Extraction, Characterization and Antimicrobial study of *Mentha spicata* (spearmint) Essential oil

A dissertation submitted in the partial fulfillment of
The requirement for the degree of
Master of Science
In
Chemistry

Submitted by:
Vaishali Sharma
(SAP ID: 500113110)

Under the supervision of
Dr. Shefali Arora

Department of Chemistry, Applied Science Cluster
School of Engineering
University of Petroleum and Energy Studies
Dehradun, Uttarakhand-248007, India
May, 2024

DECLARATION

I declare that the thesis entitled "**Extraction, Characterization and Antimicrobial study of *Mentha spicata* (spearmint) Essential oil"** has been prepared by me under the supervision of **Dr. Shefali Arora and Dr. Mamta Latwal** from **Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies (UPES), Dehradun, India.**

Vaishali Sharma

Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

CERTIFICATE

I certify that, **Vaishali Sharma** has prepared his project entitled "**Extraction, Characterization and Antimicrobial study of *Mentha spicata* (spearmint) Essential oil"** for the award of **M.Sc. Chemistry,** under my guidance. She has carried out the work at the **Department of Chemistry, School of Engineering, University of Petroleum & Energy Studies (UPES), Dehradun, India.**

Dr. Shefali Arora
Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, Indi

Dr. Mamta Latwal
Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

PLAGIARISM CERTIFICATE

I, **Vaishali Sharma**, here by certify that the research dissertation titled "**Extraction, Characterization and antimicrobial study of Mentha spicata (spearmint) essential oil**" submitted for the partial fulfillment of a M.Sc. degree from University of Petroleum Energy &Studies, Dehradun, India is an original idea and has not been copied/taken verbal in from any one or from any other sources.

I further certify that this dissertation has been checked for plagiarism through a plagiarism detection tool "**TURNITIN**" approved by the university and "**8% (should be below 15%)**" percentage similarities have been found as shown in the attached report (given in the next page).

Vaishali Sharma
Department of Chemistry
School of Engineering
University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

Approved by:

Dr. Shefali Arora
Department of Chemistry
School of Engineering

University of Petroleum & Energy Studies
Dehradun, Uttrakhand-248007, India

PLAGIARISM REPORT SUMMARY

Curiginal

Document Information

Analyzed document	B.sc Final report Modelling of Fluid Flow in Porous Media.docx (D135872005)
Submitted	2022-05-09T08:35:00.0000000
Submitted by	
Submitter email	
Similarity	7%
Analysis address	ganand.upes@analysis.urkund.com

Sources included in the report

SA	**ME_SAFA_SABET_Thesis_V3.pdf** Document ME_SAFA_SABET_Thesis_V3.pdf (D14674671)	2
W	URL: https://en.wikipedia.org/wiki/Fluid_flow_through_porous_media Fetched: 2019-09-25T08:18:57.3470000	1
SA	**GULCE_thesis_First Draft Submission.pdf** Document GULCE_thesis_First Draft Submission.pdf (D29020870)	1
W	URL: https://link.springer.com/article/10.1007/BF01036523 Fetched: 2021-03-01T07:03:13.4370000	4
W	URL: https://search.spe.org/i2kweb/SPE/doc/onepetro:07FFEF23 Fetched: 2022-05-09T08:35:50.3570000	2
W	URL: https://www.mdpi.com/2227-7390/8/7/1057/pdf Fetched: 2021-01-28T08:24:17.1870000	1
SA	**manjit Singh.pdf** Document manjit Singh.pdf (D35792844)	1

Replace this summary reports with yours

Abstract

Mentha spicata (spearmint), aromatic species is being utilised in traditional medicines and culinaries, prominent therapeutic potential due to their diverse secondary metabolites [1]. These encompassing volatile oils and flavonoids, empower it with potent biological activities such as antioxidant, antimicrobial, biopesticidal, anticancer, antiviral, antibacterial, antifungal, antiallergic, anti-inflammatory, antihypertensive, and urease inhibitory activity have been ascribed to spearmint. It is a rich source of bioactive phytochemical such as methanols, α-pinene, carvone, carveol among others [2]. Spearmint is prized for its refreshing and minty flavour, making it a popular ingredient in culinary preparations such as teas, beverages, sauces, and desserts. It is also used as a natural flavouring agent in the nutra-pharmaceutical and cosmo-nutraceutical industry also. *Mentha* are well recognized for their folk medicinal uses, especially to treat cold, fever, and digestive and cardiovascular disorders. The volatile components from fresh and dried spearmint samples were isolated by simultaneous distillation-extraction (Hydro-distillation and steam distillation) and analyzed by gas chromatography-mass spectrometry (GC-MS) [3]. The physiochemical Moreover, the oils along with their major component (carvone), exhibited moderate to good antimicrobial activity against selected bacterial (*E. coli, Klebsiella s, Listeria sp.*) and fungal (*Penicillium chrysogenum, Rhizopus oryzae, Alternaria alternata, Aspergillus fumigatus)* [4].

Keywords: Aromatic species, traditional medicines**,** biological activity, nutraceuticals.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to **Dr. Shefali Arora**, my dissertation supervisor, for their invaluable guidance, encouragement, and insightful feedback throughout the research process. Their expertise and dedication played a pivotal role in shaping the direction and quality of this dissertation.

I am also thankful to my co-supervisor Dr. Mamta Latwal, whole chemistry department, lab assistant, and PhD scholars (Sukanya Chettri, Pankaj Bhandari) for their constructive criticism and valuable suggestions, which significantly enriched the content and methodology of this study.

Furthermore, I extend my appreciation to UPES for providing the necessary resources and facilities essential for conducting this research.

My heartfelt thanks go to my parents Mr. Lekhraj Sharma and Mrs. Girisha Devi for their unwavering support, patience, and understanding during this demanding journey. Their encouragement has been my source of strength throughout.

Lastly, I am grateful to all participants who generously shared their time and insights, without which this study would not have been possible.

