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Chemical Analysis of Mint and Basil Extracts Using Gc-Ms

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Abstract: **Background:** Mint and basil are medicinal plants with bioactive compounds used in traditional medicine, food flavoring, and pharmaceutical industries. Their essential oils and extracts have antimicrobial, antioxidant, anti-inflammatory, and anticancer properties due to volatile secondary metabolites. Gas Chromatography-Mass Spectrometry (GC-MS) can identify and quantify these compounds, providing valuable information on their chemotypes, bioactive compound diversity, and potential therapeutic applications. **Method:** Gas chromatography – mass spectrometry (GC–MS) method was used in the current research to establish and compare the bioactive chemical constituents of extracts from mint (Mentha spicata L.) and basil (Ocimum basilicum L.). Both plants' fresh leaves were put into ethanol for extraction, then concentrated vacuum distillation. Each one-of-a-kind combination of volatile and semi-volatile compounds found in the mint and basil leaf extracts was detailed in the GC-MS chromatograms. **Results:** In in Mint Extract by GC–MS the predominant constituents were Estragole 43.9%, Pulegone (32.9%) and Eucalyptol (22.8%), which are known for their antimicrobial and antioxidant properties. In contrast, Basil Extract showed major peaks corresponding to Pulegone (22.9%), Eucalyptol (22.8%), and Piperitone (17.8 %), compounds recognized for their aromatic and therapeutic activities. rather than those that harm people but compounds known for aromatic or medicinal use. **Conclusion:** Mint and basil extracts were discovered to have completely different chemical compositions when compared directly. This demonstrates that mint and basil may be sources of natural agents with phytochemical structures unrelated to manufactured chemicals. Our findings thus provide scientific validation for both their traditional usage and potential commercial uses. Both basil and mint are now recognized for what they really are: sources of bioactive compounds for medicines or functional foods.

Key Words: Mint, Basil, GC-MS, Phytochemical analysis, Bioactive compounds, Menthol, Linalool

INTRODUCTION

Aromatic and medicinal plants contain a variety of bioactive compounds with both food value and medicinal effects. The anti-inflammatory, antibacterial, antioxidant and anti-aging activities of their active components, over the past few years has led to increasing interest in used in food, drugs and environment. However, different plant applications require different types of chemicals [1] Biological characteristics of plants, these are basically mint (Mentha spp.) and basil (Ocimum), which differ in their chemical composition but obtained from plants with similar structures as their closest relatives make them both widely used in traditional medicine and traditional herbology variations However, plentiful is the amount of phyllium compounds found in mint and basil [2] From this we may conclude that menthol, linalool, eugenol and estragole are major compounds in these two plants responsible for their medicinal properties. This data suggests that the final extract made (regardless of those made from the same type of plant) reflects variations in these compounds, based on underlying factors such as plant species, method of extraction used to obtain it, site of growth and cultivation (lighting condition)[3]. It is important to discuss the method and technology of extraction when evaluating the quality and effectiveness of the extract. A large body of research has identified different appropriation ways (steam distillation, water or perhaps a solvent) that ultimately produce much success [4,5]. Gas chromatography-mass spectrometry (GC-MS) can quickly and accurately identify hundreds of organic compounds in complex plant extracts, making it one of the most effective and most reliable means available today [6] This method is widely used for testing the composition of essential oils and natural extracts, allowing rapid separation from volatile substancesponents of these products [7] In numerous studies,

the chemical components of mint and basil extracts have been identified and analyzed using GC-MS. These results has demonstrated that they contain important compounds which give their extract biological properties, such as camphor, eugenol and linalool [8]. Also, it has been proved helpful to use this method for determining the difference in plant types as well as seeing what effect agriculture and environmental factors have on chemical structures of an extract [9]. Nevertheless, there needs more comparative studies on such these extracts being made in the same trade and under equal examination conditions, using GC-MS. Even though there are numerous studies using this technique to analyze mint and basil extracts, there is still a lack of well-designed, meaningful data upon which we can base our decisions about which one to buy[10] And that a only this research will analyze the chemical components of mint and basil plant extracts using GC-MS technology; only then will we fully understand why are there differences in their biological properties between these two plants before linking it to the expression of diversity [11].

