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Original Research

Physicochemical, Biochemical, and Essential Oil Diversity among Peppermint (*Mentha* × *piperita* L. "Almira", *Mentha* × *piperita* L. "Granada", and *Mentha* × *piperita* L. "Multimentha") Cultivars

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Abstract

Three mint varieties (*Mentha* × *piperita* L. "Almira", *Mentha* × *piperita* L. "Granada", and *Mentha* × *piperita* L. "Multimentha") grown in the Medicinal and Aromatic Plants Garden of Van Yüzüncü Yıl University were subjected to biochemical, physiological, and essential oil analyses. The present study revealed variations in the examined parameters among the varieties. The values of NBI (10.66-17.83 dx), chlorophyll (19.53-28.60 dx), flavonol (1.41-1.83 dx), anthocyanin (0.06-0.10 dx), total flavonoid content (353.75-288.12 mg QE 100 g^{-1}), total phenolic content (163.43-185.00 mg GAE g^{-1}), total antioxidant activity (86.95-154.45 μmol TE g^{-1}), total dry matter (23.0-24.20%), total ash (11.07-13.59%), macronutrient elements (Mg (3.45-5.83 g kg^{-1}), K (21.34-30.0 g kg^{-1}), Ca (16.93-22.06 g kg^{-1})), micronutrients (Fe (593.30-991.12 mg kg^{-1}), Mn (90.14-110.21 mg kg^{-1}), Zn (32.39-48.01 mg kg^{-1}), Cu (9.34-16.75 mg kg^{-1})), and heavy metal contents (Ni (0.16-4.68 mg kg^{-1}), and As (0.16-4.68 mg kg^{-1})). The dominant essential oil components were piperitenone oxide (43.38%) and piperitenone (39.4%) for *Mentha* × *piperita* L. "Almira", linalool (54.19%) and linalyl acetate (20.61%) for *Mentha* × *piperita* L. "Granada", and menthone (71.50%) and neomenthol (4.90%) for *Mentha* × *piperita* L. "Multimentha".

Keywords: dualex index, essential oil, heavy metal, mint, Van

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Introduction

The Mentheae tribe of the Nepetoideae subfamily belongs to the Lamiaceae family. In the 65 genera of the tribe Mentheae, more than 3000 names of the genus Mentha have been published, and most of them are illegitimate names. Their taxonomy is challenging because the nature of hybridization of the genus is easy. Hybrid seeds yield variable amounts of offspring; they can multiply via vegetative propagation. This variability has come with an outbreak of species and subspecific taxa. From 1911 to 1916 in Central Europe, one taxonomist published 434 new mint taxa. Fresh sources include between 18 and 25 species [1-3].

Peppermint (*Mentha × piperita* L.) is a perennial aromatic herb native to Europe that is cultivated in the northern USA, Canada, Asia, and many other parts of the world. As a hybrid of spearmint (*M. spicata* L.) and water mint (*M. aquatica* L.), peppermint grows particularly well in areas with soil with a high water-holding capacity. It is best known for its flavor and fragrance properties; peppermint leaves (fresh and dried) and the essential oils extracted from the leaves are used in many cosmetic, pharmaceutical, and food products [2, 4-8].

Some minerals have specific biological activities, while others can be toxic, and determining the mineral content of herbal teas and medicinal plants is necessary to understand their nutritional and health effects as well as their role in the human diet. Generally, herbal teas and medicinal plants contain high amounts of essential minerals (zinc (Zn), manganese (Mn), sodium (Na), potassium (K), calcium (Ca), iron (Fe), magnesium (Mg), and phosphorus (P)). However, the concentrations of these minerals vary widely depending on the plant material. While herbal tea is obtained from medicinal plants, minerals and the organic matrix components in the structure are extracted separately into the liquid medium; thus, herbal tea becomes a major, minor, and trace mineral source that can have some health effects on the human diet [9, 10].

