol (0.14–22.90%), 1, 8-cineole (0.97–22.54%), p-menthone (0.17–15.42%). In a large number of recent papers, the major ingredients of *Mentha longifolia* essential oil recorded in different regions are piperitenone oxide^{64–67}; 1,8-cineole^{13, 64–65, 68–71}; cis-piperitone oxide^{64, 71–72}; pulegone^{13, 15, 65, 69–71, 73}; piperitone^{15, 65, 70, 74}; thymol^{14, 68, 75}; menthone^{15, 65, 70}; caryophyllene oxide⁶⁷; carvacrol⁶⁸; carvon^{71, 75}. The results of the present study show that the essential oils of *M. longifolia* genotypes have a wide range of chemical compounds. The presence of these different components in the essential oil may be influenced by factors such as geographical location, environmental conditions and harvest time in addition to genetic effect⁷⁶.

Table 3
Yield and ingredients essential oil of *Mentha longifolia* genotypes

Essential oil compound (%)													
Genotypes	EO yield	Alpha-pinene	Sabinene	beta-Pinene	beta-Myrcene	Ethyl amyl carbinol	o-Cymene	Limonene	1,8-cineole	Cis-ocimene	Gamma-terpinene	p-menthon	Puleg
G1	0.38	0.83	ND	1.52	0.53	ND	2.05	ND	3.32	ND	0.80	4.02	16.39
G2	0.28	1.86	1.12	2.04	1.12	1.19	4.88	2.12	2.44	0.17	1.52	6.77	20.62
G3	0.30	1.94	1.66	2.62	1.36	0.53	ND	2.20	5.10	0.12	ND	ND	11.89
G4	0.50	0.68	0.61	1.02	0.61	0.92	1.22	1.27	1.68	0.21	0.35	0.87	4.93
G5	0.26	0.32	0.36	0.7	0.34	ND	0.24	1.12	0.97	ND	0.17	3.44	49.19
G6	0.40	0.85	0.86	1.31	0.70	ND	0.82	1.74	3.09	ND	0.49	1.53	22.74
G7	0.30	0.35	0.47	0.57	0.32	0.78	0.46	0.60	1.16	ND	0.17	11.16	55.97
G8	0.25	0.42	0.65	0.76	0.64	0.59	3.35	0.76	7.70	0.62	3.04	12.41	32.56
G9	0.22	ND	0.14	0.22	0.18	ND	0.14	0.31	2.02	0.14	0.90	1.60	20.96
G10	0.50	0.70	0.62	0.90	0.56	0.58	ND	3.95	3.00	0.28	ND	15.30	24.42
G11	0.42	0.35	0.26	0.48	0.26	0.59	ND	1.47	1.05	ND	ND	15.42	50.60
G12	0.47	0.61	0.54	0.80	0.27	1.03	0.21	2.12	1.74	ND	0.18	9.95	50.18
G13	0.12	0.60	0.62	0.78	0.45	0.72	0.84	1.04	2.20	0.18	0.69	13.66	46.45
G14	0.20	0.66	1.39	1.28	1.21	0.32	7.72	ND	22.54	0.76	8.82	2.46	8.59
G15	0.15	0.54	0.50	0.72	0.42	0.77	0.17	0.72	2.16	0.21	0.21	8.55	43.48
G16	0.37	0.46	0.42	0.62	0.22	0.80	ND	0.36	2.55	ND	ND	13.40	63.32
G17	0.22	0.46	0.41	0.62	0.24	0.72	ND	0.32	2.52	ND	ND	13.44	67.82
G18	0.18	1.10	1.63	1.81	1.46	0.35	7.40	ND	17.56	ND	7.07	0.50	14.26
G19	0.23	1.10	1.65	1.84	1.50	0.34	7.65	ND	17.75	ND	7.3	2.09	12.06
G20	0.27	0.57	0.41	0.70	0.32	0.56	0.46	0.72	2.82	ND	0.47	8.79	57.99
G21	0.30	0.31	0.34	0.48	0.29	0.50	0.88	0.50	3.68	ND	0.94	2.13	60.08
G22	0.47	0.87	0.85	1.29	0.82	0.80	ND	1.90	2.42	0.33	ND	7.78	31.40
G23	0.53	0.59	0.38	0.81	0.40	0.48	0.75	0.77	2.36	ND	1.03	5.14	53.08
G24	0.49	0.47	0.38	0.62	0.32	0.38	ND	0.76	1.32	0.32	ND	4.68	40.66
G25	0.39	0.41	0.30	0.55	ND	0.49	ND	0.84	1.37	ND	ND	6.92	46.69
G26	0.33	ND	ND	0.20	ND	0.97	ND	0.41	1.72	ND	ND	4.39	43.46
G27	0.41	ND	0.48	0.59	0.42	0.36	2.48	0.47	9.35	ND	2.92	4.22	35.77
G28	0.56	0.49	0.62	1.09	0.96	0.74	ND	1.02	1.34	0.46	ND	0.87	10.10
G29	0.51	0.6	0.56	0.82	0.48	0.71	ND	1.19	2.32	0.19	ND	0.64	13.49
G30	0.50	0.42	0.44	0.72	0.47	0.62	ND	1.02	1.39	0.21	ND	0.17	2.97
Sig	**	**	**	**	**	**	**	**	**	**	**	**	**
Sig: Significance													
** <i>P</i> < 0.01													