DEDICATED

TO MY

PARENTS

TABLE OF CONTENTS

Name of the Content	Page no
1. **Abstract**	5-6
2. Introduction	11-12
3. Literature review	12-20
4. Materials and methodology	21-27
5. Result and Discussion	28-32
6. Conclusion	33
7. References	34-39

LIST OF TABLES

Table1: Qualitative analysis of extracted oil

Table2: Antibacterial activity against extracted oil

Table3: Antifungal activity against extracted oil

LIST OF FIGURES

Fig1:*Mentha spicata Essential oil*

Fig2:*Mentha spicata roots*

Fig3: *Mentha spicata stem*

Fig4: *Mentha spicata leaves*

Fig4: *Mentha spicata flowers*

Fig5: *Mentha spicata seeds*

Fig6: *Collection of plant material*

Fig7: *Pre-treatment of plant*

Fig8: *Oil extraction unit*

Fig9: *Plate I Klebsiella sp .bacteria*

Fig10: *Plate II E.Coli bacteria*

Fig11: *Plate III Listeria sp. bacteria*

Fig12: *Penicillium fumigatus fun gi*

Fig13: *Rhizopus oryzae fungi*

Fig14: *Alternaria alternata fu ngi*

Fig15: *Aspergillus fumigatus f ungi*

INTRODUCTION

CHAPTER 1

INTRODUCTION

Compared to pharmaceutical drugs, natural medicinal products are increasingly valued for t heir lack of side effects in clinical studies. Throughout history, natural remedies have held significant importance in medicine and health, often being the primary treatment for ailments and injuries. For instance, ancient civilizations chewed herbs for pain relief and applied certain leaves to wounds for healing. Natural products continue to play a growing and stable role in human and veterinary medicine, agriculture, the food industry, and cosmetics. Both traditional and modern medicines rely on natural products with high therapeutic potential for maintaining health and treating diseases. This reliance underscores the importance of natural products as a valuable resource for developing potential drugs. In fields like pharmacotherapy, particularly in treating cancer and infectious diseases, natural products and their structural analogues make substantial contributions. Every culture has accumulated knowledge and experience regarding the diverse biological activities and medicinal potentials of natural products. In addition to medicinal purposes, natural products and their derivatives are utilized as antibacterial agents and antioxidants to preserve food and promote longevity.

Among the array of natural substances, we focus our attention on *Mentha spicata* study which is commonly known as Spearmint in Hindi it is termed as Pudina due to its various properties like anti microbial, anti oxidant and anti inflammatory etc. *Mentha spicata* belongs to family Lamiaceae and has more than 30 species. It is a perennial herb that can grow up to 2 feet high and wide, with bright green leaves and shoots [1]. The foliage has a strong minty fragrance. Spearmint is well-known for its extensive use in treating a wide range of ailments in traditional Ayurvedic literature. Researchers are very interested in *Mentha spicata* because of its huge medical qualities, which include antiviral, antibacterial, antifungal, antitumor, and

anti carcinogenic effects [2]. This plant's leaves are high in minerals and vitamin A, and its starch is high in iron. This can help manage stomach aches and common colds. In addition to that, it helps with digestion and boosts immunity.

In today's time, there is a notable demand for *Mentha spicata*. This demand is driven by various factors including its versatile applications in food, beverages, pharmaceuticals, cosmetics, and aromatherapy. Additionally, the growing interest in natural and herbal products has further boosted the demand for *Mentha spicata* due to its perceived health benefits and lack of synthetic additives. Furthermore, its cultivation is also economically lucrative for farmers, contributing to its continued demand in the market.

Figure-1: *Mentha spicata Essential oil*

LITERATURE

SURVEY

- **CLASSIFICATION :**

  Scientific name: *Mentha spicata*

  Family: *Lamiaceae*

  Kingdom: *Plantae*

  Phylum: *Tracheophyta* (Vascular plant)

  Class: *Magnoliopsida*

  Sub class: *Asteridae*

  Order: *Lamiales*

  Genus: *Mentha*

  Species: *M. Spicata*

  Common names: Spearmint, Pudina

  Property: Immunotherapeutic properties (anti microbial, anti cancer, anti inflammatory etc).

- **ORIGIN AND CLASSIFICATION:**
  - *Mentha spicata* (spearmint) is a perennial herbaceous plant which grows in clumps.
  - This plant is native to India but also found in Europe and southern temperate Asia, extending from Ireland in the west to southern China in the east.
  - This plant is distributed mostly in the Northern hemisphere. In India, it is largely confined to North India in the States of Uttar Pradesh, Punjab and Haryana.
  - It is indigenous to the areas of India, Ireland, China, Europe, North America, South America, Africa, and Australia.

- It is temperate to tropical climate and sunny weather with moderate rain is conducive to its luxuriant growth.
- A deep soil, rich in humus which can retain moisture, is suitable for mint cultivation.
- It is 30–100 cm (12–39 in) tall, with variably hairless to hairy stems and foliage, from which it grows. The leaves that are 5–9 cm (2–3+½ in) long and 1.5–3 cm (½–1+¼ in) broad, with a serrated margin.

- **HARVESTING AND YIELD:**
  - Spearmint leaves can be harvested throughout the growing season, but they are most flavourful just before the plant flowers, typically in early to mid-summer.
  - Choose healthy, vibrant leaves for harvesting which are dark and light green in colour.
  - Spearmint is a low-growing herbaceous plant with square stems, typical of plants in the mint family.
  - It can reach a height of 30 to 100 centimetres (12 to 39 inches) depending on growing conditions.
  - This plant is adaptable to a wide range of soil types but does best in fertile, loamy soil. Spearmint can tolerate a variety of climates but grows most vigorously in temperate regions.
  - Commonly used as a flavouring agent in food and beverages, as well as in herbal teas, sauces, and desserts.

- **BOTANICAL DESCRIPTION:**

Different parts of plants exhibit different types of morphology which are described below.

4.1) Root:

The root system of *Mentha spicata*, or spearmint, is primarily comprised of underground structures that support the plant's growth and development.

- **Rhizomes:** Spearmint spreads primarily through underground rhizomes, which are horizontal, underground stems. These rhizomes send out roots and shoots at nodes along their length, allowing the plant to form dense colonies over time. Rhizomes enable spearmint to propagate vegetatively and contribute to its ability to spread rapidly in suitable growing conditions.
- **Fibrous:** Along with rhizomes, spearmint also develops fibrous roots. These roots branch out from the base of the stem and extend into the surrounding soil, anchoring the plant and absorbing water and nutrients. Fibrous roots are important for supporting the overall health and vigor of the plant, as they facilitate water and nutrient uptake from the soil.
- **Adaptations**: Spearmint's root system is well-adapted to its natural habitat, allowing it to thrive in a variety of soil conditions. The rhizomatous nature of spearmint enables it to tolerate disturbances and regenerate from underground reserves, making it resilient and persistent in garden settings.

