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DEDICATION

I dedicate this work to my mother Fatima Nashota, may her soul rest in peace and may this booklet be as a source of Sadakatun Jariyahtun to her.

I also dedicate this project to my tender father Mustafa Aburwais, may Allah bless him.

Abstract

Zosima absinthifolia (Vent.) Link is a perennial herb which belongs to the Apiaceae family. It is distributed from Turkey to Iran, Pakistan and Central Asia. *Z. absinthifolia* is an aromatic plant and contains essential oils which can be obtained by distillation. The plant has traditional uses in Turkey and Iran.

In this study dried fruits of *Z .absinthifolia* collected from two locations in Northern Cyprus have been evaluated for essential oil yield and composition.

The Girne/ Alevkayası Armenian Monastery sample showed 32 compounds which have been identified representing 99.5% of the total oil with octyl acetate (63.2%), octyl hexanoate (18.6%), octyl octanoate (9.2%) and octanol (2.2%) as main constituents. Girne/ Alevkayası sample similarly showed 14 compounds which have been identified representing 99.6% of the total oil. Octyl acetate (59.5%), octyl hexanoate (19.8%), octyl octanoate (9.9%) and octanol (7.1%) were its major constituents.

Keywords: *Zosima*, essential oil, Apiaceae, octyl acetate

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Chapter 1: Introduction

Zosima absinthifolia (Vent.) Link is a perennial herb found in the family Apiaceae. This plant is a widely distributed from Iran to Turkey, Central Asia, Afghanistan and Pakistan. It normally grows in fields, steppe, and lime stone slopes at an altitude of the range 400-2000 m (Davis, 1972).

Zosima absinthifolia is known as peynir otu or ayi eli in Turkey, the plant serves as a spice in Iran and Turkey (Razavi et al., 2008). It can also be used for medicinal purposes as a digestive, anti-inflammatory and carminative agent in the Turkish traditional folk medicine (Tedavi, 2008). It is also mixed to a local cheese produced in East Anatolia (Aksakal and Kaya, 2008). The aerial parts of this plant can be eaten after been cooked in this same area (Ozcelik, 1994). Aerial parts of *Zosima absinthifolia* possess various medicinal value in Pakistan folk medicine to give relief against ingestion, bowel disorders and in the treatment of cough (Goodman and Ghafoor, 1992).

Some researchers have shown that the ethanolic extract of *Zosima absinthifolia* showed antimicrobial activity (Al-Shamma and Mitscher, 1979), likewise the methanolic extract showed anti-oxidative, phytotoxic and anti-proliferative properties (Razavi et al., 2008). Moreover, the antibacterial effects of these essential oils has been reported together with the isolation of three coumarin derivatives. The three coumarin derivatives auraptene, imperatorin and 7-prenyloxycoumarine having allelopatic effect were isolated from the *n*-hexane extract of the plant (Najed-Ebrahim and Razavi, 2008).

Crowden et al., (1969) reported that coumarin derivatives such as deltoin, columbiandin, imperatoin, pimpinelin, umbeliferone, bergapten and sophodin together with some alkaloids were isolated from *Z. absinthifolia* (Crowden, Harborne and Heywood , 1969).

Başer et al., (2000) reported that the essential oil distilled from dried plants were analyzed using Gas chromatography/ Mass spectrometry (GC-MS) and sixteen compounds were characterized representing 95.8% of the oil (Başer et al., 2000).

In these studies, we have investigated the composition of the essential oils present in *Zosima absinthifolia* fruits collected from two localities in Northern Cyprus with the use of GC-MS (Gas Chromatography Mass Spectrometry), and GC-FID (Gas

Chromatography Flame Ionization Detector) techniques. Yields of the essential oils were also determined.

1.1 Essential oils

The first person to systematically investigate on the constituents present in essential oils was M.J Dumas (1800-1884) a French chemist that studied some hydrocarbons, sulphur, oxygen and nitrogen-containing compounds. Later on, a French researcher known as M. Berthelot (1859) using optical rotation characterized several natural products and their derivatives. The use of ultraviolet (UV) spectroscopy in the elucidation of structure of terpene and other substances that occur naturally was applied extensively by R.B Woodward in the early forties. He later developed a rule (called the Woodward rules) that can be applied in investigating the structures of new natural products through correlation between the position of UV maximum absorption and the substitution pattern arrangement of α, β-unsaturated ketone or a diene (Woodward, 1914).

The introduction of chromatographic separation methods and spectroscopic methods such GC-MS, NMR, FT-IR into medicinal chemistry helped a lot in the investigations of the structures of terpenes. The advancement of analytical methods in last fifty years helped in the rapid growth of knowledge in the field of essential oil constituents. In the last century, many methods were developed and applied in the elucidation of essential oils.

Essential oils (EOs) are natural volatile compounds comprising such as mono and sesquiterpenoids, phenylpropanoids and benzenoids, etc. which possess bioactivities.

1.1.1 Sources of Essential Oils

Essential oils are mixtures of volatile complex compounds which are produced by living organisms and are isolated through distillation and expression in the case of citrus oils from an entire plant or certain parts of the plant of a well-known and recognized taxonomic origin. The major components are derived generally through biosynthetic pathways, which consists of the *methyl-erithrytol-pathway* which leads to mono and diterpenes, the shikimic acid pathway leading to phenylpropenes and *mevalonate pathway* that leads to sesquiterpenes. There are numerous number of single aroma substances and many diverse volatile chemicals as components of these Eos. Most of these volatile compounds possess different ecological importance. They serve as defense against herbivores, internal messengers or as volatiles that not only directly act as natural enemies to these herbivores but also serves as attracting pollinating insects to their hosts (Harrewijin et al., 2001).

