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Distillation and extraction of herbs from Lamiaceae family

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TFG REALIZADO EN PROGRAMA DE INTERCAMBIO

TÍTULO:	Distillation and extraction of herbs from Lamiaceae family
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RESUMEN

En este trabajo de fin de grado se llevó a cabo la destilación y extracción de cuatro plantas de la familia Lamiaceae: lavanda (Lavandula angustifolia), tomillo (Thymus vulgaris L.), menta verde (Mentha spicata L.) y menta piperita (Mentha piperita L.). Se realizaron dos extracciones: Soxhlet y extracción asistida por ultrasonidos.

La hidrodestilación se llevó a cabo con un aparato Clevenger, para obtener los aceites esenciales.

La extracción Soxhlet se probó con dos disolventes: n-pentano y etanol al 96%. En cada caso, el etanol al 96% fue más eficaz. La extracción asistida por ultrasonidos se llevó a cabo por pasos. Se realizaron tres extracciones en cada experimento. El disolvente utilizado para este tipo de extracción fue etanol al 96% y una mezcla de agua y etanol (70% de etanol). Se obtuvieron mayores rendimientos con el etanol al 70%. En todos los experimentos, los rendimientos más altos se obtuvieron con la lavanda.

Palabras clave: Lamiaceae, hidrodestilación, Soxhlet, extracción asistida por ultasinidos, aceite esencial

ABSTRACT

Plant products are present in many industries due to their numerous applications. In this thesis, the distillation and extraction of four plant materials belonging to the Lamiaceae family were carried out: lavender (*Lavandula angustifolia),* thyme *(Thymus vulgaris* L.*),* spearmint *(Mentha spicata* L.*) and* peppermint *(Mentha piperita* L.*).* Two different extractions were tested: Soxhlet and ultrasonic-assisted extraction.

Firstly, the moisture content of the herbs was determined. Hydrodistillation was carried out, using a Clevenger apparatus, to obtain the corresponding essential oils.

Soxhlet extraction was tested with two different solvents: n-pentane and 96% ethanol. In every case, the 96% ethanol was more effective. The ultrasonic-assisted extraction was carried out by the step-wise method. Three extractions were performed in each experiment. The solvent used for this type of extraction was 96% ethanol and ethanol water mixture (70% ethanol). Higher yields were obtained with 70% ethanol. In every experiment, lavender obtained the highest yields.

Key words: Lamiaceae family, Hydrodistillation, Soxhlet extraction, Ultrasonic assisted extraction, essential oil

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1 INTRODUCTION

In ancient times, humans turned to plants and herbs to find natural remedies that could alleviate pain and illness. A large number of medicinal herbs were highly valued for their properties. They are mentioned in books and ancient writings in places such as India, Egypt, Ancient Rome, Babylonia and China [1].

Over the years, technological progress has made it possible to increase our knowledge of these plants and to develop methods for obtaining their value-added essential oils, bioactive compounds on a large scale. Today, plant products are present in many industries and have a high commercial value due to their numerous applications. Moreover, a significant interest in natural products has re-emerged in all parts of the globe [2]. Natural products are more affordable, may have minimal side effects and they are much more compatible with human physiology than synthetic products, as the former are of natural origin. The essential oils, which are secondary metabolites produced by plants, are particularly noteworthy for their great biological usefulness [3]. In addition, environmental concerns have led to a search for environmentally friendly alternatives.

Many of the most interesting plants for the above-mentioned purposes belong to the family of Lamiaceae, which contains about 236 genera and includes from 6900 to 7200 species, mainly located in the Mediterranean area and southwest Asia [4]. In this thesis, the species that have been studied belong to Lamiaceae family: lavender (*Lavandula angustifolia)*, thyme (*Thymus vulgaris* L.*),* spearmint *(Mentha spicata* L.*)* and peppermint *(Mentha piperita* L.*).*

The main objective was to carry out distillation and extraction of different species belonging to the same family, observe the results, evaluate the efficacy of each method with each plant and to see which solvent is the best in each case. The herbal essential oils were obtained by hydrodistillation using a Clevenger apparatus. The corresponding yields were calculated and compared.

Plant materials can be subjected to different extraction methods like maceration, Soxhlet extraction, ultrasonic assisted extraction or supercritical fluid extraction, among others. In this work, Soxhlet extraction and ultrasonic assisted extraction (UAE) were tested. The solvents used were n-pentane and 96% ethanol. The extracts and the yields obtained with each solvent and each herb were compared. On the other hand, the UAE is a non-conventional, environmentally friendly method. Ultrasound waves are generated and the phenomenon of cavitation takes place, which facilitates the penetration of the solvent into the plant and thus leads to better extraction. The solvent used for this type of extraction was 96% ethanol and ethanol water mixture (70% ethanol). The extracts obtained, and the yields of each plant were calculated and compared.

2 OBJECTIVES

The aim of this thesis was to fulfil the following points:

1) Determinate dry content and moisture content of plant material samples.

2) Obtain essential oil from *Lavandula angustifolia*, *Thymus vulgaris* L., *Mentha spicata* L. and *Mentha piperita* L. by hydrodistillation, and calculate their yields.

3) Extraction of target analytes from the ground herbs using Soxhlet extractor with different solvents and calculate the yields. The solvents were:

    - n-pentane
    - 96% Ethanol

4) Extraction of the same ground plant materials by Ultrasonic Assisted Extraction UAE), calculate the yield and plot the important parameters in UAE extraction. The following solvents were used:

    - 96% Ethanol
    - 70% Ethanol

5) Draw conclusions

3 THEORICAL BACKGROUND

The plant material that has been used in this thesis belongs to Lamiaceae family. The Lamiaceae are herbaceous plants or shrubs, characterised by their flavours and aromas. Many of them are used as condiments, cosmetics, perfumes or traditional medicines due to their multiple properties.

Genera such as *Salvia*, *Nepeta* or *mentha*, among many others, are found in this family. In this thesis, the experiments have been carried out with: lavender (*Lavandula angustifolia)*, thyme (*Thymus vulgaris L.),* spearmint *(Mentha spicata L.)* and peppermint *(Mentha piperita L.),* which belong to Lavandula, Thymus and Mentha genera, respectively.

3.1 *Lavandula angustifolia* (Lavender)

Lavandula angustifolia is a species of the *Lavandula genus*, which includes about 39 species that are native to the Mediterranean but currently they are cultivated in almost all parts of the world [5]. These species are evergreen shrubs, characterized by the purple colour of their flowers arranged in spikes. Some known species are L*. lanata, L. stoechas, L. latifolia* and *L. angustifolia*.

Lavandula angustifolia is a perennial plant original from France and typical of Mediterranean climates, but it is also native to the Arabian Peninsula, Russia, and Africa. Its dried flowers have been commonly used in cloth sacks to scent and repel moths in closets. The Ancient Egyptians used lavender for mummification and other rituals. It was also widely used by the Romans, for example they added it in their baths for relaxing purposes [6].

It is a very easy plant to grow, as it is hardy and generally adaptable. They need a lot of sun and good drainage, so they prefer rather dry soils with a high pH. This aromatic plant can grow up to 1 or 2 meters high, and its stems and linear leaves are silvery green with purple flowers that are arranged in spikes and produce small fruits [7].

Lavender is a common household herb that has soothing properties, better known for its delicate perfume than for its therapeutic properties, but over the years it has been used in both cosmetics and medicine. It is noted for being a relaxing herb for the nervous system and beneficial for migraines. Its main effects include releasing gas, relieving muscle contractions, antidepressant, antiseptic and antioxidant effect, and it stimulates menstrual flow as well [5]. *Lavandula angustifolia* is known as true lavender or English lavender, being one of the most common species of lavender.

Figure 1. ***Lavandula angustifolia*** **[8].**

In perfumery and cosmetics, the species most commonly used alongside *Lavandula angustifolia* is *L. Latifolia*. These two species give rise to a hybrid known as *lavandins* that is also widely used in this field [9].

Lavender has been shown to have pharmacological uses. In vitro tests have proven its anaesthetic and analgesic effects. In addition, its scent is good for the memory and health of people with Alzheimer's disease [10]. The drug may be used in the composition of phytomedicines, to treat minor wounds and symptoms of neurotonic disorders. Most of lavender's properties are due to its essential oil, which is the most valuable product obtained from this plant.

3.1.1 Lavender essential oil

Lavender essential oil is one of the most precious aromatherapy oils. It is usually extracted from the flowers, stems and leaves of lavender by steam distillation, hydrodistillation and other techniques. It is the main reason why the cultivation of lavender has spread all over the world. It is responsible for the characteristic smell of the plant. Glands that are found in all parts of the plant, although they are most abundant in the flowers, produce this oil. The oil molecules are released during steam distillation when they come into contact with boiling water. According to The French Pharmacopoeia, lavender must contain at least 8ml/kg of essential oil [9].

The oil is a pale yellow coloured liquid with a fresh, sweet, floral and herbaceous odour. Its density can vary from 0.880 to 0.890 g/ml, its refractive index from 1.458 to 1.464; optical rotation from -11.5^{o} to -7^{o}, solubility: 1 vol in max. 2 vol of 75% ethanol, acid value: max. 1.0; ester value: 102.5-165 (ester content calculated as linalyl acetate 35.8-58%). In Haute Proven e, it is produced in a yield of 10-25kg/ha. Worldwide total production is ca. 100-200 t/a. Some varieties or clones of lavender produce more quantity but poorer quality [9]. According to literature, the essential oil yield using steam distillation ranges from 0.5 to 6.8% [11].

Lavender oil is interesting for a wide variety of industries, such as food, aromatherapy, and pharmaceuticals, since it has both medicinal properties and biological activity. Its intense aroma is very pleasant to use in personal hygiene products such as soaps, colognes or cosmetics. In food, it can be used as a flavouring agent in some beverages or sweets. In the field of aromatherapy it stands out for its beneficial effects on the nervous system and as a

drug it plays an important role due to its anti-inflammatory, antiviral and antidepressant properties. As an antiseptic compound, is able to kill many bacteria [12]. It is mainly used externally since it can be applied directly to the skin to heal wounds and soothing headaches [5].

The biological activity of the oil is closely related to its chemical composition. Mixtures of volatile compounds constitute the oil and the composition may be different depending on environmental factors and cultivation. Some of the main components are linalool, linalyl acetate, terpinen-4-ol, acetate lavandulol, ocimene and cineole. Most of them have proven analgesic and anaesthetic effects [13].

According to The French Pharmacopoeia, lavender oil must contain the following percentage in composition [14]:Linalool: 25-38%, linalyl acetate: 25-45%, Cineole: 0.3-1.5%, limonene: 0.1-0.5%, camphor: 0.2-0.5% and ∝-terpinol: 0.3-1%.

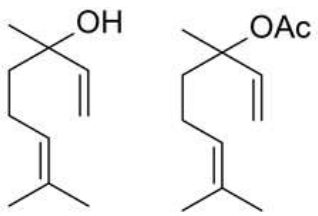

Figure 2. Linalool and Linalyl acetate molecules. [15]

The antioxidant activity of Lavender essential oil is probably its most interesting property. A recent study found that lavender oil increased the activity of the body's most powerful antioxidants such as glutathione, catalase and superoxide dismutase (SOD) [16].

3.2 *Thymus vulgaris* L. (Thyme)

Thymus genera consist of 400 species approximately, native to Europe, North Africa and Asia [17]. These species are mainly perennial subshrubs and herbaceous plants. The species include *Thymus moroderi, Thymus herba-barona* and *Thymus vulgaris*, among others. *Thymus vulgaris* is currently cultivated all over the world and is the most common species of thyme plant. It has also been used since ancient times. The Egyptians used it for washing the dead, and the Greeks already cited it as a medicine. It is native to the Mediterranean, Balkan countries, Caucasus and Africa. It is an antibacterial and spasmolytic, as well as a drug that contains essential oil, whose content ranges from 5 to 25 ml/kg (oil/plant) [14].

It is a small shrub that can reach up to 50 cm in height and has woody and strongly branched stems. The leaves are usually small and oval, and the flowers can be yellow, purple or white. This plant prefers places with dry and rocky soils. It needs to be exposed to the sun and does not tolerate cold winters [18].

