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CHEMICAL COMPOSITION AND THERAPEUTIC PROPERTIES OF THE DRY EXTRACT OF PEPPERMINT (MENTHA PIPERITA)

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Abstract
This article presents a comprehensive analysis of the botanical characteristics, chemical composition, and therapeutic significance of the Mentha piperita (peppermint) plant. The study focuses on the preparation of aqueous and alcoholic dry extracts and the quantification of their vitamin and flavonoid content. Using the High-Performance Liquid Chromatography (HPLC) method, it was found that among the vitamins, vitamin B3 is present in the highest concentration. Among the flavonoids, salicylic acid and kaempferol were identified as dominant compounds. The study emphasizes the potential health benefits of these bioactive constituents, including their antioxidant and anti-inflammatory properties, making peppermint extract a valuable resource in phytotherapy and nutraceutical development.

Keywords: Mentha piperita, dry extract, vitamin, menthol, essential oils, phenolic compounds, rutin, gallic acid, salicylic acid, kaempferol.

Introduction

Mentha piperita, commonly known as peppermint, is a perennial herbaceous plant belonging to the Lamiaceae family. It typically grows between 30 cm to 100 cm in height. The plant features a highly branched rhizome system, with lateral underground stems and fibrous roots emerging from the nodes. The stems, which are tetrahedral in cross-section, exhibit strong branching from the base and are densely covered with leaves. The leaves are short-petioled, lanceolate-ovate in shape, with serrated edges, measuring up to 8 cm in length and 2 cm in width. Both surfaces of the leaves are covered with essential oil glands. Flowering occurs from June to August, while fruit maturation takes place in September and October. The small, pink to light purple flowers are arranged in

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spike-like inflorescences, and the fruit is composed of four nutlets [1].

More than 40 different chemical compounds have been identified in peppermint, including menthol, menthone, and menthyl acetate. The safety of its consumption has been confirmed by numerous toxicological studies [2]. Mentha piperita is especially rich in menthol and its derivatives, which form the major constituents of its essential oil. This rich phytochemical composition enables its broad application across pharmaceuticals, cosmetics, food processing, and the perfumery industry. The presence of flavonoids, tannins, and phenolic compounds in the plant contributes to its well-documented antioxidant and anti-inflammatory effects. The principal active ingredient in peppermint-based medicinal products is menthol, which is primarily found in the plant's essential oil [3].

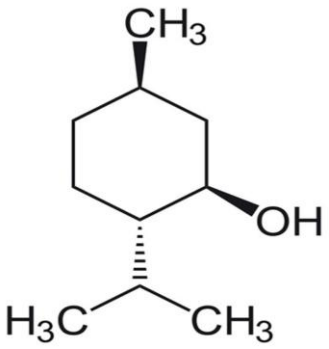

Figure 1. Chemical structure of menthol

In modern medicine, peppermint-derived preparations are widely used to relieve gastrointestinal discomfort, including abdominal pain, bloating, nausea, vomiting, cholecystitis, gallstones, and hepatitis. They are also commonly applied in the treatment of inflammatory conditions of the upper respiratory tract such as laryngitis, tracheitis, and bronchitis. Topical formulations containing 2% menthol solution or 10% oil-based emulsions are used in the treatment of migraines and neuralgia due to their analgesic and cooling effects. When ingested, menthol acts reflexively to dilate coronary vessels, enhancing cardiac blood flow.

Menthol is also a key component in antitussive lozenges such as Pectusin, and is included in various complex pharmaceutical formulations. For instance, in herbal remedies used for diabetes management, menthol-containing essential oils are incorporated for their regulatory effects. Additionally, menthol is a crucial active ingredient in widely used cardiac medications such as Corvalol, Validol, and Valocordin, further underscoring its therapeutic versatility and pharmacological importance [4].

