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**CHEMICAL COMPOSITION OF *SCHINUS MOLLE* L.  
ESSENTIAL OILS GROWN IN NORTH CYPRUS**

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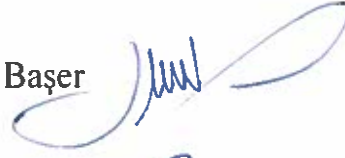
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## ABSTRACT

Alnawari, H. (Hisham). CHEMICAL COMPOSITION OF *SCHINUS MOLLE* L. ESSENTIAL OILS GROWN IN NORTH CYPRUS. Near East University Faculty of Pharmacy

MSc Thesis, Nicosia, 2018

Essential oils are a combination of highly complex volatile compounds in the form of hydrophobic liquids. Due to their interesting properties and important activities, essential oils have gained large attention and big popularity in cosmetic, food, and pharmaceutical industries. *Schinus molle* L. (Anacardiaceae) is a plant containing volatile oil. Although the chemical composition of this plant have been studied in some countries over the world, still no study has been done so far on *S. molle* which is grown in Cyprus. Therefore, the main goal of this study has been to determine the chemical constituents of *S. molle* essential oils grown in North Cyprus. The essential oil of dry leaves and fruits were obtained by hydrodistillation using a Clevenger type apparatus. Sixty seven compounds were identified by a combination of gas chromatography/ flame ionization detector (GC/FID) and gas chromatography/ mass spectrometry (GC/MS). The main abundant components in both leaves and fruits from two locations (Küçük Kaymakli and Near East University Campus) were  $\alpha$ -phellandrene (31.5%-31.6%), (26.7%-36.3%), limonene (10.1%-11.4%), (12.5%-13.5%),  $\beta$ -phellandrene (9.9%-10.9%), (10.3%-12.2%) respectively. Moreover, myrcene was a main constituent only in fruits (18.5%-19.9%). While, bicyclogermacrene was a main component in the leaf oil (12.0%-11.1%).

**Key Words:** hydrodistillation, essential oil,  $\alpha$ -phellandrene,  $\beta$ -phellandrene, limonene, *Schinus*, Anacardiaceae.



# **CHAPTER1**

## **INTRODUCTION**

## **1.1. Essential oils**

Essential oils also known as volatile oils, essences, aetheroleum, or etheric oils are valuable natural products (Baser and Demirci, 2007; Sangwan., 2001). According to European Pharmacopeia (Council of Europe 2004) and International Standard Organization (ISO9235: 2013), essential oils are products produced from aromatic plants by hydrodistillation, dry distillation or steam distillation or by mechanical process (Cold Pressing) in the case of citrus fruits. Furthermore, volatile oils are frequently associated with resins and gums that are separated by the distillation process (Baser and Demirci 2007). Essential oils are volatile oils extracted from plants bearing strong aromatic components that are made up of different volatile chemicals; for instance, hydrocarbons, alcohols, phenols, esters, ketones and aldehydes (Younis *et al.*, 2008). Essential oils are a mixture of hydrophobic and highly volatile secondary metabolites which can physically be separated from other plant components (Grassmann and Elstner 2003; Protzen 1993). Plant essential oils have many important applications, mainly in the health, cosmetics, and agriculture and food industries. Essential oils may generally constitute around 20-100 different secondary metabolites belonging to several chemical classes (Hammer and Crason, 2011). About 3000 essential oils have been obtained from at least 2000 plant species, out of which 300 are important in terms of commerce (Djilani and Dicko., 2012). They are usually stored in oil ducts, glands, resin ducts or trichomes (glandular hairs) of aromatic plants (Berger, 2007). Essential oils can be extracted from several plant parts, including leaves (peppermint), flower (rose), seeds (cardamom), fruits (fennel), grasses (lemongrass), roots (vetiver), bark (cinnamon), wood (cedar), rhizomes (ginger), tree blossom (ylang-ylang), bulbs (garlic), and dried flower buds (clove) (Tisserand and Young., 2013).

### **1.1.1. Physical properties of essential oils**

Essential oils at room temperature are usually mobile liquid and lucid, but some are solid, such as orris, or semisolid, such as guaiac wood oil. The majority of essential oils are pale yellow or colorless even a few are strongly colored, such as blue chamomile, and European valerian, which is green (Tisserand and Young, 2013). The odor of essential oils may depend on the organs, origins, and species of plants. They are volatile with high refractive index and optical rotation as a result of many asymmetrical compounds. Commonly, the relative density of essential oils is lower than the relative density of water. Therefore, they float on water, but some like clove oil sink to the bottom of water. Essential oils are usually considered as hydrophobic, they are soluble in fats, alcohols, and most organic solvents. Furthermore, they can be oxidized easily to form resinous products by polymerization (Li *et al.*, 2014).

### 1.1.2. Biosynthetic pathways

In the nature, there are two main groups of metabolites. They are primary and secondary metabolites. Primary metabolites are present in all living organisms; include carbohydrates, proteins, lipids, and nucleic acids. Secondary metabolites are classified as terpenoids, shikimates, polyketides, and alkaloids, etc. The first two are considered the most common in essential oils. In addition, some species contain high amounts of shikimates known as flavonoids, phenylpropanoids. These compounds are providing specific flavor and color to the plants (Sangwan *et al.*, 2001).

