



## Phytochemical and GCMS analysis on the ethanol extract of *Foeniculum Vulgare* and *Petroselinum crispum* leaves

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Received: 8 April 2021; Revised: 26 October 2021; Accepted: 27 October 2021

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**Citation:** Mohammed, Abubakar, J.; Iruoghene, Edo, G.; Paşaoğluları, Aydınlik, N. *Int. J. Chem. Technol.* 2021, 5 (2), 107-124.

### ABSTRACT

*Petroselinum crispum* (Parsley) and *Foeniculum vulgare* (Fennel) are aromatic herbs belonging to Apiaceae and Lamiaceae family. Phytochemical, GC-MS and FTIR properties of ethanolic extract of *Foeniculum vulgare* and *Petroselinum crispum* leaves investigated. Plant leaves were extracted based on separation using ethanol and subjected to phytochemical testing that revealed the presence of biologically active substances including terpenoids, steroids, flavonoids, alkaloids, tannins and cardiac glycosides. GC-MS evaluation of *Foeniculum vulgare* revealed two bioactive compounds (1,4 Cyclohexadiene and Metronidazole) and *Petroselinum crispum* revealed six bioactive compounds (Cineole, I-Limonene, Cyclohexane, Phenol, Neophytadiene and 9,12,15 octadecatrienoic). FTIR analysis of parsley displayed strong bands at 2915.50 cm<sup>-1</sup> which corresponds to C–H stretching and medium band at 1476.80 cm<sup>-1</sup> which corresponds to N-H stretching vibrations due to the presence of amino acids. Fennel displayed strong bands at 2832.61 cm<sup>-1</sup> which is equivalent to C–H showing saturated and unsaturated compounds and medium band at 1029.98 cm<sup>-1</sup> corresponds to C–O present in esters. Antibacterial activity of these plants confirmed their effectiveness in the traditional medicine.

**Keywords:** *Petroselinum crispum*, *foeniculum vulgare*, FTIR, medicinal plant, phytochemical, GC-MS.

### *Foeniculum vulgare* ve *Petroselinum crispum* yapraklarının etanol özündeki fitokimyasal ve GCMS analizi

#### ÖZ

*Petroselinum crispum* (Maydanoz) ve *Foeniculum vulgare* (Rezene) türleri sırasıyla Apiaceae ve Lamiaceae familyasına ait aromatik bitkilerdir. Bu çalışmada *Foeniculum vulgare* ve *Petroselinum crispum* yapraklarının etanolik ekstraktının fitokimyasal, GC-MS ve FTIR özelliklerinin araştırılması amaçlanmıştır. Her iki bitkinin yaprakları etanol kullanılarak ekstrakte edilip ayrıldı. Ayrılan bu ekstraktlar terpenoidler, steroidler, flavonoidler, alkaloidler, tanenler ve kardiyak glikozitler dahil biyolojik olarak aktif maddelerin varlığını ortaya çıkaran fitokimyasal testlere tabi tutuldu. GC-MS ölçümünde *Foeniculum vulgare*'nin değerlendirmesinde iki biyoaktif bileşik (1,4 Cyclohexadiene ve Metronidazole) ve *Petroselinum crispum* altı biyoaktif bileşik (Cineole, I-Limonene, Cyclohexane, Phenol, Neophytadiene ve 9,12,15 octadecatrienoic) ortaya çıkmıştır. Maydanoz'un FTIR analizinde, C–H gerilmesine karşılık gelen 2915.50 cm<sup>-1</sup>'de güçlü bantlar ve amino asitlerin varlığından dolayı N-H gerilme titreşimlerine karşılık gelen 1476.80 cm<sup>-1</sup>'de orta bantlar saptanmıştır. Rezenede sırasıyla doymuş ve doymamış bileşikler gösteren C–H'ye eşdeğer olan 2832.61 cm<sup>-1</sup>'de güçlü bantlar saptanmış ve 1029.98 cm<sup>-1</sup>'deki orta bant esterlerde bulunan C–O'ya karşılık gelmiştir. Bu bitkilerin antibakteriyel aktivitesi, geleneksel tıptaki etkinliklerini doğrulamıştır.