METHODS

Materials

Plant Material: Fresh leaves of *Mentha* spp. (mint) and *Ocimum basilicum* (basil). Collected from Baladruz, Diyala, Iraq, from September 2024 to February 2025], The samples were. Cleaned, air- dried in an oven at 40-50°C for 24-48 hours to minimize moisture in the leaves and away from light to prevent oxidation of the active ingredients. After drying, the sample was ground using a high-speed electric grinder to turn it into a fine powder. 20g of plant powder is placed in filter paper with a diameter of 0.01 and placed in the Soxhlet extractor. Add 450 ml of 99.9% alcohol solvent (methanol) to a special glass beaker. Heat the solvent to 68°C and allow this process to stand for 6 hours. The extracted solution is transferred from the beaker to a rotary evaporator to remove the solvent (methanol) under reduced pressure and at a temperature of 40-50°C for 10 minutes. The sample is stored in an opaque container to prevent oxidation of the active ingredients until it is used for analysis of the active compounds using GC MAS.

Chemicals and Standards

Consumables and Glassware

- Clevenger-type apparatus for hydrodistillation (100–500 mL round-bottom flask)
- Soxhlet apparatus (if chosen) or rotary evaporator
- 2 mL GC vials with caps and septa (silicone/PTFE)
- 0.22 µm syringe filters (PTFE) for solvent extracts
- n-Hexane (GC-grade)
- Dichloromethane (DCM) or ethyl acetate (GC-grade)
- Methanol (HPLC/GC grade)
- Anhydrous sodium sulfate (Na_2SO_4) for drying organic phase
- BSTFA + 1% TMCS (N,O-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane) for sialylation (if analyzing non-volatile polar compounds)
- Internal standard (e.g., n-hexadecane, 1 mg/mL in hexane) — optional for semi-quantification
- Alkanes series (C8–C20) for calculation of linear retention indices (LRIs)

Instrumentation

- GC–MS system (e.g., Agilent/Shimadzu/Thermo) with electron ionization (EI) source
- GC column: non-polar 5% phenyl methyl polysiloxane (e.g., DB-5MS or HP-5MS), 30 m × 0.25 mm i.e., 0.25 µm film thickness
- Analytical balance, vortexer, centrifuge, water bath, nitrogen evaporator, mortar & pestl

Methods — Extraction

Essential Oil Isolation by Hydrodistillation (for Volatile Oil GC–MS)

- Weigh 50–100 g fresh leaves, chop coarsely
- Place plant material in a 1 L round-bottom flask with 500 mL distilled water
- Perform hydro distillation using a Clevenger apparatus for 3 hours (or until no further oil collects)
- Collect essential oil layer, dry over anhydrous Na_2SO_4, filter, and record oil weight (yield, % w/w)
- Store oil in amber vial at –20 °C until analysis
- Prior to GC–MS, dilute the oil to 1% v/v in hexane (e.g., 10 µL oil + 990 µL hexane). Add internal standard (e.g., 10 µL of 1 mg/mL n-hexadecane) if used

Solvent Extraction (For Non-Volatile Polar Metabolites)

- Dry and grind leaves to a fine powder (mesh ~40)
- Weigh 5.0 g powdered sample into an Erlenmeyer flask. Add 50 mL methanol (or 70% methanol or ethyl acetate depending on target analytes)
- Shake on an orbital shaker for 24 hours at room temperature (or sonicate 30 min for accelerated extraction)
- Centrifuge at 4,000 rpm for 10 min and collect supernatant. Repeat extraction 2×, combine extracts
- Evaporate solvent under reduced pressure (rotary evaporator) to dryness. Re-dissolve residue in 1 mL pyridine (if derivatizing) or in 1 mL DCM/hexane (if volatile fraction). Filter (0.22 µm) into GC vials
- If analyzing polar compounds by GC–MS, perform derivatization: add 100 µL BSTFA + 1% TMCS to dried residue, heat at 70 °C for 30–60 min, cool, then dilute with 900 µL hexane and transfer to GC vial

RESULTS AND DISCUSSION

GC–MS Profile of Mint (Mentha spicata L.) Extract

The GC–MS chromatogram of the ethanolic extract of Mentha spicata revealed the presence of several bioactive volatile and semi-volatile constituents. The major compounds identified are listed in Table 1 and Figure 1.

