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Investigation Of The Chemical Composition Of Peppermint Essential Oil Using Gas-Liquid Chromatography

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Abstract
This study investigates the chemical composition of essential oil extracted from *Mentha asiatica* using gas-liquid chromatography (GLC). The method enabled the qualitative and quantitative identification of volatile compounds present in the plant's essential oil. Samples were collected at various stages of plant vegetation, allowing for the assessment of changes in the concentration of key biologically active constituents. The most prominent compounds identified in the oil were menthol, pulegone, carvone, and other terpenoids, which are known for their anti-inflammatory, antiseptic, and antispasmodic properties. The findings emphasize the pharmacological potential of peppermint essential oil and support the need for further research aimed at its application in medicine, cosmetology, and the food industry.
Keywords: *Mentha asiatica*, essential oil, gas chromatography, gas-liquid chromatography, column, flow rate, oil composition, menthol, pulegone, carvone, biologically active compounds, chemical analysis, medicinal plants.

INTRODUCTION

Currently, in Uzbekistan, active efforts are underway to establish a unified scientific foundation for the cultivation and processing of medicinal plants across the country. These efforts include studying advanced international research, fostering collaboration with leading scientific institutions, implementing modern technologies, and efficiently utilizing available resources.

Essential oils derived from plants, including peppermint, are complex multicomponent mixtures of biologically active substances with a wide spectrum of pharmacological, antiseptic, and aromatherapeutic properties. *Mentha asiatica* (Asian peppermint) is of particular interest due to its potentially unique chemical composition, which may differ from traditional peppermint species as a result of specific climatic, soil, and genetic factors.

Given the growing demand for natural pharmaceuticals and cosmetic products, as well as increasing interest in plant-based remedies and natural antiseptics, the scientific study of essential oils has become especially significant. Investigating the chemical composition of *Mentha asiatica* essential oil not only enables the identification of its biologically active compounds but also facilitates the assessment of its potential applications in the pharmaceutical, cosmetic, and food industries.

Gas chromatography–mass spectrometry (GC-MS) is one of the most precise and informative analytical techniques available for reliably identifying and quantifying the constituents of complex mixtures [1]. Applying this method to the study of *Mentha asiatica* essential oil opens up new possibilities for the standardization of plant materials, the development of new herbal preparations, and the scientific evaluation of its therapeutic value.

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One of the most widespread medicinal plants in Uzbekistan is local peppermint (*Mentha asiatica* Boriss), a perennial herbaceous plant belonging to the Lamiaceae family. This plant has traditionally been used in folk and conventional medicine for treating headaches, respiratory conditions, and gastrointestinal disorders [2,3].
The therapeutic properties of medicinal plants are directly linked to the presence and concentration of biologically active compounds. The phytochemical composition of a plant may vary depending on its developmental (ontogenetic) stage [4,5]. Since the aerial parts of *Mentha asiatica* are the main raw material for essential oil extraction, it is particularly interesting to analyze the chemical profile of oils obtained at different stages of plant development.

Traditional Uses and Therapeutic Properties

Peppermint oil is known for its pleasant aroma and skin-toning properties, which is why it is often added to bath water. In cases of dizziness or fainting, the scent of peppermint oil is used to aid recovery. For arthritis and gout (a condition related to metabolic imbalances), freshly crushed peppermint leaves are applied directly to the affected areas.
The essential oils in peppermint—particularly camphor and menthol—serve as potent antiseptics, preventing infections in areas affected by colds, cuts, or abrasions. In respiratory illnesses, inhalation of the oil is beneficial, and gargling with diluted oil is helpful for sore throats. The oil is also effective in relieving toothaches and, when applied as a drop, in alleviating ear pain.
The renowned medical practitioner Avicenna (Abu Ali Ibn Sina) praised peppermint for its ability to strengthen the stomach, warm the body, improve digestion, and stop vomiting of mucus or blood. He also noted its effectiveness in treating jaundice and internal bleeding.
Peppermint is widely used in cooking, confectionery, and the beverage industry. Its oil is added to sweet pastries and alcoholic beverages, while fresh leaves are used in salads, sour soups, and meat dishes. Dried leaves are used to prepare tea and refreshing drinks [6].
Thus, the study of the compositional makeup of peppermint essential oil using GC and GC-MS methods is a relevant scientific direction that combines analytical precision and practical value for medicine, pharmacy, and related fields.
The aim of the research was to determine the compositional makeup of local peppermint essential oil during the budding and flowering stages.

Materials and Methods of the Study

Samples of essential oil obtained from local peppermint plants at different growth stages were selected as the objects of the study. Peppermint plant samples were collected in 2024 from the Samarkand region in accordance with the generally accepted guidelines for the collection of medicinal plant materials [7].
The essential oil of the local peppermint [8] was obtained by hydrodistillation of the samples using Method 1 described in Figure 14. A Ginsberg apparatus was used to extract the essential oils.