Lots of plants have the ability of synthesizing volatile compounds but most times in little amounts. But "Essential oil bearing plants", particularly are those species that deliver an essential oil for commercial purpose. Generally, two principles are to be considered before a plant can be considered as an essential oil plant.

a) An extraordinary blend of volatiles such as the scents of flower in rose (*Rosa X damascena Mill,*), tuberose (*Polyanthes tuberosa* L.) or Indian Jasmine (*Jasminum sambac* (L.) Aiton. Such flowers immediately produce and emit the volatiles by the epidermal layers of their petals (Bergougnolix et al., 2007). Apart from distillation other special techniques such as enfleurage or extraction with solvents can be utilized for recovering the volatile fragrance substances from such products.

b) The releasing and accumulation of volatiles in a highly specialized anatomical structures. These anatomical structures that store essential oils can be cavities/ducts, glandular trichomes or secretory idioblasts known as secretory cells. From such drugs essential oils are extracted by distillation or expression in the case of fresh citrus fruits (Fahn, 1979; Svoboda et al., 2000).

The chemical contents of essential oils usually vary between different plant organs. Phytochemical polymorphism is a usual case between different plant parts. For example in the case of *Origanum vulgare* spp. *hirtum,* the polymorphism within a plant can be found at the lower level, specifically between different oil glands of a leaf (Johnson et al., 2004). This kind of polymorphism do not occur frequently, mostly differences in the compositions of the plant are due to the age of the plant (Grassi et al., 2004; Johnson et al., 2006; Schimederer et al., 2008).

Mostly, these differences are due to genetic factors, not only to environmental conditions. Numerous intraspecific polymorphisms are yet to be discovered or have been discovered recently, likewise in the essential oil crops that have been used widely such as sage (Novak et al., 2006; Nemeth-Zamborine, 2016).

1.1.2 Production of essential oils

Natural volatiles have been known to man for centuries, Assyrians and Egyptians knew how to extract them with fixed oils, Indians had discovered how to distill them using terracotta stills 5000 years ago (Schmidt, 2016).

As the industrial revolution started, the synthetic chemicals came into being during the nineteenth century. The production of aroma chemicals made an impact in our life style. Production of essential oils is carried out worldwide. In Europe, the main producing countries are located in areas bordering the Mediterranean Sea, such as countries like Spain, Italy, Portugal, France, Greece, Albania, Turkey and Croatia. They all produce essential oils in significant quantities, mainly for industrial purposes. Also, in Central European countries; Romania, Bulgaria, Ukraine and Hungary can be mentioned. South Asian countries due to their diverse climates are suitable for essential oils production. India and China play the main role proceed with Indonesia and Vietnam. The countries that produce essential oils in Africa consists of Egypt, Tunisia, Morocco and Algeria. While Ivory Coast, Ghana, Kenya, South Africa, Tanzania, Ethiopia and Uganda produce essential oils in smaller quantities. In the American continent, the major producing countries are Canada, U.S and Mexico. They have high amount of essential oil plant materials. But in South America, essential oils are produced in Argentina, Brazil, Paraguay, Uruguay, Haiti and Guatemala. Other essential oil producing countries include Taiwan, Japan, Germany, Philippines and Jamaica.

Essential oil productions differs widely and it is not easy to predict. The highest yields are mainly associated with balsams and other resinous plant exudates, like copaiba, gurjun, Peru balsam, tolu balsam and elemi which ranges between 30-70%. Nutmeg and clove buds can yield essential oils to the percentage of 17% and 15% and other important examples such as cardamom (about 8%), fennel, caraway seed, star anise and cumin seed (1-9%). Most of the oils with low yield are produced with juniper berries, whereby 75kg of the berries are needed to yield 1 kg of oil, other leaf oils e.g. geranium (about 0.15%) and sage (about 0.15%). Likewise, 1000 kg of bitter orange flowers can be used to yield 1 kg of oil and 3.5 to 4 tons of fresh Damask rose flowers are needed to distill 1 kg of rose oil. Also, the yield of peel oil of expressed fruit peels like bergamot, lemon and orange ranges between 0.2 to almost 0.5% (Susan et al., 2004).

There are certain agronomic factors that have to be looked upon prior to the production of essential oils, which consists of climate, soil type, cultivation practices, drought and water stress, influence of insects and microorganisms and seed propagation. Others are the position of the cells that produces oil in the plant, method of cultivation and the preparation of biomass before the essential oil extraction.

1.1.3 Chemical composition of essential oils

Generally, chemicals that are produced naturally are classified into two classes, which comprise the primary and secondary metabolites. The primary metabolites are the metabolites which can be found universally around the plants and animals and comprise the primary blocks of builders of life. There are four sub-groups of these primary metabolites which include carbohydrates, proteins, lipids and nucleic acid. These classes contribute less to essential oils even though some essential oils consists of the smallest unit of these groups, especially the lipids which is also the most significant. While secondary metabolites are the most significant and backbones of essential oils which are normally classified into; shikimates, polyketides, terpenoids and alkaloids. The most vital secondary metabolites as far as essential oils are concern are the terpenoids followed by shikimates.

The general biosynthetic reactions of these compounds are via photosynthesis whereby, green plants convert water and carbon dioxide to give glucose (Bu’ lock, 1965; Mann et al., 1994).

Chemical breakage of glucose give phosphoenolpyruvate and it is the key and building block of shikimate family of natural compounds. Acetyl CoA is produced from the esterification of the two carbon unit of acetate produced from through decarboxylation of phosphoenolpyruvate with the help of coenzyme-A. Self-condensation of these species results in the production of lipids and polyketides. Acetyl CoA serves also as the starting material in the production of mevalonic acid that is the main starting material for the terpenoid synthesis (Sell, 2003).

Most of the enzymes involved in these reactions require cofactors as energy providers or reagents as seen in the case of Coenzyme-A above, which is a thiol used in the formation of thioesters with carboxylic acids. This affects the acid in two ways. First, the thiolate anion is a good leaving group when compared with the alkoxide and therefore the carbonyl carbon of the thioester can react toward nucleophiles. Second, the acidity of the proton adjacent to the carbonyl is increased by the thioester group and these helps in the synthesis of the corresponding carbanions.