4.2) Stem:

The stems of *Mentha spicata*, or spearmint, are square, hairless, and light green to red tinged. They are usually branched and can be erect or creeping, ranging from 30–100 cm tall. The stems form colonies from stout, whitish underground stems called rhizomes.

- **Structure:** The stem of spearmint is typically square-shaped, a characteristic common to many plants in the mint family (Lamiaceae). This square shape is easily noticeable when rolling the stem between your fingers.

- **Texture:** Spearmint stems are often smooth and slightly hairy, particularly at the corners where the edges meet. The hairs may be more prominent on younger stems and may diminish as the stem matures.
- **Colour:** The colour of spearmint stems is usually green, similar to the colour of the leaves. However, the stems may have slightly darker or lighter shades of green depending on factors such as age, sunlight exposure, and nutrient levels.
- **Nodes:** Spearmint stems have distinct nodes, where leaves emerge from the stem in pairs opposite each other. These nodes give the stem a segmented appearance and are important sites for leaf and flower growth.
- **Internodes:** The sections of the stem between the nodes are called internodes. These internodes vary in length and contribute to the overall height of the plant.
- **Growth Habit:** Spearmint stems arise from a central crown or cluster of rhizomes, which spread horizontally underground. From these rhizomes, multiple stems emerge, forming a dense and bushy growth habit.
- **Branching:** Spearmint stems may branch out as the plant matures, especially in response to pruning or harvesting. This branching contributes to the plant's bushy appearance and can increase the overall yield of leaves for harvest.

4.3) Leaves: The leaves of *Mentha spicata*, commonly known as spearmint.

- **Shape:** Spearmint leaves are typically lance-shaped or narrowly elliptical, with pointed tips and tapered bases. The leaves are attached to the stems via short petioles
- **Texture:** Spearmint leaves have a smooth, slightly glossy texture on the upper surface, while the underside may be slightly hairy or textured. Margins: The edges of spearmint leaves are serrated or toothed, with small, pointed teeth along the margins. This serration gives the leaves a slightly jagged appearance.

- **Arrangement:** Spearmint leaves grow in pairs opposite each other along the stems. They are arranged in an alternating pattern along the stem, with one pair of leaves emerging from each node.
- **Colour:** The colour of spearmint leaves is typically a vibrant shade of green, ranging from light green to dark green depending on factors such as age, sunlight exposure, and nutrient levels.
- **Size:** Spearmint leaves vary in size but are generally small to medium-sized. They typically range from 2 to 5 centimetres (0.8 to 2 inches) in length and 1 to 2 centimetres (0.4 to 0.8 inches) in width.
- **Aroma:** When crushed or bruised, spearmint leaves emit a refreshing and aromatic fragrance that is characteristic of mint plants. This aroma is often described as sweet and slightly minty, with a hint of citrus.

4.4) Flowers:

- **Appearance**: Spearmint flowers are small and clustered in dense spikes at the apex of the stem. The spikes are cylindrical in shape and can reach several inches in length. The flowers are typically pale purple to pinkish in colour, although they can sometimes appear white or light blue.
- **Structure**: Each individual flower consists of a tubular corolla with four lobes, giving it a distinctly lipped appearance. The upper lip is hooded, while the lower lip is divided into three lobes, with the central lobe usually being larger and more prominent.
- **Fragrance**: The flowers of *Mentha spicata* emit a delicate and pleasant fragrance, similar to the aroma of the leaves. This fragrance is often described as refreshing, cool, and slightly sweet.

- **Blooming Period**: Spearmint typically blooms in mid to late summer, although the exact timing can vary depending on factors such as climate and growing conditions.
- **Pollinators**: Like other members of the mint family, spearmint flowers are highly attractive to bees, butterflies, and other pollinators. The nectar and pollen of the flowers provide a valuable food source for these insects.

4.5) Seeds:

- The seeds of *Mentha spicata*, commonly known as spearmint, are small, round, and brown in colour. They are typically found within the dried fruits or nutlets of the plant, which develop after flowering. These seeds are tiny, measuring only a few millimetres in diameter.
- *Mentha spicata* seeds contain the genetic material needed to grow new spearmint plants. They are often used for propagation purposes, allowing gardeners and farmers to cultivate new plants from existing ones.
- Spearmint seeds can be collected from mature plants by harvesting the dried fruits or nutlets, which contain the seeds within. When planting spearmint seeds, it's important to provide them with a suitable growing environment, such as well-drained soil and adequate sunlight.
- Spearmint is known for its vigorous growth and ability to spread, so it's essential to plant it in a location where it won't overcrowd other plants. Overall, the seeds of *Mentha spicata* play a vital role in the reproduction and propagation of this aromatic herb, allowing for the cultivation of new plants to enjoy its culinary and medicinal benefits.

Figure: *M. Spicata roots*

Figure: *M. Spicata stem*

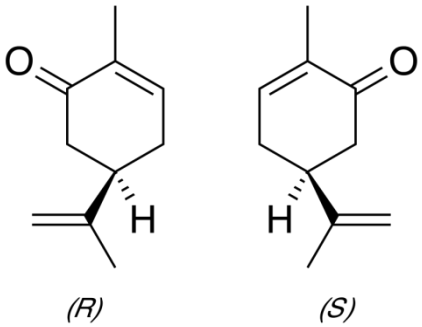
Figure: *M. Spicata flower*

Figure: *M. Spicata seed*

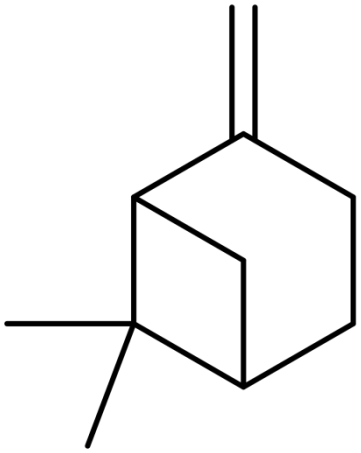
Figure: *M. Spicata leaves*

CHEMICAL CONSTITUENTS OF MENTHA SPICATA:

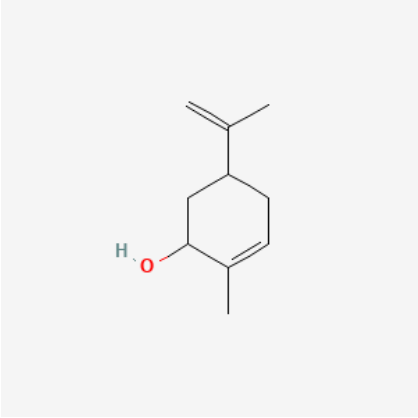
A large number of chemical constituents have been isolated from Mentha spicata belonging to different classes carvone, carveol, germacrene-D, Limonene, 1,8 -cineol, β- pinene, monoterpene, dihydrocarveol, pulegone, elemene, sabinene, aliphatic compounds and so on. Leaves are rich in carbohydrates (34.13%), proteins are (9.80%) and also contain fat, fibre, potassium and calcium



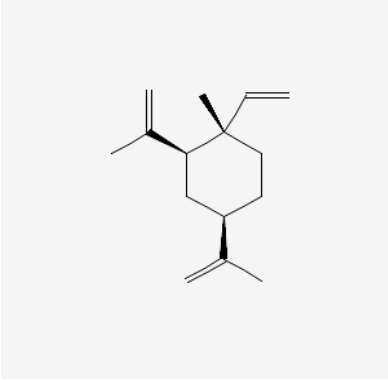
Carvone



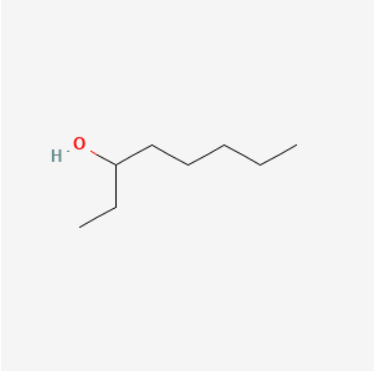
β- Pinene



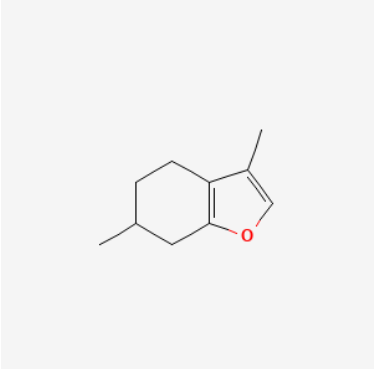
Carveol



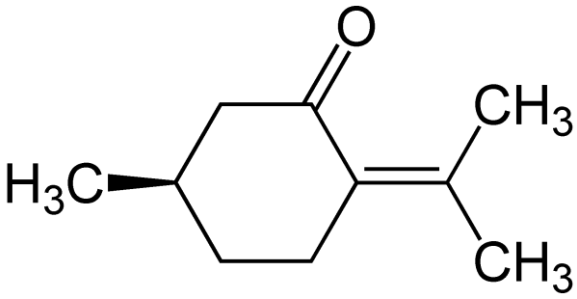
Elemene



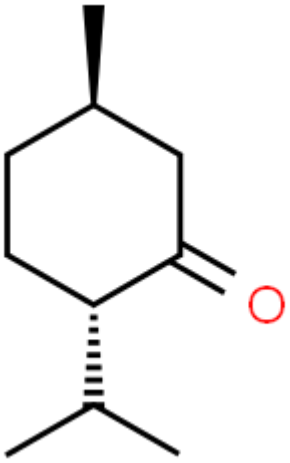
3-Octanol

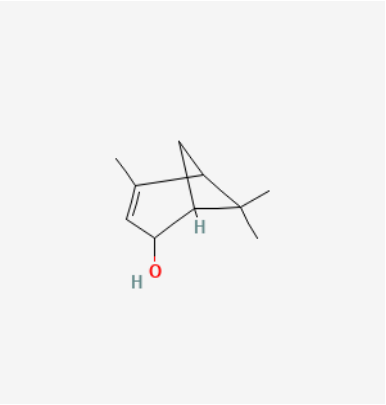


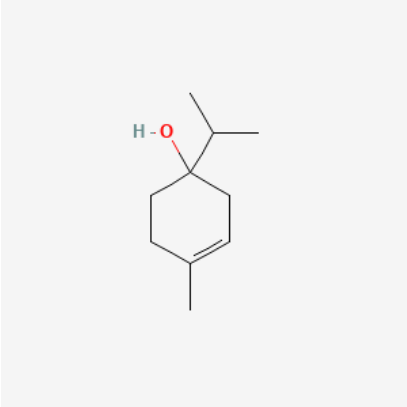
Menthofuran



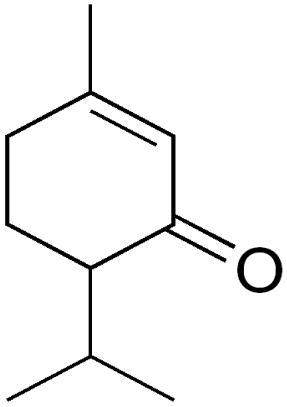
Pulegone







Terpene-4-ol



Piperitone

MATERIALS AND METHODS

CHAPTER 2
Materials and method

1) MATERIALS

i. Plant sample:

Mentha spicata (Spearmint)

ii. Chemicals/Reagents:

1. Potassium hydroxide
2. Hydrogen chloride
3. Iodine chloride
4. Diethyl ether
5. Anhy. Sodium sulphate
6. Ethanol
7. Phenolphathein
8. Tetrachloroethane
9. Potassium iodide
10. Sodium thiosulphate
11. Starch

2) METHODOLOGY:

Under the following circumstances, an experiment was conducted on *Mentha spicata* leaves:

2.1) Phytochemical investigation of extracted oil

2.2) Qualitative physiochemical characteristics.

2.3) GC-MS Analysis.

2.4) Evaluate Antimicrobial activity.

2.1) Oil extraction by phytochemical investigation:

2.1.1 Collection of plant material:

The plant was collected from (Chokha) chamoli district Uttarakhand in January month.

Figure-6: *Collection of plant material*

2.1.2 Pre treatment of plant:

First, the leaves of *Mentha spicata* was separated out from foreign residues and then cleaned with water. After that these wet leaves were dried with cotton cloth.