Essential oils are volatile, strong-smelling, and oily mixtures obtained from aromatic plants by water vapor distillation; they are liquid at room temperature and can sometimes freeze. Since essential oils can evaporate even at room temperature when left open, they are called essential (etheric) oils [11]. In addition to the pharmaceutical, food, beverage, perfume, and cosmetic industries, the demand for essential oils has increased, especially in recent years, as essential oils are used in aromatherapy applications and agricultural production. In addition to its use as an insecticide, fungicide, herbicide, and nematocide and as a natural control source, it has recently been found to be useful in animal production, poultry, and beekeeping [12, 13].

Due to legal restrictions on the chemicals used in food preservation, the demand for essential oils obtained from plants and used as antioxidants is increasing daily. Mint, orange, lemon, and other citrus fruits are the most important imported essential oils [11]. What makes peppermint oil important in world trade is that it is the source of menthol production. For this reason, the production and trade of the species whose main component is menthol are greater. *M. piperita* contains the most menthol after *M. arvensis*, and its cultivation is widespread, especially in European countries in the Northern Hemisphere [14-16].

This study aimed to determine the physiological, biochemical, and essential oil components of three mint varieties (*Mentha × piperita* L.): "Almira", "Granada", and "Multimentha".

Materials and Methods

The study was performed on three varieties of *Mentha × piperita* L.: "Almira", "Granada", and "Multimentha". The plants were taken from the collection of the Medicinal and Aromatic Plant Garden, Van Yuzuncu Yil University, Agriculture Faculty, located in the Van region of Turkey. The soil results of the trial area are given in Table 1. The plants were grown in lessive soil, which was slightly alkaline (pH 7.72).

The total ash, dry matter, and minerals (including macro elements K, Ca, and Mg; micro elements Fe, Zn, Cu, and Mn) and some heavy metals (As, Cd, Co, Ni, Cr, and Pb) were determined. Dry matter was determined by drying the samples at 105ºC for 24 h in an oven. For the total ash (inorganic matter) determination, an electrical muffle furnace set at 550ºC was used. The mineral constituents of the plant samples were investigated as follows: Dried samples were ashed in a furnace with hydrochloric acid and nitric acid (AR) (AOAC 2000). Then, distilled water (50 ml) was added to the samples in a volumetric flask. All assays were performed in triplicate, and the standard materials were utilized for chemical analyses. Atomic absorption spectrometry (AAS) was used to estimate the K, Ca, Mg, and Fe concentrations. Inductively coupled plasma optical emission spectroscopy (ICP–OES) was used to determine the concentrations of other micronutrients and heavy metal constituents (Mn, Zn, Cu, Ni, As, Cd, Co, Cr, and Pb).

The total phenolic compound content was measured according to the method of [17]. The antioxidant activity was also determined based on the antioxidant power (FRAP) (iron (III) antioxidant power reduction) method (Benzie, Strain 1996), followed by readings of the absorbance at 593 nm, and the antioxidant activity values were recorded as Trolox equivalents (TE) mg^{-1}. The total flavonoid content was determined according to the method developed by Quettier-Deleu et al. (2000) with some modifications [18]. The total amount of flavonoid was measured at 415 nm and calculated in mg quercetin equivalent (QE) 100 g^{-1} DM by using the calibration curve prepared using standard quercetin.

The nitrogen balance index (NBI) and chlorophyll, flavonol, and anthocyanin contents were measured

Table 1. The soil results of the trial area.

	Texture	pH	EC (dS m^{-1})	Lime (%)	Organic Matter (%)	Phosphorus (ppm)
Trial area	%52.40 Sand	7.72	0.091	18.4	1.44	5.31
	%14.60 Clay					
	%33.00 Silt					

in nondestructively harvested leaves using a Dualex Scientific+ (FORCE-A, France) device in real time before harvesting.

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h using a clevenger-type apparatus [19]. The obtained oils were dried over anhydrous sodium sulfate and stored at +4ºC in the dark until analysis and testing.