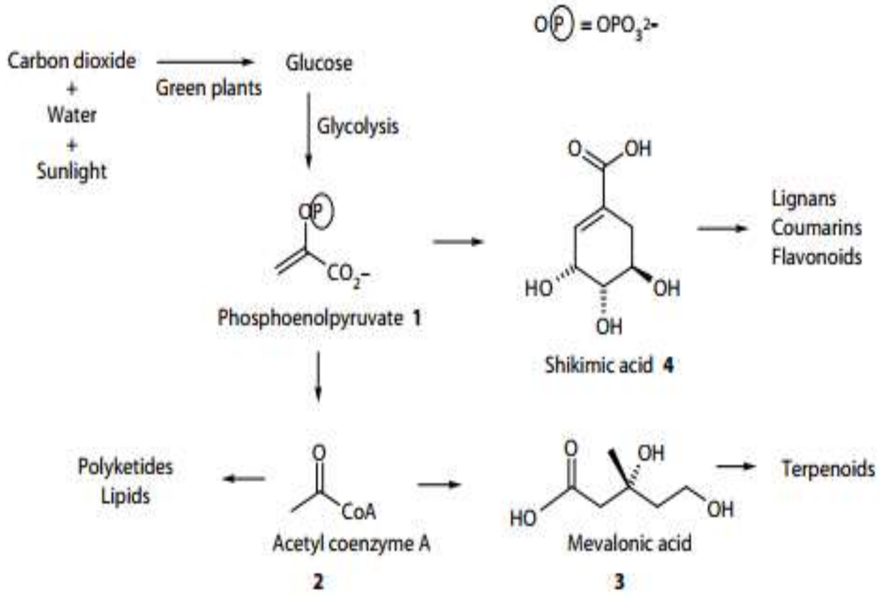

Fig. 1.1: Biosynthesis of the formation of an Essential oil

1.1.3.1 Terpenoids

These are the most vital group of natural products especially in the aspects of essential oils. Previous years in the older literatures they are referred to as 'terpenes' but nowadays this is only strained to monoterpenoid hydrocarbons. These compounds consist of isoprene (2 methyl butadiene) units. Isoprene is not normally found in essential oils and it is not an intermediate in biosynthesis.

The coupling direction of isoprene units is normally in one direction, which is the head-to-tail coupling. This is shown in fig. 1.2. The branched end of the chain is considered as the head while the other as the tail of the molecule (Bu' Lock, 1965; Croteau, 1987; Mann et al., 1994).

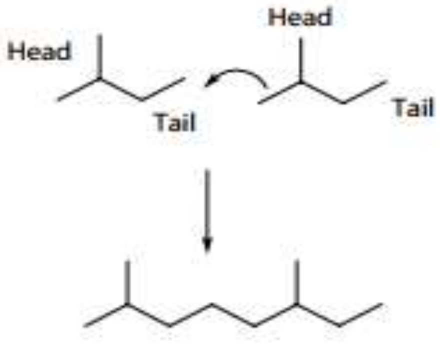

Fig. 1.2: Head-to-tail coupling of two isoprene unit

The main intermediate is mevalonic acid that is produced from three molecules of acetyl CoA. Phosphorylation of mevalonic acid proceeded by elimination of the tertiary alcohol and the concomitant decarboxylation of the adjacent acid group yield isopentenyl pyrophosphate. This can be isomerized to produce prenyl phosphate. The coupling of these two 5-carbon units gives a 10-carbon unit known as geranyl pyrophosphate. The continuous addition of isopentenyl pyrophosphate results in the formation of 15, 20-, 25- and so on carbon units.

Terpenoids generally consist of multiple of five carbon atom at the time of their first formation. Monoterpenoids have been the first terpenoid to be under study and it consists of 10 carbon atoms per molecule. While those having five carbon atoms are called hemiterpenoids, and those with 15-carbon atoms are called sesquiterpenoids and those having 20-carbon atoms are known as diterpenoids, etc. In conclusion, it is only the monoterpenoids, hemiterpenoids, sesquiterpenoids and some diterpenoids which are found to be sufficiently volatile are the components of essential oils (William 1996).

1.1.3.1.1 Hemiterpenoids

Minor components of essential oils constitute of many aldehydes, alcohols and esters having 2-methyl butane skeleton. In biosynthesis of common essential oils, mostly the oxidation pattern is that of prenol (3-methylbut-2-en-1-ol). The typical example is that the acetate of such alcohols exists as ylang ylang and other oils. Moreover, oxidation has been identified at all positions. Most esters *e.g.* prenyl acetate produce fruity high notes to oils containing them while the corresponding thioesters participate in the characteristic odors of galbanum.

1.1.3.1.2 Monoterpenoids

The main precursor of monoterpenoids is *geranyl pyrophosphate*. The chemical breakage of its carbon-oxygen bond produces the geranyl carbocations. But in the natural systems, this and other carbocations do not exist as free ions rather in the form of incipient carbocations which are held in the active sites of enzyme and are used primarily in cationic reaction through the use of a suitable reagent. But in this research, for simplicity, we are considering them to be carbocations. All the reactions are under enzymic control and the enzymes presents in any given plant will determine the kind of terpenoids it will produce.