**Anahtar Kelimeler:** *Petroselinum crispum*, *foeniculum vulgare*, FTIR, tıbbi bitki, fitokimyasal, GC-MS.

### 1. INTRODUCTION

Plants and their derivatives have always been an important source of medication for our ailing conditions for centuries in the history of mankind.<sup>1</sup> The first understanding and the discovery of different healing

effects of plants was from ancient times. Over time, humans became interested in knowing the exact origins and what was responsible for most of the healing properties of the components of the plant.<sup>2</sup> Plants are the primary basis of pharmaceutical drugs, with a broad range of biological actions including antimicrobial,

antioxidants and anti-fungal properties.<sup>3,4</sup> Although, several microorganisms have formed resistance against antibiotics, leading to healing deficiency.<sup>5,6</sup> Current antibiotic treatments are also very costly. Plants can produce a large number of diverse bioactive compounds.<sup>7</sup> High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables. Plants containing valuable phytochemicals can complement the needs of the human body by acting as natural antioxidants.<sup>8</sup> Several studies have shown that many plants are rich source of antioxidants. This resulted in an increase in the use of plant extracts and their derivatives.<sup>9</sup> In early research, some botanical products have demonstrated the curative ability of severe illnesses including cancer, blood glucose, and inflammation.<sup>10,11</sup> These reports reveal that the plants however constitute significantly for the detection of innovative drugs and medicinal substances. *Foeniculum vulgare* is commonly referred to as fennel plant belonging to the *Lamiaceae* family originally cultivated mainly in the Mediterranean region, although it is currently being adapted and planted in most part of the world. Research has indicated that the fennel plant particularly its leaves contains various biologically active and phytochemical constituents.<sup>12</sup> In highly European countries, fennel is traditional utilized as a therapeutic herb with a recipe in local dishes.<sup>13</sup> In Cyprus, the leaves and flowers are also being used as dyes called Turkish brown or yellow dye.<sup>14</sup> It is an indigenous plant of Mediterranean region in southern Europe to be precise, but then owing to its therapeutic benefit and huge bioactive constituent, fennel become adopted virtually everywhere on the world right now.<sup>12</sup> Its fresh or dried leaves, roots, seed and fruit are used in cosmetic products, pharmaceutical and food industries.

*Petroselinum crispum* (Parsley) exists as a herb from the *Apiaceae* family, which is being used in food, medicinal products, cosmetic and perfume industry.<sup>15</sup> *Petroselinum crispum* may prove to be one of the world's most ancient medicinal plants used as a condiment in food.<sup>16</sup> Earlier research on the biochemical makeup of *Petroselinum crispum* have showed the existence of flavonoid compounds, terpenoids and 2H-chromen-2-one.<sup>17</sup> It is used for treating different illnesses like strokes, clotting, alzheimer and cardiovascular diseases. In traditional medicine, *Petroselinum crispum* is used as a treatment for hemorrhoids, the roots for treating urethral infection, kidney stones and enhancing brain operation and memory.<sup>18</sup> Furthermore, Parsley is being utilized as a hypoglycemic, abortifacient, hypolipidemic, carminative, anticoagulant, emmenagogic agent and antimicrobial agent.<sup>19</sup>

This research was aimed to investigate the phytochemical, GC-MS and FTIR properties of ethanolic extract of *Foeniculum vulgare* and *Petroselinum crispum* leaves.

## 2. MATERIALS AND METHODS

### 2.1. Extract preparation

*Foeniculum vulgare* and *Petroselinum crispum* leaves were acquired from a vegetable garden in Iefkosa, Cyprus. Both plant leaves were identified by a botanist from the Cyprus ministry of agriculture and natural resources. The collected leaves from both plant material was left dry at room temperature and later grounded in an electric power grinder. Consequently, 45 g of both plant leaves powder were extracted using 200 ml of ethanol in a Soxhlet system and both extracts were filtered using a whatman filter paper. The amount of extracts obtained was weighed and the residue were kept in dark to be used further during the experiment.<sup>20</sup> The residue was kept in the dark to prevent changes in the nature of the plant's constituents.

