Research Article

Identification of Volatile Oils From *Mentha Spicata* Plant Based on GC Gas Chromatography Technology

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Abstract | The aim study is identificationthe medically effective volatile oils in *Mentha spicata* and to know their proportions and concentration. *Mentha spicata* is one of the most important medicinal plants in both Iraq and the Arab world. Cultivated in almost all types of soil, it is however salt-alkaline resistant. Abstract recent revisions within the genus *Mentha* have identified 18 recognised species within this group. The high importance of plants of the genus in the pharmaceutical and food industries is due to its high medicinal and culinary value, which is also largely connected with the chemical composition of the essential oils. Variability of *Mentha* essential oils (yields and composition) are significant and dependent on the environment (recent studies). Laboratory results for plant extracts showed to the impact of the extraction techniques on the chemical profile of *M. spicata* essential oils, methanolic extraction using a Soxhlet apparatus and Hydrodistillation using a Clevenger-type device were applied. They were the first two methods of comparison. The absence of volatile oil from Soxhlet methanolic extract may be due to drying mint leaves; drying mint leaves usually leads to loss of volatile components. In comparison to essential oil obtained by Hydrodistillation of fresh plant material with the Clevenger apparatus shallowed pale yellow shaped. The duration of extraction was highlighted as an important parameter influencing oil production, as prolonged extraction time provides a greater disturbance of plant tissues and a better release of volatile compounds, especially essential oils. Gas chromatography examination showed carvone as the most abundant compound in the *M. spicata* essential oil (44.38% of total composition). Menthone (17.69%), cineole (5.95%), limonene (3.69%), linalool (3.00%), and geraniol (2.79%) were other principle constituents. In conclusion, these results show the considerable variation of the chemical composition of *M. spicata* essential oils which have been cited worldwide. Moreover, geraniol was the most retained of the identified compounds (9.682 minutes).

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Introduction

A wide range of plants employed in herbalism are referred to as "medical plants," some of which have therapeutic qualities. Certain plants are recommended for their therapeutic qualities because they are thought to be essential sources of sustenance. They include green tea, walnuts, ginger, and a few more plants. Other plants and their derivatives are thought to be important sources of the active ingredients in toothpaste and aspirin (Süntar, 2020). Additionally, it is believed that these medicinal plants provide a rich source of ingredients for the development and synthesis of new medicines. and the development of human cultures worldwide depends on these plants (Akram and Mahmood, 2024).

At least 99 percent of them belong to the kingdom of plants. It is interested in researching the phenotypic, taxonomic, synthetic, and chemical elements of medications' natural or raw plant origins, as well as how to extract and identify their active components and demonstrate how they affect both people and other living things (Abboud and Waheed, 2017).

Volatile oils also known as aromatic oils, are oil extracts that, under normal circumstances, can evaporate and volatilize. They are derived from plants or parts of them and give plants their unique scent. These essential oils have a great deal of potential to eradicate Salmonella spp., which cause diarrhea in chickens. Furthermore, a number of studies have shown that there might be synergistic effects when herbal essential oils and antibiotics are combined. By breaking down the bacterial cell membrane, the essential oils let the antibiotics penetrate through the barrier and more quickly reach effective concentrations (Vui *et al*., 2025).

Chemically volatile oils consist of a mixture of isoprenoids group compounds. Isoprenoides, volatile oils are found in several plant families, such as Lamiaceae Labiatae, Piperaceae, Rutaceae, Pinaceae, Apiaceae, Rosaceae, etc.

The Lamiaceae family is one of the most distinctive families of flowering plants with more than 7,000 species and around 236 genera worldwide (Bendif, 2021). Compounds produced by members of this family, which are commonly known for their fragrant nature, are characterized by the production of bioactive secondary metabolites. Many species of the Lamiaceae have been researched for their biological and pharmacological characteristics, and they are extensively utilized in traditional and modern medicine. The most popular members of this family include many aromatic and culinary plants belonging to Thymus, Mentha and Ocimum (Asghari *et al*., 2017).

