T.C.
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ESSENTIAL OIL ANALYSIS OF SOME PLANT SPECIES AND ANTIMICROBIAL ACTIVITIES

MASTER'S THESIS

BÜŞRA UMUT OYMAN

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SUPERVISOR
Assit. Prof. Dr. Hülya DEMİR

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ONAY

Bu tez Yeditepe Üniversitesi Lisansüstü Eğitim-Öğretim ve Sınav Yönetmeliğinin ilgili maddeleri uyarınca yukarıdaki jüri tarafından uygun görülmüş ve Enstitü Yönetim Kurulu'nun 22./12./2017.. tarih ve 2017/26-10..... sayılı kararı ile onaylanmıştır.

Prof. Dr. Bayram YILMAZ
Sağlık Bilimleri Enstitüsü Müdürü

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Foremost, I would like to thank my parents for their continued support and encouragement.

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ABSTRACT

Oyman B.U.(2017). Essential Oil Analysis of Some Plant Species and Antimicrobial Activities

Yeditepe University, Institute of Health Sciences, Department of Nutrition and Dietetics, MSc thesis. İstanbul.

The present study was conducted to evaluate the chemical composition and antimicrobial activities of essential oils of *Ocimum basilicum* var. album(L) Benth, *Lavandula angustifolia* subsp. angustifolia, *Melissa officinalis*, *Thymus vulgaris* L., *Mentha piperita*, *Salvia officinalis*. The chemical composition of a hydrodistilled essential oils of *Ocimum basilicum* var.album(L) Benth., *Lavandula angustifolia* subsp. angustifolia, *Melissa officinalis, Thymus vulgaris* L., *Mentha piperita*, *Salvia officinalis* was analyzed by a GC/MS system. *Ocimum basilicum* var .album(L) Benth essential oil contains 1,6-octadien-3-ol,3,7-dimethyl (53.79%), *Lavandula angustifolia* subsp. angustifolia essential oil 1,6-octadien-3-ol,3,7-dimethyl(42.07%), *Melissa officinalis* essential oil d-limonene (26%). *Thymus vulgaris* L. essential oil contains eucalyptol(80.30%), *Mentha piperita* essential oil (28.75%), *Salvia officinalis* essential oil contains (1R)-2,6,6-trimethylbicyclo(33.62%). Antimicrobial screening of the essential oils was made by disc diffusion. The antimicrobial test results showed that the *Ocimum basilicum* var. album(L) Benth, *Melissa officinalis, Lavandula angustifolia* subsp. angustifolia, *Thymus vulgaris, Mentha piperita, Salvia officinalis* essential oils have great potential of antimicrobial activity against all three (*Staphylococcus aureus* (ATCC 6338) Gram positive, *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442) Gram negative bacteria), one fungi(*Aspergillus niger*), one yeast (*Candida albicans*) species tested.

Keywords*:* Essential oil, GC/MS composition, antimicrobial activity

ÖZET

Oyman B.U.(2017). Bazı Bitki Türlerinin Uçucu Yağ Analizleri ve Antimikrobiyal Aktiviteleri. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Bölümü, MSc Tezi. İstanbul.

Bu çalışma *Ocimum basilicum* var. album(L) Benth, *Lavandula angustifolia* subsp. angustifolia, *Melissa officinalis*, *Thymus vulgaris* L., *Mentha piperita*, *Salvia officinalis* uçucu yağlarının kimyasal kompozisyonu ve antimikrobiyal aktivitelerini değerlendirmek için yürütülmüştür. *Ocimum basilicum* var.album(L) Benth., *Lavandula angustifolia* subsp. angustifolia, *Melissa officinalis, Thymus vulgaris* L., *Mentha piperita*, *Salvia officinalis* 'in hidrodistilasyonla elde edilen kimysal kompozisyonu GC/MS sistemiyle analiz edildi. *Ocimum basilicum* var .album(L) Benth uçucu yağı 1,6-octadien-3-ol,3,7-dimethyl (53.79%), *Lavandula angustifolia* subsp. angustifolia uçucu yağı 1,6-octadien-3-ol,3,7-dimethyl(42.07%), *Melissa officinalis* uçucu yağı d-limonene (26%), *Thymus vulgaris* L. uçucu yağı eucalyptol(80.30%), *Mentha piperita* uçucu yağı p-cymene (28.75%), *Salvia officinalis* uçucu yağı (1R)-2,6,6-trimethylbicyclo(33.62%) içermektedir.

Uçucu yağların antimikrobiyal taraması disk difüzyon metodu ile yapıldı. Antimikrobiyal test sonuçları *Ocimum basilicum* var.album(L) Benth., *Lavandula angustifolia* subsp. angustifolia, *Melissa officinalis, Thymus vulgaris* L., *Mentha piperita*, *Salvia officinalis* uçucu yaglarının karşı (*Staphylococcus aureus* (ATCC 6338) Gram pozitif, *Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (ATCC 15442) Gram negatif, fungus(*Aspergillus niger*), mayaya (*Candida albicans*) kuvvetli antimikrobiyal aktivite gösterdiğini ortaya çıkarmıştır.

Anahtar Kelimeler: Uçucu yağ, GC/MS kompozisyonu, antimikrobiyal aktivite

1. INTRODUCTION and PURPOSE

1.1. Definition of Nutrition

Nutrition is that human begins utilize necessary food components to growth of body, healthiness and being productive to live long time. When food components are taken too much or taken less than need, it is proven scientifically that growth and development will stop and health will stop and health will be affected negatively. It should not be forgotten that nutrition is a phsyiological, sociological and psychological event(1).

Nutrition comes first of the basic requirements of living things. Human beings cannot grow and live healthy without nutrition. The importance of nutrition in human health and life is understood better day by day. Nutrients are composed of organic compounds such as protein, carbohydrate, fat, vitamins and minerals and inorganic compounds. These compounds are called 'food compounds'. Healthy eating means eating a diversity of foods from the range groups that give the nutrient person need to maintain health, strong and look fit. Eating right is choosing a diversity foods in the right proportions that consume right proportions for person's age, activity level, gender and refuse foods and drinks that are high in sugar, calories, fat, sodium and also many preservatives(2).

Healthy diet is essential for development and well-being. A healthful diet can reduce major risk factors for chronic diseases such as obesity, high blood pressure, and high blood cholesterol (3). Malnutrition may appear due to not eating enough food to meet dietary needs, but it is also caused by over-eating which can lead to obesity (4).

A balanced diet provides all the necessary nutrients in the appropriate proportions and quantities to meet a person‘s needs. One way to follow a balanced diet is to eat a variety of foods which supply a range of nutrients. Dietary needs vary form person to person, depending on age, sex, and level of activity and lifestyle (5).

Figure 1. Definition of Healthy Nutrition

1.2. Usage of Medicinal and Aromatic Plants

The plants synthesize substances that are beneficial for the health of humans and animals. These aromatic substances which are phenols or tannins. In many areas these aromatic substances work as plant defense mechanism against microorganism, insects and herbivores. Many herbs and species which are used by humans to food yield and medicinal compounds. The aromatic substances which can be found everywhere, in the kitchen or in the wild places. Many unknown benefits of plants are found by people and use them into their meal.

Thanks to treatment, people can feel better for many diseases. The researchers aim to provide the most innovative and meticulous development in research and industry. Plants are irreplaceable source of life since the existence of humanity. People have been using plants for lots of various purposes. Besides the fact that are source of food, they also used as scent, sweetener and therapeutic (6).

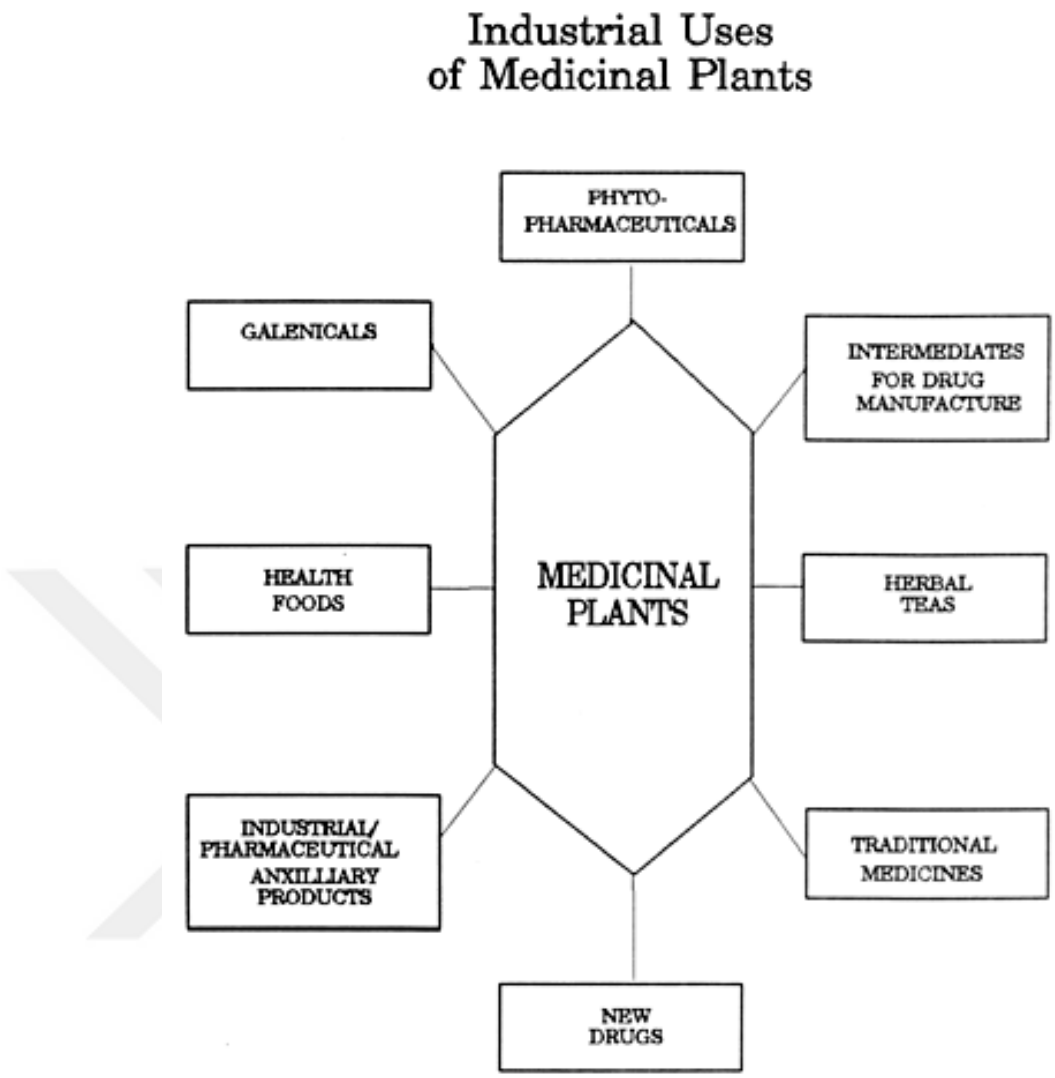


Figure 2. Industrial Use of Medicinal Plants

1.2.1. Usage Area of Plants

The many plants that are tasteful directly related with their essential oil composition. Some of these are alcohol, ester, phenol and ether but every plant species have more essential oil composition than the other. Plants are main source for human and they provide basic nutritional requirements that are carbohydrate, protein, fat. Except those, wood, selulose, gum, tire and similar helpful substances are obtained from plants. They are usefull not only food sector, but also cosmetic, chemistry, pharmaceutical industry (7).

Many civilizations have been using herbs as medicine to overcome lots of diseases. People have been prefering herbs medicine instead of modern medicine because modern medicine have not the ability for treatment every diseases and also it is more expensive than the other. In addition modern medicine causes some side effect. Nowadays, herbs medicine is used for alternative treatment or as complement beside the modern medicine. Nutrients are kept unpackaged in the stores but this sittuation cause contamination, so that foods decay and microorganisms are relicate. For this reason, chemical materials are have to used for food endustry to prevent break down and accrue the microorganisms but today instead of chemical metarials, essential oil compositions are used for food to keep them(8).

2. ESSENTIAL OILS

2.1. Definition and Features of Essential Oils

Medicinal and aromatic plants can be processed by distillation to become essential oils. They are used in cosmetology, pharmacy, perfumes and food industry. Essential oils get from plants. Essential oils get from plants, liquid room temperature and even it can evaporate at room temperature, so they callad essential or essence. Essential oils are not mix with water, but they dissolves in water at a certain level to give its smel to water. Essential oils can soluable in ethanol, ether, benzene and benzine easily. Most of volatile oils are lighter than water and they are active optically. They are located anywhere in plants, maybe in all tissues or some cells. There is no reason why these oils occur in plants but thanks to them plants are protected against animals and pollinate each other easily(9).

2.2. Terpenes

People isolate organic compounds from plants from past day to day. When the plant is slowly heated or subjected to stem distillation, a mixture of fragrant compounds known as volatile oils is obtained. Volatile oils are widely used in perfumery. When the structure of volatile oils is enlightened, the most important components which are monoterpenes are obtained (10).

Terpenes which are also known as terpenoids are a group of compounds commonly found in the plant kingdom(11). They are composed of five carbon isoprene

molecules. In volatile oils, containing carbon and hydrogen, oxygen-free terpenic substances are said hydrocarbons. There are also terpenes containing oxygen species that are also found natural and there are mainly alcohol, aldehyde or ketone forms. They are either in the form of branched chains or cyclic form. It is known today that there are more than 20 000 terpen structures with various functional groups which are either open chain or ring structure (12).

2.2.1. Terpenoid Compounds

2.2.1.1. Classification of Terpenoids

They are categorized acording to number and structural organization of carbons that they contain. 10 carbons molecules formed by biosynthesis of two isoprene units which are called monoterpenes, if they have 15 carbons, they called sesquiterpenes and if molecules have 20 carbons, they called diterpenes. A generality of these compounds are found only in plants, but some of the complex terpens (e.g. Lanosterol & Squalene) consist in animals(13).

Some volatile oils contain monoterpenes and some sesquiterpenes. Some of the sesquiterpenes and diterpenes are non- volatile oil in the plants, but they cannot pass to essential oil during acquisition because they cannot drift with water vapor. They may be found free in the structure of plants, as well as with glycosides, organic acid esters and proteins(13).

2.2.1.2. Biosynthesis of Terpenoids

The mevalonic acid which plays an important role in tyhe biosynthesis of terpenoids, is formed by the condensation of 3 mole of coenzyme A. The isoprene units forming terpenes with the loss of water and carbondioxide of mevalonic acid. Acetyl CoA which is the result of the oxidation of sugar, is used as a starting material of mevalonic acid as well as in many other natural compounds(14).

The Acetyl CoA obrained in the condensation of two moles of Acetyl CoA is combined with another two moles of Acetyl CoA give 3 hydroxy 3 methylglutaryl CoA. After that the reduction of the enzymatic heterolytic and thiol ester gorup with NADPH yields mevalonic acid. The reaction is irreversible(14).

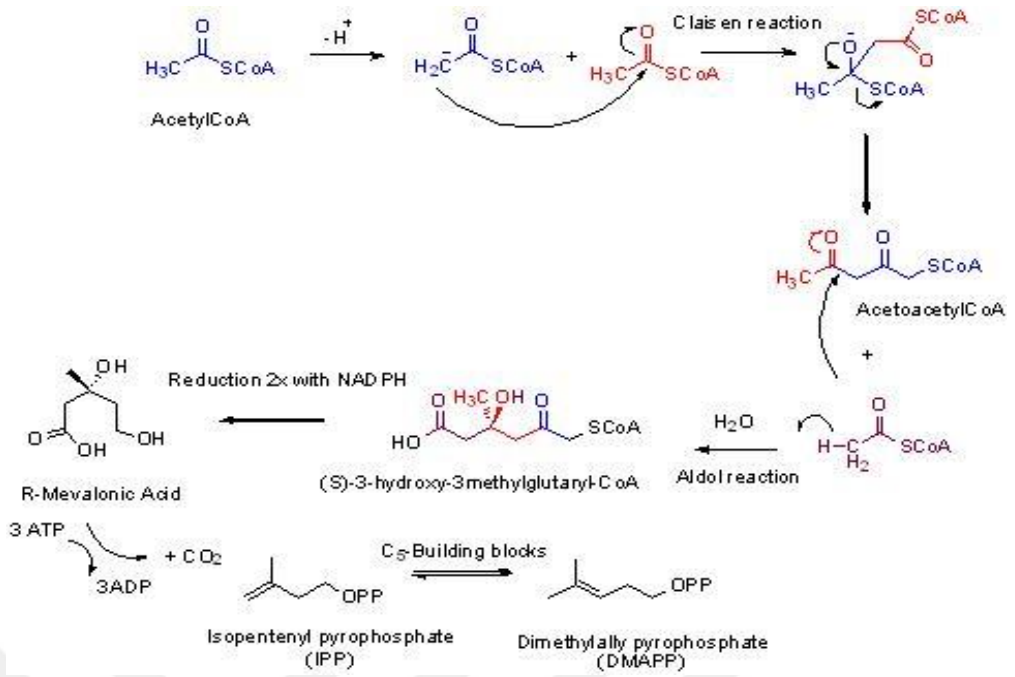


Figure 3. Formation of Mevalonic Acid.

The phosphatization of mevalonic acid with 2 moleucles of ATP results in the formation of the mevalonic acid 5 pyrophosphate compound. The hydroxyl group of this compound becomes a group which is more readily cleavoble by phosphatizing with a molar ATP. After that, isopentyl pyrophosphate molecule is formed by extraction of water and carbondioxide (15).

