

# 1-Introduction

## 1.1-The target species *Petroselinumcrispum*

*Petroselinumcrispum*(Mill)Nyman Ex AW Hill (Apiaceae) is a herb that grows up to 30-100 cm high<sup>1</sup>. Nowadays, the plant is worldwide cultivated<sup>2</sup> for its economic value. Different parts of the herb find many applications in pharmaceutical, cosmetic and food industries<sup>3</sup>.



*Petroselinumcrispum* is used traditionally to treat an array of human disorders including hemorrhoids, inflammation and kidney stones<sup>4</sup>. The plant is claimed to improve memory and brain function<sup>5</sup>. The herb is also used as emmenagogic, carminative and abortifacient<sup>6</sup>. Several investigations demonstrated the potential hypoglycemic, diuretic and hypolipidemic properties of this species<sup>7</sup>. Also the plant is claimed to possess antimicrobial, anticoagulant and hepatoprotective activities<sup>7</sup>.

The plant is reported to contain: flavonols, luteolin and myrecitin beside caretenoids, pthalides, terpenes, coumarins,

tocopherol, apin ,apiol and ascorbic acid<sup>8,9</sup>. In model animal studies, supplementation of diets with *Petroselinumcrispum* leaves enhanced plasma radical scavenging capacity<sup>10</sup>. A major constituent of *Petroselinumcrispum* essential oil- benzo[ $\alpha$ ]pyrene- inhibited tumorigensis in lungs of model animals<sup>11</sup>. The essential oil which is manipulated in the production of soaps, perfumes and creams<sup>12</sup>. *Petroselinumcrispum* volatiles include ;  $\alpha$ -pinene,  $\alpha$ - and  $\beta$ - phellandrenes,  $\beta$ -myrcene,cis-ocimene,isopropenyl-4-methylbenzene,  $\alpha$ -terpinolene, p-mentha-1,3,8-triene,  $\alpha$ -copaene, caryophyllene,  $\beta$ -famesen,  $\beta$ -selinene,  $\gamma$ -cadinene,  $\beta$ -bisabolene,  $\beta$  –sesquiphellandrene<sup>12</sup>.

With view of the titivation of phenyl propanoidmetabolism<sup>13</sup>, *Petroselinumcrispum* is used as system for investigating non-host plant/pathogen interactions. Antioxidant and antibacterial properties of *Petroselinumcrispum* make it propitious in food systems<sup>14</sup>.

Culinary herbs<sup>15</sup>, like *Petroselinumcrispum*, have been used to reduce food spoilage and controlling the growth of food-borne pathogens. Lipid peoxidation seems to deteriorate food quality. In vitro studies demonstrated that constituents of *Petroselinumcrispum* leaves scavenge superoxide anion<sup>16</sup> while the methanolic extract scavenge hydroxyl radical<sup>17</sup>. Nielsen et. al.<sup>18</sup> claimed that *Petroselinumcrispum* leaves decreased oxidative stress in human subjects<sup>15</sup>.

Freez-dried and irradiated *Petroselinumcrispum* leaves and stems were evaluated for antimicrobial and antioxidant activities<sup>15</sup>. Several mechanisms of antioxidant activity including ion chelating and free radical scavenging capacity as well as reducing power were examined<sup>15</sup>.

Different fractions of *Petroselinumcrispum*were evaluated for their DPPH radical scavenging capacity<sup>19</sup>. The dichloromethane fraction showed significant protection against H<sub>2</sub>O<sub>2</sub>-induced DNA damage in model animals suggesting a potential in cancer prevention. The extract also inhibited H<sub>2</sub>O<sub>2</sub>-induced MCF-7 cell migration which is necessary for metastasis of cancer cells<sup>1</sup>. This suggests that *:Petroselinumcrispum* has health-promoting properties with the potential to prevent oxidative stress-related diseases and can be developed into functional food.

In ADP-induced platelet aggregation, two constituents of *Petroselinumcrispum* leaves –apigenin and cosmosiin – exhibited strong antiplatelet aggregation activity<sup>20</sup>.

## **1.2- Essential oils**

Essential oils are complex mixtures of volatile organiccompounds produced as secondary metabolites in plants; theyare constituted by hydrocarbons (terpenes and sesquiterpenes)and oxygenated compounds (alcohols, esters, ethers, aldehydes,ketones, lactones, phenols and phenol ethers)<sup>21</sup>. Essential oils are odiferous bodies of an oily nature,

obtained almost exclusively from plant organs: flowers and buds, leaves, bark or wood, roots, stems, rhizomes, fruits and seeds<sup>22,23</sup>. Essential oils are generally liquid, and their pleasant odour and essence is responsible for the strong characteristic smell or fragrance of aromatic plants<sup>24</sup>.