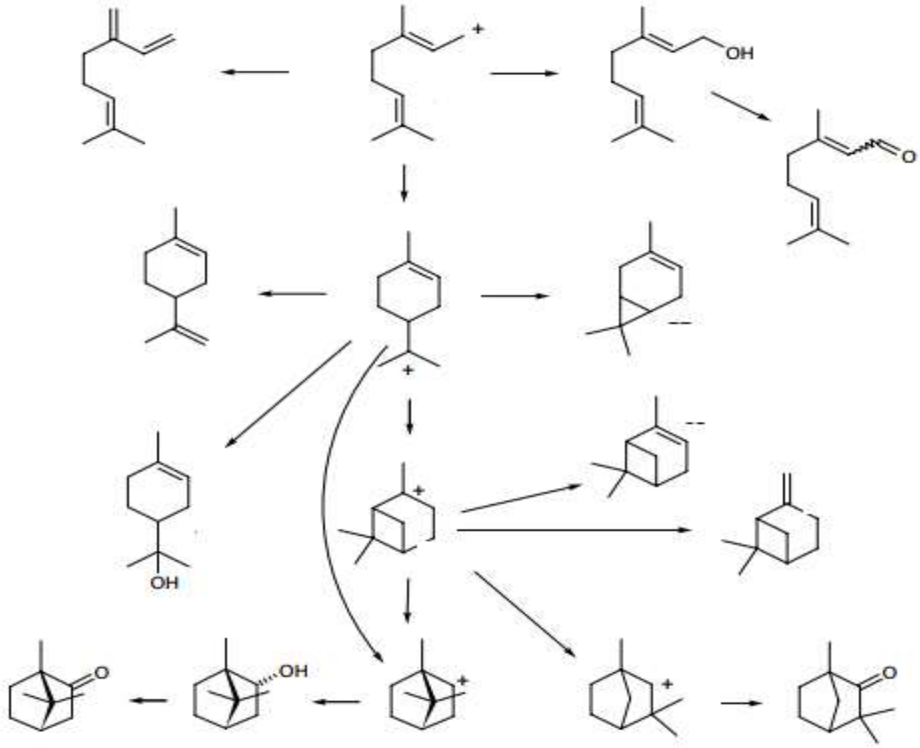

Fig 1.3: Formation of monoterpenoid skeletons

1.1.3.1.3 Sesquiterpenoids

From the definition, these terpenoids contain 15 carbon atoms. This leads to lower volatility and hence have higher boiling points compared to monoterpenoids. Little percentage of sesquiterpenoids contributes to aromatic fragrance of essential oils though those with low-odor thresholds contribute immensely as end notes. They are equally vital as fixatives for more volatile compounds.

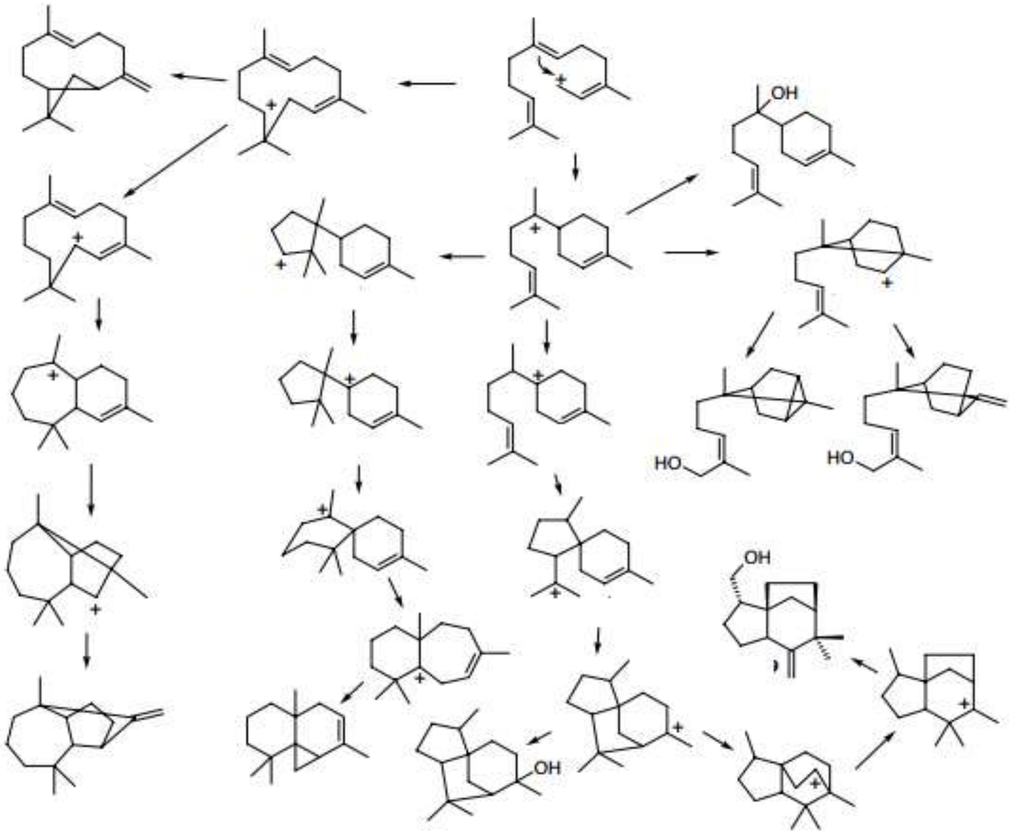

Fig. 1.4: Some biosynthetic pathways from (Z, E)-Farnesol

The main precursor of sesquiterpenoids is referred to as farnesol. Its pyrophosphate is produced naturally through the addition of isopentenyl pyrophosphate into geranyl pyrophosphate. The hydrolysis of that also gives farnesol. Incipient heterolysis of the carbon-oxygen bond of the phosphate gives the nascent farnesyl carbocations and this results in the formation of other sesquiterpenoids as in the case of the geranylcarbocations to monoterpenoids. Starting from farnesyl pyrophosphate, there is high tendency for the formation of many possible cyclic structures when compared with geranyl pyrophosphate due to the presence of three double bonds in farnesyl pyrophosphate molecule. The geometry of farnesol's double bond in position 2 is important especially in determining the pathway applied for the next cyclization reactions (Devon and Scott, 1972).

1.1.3.2 Shikimic acid derivatives

Shikimic acid is the vital biosynthetic precursor in plants, because it is the main intermediate for both the lignin and flavonoids. The flavonoids are so significant to plants as protection against ultraviolet light, colors and antioxidants etc. while lignin is the building block for the structural materials of the plants, more especially the woody tissues. Phosphoenolpyruvate and erythrose-4-phosphate are used in the synthesis of shikimic acid. Its biosynthetic pathway starts from carbohydrate. Its derivatives can easily be recognized through the characteristic shikimate pattern of the six-membered ring having either one- or- three carbon substituent at the first position and oxygenation at the third position and or fourth and fifth positions. But worthy of note, the oxygen atoms present at the final product are not the ones present at the starting shikimate because they are already lost at the beginning and then replaced (Bu' Lock, 1965; Mann et al., 1994).