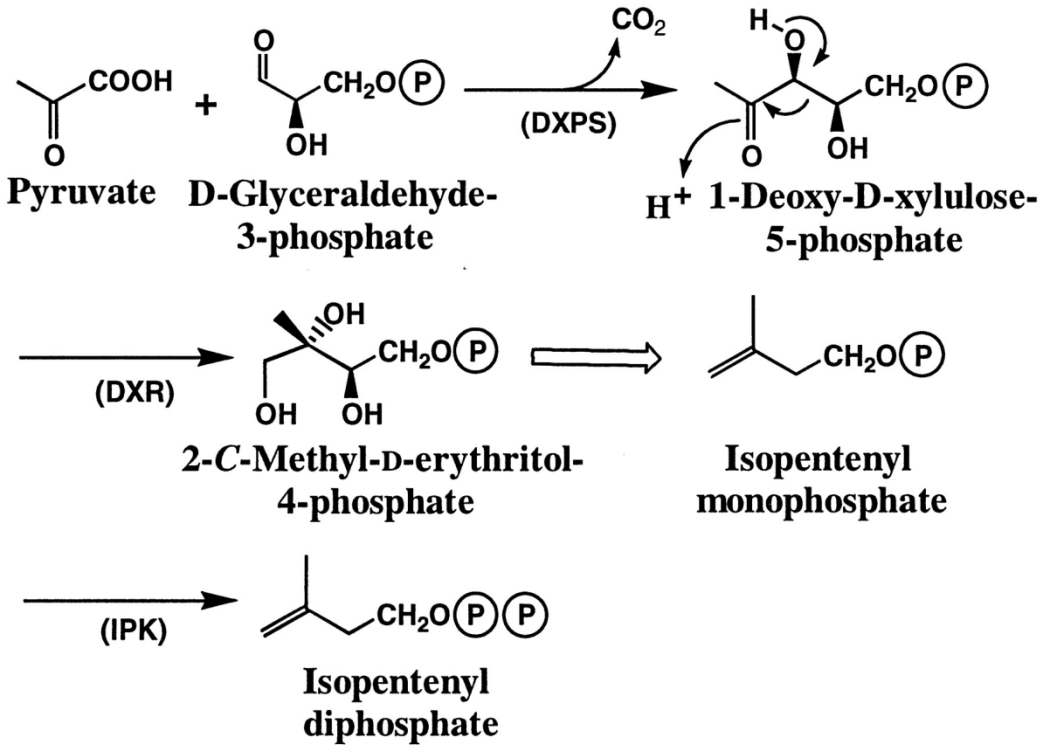


Figure 4. Formation of Isopentyl Pyrophosphate

The enzyme isomerization of the resulting isopentyl pyrophosphate result in the formation of the dimethyl allyl ester. The condensation of these two isomers to form geranyl- pyrophosphate. This compound brings the monoterpen to the field (16).

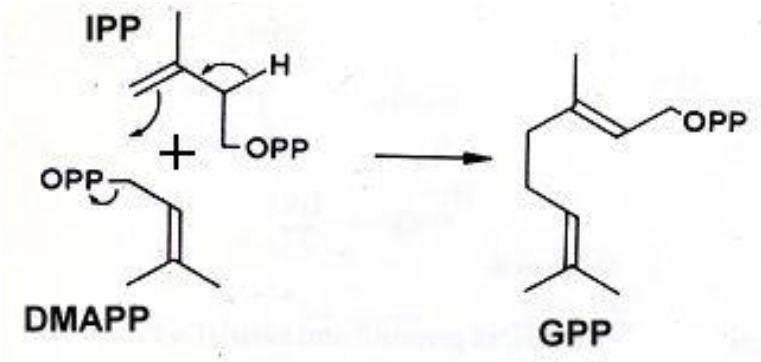


Figure 5. Formation of Isopentyl Pyrophosphate

Condensation of geranyl pyrophosphate with isopentyl pyrophosphate forms farnesyl pyrophosphate.

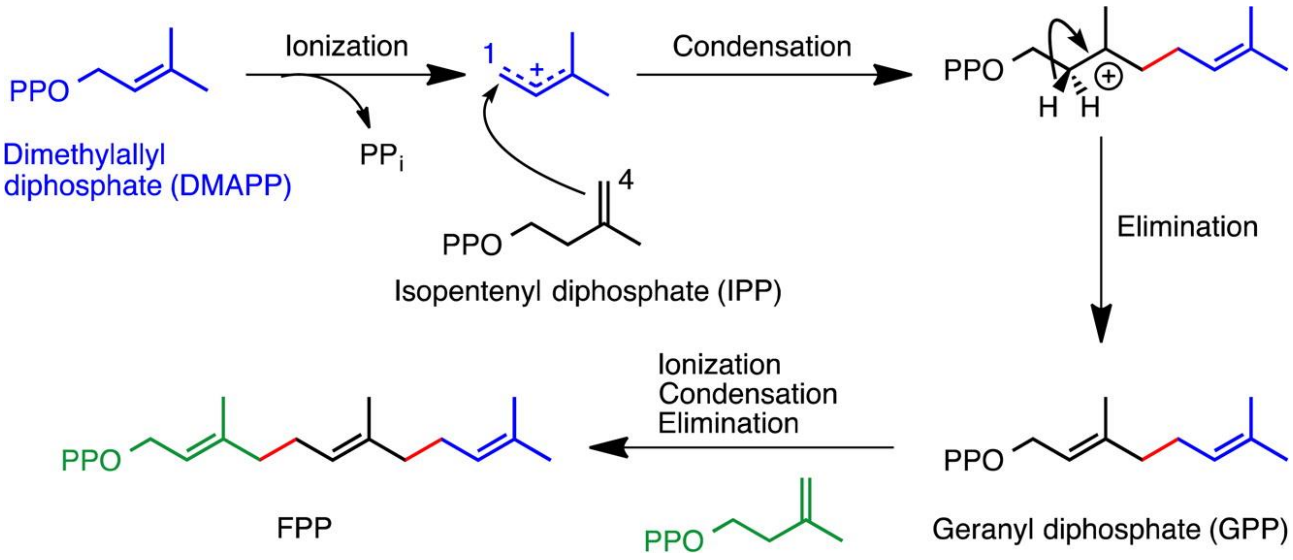


Figure 6. Formation of Farnesyl Pyrophosphate

Condensation of farnesyl pyrophpsphate and isopentyl pyrophosphate forms the geranyl-geranyl pyrophosphate complex, which is building block of condensation deterpenes and carotenoids(16).

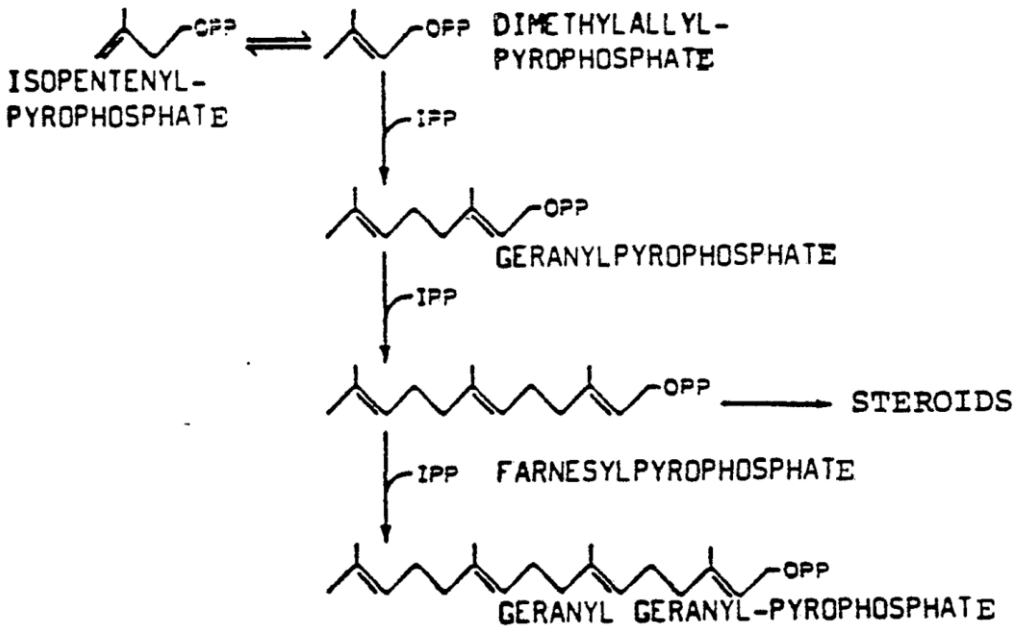


Figure 7. Formation Geranyl- Geranyl Pyrophosphate

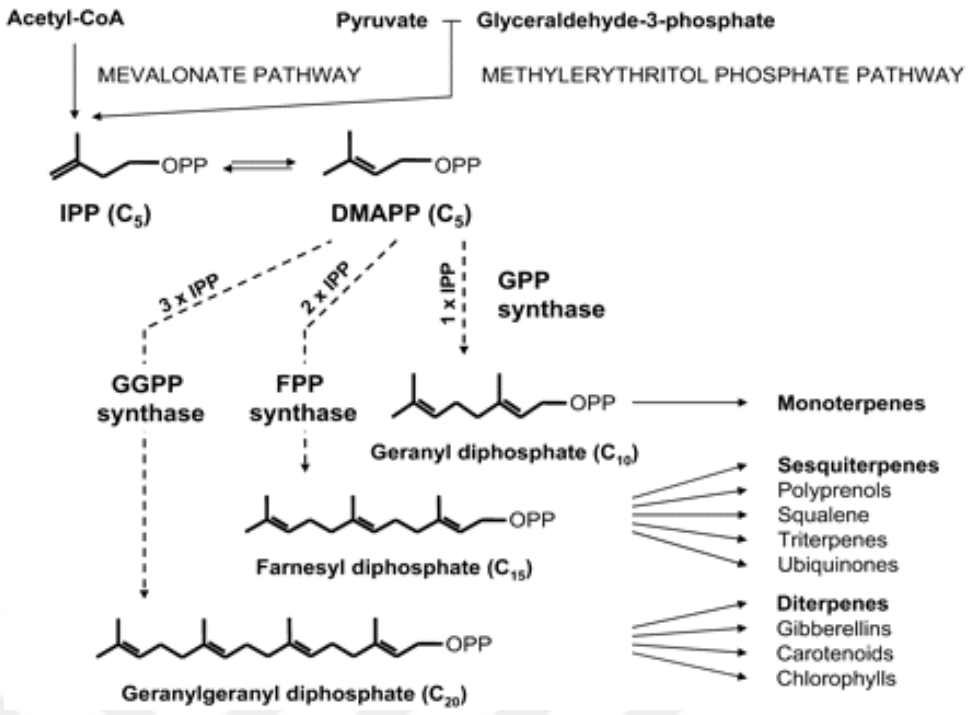


Figure 8. Formation of Terpenes Compounds

2.2.1.3. Isolation of Terpenes

Small molecules terpenoids such as monoterpenes and sesquiterpenes can be searated by water vapor distillation and larger molecular terpenoids can be separated by extrusion methods. The material from which the terpenes are obtained is dried and powered and then extruded with different polarity solutions. For purification purposes, column and preparative thin-plate chromatographic methods can be used, as well as MLPLC, HPLC, VLC and many chromatographic methods can be used(17).

2.2.2. Monoterpenes

There are over 100 known monoterpenes isolated from a number of hogh plants. Monoterpenes are also found in teh protective and pheromonal secretions of some insects. Monoterpenes are structurally different and there are about 35 different structures. Building types are acyclic, myracene, monocyclic p- menthone, bicyclic bornane, carane, fechane, pinane and thujane.

Although some optically pure forms of many monoterpenes in this group are not found naturally, some enantiomers may be present in some plants(18).

Monoterpenes, the smallest unit of terpenoids, are formed by the condensation of two isoprene isomers, geranyl pyrophosphate, which plays a specific role in the skeleton of monoterpenes(18).

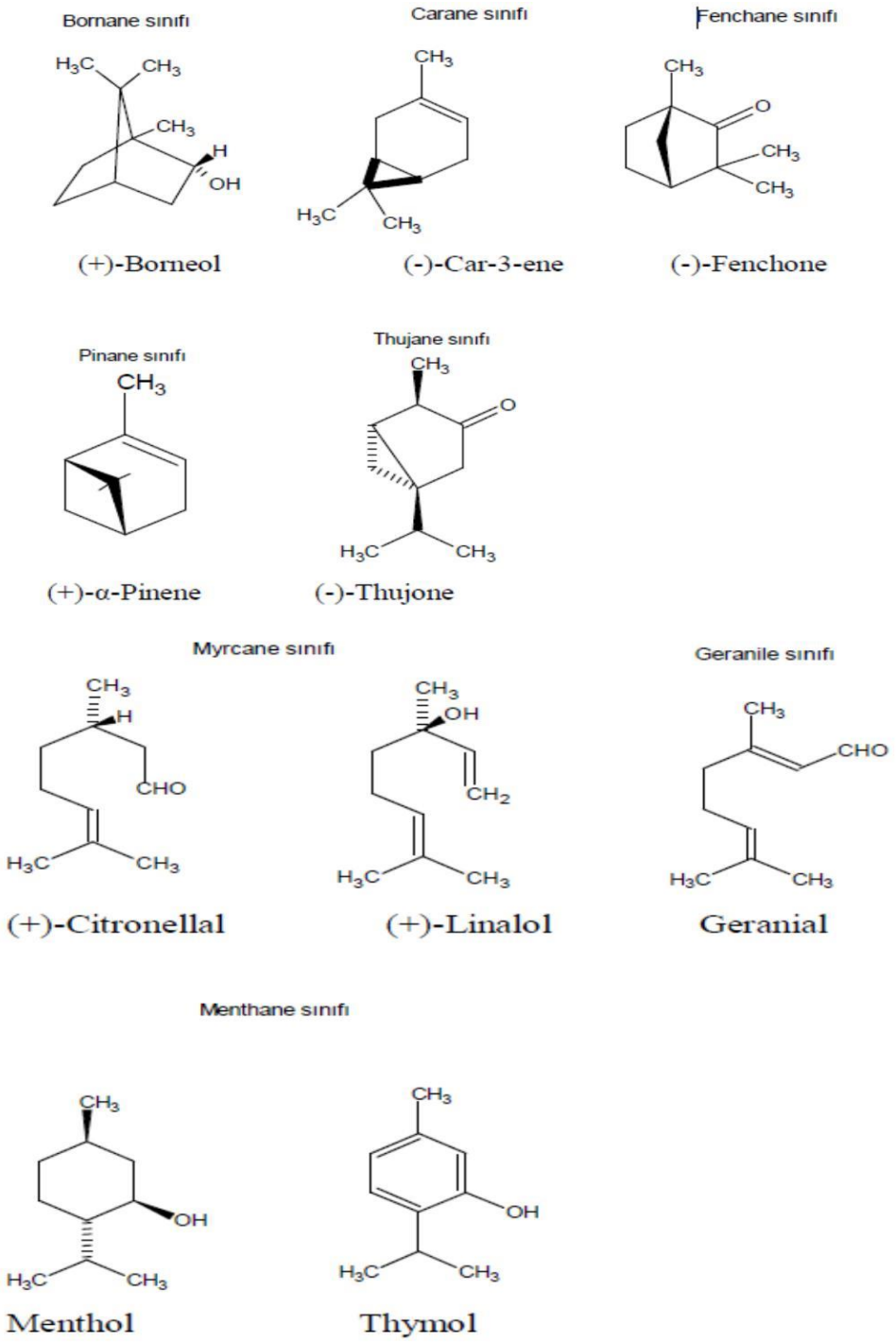


Figure 9. Monoterpenes

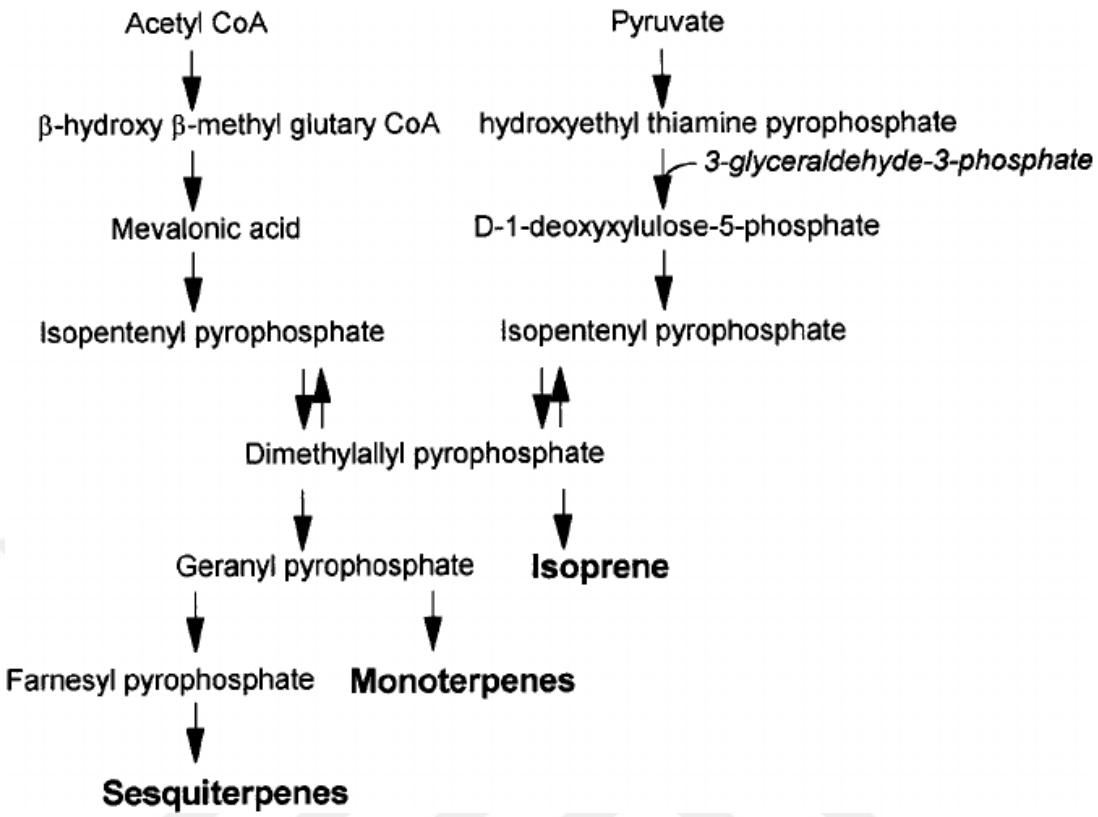


Figure 10. Sesquiterpenes

2.2.2.1. Acyclic Monoterpenes

Acyclic monoterpenes have 2-6 dimethicone skeleton and there are 3 pairs. Oxyfenateol derivatives are more important than pharmacognosy. These derivatives carry primary alcohols, tertiary alcohols, esters and aldehyde groups, may be monoethylenic or diethylenic. (19)