The essential oil composition of the samples was analyzed by gas chromatography (Agilent 5975C) coupled to a flame ionization detector and mass spectrometry (Agilent 5975C) using a capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). The essential oils were diluted 1:50 with hexane. GC–MS/FID analysis was carried out in split mode at a 50:1 ratio. The injection volume and temperature were adjusted to 1 µl and 250ºC, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 1 ml/min. The oven temperature was programmed as follows: 60ºC for 10 min, increased at 20ºC/min to 250ºC, and held at 250ºC for 8 min. MS spectra were monitored between 35 and 450 amu, and the ionization mode used was electronic impact at 70 eV.

Identification of Compounds: The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by Wiley 7n, NIST 05, and Flavor and Fragrance Natural and Synthetic Compounds (ver. 1.3) Libraries.

All analyses carried out in the study were performed in 3 repetitions, and standard deviations were determined.

Results and Discussion

The biochemical parameters of the three *Mentha* L. varieties studied in this study are summarized in Table 2. The total antioxidant activity and total phenolic and total flavonoid contents of the Mentha varieties are given in Table 3.

The total flavonoid content ranged from 288.12 mg QE 100 g^{-1} (*Mentha* × *piperita* L. "Almira") to 353.75 mg QE 100 g^{-1} (*Mentha* × *piperita* L. "Granada"), the total phenolic content ranged from 163.46 mg GAE g^{-1} (*Mentha* × *piperita* L. "Multimentha") to 185.00 mg GAE g^{-1} (*Mentha* × *piperita* L. "Granada"), and the total anthioksidan activity ranged from 86.95 µmol TE/g (*Mentha* × *piperita* L. "Multimentha") to 154.45 µmol TE g^{-1} (*Mentha* × *piperita* L. "Almira").

The Dualeks index (NBI) values ranged from 10.66 (*Mentha* × *piperita* L. "Granada") to 17.83 (*Mentha* × *piperita* L. "Almira"), the chlorophyll values ranged from 19.53 (*Mentha* × *piperita* L. "Granada") to 28.60 (*Mentha* × *piperita* L. "Multimentha"), the flavonol values ranged from 1.41 (*Mentha* × *piperita* L. "Almira") to 1.83 (*Mentha* × *piperita* L. "Granada" and *Mentha* × *piperita* L. "Multimentha"), and the anthocyanin values ranged from 0.06 (*Mentha* × *piperita* L. "Multimentha") to 0.10 (*Mentha* × *piperita* L. "Granada").

The total ash content of the three Mentha varieties ranged between 11.07% and 13.59%, with *Mentha* × *piperita* L. "Granada" having the highest value. The dry matter content ranged from 24.2% (*Mentha* × *piperita* L. "Granada") to 23.0% (*Mentha* × *piperita* L. "Multimentha") (Fig. 1a)).

Among the Mentha varieties examined, the greatest average magnesium content was detected in the *Mentha* × *piperita* L. "Multimentha" (5.83 g kg^{-1}) variety, followed by the *Mentha* × *piperita* L. "Granada" (5.72 g kg^{-1}) and *Mentha* × *piperita* L. "Almira" (3.45 g kg^{-1}) varieties. The highest average potassium concentrations were detected in *Mentha* × *piperita* L. "Multimentha" (30.00 g kg^{-1}) plants, followed by *Mentha* × *piperita* L. "Granada" (27.69 g kg^{-1}) and *Mentha* × *piperita* L.

Table 2. Biochemical content of three Mentha varieties.

Mentha × *piperita* L.	Flav. (mg QE/100 g)	Phen. (mg GAE/g)	Ant. (µmol TE/g)	NBI (dx)	Chlorophyll (dx)	Flavonol (dx)	Anthocyanin (dx)
Almira	288.12±24.9	180.62±3.5	154.45±11.7	17.83±0.7	25.16±0.7	1.41±0.09	0.08±0.01
Granada	353.75±61.7	185.00±12.8	144.90±0.6	10.66±1.4	19.53±2.8	1.83±0.09	0.10±0.02
Multimentha	319.72±25.0	163.43±3.0	86.95±17.0	15.80±2.7	28.60±1.5	1.83±0.20	0.06±0.01

Flav.: Total Flavonoid content, Fen: Total Phenolic content, Ant: Total Antioxidant activity, NBI: Nitrogen balance index, Dx: Dualex index.