Fig1.5. shows some important biosynthetic intermediates that stem from shikimic acid and are important in generating volatile materials that can be used as parts of essential oil components. Elimination of one of the alcohols in the ring and the reaction with phosphoenolpyruvate yields chorismic acid that can be further undergo oxy-cope reaction to produce prephenic acid. Elimination and decarboxylation of the ring alcohol produces the phenylpropionic acid skeleton. Amination and the reduction reaction of the ketonic group yield an essential amino acid phenylalanine. But the reduction and elimination can give cinnamic acid, and the ring hydroxylation of cinnamic acid to give the isomeric ortho and para coumaric acids respectively. Further hydroxylation results in formation of caffeic acid and further methylation results to the formation of ferulic acid. The oxidation of methyl ether of ferulic acid and further cyclization produces methylenecaffeic acid.

Aromatization of shikimic acid in the absence of adding the additional three carbon atoms from the phosphoenolpyruvate, produces benzoic acid derivatives. Benzoic acid by itself exists in various essential oils and its esters are widely spread. Methyl benzoate for example is found in ylang ylang, tuberose and many lilies. The simpler ones consist of benzaldehyde, benzyl alcohol and their derivatives (Arctander, 1960; Gildemeister and Hoffman 1956; Gunther, 1948). Benzyl alcohol can be found in jasmine and narcissus. More also, its acetate is the main component of jasmine oils. The main sources of benzaldehyde are apricot kernels and almond but it is widely found in flowers such as lilac and in some oils like cinnamon and cassia. Hydroxylation and or amination of benzoic acid result to further series of some natural products and the most significant and important, in terms of odor of essential oils (Başer and Buchbauer, 2010).

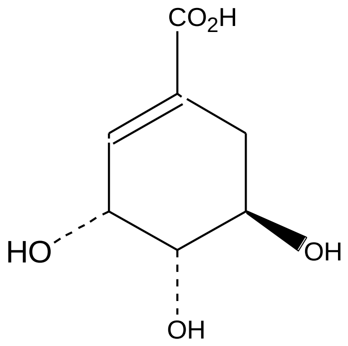

Fig. 1.5: Chemical structure of shikimic acid

1.2 Taxonomy of ***Zosima absinthifolia*** (Vent.) Link

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae

Genus: *Zosima*

Species: *Zosima absinthifolia*

Fig. 1.6 Image of *Zosima absinthifolia*

1.3 Apiaceae family

Apiaceae (Umbelliferae) family is outstanding amongst other known families of flowering plants, which contain 300-450 genera and 3000-3700 species. Plants having a place with the family Apiaceae are broadly utilized for sustenance and medicinal purposes. A few plants of this family, for example, carrots, parsley, and celery are basic vegetable products, while others like anise, coriander, cumin, fennel, and dill are well known for their restorative and fragrant properties. Apiaceae plants are aromatic plants that have a distinctive flavour due to diverse volatile compounds in their fruits, roots and leaves.

The plants of Apiaceae (Umbelliferae) family occur world-wide, yet it is most normal in mild areas and uncommon in the tropics. The most evident particular element of the family is the inflorescence, which is a straightforward or compound umbel. Umbelliferae alludes to the trademark umbellate inflorescence. Having reciprocally symmetrical blossoms towards the outside of the inflorescence tends to influence the inflorescence all in all to look like a solitary bloom (Berenbaum, 1990; Christensen and Brandt, 2006).

Apiaceae is an interesting family comprising many aromatic taxa including commercialized herbs and spices such as anise, fennel, cumin, caraway, coriander, chervil, parsley, carrot, asafoetida, galbanum, etc. Some are rich in essential oils. The most interesting feature of the family is that many of its members have high chemical diversity containing different aromatic chemicals in different organs. Therefore, it is advisable to study all parts (*e.g.*, fruit, flower, stem, leaf, and root) of an Apiaceae plant separately in order to get a complete chemical profile of that taxon (Başer and Kirimer, 2014).

The family is rich in secondary metabolites and embodies numerous genera of high economic and medicinal value. Numerous compounds, belonging to different chemical classes, have been isolated from essential oils of umbelliferous crops (Rechinger 1972; Margaris, 1999; Heywood, 1999; Ebadollahi, 2013). Many umbelliferous crops have medicinal uses for gastrointestinal complaints, cardiovascular ailments, and as stimulants (Heywood, 2007).

Fruit type in Apiaceae is *schizocarp* meaning that seed is fused with the fruit in such a way that it is impossible to separate them from each other. This is the reason why Apiaceae fruits are often called *seeds* such as aniseeds, cumin seeds, coriander seeds, fennel seeds, etc. (Başer and Kirimer, 2014).

The plants of the family Apiaceae have been utilized for different purposes in various areas around the world. For instance, in Eastern Anatolia, Van herby cheddar is a renowned dairy item containing different plant species from this family (Özçelik, 1989).

1.4 The genus *Zosima*

The genus *Zosima* is a member of the family Apiaceae which also belongs to the major group Angiosperms (flower bearing plants), this genus was first introduced in 1814 by Hoffman, who described it based on *Sphondylium orientale* which is a name written on a label of herbarium. He later differentiated genus *Zosima* from the genus *Heracleum* based on the fruits possessing hyaline wings, and round the lateral ridges form a thickened rim. (Hoffman, 1814). The genus *Zosima* was later studied by Candole in the year 1830, Bentham in the year 1867 and Boissier in the year 1872. This genus is also recognized for possessing four species namely; *Z. absinthifolia* (Vent) Link, *Z. korovimi* Hirde, *Z. gilliana* Hirde and *Z. radians* Boissier. *Z. gilliana* is found mainly at Afghanistan and North-Western of Pakistan. *Z. radians* is found in Iran, *Z. korovini* at Kirghizstan and lastly *Z. absinthifolia* is found growing widely in Central Asia and south west (Menemen and Jury, 2001).