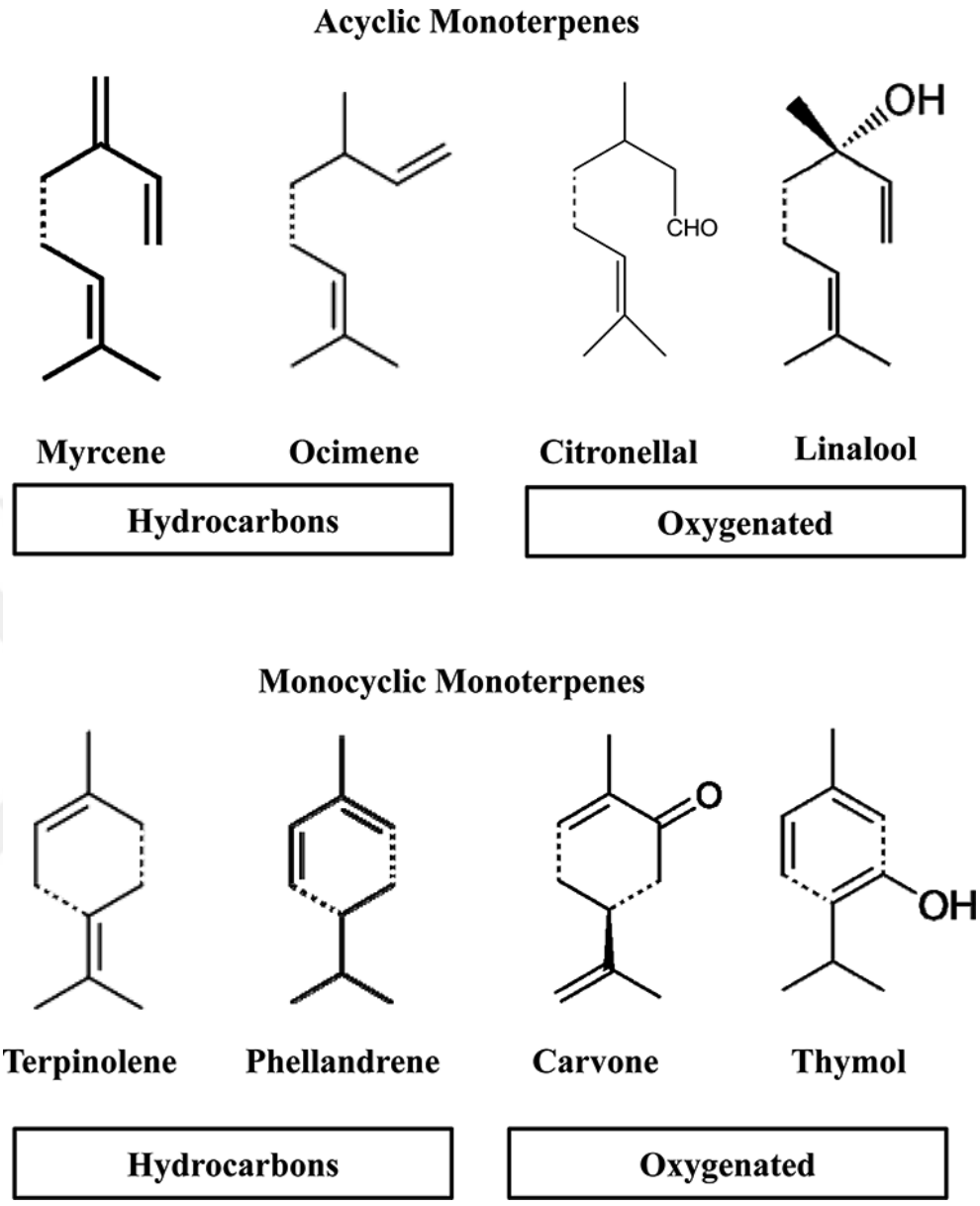


Figure 11. Acyclic Monoterpenes

2.2.2.2. Monocyclic Monoterpenes

Mostly have p- menthol skeleton. Oxygenated derivatives carry secondary or tertiary alcohols, esters, ketone, epoxides and peroxide groups. These compounds may be diethylenic, monoethylenic or saturated.

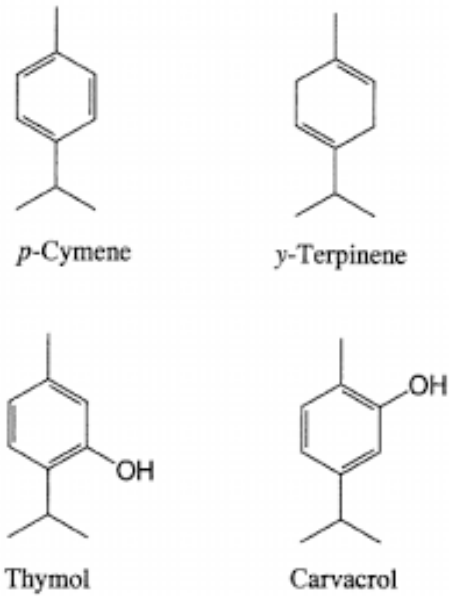


Figure 12. Monocyclic Monoterpenes

2.2.2.3. Bicyclic Monoterpenes

Bicyclic monoterpenes are derived from pinon or sabinon or karan or kamfon skeleton. They have one double bond. Oxygenated derivatives possess secondary alcohol ester or ketone groups. These compounds can be monoethykenically or saturated(20).

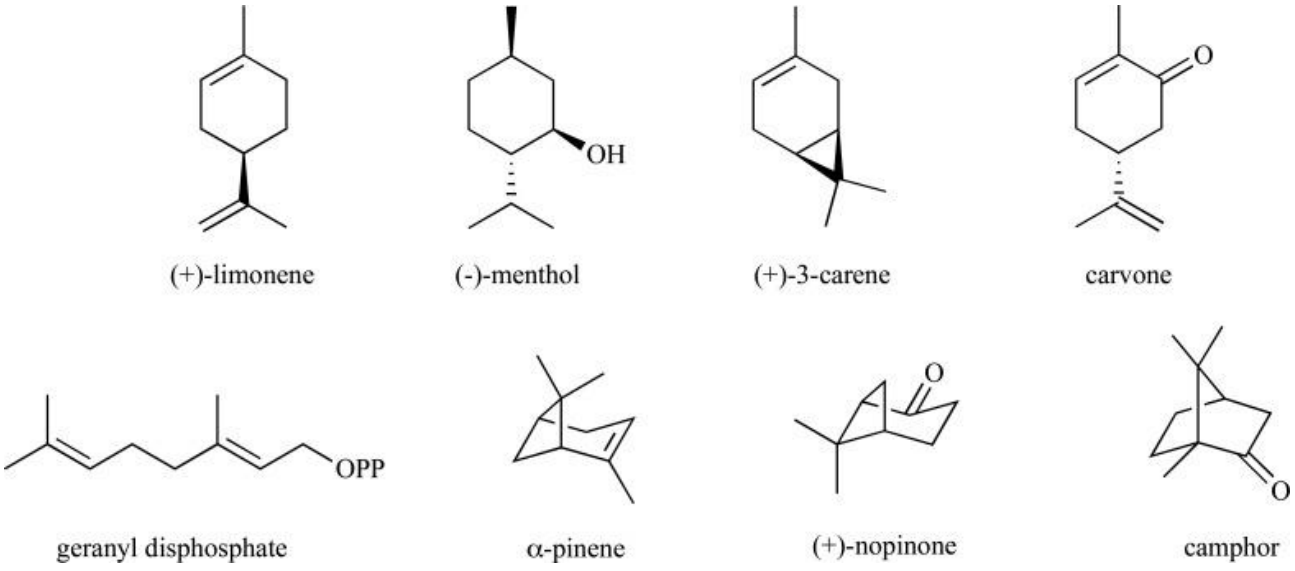


Figure 13. Bicyclic Monoterpenes

2.2.3. Sesquiterpenes

Sesquiterpenes has 15 carbons. It has extensive distrubution and it is wide-ranging class of terpenes. Geranyl pyrophosphate that is building block of monoterpenes, condensation with isopentyl pyrophosphate and they create sesquiterpenes. Sesquiterpenes has 5 different form, they called; asiclic, monocyclic, bicyclic, tricyclic, tetrocyclic. They have wide biologic activity spectrum. Finally, antimicrobial and antitumour activity of sesquiterpenes are toxic for mammals(20).

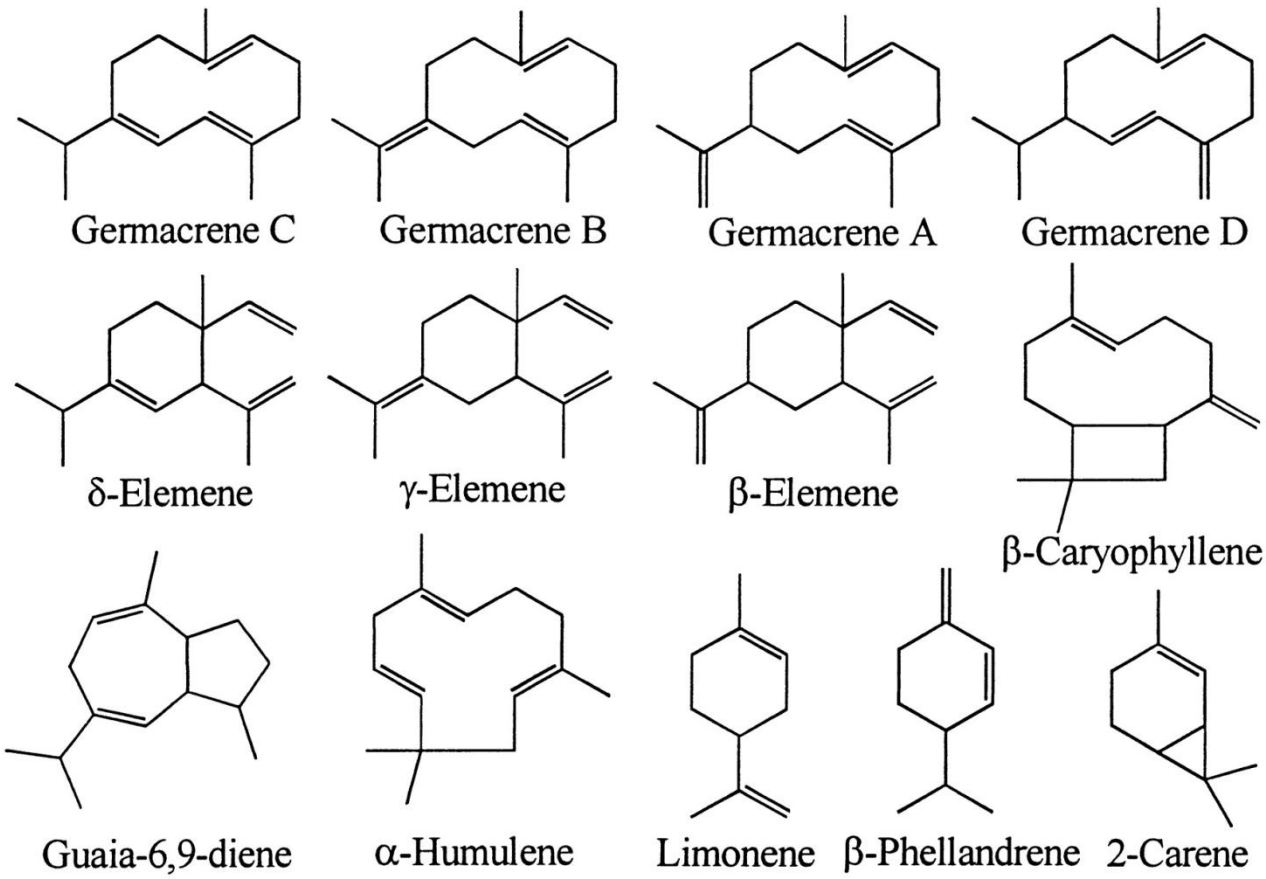


Figure 14. Some Sesquiterpenes Structures

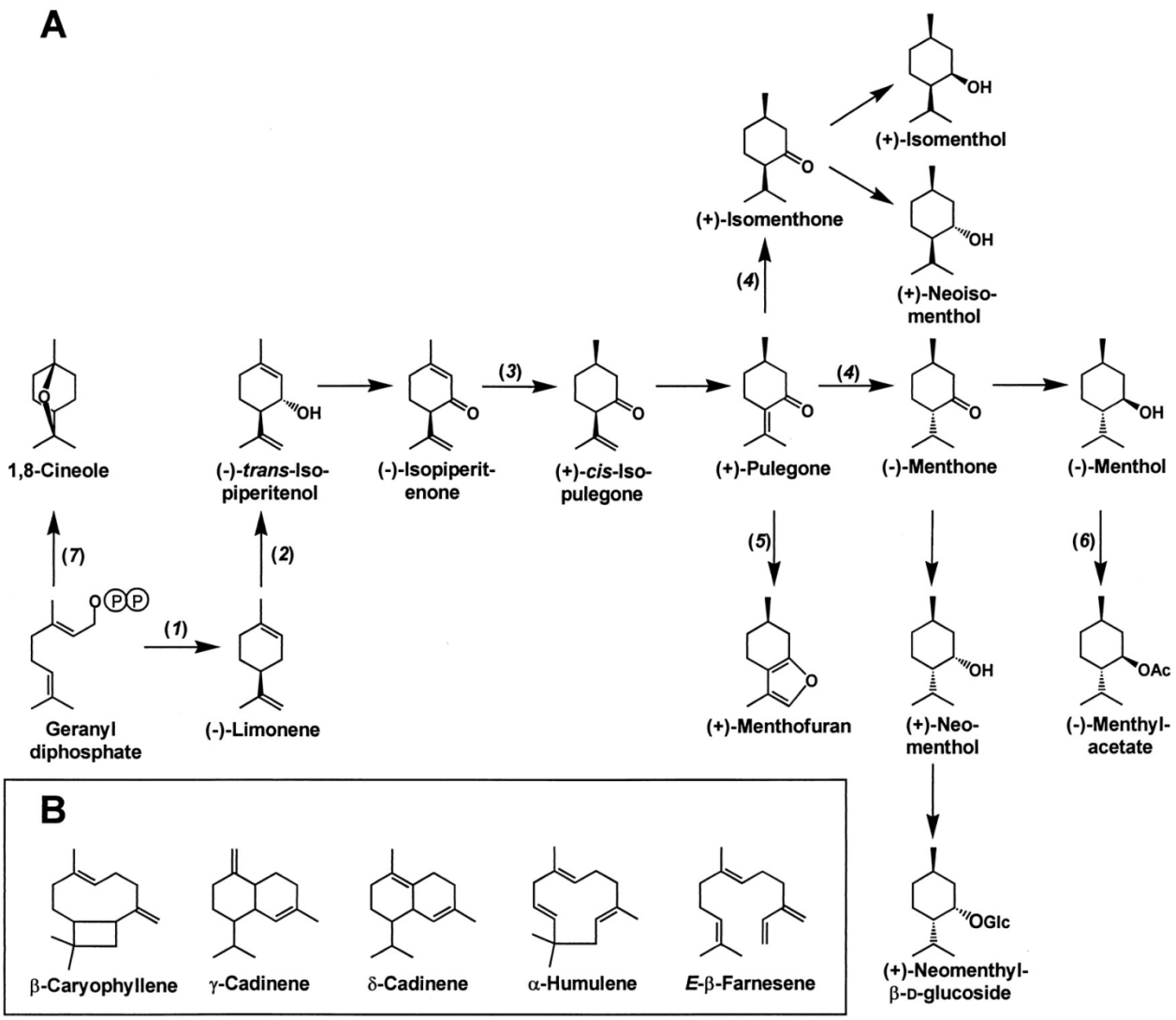


Figure 15. Biosynthesis of Sesquiterpenes

2.2.3.1. Asiclic Sesquiterpenes

It is sample for farnesol asiclic sesquiterpen. It is found in the plants' seed that called ambrette. It smeels as a lilac. In addition farnesol works as a hormone in some insects(20).