Table 3. Macro-micro nutrition element and heavy metal content of three Mentha cultivars.

	Mentha × *piperita* L. "Almira"	*Mentha* × *piperita* L. "Granada"	*Mentha* × *piperita* L. "Multimentha"
Mg(g/kg)	3.45±0.27	5.72±0.61	5.83±0.07
K(g/kg)	21.34±2.07	27.69±0.04	30.00±2.17
Ca(g/kg)	16.93±1.78	22.06±0.77	17.63±0.26
Fe (mg/kg)	991.12±23.05	812.03±4.89	593.3±7.83
Mn (mg/kg)	110.21±2.10	108.0±0.95	90.14±0.66
Zn (mg/kg)	48.01±0.31	32.39±1.80	41.32±2.13
Cu (mg/kg)	9.34±0.25	12.4±0.20	16.75±0.01
Ni (mg/kg)	4.68±0.11	0.16±2.50	2.05±0.13
As (mg/kg)	1.25±0.11	0.59±0.15	0.79±0.07
Co (mg/kg)	0.55±0.03	0.11±0.20	0.30±0.01
Pb (mg/kg)	3.92±0.46	1.56±1.49	2.20±0.39
Cr (mg/kg)	4.27±0.40	-	2.78±0.43
Cd (mg/kg)	0.04±0.01	-	0.011±0.003

"Almira" (21.34 g kg^{-1}) plants. Calcium contents were determined to be 16.93 g kg^{-1}, 17.63 g kg^{-1} and 22.06 g kg^{-1} in *Mentha* × *piperita* L. "Almira", *Mentha* × *piperita* L. "Multimentha" and *Mentha* × *piperita* L. "Granada" plants, respectively.

The micronutrients ranged from 593.30 mg kg^{-1} (*Mentha* × *piperita* L. "Multimentha") to 991.12 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), the Mn concentration ranged from 110.21 mg kg^{-1} (*Mentha* × *piperita* L. "Almira") to 90.11 mg kg^{-1} (*Mentha* × *piperita* L. "Multimentha"), the Zn concentration ranged from 32.39 mg kg^{-1} (*Mentha* × *piperita* L. "Granada") to 48.01 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), and the Cu concentration ranged from 9.34 mg kg^{-1} (*Mentha* × *piperita* L. "Almira") to 16.75 mg kg^{-1} (*Mentha* × *piperita* L. "Multimentha").

The heavy metal contents were determined as Ni, from 0.16 mg kg^{-1} (*Mentha* × *piperita* L. "Granada") to 4.68 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), As from 0.59 mg kg^{-1} (*Mentha* × *piperita* L. "Granada") to 1.25 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), Co from 0.11 mg kg^{-1} (*Mentha* × *piperita* L. "Granada") to 0.55 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), Pb from 1.56 mg kg^{-1} (*Mentha* × *piperita* L. "Granada") to 3.92 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), Cr from 2.78 mg kg^{-1} (*Mentha* × *piperita* L. "Multimentha") to 4.47 mg kg^{-1} (*Mentha* × *piperita* L. "Almira") and Cd from 0.011 mg kg^{-1} (*Mentha* × *piperita* L. "Almira"), Cr from 2.78 mg kg^{-1}. Cr and Cd, which are heavy metals, could not be detected in the *Mentha* × *piperita* L. "Granada" variety. The lowest concentrations of heavy metals were detected in the *Mentha* × *piperita* L. "Granada" variety, and the highest concentrations were detected in the *Mentha* × *piperita* L. "Almira" variety.

The essential oil ratios of the three Mantha varieties are given in Fig. 1b). The essential oil ratio of the varieties ranged between 0.73% and 2.09%, with *Mentha* × *piperita* L. "Multimentha" having the highest ratio.