Figure7: *Pre treatment of plant*

2.1.3 Preparation of extraction of oil:

These dried leaves were placed into round bottom flask with adding water. This round bottom flask is attached with a clevenger and condenser. For increasing the temperature of water and sample, provide heat with placing under the unit. After that it had to be start.

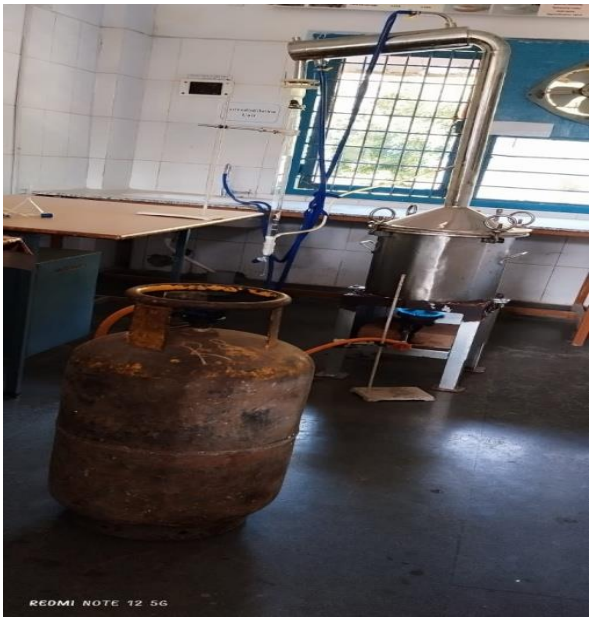


Figure8: *Oil extraction unit*

2.2) Qualitative physiochemical characteristics:

The extracted oil of the leaves of *Mentha spicata* was tested for the quality of oil by using different values as follows:

A. Acid value:

1. In 15 ml of 95 % ethanol has dissolved with one ml of the essential oil was in a conical flask.

2. Few drops of 1% phenolphthalein were added to the flask and then titrated against 0.1 N potassium hydroxide solutions.

3. The first appearance of pink colouration that did not fade with in 10 second was considered as the end point.

4. In order to determine the acid value of the oil, another set without oil was also run concurrently with the treatment. The difference between the amount of alkali needed to titrate the treatment and the set without oil revealed how much alkali was consumed.

5. The acid value was calculated by the following formula:

Acid value

Volume of 0.1 N Alkali consumed÷ Weight of 1ml essential oil×5.61

(*The weight of 1 ml essential oil of *Mentha spicata* = 0.852 gm)

B. <u>Saponification value:</u>

1. One ml of the essential oil was taken into a 100 ml Saponification flask. Ten ml of 0.5 N alcoholic potassium hydroxide solutions was added to the flask and an air cooled glass condenser was attached to it.

2. After refluxing the mixture over a water bath for an hour, it was let to cool to room temperature.

3. The contents were the titrated against 0.5 N aqueous hydrochloric acid using 3 drops of 1% phenolphthalein solution as the indicator.

4. In order to determine the Saponification number of the treatment set, another set without oil was also run parallel to it. The difference in the amount of acid consumed.

5. The Saponification value is calculated by the following formula:

Saponification value:

Volume of 0.5 N Acid consumed ÷ Weight of 1ml essential oil×28.05

(* The weight of 1 ml essential oil of *Mentha spicata* = 0.940 gm)

C. <u>pH value:</u>

1. One gram of the material was weighed by an electric balance which was then put into a 25 ml conical flask. 25 ml of distilled water was then added, heated to a boil on a hot plate, and allowed to cool.

2. Using a pH meter that was calibrated, the aqueous herbal extracts were filtered and then added distilled water to fill a 25 ml volumetric flask to the appropriate level.

D. **Iodine value:**

1. One ml of the essential oil was dissolved in Tetrachloroethane and then treated with excess amount of iodine monochloride solution in a conical flask.
2. Placed it in dark for 30 min.
3. 0.1 $Na_2S_2O_3$ is titrated this solution and in the remaining solution add some drops of starch.
4. The iodine value was calculated by the following formula:

   Iodine value:

   Volume of 0.1 N alkali consumed ÷ Weight of 1ml of essential oil × 12.69

2.2) Evaluate Antimicrobial Activity:

- **Antibacterial study:**

Extracted oil of the leaves of Mentha spicata is said to show antimicrobial activities, we perform work on antibacterial and antifungal activities. Observe anti-bacterial behavior with extracted oil. Antibacterial and Antifungal activity of the leaves works against pathogens in which inhibition against various microorganisms takes place. First choose the bacteria and fungi on which we want to study against antimicrobial, some bacterial species are *Klebsiella sp., E.Coli, Listeria sp.* and fungal species are *Penicillium chrysogenum, Alternaria alternata*, Rhizopus oryzae, Aspergillus fumigatus. These bacteria and fungi are chosen according to the objectives of oil. After that prepare a food of bacterial and fungal species to grow them and inoculate the species on the plates. Then to grow the bacteria and fungi placed in the incubator with a respected temperature. Now assess the antibacterial and antifungal growth of bacteria and fungi how much it inhibits. Hence, the potential against microbial infections has been reported in the extracted oil of the leaves of *M. spicata.*

CHAPTER 3
Result and discussion

1. Qualitative physiochemical characteristics:

Information regarding the values of the extraction of oil tested by different methods.

S.No.	Analysis	Value of E.O.	Literature review
1	Acid value	1.2	1.00-1.54
2.	Saponification value	105.1	180-190
3	Ph value	3.9	5.00-6.00
4	Iodine value	47.66	103-111

Table-3: Percentage yield of different analysis of extracted oil

The oil of the leaves of *Mentha spicata* undergoes various qualitative physiochemical tests. They represent the quality of extracted oil and showed the presence of the acidity, soap or triglycerides, alkalinity, and iodine number.

2. GC-MS Analysis:

Extracted oil of the sample was analyzed by using Perkin Elmer Gas Chromatography Clarus-590 equipped with a PE5 capillary column (30m × 0.25mm with a 0.25µm). Helium was used as the carrier gas. The GC injector temperature was maintained at 250° C and operated in the mode with slit= 1 with helium flow rate of 1.15ml\min. The initial GC column temperature was set to the boiling point of each solvent

(diethyl ether), held for 4 minutes, and then ramped to 250° C at 5° C /min hold at 250° C. The MS was operated in EI+ mode. The source and transfer line temperatures were maintained at 250° C. Mass spectra were obtained in the full scan mode, with the mass range 40-450 amu. The multiplier here was being 1940 V with a trap emission current of 100. The presence of different chemical constituent was analysed.