Mentha spicata L. is cultivated on a variety of soil types, is very resistant to alkalinity and salt, and is important as a primary source of therapeutic herbs in many Arab countries including Iraq and the. 18 species have recently been identified in the genus *Mentha*. In addition to a peppermint component, the essential oil of peppermint leaves includes a menthol compound at a concentration of 01–41%. There are 41-31% of mentone, 4-43% tanning materials, 8% flavonoids and esters, and aromatic acids. According to (AL- Ibraheemi, 2019), 45–65% of green mint leaves are carvone. Historically, dried and fresh leaves of *Mentha* were used with a wide range of herbs. Biologically active ingredients from *Mentha* species are used in traditional treatments (Mahendran *et al*., 2021).

The frequency and quantities of these components also vary with the plant organ collection and geographic origin which will help explain the differences in traditional uses, and efficacy described for different regions and medicinal practices. For example, the recovery of these compounds is dependent on the extraction methods used, which might further elucidate disparities in the application of these traditional medicines based on how the pharmaceutical preparation is conducted. In vitro and in vivo studies showed that *M. spicata* extracts and essential oils carry significant biological activities, including Anticancer, anti-inflammatory, antidiabetic, antiparasitic, and antimicrobial activities. Moreover, different organic extracts infused with biochemically active contents have shown remarkable antifungal activity by inhibiting the proliferation of pathogenic strains related with infections of human such as *Cryptococcus neoformans, Microsporum audouinii, Candida albicans, Aspergillus niger* (Hussain *et al*., 2010). In addition to that, *M. spicata* has demonstrated antibacterial proprieties against a number of clinical isolates and reference strains (Franciscato *et al*., 2018). The *M. spicata* extracts have also been reported to have therapeutic effectiveness against few of these complex human diseases, especially chronic inflammatory diseases, diabetes mellitus, and cancer (Rakebizadeh *et al*., 2018).

Medical uses plants of mint species (*Mentha genus*) have been employed for the treatment for nausea, sinus inflammation and pressure, fever, coughs, common cold and for gastrointestinal disorders like dyspepsia susceptibility or irritable bowel disease. A diverse amount of pharmacological activities have been reported in those traditional uses such as antioxidant, insecticidal, antimicrobial, antispasmodic and antifungal effects (Mahendran *et al*., 2021). Besides their medicinal importance, *Mentha species* are widely used in food dishes, cosmetics and ethnomedicine of different societies.

Phytochemical composition of mentha

Mentha's phytochemical composition includes a high concentration of polyphenols, in addition to caffeic acid and its analogs, including caftaric acid, cinnamic acid, ferulic acid and oleanolic acid (Wu *et al*., 2019). These plants also contain thymonin, salvigenin, diosmin, acacetin, apigenin, and luteolin and its analogs, In addition to coumarins such as scopoletin and esculetin, and flavonols such as catechin and epicatechin which make up approximately 10% to 70% of the complete phenolic compounds. The essential oils are a primary focus when it comes to mint compositions. Their primary constituents in *Mentha* species include alcohols, ketones, esters (Naureen *et al*., 2022).