#### Terpenes

Terpenes are produced from the condensation of a pentacarbonate unit with two unsaturated bonds (2-methyl-1,3-butadiene) or isoprene, for this reason they are called isoprenoids. Kekule was the first one who used the designation "terpenes" in 1880 to name  $C_{10}H_{16}$  compounds found in turpentine (Baser and Demirci 2007). In 1887, his assistant whose name is Otto Wallace formulated the isoprene rule predicting that the terpenes were produced by two or more isoprene units. Later on, Robinson suggested that the isoprenes were connected in a tail to head way. Leopold Ruzicka in 1950 switched this rule by the "biogenetic isoprene rule" which states, the compound is considered as isoprenoid if it is derived biologically from an isoprenoid precursor with or without rearrangements (Little and Croteau 1999). To sum up, terpenoids are produced from aliphatic precursor such as geraniol to form monoterpenes, farnesol to form sesquiterpenes, and geranylgeraniol to form diterpenes (Baser and Demirci 2007). Classification of terpenes is different according to their structural and functional classes. According to the number of isoprene units in their structure, hemiterpenes (one isoprene unite), monoterpenes (two isoprene units), sesquiterpenes (three isoprene units), diterpene (four isoprene units), so on are formed. Monoterpenes ( $C_{10}H_{16}$ ) and sesquiterpenes ( $C_{15}H_{24}$ ) are common terpenes found in essential oils.

The biosynthesis of terpenes requires two precursors, (IPP) isopentyl pyrophosphate and (DMAPP) dimethylallyl diphosphate. IPP is biosynthesized in higher plants by two pathways: mevalonate pathway and deoxyxylulose phosphate pathway, or non-mevalonate pathway.

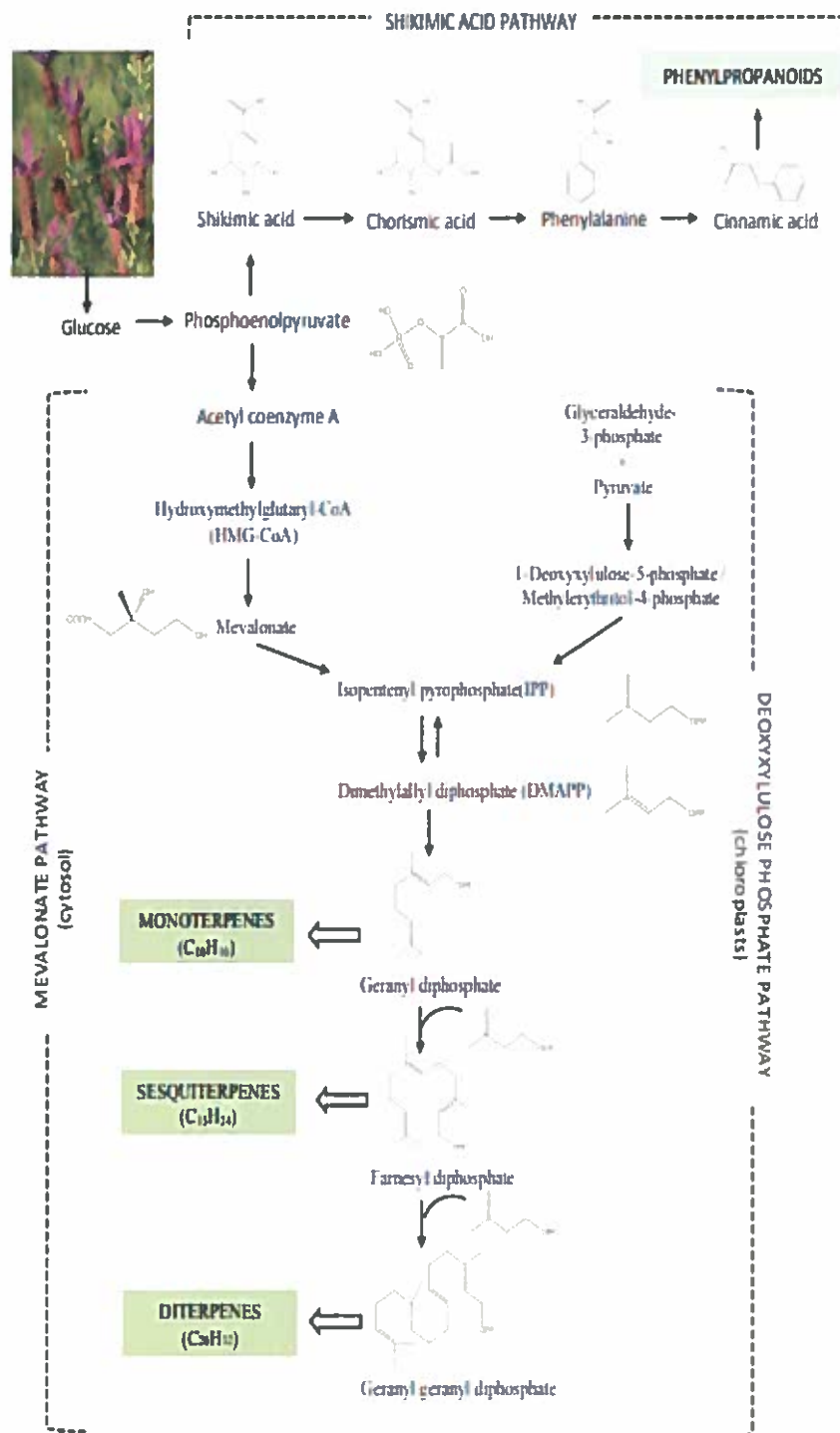


Figure 1.1. Biosynthetic Pathways of Isoprenoids of Essential Oils (Zuzart and Salgueiro, 2015).

IPP in mevalonate pathway is formed by mevalonic acid which results in from the condensation of three acetylcoenzyme-A moieties. Whereas, in non- mevalonate pathway, 1-deoxy-D-xylulose-5-phosphate(DOXP) and 2 C-methyl-D-erythritol-4-phosphate (MEP) are involved which results from the condensation of pyruvate and glyceraldehyde phosphate (Baser and Demirci 2007). The former takes place in the cytoplasm and leads to the production of most sesquiterpenes. While, the latter occurs in the chloroplasts leading to the formation of primarily monoterpenes and diterpenes. IPP and DMAPP produce (GPP) geranyl diphosphate which is the immediate precursor of monoterpenes. Condensation of GPP with IPP forms farnesyl diphosphate (FPP), the precursor of sesquiterpenes, and condensation of IPP with FPP leads to geranyl diphosphate, the precursor of diterpenes. The main compounds in essential oils are monoterpenes and sesquiterpenes (Bakkali *et al.*, 2008). In addition, the heavier terpenes like diterpenes may be found but usually they are not responsible for the odor of essential oils (Hunter 2009).