The chemical composition of the *Mentha* × *piperita* L. "Almira" essential oil is summarized in Table 4.

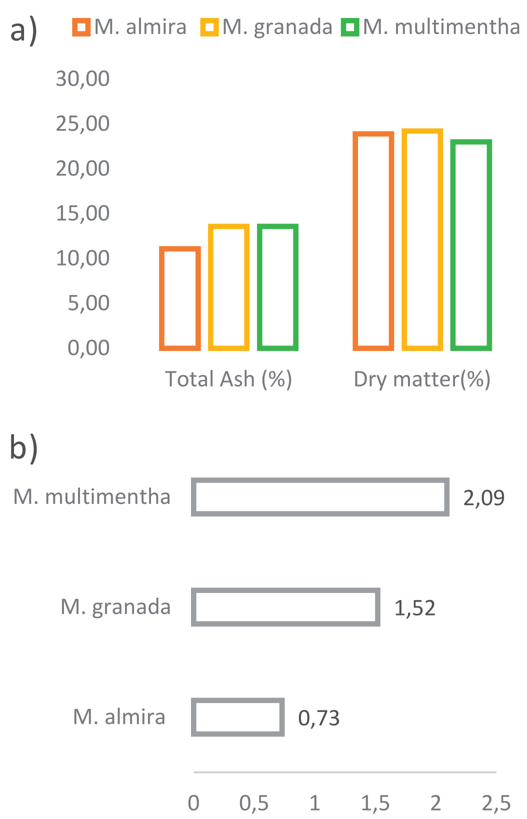

Fig. 1. Total ash and dry matter of three mentha cultivars a) and the essential oil ratio of three Mantha varieties b).

Table 4. The essential oil components of *Mentha × piperita* L. “Almira”.

RI	RT	Component name	Component quantity (%)
1016	10.958	α-pinene	0.65
1102	14.262	β-pinene	0.89
1192	18.265	Limonene	3.87
1226	19.731	β-ocimene	1.99
1437	28.054	1-octen-3-ol	0.35
1534	31.353	Linalool	1.75
1694	36.272	Borneol	0.75
1712	36.744	Germacrene	0.96
1786	38.979	Car-3-en-2-one	0.43
1843	40.547	isophorone	2.46
1932	42.778	Piperitenone	39.24
1943	43.152	*cis*-jasmone	0.70
1958	43.532	Piperitenone oxide	43.38
1972	43.902	Shisofuran	0.60
2161	48.193	Eugenol	0.38
2324	52.374	isopimaradiene	0.70
		Unidentified	0.92

RI: Retention index, RT: Retention time

Table 5. The essential oil components of *Mentha × piperita* L. “Granada”.

RI	RT	Component name	Component ratio (%)
1155	16.737	Myrcene	1.05
1204	18.863	1,8-cineole	4.92
1226	20.59	β-ocimene	0.68
1328	24.105	3-octanol acetate	2.04
1369	25.691	1-octen-3-ol acetate	1.52
1534	31.511	Linalool	54.19
1547	31.911	Linalyl acetate	20.61
1597	33.446	β-caryophyllene	0.70
1690	36.275	α-terpineol	4.22
1712	36.861	Germacrene	2.10
1714	36.98	Neryl acetate	1.05
1744	37.82	Geranyl acetate	2.20
1785	38.971	Nerol	1.09
1830	40.182	Geraniol	2.18
2092	46.637	Viridiflorol	1.45

RI: Retention index, RT: Retention time

A total of 16 compounds were identified, representing 100% of the total composition. The oil was dominated by piperitenone oxide (43.38%), and the other major component was piperitenone (39.4%).

The chemical composition of the *Mentha × piperita* L. “Granada” essential oil is summarized in Table 5. A total of 15 compounds were identified, representing 100% of the total composition. The oil was dominated by linalool (54.19%), and the other major component was Linalyl acetate (20.61%).

The chemical composition of the *Mentha × piperita* L. “Multimentha” essential oil is summarized in Table 6. A total of 13 compounds were identified, representing 100% of the total composition. The oil was dominated by Menthone (71.50%), and the other major component was neomenthol (4.90%).