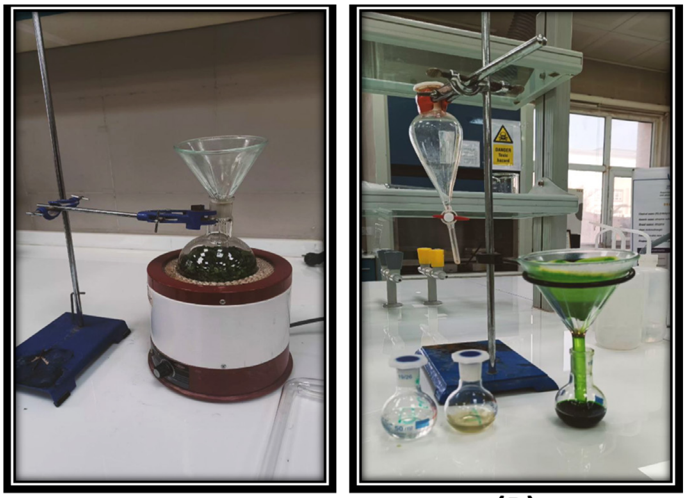

Figure 1: *Mentha spicata plants extract A: (SEM),B: (HDC)*

Materials and Methods

Preparation of samples for studies

The current study was based on samples obtained from farms in Najaf Governorate. where good leaves were chosen that were not infected with any bacterial or viral diseases and in the vegetative stage, and were cleaned and washed with running water several times to clean them of dirt and dust, It was divided into two parts, and the fresh samples were preserved, while the other part was left to dry at room temperature, and after making sure that It was completely dried and ground in an electric mill to obtain a fine powder, and kept in plastic containers until use.

Extraction of the volatile oil

Dry sample

Soxhlet extraction method (SEM)

At peak blooming, the above-ground portions of spearmint were collected, shade-dried, and milled into a fine powder. The alcoholic leaves extract was prepared in the Pharmaceuticals Laboratory, College of Pharmacy, Al-Kafeel University, from the powder of plant leaves according to the (Al-Isawi, 2020) with the modification as follows: 150 g of dried leaf powder was taken put in the Soxhlet apparatus and mixed with 300 ml of methanol and extracted for 2 hours at temperature between 30 and 60°C. It appeared white extract and the obtained collected then ready for GC examination (Alshibly and Alzamily, 2022, Figure 1A).

Fresh sample

Hydrodistillation with the Clevenger (HDC)

Put 200g from fresh *Mentha* leaves was put together with 0.4 L of DW and the process of distillation is started for 3:30 hours by the Clevenger under optimal operational conditions with a temperature of 40°C as described by (Fagbemi *et al*., 2021) When the solution in the thimble was clear, it signified that the oil was completely extracted from the raw plant leaves and the apparatus was turned off, and the produced oil was golden yellowish with high viscosity. Collect the oil and add 30 ml of hexane to it to isolate the water droplets from the oil. This is followed by collection and keeping of oil at 4°C to be analyzed by GC, Figure 1B.

Gas chromatography (GC-FID) analysis

Gas chromatographic analysis was performed with gas chromatograph (Shimadzu 2010 - Japan) with an Flame ionization detectors (FID), and a "capillary separation column type (DM-5Ms)", This test was conducted at the laboratories of the Environment and Water Department of Ministry of Science and Technology. The conditions (i.e. Temperature) of the test were C 280 for injection environments and 340 C for the reagent, while separation column were rose step by step from (100 until 300) C at a speed of ten degrees per minute. The use of "inert nitrogen gas" at a pressure of 100 kPa as the carrier medium (Hcini *et al*., 2013) Figure 2.

Results

The evaluation of the effect of various extraction methods on the phytochemical constituents of EOS from *Mentha spicata*, Methanolic extract by Soxhlet and Hydrodistillation with the Clevenger were used. For each extraction technique, Techniques used are first compared.

The identification of volatile oils from dry sample by soxhlet extraction method (SEM)

The analysis resulted not found volatile oil in extraction by Soxhlet with methanol we think this relates use dry leaves of peppermint.

The identification of volatile oils from fresh sample hydrodistillation with the clevenger (HDC)

The essential oils obtained by this method were pale yellow in color and of unpleasant odor. The extraction time is one of the key parameters often considered in obtaining maximum yield as it allows sufficient disruption by breakdown of plant cells walls and short dispersion phase for effective release of volatile principles such as essential oils (Table 1). Similar findings have been reported in previous studies as well (Idris and Mohd, 2019).