Materials and methods

2.1. Determination of Vitamin Content in the Sample Extract

Chromatograms of the sample extract (Figures 3–4) were obtained using high-performance liquid chromatography (HPLC), and based on the peak areas, the content of vitamins in 100 g of dry peppermint extract was calculated using the following formula:

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$$X = \frac{C_{vit} \cdot V_{extract}}{m_{sample}} \cdot 100\ g$$

Where:

- X = Content of the vitamin in 100 g of dry extract (mg)
- C_{vit}= Concentration of the vitamin determined by HPLC (mg/L)
- $V_{extract}$ = Volume of the extract (L)
- m_{sample} = Mass of the plant material used for extraction (g)

2.2. Reagents and Equipment

The following analytical-grade reagents and equipment were used:

- Vitamin B12 (Rhydburg Pharmaceuticals, Germany)
- Vitamin C (Carl Roth GmbH, Germany)
- Vitamin B9 (DSM Nutritional Products GmbH, Germany)
- Vitamins B1, B2, B3, B6, and PP (BLDPharm, China)
- HPLC-grade water, acetonitrile, glacial acetic acid, and sodium hydroxide

Vitamin analysis was carried out on a Shimadzu LC-40 Nexera Lite high-performance liquid chromatograph (HPLC), manufactured in Japan.

2.3. Preparation of Standard Solutions

Standard solutions (100 mg/L) of the following vitamins were prepared:

- Vitamin C (CAS 50-81-7), B1 (CAS 59-43-8), B6 (CAS 58-56-0), B3 (CAS 59-67-6), B12 (CAS 68-19-9), and PP (CAS 98-92-0) were prepared by dissolving 5 mg of each in 50 mL of 0.1 N HCl.
- Vitamin B2 (CAS 83-88-5) and B9 (CAS 59-30-3) were dissolved in 50 mL of 0.025% NaOH solution.

From these, working solutions with concentrations of 14.286, 7.143, 3.571, and 1.786 mg/L were prepared. Vitamin C standards were prepared at concentrations of 286, 143, 71.5, and 57.2 mg/L. Distilled water was used as a blank (0 mg/L) for calibration.

2.4. Preparation of Peppermint Extract

To extract water-soluble vitamins, 1 g of dried peppermint sample was placed into a 50 mL conical flask, followed by the addition of 25 mL of 0.1 N HCl. The mixture was treated in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After cooling, the mixture was filtered and diluted to 25 mL with distilled water. A 1.5 mL aliquot of the extract was passed through a 0.22 µm syringe filter, transferred into a vial, and analyzed.

2.5. Chromatographic Conditions

Sample and standard solutions were analyzed using a Shimadzu LC-40 Nexera Lite HPLC system, equipped with LC-40D pump, SIL-40 autosampler, and SPD-M40 photodiode array (PDA) detector, controlled via LabSolutions software (v6.92). A Shim-pack GIST C18 reverse-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan) was used. The mobile phase consisted of a gradient of acetonitrile (A) and 0.25% aqueous acetic acid (B), as shown in Table 1.

- Injection volume: 10 µL

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- Flow rate: 0.6 mL/min
- Column temperature: 40°C
- Detection wavelengths: 265, 291, and 550 nm for most vitamins
- For vitamin C, a 15-minute gradient and 265 nm detection wavelength were used (Table 1).

Table 1. Mobile phase gradient program.

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	0	100
3	0	100
14	20	80
17	50	50
18	0	100
25	Tugatish	