Essential oils can be found in various plant organs, being produced and stored in secretory structures that differ in morphology, structure, function, and distribution. These specialized structures minimize the risk of auto toxicity and can be found on the surface of the plant organs or within the plant tissues, being classified as external or internal secretory structures, respectively. In nature, essential oils play very important roles in plant defense and signaling processes. For example, essential oils are involved in plant defense against microorganisms, insects, and herbivores, attraction of pollinating insects and fruit-dispersing animals, water regulation and all elopathic interactions<sup>25</sup>. Essential oils are hydrophobic, are soluble in alcohol, non polar or weakly polar solvents, waxes and oils, but only slightly soluble in water and most are colourless or pale yellow, with exception of the blue essential oil of chamomile (*Matricaria chamomilla*) and most are liquid and of lower density than water (sassafras, vetiver, cinnamon and clove essential oils being exceptions)<sup>26</sup>.

### **1.2.1-Classification of essential oil**

Essential oils may be classified using different criteria: consistency, origin, and chemical nature of the main components<sup>27-30</sup>.

Essential oils depending on their consistency are classified as :

#### **i) Essences**

Fluid essences are liquids which are volatile at room temperature.

#### **ii) Balsams**

Balsams are natural extracts obtained from a bush or tree. They usually have a high benzoic and cynamic acid content with their corresponding esthers. They are thicker, not very volatile, and less likely to react by polymerising. Examples of balsams are copaiba balsam, Peruvian balsam, Banguy balsam, Tolu balsam, Liquid amber<sup>30</sup>.

#### **iii) Resins**

Within the resin group we find a number of possible combinations and mixes:

##### **a) Resins**

These are amorphous solid or semi-solid products of a complex chemical nature. They are physiological or physio-pathological in origin. Colophony, for example, is obtained by separating trementine an oleoresin. It contains abietic acid and derivates<sup>30</sup>.

## **b) Oleoresins**

These are homogeneous mixes of resins and essential oils. Trementine, for example, is obtained by making incisions in the trunk of different pine species. It contains resin (colophony) and essential oil (trementine essence) which are separated by steam drag distillation. The term oleoresin is also used to refer to vegetable extracts obtained using solvents, which should be virtually free of said solvents. They are frequently used instead of spices in foodstuffs and pharmacy because of their advantages (stability, microbiotic and chemical uniformity, and easy to add)<sup>30</sup>.

## **c) Gum-resins**

These are natural plant or tree extracts. They are a mix of gums and resins.

Essential oils depending on origin are classified as:

### **i) Natural oils**

Natural oils are obtained straight from the plant and are not modified physically or chemically afterwards. However, they are expensive because of their limited yield<sup>30</sup>.

### **ii) Artificial oils**

Artificial oils are obtained using processes of enriching the essence with one or several of its components. For example, essences of rose, geranium, and jasmine are enriched with linalool, and aniseed essence with athenol<sup>30</sup>.

### iii) Synthetic oils

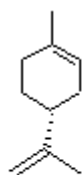
As the name suggests, are usually produced by combining their chemically synthesised components. These are the cheapest and are thus much more commonly used as fragrance and taste enhancers (vanilla, lemon and strawberry essences...) <sup>30</sup>.

### 1.3. Constituents of essential oils

Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols and phenol ethers) <sup>31</sup>.

#### 1.3.1. Hydrocarbons

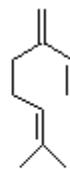
The majority of essential oils fall into this category these contain molecules of hydrogen and carbon only <sup>32,33</sup> and are classified into terpenes (monoterpenes: C<sub>10</sub>, sesquiterpenes: C<sub>15</sub>, and diterpenes: C<sub>20</sub>). These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic or tricyclic) or aromatic. Limonene(1),  $\alpha$ -pinene (2), myrcene (3) <sup>34-36</sup>.



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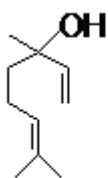
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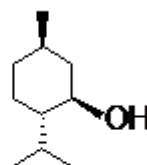
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### 1.3.2 .Alcohols

Alcohols have the hydroxile group (OH) bonded to a C<sub>10</sub> skeleton. Their names end in –ol. They are highly sought after for their aroma. In addition to their pleasant fragrance, alcohols are the most therapeutically beneficial of essential oil components. Linalool (4) gives tea taste , menthol(5) , another compound found in this group, is responsible for the smell and taste of mint<sup>34-38</sup> .



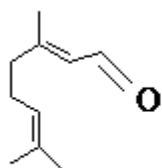
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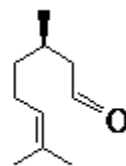
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### 1.3.3. Aldehydes

are highly reactive compounds. Their names end in –al. Many of them, such as those found in citrus fruits, match their respective alcohol. For example: geranial (6), and citronelal (7)<sup>34,39</sup>.



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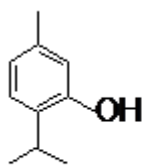


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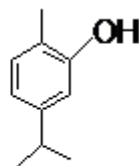
### 1.3.4. Phenols

They are only found in a few species These aromatic components are among the most reactive, potentially toxic and irritant, especially for the skin and the mucous membranes. The most important are thymol (8) and carvacrol (9)<sup>34,38</sup> .