### 2.2. Chemicals and materials

The chemicals used were all analytical grade reagents. The chemicals used were purchased from the sigma-Aldrich chemical company (St. Louis, MO, USA). The Milli-Q purification system (Millipore, Bedford, MA, USA) was used for the refinement of water used in the research analysis.

### 2.3. Phytochemical screening

The qualitative phytochemical screening of the ethanolic extract of both plant leaves were subjected for the discovery of various phytochemicals produce in the ethanol extracts by using standard method proposed by Shahmokhtar and Farzaei.<sup>21,22</sup> The ethanol extract was evaluated for the presence and absence of Flavonoids, Tannins, Saponins, Steroids, Terpenoids, Cardiac glycosides and Alkaloids.

### 2.4. Saponins

5ml of distilled water (5ml) was mixed with 2 g of extract and shaken vigorously in a test tube for 45 s. The test tube was let to stand for 30 minutes in a vertical position. The honeycomb froth that persists for 15-20 minutes demonstrates the presence of saponins.

### 2.5. Alkaloids

2ml of 1% HCL was added to 1 g of extract and then moderately heated, then the reagents (Mayer and Wagner) were added at the same time to the mixture. Darkening of the resulting precipitate was regarded as the evidence for the presence of alkaloids.

### 2.6. Tannins

The ethanol extract (1g) was mixed with distilled water (15ml) inside a test tube and heated simultaneously with

the addition of ferric chloride solution (in drops) showing the presence of tannins when a brownish green color is observed.

### 2.7. Flavonoids

The ethanol extract (1g) was mixed with sodium hydroxide (4ml) and H<sub>2</sub>SO<sub>4</sub> (2 drops) showing the presence of flavonoids when a yellow coloration is formed.

### 2.8. Glycosides

Acetic acid (4ml) and chloroform (2ml) were mixed with ethanol extract (1g) forming a solution which was cooled and the addition of H<sub>2</sub>SO<sub>4</sub> showing the presence of glycosides when a green coloration is formed.

### 2.9. Steroids

Chloroform (2ml) was mixed with ethanol extract (1g) and then H<sub>2</sub>SO<sub>4</sub> (4ml) showing the presence of steroids when a reddish coloration is formed.

### 2.10. Terpenoids

Chloroform (4ml) and acetic anhydride (1ml) were mixed with ethanol extract (1g) and the addition of H<sub>2</sub>SO<sub>4</sub> (2ml) showing the presence of terpenoids when a reddish violet coloration is formed.

### 2.11. GC-MS analysis

GC has the ability to detect and resolve complex mixtures extracts containing many different compounds. Immediately the components exit the GC column, they are then ionized and separated as fragment by the mass spectrometer (MS) using chemical ionization sources. The *Foeniculum vulgare* and *Petroselinum crispum* leaves ethanol extracts were analyzed using a GC-MS system (GC-MSQP2010 SE plus Shimadzu Technology Japan) equipped with an HP-5MS capillary column (30m x 0.25 mm) to determine the active compound. The injection volume of each sample was 1 µL. And Helium was used as a carrier gas with flow rate of 1 mL/min, the injection port temperature was 250°C and the program of the sample was set to a temperature ranges from 50°C to 300°C at a rate of 50°C/min and 10min hold at 300°C for non-volatile constituents. The GC-MS analysis was carried out in Chemistry Laboratory of Cyprus International University.

### 2.12. Identification of Compounds

The Identification details of the separation between the volatile compounds was carried out via retention indices and mass spectrometry through a comparison using database of National Institute Standard and Technology (NIST), library 2008.