3.8. Hierarchical cluster heat map and principal component analysis

Hierarchical cluster heat map analysis is a visualization method for a set of samples and measured compounds. In the present study, Hierarchical cluster heat map analysis was performed using essential oil constituents and phytochemical compounds of 30 *Mentha* genotypes. Based on the analysis, they were divided into four classes (Fig. 3). The first class is identified by the high amount of alpha-pinene, beta-pinene, trans-sabinene hydrate, thymol, o-cymene, gamma terpinene and 1,8 cineole (including genotypes G14, G19 and G18), Second class by the average amount of Chlorophyll a, Chlorophyll b, total carotenoid content, β -carotene (including genotypes G8, G27, G15, G13, G17, G16, G21, G26 and G25), and the third class by a very weak value of p-menthone, caryophyllene oxide and pulegone (including genotypes G3, G2, G10, G5, G28, G4, G6, G30, and G9), and the fourth class by weak amount of most compounds

(including genotypes G22, G12, G20, G7, G1, G24, G9, G23, and G11). Estimating the degree of similarity and distance of classes can be used as a suitable screening pattern for plant breeding works. Figure 3 presented that alphapinene, o-cymene, trans-sabinene hydrate, and pulegone had the greatest role in the classification compared to other evaluated traits. In this research, the quantity of essential oil compounds, plant pigments and bioactive compounds are expressed by color, genotypes in the rows, and the assessed traits in the columns. A number of reports have documented that Hierarchical cluster heat map analysis can be a suitable method for grouping genotypes based on the natural product or essential oil ingredients^{77–79}.

Principal component analysis (PCA) expresses the variance of the data by a two-dimensional map. PCA was performed to identify the classification pattern among the genotypes based on phytochemical traits, plant pigments and essential oil components in *M. longifolia* (Fig. 4.A). The first component showed 28.6% and the second component 21.2% of the total variance (Fig. 4.B). The first component is highly strongly correlated with beta myrcene, sabinene, o-cymene, 1, 8-cineole, gamma terpinene. The second component separates the genotypes based on plant pigments (Chlorophyll a, Chlorophyll b, β -carotene and total carotenoid content.) and pulegone.

3.9. Correlation analysis

Figure 4 shows the results of the correlation analysis of investigated traits in 30 *Mentha* genotypes. The blue color indicates a strong relationship between two variables (positive correlation), while the red color refers to a negative correlation, which means that the variables have a low correlation. The high or low correlation coefficient depends on the intensity of the color and the size of the circles (Fig. 5). Quantitative data analysis demonstrated a positive correlation between gamma terpinene, 1,8-cineole, o-cymene, and thymol, followed by chlorophyll a, chlorophyll b and β -carotene content, and beta myrcene, alpha pinene, beta pinene and sabinene (Fig. 5). Antioxidant activity by DPPH method had a high correlation with total phenol content and total flavonoid content. Modareskia et al.⁽⁸⁰⁾ mentioned that there is a strong correlation between alpha-terpinene, alpha thujene, beta myrcene, alpha pinene, beta pinene and limonene, while there is a weak correlation between thymol and p-cymene. In another study, a high correlation was reported between neomenthol with menthone and gamma-terpinene, while a low correlation was reported with isopulegol⁸¹. A positive correlation between total phenol content, total flavonoid content and antioxidant activity has been presented in several reports^{82–84}. According to the above works, it can be said that providing the correlation coefficient between phytochemical compounds and essential oil ingredients is likely to help breeding and domestication programs for progress^{80,85}.