PerkinElmer
For the Better

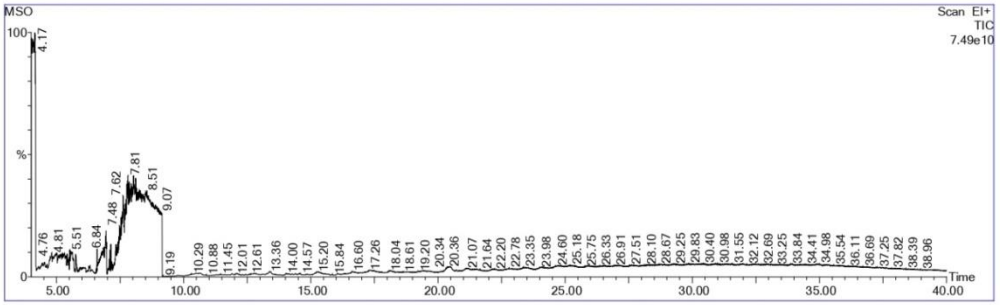
UPES- CIC

Qualitative/Quantitative Report

UPES
UNIVERSITY OF TOMORROW

File Name: C:\TurboMass\UPES CIC FEB 2022.PRO\Data\MSO.raw
Sample Run At: 29-Apr-24 11:21:32 AM
GC/MS Method: GC: MS: IPA MS Method.EXP

PerkinElmer GCMS - SQ8



#	RT	Height	Area	Area %
1	4.15	73,629,835,264	11,921,297,408.0	11.93
2	7.62	23,062,054,912	2,106,933,760.0	2.11
3	7.81	29,390,489,600	1,990,230,656.0	1.99
4	7.87	27,739,068,416	1,143,013,248.0	1.14
5	7.93	27,244,126,208	1,841,741,440.0	1.84
6	8.03	29,800,931,328	3,111,942,912.0	3.11
7	8.11	29,056,819,200	9,654,293,504.0	9.66
8	8.56	25,665,906,688	14,750,444,544.0	14.76
9	24.83	2,551,417,856	1,347,184,640.0	1.35

INSTRUMENT PARAMETERS Column : PE5 id, 0.25 micron
Manual Inj V=1.0 µL, Split=:1; Carrier Gas=, ; Inj=°C
;
EI+ Mode ; MS Scan = 40 to 450 amu; MS Solvent Delay=4.00 min; GCMS Transferline Temp=250°C; MS Source Temp=250°C; Multiplier = 1940 V; Electron Enervgy 70.00 eV; Repeller = 9.600 V; Trap Emission Current = 100.0

File Name: C:\TurboMass\UPES CIC FEB 2022.PRO\Data\MSO.raw
Sample Run At: 29-Apr-24 11:21:32 AM
GC/MS Method: GC: MS: IPA MS Method.EXP

PerkinElmer GCMS - SQ8

#	RT	Height	Area	Area %
10	25.20	2,591,177,216	1,613,865,600.0	1.61
11	25.95	2,452,370,944	1,146,736,640.0	1.15
12	26.55	2,493,427,456	1,380,742,528.0	1.38
13	27.00	2,560,791,552	1,040,007,872.0	1.04
14	27.69	2,622,762,240	1,279,747,072.0	1.28
15	28.30	2,681,996,288	1,021,352,704.0	1.02
16	28.91	2,748,326,656	1,398,316,416.0	1.40
17	29.33	2,802,453,760	1,295,195,392.0	1.30
18	29.89	2,854,922,240	1,350,911,488.0	1.35
19	30.44	2,888,572,672	1,326,911,744.0	1.33
20	30.90	2,855,547,136	1,126,197,760.0	1.13
21	31.99	2,742,376,704	1,508,147,584.0	1.51
22	32.38	2,677,321,472	1,103,212,032.0	1.10
23	32.93	2,647,346,176	2,274,292,224.0	2.28
24	34.93	2,534,352,896	1,163,755,392.0	1.16
25	35.27	2,320,492,800	1,251,806,208.0	1.25

INSTRUMENT PARAMETERS Column : PE5 id, 0.25 micron
Manual Inj V=1.0 µL, Split=:1; Carrier Gas=, ; Inj=°C
;
EI+ Mode ; MS Scan = 40 to 450 amu; MS Solvent Delay=4.00 min; GCMS Transferline Temp=250°C; MS Source Temp=250°C; Multiplier = 1940 V; Electron Enervgy 70.00 eV; Repeller = 9.600 V; Trap Emission Current = 100.0

Table-2 The chemical constituent present in extracted oil in *Mentha spicata*.

S.No.	Name of Compound	Molecular formula	Molecular weight	RT	Area% (Peak)
1	Cyclopropane,(R,R)-1-((Z),(Z)-hexa-1',3'-dienyl)-2-ethenyl-	$C_{11}H_{18}$	150.2606	4.01	4.78
2	2-Butenal, (Z)	C_4H_6O	70.0898	4.38	1.90
3	1,3-Methanopentalene, octahydro-	C_9H_{14}	122.2075	5.89	2.24
4	Benzene sulphonamide, 5-amino-2-methyl-n-phenyl	$C_{13}H_{14}N_2O_2S$	262.32746	7.19	24.33
5	Tricyclo[3,2,1,0,(2,4)]octane,-8-methylene-, (1a,2a,4a,5a)-	C_9H_{12}	120.1916	7.27	4.68
6	3-Aminoacrylonitrile	$C_3H_4N_2$	60.077	21.29	1.10
7	1R,2c,3t,4t-Tetramethyl-cyclohexane	$C_{10}H_{20}$	140.266	21.51	1.72
8	9-Thiabicyclo[3,3,1]nonan-3-one 9,9-dioxide	$C_8H_{12}O_3S$	174.264	22.50	1.48
9	Cyclobutanone, 2-methyl-2methyl-2-oxiranyl	$C_7H_{10}O_2$	126.06808	22.94	2.94
10	2H-Azepin-2-one,hexahydro-1-(2-propenyl)-	$C_9H_{15}NO$	153.22	23.54	2.13
11	(3R,2E)-2-(Hexadec-15-ynylidene)-3-hydroxy-4-methylenebutanolide	$C_{11}H_{23}NO_9$	313	24.21	1.08