Flavonoids are ubiquitous secondary metabolites in plants that help to protect plants from abiotic and biotic stresses, while anthocyanins reduce the damage caused by free radical activity. Both anthocyanin and flavonoid compounds are responsible for antioxidant activity in plants [20, 21].

Studies on Satureja hortensis, a plant belonging to the same family as Mentha, have shown that the amount of flavonoid substances is 26.52 mg QE g^{-1} [22], 44.91 mg QE g^{-1} [23], and 5.23-28.42 mg RE g^{-1} [24]. The total flavonoid content of *Mentha × Peperita* from different countries is 15.70 mg RE g^{-1} DW in Algeria [25], 47.19 mg CE g^{-1} d.m in Chile [26], and 125.57 mg CTE L^{-1} in Brazil [27]. These findings are consistent with the literature.

It is believed that some foods, especially those containing bioactive compounds, have beneficial effects on degenerative diseases, primarily due to these compounds. Among these bioactive compounds, phenolic compounds are the most common and possess strong antioxidant activity. Additionally, polyphenolic compounds, which play a significant role in plant physiology, are the most important bioactive compounds that impart characteristics such as color, taste, and aroma to plants [28- 30].

Salihoğlu et al. (2017) determined that the total phenolic content of *M. piperita* from different drying methods was 58.20 mg GAE g^{-1} for the air-dried method and 59.91 mg GAE g^{-1} for the freeze-dried method [31]. In another previous study, the total phenolic content was found to be 75.31 mg GAE g^{-1} for the aqueous extract of the leaves of *Mentha piperita* [32]. Safaiee et al. (2019) reported a maximum TPC of 0.2451 mg g^{-1} for *Mentha aquatica* [33]. The total phenolic content of *Mentha × Peperita* from different countries was determined to be 31.40 mg GAE g^{-1} DW in Algeria [25], 12.43 mg GAE g^{-1} d.m in Chile [26], and 123.2 mg GAE g^{-1} DW in İran [34]. In our study, it was determined that the total phenolic content was greater.

Table 6. The essential oil components of *Mentha × piperita* L. "Multimentha".

RI	RT	Component name	Component ratio (%)
1102	14.26	β-pinene	0.54
1155	16.631	Myrcene	0.38
1204	18.746	1,8-cineole	2.93
1457	28.8	*trans*-sabinene hydrate	0.97
1468	29.143	Menthone	71.50
1480	29.561	Menthofuran	2.84
1534	31.349	Linalool	0.38
1554	32.129	neomenthyl acetate	0.46
1588	33.089	neomenthol	4.90
1597	33.36	β-caryophyllene	1.06
1658	35.003	*trans*-β-farnesene	1.97
1712	36.747	Germacrene	0.86
1735	37.458	Piperitone	1.93
		Unidentified	9.29

RI: Retention index, RT: Retention time

The natural antioxidants in plants are of great interest in natural product science, and many herbs have significant antioxidant potential. Antioxidants decrease oxidative stress in cells and are, therefore, very useful in the treatment of many diseases and protect plants from many stress factors [35, 36].

In this study, the antioxidant activity of Satureja hortensis was determined to be 45.24 μg TE mL^{-1} [22], while that of *Thymbra spicata* var. *spicata* (zahter) was reported to be 62.13±4.04 mg TE, and that of *Origanum onites* (İzmir kekiği) was reported to be 109.67±3.26 mg TE [37]. This value was greater for species belonging to the same family in the present study.