### **Phenylpropanoids**

Phenylpropanoids include one or more  $C_6.C_3$  units.  $C_6$  is benzene esters. Most of the phenylpropanoids present in essential oils are phenol ethers and in some cases, the side chain is reduced ( $C_1$ ) such as in the case of vanillin and methyl salicylate. Phenylpropanoids are produced by shikimic acid pathway. Cinnamic acid and p-hydroxycinnamic acid are the main precursors which originate from aromatic amino acids, phenylalanine and tyrosine (Sangwan *et al.*, 2001). Shikimic acids are produced from phosphoenolpyruvate and erythrose 4-phosphate. Furthermore, aromatization of shikimic acid leads to benzoic acid derivatives which are present in many essential oils (Sell 2010).

## **1.2. Distillation of essential oils**

Extraction techniques can be classified as Classical methods and conventional methods.

### **1.2.1. Classical methods**

#### **Hydrodistillation**

It is a well known traditional technique for essential oil extraction. Water distillation (or hydrodistillation) is considered as one of the oldest methods (Meyer-Warnod *et al.*, 1984). The principle of technique is that essential oil evaporates by heating aromatic material with water, and then the essential oil is condensed on a cold trap of water and accumulate in the collector.

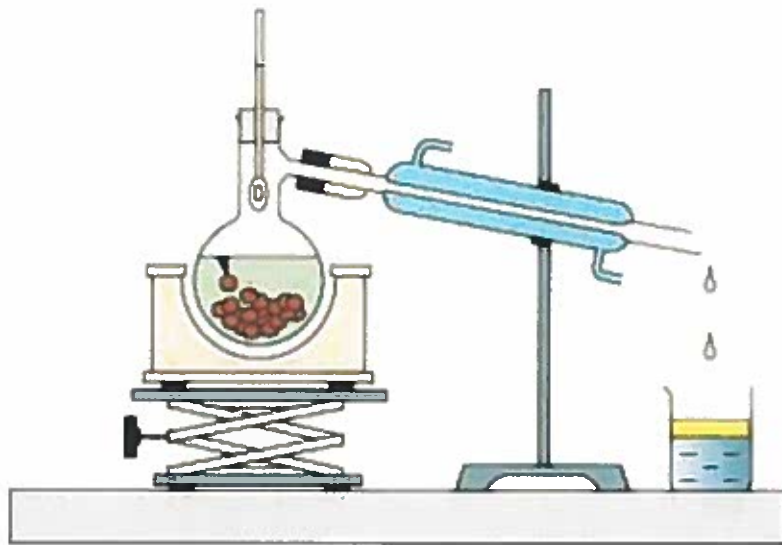


Figure. 1.2. Hydrodistillation (Hesham *et al.*, 2016).

**Steam distillation:**

It is a type of distillation which is used for heat-sensitive materials. It is used for the distillation of essential oils in industrial scale (Fahlbusch *et al.*, 2003). In steam distillation, the steam generated externally passes through the plant from the base to the top of an alembic still and produces a mixture of desired essential oil and the vapor. Then, the vapor is condensed and essential oils are collected according to their density either on top or bottom of the water layer (Rai and Suresh , 2004).

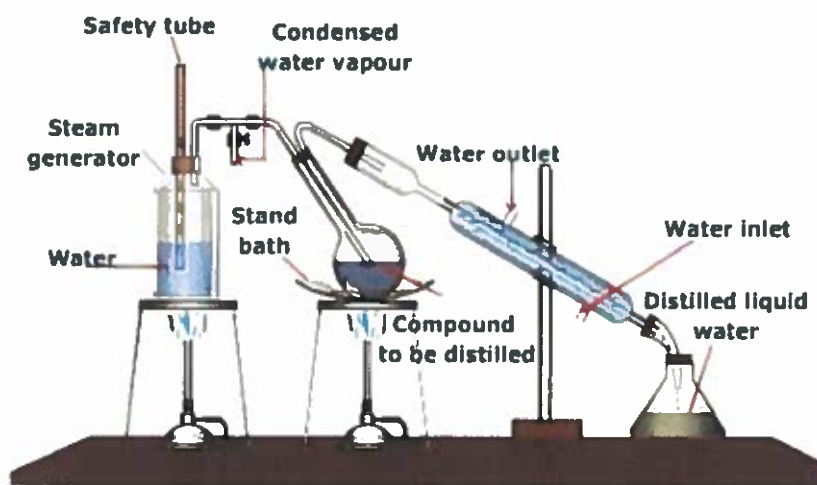


Figure. 1.3. Steam distillation (Hesham *et al.*, 2016).

## Cold pressing method

Cold pressing theoretically means the oil is expeller-pressed at low temperature and pressure. It is one of the best methods to separate essential oils especially from fresh citrus fruit peels. It is a mechanical extraction technique (Arnould *et al.*, 1981). In this method, the outer layer of citrus fruits which contains oil is scrubbed and washed with water to recover oil by centrifugation.

### 1.2.2. Innovative techniques for essential oil distillation

One of the drawbacks of conventional techniques is the use of high heat, which affects in thermolability of essential oils compounds causing alterations (isomerization, oxidation, hydrolyses). As a result, the quality of oil suffers if the time of extraction is prolonged. It is important that the extraction technique should maintain chemical composition and natural proportion of essential oil components in their original state.

### Supercritical Fluid Extraction (SFE)

It is a process of separating a component from another matrix by using supercritical fluids as extracting solvent. Supercritical fluids have been used as solvent for several applications such as metal cation extraction and aromatic extractions more than 90% of all analytical supercritical fluid extractions carbon dioxide is used as solvent (CO<sub>2</sub>). CO<sub>2</sub> is relatively nonflammable, nontoxic, non-corrosive, safe, easily removed from the extract (Rozzi *et al.*, 2002). The main disadvantage of CO<sub>2</sub> is lacking in polarity for extraction of polar analytes (Pourmortazavi *et al.*, 2007). This method leads to high yield and high diffusion coefficient, and lower viscosity. However, this method is very expensive because of the high cost of investment (Capuzzo *et al.*, 2013).