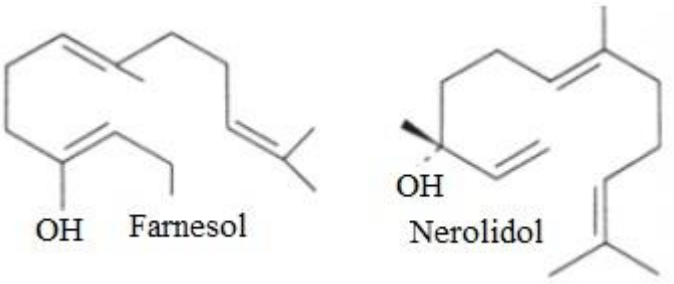
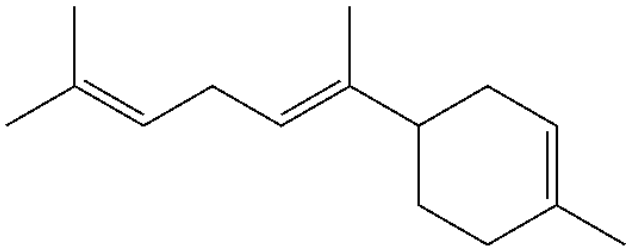


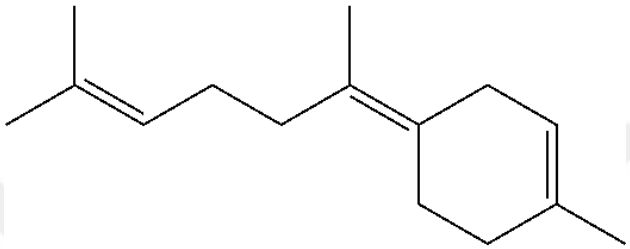
Figure 16. Some Sesquiterpenes Structures

2.2.3.2. Monocyclic Sesquiterpenes

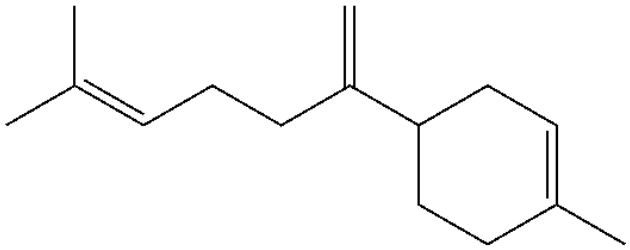
There are 4 types of monocyclic seswuiterpenes. Bisabalane, elemane, humulanc, germacrqane. They are available in essential oild. It is possile to show 3 types for bisobolene, they are alpha bisobolene, beta bisobolene and gamma bisobolene(21).



E-α-bisabolene



β-bisabolene



γ-bisabolene

Figure 17. Structures of Bisabolene

2.2.3.2. Bicyclic Sesquiterpenes

Bicyclic sesquiterpenes are examined into three groups. These groups are cadinane, evdesmane and perhidroazulene. Chamazulene is obtained from matricaria chammilla. It is a bicyclic sesquiterpene and is used like mouthwash for throat ache(21).

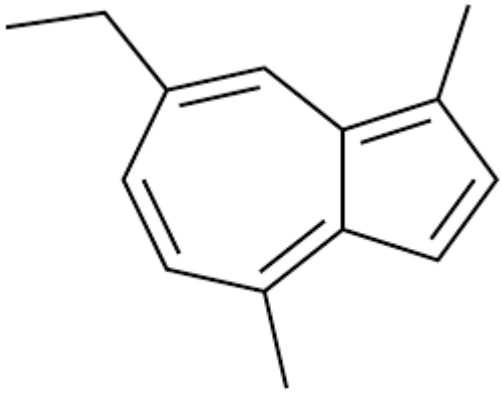


Figure 18. Chamazulene

2.2.3.4. Tricyclic Sesquiterpenes

They occur from two groups which are cedrene and longifolene(21).

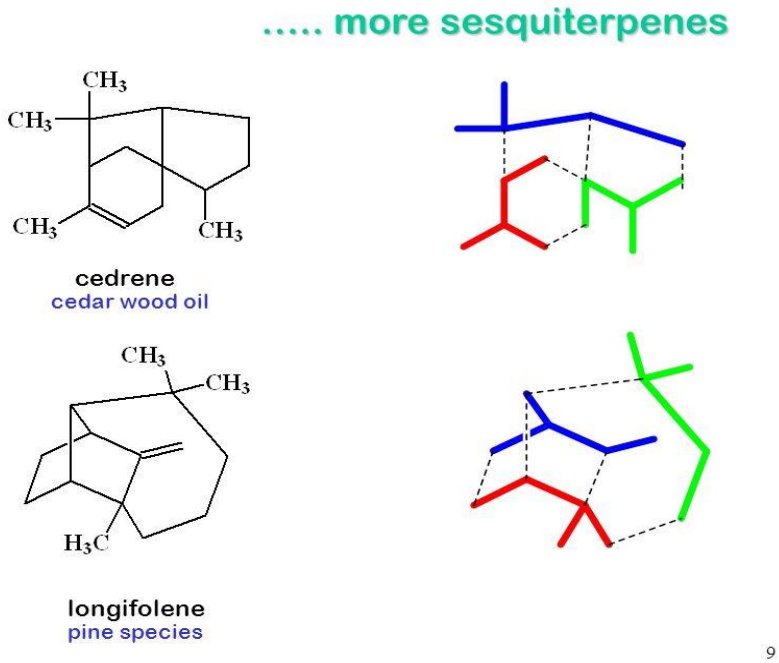


Figure 19. Cedrene and Longifolene

2.2.4. Diterpenes

Diterpenes are the most present members of the natural isoprenoid substances that are derived from 2E, 6E, 10E geranyl- geranyl pyrophophate. Despite they are plant and fungus origined, they are also present in the sea and insect world. Diterpens are easily

oxidizable, differing from triterpenes and steroids. For this reason, because of their neighbour group support many differences in the constructural reactions of diterpenes.

Of all natural substances, diterpenes are the compounds that have the broadest biological activity. Diterpenes are found in plants that develop hormones. E.g. Gibberalin; plant growth hormone, Podolacton; plant growth inhibitor, Antifeedants; insects, Antitumor; cancer effect and qualities that are similar to lower high blood pressure, solerol substances are used in the industry of perfumery.

The difference in the structure types of d,terpenes allows phytıchemistry to improve as well. In reality, the majority of diterpenes have been found as a result of chemical separations and examinations. Furthermore, biological activity is observed more in cyclic diterpenes(21).

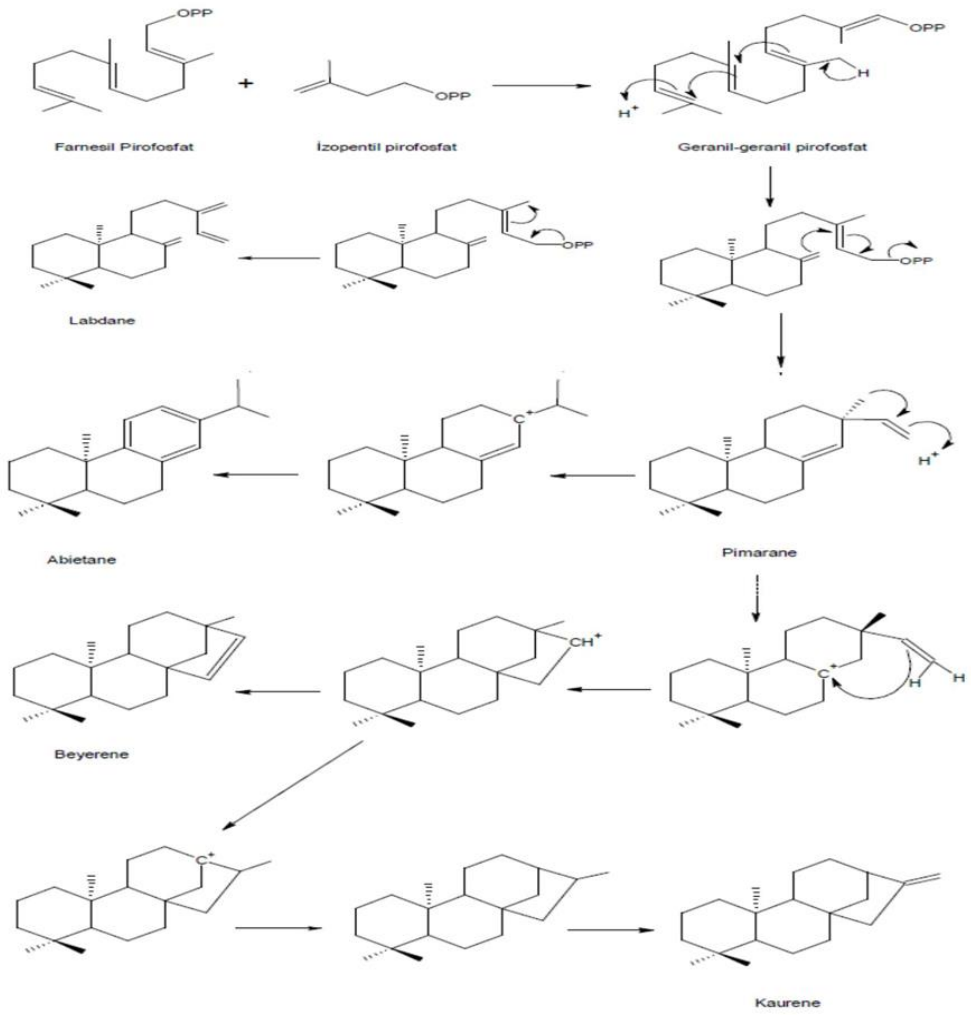


Figure 20. Diterpenes

Biogenetic variety is also the case for diterpenes. There are 70 different diterpene skeletons that have been reported in the literature. 20 of these are classified as main diterpene skeletons and the rest 50 are classified as uncommon diterpene skeletons(21).

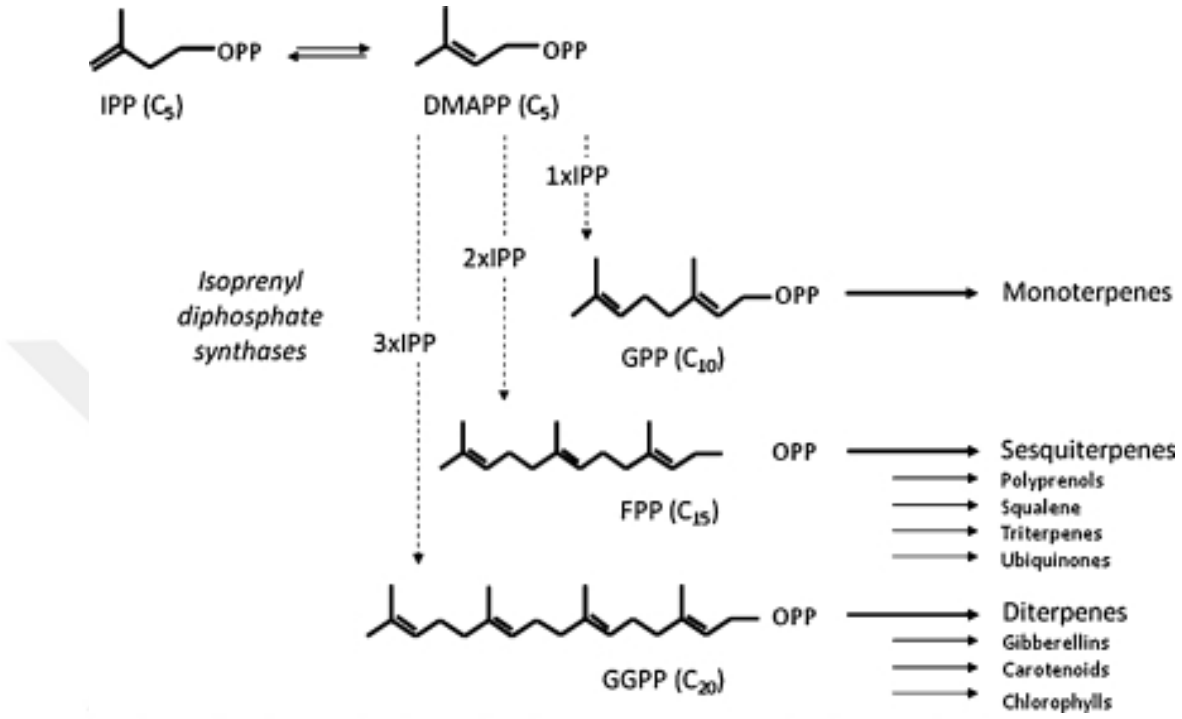
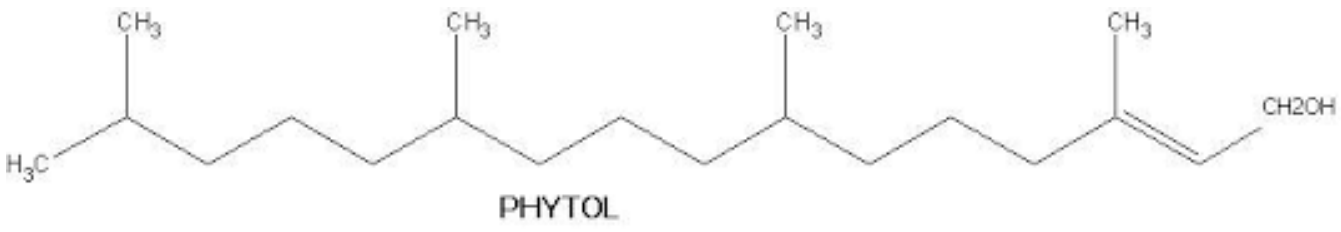


Figure 21. Mechanisms Proposed For Biosynthesis of Diterpenes

2.2.4.1. Acyclic Diterpenes

These diterpenes are saturated or unsaturated compounds that have 20 or more carbons. An example of acyclic diterpenes might be Phytol.



PHYTOL

Figure 22. Phytol

2.2.4.2. Monocyclic Diterpenes

In addition to being not so common amongstbearth plants, this type of diterpenes are especially obtained from seaweed. Epimeric molecules viridolis A obtained from Laurencia viridis, a red alp, is an example of these rare structures.

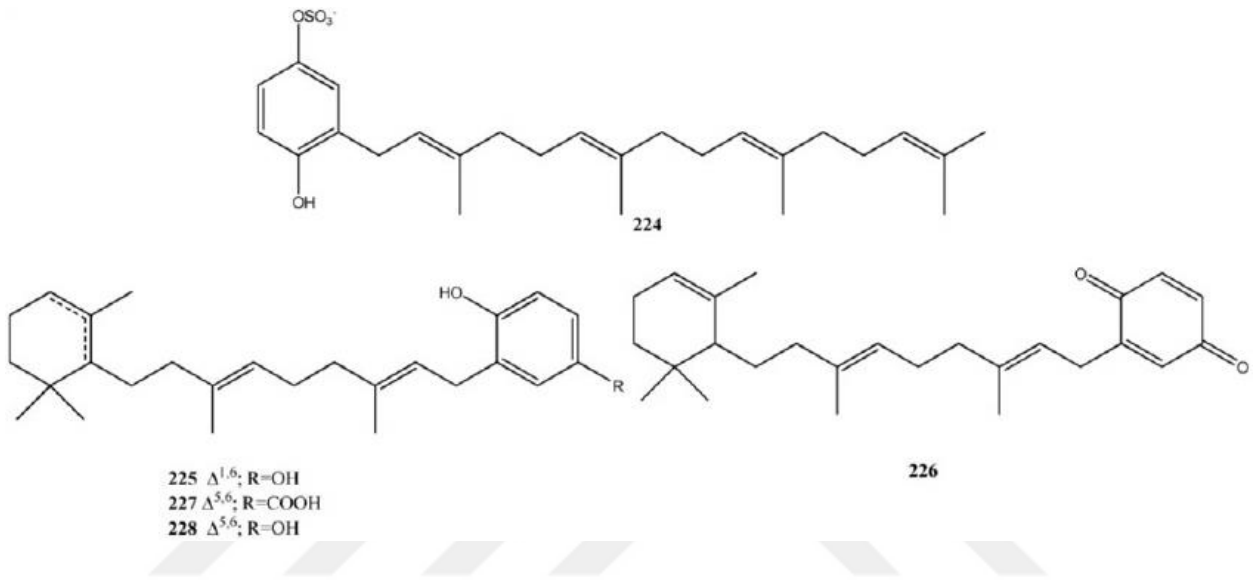
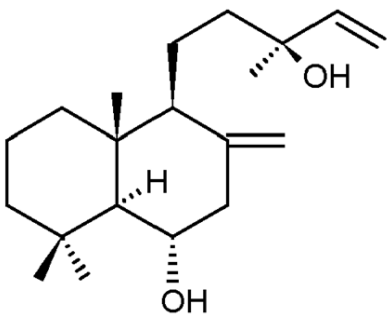


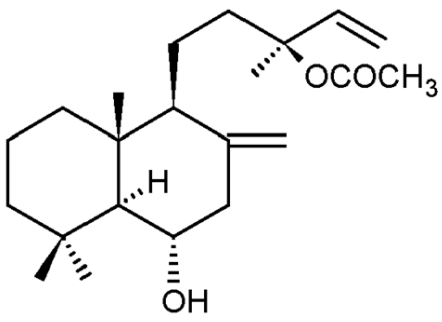
Figure 23. Monocyclic Diterpenes

2.2.4.3. Bicyclic Diterpenes

Bicyclic Diterpenes are examined in two skeletal structures named labdan and chlorenedans. Sludge obtained from the water vapor distillation oof oleresin, leaking from the bark of pine trees, rosin is an acid mixture. A few of these acids are diterpenes. Labdonoic acid can be given as an example(22).



larixol **(3)**

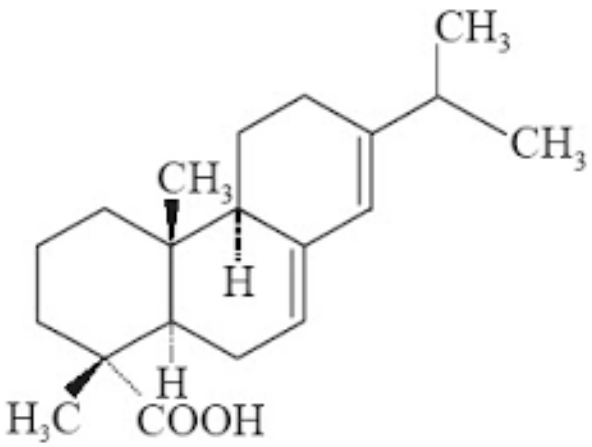


larixol acetate **(4a)**

Figure 24. Bicyclic Diterpenes

2.2.4.4. Tricyclic Diterpenes

Tricyclic diterpenes have four skeletal structures including primarans, abietansi kasans, rosans. Abietic acid can be given as an example(22).



