ORIGINAL ARTICLE

Secondary Metabolism Compounds Study of Essential Oils for the *Mentha spicata* L. and *Ocimum basilicum* L.

Raghad Khalil Alarkwazi[1] , Gasaq Reaihd Abdulreda[1] , Ali Salam Ali[1,*]

[1] **College of Pharmacy, University of Al-Kafeel, Najaf, Iraq.**

Corresponding author: **College of Pharmacy, University of Al-Kafeel, Najaf, Iraq, E-mail: qzf2020ali@gmail.com,**

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Abstract

Background: The mint and basil plants (M. spicata L. and O. basilicum L.) of the labiate family were used in the study.

Methods: A qualitative chemical detection regarding several active chemicals in the leaves of M. spicata L. and O. basilicum L. was part of the investigation.

Results: The findings revealed that both species include flavonoids and glycosides, yet tannins and saponins were not found in the leaves extract of O. basilicum, and volatile oils were found in the alcoholic extract at a rate of 4.5% in the leaves of O. basilicum. Fourier transform infrared spectrometry (FT-IR) was used to detect six chemical active groups include (O-H, C=H, C=C, C=N, C-O, C-N). Our findings revealed the presence of alkaloid; the extraction yield for volatile oils in M. spicata species is 3%. As for the presence of nutrients in the type, the highest percentage of Calcium is in species *O. basilicum*.

Conclusions: The presence of effective chemical compounds in *O. basilicum* and *M. spicata* leaves indicates its importance as a source of useful drugs and the importance of chemical components in the pharmaceutical industries, as well as enhancing the importance of its use in folk medicine as a safe treatment for many diseases.

KEYWORDS

Ocimum basilicum L., *Mentha spicata* L., Volatile oil, FT-IR

INTRODUCION

Medicinal plants are those plants that are used in the treatment of diseases and pain, because they contain effective substances and a physiological effect known as active constituents, including plants that contain volatile oils such as peppermint, anise, cumin and lemon oil, which are added to medicines to improve their taste and flavor. The volatile oils are called oils volatile, aromatic oils for their distinctive aromatic smell, or oils ethereal oils because of their ease of dissolving with ether. It is used as raw materials in many industries of scientific, medical and economic importance (1). *Ocimum basilicum* L., is one of the aromatic medicinal plants belonging to the family Lamiaceae (Labiat), also it is a small annual shrub plant that is grown in gardens as an ornamental plant. It has a fragrant aroma and a pungent taste. The plant is covered with a soft fluff and has simple ovate neck-tufted leaves. The flowers are large, somewhat lateral to symmetry, and clustered in inflorescences. White or slightly reddish, the height of the plant reaches between 25-40 cm or more. As for the spread of the plant, its original home is India and the Middle East, and its cultivation has been known in the hot regions of Africa and Asia for many centuries and spread in America and European countries (2). The properties regarding such genus' species include flower color, leaf size, flavor, and phenotypic properties. O. viride shrub, O. basilicum, O. americanus, O. gratissimum, and O. tenuiflorum are among the species. It is one of the significant sources regarding essential oil used in perfumes, food, and cosmetics, and a few Ocimum types are utilized as a folk remedy in different cases, particularly in African and Asian nations (3). In chemical composition and pharmacological effect, it is a beautiful mellow plant with beautiful leaves and flowers of different colors, including white and bright violet., has been used as a spice for centuries, and Ibn Sina said about it (it is useful from hemorrhoids as a coating after it is crushed fresh, or its fat is taken and becomes an ointment, as it is useful for accidental inflating of the stomach), and it was known in Europe and called it the royal herb and was used at that time as a medicine with many advantages, *O. basilicum* is used as a medicinal plant to treat headaches, coughs, worms, warts, fever reliever, anti-malarial, bronchitis, and as an ointment for insect and snake bites (4).

The leaves are used to strengthen the hair and prevent its loss. The leaves and flowers drenched are used as a gas repellent and a remover for intestinal colic. It is also a diuretic. Dysentery is treated with boiled seeds in water, and chronic diarrhea is treated with it in India. The plant is also used in the preparation of perfumes and toothpastes and is used to relieve joint pain. It is used to cleanse the intestines and against abdominal cramps, nausea, dysentery, depression and insomnia. As for its effect against microorganisms, it was found to be a killer and an inhibitor of many types of bacteria, fungi, viruses and yeasts (5).