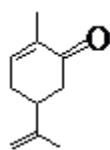
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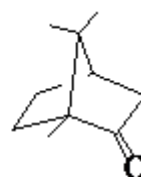
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### 1.3.5. Ketones

These are produced by the oxidation of alcohol and fairly stable molecules they end-one example Carvone (10). Ketones are not very common in the majority of essential oils are not particularly important as fragrances or flavor substances. In some cases, ketones are neurotoxic and abortifacients such as camphor (11)<sup>34,38,40</sup>.



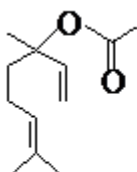
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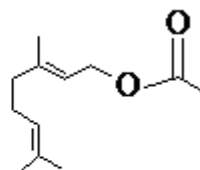
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### 1.3.6. Esters

Esters are sweet smelling and give a pleasant smell to the oils and are very commonly found in a large number of essential oils. They include for example, linalyl acetate (12), geranyl acetate (13)<sup>34,37,38,41</sup>.



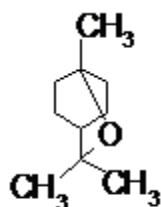
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### 1.3.7. Ethers

Ethers or monoterpene oxides are reactive and unstable. One example common ether is 1,8-cineol (also known as eucalyptol (14)<sup>27,30</sup>).



14

### 1.4 .Biological uses

Essential oils are natural volatile substances obtained from a variety of plants. Approximately 3000 essential oils are known, and 10% of them have commercial importance in the cosmetic, food, and pharmaceutical industries<sup>42,43</sup>. In general, essential oils have a nice smell, that is why they are used in different industries, especially in odors in perfumes (fragrances and lotions), in foodstuff (like flavoring and preservatives) and in pharmaceutical products (therapeutic

Essential oils exhibit a wide spectrum of pharmacological activities such as infection control, wound healing, pain relief, nausea, inflammation and anxiety<sup>44,45</sup>. Also, particular emphasis has been placed on the antibacterial, antifungal and insecticidal activities of essential oil from plants<sup>46,47</sup>.

Traditional medicines containing essential oils have been scientifically proven to be effective in treating various ailments

like malaria and others of microbial origin <sup>48-49</sup>. It has been observed that different essential oils overlap in their actions although they may differ in their chemical composition<sup>49</sup>. The biological activities of essential oils have been attributed to the composition or specific essential oil constituent.

### **1.5. Extraction of the oils**

Oils can be extracted via two key methods: Distillation (includes hydrodistillation) and Expression. Also, can be extracted via solvent extraction or enfleurage, although enfleurage is rarely performed in nowadays.

#### **i)The Distillation Process**

During distillation the plant material is placed upon a grid inside the still. Once inside, the still is sealed, and, depending upon the above methods, steam or water/steam slowly breaks through the plant material to remove its volatile constituents. These volatile constituents rise upward through a connecting pipe that leads them into a condenser. The condenser cools the rising vapor back into liquid form. The liquid is then collected in a vehicle below the condenser. Since water and essential oil do not mix, the essential oil will be found on the surface of the water where it is siphoned off. Occasionally an essential oil is heavier than water and is found on the bottom rather than the top<sup>50</sup>.

The three types of distillation include:

### **ii) Water Distillation**

The plant material comes into direct contact with the water. This method is most often employed with flowers (rose and orange blossoms), as direct steam causes these flowers to clump together making it difficult for steam to pass through<sup>50</sup>.

### **iii) Water and Steam**

This method can be employed with herb and leaf material. During this process, the water remains below the plant material, which has been placed on a grate while the steam is introduced from outside the main still (indirect steam)<sup>50</sup>.

### **iv) Steam Distillation**

This method is the most commonly used. During this process, steam is injected into the still, usually at slightly higher pressures and temperatures than the above two methods<sup>50</sup>.

### **v) Percolation or Hydrodiffusion**

This is a relatively recent method and is very similar to steam distillation except that the steam comes in through the top rather than the bottom, and there is a shorter distillation time. It is useful in extracting essential oils from woody or tough material or seeds.

Hydrosols, also known as hydrolats, are the by-product or product (depending on the distiller purpose) of the distillation process. Hydrosols contain the water-soluble constituents of the aromatic plant and retain a small amount of essential oil. Every liter of hydrosol contains between 0.05 and 0.2 milliliter of dissolved essential oil, depending on the water solubility of the plant's components and the distillation parameters<sup>50</sup>.

#### **vi) Expression Extraction**

Expression, also referred to as cold pressing, is a method of extraction specific to citrus essential oils, such as tangerine, lemon, bergamot, sweet orange, and lime. In older times, expression was done in the form of sponge pressing, which was literally accomplished by hand. The zest or rind of the citrus would first be soaked in warm water to make the rind more receptive to the pressing process. A sponge would then be used to press the rind, thus breaking the essential oil cavities, and absorb the essential oil. Once the sponge was filled with the extraction, it would then be pressed over a collecting container, and there it would stand to allow for the separation of the essential oil and water/juice. The essential oil would finally be siphoned off<sup>50</sup>.