Figure 3. *Thymus vulgaris* L. [19]

Thymus vulgaris L. is especially known for its aromatic qualities, it is used as ornamentals and condiments. It also has many medicinal properties, which are principally due to its essential oil. However, the constituents of the oil are not exclusively responsible for the spasmolytic activities. Thyme relieves many ailments, especially respiratory disorders [5]. The plant is useful to treat cough, diabetes, and cold and chest infections. It is also soothing for sore throat. Thyme is disinfectant and tonic, and has antiseptic, antibiotic, and antifungal properties [4].

3.2.1 Thyme essential oil

Thyme essential oil is derived from the leaves of thyme plant and is one of the principle volatile oils used in the food and cosmetic industries. Nowadays there is an increase in thyme demand owing to its essential oil multiple applications [20].

The colour of the oil may vary from colourless to pale yellow. In some cases it can even be a darker reddish colour. It has a very characteristic aromatic odour, herbaceous and slightly spicy. As for its physical-chemical properties, its density varies from 0.910 to 0.937 g/ml, refractive index from 1.494 to 1.504; optical rotation from -6^{o} to -1^{o}, solubility: 1 vol in max. 3 vol of 80% ethanol, total phenol content: 38-56%. The main producer of thyme oil is Spain [9]. In a recent study, thyme oil was obtained with 1.25% yield by steam distillation [16].

It is used to add flavour to a variety of foods, enriching cooking recipes and protecting the body from pollutants. Due to its multiple properties is not only used as a food preservative but also as a cleansing or skin care product [21]. Traditionally, it has been used orally for gastrointestinal disturbances such as bloating and flatulence, as well as for treating coughs. Topically, thyme oil relieves nasal congestion, heals minor wounds and is used in mouthwashes for oral hygiene [14]. Recent studies have shown its ability to minimize foodborne bacteria, inflammation in the body and its contribution to the proper functioning of the heart, among other benefits [22].

Thyme oil owes its antioxidant, antiseptic and antifungal properties to its chemical composition, which is composed by a mixture of volatile compounds that is very rich in phenols. Although all the chemotypes are active, the bactericidal activity is greater for thymol –a natural terpenoid that is the main compound of the oil. Other compounds like flavonoid aglycones and glycosides or phenolic acids are present in considerable quantities. Some of these are carvacrol, gamma-terpineol and linalool [23]. However, it has been demonstrated

that phenol concentration in aqueous preparations of the drug is not enough for the spasmolytic activity. The latter, is linked to polymethoxyflavones and di-, tri-, and tetramethoxylated flavones [14].

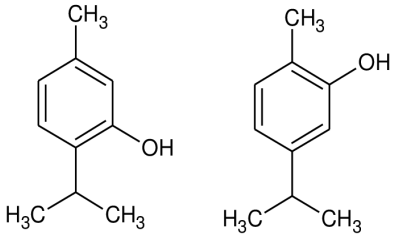

Figure 4. Thymol and carvacrol molecules [24]

It must be taken into account that the composition of the essential oil of the same herb may vary depending on external factors such as growing conditions, genotype, cultivation, etc. [2]. The essential oil "of thyme, containing thymol, Spain type" is the subject of a French Standard (NF T 75-349 (1993). The profile covers 13 constituents, mainly: thymol (36-55%), p-cymene (15-28%), linalool (4-6.2%) and gamma-terpinene (5-10.3%) [14].

3.3 *Mentha spicata* L. (Spearmint)

Mentha spicata L. is a widely known species and the oldest one from Mentha genus. The latter can be classified into 42 species, 15 hybrids and hundred of subspecies and varieties. Most of them are perennial plants that are cultivated all over the world: Europe, Africa, Asia, North America and Australia. Some well known species are *Mentha aquatica L.* and *Mentha Spicata L.*, whose hybrid is *Mentha piperita L.* [25].

Spearmint is an aromatic perennial herb, originally from Europe and Asia. Humans have used it since ancient times. In Greco-Roman civilizations, it was commonly used as appetizer and herbal medicine. According to historical record, Greeks added spearmint to their baths and it was used to treat sexually transmitted diseases, whiten teeth and heal mouth sores [26].

This plant is easily grown, as it is adaptable to most types of soils from sandy to clay ones. It needs moisture, and grows better under sunlight to yield more essential oil but it can also be cultivated in shaded areas [27]. It can reach 30 to 100 cm in height. Its stems are square-shaped and the leaves are sharply serrated, wrinkled, and bright green. The flowers grow in spikes and are white or pinkish in colour [28]. Spearmint is also known as garden mint or common mint.

Figure 5. *Mentha spicata* L. [29]

The leaves, flowers and stems are widely used in the food industry, for example as a tea-flavouring agent or other food preparations. It is very popular in toothpaste, chewing gum and pharmaceutical industries as well.

In folk medicine, it is used for treating cold, stomach ache, and indigestion, among other disorders. That is because it possesses several biological activities, which are beneficial for the cure or soothing of respiratory and digestive diseases. In a recent study of Iranian Traditional medicine, *Mentha spicata* L. turned out to be one of the plants that are effective in dyspepsia symptoms [30].

3.3.1 Spearmint essential oil

Spearmint essential oil is characterized by a yellow-green colour, an oily liquid appearance and an herbaceous fresh fragrance that is uplifting and calming [31]. As in all aromatic plants, herb's odour is due to the essential oil found in the glands of the plant.
The spearmint oil density ranges from 0.921 to 0.938 g/ml, its refractive index from 1.484 to 1.491; optical rotation from -59^{o} to -48^{o}, solubility: 1 vol in 3 vol of 70% ethanol at 20ºC, carbonyl number: min. 224; corresponding to a (-)-carvone content of 61%. The largest amount of spearmint essential oil is produced in the United States of America, specifically in Washington. The total production in USA is about 1200 t/year, whilst in other cultivation areas, such as China and India, they produce together around 1000 t/year [9].

The essential oil is one of the most appealing parts of the herb because of its multiple applications. It has been successful in the candy, chewing gum and dental care products, as it is ideal to freshen breath and cleaning the mouth, offering a sweet and fresh taste. Like the leaves, the essential oil is also used for cooking or added to food for flavouring. Some drops can be added to the meal or beverage to promote digestion and protect the stomach. It is also used as flavouring other products such as candles or jellies. Moreover, the oil is beneficial for the memory and it may be helpful to stay focused and energized. This oil is milder than the one from other types of mint, making it a suitable choice for application to the skin [32].

The chemical composition of *Mentha spicata* L. oil has been addressed in several studies. The percentages of each component of the oil are variable in each case, as the composition depends directly on the growing conditions, harvesting time and other environmental factors. However, in all investigations, carvone has been found to be the most abundant component, followed by limonene in most cases [33].

According to a French Standard NF T 75-245 [1986], only the varieties of *Mentha spicata* that yield oil rich in carvone can be considered sources of spearmint oil. The official essential oil must contain carvone (55-67 %) and limonene (2-25 %), while menthone, isomenthone, menthol, menthofuran, menthyl acetate and cineole have to be less than 2%. The level of pulegone must be not more than 0.5% [14].

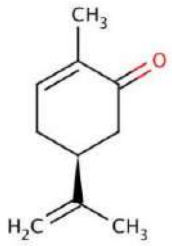

Figure 6. Carvone molecule. [34]

3.4 *Mentha x piperita* L. (Peppermint)

Mentha piperita L. is the natural hybrid from *M. aquatica* L. and *M. spicata* L., belonging to the mentha genus. It can also be referred as *Mentha balsamea* Wild [14].

Peppermint is an herbaceous perennial plant that grows in Europe and the Middle East. Remnants of dried leaves have been found in the pyramids of Egypt, indicating that this plant has been used for many years. It was highly valued by the Greeks and Romans for its soothing effect on the digestive system, nausea and bloating [5].

There are three varieties of peppermint, of which two are cultivated today all over the world. These varieties are black mint and white mint. The former is the most widely cultivated as it produces more oil than the latter. Although the white mint is less resistant and less productive, its oil obtains a higher price and its odour is more graceful.

The plant grows from 45 to 80 cm in height. It grows best in moist and requires sunlight and temperate climates. The stem is purplish and hairy, whilst the leaves are oval-shape and opposite, pointed, and of a dark green colour. The flowers are small, purple, and are arranged in spikes [35].

Figure 7. *Mentha piperita* L. [36]

Like other aromatic plants, peppermint is used as a flavouring and culinary herb because of its sweet smell and warm, spicy and refreshing taste. It is also widely used as a medicinal herb, stimulating the secretion of digestive juices and bile and relaxing intestinal muscles. It is

beneficial for both diarrhoea and constipation. Infusions of this plant are commonly consumed for digestive purposes. In fact, several studies have demonstrated that this plant is highly effective for the treatment of colon inflammation. Peppermint soothes pain, such as headaches, and can be applied directly to the skin. It is also effective for respiratory problems [5].

3.4.1 Peppermint essential oil

The appealing and pleasant aroma of mint is due to the presence of volatile essential oils in the leaves and other parts of the plant. The oil is mainly obtained from the fresh leaves of the plant, by steam distillation. Peppermint oil represents from 10 to 30 ml/kg of the weight of the dried drug [14].

Peppermint oil is colourless to pale yellow-greenish liquid. Its odour is strongly aromatic and fresh, while its taste is sweet, balsamic, and pungent. Both of them are followed by a sensation of cold. Its density ranges from 0.898-0.918 g/ml, its Index of refraction from 1.459 to 1.465; the specific optical rotation from -30º to -14º, solubility: 1 volume in 5 volumes of 70% ethanol at 20 °C and very slightly soluble in water; ester number: 12-30. According to literature, the yield on essential oil ranges from 0.5 to a maximum of 0.9% [37]. The United States is also the leader producing this type of oil, with 3500 t/year. India and China produce between 1000 and 1500 t/year, approximately [9].

Like spearmint, peppermint oil is used for flavouring products in dentifrices, para-pharmaceutical, chewing gum or candy industries. Food technology is the main consumer for confectionery, liquors and other products. Beyond that, the oil is also used for pharmacological purposes, as it is very good for treating different diseases. Internally, it relieves discomfort of the gastrointestinal tract, irritable bowel, colds and inflammation of the oral mucosa. Its external use is also indicated for colds, urticaria, pain such as myalgia and neuralgia, and skin irritation [38]. Peppermint oil is so beneficial because it is strongly antibacterial, since its components are antiseptic, fungicidal, refreshing and anaesthetic on the skin [5]. However, peppermint oil is not devoid of toxicity: high doses of the oil to rats proved to induce histopathological changes in the brain, due to certain constituents. Menthol itself does not induce serious side effects [14].

The composition of peppermint essential oil may vary depending on multiple factors, but its chief constituent is always (-)-menthol (30-40%, sometimes more than 50%). It occurs alongside menthone (7.5-12.5% in the case of white peppermint, twice as much in the case of the black type) (Bruneton, 1999). Further components are (-)-menthyl acetate, 1,8-cineole, limonene, beta-pinene and beta-caryophyllene [39].

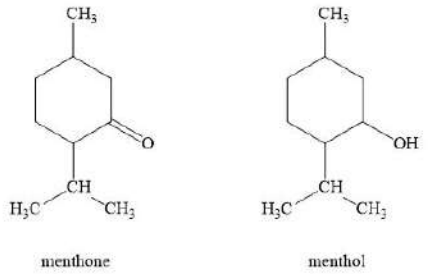

Figure 8. Menthone and menthol molecules. [40]

Peppermint oil must pass the Pharmacopoeial tests to be official: TLC identification of the essential oil, determination of the acid value (<1.4), refractive index (1.457 to 1.467) and specific optical rotation. Finally, the oil must contain 30-55% menthol, 14-32% menthone, 1-9% menthofuran, 2.8-10% menthyl acetate, not more than 4% pulegone and not more than 1% carvone. It must also contain 1-5% limonene, 3.5-14% cineole and 1,5-10% isomenthone; the cineole (%) / limonene (%) ratio must be more than 2 [14].