4. Conclusions

In the present work, 30 *Mentha* genotypes from two provinces of Iran (West and East Azerbaijan) were compared in terms of plant pigments, phytochemical compounds and essential oil compounds. Among the 30 evaluated genotypes, G26 had the highest amounts of chlorophyll a, chlorophyll b and carotenoid, but the highest amount of beta-carotene was found in G27. Genotypes G24, G7, G10, and G29 were introduced as potential sources of total phenol and flavonoid content, antioxidant activity, total anthocyanin content, and total soluble carbohydrate, respectively. Various genotypes of *M. longifolia* were introduced as potential sources of phenolic and flavonoid compounds (G24), antioxidant activity (G7), total anthocyanin content (G10) and total soluble carbohydrates (G29). The highest yield of essential oil was related to G28. Piperitenone oxide (G30), pulegone (G17), cis-piperitenone oxide (G28), thymol (G19). 1, 8-cineole (G14), p-menthone (G11) were revealed as the major ingredients of the essential oil. In general, the results of this work demonstrated the existence of diversity, according to Hierarchical cluster heat map and principal component analysis, in natural compounds, plant pigments and essential compounds of different *Mentha longifolia* genotypes. Therefore, evaluating the diversity among different genotypes of *Mentha longifolia* provides the possibility of selecting the ideal genotype for use in the food, pharmaceutical and cosmetic-health industries.

Abbreviations

Cha, chlorophyll a; Chb, chlorophyll b; TCC, total carotenoid content; β -carotene, beta-carotene; A, absorbance; TPC, total phenolic content; GAE, gallic acid; TFC, total flavonoid content; QUE, quercetin; DPPH, 2,2-diphenyl-1-picryl-hydrazyl; FRSA, free radical scavenging activity; AC, the absorbance of control; AS, the absorbance of sample; AA, ascorbic acid; FW, fresh weight; DW, dry weight; TAC, total anthocyanin content; A, sample absorption; ϵ , extinction coefficient; B, Cuvette width; C, amount of anthocyanin; SCC, soluble carbohydrate content; EO, essential oil; EOYS, essential oil yields; VEO, volume of essential oil; DWPM, dry weight of plant material; GC-MS. gas chromatography-mass spectrometry; LSD, least significant difference test; CRD, completely randomized design.

Declarations

Funding

This paper was advocated by Shahid Bakari Miandoab Higher Education Center, Urmia University, Urmia, Iran

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Competing interests

The authors declare no competing interests.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Figures

Figure 1



Figure 1
Picture of aerial parts of 30 *Mentha longifolia* genotypes.