#### **vii) Solvent Extraction**

Some plant material is too fragile to be distilled and an alternative method must be employed. Solvent extraction is the

use of solvents, such as petroleum ether, methanol, ethanol, or hexane, to extract the odoriferous lipophilic material from the plant. The solvent will also pull out the chlorophyll and other plant tissue, resulting in a highly colored or thick/viscous extract. The first product made via solvent extraction is known as a concrete. A concrete is the concentrated extract that contains the waxes and/or fats as well as the odoriferous material from the plant. The concrete is then mixed with alcohol, which serves to extract the aromatic principle of the material. The final product is known as an absolute<sup>50</sup>.

After the solvent extraction process has been completed, the resulting absolute will have an extremely low concentration of solvent residue, approximately 5 to 10ppm (parts per million). The current European Union standards are for less than 10 parts per million solvent residues in a finished absolute. However, even with such a potentially small residue (less than .0001%), many aromatherapists disagree with the use of absolutes for individuals with a compromised immune system due to the potential effect of the residual pesticide<sup>51</sup>.

## **1.6- Chromatography**

Chromatography from Greek chroma which means "color" and graphein "to write" is the collective term for a set of laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries it

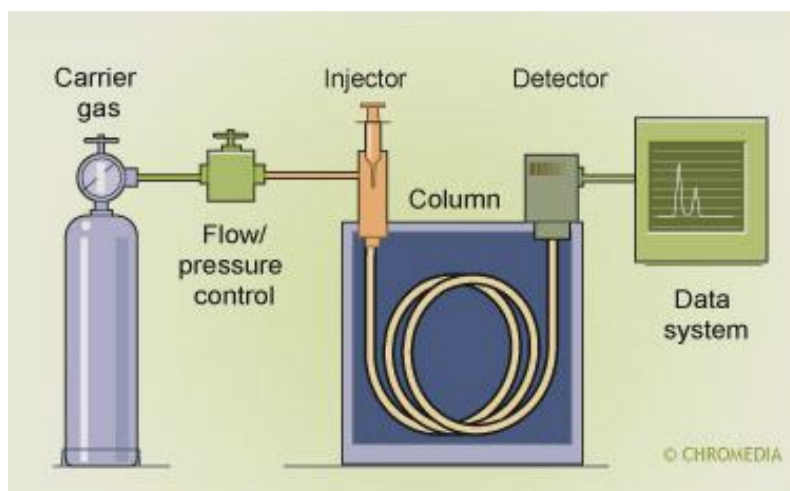
through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus changing the separation<sup>51</sup>.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for more advanced use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture<sup>51</sup>.

The chromatographic technique is used for the separation of amino acids, proteins and carbohydrates. It is also used for the analysis of drugs, hormones, vitamins. It is helpful for the qualitative and quantitative analysis of complex mixtures. The technique is also useful for the determination of molecular weight of proteins. Types of Chromatography include; Paper Chromatography, Thin Layer Chromatography(TLC), Gel Chromatography, Column Chromatography, Ion Exchange Chromatography, Gel Filtration Chromatography, Gas Liquid Chromatography, Affinity Chromatography<sup>52</sup>.

## 1.6 .1-Gas chromatography (GC)

Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations, GC may help in identifying a compound in preparative chromatography; GC can be used to prepare pure compounds from a mixture<sup>53</sup>.



GC system

A gas chromatograph is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the



column, through which different chemical constituents of a sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature<sup>53</sup>.

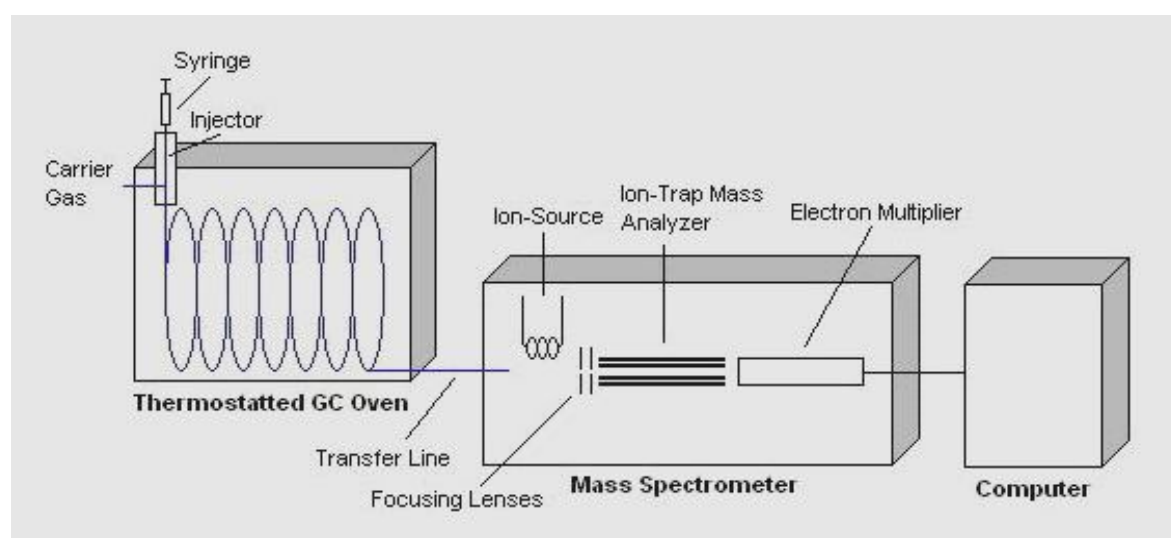
In general, substances that vaporize below 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard<sup>53</sup>.

Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS. Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR is connected to an infrared spectrophotometer which acts as a backup detector. This

combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS<sup>53</sup>.

### 1.6.2- Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification<sup>53</sup>.



GC/MS system

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s after being originated by James and Martin in 1952.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (i.e. have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample. For the analysis of volatile compounds, a purge and trap (PT) concentrator system may be used to introduce samples. The target analytes are extracted and mixed with water and introduced into an airtight

chamber. An inert gas such as nitrogen (N<sub>2</sub>) is bubbled through the water; this is known as purging. The volatile compounds move into the headspace above the water and are drawn along a pressure gradient (caused by the introduction of the purge gas) out of the chamber. The volatile compounds are drawn along a heated line onto a 'trap'. The trap is a column of adsorbent material at ambient temperature that holds the compounds by returning them to the liquid phase. The trap is then heated and the sample compounds are introduced to the GC-MS column via a volatiles interface, which is a split inlet system. PT/ GC-MS is particularly suited to volatile organic compounds (VOCs) and aromatic compounds associated with petroleum)<sup>53</sup>.

## **Aims of this study**

This study was designed to :

- Screen the medicinally important species *Petroselinum crispum* for major secondary metabolites.
- Conduct a GC-MS study on the oil.
- Evaluate antimicrobial activity of oil.

## 2-Materials and Methods

### 2.1-Materials

#### 2.1.1-Plant material

Seeds of *Petroselinum crispum* were purchased from the local market-Khartoum and authenticated by direct comparison with a herbarium sample.

#### 2.1.2- Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25  $\mu$ m, thickness) was used.

#### 2.1.3-Test organisms

*Petroselinum crispum* oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table (2.1).

**Table 2.1: Test organisms**

Ser. No	Micro organism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
5	<i>Aspergillus niger</i>	fungi
6	<i>Candida albicans</i>	fungi

## **2.2- Methods**

### **2.2.1- Preparations of reagents for phytochemical screening.**

#### **2.2.1.1-Flavonoid test reagents**

##### **- Aluminium chloride solution**

(1 g ) of aluminum chloride was dissolved in 100 ml methanol

##### **- Potassium hydroxide solution**

(1 g) of potassium hydroxide was dissolved in 100 ml water.

##### **-Ferric chloride solution**

(1 g) of ferric chloride was dissolved in 100 ml methanol.

#### **2.2.1.2- Alkaloid test reagents**

##### **Maeyer reagent**

- **Mercuric chloride solution:** 1.36 g in 60 ml. water.

- **Potassium iodide solution:** 5 g in 10 ml. water

The two solutions were combined and then diluted with water up to 100 ml.

##### **-Wagner reagent**

(1.27 g) iodine and ( 2 g) of potassium iodide in (100 ml) water.

### **2.2.1.3- Preparation of plant extract for phytochemical screening**

(100 g) Of powdered air- dried fruit pulp of *Petroselinum crispum* were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract (PE) was used for phytochemical screening.

### **2.2.2- Phytochemical screening**

The prepared extract of the plant was screened for major secondary constituents.

#### **2.2.2.1 Test for unsaturated sterols and for triterpenes**

(10 ml ) of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chloroform solution was dehydrated over anhydrous sodium sulphite . (5 ml ) portion of the solution was mixed with( 0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

#### **2.2.2.2- Test for flavonoids**

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:



- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.
- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

#### **2.2.2.3- Test for alkaloids**

(10 ml) of the (PE) were evaporated to dryness on a water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into two portions:

To one portion a few drops of Maeyer reagent were added, to the other portion few drops of Wagner reagent were added.

#### **2.2.2.4- Test for tannins**

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled, filtrated and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

#### **2.2.2.5 -Test for saponins**

(1g) of dried powdered plant material was placed in a clean test tube. (10 ml) of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand.

#### **2.2.3-Extraction of oil from seeds of *Petroselinumcrispum***

Powdered shade-dried seeds of *Petroselinumcrispum* (500g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

#### **2.2.4-Esterification of oil**

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol.A stock solution of methanolic sulphuric acid was prepared by mixing (1ml )of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml of alcoholic sulphuric acid.The tube was stoppered and shaken vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added,then (2ml) of normal hexane were added and the tube was vigorously shaken for five minutes. The hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether . The solution was filtered and the filtrate (1µl) was injected in the GC-MS vial.