12	Cis-1,3-Cyclohexanedicarbonitrile	$C_8H_{12}O_4$	172.178	24.97	3.48
13	Cyclobutane, 2-methyl-2-oxiranyl	$C_7H_{10}O$	110.157	25.52	1.70
14	3-Hexane, 2-methyl-	C_7H_{14}	98.19	26.76	4.67
15	Cyclododecanepropnenitrile	$C_{15}H_{25}NO$	235.37	27.01	2.58
16	3-Heptayne, 5-methyl-	C_8H_{14}	110.1969	28.85	1.22
17	2,6,10- Cyclotetradectrien-1-one, 3,7,11-trimethyl-14-(-1-methylethyl)-	$C_{20}H_{32}$	272.05	30.61	2.76
18	Cyclobutane,2,2-dimethyl-	$C_6H_{12}O$	100.16	30.94	1.57
19	1H-1, 2,4- Triazole , 3-ethyle-	$C_4H_7N_3$	97.12	34.85	2.11
20	1,3- Cyclopentanedione, 4,-4-dimethyl-	$C_7H_{10}O_2$	126.155	37.40	1.04
21	1,- Cyclopentadione, 4,4-dimethyl-	$C_7H_{10}O_2$	126.155	37.75	3.30
22	2-Butene, (Z)	C_4H_8	56.106	38.12	1.39
23	1H-Imidazole, 1-(2-propenyl)-	$C_6H_8N_2$	108.14	38.29	1.13
24	6-Methoxy-2,6-dihydropyran-3-one	$C_6H_8O_3$	128.13	38.56	1.64
25	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	98.0999	38.83	2.50

3) Evaluate Antimicrobial activity:

The extracted oil of the leaves of *Mentha spicata* was screened for antibacterial and antifungal activities.

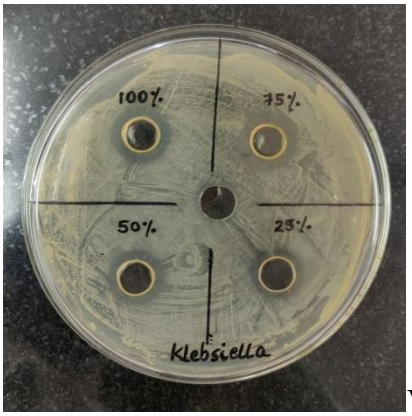
- **Antibacterial study:**

In table-3 and antibacterial activities of extracted essential oil against tested microorganism are shown. Extracted oil of *Mentha spicata* leaves showed antibacterial activity against all tested microorganisms. Among all the bacteria *Listeria sp.* showed the highest activity against extracted essential oil. Here, *Mentha spicata* essential oil of leaves is treated by three bacterial species. These are

Klebsiella sp. E.Coli Listeria sp.

Listeria sp. showed highest activity against extracted oil as well as *Klebsiella sp.* showed lower anti activity.

S.No	**Bacteria**	**Chloroamphenicol**	**Zone of Inhibition**			
			100% oil conc. (mm)	**75% oil conc. (mm)**	**50% oil conc. (mm)**	**25% oil conc. (mm)**
1	*Klebsiella sp.*	28	18	17	16	14
2	*E.Coli*	30	19	17	16	15
3	*Listeria sp.*	32	19	17	16	15



V

Figure9: *Plate I Klebsiella sp.*

DMSO

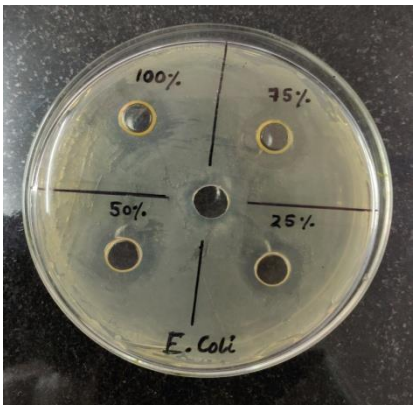


Figure10*: Plate II E.Coli*

DMSO

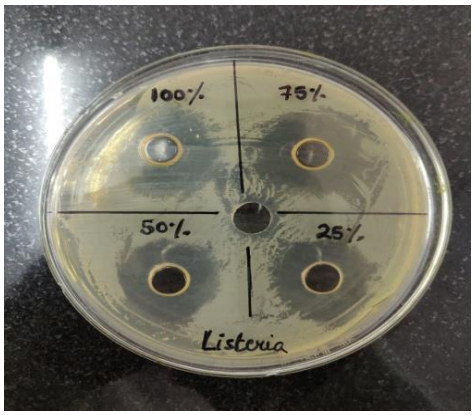


Figure11: *Plate 3 Listeria sp.*

DMSO

- **Antifungal study:**

Antifungal activities of different fungi test against extracted oil of the leaves of *Mentha spicata* are given in table - 4. Among all fungi *Rhizopus oryzae* showed higher activity against extracted oil of the leaves of *Mentha spicata*. After that *Penicillium chrysogenum* acetone also showed good activity. *Alternaria alternata*, *Aspergillus fumigatus* showed almost similar activity

against the extracted oil of leaves of *Mentha spicata*. Here, extracted oil of leaves of *Mentha spicata* is treated against four fungal activities. These are:

Penicillium chrysogenum

Alternaria alternata

Rhizopus oryzae

Aspergillus fumigatus

S.No	**Fungi name**	**Chloroamphenicol**	**100% oil conc. (mm)**
1	*Penicillium chrysogenum*	41	17
2	*Alternaria alternata*	30	16
3	*Rhizopus oryzae*	39	13
4	*Aspergillus fumigatus*	40	14