### 2.12. FTIR analysis

The Fourier-transform infrared spectroscopy (FTIR) method and technique was used for the determination and identification of various types of functional groups in each powdered leaf ethanol extract of the two plant used in the analysis as described by Hussein.<sup>4</sup> The absorbed light wavelength is a function of the chemical bond. By reading the infrared absorption spectrum, the chemical bonds within a molecule are determined. The infrared spectroscopy spectrum (IR) was obtained using the Fourier-transform infrared spectroscopy (Shimadzu Japan). The ethanol extract powdered (10mg) of both plant leaves was toiled in an agate mortar, encapsulated in 100mg of KBr pellet, to prepare translucent sample disc and pestle in order to obtain a fine powdered sample and the obtained fine powdered was subsequently used for the FTIR analysis.

## 3. RESULTS AND DISCUSSION

Qualitative analysis of some secondary metabolites was studied in *Foeniculum vulgare* and *Petroselinum crispum* leaves ethanol extract. Generally, the medicinal properties of pharmaceutical plants may be ascribed to the existence of a variety of phytochemicals such as Steroids, Tannins, Terpenoids etc. The various biological activities of all known phytochemicals including antioxidants, antifungals and antibacterial activity are recognized. The results showed that both plant extracts contain flavonoids, tannins, saponins, steroids, terpenoids, alkaloids and glycosides. Likewise, terpenoids was not present in *Petroselinum crispum* leaves ethanol extract. These phytochemicals indicated to offer exceptional pharmaceutical activities in both the conventional and traditional medicine. The unique healing and medicinal effects of plants is also dependent on the presence of their secondary metabolites. *Foeniculum vulgare* and *Petroselinum crispum* ethanol leaves extract, in accordance with the previous phytochemical study, have the same secondary metabolites such as flavonoids, tannins, saponins, steroids, terpenoids and glycosides.<sup>12,22</sup> The presence of organic nitrogen compounds with antibacterial properties is alkaloids. The core group of phenolic compounds acting as anti-inflammatory, antimutagenic, antioxidants and anticarcinogenic properties are flavonoids and tannins. hepatoprotective, antipyresis, antidiabetic, pain alleviation and relaxing therapies are found in terpenoids. Both plants have often been used as nausea, laxative and antitumor treatments for fever medications.<sup>23,24</sup> Analysis of phytochemistry of both leaves showed that both plants contains rich bioactive compounds as shown in Table 1 and Table 2. However, the leaves and stems are currently used in a limited number of current uses in conventional medicine, the lack of science reports on the leaf prompted us to carry out a methodical phytochemical analysis of the plant. Potential future research would certainly allow the positive properties to be highlighted, which may open

new pathways so that they can make effective use of the plant as a rich source of bioactive compounds in the pharmaceutical industry.

**Table 1.** phytochemical analysis results of *Foeniculum Vulgare* leaves ethanol extract.

Phytochemicals	<i>Foeniculum Vulgare</i> leaves
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Terpenoids	+
Alkaloids	+
Glycosides	+

+ (present), - (absent)

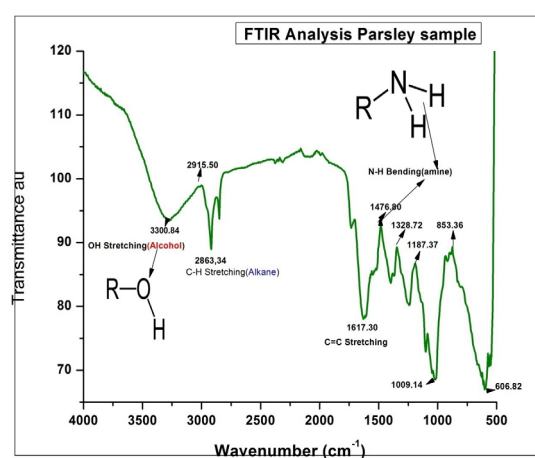
**Table 2.** Phytochemical analysis results of *Petroselinum crispum* leaves ethanol extract.