Table 1: Taxonomic treatment of the genus *Zosima* by some scientists

Author	Tribe and subtribe	Taxa
HOFFMAN (1814)		*Z. orientale* Hoffman
LINK (1821)		*Z. absinthifolia* Vent Link
DE CANDOLE (1830)	Peucedanea	*Z. anethifolia, Z .absinthifolia* De Candole
BENTHAM (1867)	Peucedanea	*Z. absinthifolia var radians* Bentham
BOISSIER (1872)	Peucedanea-Eupeucedanea	*Z. absinthifolia, Z .radians, Z. tragiodes, Z. frigida, Z .lasiocarpa, Z. dichotoma* Boissier
DRUDE (1898)	Peucedanea-Tordylinae	*Z. absinthifolia, Z. tragiodes, Z. frigida, Z. lasiocarpa, Z. dichoma* Drude
SCHISCHKIN (1951)	Peucedanea	*Z. tordyliodes, Z. absinthifolia* Schishkin
HIRDE (1979)		*Z. absinthifolia Z. tragiodes, Z. pimpinellioides, Z. bucharia, Z. depauperata, Z. heterodonta, Z. komarovii, Z. rubtzovii* Hirde
MOZAFFARIAN (1983)		*Z. radians, Z. absinthifolia, Z. frigida, Z. dichotoma* Mozaffarian
ALAVA (1987)		*Z. absinthifolia, Z. radians, Z. gillana* Alava
Eller & HEYN (1993)		*Z. absinthifolia, Z. radians* Eller & Heyn

1.5 *Zosima absinthifolia*

Z. absinthifolia is a perennial plant and its flowering period starts at the mid of April and proceed throughout July. It has a taproot system. Its height ranges from 30 to 100 cm. Its stem is straight or sometimes deeply sulcate and hairy and it produces a rigid fibrous collar, which is the remnant of the basal leaves just above the root. Its leaves are alternate and are differentiated into a distinct petiole and a pinnate lamina. The leaves are generally 2-3 pinnate. The radical leaf sizes of this plant is 150-400 X 600 mm. It possesses 3-4 primary leaf segments. The anatomy of the leaves is so much similar to that of the stem in the sense that they are hairy on both sides. It has compound umbels, but in this case if two to three lateral umbels meet and branch from the same node and at the top of the plant, it is referred as complex compound umbel, but if the lateral umbels meet alternatively, it is then referred to as simple compound umbel. But generally it has a tendency of forming simple compound umbel than a complex compound umbel. The diameter of these umbels varies from 35 to 140 mm and are equal or almost equal. Also, the number of its rays range from 15 to 40. This plant has the presence of bracts which varies from 5 to 8. Its shape is usually linear or linear-lanceolate with ciliated margin and hairy. It also possesses bracteoles which vary from 5 to 7.

The plant is characterized with the absence of sepal teeth or sometimes minute if present. Its petals are hairy on their dorsal surfaces and are whiter in colour which varies from 1 to 7 mm in length. The fruits of this plant are dorsally compressed and the mericarps elliptic. Its fruits range between 7.5 and 12 mm long and its style is curved. The apex of the mericarps is slightly or completely emarginated. The cells on the dorsal surface of the fruit form a reticulate pattern. The surface between the two ribs of the plant is striate, the margin of the mericarp surface is so much similar to that of the surface between the two ribs. The number of dorsal vitae in this plant is four. But there is just one vittae between the two dorsal ribs which totally fills it having a width range of 0.16 to 0.5 mm. This plant possesses oval pollen grains of which its outer contour is mostly straight and the polar ends are round. It has a cerebroid tectum (Menemen and Jury, 2001).

Fig. 1.7 Image of *Zosima absinthifolia*

1.5.1 Origin and distribution of *Zosima absinthifolia*

Zosima absinthifolia is found in Turkey, Iran, Iraq and different countries of the Middle East and Central Asia. *Z. absinthifolia,* and *Z. korovinii* are perennial. *Z. gilliana*, and *Z. radians* are biennial. All species have taproots. The plant has notched pubescent stems that compass up to 1 m in tallness. This far reaching plant has tripinnate leaves, 10– 25 rayed umbels, greenish to light yellow blossoms and elliptic to deter organic products with tumid edge (Davis, 1972).

The flowering time frame begins in April and proceeds all through July. Stem, indumentum and height of the plants ranges from 30 to 100 cm in *Z. absinthifolia* (Menemen and Jury, 2001).

1.5.2 Uses of *Zosima absinthifolia* (traditional and medicinal uses)

Zosima absinthifolia is an important plant due to its wider range of applications and uses especially for medicinal and other traditional uses.

This plant has been produced in Iran due to its aromatic fruits that are utilized as a condiment. They can be crushed and used as a flavouring agent in cookies, pickles and seasoning. They serve as spice for a native food called Golpar in Iran. The condiments prepared from this plant improve the flavour and taste, and make food delicious as well, it has a positive impact in the human body. It has the ability to destroy and remove pathogenic microorganisms, serves as cancer chemo-preventive agent and can also act and serve in numerous biological effects due to their potency in treating some ailments (Seidman, 2005).

It has been reported to have higher antibacterial activity against gram positive bacteria such as *Bacillus pumilus* and *Bacillus subtilis* based on a study showing that *Zosima absinthifolia* seeds with major components of octyl acetate (87.4%), octyl octanoate (5.0%) and 1-octanol (2.3%). It has also been reported to exhibit cytotoxic activity, antimicrobial and antioxidative activity (Razavi et al., 2008).