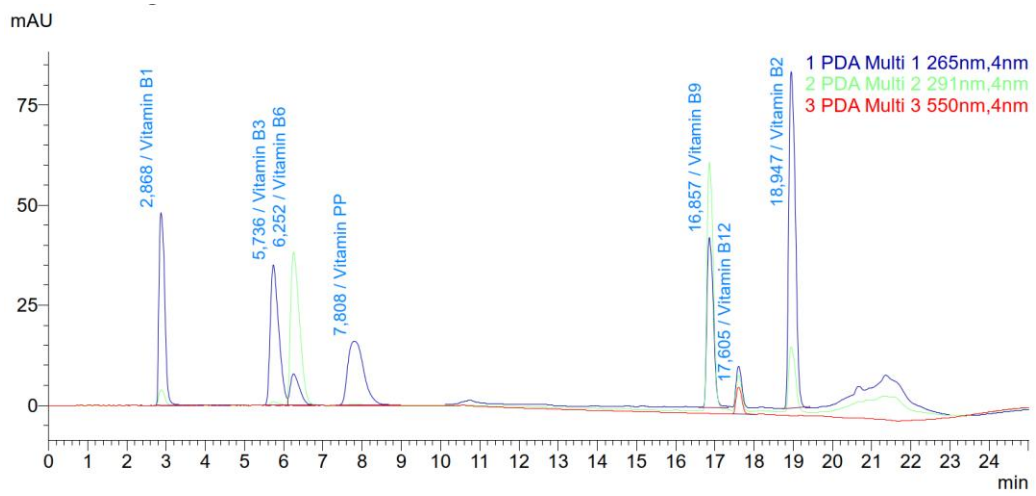

Figure 1. Chromatogram of the standard vitamin solutions obtained using high-performance liquid chromatography (HPLC).

2.6. Reagents and Equipment Used for Phenolic Compound Analysis

The following reagents were used: gallic acid (Macklin, China), salicylic acid (Rhydburg Pharmaceuticals, Germany), quercetin, apigenin, and kaempferol (Regal, China). Rutin was isolated from natural sources through extraction and column chromatography. High-purity HPLC-grade solvents such as water, acetonitrile, glacial acetic acid, and sodium hydroxide were employed throughout the analyses.

Quantification of phenolic compounds in the peppermint extract was conducted using a Shimadzu LC-40 Nexera Lite high-performance liquid chromatography (HPLC) system, manufactured in Japan.

2.7. Preparation of Standard Solutions

Standard solutions were prepared by dissolving gallic acid (5.2 mg), salicylic acid (5.2 mg), rutin (5 mg), quercetin (5 mg), apigenin (5 mg), and kaempferol (5 mg) in 96% ethanol. The solutions were treated in an ultrasonic bath for 20 minutes and transferred to 50 mL volumetric flasks, which were then filled to volume with ethanol. A mixture was prepared by combining 200 µL from each

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standard solution. From this stock, four working solutions were prepared by serial dilution. All standard solutions were filtered and transferred to vials for chromatographic analysis.

2.8. Preparation of Plant Extract for Phenolic Analysis

A 1 g sample of dried peppermint was weighed with 0.01 g precision using an NV222 analytical balance (OHAUS, USA) and placed in a 50 mL conical flask. Then, 25 mL of 96% ethanol was added. The mixture was subjected to ultrasonic extraction in a GT SONIC-D3 ultrasonic bath (China) at 60°C for 20 minutes. After cooling, the mixture was filtered and diluted to 25 mL with ethanol in a volumetric flask. An aliquot of 1.5 mL of the resulting extract was centrifuged at 7000 rpm for 5 minutes using a Mini-7 centrifuge (BIOBASE, China), and the supernatant was filtered through a 0.45 µm syringe filter prior to analysis.

2.9. Chromatographic Conditions for Phenolic Compound Analysis

Chromatographic separation of phenolic compounds was performed using a Shim-pack GIST C18 reverse-phase column (150 × 4.6 mm; 5 µm, Shimadzu, Japan). A gradient mobile phase was applied, consisting of acetonitrile (A) and 0.5% aqueous acetic acid (B), as detailed in Table 2. The injection volume was set at 10 µL, with a flow rate of 0.5 mL/min, and the column temperature maintained at 40°C. Analytical signals were detected at a wavelength of 300 nm (Figure 2).