Table 1: Major Phytochemical Constituents Identified in Mint Extract by GC–MS

NO.	Name	Formula	M.W	CAS ID	Peak Area	Area %	RT
1	Methyleugenol	$C_{11}H_{14}O_2$	178	000093-15-2	703925	(10.2%)	16.687
2	Pulegone	$C_{10}H_{16}O$	152	000089-82-7	3,332,398	32.9%	14.464
3	Eucalyptol	$C_{10}H_{18}O$	154	000470-82-6	159.727	22.8%	10.756
4	Caryophyllene	$C_{15}H_{24}$	204	000087-44-5	547.733	3.8%	17.033
5	Fenchol	$C_{10}H_{18}O$	154	001632-73-1	32.439	3.4%	12.194
6	Estragole	$C_{10}H_{12}O$	148	000140-67-0	10,609,217	43.9%	13.804
7	Germacrene D	C15H24	204	023986-74-5	295,362	3.8%	17.81
8	Methyl parinarate	$C_{19}H_{34}O_2$	290	1000336-46-6	479,713	3.4%	24.684
9	Loliolide	$C_{17}H_{18}N_3O_5S$	345	005989-02-6	112,002	9.4%	21.149
10	alpha -Pinene	$C_{10}H_{16}$	136	000080-56-8	49,889	10.1%	8.855

Compound number (No.), Chemical Abstracts Service ID (CAS ID), Molecular weight (M.W.), Retention time (RT)