3.5 Antioxidants

As mentioned above, antioxidant capacity is one of the most attractive characteristics of essential oils. The antioxidants are stable molecules, able to reduce the damage caused by free radicals. The latter, are molecules that can either donate or accept electrons from other molecules, what makes them highly reactive and hence dangerous as they can damage relevant molecules of the human body such as DNA or proteins [41]. Free radicals are produced from both essential metabolic processes and the exposure to damaging external sources such as cigarettes, air pollution, etc.

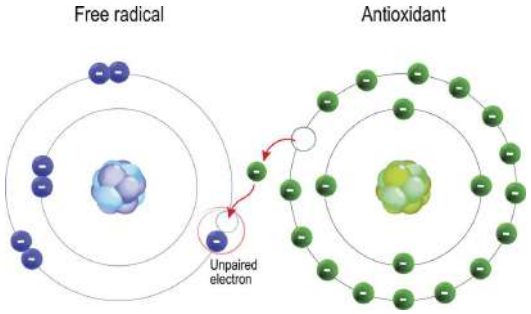

Figure 9. Free radical and antioxidant. [x]

When the balance between free radical generation and antioxidants is unfavourable, the disease known as oxidative stress arises, damaging a wide range of molecular species. In fact, oxidative stress is thought to contribute to the development of certain diseases such as cancer, inflammatory and neurological disorders, among many others [41].

There is an increasing demand for natural antioxidants due to their free radical scavenging property and also because more and more people are turning to natural remedies.

3.6 Extraction techniques

The need and relevance of active compounds in different industries has led to the search for the most appropriate method to extract these compounds, giving rise to several techniques. In fact, these extraction methods are the first stage of any study of plant materials, so they are also known as sample preparation techniques. They play an important role in analytical studies, since the quality of the results will depend greatly on the extraction performed.

There are conventional methods, but in the last decades non-conventional ones have also been established. However, none of them is regarded as the standard method for plant extraction as the efficiency of these techniques depends on each case. For example, the result of each extraction may vary depending on input parameters, the plant matrix or the chemistry of the compounds.

Despite their differences, all these methods have certain objectives in common [42]:

- To extract targeted bioactive compounds from complex plant sample
- To increase selectivity of analytical methods
- To increase sensitivity of bioassay by increasing the concentration of targeted compounds
- To convert the bioactive compounds into a more suitable form for detection and separation
- To provide a strong and reproducible method that is independent of variations in the sample matrix

3.6.1 Conventional methods

These are the classic methods; the majority of them are characterized by the use of solvents, heat and mixing. The raw material used to carry out these extractions is usually the dry inflorescences of the plant. Although these methods have some drawbacks, they are the conventional ones as they are simple, easy to operate and inexpensive.

1) Steam distillation

Distillation is the process by which a liquid is converted to vapour, being condensed back again to obtain the compounds of interest. This process can either be used for separating liquids from non-volatile solids or separating liquids with different boiling points.

Steam distillation is the traditional method to isolate volatile compounds from plant materials, especially when the raw material consist of flowers and leaves. As a result, the essential oil of the plant material is obtained. The boiling point of essential oils is very high, above the boiling point of water. With the steam distillation technique, the boiling point of the oils is close to that of water, thus avoiding their degradation due to high temperatures. This is because the boiling point of a mixture of two immiscible liquids will be the temperature at which the total

pressure is equal to the working pressure. Therefore, the resulting boiling point is lower than each substance would be separately.

Steam distillation is widely used as it presents some advantages. Firs of all, it provides products free of organic solvent and does not require the use of subsequent separation processes. A further advantage is that it is not only low-cost equipment but also has a high capacity on industrial scale. However, it requires very long extraction times, which implies a high-energy consumption. Additionally, it can lead to degradation of sensitive compounds due to long exposure at high temperatures. There are three types of steam distillation: dry steam distillation, direct steam distillation and hydrodistillation [43].

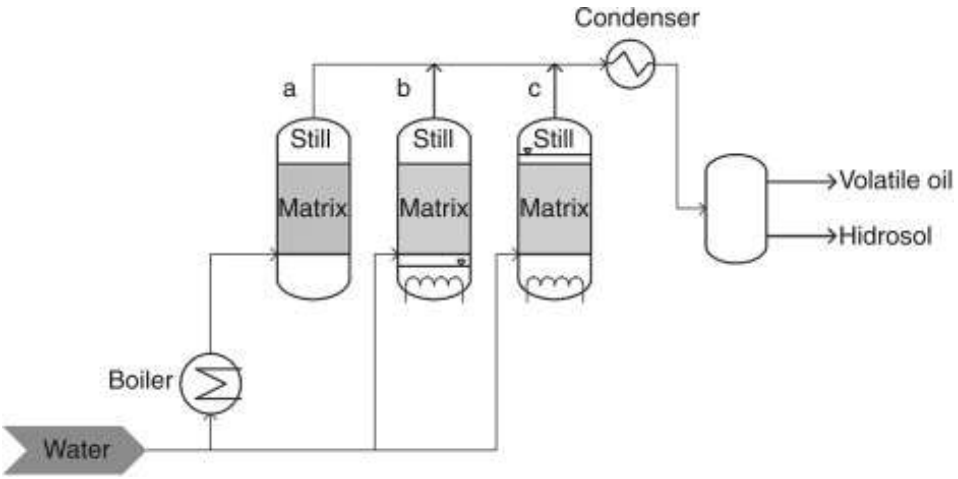

Figure 10. A type of steam distillation [43]

a. Dry steam distillation

The steam is generated outside the still and it flows through the matrix, therefore the pressure is moderate and there is no contact between water and plant material because they are in different containers.

b. Direct steam distillation

The material is suspended above the water, being supported on a perforated grid or screen. In this case, the water and matrix are not mixed either but the boiler can be inside or outside the still.

c. Hydrodistillation

The raw material and the water are mixed in the same flask or container and the boiler is inside the still.

In any of the three cases, water or steam is the most influential factor in releasing the bioactive compounds from the plant tissue. The water-oil vapour mixture is condensed and subsequently separated.

2) Maceration

This has been a popular method for a long time, but it can also be used in laboratory scale. The material from which the essential oil and / or compounds of interest are to be obtained must be previously ground to improve extraction. The solvent, which can be water or organic solvent, is added to the ground material.
This mixture remains in a closed container for a period of time at room temperature, so that the plant material is soaked in the solvent. During maceration, it is important to shake the mixture to facilitate extraction, as diffusion increases with stirring. After the required time, the liquid is strained and the solid material is drained to obtain the rest of the solution. These liquids are filtered and evaporated, obtaining the extract and recovering the solvent [42].

3) Soxhlet extraction

Soxhlet extraction is a traditional technique that has been considered the standard analytical extraction method for more than 100 years. It is widely used for many types of solid samples, such as sediments or plant tissues, in order to prepare samples for analysis, since most often it is not possible to perform a direct analysis of the starting material. This is because the target analytes may be extremely concentrated or diluted and also because not all the instruments or procedures are able to conduct such analysis [44]. It is used when the compound to be extracted has limited solubility in the solvent and the impurity is insoluble in it [45].

It is still used to extract bioactive compounds from numerous plant materials. The ground plant material is introduced into a thimble, which in turn is introduced into the Soxhlet extractor. The latter consists of the extraction chamber and the distillation flask, where the solvent is placed.

The solid material and the solvent come into contact in the extraction chamber. When a certain level has been reached the solution is aspirated through a siphon into the distillation flask. In this solution there are already solutes that will remain in the flask while the solvent is repeatedly recirculated until the process is finished [42].

Soxhlet extractor is a continuous-discontinuous technique. On the one hand, it operates as a batch system since the solvent acts in stages. But it also works the other way, as the solvent is continuously recirculated through the sample until the process is finished. This makes the operation effective since the continuous contact between the material and the fresh solvent improve the displacement of the transfer equilibrium [45]. Moreover, it is quite common to perform several parallel extractions at the same time, in order to increase sample throughput.

This technique is required to extract any compounds of interest: colours, pigments, sugars… of natural materials [46], as they are more difficult to obtain without sampling preparation. The

Soxhlet extraction tends to achieve good results and it is straightforward to carry out. A further advantage is that it does not need full time supervision and it is a well-established technique. The basic equipment required is inexpensive, what makes Soxhlet extraction a low cost operation [47].

3.6.2 Non-conventional methods

These methods have been developed to overcome the limitations of conventional methods. They are more environmentally friendly techniques as they use smaller amounts of synthetic and organic solvents. Moreover, the operation time is shorter and their operating temperatures avoid the decomposition of thermo labile compounds that can occur in classical methods. These promising new techniques include the following: ultrasound assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, pulsed electric field assisted extraction, supercritical fluid extraction and pressurized liquid extraction [42].

1) Ultrasound assisted extraction (UAE)

This method has been developed on the basis of ultrasound. It uses ultrasonic waves, which frequency ranges from kHz to MHz, to extract bioactive compounds such as carotenoids, polysaccharides, proteins, phenolic compounds, aromatic compounds, and sterols from different matrices, e.g. plant tissues [48].

This technique produces mechanical and thermal effects, as well as the phenomenon of cavitation. The latter causes bubbles to burst, causing pressure, temperature changes and damage to the plant wall due to shock waves, interparticle collisions, etc. This results in a disruption of the cells and thus in an increased transfer of matter. This is because the active compounds are forced and diffuse rapidly from the solid phase into the solvent, as there is increased penetration of the solvent into the material. As a result, there is an increase in the extraction yield of the target compounds compared to other methods [49, 50]. However, obtaining an efficient extraction depends on numerous factors such as temperature, time, pressure, moisture content of sample, grinding of the material and the solvent used.

The UAE method is considered an efficient, sustainable and green technology, characteristics that have been pursued in the last decades due to high energy costs, high greenhouse gas emissions, etc. The most remarkable advantages of UAE are the short extraction times with high reproducibility, lowering energy input and requiring less solvent consumption but obtaining higher purity of the final product [51]. Furthermore, is not expensive and can be applied in lab or in industrial scales. All this makes it an environmentally friendly method, especially when compared to other extraction methods.

2) Microwave-assisted extraction (MAE)

Microwave energy is used as a novel and efficient method for the extraction of soluble products. It is composed of an electric and magnetic field working on the hertz scale, from Mhz

to Ghz. In this way, heat is generated in the process and the bioactive compounds are extracted quickly.

Three steps govern the microwave-assisted extraction mechanism:

-Separation of the solute from the matrix by applying an increase in temperature and pressure.
-Diffusion of the solvent
-Release of the solutes from the sample into the solvent.

3) Supercritical fluid extraction (SFE)

Since its discovery, SFE has aroused great interest in different fields of science. It has been successfully used in numerous pharmaceutical, food, polymer and environmental applications.

If a gas is subjected to high pressure, it is compressed and will therefore become liquid. However, if the gas is heated beyond a certain temperature no compression will cause it to become liquid. This specific temperature is known as critical temperature (T_c) and its corresponding pressure is the critical pressure (P_c). They both define the critical point, which is unique for each substance [52]. The supercritical state is a distinctive state, which is reached when the temperature and pressure of a substance are beyond its critical point. The supercritical fluid has gas-like diffusion, viscosity and surface tension properties. While its density and solvating power are similar of those of the liquid.

Carbon dioxide is considered as an ideal solvent for SFE of natural products. Not only its critical point allows it to operate at moderate pressures and temperatures, but also it is an inert, inexpensive, easily available, odourless, tasteless, environment friendly and GRAS (generally regarded as safe) solvent [52].

Nowadays SFE is mainly used for decaffeination of coffe and tea and production of hop extracts. However, there is also a growing interest in SFE application in natural products. The use of SFE for the extraction of bioactive compounds is receiving much attention, especially from the food, cosmetic and pharmaceutical industries. For example, to extract compounds such as monoterpene and sesquiterpene hydrocarbons and oxygenated compounds from essential oils [53].

4 MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Plant materials

- Lavender (*Lavandula angustifolia*)

The lavender raw material used in this work was only the flowers of the plant. It was obtained from Herbaria Ltd (Hungary) with a label of H-136/20.

Figure 11. Lavender flowers.

- Thyme (*Thymus vulgaris* L.)