Table 2. Gradient elution program of the mobile phase used for HPLC analysis

Time, minute	Acetonitrile (A), %	0.5% acetic acid (B), %
0	5	95
5	5	95
17	40	60
22	40	60
22,1	5	95
40	Finish	

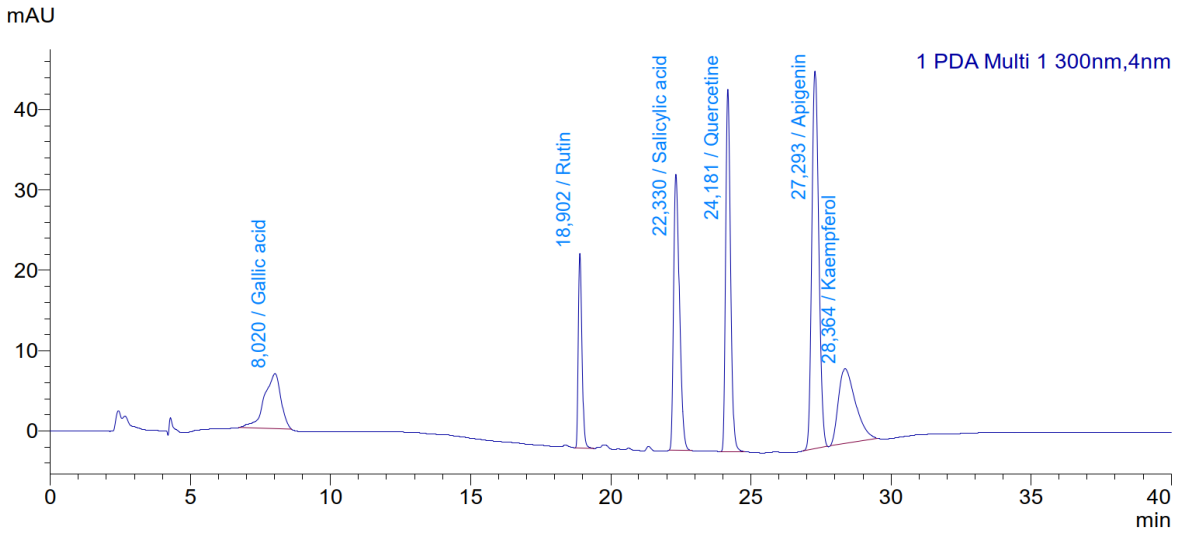

Figure 2. Chromatograms at 300 nm of the standards rutin, caffeine, gallic acid, quercetin, kaempferol, apigenin, and salicylic acid.

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Results and discussion

A research group led by A.A. Bendishev in the Russian Federation has developed reliable HPLC-based analytical protocols employing gradient elution for the quantification of water-soluble vitamins in vitamin premixes, biologically functional mixtures (BFMs), and pharmaceutical formulations [5].

In our study, we successfully applied high-performance liquid chromatography (HPLC) with gradient elution to determine the concentration of water-soluble vitamins in the dry extract of Mentha piperita cultivated in the Andijan region of Uzbekistan. The chromatogram of the peppermint extract is presented in Figure 3, and the processed data obtained from the chromatographic analysis are summarized in Table 3.