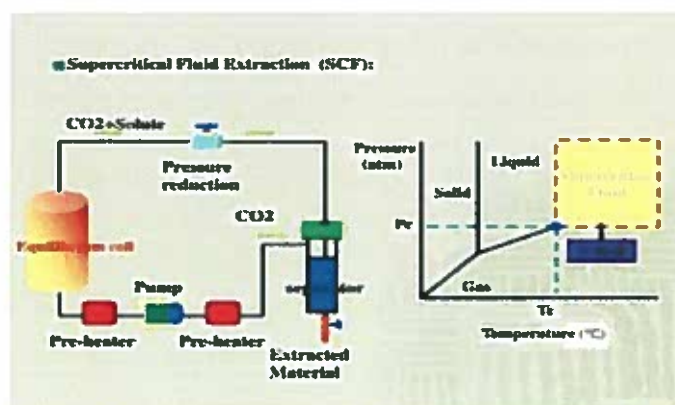


Figure 1.4. Supercritical Fluid Extraction (SFE) (Hesham *et al.*, 2016).

### Microwave-assisted hydrodistillation (MAHD)

Microwave assisted hydrodistillation is considered as an advanced extraction technique using a microwave oven in the extraction process (Golmakani *et al.*, 2008). The efficiency of the extraction process by microwave assisted hydrodistillation strongly relies on dielectrical constant of both sample and water (Brachet *et al.*, 2002). Less solvent consumption, protection that offered for thermolabile constituents and fast extraction process are the main advantages for the microwave assisted hydrodistillation technique. Its assembly consists of a Clevenger apparatus placed in a microwave oven. Microwave energy is used to boil water to affected distillation (Ferhat *et al.*, 2006; Lucchesi *et al.*, 2004).

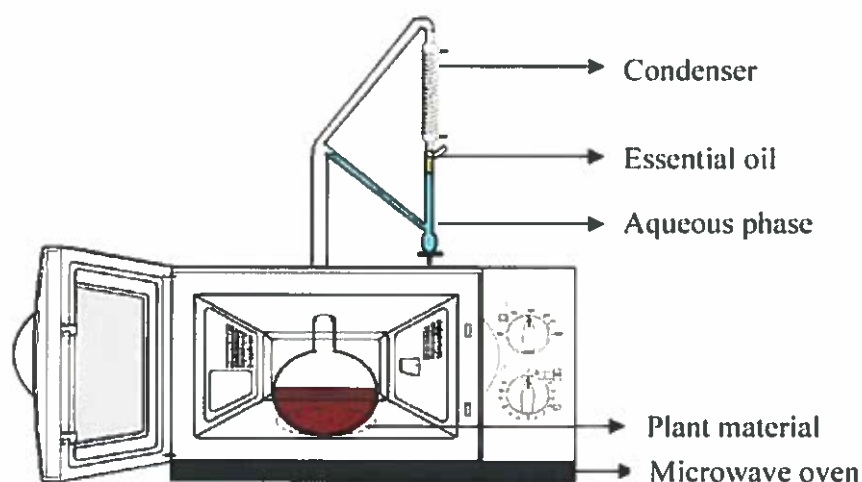


Figure. 1.5. Microwave Assisted Hydrodistillation (MAHD) (Hesham *et al.*, 2016).

### Solvent Free Microwave Extraction (SFME)

This method was developed by Cheat and Co-workers (Lucchesi *et al.*, 2004 a,b). This method is based on the integration between microwave heating energy and dry distillation. In this method, the subjected plant material is moistened before the extraction by soaking it in an amount of water for about two hours then draining off the extra water. Next step, the moistened material is put in a microwave oven and subjected to microwave radiation. The condenser will start to condense and collect the essential oil. The temperature, radiation power and extraction time are controlled via the panel of the instrument.



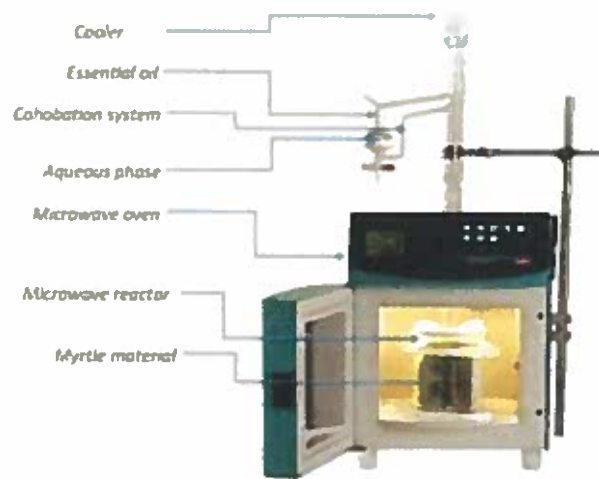


Figure. 1.6. Solvent Free Microwave Extraction (Hesham *et al.*, 2016).

### 1.3. *Schinus molle* L.

Essential oils have been recognized for many years as important ingredients of pharmaceutical agents and food additives (Dalia *et al.*, 2014). *Schinus molle* L. (Anacardiaceae) is a promising plant as a source of essential oil. *Schinus molle* also known as Brazillian pepper tree, is originally from South- America, but has been introduced to many countries around the world. It is planted on road sides and gardens as shade tree (Belhamel *et al.*, 2008). This plant plays an important role in Chilean folk medicine. The whole fruits of *S.molle* are soaked or fermented in hot water to make a drink which is used for many health purposes, such as diuretic, digestive, antiseptic, for pain relief and hemorrhoids. The plant has also been used in the treatment of menstrual disorders, respiratory infections, and urinary tract infections (Perez-Lopez *et al.*, 2011). In some traditional cuisines, *S. molle* fruits have been used as a replacement for black pepper and to prepare alcoholic beverages (Maringiu *et al.*, 2004).