Mentha spicata L. is considered one of the main sources of medicinal plants in Iraq and the Arab world, is grown in all types of lands and it is very resistant to salinity and alkalinity. The essential oil of peppermint leaves contains a Menthol compound at a concentration of 01-41%, as well as a peppermint compound. Menthone by 41-31% and contains 4-43% tanning materials, 8% esters and flavonoids, as well as aromatic acids. As for green mint leaves, they contain 45-65% Carvone (6). Mentha leaves have historically been utilized with a variety of spices, both fresh and dried. Traditional medicines make use of biologically active components found in Mentha species. Mint species might also be applied to treat major disorders like coughs, colds, fever, sinusitis, nausea, and bronchitis in traditional medicine. Antimicrobial, insecticidal, antioxidant, antispasmodic, and antifungal properties have also been documented for the mint plant (6).

Limonene, carvone, 1,8 cineol, cis-carveol, and cis-sabinene hydrate are the primary constituents of M. spicata L.'s volatile oil, with carvone being the most prominent. In literature, the antibacterial and antifungal properties regarding volatile oil components were specified. The plant produces less volatile oil molecules, yet they serve as defensive mechanisms against predators like insects and pathogens (7). The goal of this work was to figure out what chemicals were in the volatile oils of Mentha spicata L. and Ocimum basilicum L.

O. basilicum are classified as follows

Scientific name	***Ocimum basilicum* L.**
Kingdom	Plantae
Divition	Tracheobionta
Order	Lamiales
Genus	Ocimum
Class	Magnoliopsidae
Family	Lamiaceae
Sub class	Magnoliopsidae
Species	*Basilicum*

Table (1) Classification of *O. basilicum*.

Fig (1): *O. basilicum*.

Scientific name	***Mentha spicata L.***
Kingdom	***Plantae***
Divition	***Tracheobionta***
Family	***Lamiaceae***
Species	***Spicata***
Order	***Lamiales***
Class	***Magnoliopsidae***
Genus	***Mentha***
Sub class	***Magnoliopsidae***

Table (2): Classification of *M. spicata*

Fig (2) *M. spicata*

FT-IR analysis:

The emission spectrum or infrared absorption of a liquid, solid, or gas sample is obtained using FTIR.

An FTIR spectrometer, on the other hand, acquires data with great spectral resolution over a wide spectrum range [Ramana, 2014].

"FTIR screening is primarily an experimental analysis method utilized to separate organic and a few inorganic substances using infrared radiation (IR)," according to (8). The FTIR device emits infrared radiation between 10,000 and 100 cm -1 over a sample, absorb some and pass-through others. The absorbed radiation's conversion into vibrational energy produces vibrational energy (9) The primary purpose of IR spectroscopy is to identify the chemical functional groups in the sample. For compound identification and structural elucidation, IR spectroscopy is a useful and widely used method (10).

Vibrational spectroscopy can be defined as a typical technique in analytical chemistry and pharmacy that produces images of the vibrations regarding atoms in a compound. It depends on the nature of IR radiation's interaction with molecular vibrational modes. Alterations in vibrational energy are accompanied by changes in rotational energy in IR spectra (11).

MATERIALS & METHODS

1- Plant collection

In the year 2022, throughout the flowering stage, M. spicata and O. basilicum were collected from Karbala city. The plants have been air dried and powdered at a temperature of 45°C in the oven (1 and 2).

2- Soxhlet Extraction

50 gram of fin powder has been placed in a thimble as well as extracted for a period of 24 hrs with 150 ml of (70%) ether in a flask round volume (500 ml). Extract was evaporated at a temperature of 45 Celsius with the use of a rotary evaporation equipment (1 and 2) The percentage is calculated with the use of law shown in equation No (1) (3 and 4)

$$\text{Yield (wt.\%)} = \frac{\text{Weight of Oil produced}}{\text{Weight of Seed powder used}} \chi 100\%$$

3- Phytochemical study:

3-1: Saponins

The appearance of foam after stirring the plant's aqueous solution in a test tube for a long time indicated the presence of saponins (12).

3-2: Test Tannins

Lead acetate test: Ten milligrams of extract were mixed with 0.5 milliliters of 1% lead acetate solution, and the precipitate's formation indicated the presence of tannins.

3-3: Test for Glycosides

One milliliter regarding each part's extract was combined with five milliliters of Benedict reagent. The appearance of red sediment is indicating the presence of reducing sugar (13).

3-4: Alkaloids:

Wagner's Test:

Wagner reagent (which is a mixture of iodine solution together with potassium iodide) was added to the collected filtrate. The presence of alkaloids compounds in the sample is indicated by the formation of a reddish-brown precipitate.