Table-32: *Antifungal activity against extracted oil*

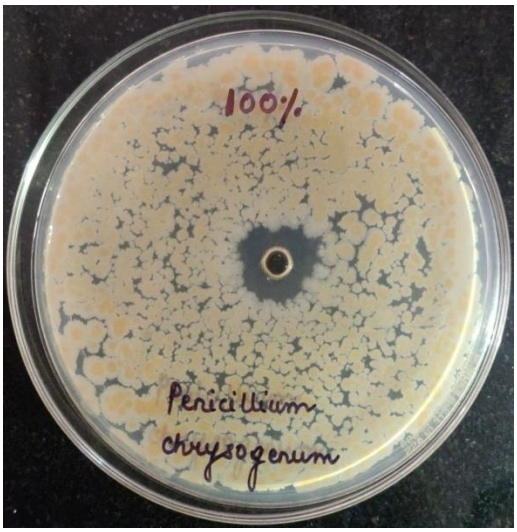


Figure12: *Plate I Penicillium chrysogenum*

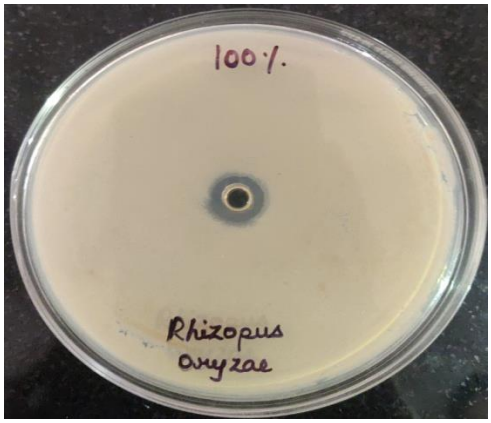


Figure13: *Plate II Rhizopus oryzae*

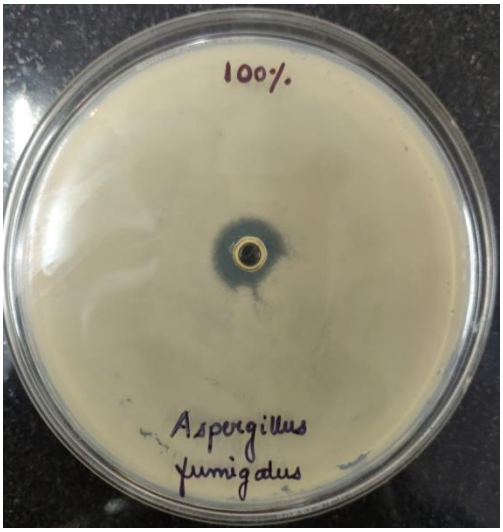


Figure14: *Aspergillus fumigatus*

Figure15: *Alternaria alternata*

CONCLUSION

- We extract essential oil of the leaves of *Mentha spicata* and continue with phytochemical investigation that is pre-treatment with the sample, extraction (hydro distillation and steam distillation).
- The qualitative physiochemical analysis has to be done to know the value of the oil such as Acid value, Saponification value, pH value, Iodine value.
- Further the detailed study of the bioactive compounds present in the oil is done through GC-MS.
- The Antimicrobial study was performed for the oil against bacteria and fungi (E.Coli, Penicillium chrysogenum and so on).
- Hence, from here we conclude that the extracted oil of *Mentha spicata* is very rich in different physiochemical properties having numerous chemical constituents due to which the oil shows great activity against different microbes that have good medicinal effect in treatment of different diseases.

REFRENCES:

[1] Farooq Anwar1 | Ali Abbas1,2 | Tahir Mehmood1,3 | Anwarul - Hassan Gilani4 |Najeeb - ur Rehman5, *Mentha*: A genus rich in vital nutra - pharmaceuticals—A review 10.1002/ptr.6423.

[2] Naoual El Menyiy,[1]Hanae Naceiri Mrabti,[2]Nasreddine El Omari,[3]Afaf EI Bakili,[4]Saad Bakrim,[5]Mouna Mekkaoui,[6]Abdelaali Balahbib,[7]Ehsan Amiri-Ardekani,[8]Riaz Ullah,[9]Ali S. Alqahtani,[9]Abdelaaty A. Shahat,[9]and Abdelhakim Bouyahya[10], Medicinal Uses Phytochemistry, Pharmacology, and Toxicology of *Mentha spicata* https://doi.org/10.1155/2022/7990508.

[3] Iram Saba & Farooq Anwar, Effect of Harvesting Regions on Physico-chemical and Biological Attributes of Supercritical Fluid-Extracted Spearmint (*Mentha spicata* L.) Leaves Essential Oil https://doi.org/10.1080/0972060X.2018.1458658.

[4] M. Consuelo Díaz-Maroto, M. Soledad Pérez-Coello, M. A. González Viñas and M. Dolores Cabezudo, Influence of Drying on the Flavor Quality of Spearmint (*Mentha spicata* L.) https://doi.org/10.1021/jf020805l.

[5] Priscilla P. Almeida & Natália Mezzomo & Sandra R. S. Ferreira, Extraction of *Mentha spicata L*. Volatile Compounds: Evaluation of Process Parameters and Extract Composition DOI 10.1007/s11947-010-0356-y.

[6] Nilufar Z. Mamadalieva1,2 Hidayat Hussain2 Jianbo Xiao3 ,Recent advances in genus *Mentha*: Phytochemistry, antimicrobial effects, and food applications, DOI: 10.1002/fft2.53

[7] Mihaela Buleandraa, Eliza Opreab, Dana Elena Popaa,*, Iulia Gabriela Davida, Zenovia Moldovana, Iuliana Mihaia and Irinel Adriana Badeaa, Comparative Chemical Analysis of *Mentha piperita* and *M. spicata*and a Fast Assessment of Commercial Peppermint Teas https://doi.org/10.1177/1934578X1601100433

[8] R. Paul Choudhury, A. Kumar, A.N. Garg ∗ Analysis of Indian mint (*Mentha spicata*) for essential, trace and toxic elements and its antioxidant behaviour, https://doi.org/10.1016/j.jpba.2006.01.048

[9] Ganesan Mahendran, Sanjeet Kumar Verma, Laiq-Ur Rahman, The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L.): A review, https://doi.org/10.1016/j.jep.2021.114266

https://www.first-nature.com/flowers/mentha-spicata.php

https://plantmaster.com/plants/eplant.php?plantnum=6701

https://www.gardenersworld.com/plants/mentha-spicata/

https://thewholesaler.in/products/spearmint-mentha-spicata

https://www.minnesotawildflowers.info/flower/spearmint

https://www.minnesotawildflowers.info/flower/spearmint

https://commons.wikimedia.org/wiki/File:Mentha_spicata_L..jpg

https://www.first-nature.com/flowers/mentha-spicata.php