### 2.2.5- GC-MS analysis

The oil of the seeds of *Petroselinum crispum* was analysed by gas chromatography – mass spectrometry. A Shimadzu GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length ; 0.25mm diameter ; 0.25  $\mu\text{m}$ , thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 2.1, while other chromatographic conditions are depicted in Table 2.2.

**Table 2.2: Oven temperature program**

Rate	Temperature( $^{\circ}\text{C}$ )	Hold Time (min. $^{-1}$ )
-	150.0	1.00
4.00	300.0	0.00

**Table2.3: Chromatographic conditions**

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

## **2.2.6-Antimicrobial assay**

### **2.2.6.1-Preparation of bacterial suspensions**

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about  $10^8$ - $10^9$  colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable

organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

#### **2.2.6.2-Preparation of fungal suspensions**

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

#### **2.2.6.3-Testing for antibacterial activity**

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate

Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).

The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

## 3-Results and Discussion

### 3.1-Phytochemical screening

Phytochemical screening of *Petroselinum crispum* leaves gave positive reactions for: flavonoids, tannins, alkaloids, saponins and carbohydrates.

**Table 3.1: Phytochemical screening of *Petroselinum crispum***

Species	Flavonoids	Tannins	Alkaloids	Saponins	Carbohydrates
<i>Petroselinum crispum</i>	+ve	+ve	+ve	+ve	+ve

### 3.2-GC-MS analysis of *Petroselinum crispum* essential oil

GC-MS analysis of *Petroselinum crispum* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern. Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

#### 3.2.1- Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 47 components. The typical total ion chromatograms (TIC) of hexane extract are shown in Fig.3.1.

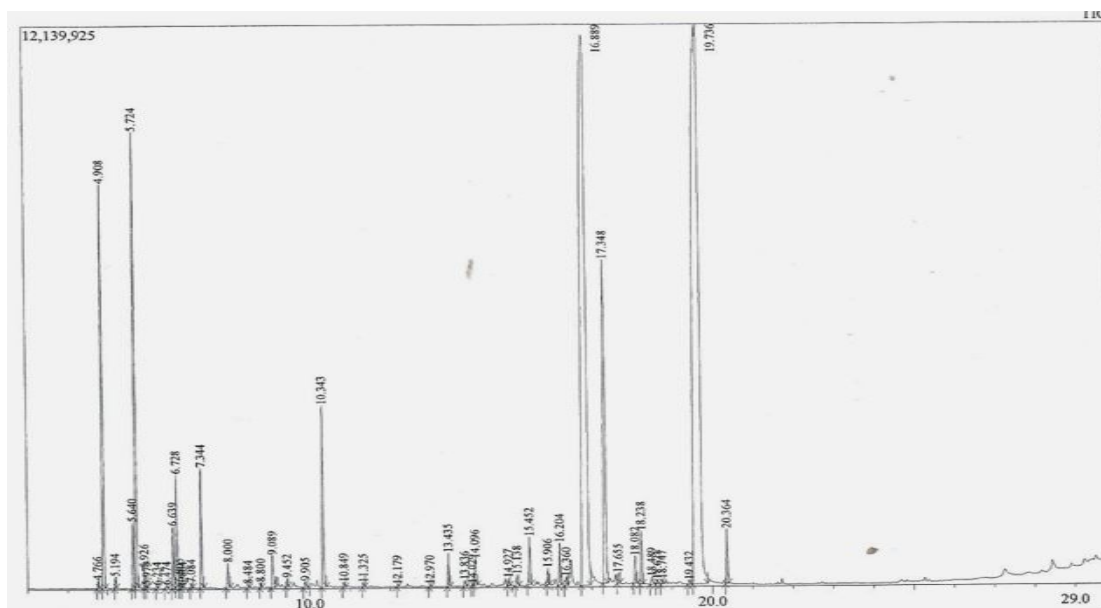


Fig.3.1: Chromatograms of *Petroselinum crispum* oil

The following major constituents were detected in the chromatograms:

### **Apiol (38.28%)**

The EI mass spectrum of apiol is shown in Fig. 3.2. The peak at  $m/z$  222, which appeared at R.T. 19.736 in total ion chromatogram, corresponds to  $M^+[C_{12}H_{14}O_4]^+$ . The peak at  $m/z$  207 corresponds to loss of a methyl function.