The thyme raw material used in this work was the leaves of the plant. It was obtained from Herbaria Ltd (Hungary) with a label of H-177/20.

Figure 12. Thyme leaves.

- Spearmint (*Mentha spicata L.*)

The spearmint raw material used in this work was the leaves of the plant. It was obtained from Herbaria Ltd (Hungary).

Figure 13. Spearmint.

- Peppermint (*Mentha x piperita L.*)

The peppermint raw material used in this work was the leaves of the plant. It was obtained from Herbaria Ltd (Hungary) with a label of H-170/20.

Figure 14. Peppermint.

4.1.2 Chemicals

The chemicals that have been used in the experiments to reach the objectives:

- Ethanol (C_2H_6O): was supplied by Molar Chemicals Kft, purity: 95.8%.
- Absolute ethanol (C_2H_6O): was supplied by Molar Chemicals Kft, purity: 99.8%.
- n-Pentane (C_5H_{12}): was supplied by Molar Chemicals Kft, purity: 98.7%.
- Distilled water: was obtained from a distillation apparatus in the DCS laboratory of BME University.

4.2 METHODS

4.2.1 Dry content determination

Many plant materials still contain around 8-12% of bound-water or moisture after air or sun-drying. Moreover, plants tend to adsorb moisture. For these reasons, it is very common to calculate the dry and moisture content in order to make the corresponding calculations, taking into account only the dry material of the starting material. In this way, it is possible to assess the results more properly.

The dry content has been determined for all the plant materials studied in this thesis according to the gravimetric method. For each of them the following steps have been followed:

1. Three samples of starting material of similar quantity were taken in petri dishes and weighed in the electronic scale.
2. The petri dishes with the corresponding material were put into the oven at 105ºC for at least 24h.
3. After the required time had elapsed, the samples were taken out and let them cool to room temperature.
4. The petri dishes were weighed again in the electronic tare scale.

From the difference in weights before and after drying, the moisture content and dry content can be calculated according to the following formula:

$$\%\ dry\ content = \frac{m2}{m1} * 100$$

$$\%moisture\ content = 100 - \%\ dry\ content$$

m2, mass of plant after drying (g)
m1, mass of plant before drying (g)

4.2.2 Grinding

The plant material was ground in a FRITSCH Cutting Mill with 1mm sieve insert. This equipment is ideal for comminution of soft to medium-hard, brittle, fibrous, tough or temperature-sensitive materials as well as plastics and for preparation of heterogeneous mixtures. The samples are ground by shearing forces and by cutting. The selected sieve plates determine the fineness of the material. It is a fast working machine with safe comminution and easily cleanable after use. Based on previous experiments, grinding was carried out with 1 mm sieve plate.

Figure 15. FRITSCH Cutting Mill

Before starting the extraction operation, it was necessary to get the particle size of the material small enough to increase the surface area for proper mixing with solvent. This makes the extraction easier and faster to perform. The ground herbs were placed into a plastic bag and were stored in a dark, cool storage area until further experiments. Ground herbs were used in Soxhlet and UAE extractions.

Figure 16. *Lavandula angustifolia* before and after grinding.

4.2.3 Hydrodistillation

Hydrodistillation was carried out in the laboratory to obtain the essential oils, using Clevenger apparatus. According to *Figure 17*, the following parts can be distinguished:

A. Round-bottomed flask, containing plant material and distilled water.

Clevenger apparatus:

C. Condensers.
A. Round bottom flask
D. Essential oil collector.
B. Volumetric cylinder.

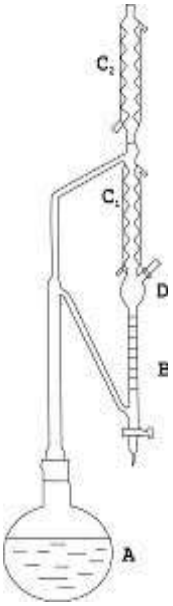

Figure 17. Hydrodistillation. Clevenger apparatus (Research Group).

Setting up hydrodistillation:

About 50-100 g of plant material was weighed and mixed with distilled water (1000 ml) in a round-bottomed flask, also some boiling rocks were added to promote smooth boiling. The round bottom flask was placed in a silicon oil bath, with the help of a support that was raised. The heater was inside the oil bath, and the thermometer was inmersed too to control the temperature of the silicon oil (to be maintained between 120 and 130°C). The main parts of the assembly were covered with aluminium foil in order to avoid heat loss. The cooling water of the condenser was opened and then the heating. From that moment, the mixture of plant material and water started to heat up. When the boiling point of the mixture was reached, the timing of distillation process started and it run for 3 hours (according to literature it needs from 1h to 5h to finish). The essential oil began to appear in the Clevenger on top of the water as time passed , which can be seen in *Figure 18*.

Figure 18. Distilled essential oil in the Clevenger apparatus.

Figure 19 shows the equipment in operation with thyme plant.

Figure 19. Hydrodistillation. Clevenger apparatus in the laboratory.

4.2.4 Extractions

4.2.4.1 Soxhlet extraction

Four different main parts can be distinguished in Soxhlet apparatus. According to *Figure 20*, these parts are: extraction chamber, siphon, condenser and flask.

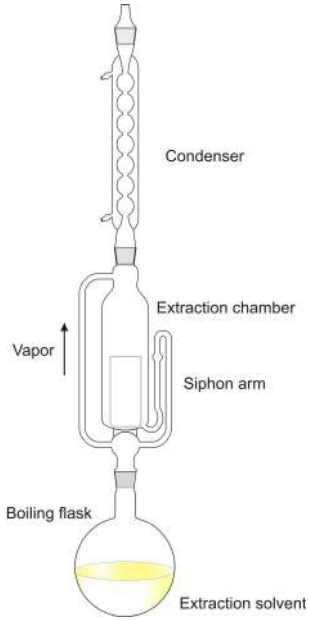

Figure 20. Soxhlet Extractor [54].

Setting up the Soxhlet extractor:

A certain amount (15-20 g) of ground herbs was introduced into a thimble, then that was plugged with absorbent cotton. The thimble was placed inside the sample holder and the solvent was weighted inside the round bottom flask by volumetric flask (220-250 mL). The corresponding parts were fixed with clamps, and the flask was immersed in a silicone oil bath, in which the heater was located. The thermometer was inside the bath to control the oil temperature.

Once the equipment was correctly set up, the fresh solvent in the flask started to heat up in the oil bath. Solvent vapours were produced and passed through the thimble containing the material, transferring the analytes to the vapour and then the whole was condensed. The liquid started to fill the sample holder part, and once it reached the overflow level the siphon aspirates the solution. The latter fell back into the round bottle. At this point the process started again, the solvent vapours evaporated again and the desired solute remained in the bottle. The process was repeated until all the solute was extracted. It usually takes 8-48 hours, depending on the plant material and solvent used.

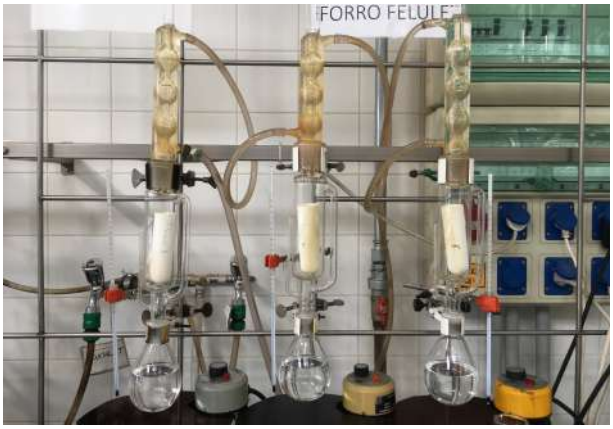

Figure 21. Soxhlet extractors

Figure 22. Soxhlet extraction in progress with thyme and 96%EtOH.

When the operation was finished, the desired analytes had already been transferred to the liquid phase. It was necessary to separate them from the solvent to complete the extraction. The solvent was evaporated from each flask using an evaporator working under vacuum. The extract was weighted back, collected from the flask into sample bottles and kept in fridge for further analysis. The extracted plant residue in the paper thimble was dried and discarded.

4.2.4.2 Ultrasonic Assisted Extraction (UAE)

Ultrasound assisted extractions were carried out using a laboratory scale Hielscher UP200ST Ultrasonic Processor which consists of an ultrasonic transducer and generator. The generator converts electric power into mechanical oscillations and transfers these to the sonotrode. Then the sonotrode transfers the mechanical oscillations to the medium.

Experimental procedure was the following:

To perform the extraction, it is necessary to determine the solid to liquid ratio at which to work (plant material (g) : solvent (ml)). In all exepriments the solid to liquid ratio was set as 1:15 (mass to volume ratio). About 15 grams of ground material was measured to a 400 ml previously selected glass beaker and the solvent (70% or 96% ethanol) was poured to the beaker gently and mixed well with the plant material using a glass rod or similar.

On the other hand, a container with distilled water was heated with an electric plate to more or less the operating temperature, which was chosen 40°C in all exepriments. Once this temperature was reached, the beaker with the sample was introduced into the water bath to keep the temperature constant through the whole experiment. Sonotrode with a temperature sensor was put approximately 1 cm from the bottom of the beaker. The amplitude and pulse parameters were selected at the maximal range in the ultrasound device and pressed start. In all experiments, the amplitude and pulse were set at 100%, without any change, as based on previous studies at these settings the highest extraction yield can be achieved. Here the ultrasonic extraction started, and ran for a set time (which was chosen 10 minutes) that had been determined before the start of the extraction. During this time, the values of power, sample temperature and bath temperature were recorded every minute. The bath water was changed to cold water if the sample exceeded the operating temperature, to avoid damaging the thermally sensitive compounds.

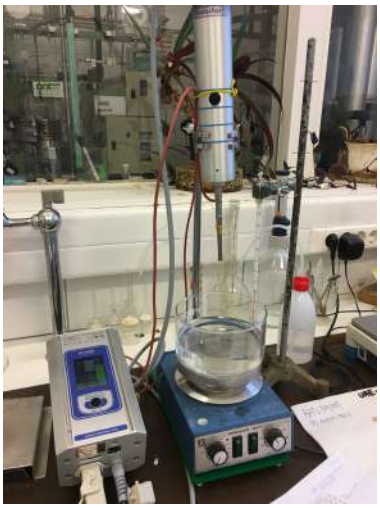

Figure 23. UAE in the laboratory.

In this work, the UAE has been carried out by the step-wise method. Three extraction steps were performed in each experiment. Once the first extraction was finished, the sample was filtered under vacuum. From there, the filtrate was collected and taken to evaporation to obtain the first extract, and the residue was re-mixed with fresh solvent and then the second extraction by UAE was performed. The process was repeated until it ended with the third extraction. In this way, the extracts were obtained at the end of evaporation, while the residue was dried out in an oven at 105°C.

Figure 24. Evaporator.

To obtain the total extraction yield (%, g/ 100 g dry mass) it is first necessary to calculate the yield of each step, which can be calculated according to the following formula:

$$YIELD\ (\%) = \frac{m_{EXTRACT}}{m_{DRY\ MASS}} * 100$$

The total cumulative yield can be either calculated by adding the yields of each stage, or using the previous formula with the total mass of the three extracts.

The mass balance can be written down as the following:

$$m_{d.m.} = \sum m_{extr.} + m_{d.r.} + m_{loss.}$$

m d.m. – mass of dried plant material (g)
m ext – mass of extracts (g)
m d. r. – mass of dried residue (g)
mloss – mass of loss during the experiment (g)
The mass balance error can be expressed as the following:

$$E = \frac{m_{loss.}}{m_{d.m.}} \cdot 100$$

E – mass balance error (%)

The mass balance error can be calculated from the ratio of mass loss during the experiment and the mass of dry material (mass of material corrected by the dry content).

5 RESULTS AND DISCUSSION

5.1 Results of dry content determination

Three repeated experiments were carried out in each case (Detailed data in *Appendix 8.2).*

5.1.1 Lavandula angustifolia (lavender)

To determinate the dry content, the average and standard deviation have been calculated and they are shown also in *Table 1*:

	Test 1	Test 2	Test 3
Dry content (%)	86.08	86.13	84.95
Moisture content (%)	13.92	13.87	15.05
Average (%)	85.72		
Standard deviation	0.67		

Table 1. Dry content measurement of *Lavandula angustifolia* (3 parallel measurements).