#### 1.3.1. Anacardiaceae

The family Anacardiaceae is commonly known as cashew family or sumac family. It comprises about 83 genera, and 860 species (Elhassan *et al.*, 2016).

#### 1.3.2. Distribution and habitat

Andes region is considered as the area for natural distribution of *S. molle*, mainly Peru. It is found at altitudes up to 900 meter above sea level, in areas with 300-700 mm rain/year. It tolerates high temperatures and once established it resists frost, but not for long periods. It is a fast growing pioneer species that is typically found in disturbed areas with secondary growth on roadsides and on agricultural lands. It grows well in drained soils and can tolerate most soil types (Orwa *et al.*, 2009).

It is native in Argentina, Bolivia, Peru, and exotic in Cyprus, Dominican Republic, Australia, Ethiopia, Greece, India, Eritrea, Haiti, Kenya, Paraguay, Mexico, South Africa, Sudan, Spain, Turkey, Tanzania, Uganda, and United States of America (Orwa *et al.*, 2009).



Figure 1.7. *S. molle* distribution (Orwa *et al.*, 2009).

### 1.3.3. Local names

Arabic (felfel kazib), Amharic (qundo berbere), English (pepper tree, Californian pepper tree, Chilean pepper tree, pepper berry tree, mastic tree, weeping pepper, Peruvian mastic, peruvian pepper tree, pink pepper), French (Faux poivrier dupervian, poivre rose), Italian ( pep del peru, albero delpepe, schino), German (Brasilianischer pfeffer, rosa pfeffer, Peruanischer pfeffer), Spanish ( pimiento, pirul, arveira), Swahili (mpilipili), Swedish ( rosepeppar), Portuguese ( aroeira-do-matto), Brazil ( aroeira-saslo, Kenya ( mugaita, Netherlands ( peperboom, amerikaanse), Somalia ( mirimiri) (Orwa *et al.*, 2009).

### 1.3.4. Botanical description

*S. molle* is considered as an evergreen tree 3-15 meters in high, trunk short, crown with equal spread, bark is brown and deeply fissured, if the bark is damaged, very sticky latex will be secreted.



Figure 1.8. *Schinus molle* in North Cyprus

#### Leaves of *Schinus molle*

Leaves of *S. molle* are imparipinnate, with winged rachis. The number is about 20-40 leaflets. Leaflets are linear-lanceolate, margins entire or dentate, about 2-5 cm and 4-8 cm.



Figure 1.9. *Schinus molle* leaf

### Leaves of *Schinus terebinthifolius*

Bright green and non-leathery in texture, the leaves are compound which means there are several leaflets arranged opposite each other around one stem.



Figure 1.10. *Schinus terebinthifolius*

### Flowers of *Schinus molle*

Flowering occurs in September to December. Flowers can grow up to 30 cm long, the long of petals is about 2 mm, drooping clusters of pale yellow, tiny flowers which develop into bunches of pink berries. Female flowers give rise to berries. Some trees have almost no berries because they have mostly male flowers (Orwa *et al.*, 2009).



Figure. 1.11. Flowers of *Schinus molle*

### Fruits of *Schinus molle*

*S. molle* fruits are small, round berries. 5-9 mm in diameter, the color of berries is bright red when mature, later turning to black. Fruits are ripe in December-January. Fruits do not mature at the same time even with the same cluster the fruits will often be at different stages of maturity. The pulp is tiny and leathery. It has sweet taste and contains one or two seeds in each fruit. Seeds are 2-4 mm in diameter, round, brown black (Orwa *et al.*, 2009).



Figure. 1.12. Fruits of *S. molle*

#### **BOTANICAL DESCRIPTON**

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Supermatophyta

Division: Magnoliophyta

Subclass: Rosidae

Order: Spindales

Family: Anacardiaceae

Genus: *Schinus*

Species: *Schinus molle* L.

#### **1.4. Literature review**

##### **1.4.1. Pharmacology**

The essential oil of *S. molle* showed the maximum fungitoxic activity as compared with some other essential oils during the screening against common storage and animal pathogenic fungi (Dikshit *et al.*, 1986). Some oils of *S. molle* showed potential anti-tumoral effects, either alone or in combination (Diaz *et al.*, 2008).

*S. molle* essential oils showed insecticidal activity against *Trogoderma granarium* and *Trogoderma castaneum* (Abdel-sattar *et al.*, 2008). The essential oil and hexane extract of *S. molle* showed potential result in term of antimicrobial and insect repellent activity (Onder *et al.*, 2010). The essential oils extracted from *S. molle* leaves showed inhibitory effects, reduced growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella setubals*, and *Candida albicans* (Eucles *et al.*, 2011). The essential oil isolated from the fruits showed higher antioxidant activity than the oil isolated from leaves of *S. molle* (Abir *et al.*, 2016). Five terpenes were isolated from the bark resin of *S. molle* provided significant growth inhibitory effect against human colon carcinoma (Gonzalo *et al.*, 2017).