Table 1: *Classification of M. spicata (12)*

Scientific name	Mentha spicata L.
Kingdom	"Plantae"
Phylum:	"Angiospermophyta"
Class	"Magnoliopsida"
Order	"Lamiales"
Family	"Lamiaceae"
Genus	"Mentha"
Species	*"Spicata"*

Table 2: *The chemical constituents of M. spicata*

No.	Carvone %	Menthone %	Cineole %	Limonene %	Linalool %	Geraniol %
1	44.38	17.69	5.95	3.69	3.00	2.79

Principal component analysis results

The findings of GC test the major constituents of *M. spicata* have been shown in Table 2. It shows that the main volatile oil was Carvone (44.38%). The second compound was Menthone (17.69%), Cineole (5.95%), limonene (3.69%), Linalool (3.00%) and Geraniol (2.79%), as shown in Table 2. There is broad variation in the constituents of *M. spicata* plants seen worldwide. These results are consistent with the study (Jamshidi-Kia *et al*., 224).

Identification of *volatile oils* by Gas Chromatograph (GC)

Menthone

The results shown in Figure 3 and Table 3 content *M. spicata* of Menthone with Retention time 6.562 min.

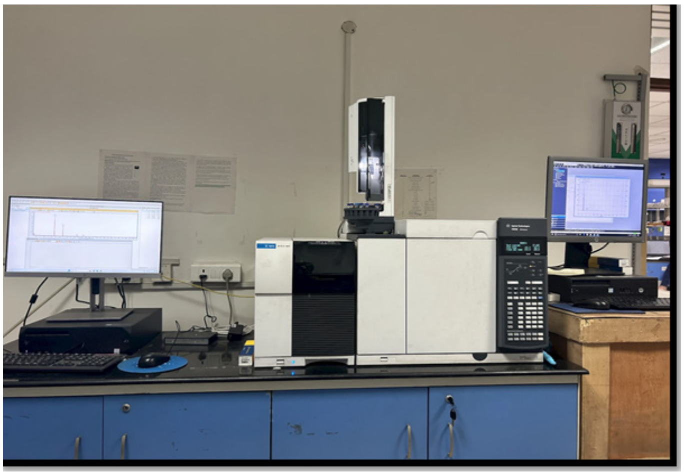

Figure 2: *Gas chromatography*

Table 3: *GC of Menthone*

No.	Retention time	Area	Area (%)	Height	Compound
1	6.562	235698	100.0000	284758	Menthone
Total		235698	100.0000	284758	

Table 4: *GC of Geraniol*

No.	Retention time	Area	Area (%)	Height	Compound
1	9.682	26893994	100.0000	13947891	Geraniol
Total		26893994	100.0000	13947891	

Table 5: *GC of Carvone*

No.	Retention time	Area	Area (%)	Height	Compound
1	1.773	4304196	50.8871	4794741	Carvone
2	4.951	23569	49.1129	4625920	
Total		4327765	100.0000	9420661	

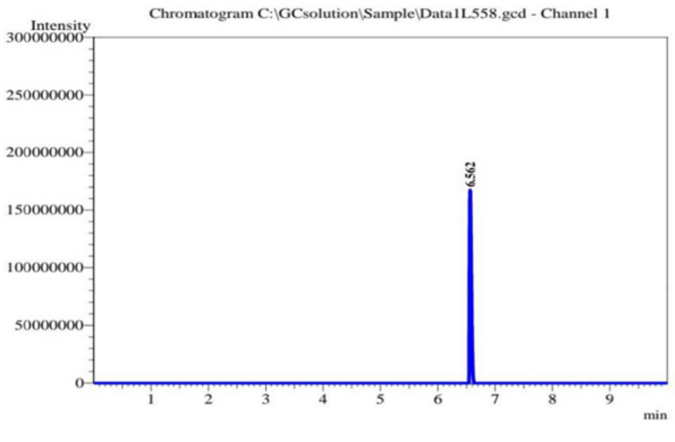

Figure 3: *GC of menthone*

Geraniol

The results shown in Figure 4 and Table 4 content *M. spicata* of Geraniol with Retention time 9.682 min.

Carvone

The results shown in Figure 5 and Table 5 content *M. spicata* of Carvone with Retention time 4.951 and 1.773 min.