Phytochemicals	<i>Petroselinum crispum</i> leaves
Flavonoids	+
Tannins	+
Saponins	+
Steroids	+
Terpenoids	-
Alkaloids	+
Glycosides	+

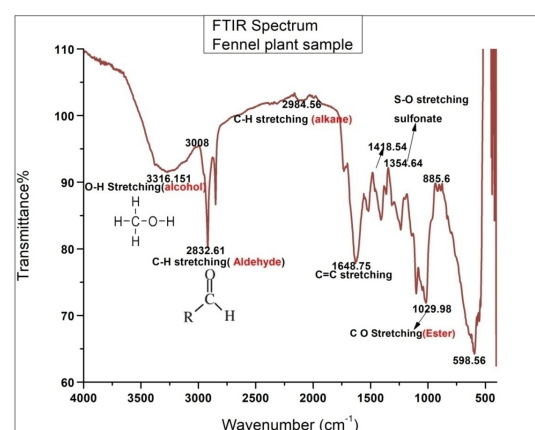
+ (present), - (absent)

Fourier Transform Infrared Spectrophotometer (FTIR) is one of the most important analytical tools for the determination and identifying the types of the functional group presents in a given compound. It is also a rapid technique used to synthesize and characterize organic cell properties and identify their functional group in molecules depending on their vibrating frequency at different wave number. The FTIR spectroscopy is an analytical tool used in identification of several functional groups responsible for medicinal properties in both plants. FTIR spectroscopy was used to determine some qualitative aspects of the organic compounds in *Foeniculum vulgare* and *Petroselinum crispum* leaves ethanol extract. The FT-IR spectrum shows the characteristics of the fingerprint. The infrared spectrum

can recognize and detect certain variations not only of the main inorganic materials. We confirm the presence of many characteristics functional groups as detected at different vibrational frequency band in the IR spectrum as shown in Figure 1 and Figure 2. The various functional groups observed using FTIR spectrum indicates the presence of O-H group (alcohol), carboxylic acid, amine, Sulphur derivatives, amino acid, and nitro - compounds among others as recorded in Table 3 and Table 4. The FTIR spectrum of *Petroselinum crispum* leaves displayed four different bands across the entire range observed. The frequency bands at 2915.50  $\text{cm}^{-1}$  and 3300.84  $\text{cm}^{-1}$  corresponds to C-H and O-H stretching present in hydrocarbons and benzene ring compounds (like ascorbic acid) respectively.<sup>25,26</sup>



**Figure 1.** FTIR Spectrum of *Petroselinum crispum* leaves sample at solid state.



**Figure 2.** FTIR Spectrum of *Foeniculum Vulgare* leaves sample at solid state.

The absorption bands at 1476.80  $\text{cm}^{-1}$  which corresponds to N-H stretching vibrations may be due to the presence of amino acids.<sup>27</sup> The FTIR spectrum of *Foeniculum vulgare* leaves displayed six different bands across the entire range observed. The bands at 2832.61  $\text{cm}^{-1}$  and 2984.56  $\text{cm}^{-1}$  corresponds to C-H present in alkenes and

alkanes respectively and bands at 1029.98  $\text{cm}^{-1}$  corresponds to C–O present in esters.<sup>28</sup> The frequency bands at 3316.15  $\text{cm}^{-1}$  and 1354.64  $\text{cm}^{-1}$  corresponds to O–H stretching and SO stretching present in alcohol.<sup>25</sup>

**Table 3.** Absorption peak and functional group of *Petroselinum crispum* leaves sample (at solid state).

Absorption ( $\text{cm}^{-1}$ )	Functional Group	Peak Appearance
3300.84	OH Stretching (alcohol)	Medium
2915.50	CH Stretching (alkane)	Strong
1476.80	N-H Bending (amine)	Medium
1617.30	C=C Stretching	Medium