According to the data, flowers of lavender (*Lavandula angustifolia*) had 85.72 ± 0.67 % of dryness, the rest was moisture.

5.1.2 Thymus vulgaris L. (thyme)

To determinate the dry content, the average and standard deviation have been calculated and they are shown in *Table 2*:

	Test 1	Test 2	Test 3
Dry content (%)	90	89.50	89.71
Moisture content (%)	10	10.50	10.29
Average (%)	89.74		
Standard deviation	0.25		

Table 2. Dry content measurement of *Thymus vulgaris* L. (3 parallel measurements).

According to the data, leaves of thyme (*Thymus vulgaris* L.) have 89.74 ± 0.25 % of dryness, the rest was moisture.

5.1.3 Mentha spicata L. (spearmint)

To determinate the dry content, the average and standard deviation have been calculated and they are shown in *Table 3*:

	Test 1	Test 2	Test 3
Dry content (%)	90.16	90.12	90.00
Moisture content (%)	9.84	9.88	10.00
Average	90.09		
Standard deviation	0.08		

Table 3. Dry content measurement of *Mentha spicata* L. (3 parallel measurements).

According to the data, leaves of spearmint (*Mentha spicata* L.) have 90.09 ± 0.08 % of dryness, the rest was moisture.

5.1.4 Mentha x piperita L. (peppermint)

To determinate the dry content, the average and standard deviation have been calculated and they are shown in *Table 4*:

	Test 1	Test 2	Test 3
Dry content (%)	88.89	90.12	89.66
Moisture content (%)	11.11	9.88	10.34
Average	89.56		
Standard deviation	0.62		

Table 4. Dry content measurement of *Mentha piperita* (3 parallel measurements).

According to the data, leaves of peppermint (*Mentha piperita* L.) have 89.56 ± 0.623 % of dryness, the rest was moisture.

5.1.5 Comparison of the dry content of the four herbs

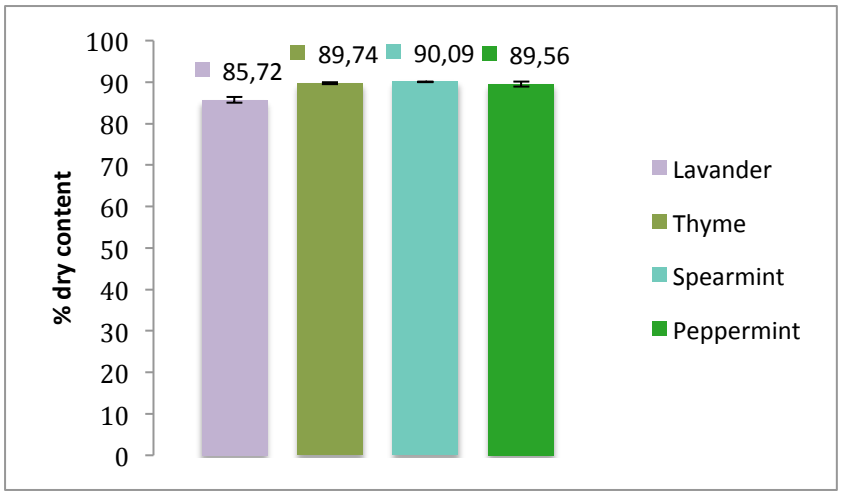

Figure 25. Dry content of 4 herbs.

According to the results obtained, which are shown in *Figure 25* more clearly, lavender contained the highest amount of bound moisture, while thyme, spearmint and peppermint contained almost the same moisture. This may be due to the species, but also to other external factors, such as harvest, drying technique applied or storage.

5.2 Results of hydrodistillation

Below are the results (Detailed data in *Appendix 8.2)* of the steam distillation of the four plants used in this thesis. All distillations lasted for 3 hours, at the boiling temperature of water.

5.2.1 Lavender *(Lavandula angustifolia)*

Three distillations were carried out with lavender to determine the amount of oil obtained and the yield. The results are shown in Table 5 (More data and results are in Appendix in *Table 30*):

	1	2	3
Plant mass (g)	100.08	30.01	30.01
Essential oil volume (ml)	3.5	1.6	1.15
Yield (%, ml/100 g dry material))	4.08	6.22	4.47

Table 5. Results of *Lavandula angustifolia* hydrodistillation

In the first distillation, 100 grams of lavender were used and 3.5 mLs of oil were obtained, quantity sufficient to make calculations. However, this amount of oil could not be quantified in the Clevenger apparatus, as the calibrated volume of it set for 1 ml maximum. For this reason, in the following distillations less amount of plant material was weighted - 30 grams were used to obtain a considerable amount of oil.

In *Figure 26,* the obtained lavender essential oil can be seen. It was light pale yellow, highly scented with a strong lavender scent.

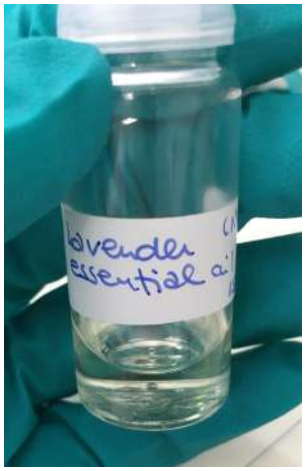

Figure 26. Lavender essential oil.

The average and standard deviation of the 3 attempts are shown below:

	Average	Standard deviation
EO Yield (%)	4.92	1.14

Table 6. Average EO yield of *Lavandula angustifolia*

The essential oil yield of *Lavandula angustifolia* is 4.92 ± 1.14 %. It was difficult to read the volumetric cylinder of Clevenger apparatus so the data extends over a wider range of values. Also two different volumetric cylinders were used.These might contribute and explain the large standard deviation.

5.2.2 Thyme (*Thymus vulgaris* L.*)*

In the same way, three distillations were also carried out with thyme. The results are shown in *Table 7* (More data and results are in Appendix in *Table 31*):

	1	2	3
Plant mass (g)	50.02	50.04	49.99
Essential oil volume (ml)	0.22	0.52	0.65
Yield (%)	0.49	1.16	1.45

Table 7. Results of *Thymus vulgaris* L. hydrodistillation.

In this case, approximately 50 grams of thyme leaves were used in every experiment, obtaining enough amount of oil in each distillation. The yield of the first attempt is much lower than the others, since a large amount of oil was lost when it was collected. Therefore, this data has not been taken into account for calculating the average and standard deviation, as it is not sufficiently accurate.

In *Figure 27*, the obtained thyme essential oil can be seen. It was dark pale yellow with a strong thyme scent. It was more dense than lavender oil.

Figure 27. Thyme essential oil.

The average essential oil yield with standard deviation are shown below:

	Average	Standard deviation

EO Yield (%)	1.30	0.21

Table 8. Table 12. Average EO Thymus Vulgaris yield.

The essential oil yield of *Thymus vulgaris* is 1.30 ± 0.21 %.

5.2.3 Spearmint (*Mentha spicata L.)*

The results of spearmint are shown in *Table 9* (More data and results are in Appendix in *Table 32*):

	1	2	3
Plant mass (g)	50.09	50.17	50
Essential oil volume (ml)	0.34	0.39	0.39
Yield (%)	0.75	0.86	0.87

Table 9. Results of *Mentha spicata* L. hydrodistillation.

For spearmint, approximately 50 grams were used for each distillation. In *Figure 28*, spearmint oil is shown. It was light pale yellow, less dense than thyme and lavender oils, and it had mild mint scent.

Figure 28. Spearmint essential oil.

The average and standard deviation of the 3 attempts are shown below:

	Average	Standard deviation
EO Yield (%)	0.83	0.06

Table 10. Average EO yield of Mentha spicata

The essential oil yield of *Mentha spicata* L. is 0.83 ± 0.06 %. Regular results have been obtained, so the standard deviation is very small in this case.

5.2.4 Peppermint (*Mentha x piperita L.)*

The results of peppermint distillation are shown in *Table 11* (More data and results are in Appendix in *Table 33*):

	1	2	3
Plant mass (g)	50.02	50.16	49.99
Essential oil volume (ml)	0.74	0.78	0.81
Yield (%)	1.65	1.74	1.81

Table 11. Results of Mentha Piperita L. hydrodistillation.

For peppermint, approximately 50 grams were used in each distillation. Peppermint oil is shown in *Figure 29*. It can be seen that it was yellowish but almost colourless, and it had a stronger and fresh mint smell than spearmint.

Figure 29. Peppermint essential oil.

The average and standard deviation of the 3 attempts are shown below:

	Average	Standard deviation
EO Yield (%)	1.73	0.08

Table 12. Average EO yield of *Mentha Piperita*

The essential oil yield of *Mentha piperita* L. is 1.73 ± 0.08%. As with spearmint, the results are regular and then the standard deviation is very small.

The yields obtained for each herb are summarised in *Table 13*, and can be seen in *Figure 30*.

HERB	EO YIELD (%)	S.D
Lavender	4.92	1.14
Thyme	1.30	0.21
Spearmint	0.83	0.06
Peppermint	1.73	0.08

Table 13. Essential oil yields – 4 herbs.

It can be seen that the yield increased with the herbs in the following order: Lavender > Peppermint > Thyme > Spearmint.

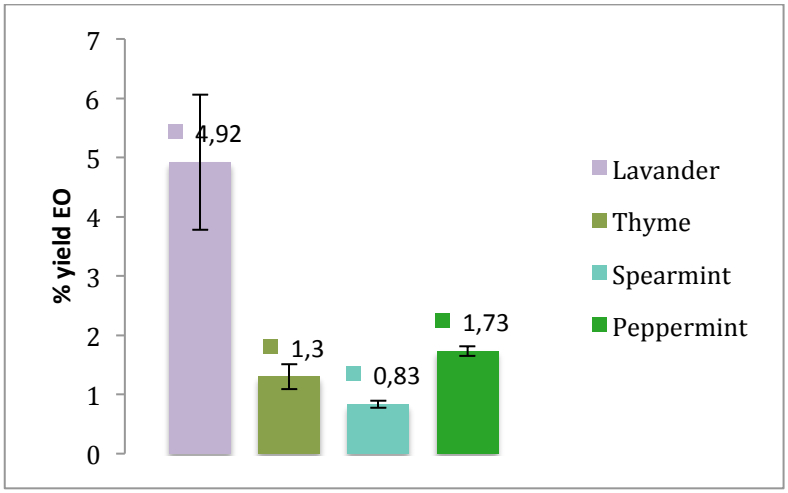

Figure 30. Essential oil Yield of 4 herbs.

Lavender essential oil yield is remarkably higher than the others. For lavender and thyme, the values obtained coincide with those found in the literature. According to the latter, lavender ranges from 0.5 to 6.8% [11] and thyme oil yield was 1.25% [16]. While for mints, the literature value is 0.5 to 0.9% [37]. It is shown here that the results depend on different factors such as their origin, cultivation method or environmental factors.

5.3 Results of Soxhlet extraction

The solvent extraction yield can be calculated from the quantity of each extract, representing as g of extract/ 100 g dry plant material. In Soxhlet experiments, for every solvent and plant material, three parallel measurements took place in each case (Data in *Appendix 8.3).*

5.3.1 Lavender (*Lavandula angustifolia)*

The numeric data of Soxhlet extraction yield with different solvents (Detailed Results in Appendix in *Table 34* and *Table 35*):

	Average Yield (%)	Standard deviation
N-pentane	9.62	0.21
96% ETOH	37.69	1.16

Table 14. Results of *Lavandula angustifolia* Soxhlet extraction.

Figure 31. Lavender extracts – soxhlet extraction.

According to the data shown in *Table 14,* almost four times more extract was obtained with 96% ethanol, than with n-pentane from lavender. The Soxhlet extracts of n-pentane and 96% EtOH are shown respectively in the previous picture (*Figure 31*). As can be seen, both extracts are very dark in colour. The first one, n-pentane extract, has a very dark green-brownish colour and it is very scented, with a strong smell of lavender. Its texture is creamy and viscous. The EtOH extract is very sticky, has high viscosity and it is almost black. It has even a stronger smell than the n-pentane extract. More extract was obtained with EtOH than with n-pentane.