#### 1.4.2. Chemical composition

There are several studies from different places around the world revealing the variation in the chemistry of *S. molle* essential oil. Water distilled oils from dried fruits and leaves of *S. molle* grown in Turkey revealed 80 components representing 98.2% to 99.8% of the oil. Fruit oil contained  $\alpha$ -phellandrene (22.1% and 38.1%),  $\beta$ -phellandrene (10.4% and 11.8%) and limonene (9.6%). Whereas, the main components in the leaf oil were  $\alpha$ -phellandrene (45.7%),  $\beta$ -phellandrene (13.6%) and limonene (13.4%) (Baser *et al.*, 1997). In Algeria, the main components were  $\alpha$ -phellandrene (26.5%), limonene (8.6%),  $\beta$ -phellandrene (12.4%), elemol (8.6%) and  $\alpha$ -eudesmol (6.1%) (Belhamel *et al.*, 2008). On the Costa Rican material the main components of the essential oil extracted from *S. molle* leaves were  $\beta$ -pinene and  $\alpha$ -pinene (Diaz *et al.*, 2008). The main components of the leaf extracts from *S. molle* leaves grown in Izmir, Turkey were germacene D (20.7%) and  $\beta$ -caryophyllene (13.4%) (Onder *et al.*, 2010). The main components of essential oil extracted from *S. molle* fruit grown in Syria (Damascus) were  $\alpha$ -phellandrene (24.8%),  $\beta$ -pinene (14.7%),  $\beta$ -phellandrene (11%) and limonene (10.5%) (Ibrahim and Al-naser, 2014). The main components of essential oils obtained from leaves, stems and fruits of *Schinus molle* grown in Tunisia were  $\beta$ -eudesmol (14.8%), elemol (13.7%),  $\alpha$ -eudesmol (12.7%), limonene (9.2%) and spathulenol (7.2%). Whereas, the main components in stem were elemol (20.7%), 6-epi-shyobunol (20.3%) and  $\alpha$ -eudesmol (7.0%). Fruit essential oils were characterized by 6-epi-shyobunol (16.2%), limonene (15.3%), spathulenol (8.1%) and 4-epi-cubebol (7.8%) (Abir *et al.*, 2016).

The main goal for this study was to determine the chemical composition for *S. molle* essential oils collected in different locations in North Cyprus.

## **CHAPTER 2**

### **MATERIALS AND METHODS**



## 2. MATERIAL AND METHODS

### 2.1. Plant material

Leaves and fruits of *Schinus molle* L. were collected on 6/10/2017 from Near East University Campus, Nicosia, North Cyprus. While, the other *Schinus molle* leaves and fruits were collected on 10/11/2017 from Kucukkaymakli area, Sehit Mehmet street. Leaves and fruits were left to dry for about 15 -20 days before distillation. The plants were identified by Prof. Dr. K. Husnu Can Baser, Head of Pharmacognosy Department. Herbarium voucher numbers were given as 6896 for Near East University sample, and 6897 for to Kücükaymakli sample and stored at the herbarium of the university.



Figure. 2.1. *S. molle* in NEU Location



Figure. 2.2. *S. molle* in KK Location

## 2.2. Distillation of essential oil

50 g of dry *Schinus molle* leaves (NEU) were subjected to hydrodistillation for 3 hours using a Clevenger apparatus at the laboratory of the Pharmacognosy Department. The color of the oil was yellowish with a strong odor. The oils were kept in amber flasks at 4 °C until the analysis. The percentage of oil yield was 1.88%. While, 50 g of dry *S. molle* fruits were subjected to hydrodistillation for 3 hour just after two days of the leaf experiment. The oil was collected and stored at the same freezer. The percentage yield was 0.48%. 50 g of dry *S. molle* leaves (K.K) were hydrodistilled by using Clevenger apparatus for 3 hours. The oil was yellowish color with a strong aromatic smell. The percentage yield was 1.62%. 50 g of dry *S. molle* fruits (K.K) were hydrodistilled by using a Clevenger apparatus for 3 hours. The oil was almost identical to the previous oils in terms of physical appearance and odor. The percentage oil yield was 0.62%.

Table 2.1. Percentage yield of essential oils in leaves and fruits of *Schinus molle*

<i>Schinus molle</i>	NEU%	KK%
Leaf oil	1.88	1.62
Fruit oil	0.48	0.62



Figure. 2.3. Hydrodistillation

### 2.3. Gas chromatography analysis

The essential oils of *S.molle* were analyzed by GC/MS and GC/FID, simultaneously

#### GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MS system. Innowax FSC column (60 m\*0.25 mm film thickness) was used with helium as carrier gas (0.8 ml/min). GC oven temperature was kept at 60 C° for 10 min and programmed to 220 C° at a rate of 4 C°/min, and kept constant at 220 C° for 10 min. Then, programmed to 240 C° at a rate of 1 C°/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 C°. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 450.

#### GC analysis

The GC analysis was carried out using an agilent GC system. FID detector temperature was 300 C° to obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are given in table 3.1

Identification of the essential oil components was carried out by comparison of their retention times with those of authentic samples or by comparison of their Linear Retention Indices (LRI) to a series of n-alkanes. Computer matching against commercial (Wiley GC/MS library, MassFinder 3 Library) (MacLafferty and Stauffer, 1989 : Koenig *et al.*, 2004) and in house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (Joulain and Koenig, 1998: Eso 2000), was used for the identification.

# **CHAPTER 3**

## **RESULTS**

### 3.1. Result

#### 3.1.1. Chemical Composition of Essential Oils

The chemical composition of essential oils of leaves and fruits growing in North Cyprus are presented in Table 3.1. In total, 67 constituents were identified. The abundant component was  $\alpha$ -phellandrene in leaves and fruits of both NEU and K.K locations as 31.6% and 36.3%; 31.5% and 26.7%, respectively. The second main component for leaves and fruits of both locations were limonene by 11.4%, 13.3% for NEU, and 10.1%, 12.5% for KK, respectively. While,  $\beta$ -phellandrene took the third place in both leaves and fruits of both samples 10.9%, 12.2% for NEU, and 10.3%, 9.9% for KK, respectively. In addition, the fruits of both locations were rich in myrcene by 19.9% for NEU and 18.5% for KK. In the leaf oils of both locations, the percentage of myrcene was low (2%). On the other hand, bicyclogermacrene was a major constituent in the leaf oils of both locations by 11.1% for NEU and 12.0% for KK. However, the percentage of bicyclogermacrene in the fruit oils of both plants was not more than 2%. Moreover, fruits of KK contained the highest percentage of p-cymene (8.1%). In the other samples, the percentages were not more than 4.2%. Leaf oils of KK had the highest percentage of elemol (9.3%), while in leaf oils of NEU it was 6.3%.