The elevated parts of the plant are devoured as vegetable prior to being well cooked. Its fruit has ethnobotanical use in Turkish folk medicine due to its carminative, digestive and anti-inflammatory properties. Furthermore, its aerial parts are used in the treatment of indigestion, cough, stomach gas and bowel disorders (Goodman and Ghafoor, 1992).

Recent phytochemical analyses have shown the presence of coumarins such as zosimin, deltoin, bergapten, pimpinelin, isobergapten, isopimpinelin, umbelliferone and sphondin in *Z. absinthifolia*. These coumarins are natural compounds that can be found in plants, some animal species and microorganisms (Lake, 1999).

They can be used as food and cosmetics additives for products such as soap and perfumes and in certain alcoholic beverages and tobacco products (Al-Haiza et al. , 2005).

Previous studies have shown that ethanolic extract of the aerial part of this plant has shown antimycobacterial activity (Al-Shamma and Mitscher, 1979) and the methanolic extract of its fruits has shown significant antioxidant, phytotoxic and antiproliferative activities (Razavi et al., 2008).

1.5.3 Chemical composition of *Zosima absinthifolia*

Başer *et al.,* (2000) reported that the hydrodistilled essential oil of dried fruits from *Zosima absinthifolia* was analysed using GC-MS. The result showed sixteen components, representing 95.8% of essential oil of *Z. absinthifolia.* They were further characterized and the major components were octyl acetate 38.4%, octyl hexanoate 31.9%, and octanol 12.9%. Shown in the table 2 below (Başer et al., 2000).

Table 2: The composition of the essential oil of *Z. absinthifolia* (Vent.) Link

Compound	%	RI^a	RI^b
Octyl acetate	38.4	1483	1565
Octyl hexanoate	31.9	1815	1050
Octanol	12.9	1562	1759
Octyl octanoate	6.0	2020	-
Osthol	1.5	2449	975
Octanal	1.0	1296	1367
Octyl butyrate	2.0	1624	1078
Linalool	0.4	1553	1271
Carvacrol	0.4	2240	1937
Hexadecanoic acid	0.3	2931	1193
Thymol	0.2	2205	1262
α-Pinene	0.2	1118	968
Germacrene D	0.2	1726	1472
β-Bourbonene	0.2	1535	1380
Nonacosane	0.1	2900	2900
Decanal	0.1	1506	1179

Note: RI^a Retention index on polar column (Innowax)

RI^b Retention index on non-polar column (CPSil5CB)

Razavi *et al.*, (2008) reported that the hydrodistilled dried fruits of *Z. absinthifolia* yielded 2.4% (v/w) of colourless oil having a characteristics odour. Twenty-four components were identified in the fruits using GC-MS. The main component of the oil was octyl acetate (87.5%) and the minor components were octyl octanoate (5.0%), 1-octanol (2.4%), hexyl hexanoate (1.5%), and octanoic acid (1.1%) as shown in Table 3 (Razavi et al., 2008).

Table 3: Chemical composition of the essential oils of *Z. absinthifolia* (Vent.) Link

Compound	%	R1
α-Pinene	0.07	937
Camphene	0.02	953
Hexanoic acid	0.01	968
Sabinene	0.01	975
2,3-Dehydro-1,8-cineole	0.02	992
Octanal	0.61	997
Hexyl acetate	0.02	1005
p-Cymene	0.04	1024
Limonene	0.05	1030
1,8-Cineole	0.2	1033
Phenyl acetaldehyde	0.01	1040
1-octanol	2.37	1065
Octanoic acid	1.1	1182
(Z)-3-octen-1-ol	0.54	1191
Octyl acetate	87.48	1212
Bornyl acetate	0.37	1287
Octyl propionate	0.07	1294
Citronellyl acetate	0.03	1345
Octyl butanoate	0.13	1380
Decyl acetate	0.12	1400
Octyl 2-methyl butyrate	0.16	1425
β-caryophyllene	0.02	1430
Hexyl hexanoate	1.52	1572
Octyl octanoate	5.03	1771

Note: R1 = Observed retention indices.

The major compounds present in the oil are classified as; esters (94.93%), alcohols (2.9%), fatty acids and terpenes (less than 1%) shown in table 4 below (Razavi et al., 2008).

Table 4: Chemical class distribution of the essential oil components of *Zosima absinthifolia* fruits.

Compound class	% Area	NO. of compounds
Esters	94.93	10
Alcohols	2.91	1
Fatty acids	1.11	2
Aldehydes	0.62	2
Terpenes	0.43	6

1.6 The aim of the study

The aim of this research was to investigate the yield and composition of essential oils of *Zosima absinthifolia* (Vent.) Link fruits (Apiaceae family) collected from two locations in Northern Cyprus using GC-FID (Gas Chromatography Flame Ionization Detector) and GC-MS (Gas Chromatography Mass Spectrometry) techniques.

Chapter 2: Material and Method

2.1 Plant material

Fruits of two samples of *Zosima absinthifolia* were collected. Sample 1 was collected in a garden of Armenian Monastery in Girne/ Alevkayası at Turkish republic of Northern Cyprus, on the 3rd May, 2016. And sample 2 was collected at the entrance of Girne/ Alevkayası region on the 1st June, 2016. The plants were then identified through taxonomical process followed by documentation and authentication in the herbarium with herbarium numbers (6887) and (6894) for sample 1 and sample 2, respectively and deposited at Near East University Pharmacognosy Department.

2.2 Extraction of essential oils by Clevenger apparatus

Distillation process was carried out to distil the oils from the dried plant material using a Clevenger apparatus. For sample 1, 54 grams and for sample 2, 42 grams of dried fruits were used. They were placed in the Clevenger apparatus and 500 ml of water was added. The apparatus was further turned on for three hours until the required amount of oils were produced.

Finally, the samples were further analysed using GC-FID (Gas Chromatography Flame Ionization Detector) and GC-MS (Gas Chromatography-Mass Spectrometry).