3-5: Flavonoids:

To 4mL of each sample extract, 1.5mL of 50% methanol was added. After mixing, it was warmed with magnesium metal and acidified with 5-6 drops of conc. HCl until red color is formed. Red color means the presence of flavonoids (14).

4- Diagnosis using FT-IR infrared spectrum:

The identity regarding different phyto-chemical constituents involved in the stabilization and reduction of NPs can be determined using FT-IR spectroscopy. The FT-IR spectra for powdered and dried ZnO NPs has been acquired with the use of Attenuated Total Reflectance (ATR) method on a Perkin Elmer FT-IR Spectrophotometer Frontier between 4000 and 500 cm-1 (15).

Procedure

2 mg was taken from sample and mixed with 98 mg of KBr which had been dried for 24 hours at temperatures of 105 C°, isolates were analyzed at wave number 4000 cm^{-1} to 400 cm^{-1}. Baseline used was KBr (16).

5- Estimation of the proportions of some mineral nutrients: The proportions of some mineral element's Calcium, iron, Nitrogen by Atomic absorption spectro photo metric - atomic absorption spectrometer 5000 (17)

RESULTS

1. General chemical detection

It was found using a number of chemical detections for the extract of the leaves that it contains a number of basic components, The results in Table (3) indicate that the *O. basilicum* plant contains alkaloids in the leaves. As for the presence of glycosides they appeared in the alcoholic extract, but the saponins and tannins did not appear in the leaves extract, as for the flavones, they are widely present, and for the volatile oils, they were present in the alcoholic extract at a rate of 4.5% in the leaves as well Table (3). Our findings were consistent with (19-22) and none of the essential oils tested revealed the presence of alkaloid; the extraction yield for volatile oils in M. spicata species is 3% Table (4).

Compounds	*O. basilicum*	*M. spicata*
Tannins	-	+
Saponin	-	+
Alkaloid	+	-
Glycoside	+	+
Flavonoid	+	+

Table (3) Phytochemical of O. basilicum and M. spicata extract

extracted	**Percentage of oils extracted**
M. spicata	3/100 *100 = 3%
O. basilicum	4.5/100*100= 4. 5%

Table (4) Percentage of voltile oils extracted using suxhlet method

2. FTIR-Spectroscopic Analysis:

The existence of various functional groups regarding bioactive compounds in the aerial sections of M. spicata and Flavonoid extracts was discovered by FTIR spectroscopic analysis.

2-1: FTIR Spectrum of Air Parts Extract of *M. spicata*

The results of the FTIR spectrum of aerial parts extract of *M. spicata* with a peak at corresponded.

1- The peaks at 3466.32 and 3007.95 cm^{-1} confirmed the existence of alcohols, phenol O-H bonding and hydroxyl.

2- The peaks at 2923.03 and 2855.60 cm^{-1} which is allocated to C=H cm^{-1} stretching that a few alkene compounds, wax, fatty acid, carotenoid and phytosteroil are present.

3- The peak 1744.15 cm^{-1} which is allocated to the C=C stretching confirmed the existence of glycoside.

4- The peak at 1655 cm^{-1} which is allocated to the C-O cm^{-1} stretching confirmed the existence of Flavonoid, polyphenol and catechins.

5- The peak at 1456.47 cm^{-1} which is allocated to the C-C cm^{-1} stretching confirmed the existence of aromatics and flavonoid.

6- The peaks at 1368.55 cm^{-1} which is allocated to the C-N cm^{-1} stretching confirmed the existence of aromatic amines.

7- The peak at 1234.65 cm^{-1} which is allocated to C-N stretching confirms aliphatic amines.

8- The peaks at 1158.83 and 1101.87 cm^{-1} which is allocated to the C-O stretching confirmed the existence of alcohols, ester and carboxylic acids.

9- The peaks at 91368 and 720.29 cm^{-1} which is allocated to the C-N stretching confirmed the existence of aliphatic amins and secondary alcohols, figure (3),(1,10).

3. The existence of glycoside is confirmed by the peak at 1743.38.1 cm^{-1} which is allocated to C-C stretching.

4. The existence of flavonoid is confirmed by the peak at 1654.41 cm^{-1} which is allocated to C=O stretching.

5. The existence of aromatics and flavonoid is confirmed by the peak at 1456.37 cm^{-1} which is allocated to C-C stretching.

6. The aromatic amines are confirmed by the peak at 1368.62 cm^{-1} which are allocated to C=N stretching.

7. Alcohol, ester, and carboxylic acids are confirmed by the peak at 1233.97 cm^{-1} which is allocated to C-N stretching.