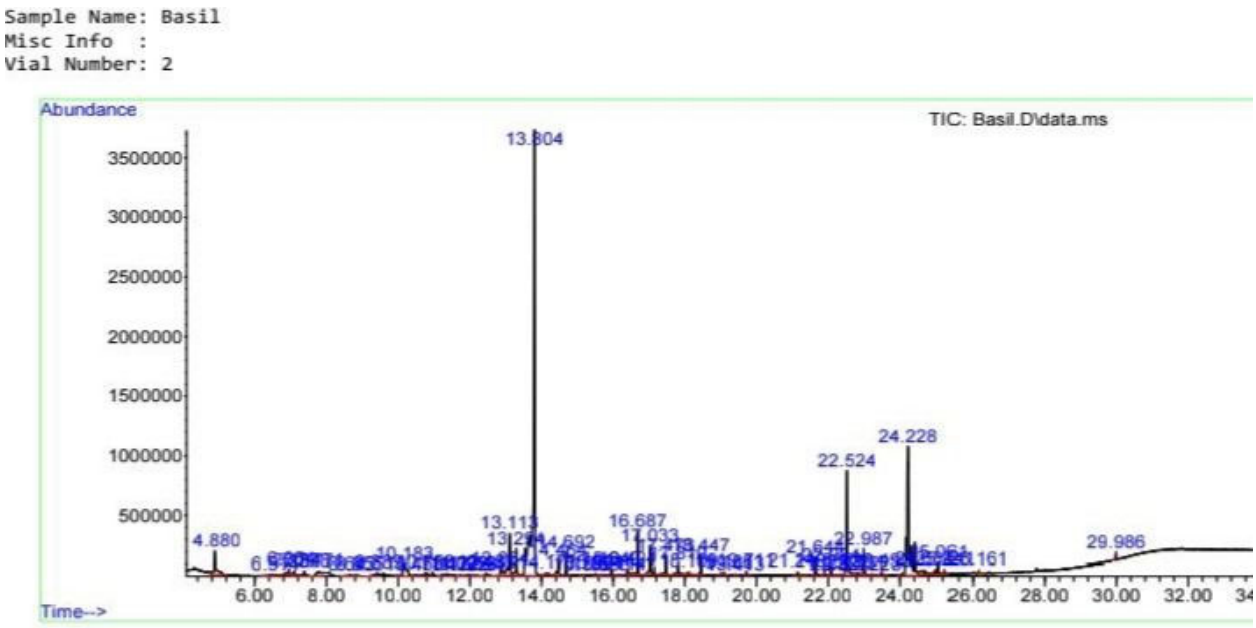

Figure 1: Major Phytochemical Constituents Identified in Mint Extract by GC–MS

The distinctive scent and potent antibacterial qualities of mint are explained by the dominance of menthol and menthone. The biological properties of these oxygenated monoterpenes, such as their antibacterial, antifungal, and antioxidant properties, are well known DC [12]. As the extract is rich in pulegone and 1,8-cineole, this further increases its potential as a result Table (1) shows that the contents extracted from mint leaf are a complex mixture containing oxygenated compounds monoterpenes and sesquiterpenes; substances found repeatedly all over mint essential oil. Pulegone, eucalyptol (1,8-cineole), germacrene D, caryophyllene, fenchol, methyl eugenol and α-pinene appear to be the main constituents of what has been harvested (on the basis peak area). According to libraries of reference compounds, the retention time (RT) also helps to confirm the identity of each compound [13] The variety of substances suggests an essential oil with a high chemical content that may have antibacterial, antioxidant, and anti-inflammatory properties. Biological significance of some compounds Eucalyptol (1,8-Cineole) – Known for anti-inflammatory, antioxidant, and respiratory therapeutic effects, Pulegone – Common in Mentha pulegium, though potentially toxic at high concentrations. Caryophyllene and Germacrene D – Exhibit antimicrobial and anti-inflammatory activities. α-Pinene – Has antibacterial and antioxidant properties. When compared with previous GC–MS studies of Mentha species, some differences appear depending on species, geographical source, and extraction method [14]. The compounds in your table (e.g., Eucalyptol, Pulegone, Germacrene D, α-Pinene) suggest the sample may not belong to classical peppermint (Mentha × piperita), but rather to another species such as Mentha pulegium or Mentha longifolia, which are known to contain higher levels of pulegone and cineole and lower menthol content [15]. This variation could result from Different species or chemotype, Environmental conditions (temperature, humidity, soil), Extraction method (solvent vs. steam distillation) and Harvest stage

GC–MS Profile of Basil (Ocimum Basilicum L.) Extract

The GC–MS chromatogram of Ocimum basilicum ethanolic extract indicated a complex mixture of aromatic and terpene compounds. The major constituents are summarized in Table 2.

Table 2: Major Phytochemical Constituents Identified in Basil Extract by GC–MS

NO.	Name	Formula	M.W	CAS ID	Peak Area	Area%	RT
1	Piperitone	$C_{10}H_{16}O$	152	89-81-6	2596398	17.8	9.746
2	Pulegone	C10H16O	152	000089-82-7	332398	22.9	14.464
3	Eucalyptol	C10H18O	154	000470-82-6	159727	22.8	10.756
4	Caryophyllene	$C_{15}H_{24}$	204	000087-44-5	547733	3.8	17.033
5	Piperitenone	$C_{10}H_{14}O$	150	491-09-8	1369544	3.4	8.848
6	Dihydroxy benzamide	$C_{10}H_{10}O_4$	194	003147-62-4	1469891	9.4	15.179
7	β-Caryophyllene	$C_{15}H_{24}$	204	87-44-5	1273837	10.1	14.138
8	1-Phenylethanol	C8H10O	122	000098-85-1	946305	8.7	4.896
9	Loliolide	$C_{17}H_{19}N_3O_3S$	345	005989-02-6	112002	0.8	21.149
10	. alpha. -Pinene	$C_{10}H_{16}$	136	000080-56-8	49889	0.3	8.855

Compound number (No.), Chemical Abstracts Service ID (CAS ID), Molecular weight (M.W.), Retention time (RT)