Table 3.1. Chemical components of *Schinus molle* essential oil

LRI	Compound Name	KK% Fruit	NEU% Fruit	KK% Leaf	NEU% Leaf
1020	$\alpha$ -pinene	3.7	4.0	3.9	5.2
1119	$\beta$ -pinene	0.2	0.2	0.1	0.1
1131	sabinene	0.1	0.1	0.3	0.2
1173	myrcene	18.5	19.9	1.9	1.4
1178	$\alpha$ -phellandrene	26.7	36.3	31.5	31.6
1181	pseudolimonene	0.1	0.1	0.1	0.1
1212	limonene	12.5	13.3	10.1	11.4
1221	1,8-cineole	0.4	-	-	-
1223	$\beta$ -phellandrene	10.3	12.2	9.9	10.9
1263	(E)- $\beta$ -ocimene	-	-	0.1	-
1288	p-cymene	8.1	4.2	2.1	4.2
1299	terpinolene	0.1	0.2	0.2	0.2
1400	methyl octenoate	0.9	0.5	-	-
1499	bicycloelemene	-	0.1	1.0	0.5
1555	$\alpha$ -gurjunene	-	-	0.2	-
1556	linalool	0.1	-	-	0.1

1580	cis-sabinene hydrate	-	0.1	-	-
1581	trans-p-menth-2-en-1-ol	0.4	-	-	-
1605	bornyl acetate	0.1	0.1	-	-
1613	$\beta$ -elemene	0.1	-	0.7	0.4
1625	terpinen-4-ol	0.2	-	-	-
1627	$\beta$ -caryophyllene	-	-	0.2	0.2
1638	aromadendrene	0.1	0.2	0.3	0.4
1661	$\gamma$ -elemene	-	-	0.3	0.2
1685	allo aromadendrene	-	-	0.1	0.1
1688	epi-zonarene	-	-	tr	-
1701	trans-piperitol	0.2	-	-	-
1702	$\alpha$ -humulene	-	-	0.2	0.1
1710	cryptone	0.3	0.1	-	tr
1715	carvotanacetone	0.1	-	-	-
1717	$\gamma$ -humulene	-	-	0.1	-
1726	ledene	-	-	0.2	0.2
1742	valencene	-	-	-	0.3
1743	germacrene D	-	-	0.3	-
1752	$\alpha$ -muurolene	0.1	-	0.5	0.3
1756	$\beta$ -selinene	-	-	-	0.2
1759	selina-4,11 diene	-	-	-	0.2
1762	phellandral	0.1	-	-	-
1769	bicyclogermacrene	1.1	2.0	12.0	11.1
1786	$\delta$ -cadinene	0.3	0.1	1.4	0.6
1692	$\gamma$ -cadinene	-	-	0.2	0.1
1802	cadina-1,4-diene (=cubenene)	-	-	0.1	-
1822	cuminaldehyde	0.1	-	-	-
1836	p-mentha-1(7),5-dien-2-ol	2.1	0.8	0.2	-
1859	carveol	0.1	-	-	-
1871	germacrene B	-	-	0.1	-
1873	p-cymen-8-ol	0.1	-	-	-
1916	epicubebol	-	-	0.1	-
1931	$\alpha$ -phellandrene epoxide	0.5	0.1	-	-
2072	ledol	-	-	0.1	-
2085	germacrene D-4-ol	-	-	-	0.3
2092	cubenan-11-ol	-	-	0.1	-
2098	cubenol	-	-	0.1	-
2108	elemol	4.0	1.4	9.3	6.3
2124	viridiflorol	-	-	0.6	-
2148	10-epi- $\gamma$ -eudesmol	-	-	0.2	-

2160	spathulenol	2.0	2.0	2.0	5.3
2205	$\gamma$ -eudesmol	0.6	0.2	1.7	1.1
2217	eremoligenol	-	-	-	0.2
2222	T-muurolool	0.1	-	0.4	0.2
2226	$\alpha$ -guaiol	0.1	-	0.1	0.2
2231	$\delta$ -cadinol	-	-	0.1	-
2244	thymol	0.7	0.2	0.2	0.2
2261	$\alpha$ -eudesmol	1.2	0.7	1.8	2.2
2268	$\alpha$ -cadinol	0.2	-	0.8	0.3
2273	$\beta$ -eudesmol	1.4	0.6	1.9	2.4
	Total %	98.4	99.7	97.8	99.0

tr= trace (<0.1%)

**CHAPTER 4**  
**DISCUSSION**



#### 4.1. DISCUSSION

The results of this study have been compared with the previous results reported from Turkey and elsewhere.  $\alpha$ -Phellandrene was a main component in leaf and fruit oils from Turkey (45.7 % and 38 %), respectively (Baser *et al.*, 1997). However, no component in the leaf oils of the other Turkey sample was more than 7 % (Onder *et al.*, 2010). The leaf oils of Algeria contained  $\alpha$ -phellandrene (26.5 %),  $\beta$ -phellandrene (12.4%),  $\beta$ -eudesmol (14.8 %) and elemol (13.7 %) as main components (Belhamel *et al.*, 2008). While, the Egyptian fruit oil contained  $\alpha$ -phellandrene (25.1 %), limonene (20.9%) and myrcene (16.4%) as main components. In contrast, the Egyptian leaf oils contained p-cymene (9.4 %) as a main component, limonene,  $\alpha$ -pinene and myrcene contents were less than 5 % (Dalia *et al.*, 2016). The Tunisian leaf and fruit oils contained different main components. 6-epi-Shyobunol (16.2 %), limonene (15.3 %) and spathulenol ( 8.1% ) were the main components in the Tunisian fruit oils. Whereas, the leaf oils contained  $\beta$ -eudesmol (14.8 %), elemol (13.7 %) and  $\alpha$ -eudesmol as the major components (Abir *et al.*, 2016). The leaf oil of Costa Rican origin contained  $\alpha$ -pinene (22.7%) and  $\beta$ -pinene (31.1%) as main components (Diaz *et al.*, 2008). The Syrian fruit oils showed  $\alpha$ -phellandrene (24.8 %),  $\beta$ -pinene (14.7 %) and  $\beta$ -phellandrene (11%) as main components (Ibrahim and Al-naser, 2014). The Uruguaian laef oil contained bicyclogermacrene (29.2 %) as main the component (Carmen *et al.*, 2011).