8. The existence of esters, alcohol, and carboxylic acids is indicated by the peaks at 1101.84 cm^{-1} and 1158.83 cm^{-1} which are allocated to C-O stretching.

9. The existence regarding alphatic and secondary alcohols is indicated by the peak at 720.15 cm^{-1} which is allocated to C=N stretching as shown in figure (4). (17).

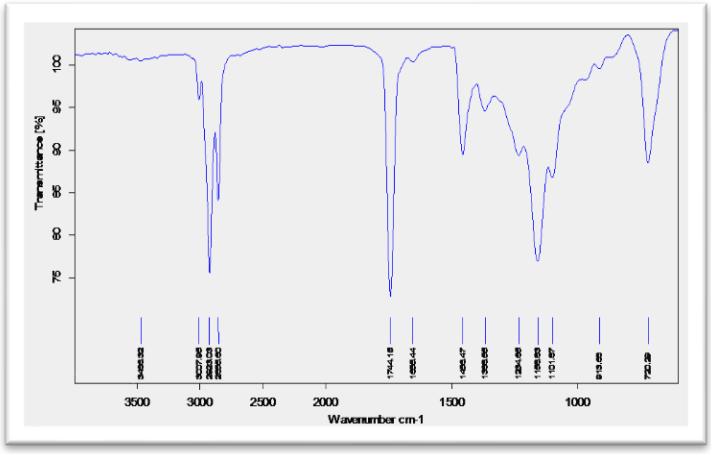

Figure (3): FTIR for M. spicata

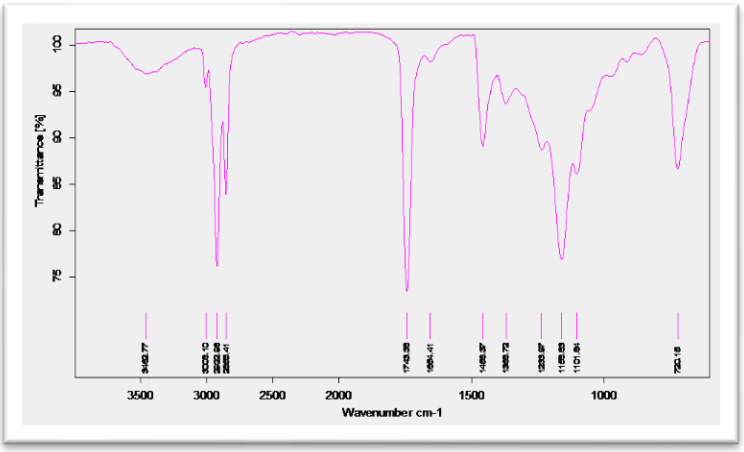

Figure (4): FTIR for *O. basilicum*

2-2: FTIR Spectrum of *O. basilicum*

The results of the FTIR spectrum of *O. basilicum*

1. The peaks at 3462.77 and 3008.10 cm^{-1} confirmed the existence of the phenols, alcohols hydroxyl and O-H bonding.

2. The peaks at 2922.96 and 2855.41 cm^{-1} and 2071.62 cm^{-1} which is allocated to C-H stretching, indicating that a few alkene compounds, fatty acid, carotenoid and phytosteroil are present.

nutrients elements	*O. basilicum*	*M. spicata*
Calcium	3.01	2.1
Iron	2.61	2.78
Nitrogen	1.98	2.34

Table (5) The proportions of some nutrient's elements

3. Estimation of nutritional ingredients

Table (2) shows the percentages of some nutrients for dry leaf powder of the two plants represented with Calcium, Iron, Nitrogen, magnesium.

DISCUSSION

This result is consistent with what was mentioned by some researchers, who confirmed that the leaves of the *O. basilicum* plant contain the above compounds in varying proportions (18).

Mint essential oils comprise the majority of phytoconstituents, such as saponins, flavonoids, reducing sugars, cardiac glycosides, steroids, and Tanin, according to phytochemical research Table (3).

The percentage of Calcium was 3.01% in species *O. basilicum* Calcium is one of the most important macro elements in plants and humans need it in building and developing bones, and it is an essential element in the safety of nerves and muscle tissue (9), While high concentration of *M. spicata* for element Nitrogen is 2.34% Table (5).

CONCLUSIONS

The presence of effective chemical compounds in *O. basilicum* and *M. spicata* leaves indicates its importance as a source of useful drugs and the importance of chemical components in the pharmaceutical industries, as well as enhancing the importance of its use in folk medicine as a safe treatment for many diseases.

Conflict of Interest: None

Ethical consideration: from ethical committee in the University of Al-Kafeel, Najaf, Iraq

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