2.3 Analysis by GC-MS and GC-FID

2.3.1 Gas Chromatography Mass Spectrometry (GC-MS)

The essential oils of *Zosima absinthifolia* were characterized using GC-MS system. The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was utilized 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 and then modified 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.

2.3.2 Gas Chromatography Flame Ionization Detector (GC-FID)

The GC analysis was carried out using an Agilent 6890N 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. Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (Mac Lafferty and Stauffer, 1989) and (Koenig, Joulain and Hochmuth, 2004) and in-house “Başer 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) and (ESO, 1999), was used for the identification.

Chapter 3: Results and Discussion

Table 5: Essential oil composition of *Zosima absinthifolia* collected from Girne/ Alevkayası in Armenian Monastery location

LRI	Compound	%
1032	α-Pinene	Tr
1048	2-Methyl-3-buten-2-ol	0.1
1093	Hexanal	0.1
1194	Heptanal	0.1
1244	2-Pentyl furan	Tr
1255	γ-Terpinene	0.1
1280	p-Cymene	Tr
1296	Octanal	0.3
1327	3-Methyl-2-butenol	Tr
1400	Nonanal	0.1
1483	Octyl acetate	63.2
1516	(Z)-4-Octenyl acetate	0.5
1535	β-Bourbonene	Tr
1562	Octanol	2.2
1612	β-Caryophyllene	0.5
1623	Octyl butyrate	1.0
1634	Octyl 2-methyl butyrate	0.1
1726	Germacrene D	0.1
1755	Bicyclogermacrene	0.1
1829	Octyl hexanoate	18.6
1856	(Z)-4-Octenyl hexanoate	0.1
1893	Octyl heptanoate	Tr
2008	Caryophyllene oxide	0.2
2020	Octyl octanoate	9.2
2084	Octanoic acid	0.3
2144	Spathulenol	0.1
2298	Decanoic acid	0.1

2300	Tricosane	0.4
2500	Pentacosane	0.1
2503	Dodecanoic acid	0.7
2670	Tetradecanoic acid	0.8
2931	Hexadecanoic acid	0.4
	Total	99.5

LRI: Linear retention indices calculated against *n*-alkanes

%: calculated from FID data tr: Trace (<0.1%)

Table 6: Essential oil composition of *Zosima absinthifolia* fruits collected from Girne/ Alevkayası location

LRI	**COMPOUND NAME**	**%**
		A
1303	Octanal	0.3
1487	Octyl acetate	59.5
1522	(Z)-4-Octenyl acetate	0.5
1567	Octanol	7.1
1611	(Z)-3-Octen-1-ol	Tr
1628	Octyl butyrate	1.7
1638	Octyl-2-methyl butyrate	Tr
1743	7-epi-1,2-Dehydro-sesquicineole	0.1
1757	Octyl angelate	0.1
1824	Octyl hexanoate	19.8
1862	(Z)-4-Octenyl hexanoate	0.1
2022	Octyl octanoate	9.9
2063	3-Octyl octenoate	0.3
2243	α-Bisabolol	0.1
	Total	99.6

A: *Zosima absinthifolia* fruits essential oil

LRI: Linear retention indices calculated against *n*-alkanes

%: Calculated from FID data tr: Trace (<0.1%)

Essential oils of *Zosima absinthifolia* fruits have been characterized by the occurrence of octanol and its esters *e.g.* octyl acetate, octyl hexanoate etc.

These are two previous reports on the essential oil composition of *Z. absinthifolia* fruits. Başer *et al.,* (2000) reported that octyl acetate (38.9%), octyl hexanoate (31.9%) and octanol (12.9%) were the main constituents in the oil of *Z. absinthifolia* collected in Turkey. Razavi *et al.,* (2009) reported the essential oil composition of *Z. absinthifolia* fruits of Iran origin as octyl acetate (87.5), octyl octanoate (5.0%), octanol (2.4%), hexyl hexanoate (1.5%) and octanoic acid (1.1%) as main constituents.

We have investigated the fruits oils of *Z. absinthifolia* collected from two localities in Northern Cyprus. The Girne/ Alevkayası Armenian Monastery sample, the essential oil yield was 0.05 (v/w), showed 32 compounds representing 99.5 % of all oil. Octyl acetate (63.2%), octyl hexanoate (18.6%), octyl octanoate (9.2%) and octanol (2.2%) were characterized as main constituents.

Girne/ Alevkayası sample, the essential oil yield was 0.04 (v/w), similarly showed 14 compounds which have been identified representing 99.6 % of the total oil with octyl acetate (59.5%), octyl hexanoate (19.8%), octyl octanoate (9.9%) and octanol (7.1%) as major constituents.

These major constituents (octyl acetate, octyl hexanoate, octyl octanoate and octanol) had previously been reported to be bioactive compounds as Hajhashemi *et al.,* (2009) reported that *Heracleum persicum* oil which contained octyl acetate (16.5%) identified as a constituent of the essential oil of the fruits and the result clearly showed analgesics and anti-inflammatory activities of the hydroalcoholic extract and essential oils of the plant fruits (Hajhashemi et al, 2009).

Miladinovic *et al.,* (2013) also studied and reported that the major constituents of *Heracleum sibiricum* L. were found to be 1-octanol (13.6%) and octyl hexanoate (8.1%) among others and showed antibacterial activity.

Iscan *et al.,* (2004) studied and reported that essential oils of *Heracleum sphondylium* L. subsp. *ternatum* (Velen) Brummit, major components were identified to be octyl acetate (93.7%) also found to be the most active compound among others and showed antimicrobial activity of the oils using microdilution broth and agar diffusion method (Iscan et al., 2004).

4. Conclusion

The major constituents of these plant samples (octyl acetate, octyl hexanoate, octyl octanoate and octanol) had previously been reported to be bioactive compounds in the essential oils of *Zosima absinthifolia* as well as *Heracleum persicum*, and *Heracleum sphondylium* L. subsp. *ternatum* (Velen) Brummit the latter had shown anti-inflammatory, analgesics, antibacterial and antimicrobial activities.

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