Figure 2

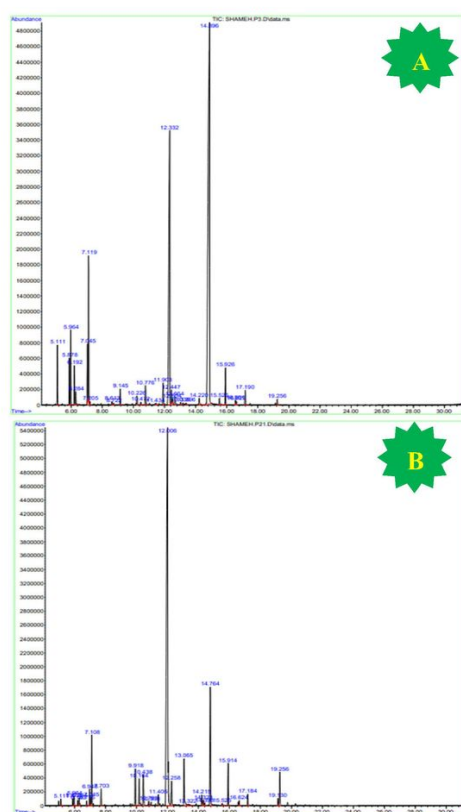


Figure 2

GC-MS chromatogram of essential oil ingredients (A: G3 and B: G21): 4.95': alpha-Thujene, 5.11': alpha-Pinene, 5.87': Sabinene, 5.96': beta-Pinene, 6.19': beta-Myrcene, 6.29': Ethylamylcarbinol, 6.35': Decane, 6.77': alpha-Terpinene, 6.95': O-cymene, 7.03': Limonene, 7.11': 1,8-Cineole, 7.20': cis-Ocimene, 7.71': gamma-Terpinene, 7.90': trans-Sabinene hydrate, 8.61': Isoamyl 2-methylbutyrate, 8.63': Linalool, 8.72': Isopentyl isovalerate, 9.72': Isopulegol, 9.92': p-Menthone, 10.17': Isomenthon, 10.34': Menthol, 10.44': Cis isopulegone, 10.47': 4-Terpineol, 10.77': alpha-Terpineol, 11.90': Pulegone, 12.01': Carvone, 12.24': Cis piperitenone oxide, 13.06': Thymol, 13.11': Carvacrol, 13.43': Cis-carane, 14.22': Piperitenone, 14.32': alpha-Ocimene, 14.73': Piperitenone oxide, 15.03': Neryl acetate, 15.90': trans-Caryophyllene, 16.56': beta-Farnesene, 16.62': α -humulene, 17.18': Germacrene D, 19.13': Spathulenol, 19.25': Caryophyllene oxide.

Figure 3

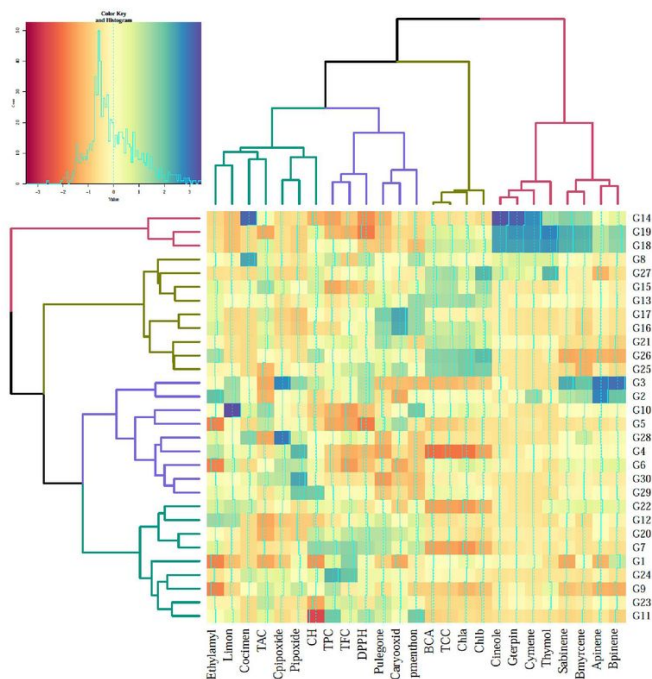


Figure 3

Hierarchical cluster heat map analysis of *Mentha longifolia* genotypes based on essential oil ingredients , plant pigment and phytochemical traits: BCA: β -carotene content, CHA: Chlorophyll A, CHB: Chlorophyll B, TCC: Total carotenoid content, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity by DPPH assay, TAC: Total anthocyanin content, SCC: Soluble carbohydrate content, EOY: Apinene: alpha-Pinene, Sabinene: Sabinene, Bpinene: beta-Pinene, Bmyrcene: beta-myrcene, Ethylamyl: Ethylamyl carbinol, Cymene, O-Cymene, Limon: Limonene, Cineole: 1,8-Cineole, Cocimen: Cis-ocimene, Gterpin: gamma-Terpinene, pmenthon: p-menthone, Pulegone: Pulegone, Cypoxide: Cis piperitenone oxide, Thymol: Thymol, Pipoxide: Piperitenone oxide, Caryoxoid: Caryophyllene oxide.