5.3.2 Thymus Vulgaris L.

The yield of extracts obtained in Soxhlet extraction is reflected in *Table 15* (Detailed Results are in Appendix in *Table 36* and *Table 37*):

	Average Yield (%)	Standard deviation
N-pentane	5.3	0.25
96% ETOH	32.38	0.85

Table 15. Results of Thymus Vulgaris L. Soxhlet extraction.

It can be seen that from thyme, six times more extract was obtained with 96% ethanol.

Figure 32. Thyme extracts. - soxhlet extraction.

In *Figure 32*, the extracts of thyme are shown. The first sample contains the n-pentane extract; it is green-brownish in colour, sticky and has strong aroma of thyme. It can be seen that a much higher amount of extract was obtained with ethanol. In this case, which is the second sample, the extract is brown-greenish and very scented. It is drier than the previous one and it is not very sticky.

5.3.3 Mentha Spicata L.

For Spearmint, the yields of extracts obtained with different solvents are in *Table 16* (Detailed results in Appendix in *Table 38* and *Table 39*).

	Average Yield (%)	Standard deviation
N-pentane	2.21	0.16
96% ETOH	23.19	0.67

Table 16. Results of *Mentha Spicata* L. Soxhlet extraction.

As with the previous plants, the yield is much higher when using ethanol as solvent. In this case with 96% ethanol 10 times more extract was obtained than with n-pentane.

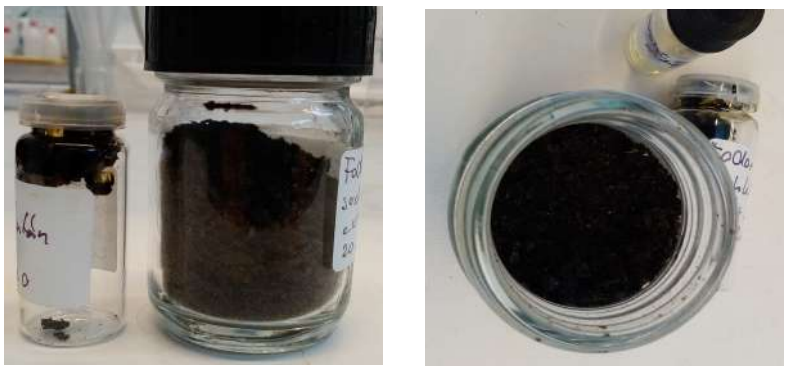

Figure 33. Spearmint extracts. - soxhlet extraction.

In the first picture (*Figure 33*), the samples of the n-pentane and ethanol extracts are shown, respectively. The n-pentane extract (left) is yellowish-green in colour, sticky (can be spread)

and it has mint smell. The ethanol extract is dark brown and its texture is dry, more like dust with big particles. This extract can be seen well in the second picture (right side of *Figure 33*).

5.3.4 Mentha piperita L.

The yield of extracts obtained in Soxhlet extraction is reflected in *Table 17* (Detailed Results are in Appendix in *Table 40* and *Table 41*):

	Average Yield (%)	Standard deviation
N-pentane	3.65	0.44
96% ETOH	25.54	1.25

Table 17. Results of Mentha Spicata L. Soxhlet extraction.

Once again, ethanol provides a higher yield in the Soxhlet extraction. In this case 7 times more extract was obtained with 96% ethanol than with n-pentane.

Figure 34. Peppermint extracts. - soxhlet extraction.

The *Figure 34* shows the Soxhlet extracts of peppermint. The n-pentane extract (left) is very dark green, gluey, scented has high viscosity. The ethanol extract (right) is much more abundant than the first one. It is green-brownish, dry and with mint scent.

5.3.5 Comparison of the Soxhlet extraction of the four herbs

In Figure 35 the results shown above demonstrate that in all cases the Soxhlet extraction using 96% ethanol as solvent gives much higher yields than those obtained with n-pentane. It is due to the fact, that the solvent power of polar ethanol is stronger than that of the non-polar n-pentane. N-pentane is only capable to solute apolar compounds, waxes, essential oils, pigments, while polar ethanol solubilizes more compounds, such as phenolics, sugars, and larger molecules weighted pigments, etc.

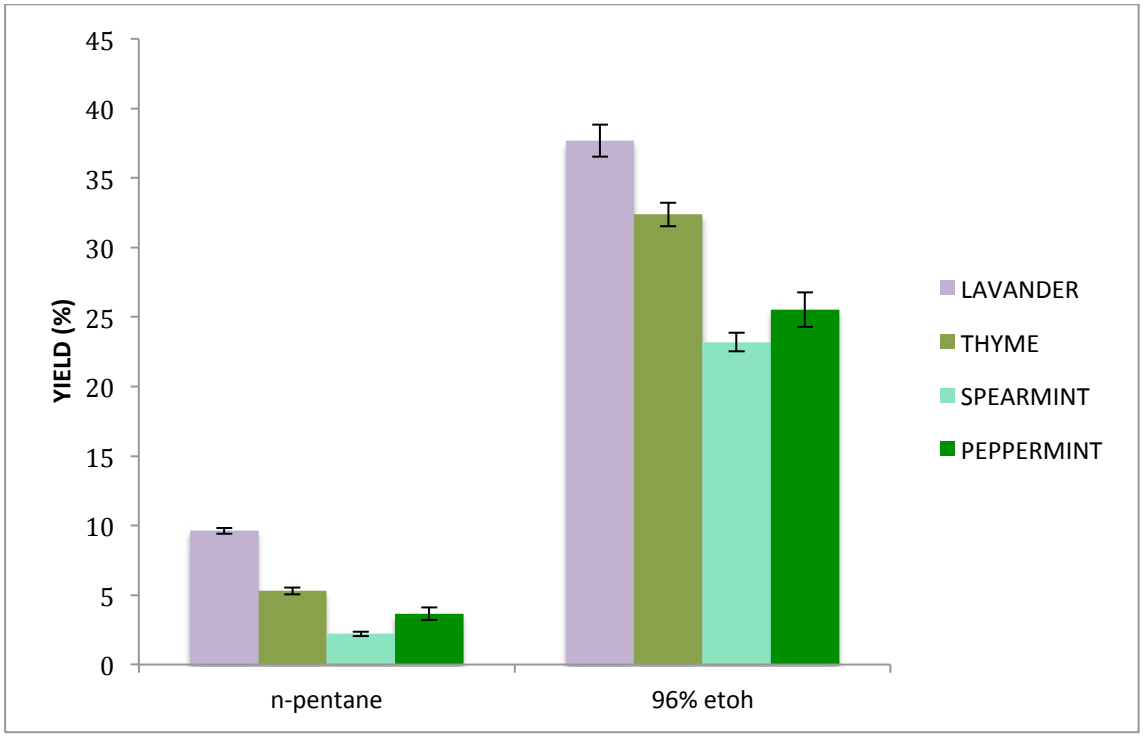

Figure 35. Results of Soxhlet extraction of four herbs

In the case of ethanol, the yields obtained range from 23.19 to 37.69 %, while in the case of n-pentane, the yields are between 2.21 and 9.62 %. It can also be observed that for both types of solvent, the yield increases with herbs in the following order: Lavender > Peppermint > Thyme > Spearmint. The highest yield was obtained with lavender and 96% ethanol, whilst the lowest yield was obtained with spearmint and n- pentane. The four extracts obtained with n-pentane turn out to be similar, as they are all sticky or creamy. On the other hand, the extracts obtained with ethanol are also similar as they are drier; with the exception of the lavender extract which is the only one that was very sticky.

5.4 Results of Ultrasound assisted extraction (UAE)

For the ultrasonic assisted extraction, the extractions were carried out according to the stepwise method, in which three extraction steps were carried out of ten minutes each at the same settings. Three extracts were obtained, while the extracted residue was re-extracted with fresh solvent at each step. . Therefore, in this part, the yield of every step can be found, as well as the total or cummulative yield (all of them are represented as g/100 g dry mass). In addition, the mass balance error for each extraction has been calculated (Additional data and Results are in *Appendix 8.4*).

The four plants (lavender, thyme, spearmint and peppermint) were extracted also using two different solvents: 96% ethanol and 70% ethanol in the same step-wise manner.

For all UAE experiments, the selected parameters were the followings:
Herb to solvent ratio (m/v) = 1:15, Tª = 40ºC, T = 10 min, Pulse & Amp = 100 %

5.4.1 Lavandula angustifolia

UAE 1 – Solvent: 96% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	1	2	3	
Yield (%)	20.28	3.70	1.79	25.77
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.01 g	13.88 g	6.72 %	

Table 18. Results of *Lavandula angustifolia* UAE 1.

UAE 2 – Solvent: 70% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	1	2	3	
Yield (%)	31.28	5.83	1.86	38.96
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	12.98 g	13.77 g	6.06 %	

Table 19. Results of *Lavandula angustifolia* UAE 2.

It can be seen in the previous tables (*Table 18 and Table 19*) and in the following graph (*Figure 36*) that the UAE2, which was carried out with 70% EtOH as solvent, obtained a higher yield than UAE1 (96% EtOH as solvent).

Regarding the mass balance, there is an error in both cases around 6-7%. Although the error should exist because the input is bigger than the output (usually some material is lost during the process), in both cases it is due to a bigger output. This may be because after carrying out the extraction, the extract needs to be evaporated. For lavender, it took a lot of time for the extract to evaporate so most probably the solvent was not completely removed. Also the

extracts were highly viscous and sticky making difficult the residual solvent traces eliminating completely. This can explain why the output is bigger than the input. Further evaporation of the extract could have been achieved by increasing the temperature of the evaporator. However, this was not done because it is not desirable to exceed a certain temperature, in order not to damage the thermolabile bioactive compounds, which are the target analytes of the extract.

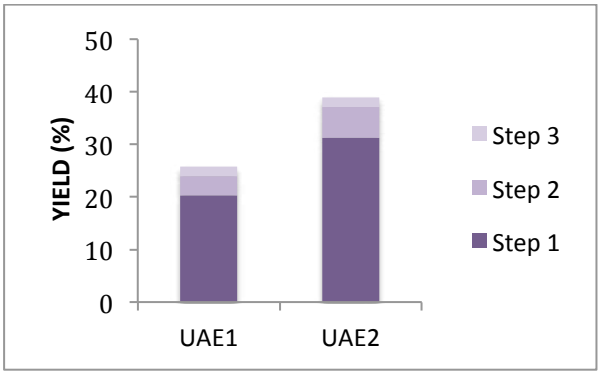

Figure 36. Yield of UAE extractions – lavender.

In *Figure 37*, the extracts obtained in UAE1 and UAE2 are shown, respectively. In the left, there are extract 1 and 2 of UAE1 when 96% ethanol used as extraction solvent. The first one is very dark green, a bit sticky and has lavender scent. In the second sample, there is the extract 2 (from Step 2) that is less dark, less sticky and less scented than the previous one. In this sample, also some extract 3 (step 3) was added, but very little as the amount obtained was so small that it was very difficult to collect. In the right side of the picture, there are the extracts obtained by UAE2 when 70% ethanol used as extraction solvent. The first one corresponds to step 1. It is brown in colour and dry, its texture is a bit like dust with some crystals, which gives it shine. The second sample contains extract 2, which is similar to extract 1 but with a greener colour and smaller particles. Both of them have a softer smell than the extracts of UAE1.

It can be also seen that the majority of extracts were obtained after the first step of extraction. If we take the cumulative yield as theoretically total extraction yield, based on the results, we can assume that 78% of total extract was obtained in the first step by 96% ethanol, while 80% of total extract was obtained by 70% ethanol.

Figure 37. Lavender UAE1 and UAE2 extracts.

It should be noted that evaporation of filtrate obtained with 70% ethanol required more time, due to a greater presence of water. In the end, these extracts have a drier appearance than those with 96% ethanol.