The result of this study showed similarity with fruit and leaf oils from Turkey, While Algerian leaf oils and Syrian fruit oils by having  $\alpha$ -phellandrene and  $\beta$ -phellandrene as main components. Whereas, the differences in main components were observed Uruguaian oil, which contained bicyclogermacrene; and Costa Rican oils, which contained  $\alpha$ -pinene and  $\beta$ -pinene as major components.

Table 4.1. Main components of *Schinus molle* essential oil in different countries.

Main components	Turkey 1	Turkey 2		Egypt		Tunisia		Algeria	Costa Rica	Syria	Uruguay
	Leaf	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf	Leaf	Leaf	Fruit	Leaf
$\alpha$ -phellandrene	6.9	38	45.7	25.1	-	-	-	26.5	-	24.8	-
$\beta$ -phellandrene	-	11.7	13.6	-	-	-	-	12.4	-	11	-
$\alpha$ -pinene	-	-	-	2.5	4.4	-	-	-	22.7	-	-
$\beta$ -pinene	-	-	-	-	-	-	-	-	31.1	14.7	-
limonene	-	-	13.4	20.9	4.3	-	-	8.6	-	10.5	-
bicyclgermacrene	-	-	-	-	-	-	-	-	-	-	29.2
$\alpha$ -eudesmol	2.3	-	-	-	-	-	12.7	6.1	-	-	-
$\beta$ -eudesmol	-	-	-	-	-	-	14.8	-	-	-	-
elemol	5.1	-	-	-	-	-	13.7	10.8	-	-	-
6-epi-shyobunol	-	-	-	-	-	16.2	-	-	-	-	-
germacrene D	6.5	-	-	-	-	-	-	-	-	-	-
$\beta$ -caryophyllene	-	-	-	-	-	-	-	-	-	-	-
p-cymene	-	-	-	6.3	9.4	-	-	-	-	-	-
myrcene	-	-	-	16.8	4.4	-	-	-	-	-	-
limonene	-	-	-	-	-	15.3	-	-	-	-	-
spathulenol	-	-	-	-	-	8.1	-	-	-	-	-
References	(Onder <i>et al.</i> , 2010)	(Baser <i>et al.</i> , 1997)		(Dalia <i>et al.</i> , 2016)		(Abir <i>et al.</i> , 2016)		(Belhamei <i>et al.</i> , 2008)	(Diaz <i>et al.</i> , 2008)	(Ibrahim and alnaser, 2014)	Carmen <i>et al.</i> , 2011)

Table 4.2. Main components of *Schinus molle* essential oil (previous studies).

Country	% of Main components	References
Turkey 1	Leaf oils : $\alpha$ -phellandrene 6.9, germacrene D 6.5 and elemol 5.1	(Onder <i>et al.</i> , 2010)
Turkey 2	Leaf oils : $\alpha$ -phellandrene 45.5, $\beta$ - phellandrene 13.6 and limonene 13.4	(Baser <i>et al.</i> , 1997)
	Fruit oils : $\alpha$ -phellandrene 38 and $\beta$ -phellandrene 11.7	
Egypt	Leaf oils : p-cymene 9.4, myrcene and $\alpha$ -pinene 4.4	(Dalia <i>et al.</i> , 2016)
	Fruit oils : $\alpha$ -phellandrene 25.1, limonene 20.9 and myrcene 16.8	
Tunisia	Leaf oils : $\alpha$ -eudesmol 12.7, $\beta$ - eudesmol 14.8 and elemol 13.7	(Abir <i>et al.</i> , 2016)
	Fruit oils : 6-epi-shyobunol 16.2 and d-limonene 15.3	
Algeria	Leaf oils : $\alpha$ -phellandrene 26.5, $\beta$ - phellandrene 12.4 and elemol 10.8	(Belhamel <i>et al.</i> , 2008)
Costa Rica	Leaf oils : $\alpha$ -pinene 22.7, and $\beta$ - pinene 31.1	(Diaz <i>et al.</i> , 2008)
Syria	Fruit oils : $\alpha$ -phellandrene 24.8, $\beta$ - phellandrene 11 and $\beta$ - pinene 14.7	(Ibrahim and al-naser, 2014)
Uruguay	Leaf oils : bicyclogermacrene 29.2	(Carmen <i>et al.</i> , 2011)

**CHAPTER 5**  
**CONCLUSION**

### 5.1. Conclusion

$\alpha$ -Phellandrene, limonene, and  $\beta$ -phellandrene were the main components in essential oil of the leaves and fruits of *S. molle* grown in two locations of North Cyprus. Myrcene was abundant in both fruit samples and bicyclogermacrene was a main component in both leaf samples. In addition, fruits of *S. molle* of KK contained p-cymene twice as much as the oils of NEU location. *S. molle* is a promising plant since some of its components have pharmacological activities. For example,  $\alpha$ -phellandrene can be used for leukemia as an apoptosis enhancer (Lin *et al.*, 2013). In addition,  $\beta$ -phellandrene had anticociceptive activity in tail-flick test in mice (Him *et al.*, 2008). Limonene can improve wound healing, skin repair, and anti-inflammation (Alessio *et al.*, 2013).  $\alpha$ - and  $\beta$ -pinene were able to inhibit growth of potent infections like endocarditis caused by gram positive bacteria (Leite *et al.*, 2007). Further clinical studies need to be done to prove the efficacy of the oils and main components of *Schinus molle*.

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