Antón-Herrero et al. (2021) conducted a study to determine the effect of biostimulant fertilizers on leaf indices in *Capsicum annuum* plants [38]. The NBI ranged from 23 to 73, the chlorophyll content ranged from 22 to 48, the flavonol content ranged from 0.61 to 1.0, and the anthocyanin content ranged from 0.042 to 0.11. Dambrauskienė et al. (2008) determined the average chlorophyll content of *Mentha piperita* species in Lithuania and Poland to be 2.8 and 3.1 mg g^{-1}, respectively [39]. Barickman et al. (2021) determined the chlorophyll content in *Ocimum basilicum* L. plants in the control group to be 21.468 $\mu g \cdot ml^{-1}$, the flavonoid content to be 0.6853 $mg \cdot g^{-1}$ DM, the anthocyanin content to be 0.1144 $mg \cdot g^{-1}$ DM, and the NBI content to be 32.415 (dualex index) [40]. The NBI was low, the flavonoid content was high, and the chlorophyll and anthocyanin contents were within the ranges reported in the relevant literature for the parameters examined.

In *Mentha piperita*, the ash content was determined to be 12%, while in our study, this ratio was greater in the other varieties except for the *Mentha × piperita* L. "Almira" variety [41]. In *Mentha longifolia* (L.) HUDSON. subsp. *typhoides* (BRİQ), HARLEY var. *typhoides* (L.) HUDSON species, and the ash content was reported to be 23% [42]. It was determined that this value was equivalent to the lowest value reported in our study. The dry matter content was found to be 24.24% for *Mentha x piperita* L. var. *citrata* Ehrh., 19.82% for *Mentha × piperita* L. var. *officinalis* Sole f. *pallescens* Camus, and 20.35% for *Mentha × piperita* L. var. *officinalis* Sole f. *rubescens* Camus [43]. This value was within the relevant range for all three varieties.

Plant nutrients are essential for the growth and normal development of plants, and they cannot be replaced by any other chemical element in terms of their functions [44]. These mineral elements are essential for plants to perform vital functions. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have identified the essential nutrients and their required quantities for human health. These elements play critical roles in various biochemical processes in the body to maintain a healthy life and prevent diseases. The chemical composition of peppermint (*Mentha Piperita*) studied by Kızıl et al. (2010) in Diyarbakır is Cu at 11.52 mg kg^{-1}, Fe at 31.5 mg kg^{-1}, Mn at 70.82 mg kg^{-1}, and Zn at 12.64 mg kg^{-1} [45]. Ceylan and Yücel (2015) determined the nutrient content in 12 plant taxa belonging to 10 families as follows: 3.05-10.13 g kg^{-1} for K, 0.436-3.526 g kg^{-1} for Ca, 21.55-1599 mg kg^{-1} for Mg, 0.5-2.10 mg kg^{-1} for Cu, 10.5-495.5 mg kg^{-1} for Fe, and 1.45-22.65 mg kg^{-1} for Mn [42]. Akgünlü (2012) determined the content of some macro- and micronutrients in wild mint species as follows: Mg (6368 mg kg^{-1}), Ca (15.044 g kg^{-1}), Cu (2.60 mg kg^{-1}), Fe (313.0 mg kg^{-1}), and Mn (6.60 mg kg^{-1}) [46]. The study concluded that the nutrient content of all three varieties generally fell within the range reported in the relevant literature. According to Yalçın (2023), the levels of Cd (0.025-0.055), Cr (0.387-1.789), Pb (4.33-8.88), and Ni (0.73-9.89) in medicinal herbs such as sage, linden, thyme, and green tea sold in herbalists ranged from ppm [47]. Arsenic (As) was not detected in these samples. In a study conducted by Acar (2019) on 15 commercial tea samples consumed in Şanlıurfa Province, the heavy metal content was determined as follows, based on average values: Cr levels ranged from 0.72 to 3.86, Co levels ranged from 0.03 to 0.16, Ni levels ranged from 4.06 to 1.35, and Pb levels ranged from 4.02 to 12.6 [48]. In a study by Bedir et al. (2010), Cr, Ni, and Pb metals in mint and thyme teas sold both in open and packaged forms in herbalists were found to range from 2 to 14 ppm, 6 to 24 ppm, and 4 to 6 ppm, respectively [49]. Cd was not detected in any of the samples in this study. Additionally, in this study, Cr and Cd were not detected in the *Mentha* × *piperita* L. "Granada" variety.