Abietic Acid

Figure 25. Abietic Acid

2.2.4.5. Tetracyclic Diterpenes

Tetracyclic diterpenes are examined in two groups consisting of kaurens and beyerens. Linearol is a tetracyclic diterpene compound.

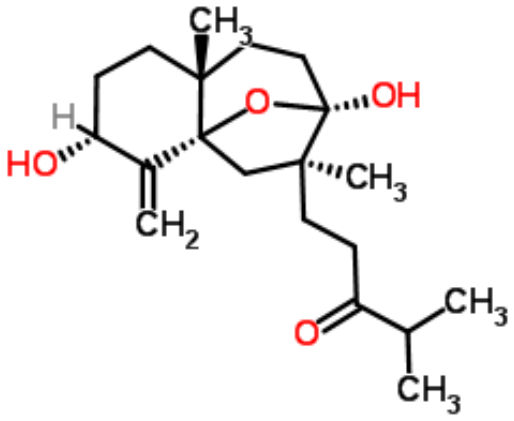


Figure 26. Linearol

2.2.5. Triterpenes

Triterpenes can be biosynthetically obtained from six isopren unity, and the first acyclic 30 carbons member is Squalene. Variation of closing of the cycles in Squalen causes triterpenes to have different variations skeleton structure (22).

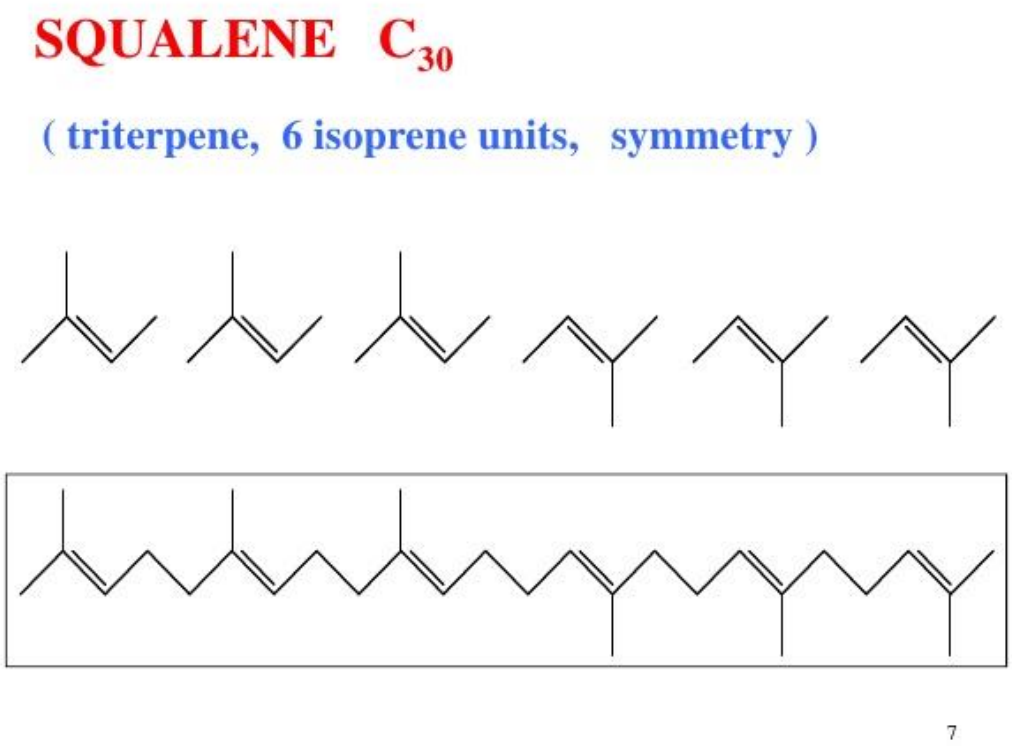


Figure 27. Squalene

2.2.5.1. Tetracyclic Triterpenes

Tetracyclic triterpenes are important compounds and that have skeletpn. This class is obtained as two groups, that called *lanosterol* and *cuphol* groups(22).

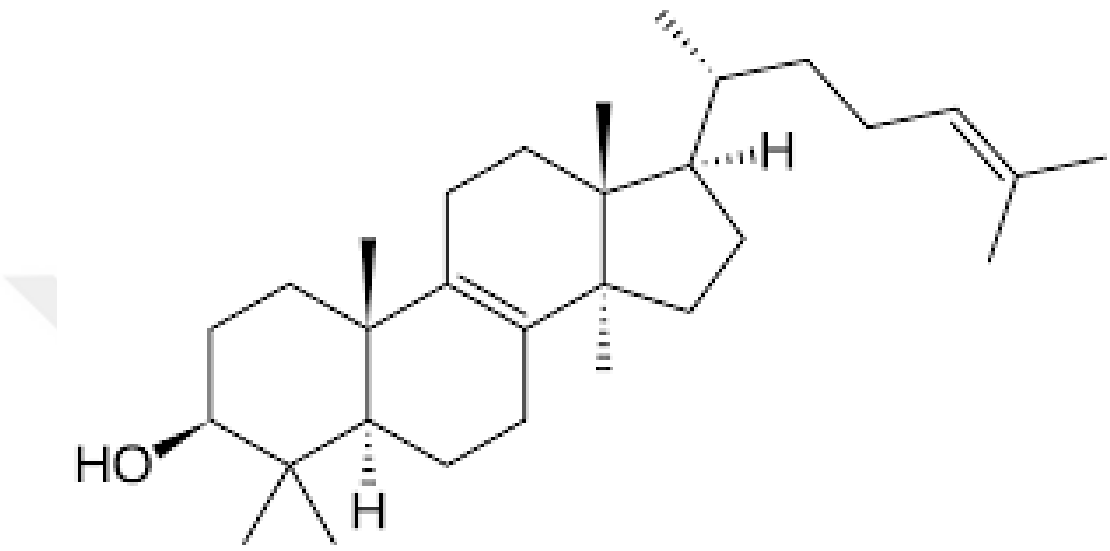


Figure 28. Lanosterol

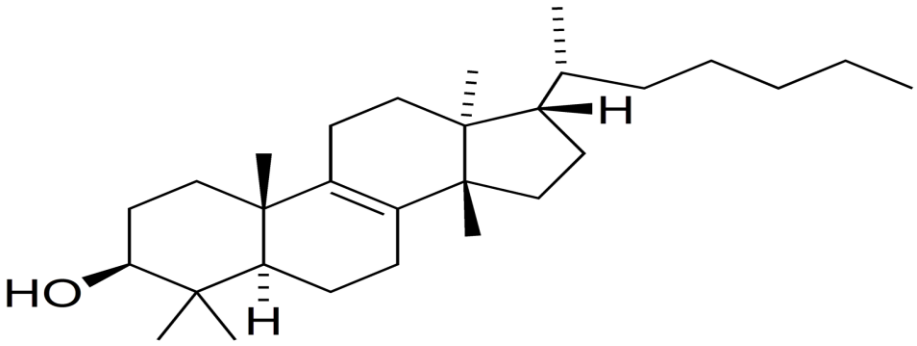


Figure 29. Euphol

2.2.5.2. Pentacyclic Triterpenes

Pentacyclic triterpenes are obtained various subgroups. For example: oleanone group, ursane group, lupanc group.

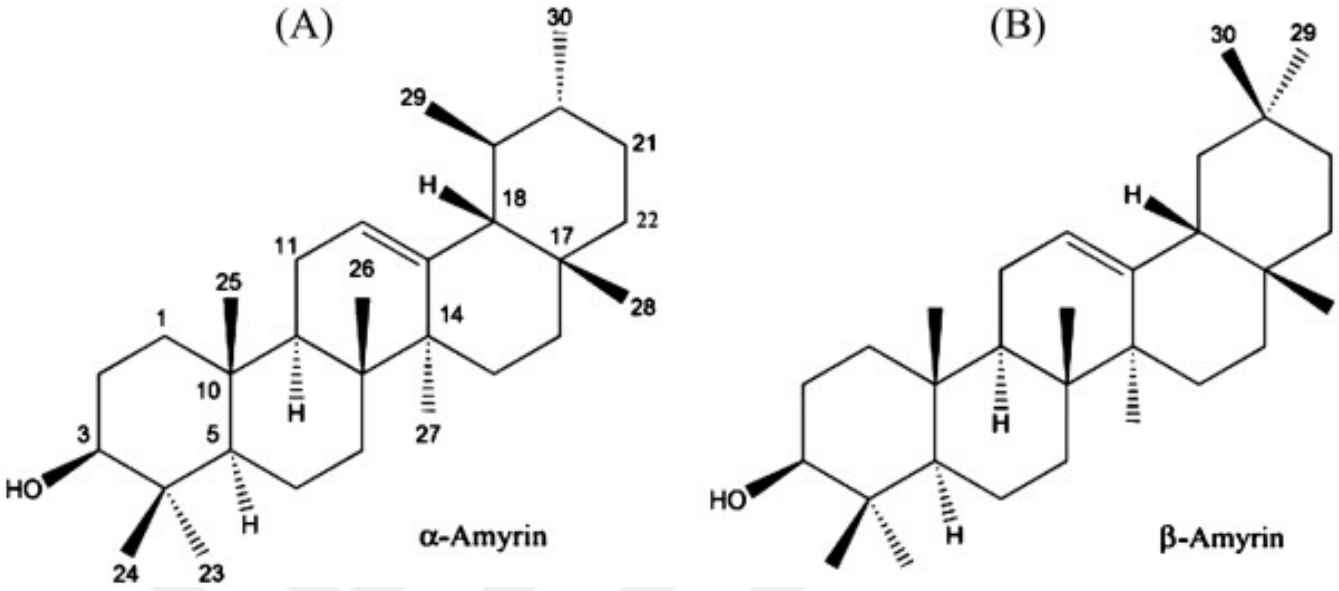


Figure 30. Alpha Amyrin - Beta Amyrin

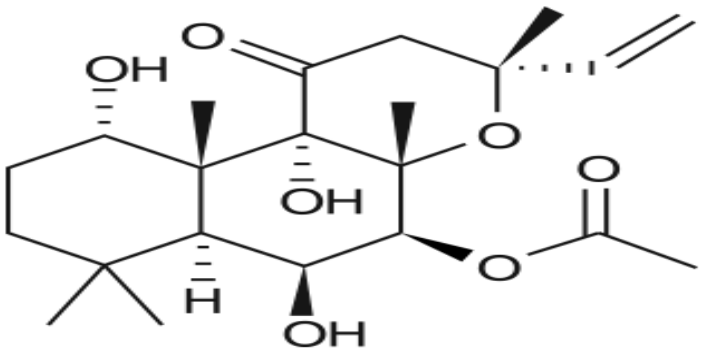


Figure 31. Lupeol

2.2.6. Tetraterpenes

Carotens are tetraterpenes. They may though two terpenes that connect tail-tail.

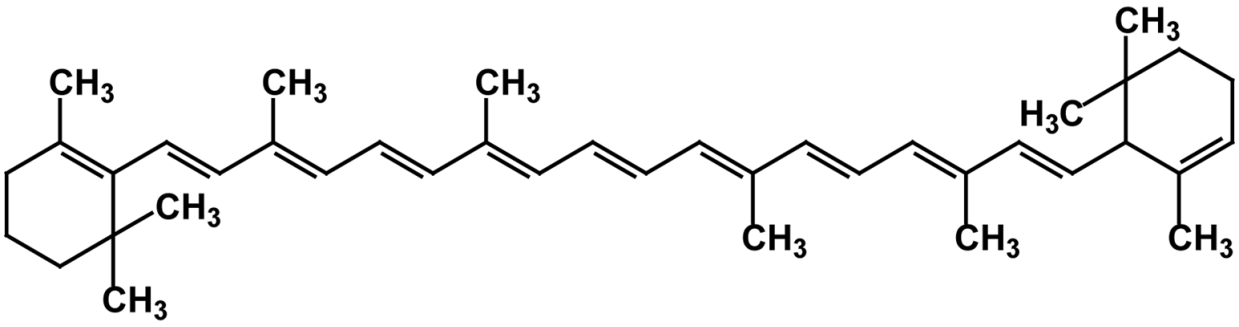


Figure 32. Alpha Carotene

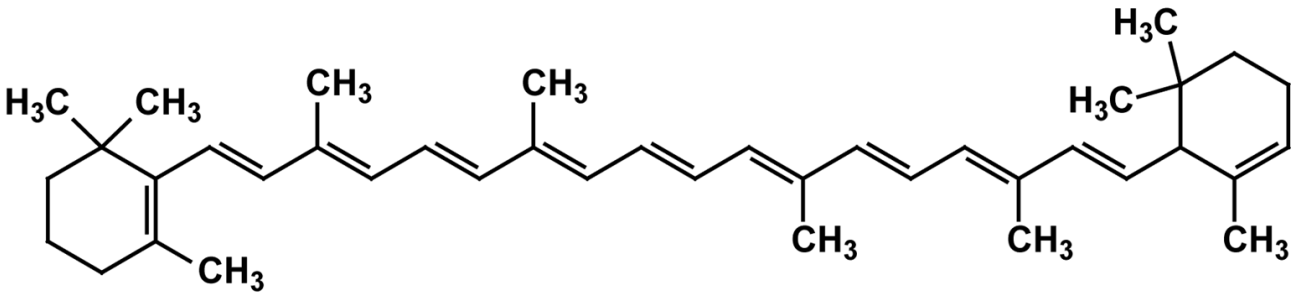


Figure 33. Beta Carotene

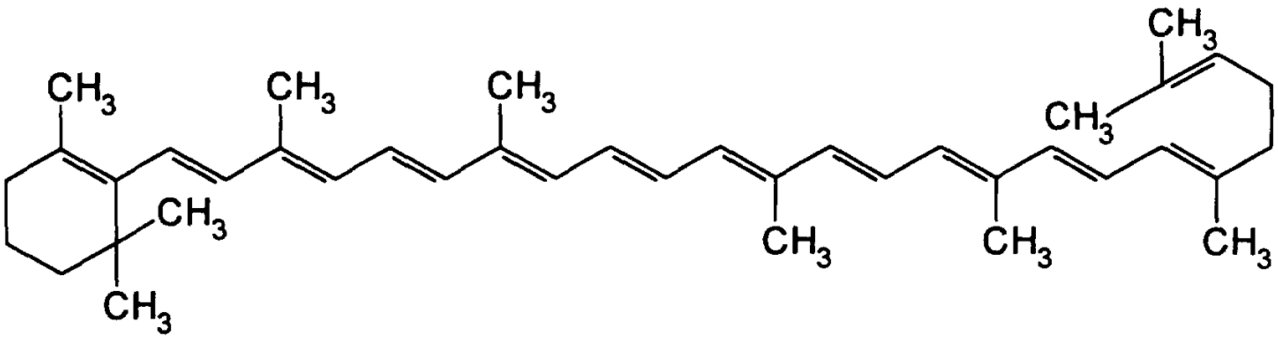


Figure 34. Gamma Carotene

2.2.7. Polyterpenes

The most known member of polyterpenes is rubber, the other name is latex.

Rubber is obtained from trees that are grown up in tropical region. When the tree' s body is cut, latex begin to leak. Asetic acid is added and rubber leaves from solution. It covered leaves and dried in warm air or smoked. The main product of this process is isoprene. So that, the molecular formula of rubber is (C5H8)n (22).