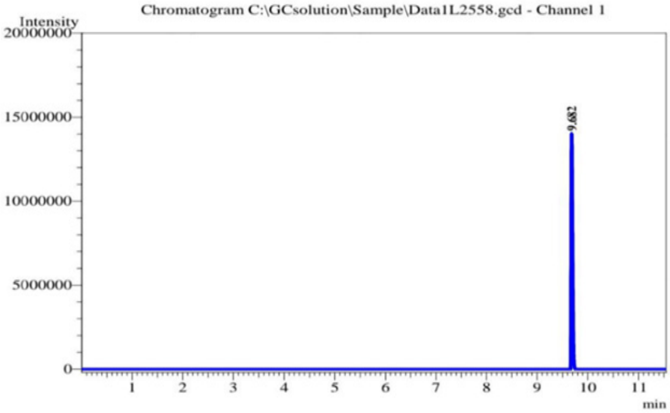

Figure 4: *GC of geraniol*

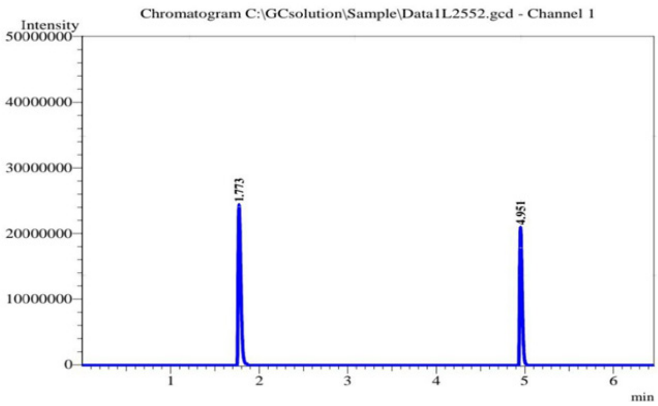

Figure 5: *GC of carvone*

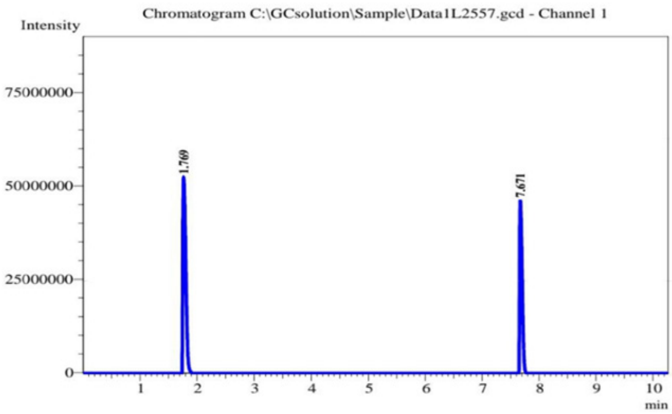

Figure 6: *GC of limonene*

Limonene

The results shown in Figure 6 and Table 6 content *M. spicata* of Limonene with Retention time 1.769 and 7.671 min.

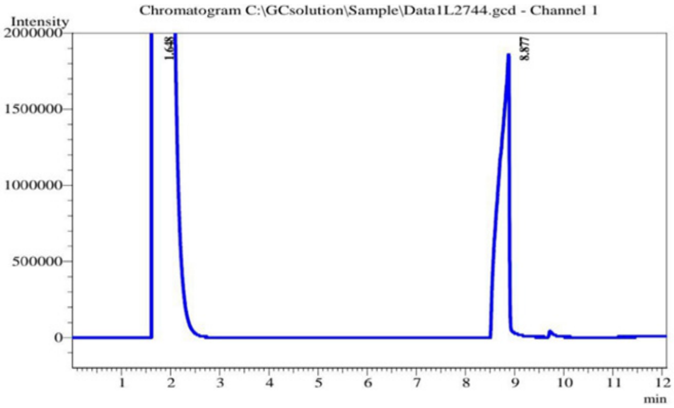

Figure 7: *GC of linalool*

Table 6: *GC of limonene*

No.	Retention time	Area	Area (%)	Height	Compound
1	1.769	195073158	57.3547	080969	Limonene
2	7.671	145044260	42.6453	141165	
Total		340117418	100.0000	222134	