5.4.2 Thymus Vulgaris L.

UAE 1– Solvent: 96% EtOH

	Step 1	Step 2	Step 3	**SUM**
Extract	**1**	**2**	**3**	
Yield (%)	11.29	3.14	2.18	16.6
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.51 g	13.4 g	0.79 %	

Table 20. Results of *Thymus vulgaris* L. UAE 1.

UAE 2– Solvent: 70% EtOH

	Step 1	**Step 2**	**Step 3**	**SUM**
Extract	**1**	**2**	**3**	
Yield (%)	23.79	5.12	1.95	30.85
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	14.12 g	14.02 g	0.76 %	

Table 21. Results of Thymus Vulgaris L. UAE 2.

Like lavender, the second extraction (UAE2) which was carried out with 70% ethanol obtained a higher yield than the first extraction in which 96% ethanol used as extraction solvent (*Figure 38*). On the other hand, the mass balance of the thyme experiments turned out to be correct in both cases. The output is smaller than the input, which indicates that some material was lost during extraction and filtration. However, the amount is very small, around 0.1 g. Thus, the error obtained is also small, between 0.76 and 0.79%.

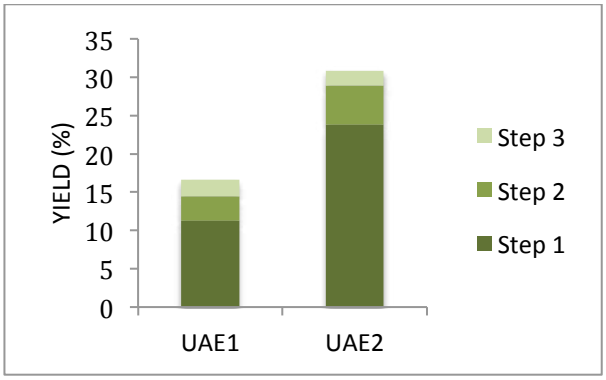

Figure 38. Yield of UAE extractions – thyme.

Thyme extracts, from UAE1 and UAE2, are shown respectively in *Figure 39*. In the left, it can be seen the sample of UAE1 (containing extract 1). This extract is very dark green, a bit sticky and with thyme smell. The second sample has extract 2 and 3, which are dark green and dry. They look a bit like dust but with bigger particles. There are some crystals so it is a bit shiny. In the right side, there are big samples containing extract 1 and extract 2 (together with extract 3). Both of them look similar because their colour is brownish-green. They also look like dust with crystals (but with smaller particles than UAE1 2/3). They are brighter than UAE1 samples.

Regarding the cumulative yield, we can assume in this case that 68% of total extract was obtained in the first step by 96% ethanol, while 77% of total extract was obtained by 70% ethanol.

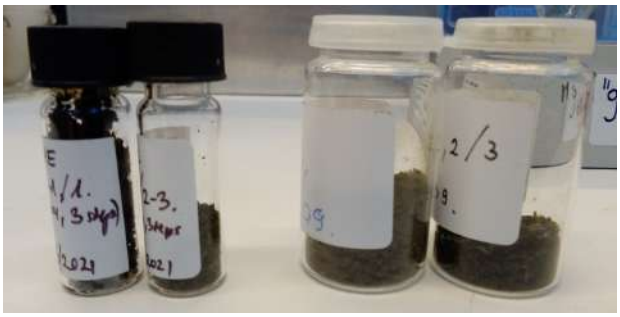

Figure 39. Thyme UAE1 and UAE2 extracts.

5.4.3 Mentha Spicata L.

UAE 1– Solvent: 96% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	**1**	**2**	**3**	
Yield (%)	7.35	2.80	1.53	11.68
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.64	13.14	3.64	

Table 22. Results of Mentha Spicata L. UAE 1.

UAE 2– Solvent: 70% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	**1**	**2**	**3**	
Yield (%)	20.87	5.67	2.01	28.55
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.93	13.69	1.74	

Table 23. Results of Mentha Spicata L. UAE 2.

Spearmint ultrasonic assisted extraction also obtained a higher yield in UAE2 than in UAE1. The difference between the yield of extraction 2 and 1 is greater for spearmint than for lavender or thyme. Regarding the mass balance, the input was the largest stream in both cases. This indicates that some material was lost during the experiment. More was lost in the first case, and therefore the error (3.64 %) is larger than in the second case (1.74 %).

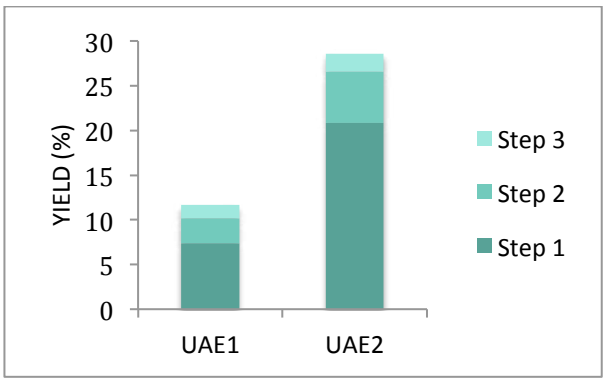

Figure 40. Yield of UAE extractions – spearmint.

The extracts from UAE1 are shown in the left of *Figure 41*, while the UAE2 extracts are in the right side. The sample containing extract 1 (UAE1) is the first one in the picture. It is dark green, a little bit creamy and a bit sticky. It smells like toothpaste. The extract 2 and 3 are in the second sample of the picture. They are green and dry, without scent. The extract 2 of UAE2 is dark brown with crystals, so it is shiny. It is a little bit sticky and smells like herb, but very little like mint. The extract 2 is yellowish-brown with crystals, and the extract 3 is dark brown.

In this case, 63% of total extract was obtained in the first step by 96% ethanol, while 73% of total extract was obtained by 70% ethanol.

Figure 41. Spearmint UAE1 and UAE2 extracts.

5.4.4 Mentha piperita L.

UAE 1– Solvent: 96% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	**1**	**2**	**3**	
Yield (%)	8.97	3.50	2.53	15.00
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.60	13.26	2.52	

Table 24. Results of Mentha Piperita L. UAE 1.

UAE 2– Solvent: 70% EtOH

	Step 1	Step 2	Step 3	SUM
Extract	**1**	**2**	**3**	
Yield (%)	20.80	5.76	1.77	28.33
Mass balance	IN	OUT	$\lvert ERROR \rvert$	
	13.51	13.58	0.53	

Table 25. Results of Mentha Piperita L. UAE 2

Once again, the second extraction was more effective than the first extraction. In the UAE1, some material was lost and the error is bigger than the error or UAE2. The latter, has a bigger output than input. This may be due to an inefficient evaporation.

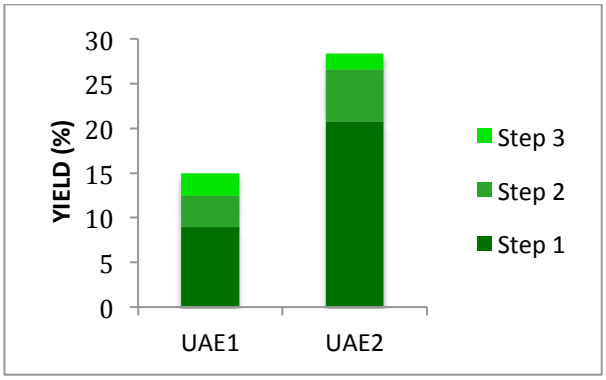

Figure 26. Yield of UAE extractions – peppermint.

The extract 1 from UAE1 (left side of *Figure 43*) is very dark green and a very scented sticky wax. The extract 2 and 3, are dark green, brownish, sticky, waxy and no scented. Regarding the extracts from UAE2 (right side of the picture), the extract 1 is dark brown with crystals (it is very shiny) and has little scent (can be seen more clearly in *Figure 44*). Extract 2 and 3 are waxy with some crystals, very little scented.

For peppermint, 60% of total extract was obtained in the first step by 96% ethanol, while 73% of total extract was obtained by 70% ethanol.

Figure 42. Peppermint UAE1 and UAE2 extracts.

Figure 43. Peppermint crystals. Extract 1 – UAE2.

5.4.5 Comparison of the UAE of the four herbs

In *Figure 45*, there is the total yield of the four herbs extracted with the two solvents using UAE method. In every case, it has been noted that the extraction carried out with 70% ethanol obtained a bigger yield than 96% ethanol. In all the experiments, the ratio (1:15) and the other parameters were the same so the only difference was the solvent used (and herb). Hence, using 70% ethanol is more effective in achieving a higher amount of extract. By introducing 30% of water in the extraction solvent, the solvetnt power increased, therefore the extraction yields were increased too compared them to the yields obtained with 96% ethanol. Moreover, the extracts obtained with this solvent tend to be drier and with crystals with less scent, while the extracts of 96% ethanol are stickier and with a stronger smell.

As previously mentioned, the most effective step is the first one, in which the largest amount of material was obtained (from 60 to 78% of total extraction with 96% ethanol and from 70 to 80% of total extraction with 70% ethanol). In the second step, a smaller amount is obtained, and the yield is much lower than in the first step. Finally, the third step always has a very low yield, around 1-2%, as almost all the material had already been extracted. In almost all cases, the amount was so small that this extract was impossible to collect from the flask.

For the two types of solvent, almost the same trend has been observed in the following order (from highest to lowest yield): Lavender > Thyme > Spearmint and Peppermint.

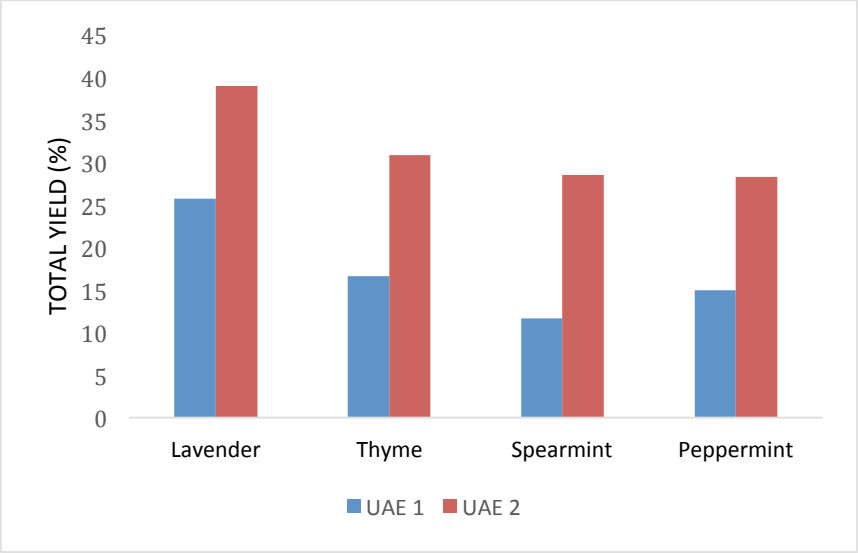

Figure 44. Results of 4 herbs. UAE.

5.4.6 Comparison of Soxhlet and UAE extraction methods

In *Figure 46*, the results of Soxhlet (blue) and UAE (green) extractions are shown together. The same trend in yield can be observed in all the extractions: Lavender > Thyme > Mints (spearmint and peppermint are very close and depend on each case).

The highest yields were reached in UAE2 (using 70 % ethanol as solvent), except from thyme that had a higher yield in 96%EtOH than in UAE2. The lowest yields were reached in n-pentane

Soxhlet extraction. Using the same solvent, 96% EtOH in Soxhlet and UAE1 extracions, it can be seen that the Soxhlet was more effective. However the Soxhlet extractions ran for 48-96 hours and a full, exhautsed extraction could have been achieved.

If a suitable solvent is chosen, better results can be achieved with ultrasonic assisted extraction. By UAE in very short extraction time high yield can be achieved when 70% ethanol solvent used. It is also a more environmentally friendly alternative to conventional methods, making it a better option.