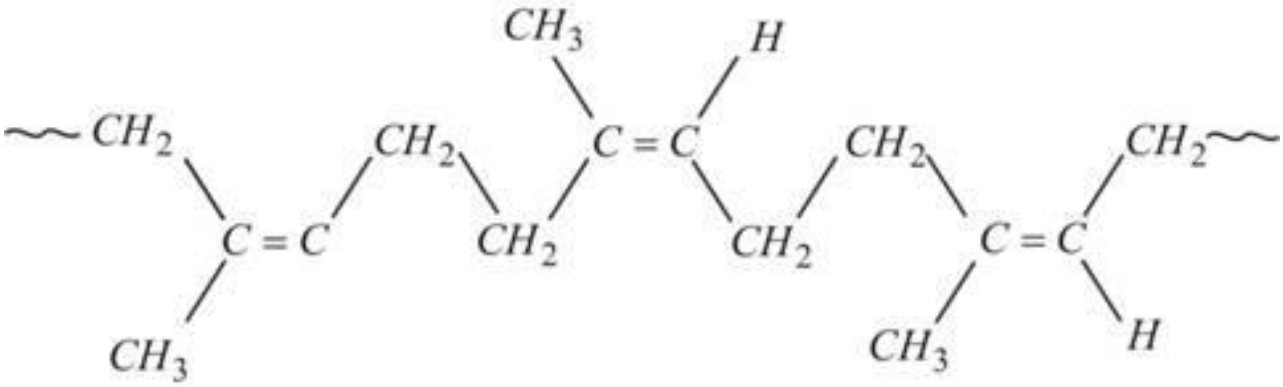


Figure 35. Nature Rubber

2.3. Methods of Obtaining Essential Oil

2.3.1. Distillation Method

Distillation is a separation procedure that' s made by utilizing the differences of boiling points of fluids. The majority of the volatile oils obtained by this procedure are compounds with a low boiling point and a minorit are water-soluable and have a high boiling point. Distillation methods include water distillation, vapor distillation and vacuum distillation(23).

2.3.1.1. Vapor Distillation

In this method, the steam applied by pressure to the plant material that is placed in the glass container brings the droplets of the oil by itself and brings them to the collection vessel. There, the oil is condensed and separated from the water(23).

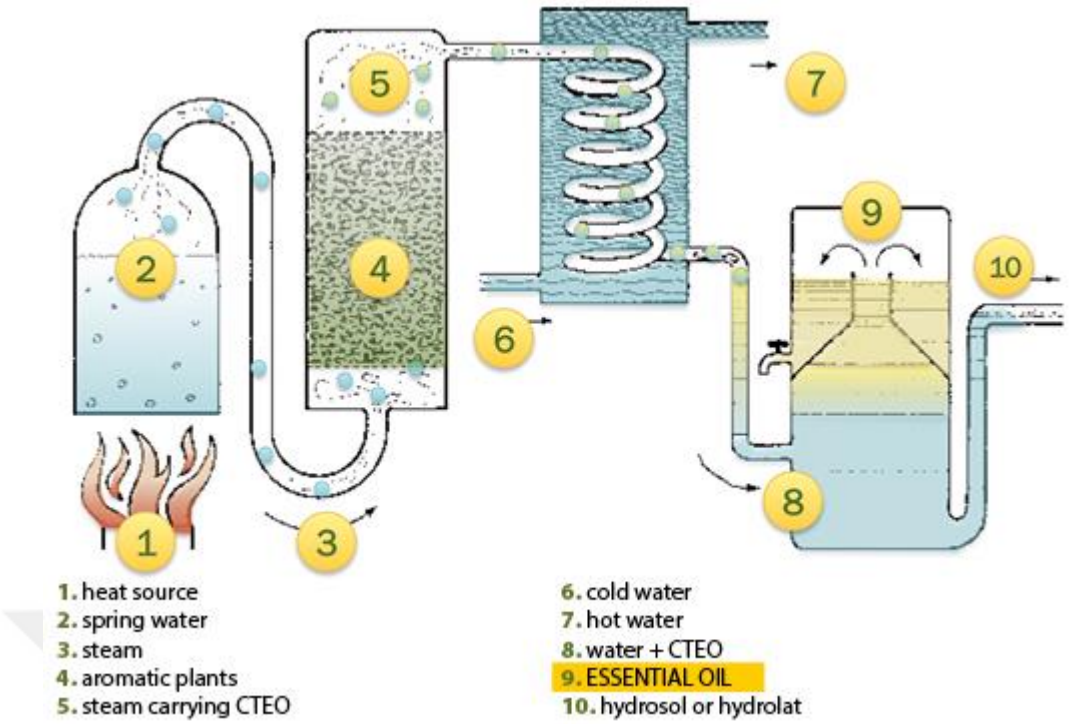


Figure 36. Vapor Distillation

2.3.1.2. Vacuum Distillation

In order to obtain compounds that have a quite high boiling point, it is more effective to lower the pressure than to increase the heat. Once the pressure is under the vapor pressure of the compound, boiling and distillation process begins.

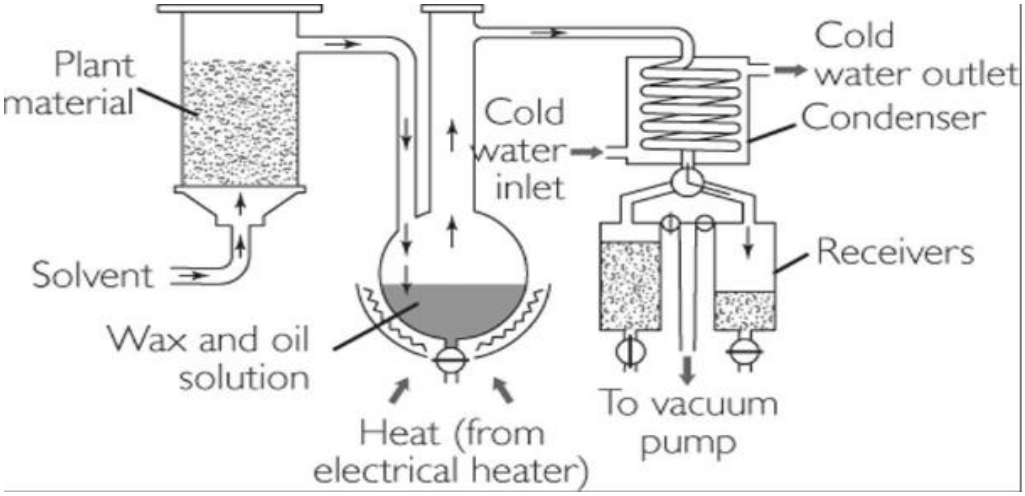


Figure 37. Vacuum Distillation

2.3.1.3. Hydrodistillation

This method is widely used in obtaining volatile compounds. The method is based on boiling the water and plant material in a glass balloon connected to the cooler and concentrating the oil molecules moving with the water vapor into the coolant separating them from the water (23).

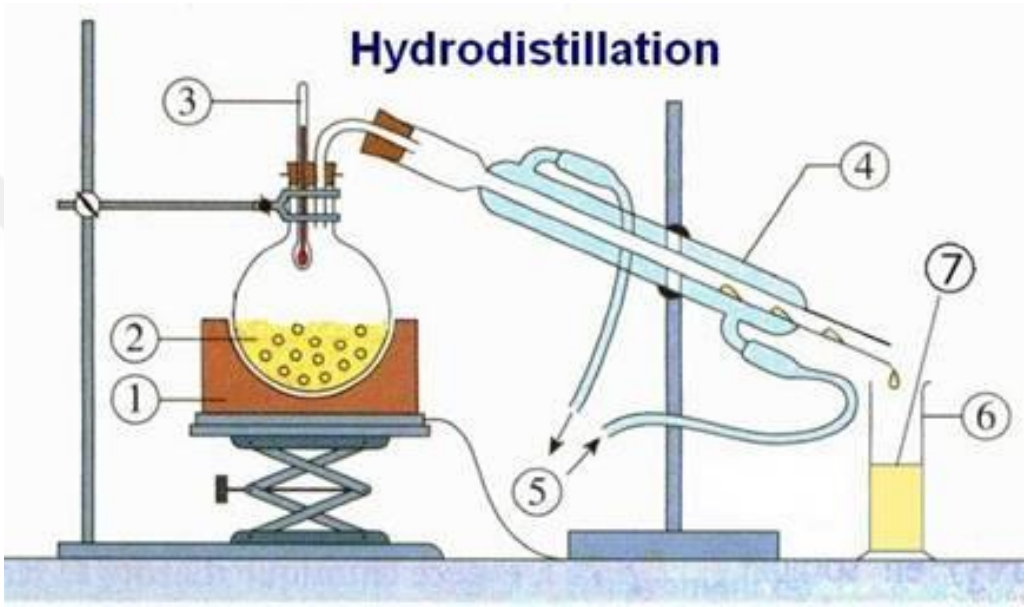


Figure 38. Hydrodistillation

2.3.2. Extraction Method

One of the research methods and the other is the extraction method. This method is divided into two groups, traditional and new methods. Supercritical fluid extraction, and microwave extraction are fast, efficient and modern methods developed in recent years. Temperature is important for efficient extraction.

2.3.2.1. Solvent Extraction

It is a traditional extraction method in which the plant sample can be placed directly into the solvent at room temperature or is boiled with organic solvent in a soxlet. Ethylene and pentane-dichlormethane are widely used in industrial studies in solvents

such as hexane and ethanol: analytical laboratories. As a result of extraction, the solvent is recovered by distillation from the medium. The remaining oily fraction contains volatile compounds(24).

This method has some advantages compared to steam distillation. The use of low temperature during extraction is one of them. Generally, the temperature is less than 60 degrees centigrade in the Soxlet apparatus and 5-25 degrees centigrade in the immersion method. The low temperature ensures that the resulting volatile oil forms a more natural content than steam distillation.

However, solvent extraction also has disadvantages. One of them is the loss of low molecular weight volatile compounds and the formation of artifacts during condensation during extraction. The other is an increasing solvent after extraction. This problem is very important both economically and in terms of environmental pollution. Pure and high-quality solvents are expensive and, if used in large quantities, bring a financial burden(24).

2.3.2.2. Supercritical Fluid Extraction

The treatment of natural products with solvent has become an undesirable phenomenon in terms of environment and health. In this sense; consuming less solvents, having a shorter extraction period and having the ability of separating compounds that are soluable in high temperatures under normal conditions, this method has attracted a great interest in the recent year

Supercritical fluid extraction is in fact a solvent extraction method. Instead of organic solvents, the supercritical fluid properties are used for solvent purposes. This substance exhibits a supercritical fluid property above the critical temperature and critical pressure point. In this case, the supercritical fluid is located between the liquid and the gas in terms of its thermo-physical properties. While the solubility of liquid solvents can solve many substances with its solubility power, it also dissolves dissolving substance rapidly by the diffusion coefficient close to the gases (25).

2.3.2.3. Microwave Extraction

Since II. World War, microwave technology has used and microwave extraction occured by this way. Microwave energy' s effectiveness depends on content of solvent. It also linked plant material and applied microwave power. If the both of polar molecules

and ionic species coexist, energy balancing is faster. The advantage of microwave method is deterioration of weak hydrogen bonds. In contrast to classic heat conduction method, microwave method provides warm up the all sample at the same time. Thanks to microwave, extraction comes true by two different systems. Container that temperature and pressure controllable closed system extraction(26,27).

2.3.2.4. Compressed Solvent Extraction

This method is imorıved as an alternative to classical methods. This extraction has many advantages; duration of extraction, solvent consumption, yield, repeability. In order to increase the effectiveness of the method, organic solvents are used at high temperature and pressure. Increasing temperature accelerates the kinetics of extraction, increasing pressure keeps solvent in liquid state. So, a safe and rapid extraction is provided. In addition, high pressure affects to the interior of the material(28).

2.2.3. Mechanic Method

The volatile compounds that found in citrus, decay by distillation method. Therefore, heels of these fruits put in a vloth bag and squeeze in cold hydraulic press. As a result volatile oils ocur(28).

2.4. Quantity of Essential Oils

The differet methods are used for this purpose. One of these name is volumetric, the other name is gravimetric. Measuring the volume of volatile oil that why volumetric methods are used.

The volatile oil is separated by water vapor distillation by gravimetric method. Water- oil solution is saturated by salt and taken with an organic solvent.

2.5. Determination of Compounds in Volatile Oil

Two different methods are used to determine the amount of essential oils, one being volumetric and the other being gravimetric. The essence of the volumetric quantity determination methods is to measure the volume by collecting in a volatile oil-leveled container, which is separated from the water vapor distillation(29).

As a result of this method, the volatile oil content is found in volume / weight. By measuring the density of the oil, the percentage by weight / weight can be calculated.

In the gravimetric method, which is the other method, the essential oil is separated by the water vapor distillation. The distillate composed of water-oil mixture is saturated with salt and withdrawn with an organic solvent. The solvent is evaporated in a sparged container and the remaining amount is measured and the percentage by weight / weight is calculated.

2.6. Gas Chromatography Mass Spectroscopy (GC / MS)

It is a system formed by combining gas chromatography mass spectroscopy. The compounds in one sample are used quite widely for identification. It is used to analyze the planet's atmosphere and soil by sending it to the airwaves at security controls and astronomical workings as well as analysis of samples, drugs, explosives, nutrients, narcotics, cosmetics and perfumes unknown by GC / MS. By GC / MS, trace elements in a sample can be assigned(30).

By using both methods together more precise results are obtained. In the analysis of a sample containing more than one compound, the exact result may not be achieved by using only one of these methods. In mass spectroscopy, very pure samples must be used. Sometimes, the ions from which two molecules are formed may be similar. In gas chromatography, the detector used can not distinguish substances with the same retention times in a mixture. When these two methods are used together, the results are quite accurate since it is extremely difficult to behave in the same way in two different molecules and in both gas and mass spectrometers.

The specimen injected into the device is transferred to the column with the carrier gas after it becomes vapor in the injection part. As they move through the column, the substances in the sample are held in the column for various periods of time. This retention time depends on the properties of the compound, such as volatility and molecular weight, and is called retention time (RT) (compounds with

low volatility and low molecular weight are generally less retained in the column). As the temperature of the column increases, the molecules retained in the column go to the gas phase, proceeds. Compounds which are separated from each other by the retention times and leave the column reach mass spectrometry. Here, they are bombarded with electrons obtained from a filament. This is called electron ionization (EI). The ions forming the electron ionization result are separated by the mass / charge ratio, and the detector is recorded and the spectrum is taken from the computer (30).

2.7. Lamiaceae family

Lamiaceae, formerly called Labiatae, the mint family of flowering plants, with 236 genera and more than 7,000 species, the largest family of the order Lamiales. Lamiaceae is distributed nearly worldwide, and many species are cultivated for their fragrant leaves and attractive flowers. The family is particularly important to humans for herb plants useful for flavour, fragrance, or medicinal properties(30).

2.7.1. Basil (Ocimum basilicum L.)

Figure 39. Basil (Ocimum basilicum L.)

Of the 9,000 plant species found in the natural flora of Turkey, 500 are used for medical purposes, and the vast majority of them grow naturally and only a few are cultivated. Basil (*Ocimum basilicum* L.), lavender (*Lavandula angustifolia* Mill.) and, melissa (*Melissa officinalis* L.) belong to Lamiaceae family and grow in Turkeys(30). The O.

basilicum essential oils exhibit a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colours, aroma and origin of the plants. The chief constituents include chavicol methyl ether or estragole, linalool and eugenol(31). There is a long tradition of using basil as a medicinal plant in treating coughs, diarrhoea, worm infestations and kidney malfunctions(32). Recent studies even suggest that basil oil displays great potential as a stress repressor(33) and it is also used as a component in drugs for leukaemia treatment(34).

2.7.2. Lavender (Lavandula angustifolia Mill.)

Figure 40. Lavender (Lavandula angustifolia Mill.)

Several Lavandula species are essential oil rich-plants showing high yield values. In food manufacturing, the essential oil of lavandin has been employed in flavoring beverages, ice cream, baked goods, and chewing gum(35). Lavender oil contains linalool, linalyl acetate, levender, geraniol tannin, flavonoids, and cineol, and has antimicrobial, antifungal, antibiotic, and antidepressant effects (36).

2.7.3. Melissa (Melissa officinalis L.)

Figure 41. Melissa (Melissa officinalis L.)

Medical authorities of ancient Greece and Rome mentioned topical Melissa as a treatment for wounds. The herb was later used orally as a treatment for influenza, insomnia, anxiety, depression, and nervous stomach(37). *Melissa officinalis* is a well-known medicinal plant species used in perfumes, cosmetics, tea and food products in many countries, and has been reported to possess sedative, spasmolytic and antibacterial properties. Herbal essential oils generally contain a variety of volatile compounds, which may have medicinal properties. It has been reported that *M. officinalis* essential oil has antimicrobial, antioxidative and antitumor properties(38). A study on *M. officinalis* showed that long-term oral administration of *M. officinalis* essential oil(at an effective dose of 0.04 mg/day) can suppress chemical hyperalgesia in diabetic rats(39).

This study aimed to extract basil (Ocimum basilicum var. album (L.) Benth), lavender (Lavandula angustifolia subsp. angustifolia), and melissa (Melissa officinalis) essential oils to identify its constituents the compounds from the oil using gas chromatography mass spectrometry (GC-MS) analysis and evaluate it’s the antimicrobial activity of the oils.