Table 7: *GC of linalool*

No.	Retention time	Area	Area (%)	Height	Compound
1	1.648	991958175	99.9982	858415	Linalool
2	8.877	18144	0.0018	21961	
Total		991976319	100.0000	856863	

Table 8: *GC of cineole*

No.	Retention time	Area	Area (%)	Height	Compound
1	1.892	912294428	99.9308	261853	Cineole
2	7.132	632042	0.0692	395384	
Total		912926470	100.0000	657237	

Linalool

The results shown in Figure 7 and Table 7 content *M. spicata* of Linalool with Retention time 1.648 and 8.877 min.

Cineole

The results shown in Figure 8 and Table 8 content *M. spicata* of Cineole with Retention time 1.892 and 7.132 min.

The oil profile is unique for each species of *Mentha*. Nevertheless, overlapping of chemical profiles in different Mentha plants is often encountered because of intensive hybridization. The ratios of these oils are variable between years and according to environmental conditions and cultivation site (Kapp, 2015).

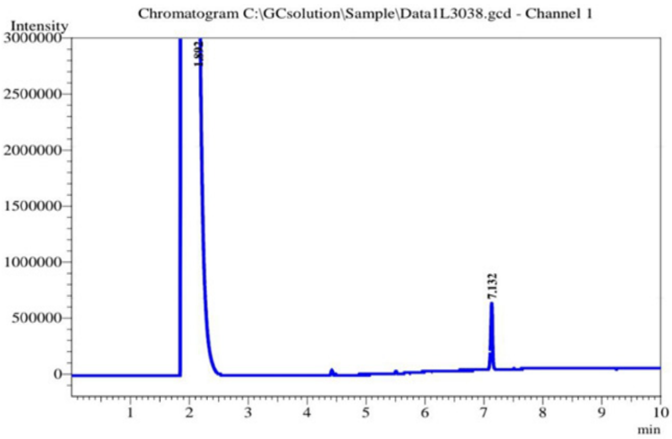

Figure 8: *GC of cineole*

Discussion

Mint varieties can be seen as some of the most interesting plants to study, sitting in a sort of semi-therapeutic-plant-fragrant-nature limbo. Although this genus has been the subject of extensive literature and many species, genotypes, and hybrids are cultivated worldwide, this study deals only with a few abundant species and a few available geographical sources. Chemical profile differences of essential oils were found in *M. spicata*, which may be due to genetic, ecological, or cultivation differences, such as methods and timing of harvesting (Khan and Ahmad, 2011).

The favorable outcomes discussed in this research highlight the effectiveness of *Mentha* species to the point that they can be regarded as promising natural extracts with obvious uses as antioxidants, vitamins, and preservatives. Mint's intended use extends beyond food preparation, elaboration, and storage; it should also be developed as a novel tool for the pharmaceutical industry (Tafrihi *et al*., 2021).

Conclusions and Recommendations

Mentha species are used for their therapeutic advantages. They are often used as a sweetener in foods and medications. Among diverse methods, hydrodistillation (HD) has been determined as the best method for extraction of essential oils from *M. spicata* leaves. It is therefore recommended to use fresh botanical material for extraction rather than dried samples, which was unsuccessful with the Soxhlet. The gas chromatography (GC) study showed that Carvone (44.38%) was the major compound of the essential oil of *M. spicata* L. In addition, the longest Retention time found for Geraniol was 9.682 minutes.

Novelty Statement

This study comparatively evaluates Soxhlet methanolic extraction and hydrodistillation methods for *Mentha spicata* essential oil recovery and confirms the superiority of hydrodistillation using fresh leaves. Additionally, it provides a detailed GC-FID profiling of major volatile constituents, highlighting carvone as the dominant compound under Iraqi cultivation conditions.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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