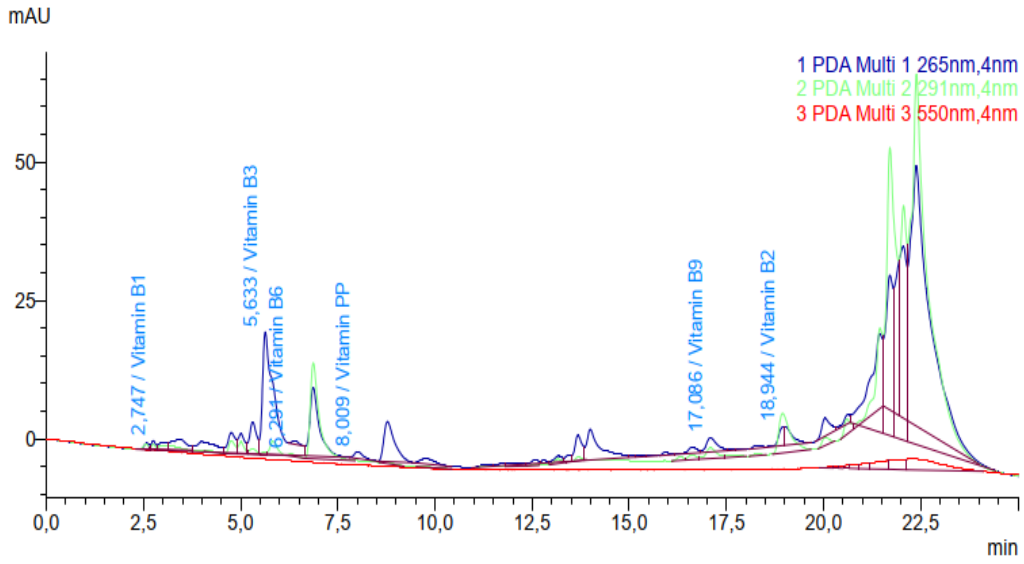

Figure 3. Chromatographic profile of water-soluble vitamins in Mentha piperita extract.

Table 3. Retention times and concentrations of vitamins detected in the peppermint extract.

Vitamin	Retention time, sec	Concentration, mg/l	Amount in 100 g sample, mg
Vitamin B_1	2,747	0,454	1,135
Vitamin B_3	5,633	13,237	33,093
Vitamin PP	8,009	0,647	1,618
Vitamin B_9	17,086	1,534	3,835
Vitamin B_2	18,944	0,737	1,843
Vitamin B_6	6,291	0,159	0,398
Vitamin B_{12}	Not specified	0	0,000
Vitamin C	4,025	4,075	10,188

Upon analyzing the chromatographic profile of vitamins in the peppermint extract (Figure 3), it was observed that among the seven detected water-soluble vitamins, the peak corresponding to vitamin B3 (niacin) was the most prominent. This indicates that vitamin B3 is the dominant compound within the extract. Conversely, the absence of a peak corresponding to vitamin B12

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suggests that this vitamin was not detected in the tested sample.

Experimental analysis of the chemical composition of Mentha piperita (Table 3) confirms that, among the seven quantified vitamins present in the aqueous extract prepared from 100 grams of sample, vitamin B3 exhibited the highest concentration. In contrast, vitamin B12 was not detected in the extract, confirming its absence under the conditions and sensitivity of the applied method.

Determination of Phenolic Compounds and Caffeine in the Peppermint Extract

A chromatogram was obtained for the extract sample prepared from 1 g of dried Mentha piperita material (Figure 4), and the analytical results were processed and summarized in Table 4.