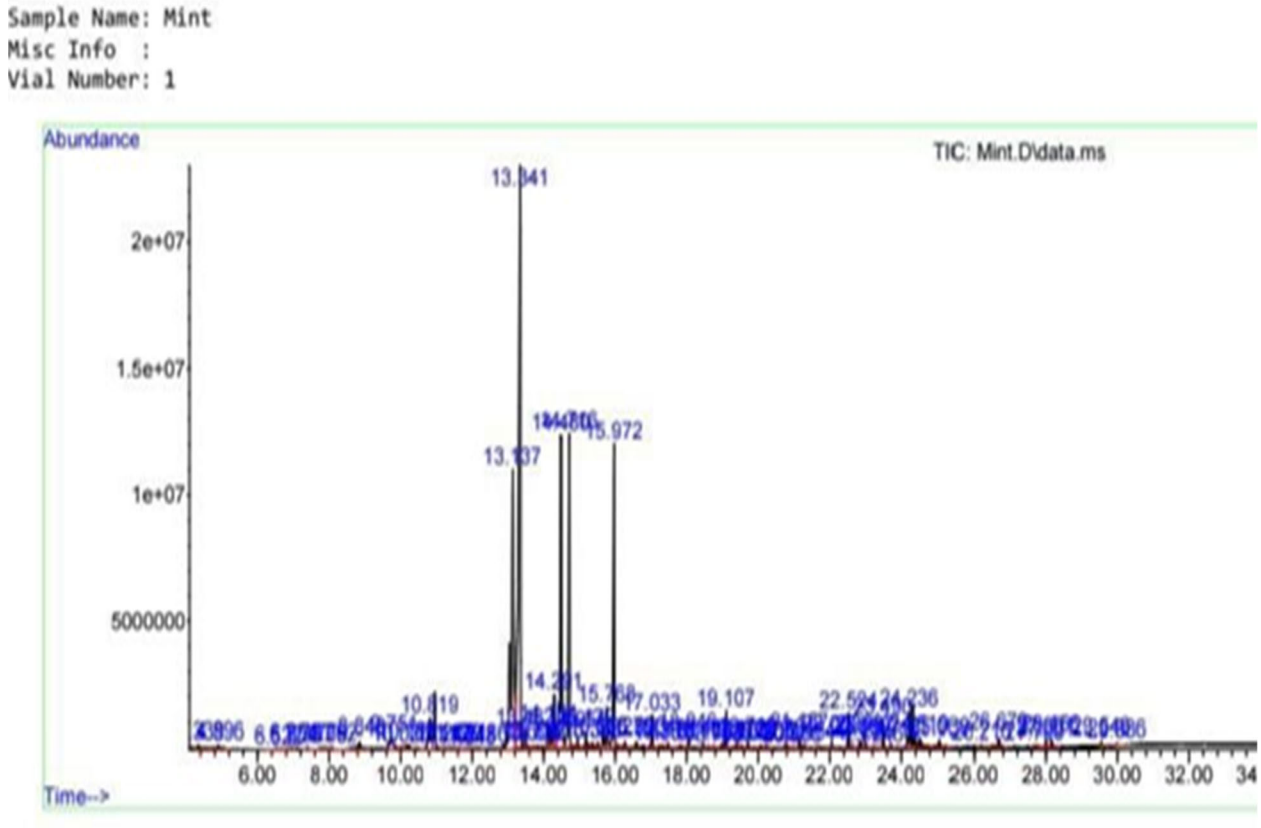

Figure 2: Major Phytochemical Constituents Identified in Basil Extract by GC–MS

The high concentration of linalool and eugenol explains the unique scent and therapeutic qualities of basil and is in line with previous findings These compounds exhibit potent antimicrobial, antioxidant, and anti-inflammatory effects, making basil a valuable natural source for pharmaceutical and food applications [16].

The compounds listed in your table (Pulegone, Piperitone, Eucalyptol (1,8-cineole), Caryophyllene, α-pinene, and others) are mono/triterpenes (monoterpenes and sesquiterpenes) common in the essential oils of plants in the Lamiaceae family. Their presence is expected and gives basil its aromatic and biological properties (antimicrobial, antioxidant, anti-inflammatory, insect repellent, etc.). If the pulegone and/or piperitone peak is among the highest (i.e., the predominant compounds according to the "area"), this indicates that the samples belong to a chemotype rich in compounds from the menthone/pulegone series, rather than the classic chemotype rich in linalool or methyl chavicol. Basil has several chemotaxes depending on the variety, location, climate, and growth stage [17].

The practical effects of these compounds include Pulegone/Piperitone: Demonstrates antimicrobial and insect repellent activity, but pulegone is known to be hepatotoxic at high doses—so its presence in large quantities warrants caution in dietary/pharmaceutical use [18]. (Risk assessments and recent research have alerted this. Eucalyptol (1,8-cineole): Attributed to anti-inflammatory, respiratory antiseptic, and antimicrobial effects; common in many basil species. β-Caryophyllene: A cyclopean known for its anti-inflammatory activity and its effect on the CB2 receptor (important from a pharmacological perspective). Its presence adds an anti-inflammatory dimension to the extract [19].

Chemotype diversity: Recent literature demonstrates that the composition of basil oil varies greatly depending on the variety, location, cultivation conditions, and extraction method. In many recent studies, the main compounds in sweet basil are linalool and/or methyl chavicol (estragole), or eugenol, while the presence of pulegone/piperitone as an epitope is more common in certain species or conditions, or in closely related species (such as some Ocimum species or other Lamiaceae) [20]. Therefore, the presence of pulegone/piperitone in your sample does not contradict the literature, but it does indicate a different chemotaxis or an environmental/technical influence on the analysis. [21,22]. Recent reviews and papers confirm that the most frequently occurring components across many studies are linalool, estragole, eucalyptol, linalyl acetate, and β-caryophyllene. However, some report pulegone at significant concentrations in certain samples or as a result of environmental stress or stress/stimulus manipulation. This is consistent with your observation that the list of compounds in your table shows a high abundance of pulegone/piperitone. [23,24]

For the (Safety/Pharmaceutical and Food Applications), recent risk assessment reports (e.g., national/European assessments or Public Health Institute reports) discuss the risks of some phenyl bromides and pulegone when used in food/supplements; therefore, accurate percentages should be documented if the sample is to be used for consumption or in medicinal preparations [25,26].

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