The fatty acid composition was studied in the form of their methyl esters. For this purpose, the oil samples were transesterified with a 2 M solution of HCl in methanol. When studying the composition of essential oil components, the samples were diluted with hexane at a ratio of 1:300, and the resulting hexane extract was used for gas chromatograph-mass spectroetry (GC-MS) analysis.

For analysis, a YL6900 GC-MS equipped with a 30 m long HP-5 capillary column with an internal diameter of 0.32 mm and a stationary phase thickness of 0.25 µm was used.
Chromatography conditions: thermostat temperature — initial temperature 60 °C for 3 minutes (isothermal mode); temperature programming at 15 °C/min up to 250 °C, then 3 minutes at 250 °C (isothermal mode). Injector temperature — 250 °C; helium gas flow — 1 ml/min; Split Ratio — 1/100.
Mass detector parameters: solvent delay — 3 minutes; emission current — 50 mA; scan range — 30–350 m/z; scan rate — 1600 m/z per second; ion source temperature — 230 °C; transfer line temperature — 280 °C. Total analysis time — 25 minutes.

Component identification was performed by comparing the obtained mass spectra with the NIST mass spectral library and retention times. The internal normalization method was used for quantitative analysis.

Table 1.
Results of the Analysis of ***Mentha asiatica*** Essential Oil During the Flowering Stage

№	Name of Component	Retention Time, min	Chromatographic Peak Height	Proportion Relative S to Total Amount, %	Proportion Relative S to Maximum, %
1	Limonene	2.94	2.44	0.565	0.64
2	Eucalyptol	2.95	5.48	1.271	1.43
3	Menthone	3.97	24.46	5.339	5.61
4	Isopulegone	4.16	10.34	2.969	3.33
5	Unidentified Component	4.51	2.32	0.612	0.689
6	Pulegone	4.69	190.6	89.26	100

According to the data presented in Table 1, five main components were identified in the essential oil of *Mentha asiatica* during the flowering stage. These components are biogenetically related in nature. The principal constituent of the essential oil is pulegone, which accounts for more than 89% of the total composition. Pulegone is a widely occurring compound in essential oils and is commonly used as a fragrance additive and as a precursor for menthol synthesis [9]. It is this same pulegone that is often associated with the antibacterial, anti-inflammatory, and antihistamine effects of medicinal plants.
Interestingly, the essential oil of peppermint contains several monoterpene compounds, particularly limonene, eucalyptol, and menthone. These biologically active substances possess a wide range of pharmacological effects—including antiseptic, anti-inflammatory, antispasmodic, analgesic,

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sedative, and antimicrobial activities [9,10]. Therefore, the essential oil derived from *Mentha asiatica* during the flowering stage can be considered a promising source for the development of new plant-based medicinal preparations.

Table 2.

Results of the Analysis of *Mentha asiatica* Essential Oil During the Budding Stage

№	Name of Component	Retention Time, min	Chromatographic Peak Height	Proportion Relative to Total Amount, %	Proportion Relative to Maximum, %
1	Limonene	2.94	37.0	6.41	9.33
2	Trans-Dihydrocarvone	4.40	45.0	9.28	13.51
3	Unidentified Component	4.42	44.5	0.86	1.11
4	Carvone	4.75	16.5	68.76	100
5	Unidentified Component	4.83	4.1	0.77	1.11
6	Beta-Bourbonene	5.91	9.3	1.96	2.81
7	Caryophyllene	6.10	48.7	8.78	12.65
8	Unidentified Component	6.49	12.6	2.36	3.42
9	Unidentified Component	6.90	2.2	0.38	0.54

During the analysis of local peppermint essential oil in the budding stage, carvone was identified as the dominant component, accounting for more than 68% of the composition. Carvone is a well-known monoterpene compound recognized for its bactericidal activity [11]. The essential oil also contains caryophyllene, which functions as an adaptogen, supporting the normal functioning of the immune and nervous systems through its pharmacological action. Moreover, there is evidence that caryophyllene possesses anti-inflammatory and analgesic properties [12]. Given the diversity of therapeutic effects observed among the detected compounds, further investigation into the pharmacological activity of *Mentha asiatica* essential oil is warranted.

Conclusion

In this study, the chemical composition of essential oils obtained from *Mentha asiatica* during both the budding and flowering stages was investigated. Gas chromatography in combination with mass spectrometry was employed to determine the oil's chemical profile.

According to the analysis results, essential oils extracted from flowering and non-flowering peppermint samples differed in both the quality and quantitative ratio of their components. Specifically, pulegone was identified as the dominant component during the flowering stage,

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whereas carvone prevailed during the budding stage. Furthermore, all identified biologically active compounds were found to exhibit beneficial pharmacological properties, including anti-inflammatory, analgesic, antispasmodic, and antibacterial effects.

Based on these findings, the essential oil of local peppermint can be regarded as a promising source for the development of future medicinal products. In-depth study of its pharmacologically active properties is therefore both relevant and scientifically justified.

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