Figure 4

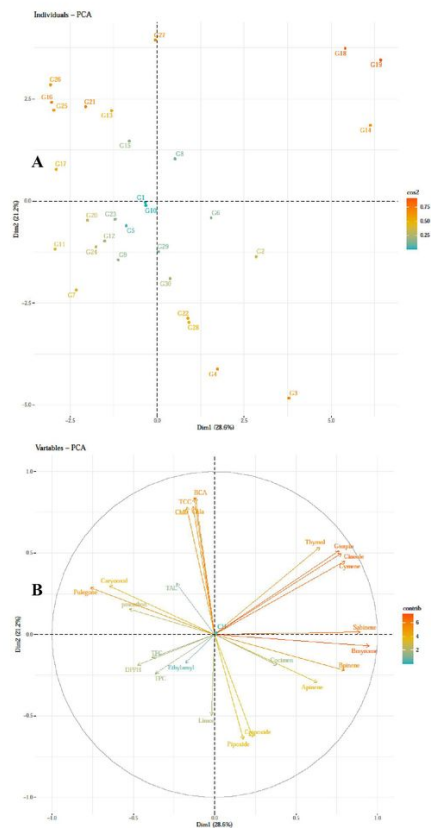


Figure 4

Principal component analysis (PCA) plot of various phytochemical traits, plant pigments and essential oil components in *Mentha longifolia* genotypes. (a) Scatter plot of the various *Mentha longifolia* genotypes represented in two major principal axes. (b) Grouping of the phytochemical traits and essential oil components in two principal components. Dim 1 represented essential oil components and PC2 represented the phytochemical traits and plant pigments.

Figure 5

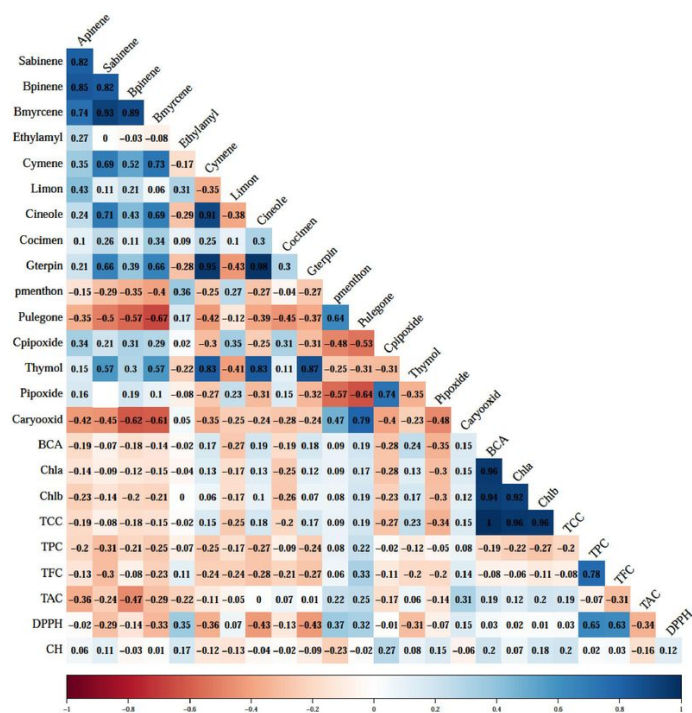


Figure 5

Correlation between essential oil ingredients, plant pigment and phytochemical traits of *Mentha longifolia* genotypes: BCA: β -carotene content, CHA: Chlorophyll A, CHB: Chlorophyll B, TCC: Total carotenoid content, TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: Antioxidant activity by DPPH assay, TAC: Total anthocyanin content, SCC: Soluble carbohydrate content, EOY: Apinene: alpha-Pinene, Sabinene: Sabinene, Bpinene: beta-Pinene, Bmyrcene: beta-myrcene, Ethylamyl: Ethylamyl carbinol, Cymene, O-Cymene, Limon: Limonene, Cineole: 1,8-Cineole, Cocimen: Cis-ocimene, Gterpin: gamma-Terpinene, pmenthon: p-menthone, Pulegone: Pulegone, Cpipoxide: Cis piperitenone oxide, Thymol: Thymol, Pipoxide: Piperitenone oxide, Caryoxoid: Caryophyllene oxide.