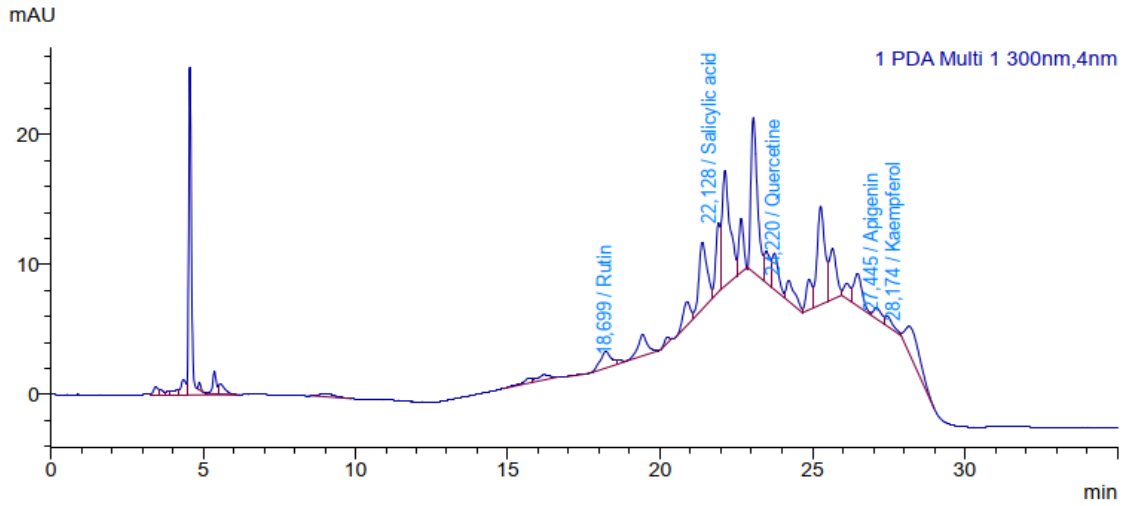

Figure 4. Chromatogram of the determination of polyphenols in peppermint extract.

Table 4. Amount of polyphenols in the extract and retention times.

Name of phenol compound	Retention time, sec	Concentration, mg/l	Amount in 100 g sample, mg
Gallic Acid	Not specified	0	0,000
Rutin	18,699	0,186	0,465
Salicylic Acid	22,128	5,692	14,230
Quercetin	24,22	0,988	2,470
Apigenin	27,445	0,298	0,745
Kaempferol	28,174	3,1	7,750

Analysis of the chromatographic profile of phenolic compounds in the 96% ethanolic extract of Mentha piperita (Figure 4) revealed five distinct flavonoid peaks. Among them, the peaks corresponding to salicylic acid and kaempferol were the most prominent, indicating that these two compounds are present in significantly higher concentrations. Conversely, the absence of a peak corresponding to gallic acid indicates that this compound was not detected in the analyzed sample.

Experimental investigation of the chemical composition of peppermint (Table 4) further confirmed that salicylic acid and kaempferol are the dominant flavonoids in the ethanolic extract prepared from 100 grams of sample material. The results also verified the absence of gallic acid in the extract under the applied chromatographic conditions.

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Conclusions

The quantitative analysis of water-soluble vitamins and flavonoids in the dry extract of Mentha piperita using high-performance liquid chromatography (HPLC) revealed that, per 100 grams of sample, the extract contained 33.093 mg of vitamin B3 and 10.188 mg of vitamin C. Additionally, in 1000 mL of ethanolic extract, 14.230 mg of salicylic acid and 7.750 mg of kaempferol were detected.

The high concentration of vitamin B3 (pantothenic acid) in peppermint is of particular interest. Vitamin B3 plays a crucial role in normalizing blood lipid levels, enhancing myocardial metabolism, and regulating adrenal gland function. Pantothenic acid is an essential component of coenzymes, notably coenzyme A (CoA). It is referred to as an acylation coenzyme due to its involvement in enzymatic reactions that activate and transfer CH_3CO- acyl radicals. The role of vitamin B3 in metabolic processes is well-established and critical for cellular function.

The significant presence of vitamin C in the extract highlights the plant's potential in supporting human health. Vitamin C contributes to collagen synthesis, slows the aging process, reduces the risk of chronic diseases, enhances immunity by protecting against infections and supporting iron absorption, and helps regulate blood pressure. It also lowers the risk of cardiovascular diseases and may aid in the prevention of gout. These findings suggest that Mentha piperita extract could be effectively used in the development of nutraceuticals and therapeutic food supplements.

The flavonoids salicylic acid and kaempferol were also found in high concentrations. Kaempferol has demonstrated multiple therapeutic effects, particularly in liver protection through various antioxidant mechanisms. Furthermore, it has been confirmed to have a preventive and curative effect against breast cancer, underscoring its pharmacological importance.

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