2.7.4. Mentha Piperita

Figure 42. Mentha piperita

Peppermint is widely used in food, cosmetics and medicines. Peppermint leaf and oil are used for folk medicine, as flavoring agents, and in cosmetic and pharmaceutical products throughout the World(40). Peppermint oil is the most extensively used of all the volatile oils. Peppermint is taken internally as a tea, tincture, oil, or extract, and applied externally as a rub or liniment. Herbalists consider peppermint an astrigent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue. Pepermint oil vapor is used as an inhalant for respiratory congestion. Peppermint tea is used to treat coughs, bronchitis, and inflammation of the oral mucosa and throat(41,42). It has traditionally been used to treat a variety of digestive complaints such as colic in infants, flatulence, diarrhea, indigestion, nausea and vomiting, morning sickness and anorexia, and as a spasmolytic to reduce gas and cramping. Pepermint is currently used to treat irritable bowel syndrome, Crohn's disease, ulcerative colitis, gallbladder and biliary tract disorders, and liver complaints. Peppermint oil is used to relieve menstrual cramps. Peppermint oil is used externally for neuralgia, headaches, migraines and chicken pox(43). *Mentha* genus contains about 25 species and some hybrids and bolong to the Lamiaceae family(44). Mints contain volatile components, flavonoids, organic acids, quinones, such as fort he digestive system, centarl nervous system, respiratory system. It was used in

antimicrobial, antiinflammatory or anesthesia. *M. Piperita* is a hybrid of spearmint (*M. spicata L.*) and water mint (*M. aquatica L.*), it grows particularly well in areas with high water-holding capacity soil(45,46).

2.7.5. Thymus vulgaris L.

Figure 43. Thymus vulgaris L.

Thymus vulgaris L. (thyme) is an aromatic plant belonging to the Lamiaceae family,used for medicinal and spice purposes almost everywhere in the World(47). In Romania, Thymus genus contains one cultivated species as aromatic plant (*Thymus vulgaris* L.) and other 18 wild species(48). *Thymus vulgaris* shows a polymorphic variation in monoterpene production, the presence of intraspecific chemotype variation being common in the genus Thymus. Each of the six chemotypes, geraniol (G), α-terpineol (A), thuyanol-4 (U), linalool (L), carvacrol (C), and thymol (T), is named after its dominant monoterpene(49). Many pharmacological in vitro experiments carried out during the last decades revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma(50).

2.7.6. Salvia officinalis

Figure 44. Salvia officinalis

Salvia officinalis or sage (Lamiaceae family) is a perennial low shrub native of the Mediterranean region and its family reported to include more than 900 species (51). Its essential oil is added to meat, sausage, poultry stuffings, fish, soups, canned foods and other food products. Sage essential oil protected liver patés from oxidation processes and could be used as alternative option to synthetic antioxidants such BHT and was used in dry fermented buffalo sausage too (51).

3. Materials and Methods

3.1. Plant Material

Ocimum basilicum var. album (L.) Benth, Mentha piperita, *Thymus vulgaris L.* plants were collected at the flowering stage from the Oltu valley in eastern Anatolia, Turkey, Lavandula angustifolia subsp. angustifolia, Melissa officinalis plants were collected in southeast of Marmara sea, Bursa, Turkey, *Salvia officinalis* were collected at the flowering from the Adana in South Anatolia, Turkey. Collected plant materials were dried in the shade, then separated from the stem of the plant.

3.2. Essential Oil Extraction Preparation

The aerial parts of the air-dried plants of basil (Ocimum basilicum var. album (L.) Benth.), lavender (Lavandula angustifolia subsp. angustifolia), Mentha piperita, *Thymus vulgaris L., Salvia officinalis* and Melissa (Melissa officinalis) were submitted for 4 h modified Clevenger collector apparatus. Obtained essential oil was dried over

anhydrous sodium sulfate (Na_2SO_4) and stored at -4 °C until tested and analyzed(30). Qualitative and quantitative analyses of the oils were performed using GC/MS.

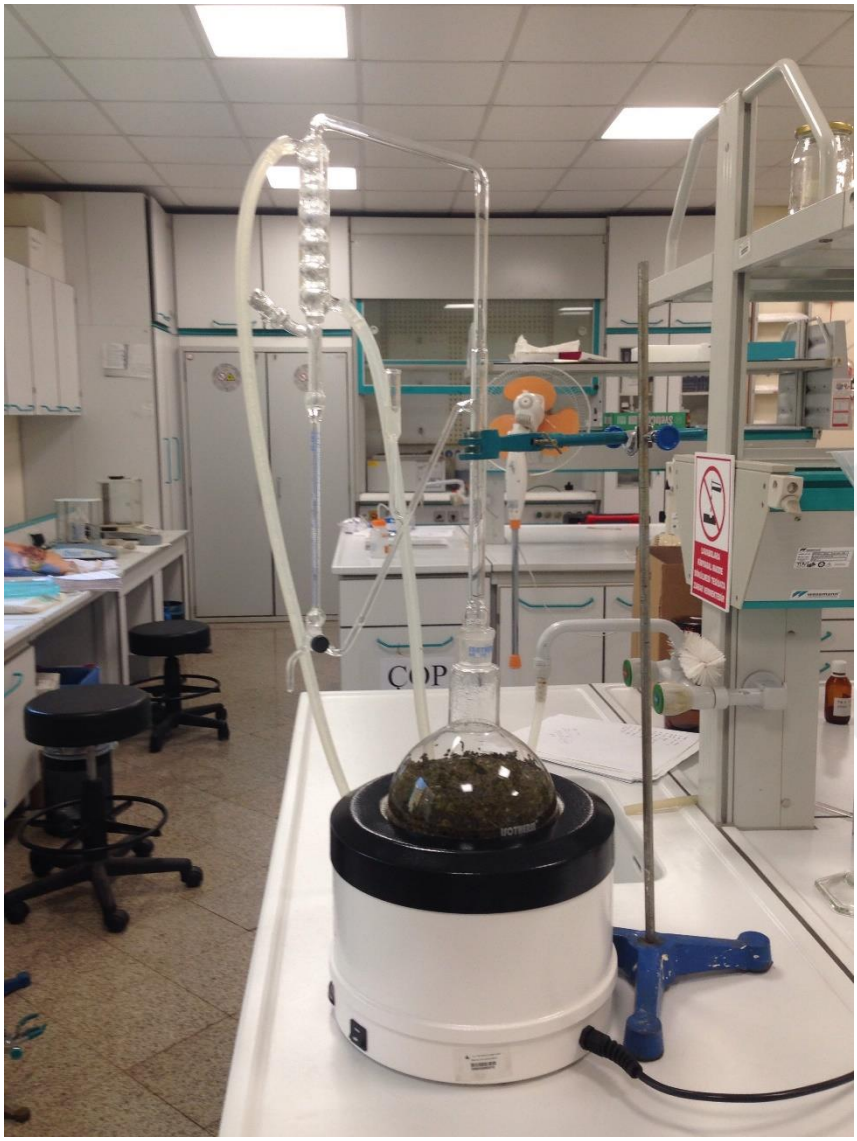


Figure 45. Clevenger collector apparatus

3.3. Test Organisms

A total of five organisms were tested on the antimicrobial activity. Namely Staphylococcus aureus (ATCC 6338) Gram positive, Escherichia coli (ATCC 10536) and Pseudomonas aeruginosa (ATCC 15442) are Gram negative, Aspergillus nigeris fungus, Candida albicans is a yeast.

3.4. Determination of Antimicrobial Activity of Essential Oil

The antimicrobial activity was tested out by disc diffusion method. Discs with 6 mm in diameter of Whatman No. 1 filter paper were used. Briefly, 100 µL suspension of individual test microorganism was spread homogenously on each plate of mannitol salt agar media. Each disc was soaked with 100 µL of pure essential oil and placed on the microbial lawn. Positive control experiments were carried out under similar condition by using ofloxacine for antibacterial activity and nystatin for antifungal activity. Negative control experiments were carried out by using sterile water. The tests were repeated three times to ensure reliability. The plates were incubated at 37 oC for 24 h and the inhibition zones were checked(52).

3.5. GC-MS Analysis

The analysis of the essential oil was performed using a Thermo Finnigan Trace GC/TraceDSQ/A1300 equipped with an SGE-BPX5 MS capillary column (30 m × 0.25 mm id, 0.25 µm). For GC/MS detection an electron ionisation system with an ionisaton energy of 70 eV was used. Helium was the carrier gas at a flow rate of 1 mL·min-1. Injector and MS transfer line temperatures were set at 220 and 290^{o}C respectively. The column temperature was raised from 50 to 150^{o}C at a rate of 3^{o}C·min-1, held isothermal for 10 min and finally raised to 250^{o}C at 10 oC·min-1. Diluted samples (1:100 v/v in methylene chloride) of 1.0 µL were injected manually in splitless mode. The components were identified by comparison of their relative retention times and mass spectra with those of standards, Wiley 7N library data of the GC/MS system and literature data(52). The results were also confirmed by comparison of the elution order of the compounds with their relative retention indices on non-polar phases reported in the literature(52).

4. RESULTS

4.1. Antimicrobial Activity of Essential Oil:

The essential oil exhibited antimicrobial activity against Gram negative, Gram positive, fungus and yeast. Discs (6 mm) containing 100 µL essential oil were subjected to five microbial strains individually. Among the tested microbial strains, strong inhibition effect was found against all of them.

4.2. Chemical Composition of Essential oil of Ocimum basilicum var .album (L) Benth

The GC-MS analysis of the Ocimum basilicum var. album (L.) Benth essential oil led to identification of 48 different compounds. The total compounds percentage was 100%. 1,6-octadien-3-ol,3,7-dimethyl (53.79%), 3-Allyl-6-methoxyphenol (12.57%), eucalyptol(1,8-cineole) (4.33%), α-muurolol (3.87%) were major compounds(Table 1).

4.3. Chemical Composition of Essential Oil of Melissa officinalis

The GC-MS analysis of the Melissa officinalis essential oil leads to identification of 48 different compounds. The total compounds percentage was 100%: D-limonene (26.00%), citral (14.93%), neral (13.60%), caryophyllene oxide (11.45%), benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl (6.97%) were major compounds (Table 2).

4.4. Chemical Composition of Essential Oil of Lavandula angustifolia subsp. Angustifolia

The GC-MS analysis of the Lavandula angustifolia L. essential oil led to identification of 48 different compounds. The total compounds percentage was 100%. 1,6-octadien-3-ol,3,7-dimethyl (42.07%), linalyl acetate (18.26%), camphor (5.89%), alpha-terpineol (4.83%), geranyl acetate (2.56%) were major compounds (Table 3).

4.5. Chemical Composition of Essential Oil of Mentha piperita

The GC-MS analysis of the *Mentha piperita* essential oil led to identification of 25 different compounds. The total compounds percentage was 100%. Eucalyptol (80.30%), Trifluoroacetyl-α-terpineol (3.61%), p-Menth-8-en-1-ol (1.4%), Bicyclo(4.1.0)heptan-2-ol (0.57%) were major compounds (Table 4).

4.6. Chemical Composition of Essential Oil of *Thymus vulgaris L.*

The GC-MS analysis of the *Thymus vulgaris L.* essential oil led to identification of 32 different compounds. The total compounds percentage was 100%. P-cymene (28.75%), benzene,1-ethyl (16.92%), benzene,1-ethyl-2,4-dimethyl (4.97%), 1,3,8-p-menthatriene (1.61%) were major compounds (Table 5).

4.7. Chemical Composition of Essential Oil of *Salvia officinalis*.

The GC-MS analysis of the *Salvia officinalis* essential oil led to identification of 32 different compounds. The total compounds percentage was 100%. (1R)-2,6,6-trimethylbicyclo (33.62%), α-pinene (8.89%), trans-α-ocimene (6.58%), and1,3,6-octatriene(5.04%) were major compounds(Table 6).

Table 1. Chemical composition of essential oil of Ocimum basilicum var. album (L) Benth.

N	R.Time	Compound	%
1	8.398	hex-2(E)-enal	0.08
2	11.744	alpha-pinene	0.33
3	12.345	camphene	0.15
4	13.458	sabinene	0.17
5	13.543	β-pinene	0.44
6	14.270	Myrcene	0.50
7	15.768	d-limonene	0.50
8	15.863	eucalyptol(1,8-cineole)	4.33
9	16.205	cis-ocimene	0.06
10	16.613	ocimene<E-beta	0.66
11	16.996	gamma-terpinene	0.06
12	17.707	linalool oxide	0.47
13	18.151	bicyclo[2.2.1]heptan-2-one,1,3,3-trimethyl-,(1R)-	1.11
14	18.328	trans-linalool oxide	0.46
15	19.109	1,6-octadien-3-ol,3,7-dimethyl-	53.79
16	20.431	(+)-2-bornanone	2.28
17	22.436	benzene,1-methoxy-4-(2-propenyl)-(CAS)	1.49
18	27.743	alfa-cubebene	0.09
19	28.185	3-Allyl-6-methoxyphenol	12.57
20	28.648	Copaene	0.18
21	28.940	beta-bourbonene	0.26
22	29.172	beta-elemene	1.47
23	29.547	benzene 1,2-dimethoxy-4-82-propenyl)-(CAS)	2.14
24	30.063	trans(beta)-caryophyllene	0.31
25	30.557	bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-	1.72
26	30.658	alpha-guaiene	0.64
27	30.909	(+)-epi-bicyclosesquiphellandrene	0.11

28	31.155	alpha-humulene	1.02
29	31.434	(+)-epi-bicyclosesquiphellandrene	0.45
30	31.547	İtalicene	0.06
31	32.009	d-dermacrene	1.15
32	32.088	farneseneE-beta	0.18
33	32.166	beta-selinene(CAS)	0.16
34	32.483	bicyclogermacrene	0.85
35	32.759	azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylet-henyl)-,[1S-	1.08
36	33.018	naphthalene 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methyl	2.09
37	33.258	naphthalene 1,2,3,5,6,8a-hexahydro-4,7-dmethyl-1-(1-methy-lethyl)	0.48
38	33.693	naphthalene1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methy-lethyl)	0.05
39	35.071	caryophyllene oxide	0.08
40	35.195	alloaromadendrene oxide	0.08
41	35.383	salvial-4(14)-en-1-one	0.06
42	35.839	12-oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl	0.18
43	37.517	alfa-muurolol	3.87
44	38.035	3-methyl-5-propyl-4-butylidene-cyclohex-2-ene-1-one	0.89
45	38.605	Tetradecanal	0.07
46	42.028	Phytone	0.08
47	44.982	n-hexadecanoic acid	0.16
48	49.122	9-octadecenoic acid	0.25

Table 2. Chemical composition of essential oil of Melissa officinalis.

N	R.Time	Compound	%
1	11.740	α-pinene	0
2	13.453	Sabinene	1
3	14.126	6-methyl-5-hepten-2one	2
4	14.275	Myrcene	0
5	15.911	d-limonene	2
6	18.563	3-methyl-2-(2-methyl-2-butenyl)-furan	0
7	18.758	z-citral	0
8	18.844	3,4,4-trimethyl-2-cyclopenten-1-one	0
9	19.944	7-Oxabicyclo[4.1.0]heptane,1-methyl-4-(1-methylethenyl)	0
10	20.127	(+)-E-limonene oxide	0

11	20.572	trans-chrysanthemal	0
12	20.724	Citronella	0
13	21.577	2-(2’,3’-Epoxy-3’-methylbutyl)-3-methylfuran	0
14	21.847	Verbenol	0
15	22.325	(S)-(-)-(4-ısopropenyl-1-cyclohexenyl)methanol	0
b16	22.452	santolina triene	0
17	24.115	Neral	1
18	24.480	Piperitone	0
19	25.197	Citral	1
20	28.197	neryl acetate	0
21	28.639	Copaene	0
22	28.848	geranyl acetate	2
23	28.941	β-bourbonene	0
24	29.838	1H-3a,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl	0
25	30.084	trans(β)-caryophyllene	0
26	31.150	α-humulene	0
27	31.377	Neoalloocimene	0
28	31.707	geranyl propanoate	0
29	31.845	γ-cadinene	0
30	32.052	benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl	6
31	32.453	Bicyclogermacrene	0
32	32.793	β-bisabolene	0
33	32.886	α-cedrene	0
34	33.005	naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-	0
35	33.267	Deltacadinene	0
36	33.697	naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	0
37	34.167	caryophyllene oxide	0
38	35.187	caryophyllene oxide	1
39	35.289	benzenebutanoic acid,2,5-dimethyl-,methyl ester	0
40	35.428	benzenebutanoic acid,2,5-dimethyl-,methyl ester	0
41	35.575	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene	1
42	35.864	12-oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-	1
43	36.684	2,6-octadienoic acid,3,7-dimethyl-,methyl ester	0
44	37.110	1H-benzocyclohepten-7-ol,2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-	0
45	42.029	Phytone	0
46	44.963	n-hexadecanoic acid	0
47	48.458	4-methyl-5-(3methyl-2-butenyl)-6-methyl-1-formyl-6-(4 methyl-3-penten)	0
48	49.105	cis-vaccenic acid	0

Table 3. Chemical composition of essential oil of Lavandula angustifolia subsp. angustifolia.