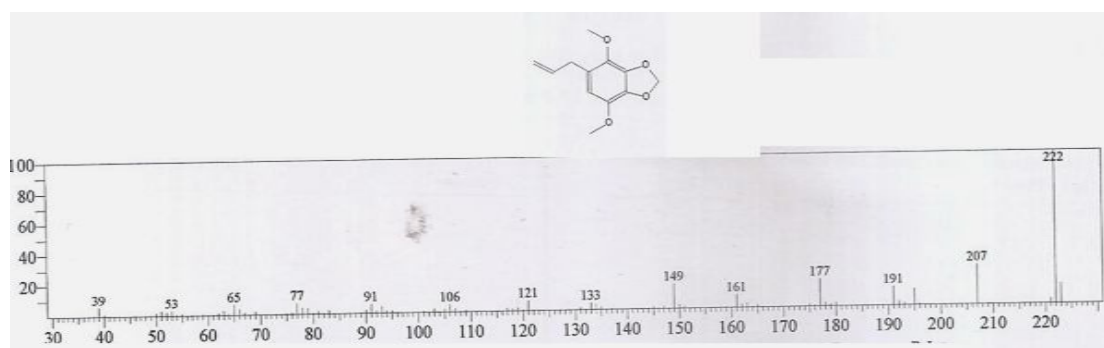


Fig. 3.2: Mass spectrum of apiol



#### 4-Methoxy-6-(2-propenyl)-1,3-benzodioxole(28.82%)

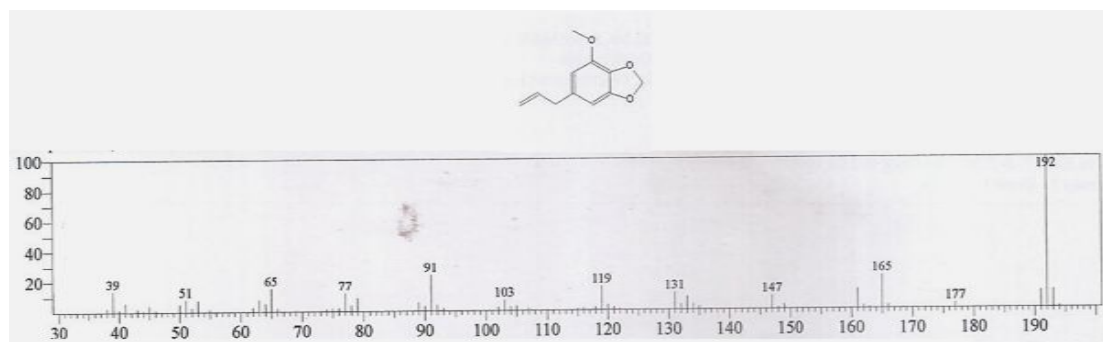


Fig. 3.3: Mass spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole

The EI mass spectrum of 4-methoxy-6-(2-propenyl)-1,3-benzodioxole is shown in Fig. 3.3. The peak at  $m/z$  192, which appeared at R.T. 16.89 in total ion chromatogram, corresponds to  $M^+[C_{11}H_{12}O_3]^+$ . The peak at  $m/z$  177 corresponds to loss of a methyl function.

#### 1,2,3-Trimethoxy-5-(2-propenyl)benzene(6.63%)

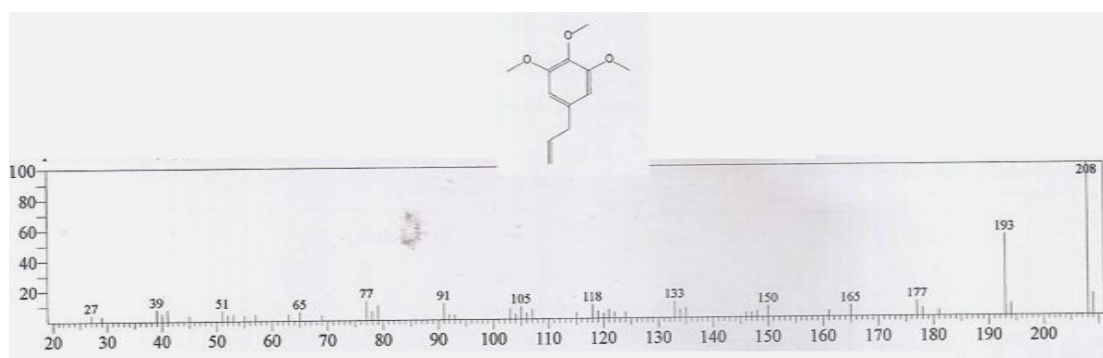


Fig. 3.4: Mass spectrum of 1,2,3-trimethoxy-5-(2-propenyl)benzene

The EI mass spectrum of 1,2,3-trimethoxy-5-(2-propenyl)benzene is shown in Fig. 3.5. The peak at  $m/z$  270, which appeared at R.T. 17.34 in total ion chromatogram,

corresponds to  $M^+[C_{17}H_{34}O_2]^+$ . The peak at  $m/z$  239 corresponds to loss of a methoxyl function.