**Table 4.** Absorption peak and functional group of *Foeniculum Vulgare* leaves sample (at solid state).

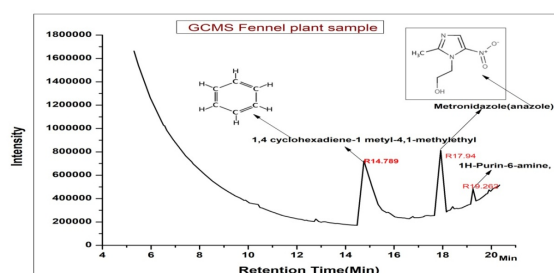
Absorption ( $\text{cm}^{-1}$ )	Functional Group	Peak Appearance
3316.151	O-H Stretching (Alcohol)	Medium
2832.61	C-H Stretching (Alkene)	Strong
2984.56	C-H Stretching (Alkane)	Weak
1354.64	SO Stretching	Medium
1648.75	C=C Stretching	Strong
1029.98	C-O Stretching(ester)	Strong

GC-MS chromatogram is still the best instrument for the separation of organic chemical compounds while at the same time the identifying of such compounds through the use of mass spectroscopy. GC-MS analytical technique is usually seen as a common confirmation test. It is best used to make an effective chemical analysis. The analysis provides a representative spectral output of all the compounds that get separated from the sample. The initial involves injecting the sample to the injected port of the Gas chromatography (GC) device. Then the GC instrument separate and vaporizes the sample and analyses the various components. Each component was ideally producing a specific spectral peak that may be recorded on a paper chart electronically. The time elapsed between elution and injection is called the retention time. The peak is measured from the base to the tip of the peak. Interpretation of Mass-Spectrum was carried out by using the database of National institute

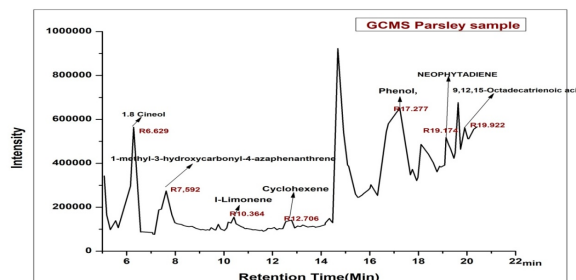
Standard and Technology (NIST) having more than 62,000 patterns. Where the spectrum of the unknown components is compared with the spectrum of known components which was stored in the NIST library. The name, chemical structure and molecular weight of the components of the test materials were determined. GC-MS analysis of ethanolic extract of *Foeniculum vulgare* and *Petroselinum crispum* leaves

In accordance with the present study, compounds of ethanol extracts of *Foeniculum vulgare* and *Petroselinum crispum* leaves have been identified with compounds name, retention time, peak area and its bio-active activities through GCMS evaluation. In the current research, some phytochemicals were identified from ethanol extracts by GCMS. *Foeniculum vulgare* and *Petroselinum crispum* leaves ethanol extract were subjected to GC-MS in order to recognize the phytochemical compounds. The biologically active compounds such as 1,4 Cyclohexadiene, Metronidazole, 1H-Purine-6 amine, I-Limonene which has been reported as hepatoprotective, antihistaminic, anti-eczemic, antimicrobial, anti-cancer, anti-arthritis, anti-asthma and antidiuretic activities.

Figure 3 and Figure 4 were the chromatogram of both extracts. The phytochemicals are believed to have been present between retention times 14.759 to 19.262 and 6.629 to 19.922 respectively. In *Foeniculum vulgare* leaves ethanol extract, two biologically active compounds have been detected and that this biologically active compounds name, retention time, peak area and its bio-active activities were presented in Table 5.



**Figure 3.** GC-MS chromatogram of Ethanol extract of *Foeniculum Vulgare* leaves



**Figure 4.** GC-MS chromatogram of Ethanol extract of *Petroselinum crispum* leaves

The bioactive compounds are 1,4 Cyclohexadiene with the retention time at 14.759 and peak area of 3.4 has anti-cancer activity.<sup>29</sup> Metronidazole (anazole) with the retention time at 17.943 and peak area of 4.2 has Anti-bacterial activity.<sup>30</sup> 1H-Purine-6 amine at a retention time at 19.262 and a peak area of 0.96 displayed no biological activity that is linked to the compound until now.