N	R. Time	Compound	%
1	7.412	hexane,1-methoxy-(CAS)	0.10
2	11.742	alpha-pinene	0.17
3	12.342	Camphene	0.24
4	13.456	Sabinene	0.05
5	14.086	beta-pinene	0.16
6	14.086	3-octanone(CAS)	0.64
7	14.280	Myrcene	1.30
8	14.923	herboxide second isomer	0.07
9	15.250	hexyl-ethanoate	0.86
10	15.590	para-cymene	0.06
11	15.770	cyclohexene,1-methyl-5-(1-methylethenyl)-	0.97
12	15.870	eucalyptol(1,8-cineole)	3.73
13	16.214	alfa-pinene	1.08
14	16.627	(E)-beta-ocimene	1.57
15	17.677	linalool oxide	2.11
16	18.178	alpha-terpinolene	0.33
17	18.325	trans-linalool oxide	1.64
18	19.113	1,6-octadien-3-ol,3,7-dimethyl	42.07
19	19.726	cyclobutanecarboxylic acid, octyl ester	0.13
20	19.847	alloocimene(CAS)	0.06
21	20.478	Camphor	5.89
22	20.604	propanoic acid,2-methyl-,hexyl ester (CAS)	0.29
23	20.680	Lilac aldehyde B	0.06
24	20.814	2H-pyran,3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)	012
25	21.966	2-cyclohexen-1-one,4-(1-methylethyl)	0.17
26	22.254	hexyl-butyrate	2.01
27	22.535	alpha-terpineol	4.83
28	22.933	acetic acid,2-ethylhexyl ester(CAS)	1.33
29	23.270	gamma-terpinene	0.32
30	23.505	isobornyl formate	0.29
31	23.840	hexyl 2-methyl butyrate	0.24
32	23.920	benzaldehyde, 4-(1-methylethyl)	0.10
33	24.013	butanoate hexyl-,3-methyl-	0.29
34	24.678	linalyl acetate	18.26
35	25.046	Citral	0.07

36	25.597	endobornyl acetate	0.08
37	25.759	lavandulyl acetate	0.08
38	27.081	hexyl tglate	0.60
39	27.793	linalool	0.08
40	28.215	neryl acetate	1.42
41	28.810	geranyl acetate	2.56
42	30.070	trans-beta-caryophyllene	0.42
43	31.160	(E)-beta-farnesene	0.28
44	31.994	D-germacrene	0.13
45	32.784	neryl(S)-2-methylbutanoate	0.18
46	35.077	caryophyllene oxide	0.47
47	44.962	n-hexadecanoic acid	0.07
48	49.121	9-octadecenoic acd,E	0.27
			100

Table 4. Chemical composition of essential oil of Thymus vulgaris L.

N	R. Time	Compound	%
1	9.22	Eucalyptol	80.20
2	8.84	Trifluoroacetyl-α-terpineol	3.61
3	7.80	Cyclohexznemethanol	1.97
4	7.87	Cyclohexanol	1.51
5	7.80	p-Menth-8-en-1-ol	1.45
6	7.69	7-Oxabicyclo(2.2.1)heptane	1.28
7	7.57	2-Cyclohexen-1-ol,1-methy	0.70
8	7.54	1-Cyclopentene-1-methanol	0.67
9	7.51	2-Cyclohexen-1-ol,1-methy	0.59
10	7.47	Bicyclo(4.1.0)heptan-2-ol	0.57
11	7.42	Bicyclo(3.1.0)hexan-2-ol	0.50
12	8.42	7-oxabicyclo(2.2.1)heptane	1.28
13	7.40	2-cyclohexen-1-ol,2-methy	0.42
14	7.39	1,7-Octadien-3-ol,2,6-dimetil	0.37
15	7.42	7-oxabicyclo(2.2.1)heptane	1.28
16	7.34	3-cyclohexen-1-ol,1-methyl	0.29
17	7.31	3-decen-2-one	0.29
18	7.34	2-cyclohexen-1-ol,1-methyl	0.59
19	7.29	ısopulegol	0.28
20	7.43	cyclohexanol,5-methyl	0.27
21	7.30	cyclopentanol,1,2-dimethyl	0.25
22	7.31	2-oxabicyclo(2.2.1)heptane	0.21
23	7.28	7-oxabicyclo(2.2.1)heptane	1.28
24	7.24	2-acetonylcyclohexanone	0.16

25	7.17	1,2-cyclohexanediol,3-methyl	0.13

Table 5. Chemical composition of essential oil of Mentha piperita

N	R.Time	Compound	%
1	9.46	p-cymene	28.75
2	9.47	o-cymene	25.41
3	8.94	benzene,1-ethyl	16.92
4	8.90	p-cymene	28.75
5	9.00	benzene,1-ethyl-2,4-dimethyl	4.97
6	8.84	benzene,1-methyl	16.92
7	8.98	benzene,2-ethyl-1,3-dimethyl	4.20
8	8.90	benzene,2-ethyl-1,4-dimethyl	3.55
9	8.48	benzene,4-ethyl-1,2-dimethyl	3.27
10	8.47	benzene,1-ethy-2,4-dimethyl	4.97
11	8.45	benzene,1-ethyl-2,3-dimethyl	2.37
12	8.85	1,3,8-p-menthatriene	1.67
14	8.35	benzene,1,2,4,5-tetramehyl	0.67
15	8.25	benzene,4-ethyl-1,2-dimethyl	3.27

Table 6. Chemical composition of essential oil of Salvia officinalis.

N	R.Time	Compound	%
1	9.46	(1R)-2,6,6-trimethylbicyclo	33.62
2	9.26	α-pinene	9.89
3	9.06	trans-α-ocimene	6.58
4	9.04	1,3,6-octatriene	5.04
5	9.01	tricyclo(2.2.1.0(2,6))heptanel	5.04
6	9.00	(1S)-2,6,6-trimethylbicyclo	5.04
7	9.17	bicyclo(3.1.1)hept-2-ene	4.45
8	8.93	α-ocimene	4.11
9	9.14	3-carene	3.79
10	8.90	α-pinene	9.89
11	8.88	(1R)-2,6,6-trimethylbicyclo	33.62
12	8.86	1,3,6-octatriene,3,7-dimethyl	5.04
13	9.11	Bicyclo(3.1.0)hex-2-ene	2.90
14	8.83	3,5-methanocyclopentapyrane	2.68
15	9.05	Bicyclo(3.1.0)hex-2-ene	2.47
16	8.90	(1S)-2,6,6-trimethylbicyclo	5.04
17	8.76	tricyclo(2.2.1.0(2,6))heptanel	1.89
18	8.93	4-carene	1.76

5. DISCUSSION

Table 1 lists the chemical composition of the essential oils of Ocimum basilicum var. album (L.) Benth. The major compound of Ocimum basilicum var. album (L.) Benthoil is 1,6-octadien-3-ol,3,7-dimethyl (53.79%). In addition to 3-Allyl-6-methoxyphenol (12.57%), eucalyptol (1,8-cineole) (4.33%), and α-muurolol (3.87%) as major constituents. However, we found very low levels of bicyclo[3.1.1n]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)-, alfa-humulene, bicyclogermacrene: 1.72%, 1.02% and 0.85%, respectively. Abou El-Sound et al. (54) reported that the content of essential oil in Egypt basil varieties was from 0.3 to 0.7%. Nineteen compounds, representing 96.7% of the total oil were identified. The main components were as follows: linalool(48.4%), 1,8-cineol (12.2%), eugenol (6.6%), methyl cinnamate (6.2%), α-cubebene(5.7%), caryophyllene (2.5%), β-ocimene(2.1%) and α-farnesene (2.0%). The tested oil showed significant antifungal activity that was dependent on the used oil concentration. Differences in basil essential oil content between this study and another report from research conducted in Egypt could be due to diffential environmental conditions Egypt and Turkey. It has been demonstrated that basil essential oil can vary depending on growth conditions(55). In a study conducted in Australia, the morphological characteristics, yield and essential oil components of 5 different varieties of basil were investigated. Researchers have reported that plant length varies from 39 to 61 cm, single plant weights vary from 80 to 499 g, and total plant weights vary from 448 to1,624 kg/d, and the components of the varieties are different according to the results of the gas chromatography analysis and that the main components of the essential oils are methyl chavicol, linalol, 1,8-sineol(56) .

In another study, the chemical compositions of essential oils of the two types of basil grown in Turkey were examined. Essential oils of over-ground parts of plants were obtained and their components were determined by GC-MS.

Korucu (57) found that the highest amounts of essential oil components in fresh basil samples were linalol (16.67%-25.58%) and eugenol (7.31%-12.64%) while Celebi [23] found that ladenein was between 11.79%-30.07%, pilosin was between 1.99%-7.24%, genkwan was between 2.38%-5.16%, salvigen was between 2.29%-4.32%, cirsiliol was between 1.42%-26.01%, and apigenin was between 4.5%-5.35%. In one study, they found that the essential oil ratio of the basil flower varies between 0.13% and 1.23%, while essential oil content of its leaf varies between 0.18% and 1.70%. The main components of essential oil were methyl chavicol and eugenol(58).

In our study, we found that S. aureus, E. Coli, P. aeruginosa, A. nigerand, C. Albicans are inhibited by the essential oils of O. basilicum L. This can be explained by the fact that the oil acts on the bacterial membrane. Monoterpenes or sesquiterpene hydrocarbons and their oxygenated derivatives exhibit a potential antimicrobial activity(59). Abou El-Soudet(54) showed that compounds of O. basilicum essential oil such as linalool, 1,8-cineol, eugenol, methyl cinnamate and α-cubebene were among the main components present. The antimicrobial activity of an essential oil is attributed mainly to its major compounds. However, the synergistic and antagonistic effect of one compound in minor percentage in the mixture has to be considered. Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the essential oils and the antimicrobial or antifungal activities(60).

Table 2 lists the chemical composition of the essential oils of Melissa officinalis. The major compound of Melissa officinalis oil is d-limonene (26.00 %), following by citral (14.93%), neral (13.60%), caryophyllene oxide (11.45%), and benzene,1-(1,5-dimethyl-4-hexenyl)-4-methyl (6.97%). However, we found very low levels of 6-methyl-5-hepten-2one, geranyl acetate, 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene: 2.22%, 2.37% and 1.94%, respectively.

In the research of Taherpour et al(61), (E)-citral (37.2%), neral (23.9%) and citronella (20.3%) have the highest percentages (81.4%) among the 14 components identified. M. officinalis L. has a strong lemon odour, which may be due in large part to (E)-citral. (E)-citral is not optically active and is a principal component of lemon grass oil. This compound has been utilized in perfumes, as a flavouring agent, and as an intermediate for other fragrances and vitamin A synthesis. The pure form of neral is a colourless liquid and has a rose-neroli odour.

This compound is utilized for perfumery and flavouring. Citronellal has both d- and l-isomers(62). Biological and aromatic effects of the main and minor compounds of the essential oil of M. officinalis L. have high importance in term of their possible use in medicine, cosmetics and foods (63).

The obtained essential oil is rich in monoterpenes and sesquiterpenes. The main compounds of volatile oil are citral (geranial, neral) and citronellal, giving the characteristic lemon odor to the oil. Other ingredients arebenzoic acids (gallic acid, protocatechuic acid), flavonoids (apigenin, luteolin), triterpenes (ursolic acid, oleanolic acid) and phenylpropanoyl glycosides(64,65). Korucu(57) reported that thirty three components

were identified representing 89.30% of the total oil in leaves composition. Six predominant components followed in the essential oils from Melissa officinalis were citronellal (14.40%), isogeraniol (6.40%), geraniol acetate (10.20%), nerolecetate (5.10%), caryophyllene (8.10%) and caryophyllene oxide (11.00%), representing 55.20% of the total oil. However, the age of Melissa officinalis plant effected the concentration of other constituents and the proportions of the following compounds were subject to especially high fluctuations: citronellal (8.7% and 0.4%), geraniol (trace amounts and 0.6%), and geranyl acetate (0.5% and 3.0%), as well as, among others, isogeranial, E-caryophyllene oxide, germacrene D, and carvacrol(65).

In the essential oil of Melissa officinalis ssp inodora, sesquiterpenes such as alpha-kubeben, beta-cariophilene and alpha-kadinol were found as main components(66). Melissa officinalis plant has sedative, carminative, antimicrobial and topical antiviral effects. It is used internally for nervous sleep problems, anxiety, unrest, irritability and functional gastrointestinal disorders(67,68). Other study showed that M. officinalis essential oil has anti-bacterial, anti-fungal, anti-parasitic and anti-histaminic activities(69). Thanks to its anti-microbial effect, it has a preventive effect on the growth of yeasts which cause the food degradation in the food industry(70).

In a study in German, N. Catariave M. officinalis essential oils have been researched for antibacterial activities against bacteria that affect the respiratory system and cause skin infections. The highest antibacterial activity in all the bacteria used belonged to M. officinalis essential oil. In particular, M. officinalis essential oil was found to be the most effective one against Streptococcus pneumoniae strain with the lowest MIC value(71). In a study using disk diffusion method on essential oil obtained by hydrodistillation from the aerial parts of the plant, it was found that essential oil was effective against all gram positive and gram negative bacterial strains tested at different ratios and showed high antimicrobial activity especially on Sigellasonei which is a very resistant bacterium and E. Coli(72).

As seen in Table 3, the major compound of Lavandula angustifolia Mill. oil is 1,6-octadien-3-ol,3,7-dimethyl (42.07%). In addition to linalyl acetate (18.26%), camphor (5.89%), alpha-terpineol (4.83%), and eucalyptol (1,8-cineole) (3.73%) as major constituents. However, we found very low levels of geranyl acetate, linalool oxide, acetic acid, and 2-ethylhexyl ester (CAS): 2.56%, 2.11%, and 1.33%, respectively. Results of other studies indicated that the essential oil of lavandula from Iranian contains 1,8-cineole(47.94%), borneol (26.14%), camphor(14.4%), while the essential oil lavandula from

Romania contains camphor(32.7%) and eucalyptol (26.9%)(73). In the other reports, linalool and linalyl acetate were the major components of the essential oil of Hungarian lavandula(74).

Essential oils of the lavandula species (lavender) exhibit various biological activities, namely anti-microbial, antimutagenic, anti-inflammatory and analgesic properties. Chemical components of plants are determined by a series of factors, including plant genetic,climate, adaptic, elevation, topography, and also by interaction of various factors. The principal compound of the essential oil from *L. angustifolia* (Croatia) is linalool (66.83%), while for the same species grown in France, this compound only represents 37.31% of the chemical composition of the oil, being curcuminaldehyde (41.32%) the major component. Moreover, the oil of the same Lavandula species (*L. angustifolia*) grown at high altitude in France also presents a great amount of linalool (23.49%), but with linalylformate (41.72%) being the most abundant compound. The main compounds of the other Lavandula species from Croatia (*L. officinalis)* are linalool (47.86%), linalyl formate (22.09%), and limonene (14.78%) (75). Linalool and linalyl acetate have maximum and great absorbing properties from skin during massage with a depression of central nervous system. Linalool shows sedative effects and linalyl acetate shows marked narcotic actions. These two actions may be responsible for its use in lavender pillow anxiety patients with sleep disputance pattern, improving the felling of well being, supporting mental alertness and suppressing aggression and anxiety.

Generally, these major components determine the biological properties of the essential oils. The main group is compose of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight.

Vukovic-Gacic et al.(2006) showed that *Salvia officinalis* and major components thyone, 1,8-cineole, camphor and limonene inhibit UVC-induced mutagenesis in salmonella typhimurium, Escherichia coli and Saccharomyces cerevisiae(76). De-Oliveira et al. (1997,1999) have demostrated that (-)-menthol,(-)-α-pinene, α-terpinene, α-terpineol, 1,8-cineole, d-limonene, camphor, citronellal and citral modulate hepatic monooxygenase activity such as CYP1A1 and CYP2B1 interacting with promutagen or procarcinogen xenobiotic biotransformation(77).

Manosroi et al.(2006) have shown an inhibition of the proliferation of murine leukemia and human mouth epidermal carcinoma cell lines by *Ocimum sanctum, Lavandula angustifolia, Ocimum basilicum, Ocimum americanum* and *Mentha spicata* essential oils(78). Khan and Abourashed reported that peppermint yields 0.1-1.0% of volatile oil

that is composed mainly of menthol (29-48%), menthone(20-31%), and menthyl acetate (3-10%)(79). Reddy et al. reported that peppermint essential oil comprising methol (36.02%), menthone (24.56%), menthyl acetate (8.95%), and menthofuran (6.88%); these are major components, and others are minor components(80). Our study; essential oil shows significant antibacterial and antifungal activity that principle components. In the other study;volatile oil obtained by steam distillation contains high amounts of thymol and p-cymene(81).

In the research of Porte et al.(82) the major constituens of the *Salvia officinalis* L. oil were α-thujone (40.90%), camphor (26.12%),α-pinene(5.85%) and β-thujone (5.62%). Many studies have assessed the antibacterial (83) and antifungal (84) activities of *Mentha piperita* L.. Thus, essential oils could make their way from the traditional into the modern medical domain.

6. CONCLUSION

Most of the essential oils obtained from plants form terpene type compounds. Terpen type compounds are used in many fields such as medicines, cosmetics, perfumery, food because of their pleasant smells and their high biological activity. Similar studies have to be done and increased to find plants containing these compounds in high proportion.

Antimicrobial resistance in bacteria is increasing rapidly. In contrast, bacteria do not gain resistance to plant and plant products that show antimicrobial properties. The reason for this is that synthetically produced medicines are made by isolating any active substance in plants. Bacteria can neutralize medicines by creating resistant breeds against synthetic drugs containing a single structure in time.

On the other hand, since the active substances in plants are in a complex structure with other substances, it is difficult for bacteria to develop resistance against this structure. Therefore, as an alternative to antibiotics, it may be appropriate to increase the number of researches for the use of plants and herbal products as traditional antibiotics. At the same time, the use of plants and herbal products is advantageous in this respect because of the higher number of side effects of synthetically derived materials. However, especially the more detailed results obtained by purely obtaining essential oil components or their main groups and testing them on microorganisms may be even more enlightening.

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