### 2,6,6-trimethyl-bicycl[3.1.1]heptanes(6.31%)

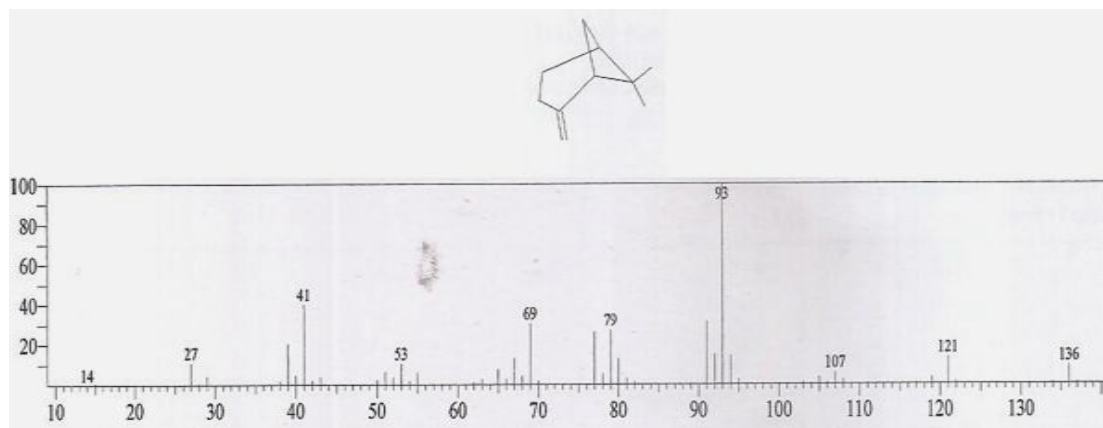


Fig. 3.5: Mass spectrum of 2,6,6-trimethyl-bicycl[3.1.1]heptanes

The EI mass spectrum of 2,6,6-trimethyl-bicycl[3.1.1]heptanes is shown in Fig. 3.4. The peak at  $m/z$  136, which appeared at R.T. 5.72 in total ion chromatogram, corresponds to  $M^+[C_{10}H_{16}]^+$ . The peak at  $m/z$  121 corresponds to loss of a methyl function.

### $\alpha$ -Pinene (5.68%)

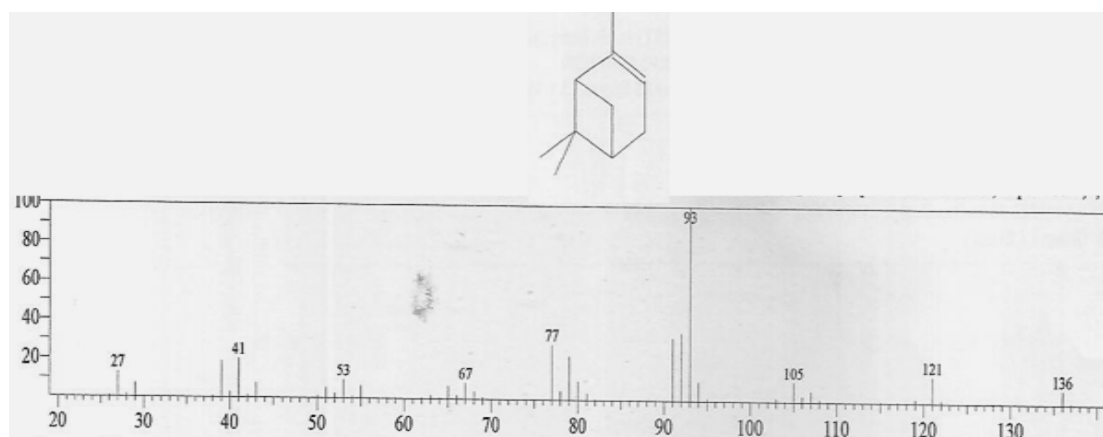


Fig. 3.6: Mass spectrum of  $\alpha$ -pinene

The EI mass spectrum of  $\alpha$ -pinene is shown in Fig. 3.6. The peak at  $m/z$ 136, which appeared at R.T.4.90 in total ion chromatogram, corresponds to  $M^+[C_{10}H_{16}]^+$ . The peak at  $m/z$ 121 corresponds to loss of a methyl function.

### 3.3-Antimicrobial activity

The oil was screened for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (3.2). The results were interpreted in terms of the commonly used terms (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Tables (3.3) and (3.4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

**Table 3.2 : Antibacterial activity of *Petroselinum crispum* oil :M.D.I.Z (mm)**

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
<i>Petroselinum crispum</i>	100	-	-	12	-	-	-

**Table 3.3: Antibacterial activity of standard chemotherapeutic agents: M.D.I.Z (mm)**

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

**Table 3.4: Antifungal activity of standard chemotherapeutic agents against standard fungi**

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.: *Staphylococcus aureus*
- Ec.: *Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- Bs.: *Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone  $\geq 15$ : sensitive,  $< 15$ : resistant.

The oil showed activity against *Staphylococcus aureus* (Table 3.2).

## **Conclusion**

GC-MS analysis of *Petroselinum crispum* oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern.<sup>47</sup> Components were detected in the oil. In the cup plate agar diffusion bioassay the oil was evaluated for antimicrobial potency and significant results were obtained.

## **Recommendations**

1-The extracted oil may also be evaluated for anti-inflammatory, antimalarial and antispasmodic activities.

2-Other bio constituents from the target species may be isolated and evaluated for their biological potential and their structures may be elucidated via spectral tools.

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