Environmental factors such as climate conditions, day length, temperature, diurnal temperature variation, and light intensity affect the levels of volatile oil constituents in plants. Research has indicated that the volatile oil component of *Mentha* × *piperita* is influenced by climate factors, particularly the coenzyme $NADPH_2$. Conditions such as day length, temperature, light intensity, and diurnal temperature variation have been found to accelerate the conversion of pulegone to menthone and menthol in conjunction with $NADPH_2$ levels, with optimal conditions resulting in high proportions of the main components. Essential oil components vary depending on the environmental conditions [41].

Mentha × *piperita* (a hybrid of *M. aquatica* x *M. spicata*) is the most widely utilized mint species for its volatile oil worldwide. The essential oil content of *Mentha* × *piperita* has been reported to be at least 0.7 ml 100 g^{-1} [41]. It was determined that the essential oil content in all three varieties was greater than the lowest rate reported. Dambrauskienė et al. (2008) determined the average volatile oil content in *M. piperita* to be 0.17% and 0.60% in Lithuania and Poland, respectively [39]. In the essential oil from *M. spicata* (EOMSP), carvone (41.215%±4.18%) was the major compound, followed by menthol (12.774%±2.48%), menthone (7.225%±1.81%), and pulegone (3.763%±1.04%). In the essential oil from *M. suaveolens* (EOMSU), piperitenone oxide (73.773%±6.41%) was the major compound, followed by germacrene-d (3.309%±1.19%) and limonene (2.969%±1.02%) [50]. Piasecki et al. (2023) emphasized that *Mentha* × *piperita* L. "Almira" contains 16 volatile oil components, with the two dominant compounds being piperitenone (26.5%) and piperitenone oxide (50.0%) [51]. Similarly, in our study, *Mentha* × *piperita* L. "Almira" contained 16 volatile oil components, the dominant of which were piperitenone (39.24%) and piperitenone oxide (43.38%). Piasecki et al. (2023) highlighted that the two dominant compounds in *Mentha* × *piperita* L. "Granada" are linalool (41.2%) and linalyl acetate (24.2%) [51]. Similarly, in our study, the dominant compounds in *Mentha* × *piperita* L. "Granada" were linalool (54.19%) and linalyl acetate (20.61%). Piasecki et al. (2023) emphasized that the two dominant compounds in *Mentha* × *piperita* L. "Granada" are menthone (48.9%) and menthol (22.0%) [51]. Similarly, in our study, the dominant compound in *Mentha* × *piperita* L. "Multimentha" was menthone (71.50%). The greater dominance of these compounds in the present study than in the relevant literature may be due to altitude, soil, and climatic factors.

Conclusions

The essential oils obtained from mint (*Mentha* spp.) species in the world rank second after citrus oils in the essential oil trade. It ranks first among the essential oils imported by Turkey. Menthol obtained from medicinal mint is a very valuable product that is used in the food, flavoring, and pharmaceutical industries. The total antioxidant activity, NBI, Fe, Mn, Zn, and all heavy metals were found to be high in the *Mentha* × *piperita* L. "Almira" variety, while the total flavonoid content, total phenolic content, flavonol content, anthocyanin content, total dry matter content, total ash content, and Ca content were high in the *Mentha* × *piperita* L. "Granada" variety. Chlorophyll, flavonol, Mg, K, Cu, and volatile oil contents were high in the *Mentha* × *piperita* L. "Multimentha" variety.

The dominant components in volatile oil constituents were determined as Piperitenone (39.24%) and Piperitenone oxide (43.38%) for *Mentha* × *piperita* L. "Almira", Linalool (54.19%) and Linalyl acetate (20.61%) for *Mentha* × *piperita* L. "Granada", and Menthone (71.50%) for *Mentha* × *piperita* L. "Multimentha".

It is important to establish medicinal mint plantations in accordance with this technique to diversify our agricultural production and ensure that our producers generate more income through the production of medicinal and aromatic plants with high added value.

Conflict of Interest

The authors declare no conflict of interest.

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