In *Petroselinum crispum* leaves ethanol extract, six biologically active compounds have been detected and that this biologically active compounds name, retention time, peak area and its bio-active activities were presented in Table 6. The biologically active compounds are Cineole, with the retention time at 6.629 and peak area of 12.8 has anti-inflammatory, antioxidants and antimicrobial activities as well as, in the treatments of cardiovascular and respiratory disease.<sup>31</sup> I-Limonene

with the retention time at 10.364 and peak area of 3.21 has anti-microbial activities.<sup>32</sup> cyclohexane with the retention time at 12.706 and peak area of 2.4 has Anti-microbial activities which helps in many drug formulation procedure.<sup>33</sup> Phenol with the retention time at 17.277 and peak area of 14.3 has anti-inflammatory and antimicrobial activities.<sup>34</sup> Neophytadiene with the retention time at 19.174 and peak area of 15.2 has anti-inflammatory and antimicrobial activities as well as, in the treatment of skin disease and headache.<sup>35</sup> 9,12,15 octadecatrienoic with the retention time at 19.922 and peak area of 13.76 has hepatoprotective, antihistaminic, anti-eczemic, antimicrobial, anti-cancer, anti-arthritis, anti-asthma and antidiuretic activities.<sup>36</sup> 1 methyl 3 hydroxylcarbonyl at a retention time at 7.59 and a peak area of 8.34 displayed no biological activity that is linked to the compound until now.

**Table 5.** Bioactivities of phytocomponents identified in the Ethanol extract of *Foeniculum Vulgare* leaves by GC-MS.

SN	Retention Time	Name of the compounds	Peak Area	Biological Activity
1	14.759	1,4 Cyclohexadiene	3.4	Anti-cancer Agents
2	17.943	Metronidazole (anazole)	4.2	Anti-bacterial activity
3	19.262	1H-Purine-6 amine	0.96	NO Biological activity

**Table 6.** Bioactivities of phytocomponents identified in the Ethanol extract of *Petroselinum crispum* leaves by GC-MS.

SN	Retention Time	Name of the compounds	Peak Area	Biological Activity
1	6.629	Cineole	12.8	Anti-inflammatory, Antioxidants and Antimicrobial activity
2	7.592	1 methyl 3 hydroxylcarbonyl	8.34	NO Biological activity
3	10.364	I-Limonene	3.21	Anti- microbial activity
4	12.706	Cyclohexane	2.4	Anti-microbial activity
5	17.277	Phenol	14.3	Anti-inflammatory agents, and antimicrobial
6	19.174	Neophytadiene	15.2	Anti-inflammatory agents, and antimicrobial
7	19.922	9,12,15 octadecatrienoic acid	13.76	Hepatoprotective, Antihistaminic, Anti-eczemic, Antimicrobial, anti-cancer Anti-arthritis, anti-asthma and antidiuretic.

#### 4. CONCLUSIONS

Various phytochemicals and pharmacological studies have been performed on two different medicinal plants (*Foeniculum Vulgare* and *Petroselinum crispum* leaves) using analytical methods (GC-MS and FTIR analytical techniques) and phytochemical screening. The GC-MS chromatogram of the ethanol extract of the two selected plant samples showed the presence of eight therapeutic bioactive compounds. phytochemical screening of the ethanolic leave extract of the sample analyzed indicates the presence of many various secondary metabolites such as tannins, flavonoids, saponins, terpenoids and steroids among others. These plants are potential source of natural antioxidants that have great therapeutic property. However, the FTIR analysis showed the presence of characteristics functional groups such as amine, O-H group and Sulphur derivatives among others. Further studies may also be conducted in other to identify more bioactive compounds in *Foeniculum Vulgare* leaves.

#### Conflict of interests

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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