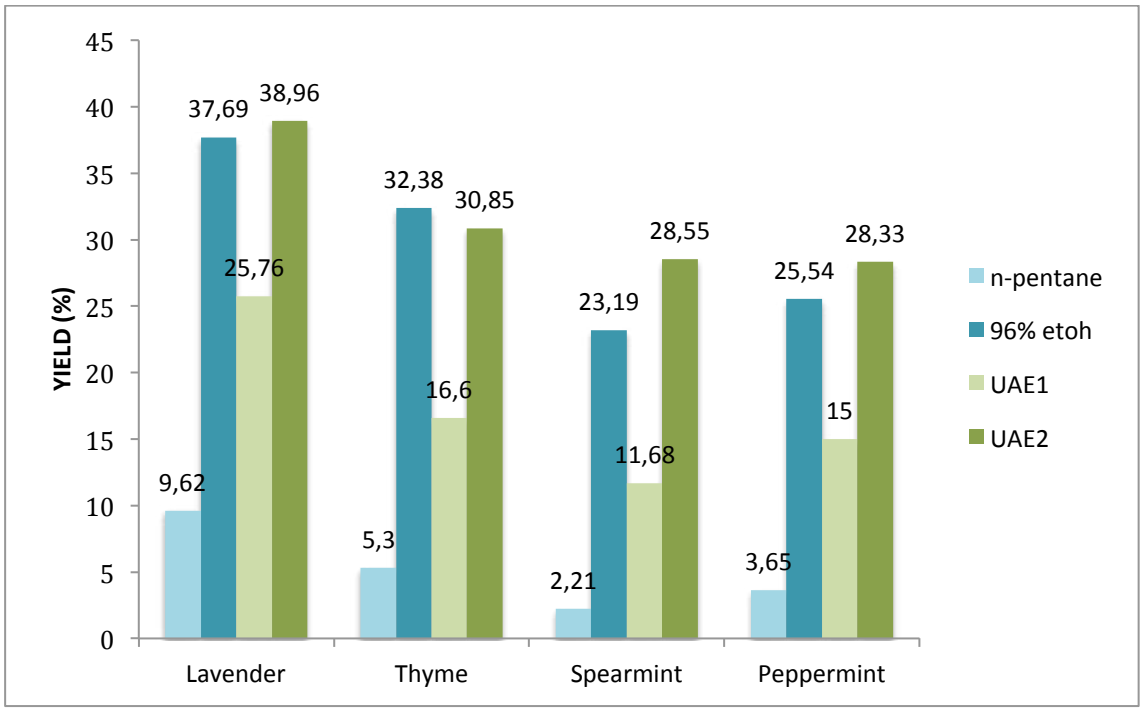

Figure 45. Soxhlet versus UAE extraction.

5.4.7 Power and temperature versus time in UAE

During ultrasonic-assisted extraction, three parameters are recorded every minute: power, sample temperature and bath temperature (Data in *Appendix 8.4*). The temperature of the sample is the most important parameter to control, since the extraction needs a certain temperature to be carried out correctly. However, this temperature cannot be too high because thermolabile compounds could be damaged.

In this thesis, the temperature set for all the UAE was 40 °C degrees. This must be controlled since heat is generated throughout the extraction. For this, the bath water must be changed to prevent the sample from overheating above 40 degrees.

In *Figure 47 and 48*, two different graphs can be seen. They show the power and temperature (of the sample) as a function of time. The first one shows UAE1 (Step 1) of Thyme. The blue line represents power over time. It grows rapidly at first, as the equipment has gone from

shutdown to start-up. Afterwards, the power value stabilizes in a range between 65 and 75 W. Several peaks are observed, but this is a normal behaviour of the function.

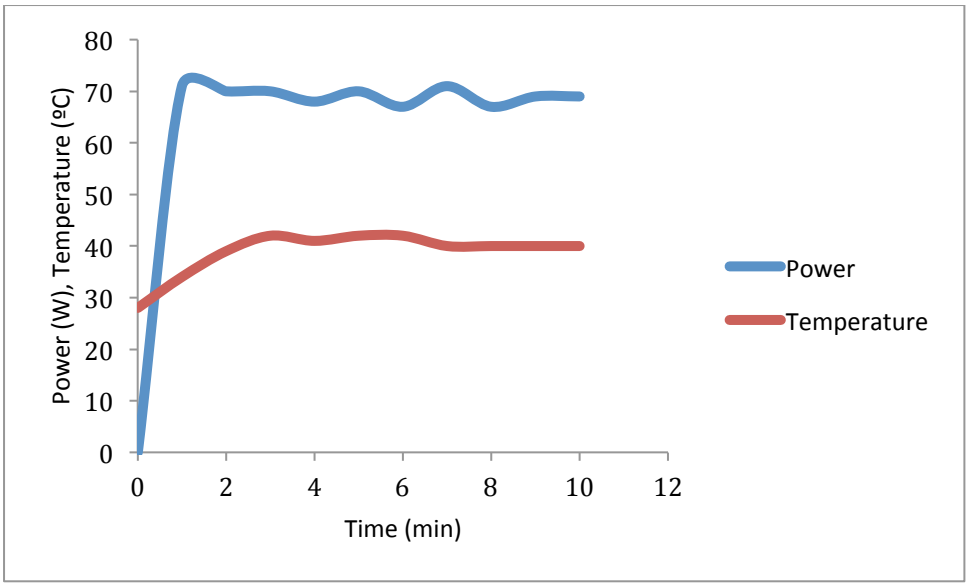

Figure 46. Power, Temperature vs time. Thyme UAE1 step 1.

In this case, the temperature (red line) is between 28 and 42 degrees. It also rises rapidly at the start of extraction. In this case it reached 40 degrees within the first two minutes. Two peaks corresponding to 42 degrees can be observed, one at 3 minutes and the next during minutes 5 and 6. At this time, the water in the bath was changed to cold water to prevent further increase and stabilize the temperature of the sample.

The following graph shows UAE1 (Step 3) of Spearmint. The power (blue line) grows rapidly at the beginning, as in all cases. Then it drops a little and stabilizes but with some oscillation. It behaves similarly to the previous case, which is to be expected. The power range is between 61 and 72 W.

Regarding the temperature (red line), it grows and reaches 40 degrees within two and a half minutes. At minute 3 is the highest peak, about 43 degrees. Once the water is changed, it remains fairly constant until the end of the extraction. Temperature ranges from 23 to 42 degrees.

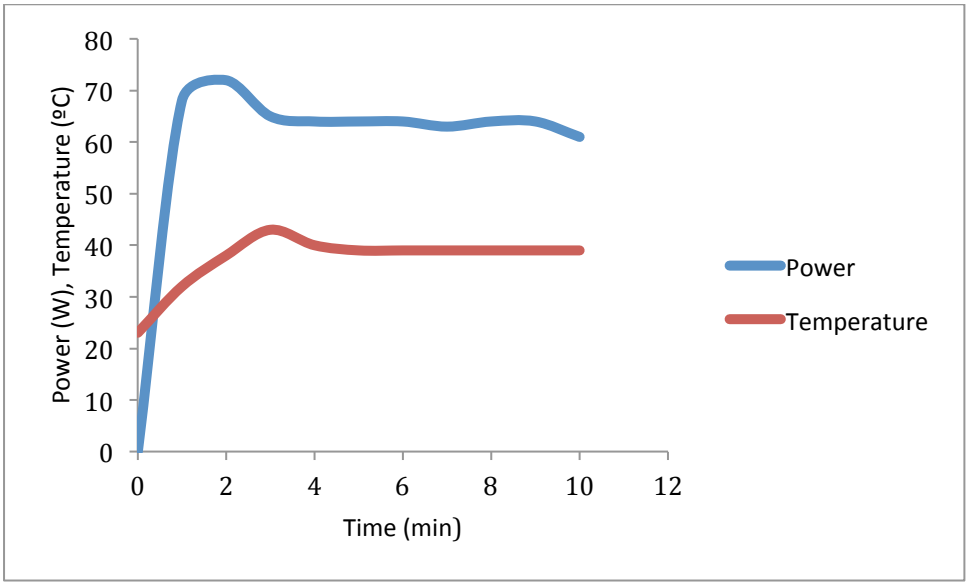

Figure 47. Power, Temperature vs time. Spearmint UAE1 step 3.

It has been done in this way in all the UAEs of this work. However, it is a bit complicated as the temperature rises very fast and it is necessary to interrupt the extraction every time the water temperature needs to be lowered. By implementing a temperature controller, or an automatic water change with inlet and outlet streams, the extraction would be better and faster to perform. The temperature value would not oscillate so much, and then it would remain more stable. In addition, the extraction would not have to be interrupted and therefore it would be continuous.

6 CONCLUSION

Different extraction methods carried out with different solvents in the laboratory, have been compared according to the yields and extracts obtained, for four plants: lavender, thyme, spearmint and peppermint from Lamiaceae plant family.

First of all, the dry content of the herbs was tested. Spearmint (90.09 ± 0.08 %) had the highest dry content, followed by thyme (89.74 ± 0.253 %**)** and then peppermint (89.56 ± 0.623 %**)**. Lavender (85.72 ± 0.669 %) was the plant with the lowest dry content.

Regarding the essential oil yield, lavender had the highest yield (4.9 ml/ 100 g dry mass). Peppermint had the second highest yield (1.65 ml/ 100 g dry mass), while thyme had the third (1.3 ml/ 100 g dry mass). Finally, the essential oil of spearmint was obtained the lowest yield (0.83 ml/ 100 g dry mass). Not for all the herbs, the essential oil yiel obtained matched with the values found in literature, which demonstrate that values may vary depending on external factors.

Two solvents were applied in Soxhlet extraction: n-pentane and 96% ethanol. Lavender obtained the highest yield in Soxhlet, with 96% ethanol (37.69 ± 1.16%). While the rest of plants obtained the following yields with the same solvent: thyme (32.38 ± 0.85 %), peppermint (25.54 ± 1.25%) and spearmint (23.19 ± 0.67 %). Using pentane as solvent, the same trend was observed as lavender (9.62 ± 0.21 %) obtained the highest yield among all plants, then thyme (5.3 ± 0.25 %), peppermint (3.65 ± 0.44 %) and finally spearmint (2.21 ± 0.16 %). In all cases, the yields obtained with n-pentane were considerably lower than those with ethanol, about 10-15% less. The extracts obtained with n-pentane were darker and more sticky, while the ethanol extracts were lighter in colour and less sticky.

In UAE, the extractions were carried out with 96% ethanol and with ethanol-water mixture (70% ethanol) . From lavender the highest yield (38.96%) was obtained with 70% ethanol as solvent. After lavender, high yields were obtained from thyme, peppermint and spearmint (respectively). Extractions with 96% EtOH followed the same trend. In this case, lavender yield was 25.77 %. The yields increased with the increase of water content in the solvent. That is, 70% ethanol obtained higher results than 96% ethanol. However, the filtrates obtained with 70% ethanol took much longer to evaporate, making this extraction more time consuming. The 70% extracts were lighter in colour, drier and contained more crystals than the 96% extracts, but had a milder smell.

With the UAE process and the extraction graphs that have been drawn, it has been observed that controlling the temperature manually hinders the process. Therefore, it would be much more appropriate to automate the temperature control to obtain a more stable temperature throughout the extraction, and to be far from damaging the thermolabile compounds.

Comparing the two different extraction methods, it can be conclude that with apolar n-pentane the smallest extraction yields were obtained resulting in waxy, greasy extracts with

strong scentes of herbs. The yields obtained with the short non-conventional extraction methods UAE are comparaeble with those obtained with Soxhlet extraction using the same solvent. With 96% ethanol the yields were between 23.2-37.7%, obtained with long fully exchausetd extraction. With UAE method in only 10 minutes of extraction the yields were between 11.7-25.8%, which are lower, but the extraction condition was milder (at 40 ^{o}C) and very short time. Once water was introduced into the extraction solvent, using 70% ethanol the yields started to increase dramatically, they were between 28.3-39.0%, resulting in less waxy, drier extracts.

Generally it can be concluded that essential oils from lavender, thyme, spearmint and peppermint were obtained in similar amounts as it can be found in literature using a lab scale Clevenger apparatus. Using solvent extraction waxy extracts were obtained with apolar n-pentane solvent in 2.2-9.6%, while with polar ethanol the extraction yields increased dramatically. The extracts are dark in colour, highly scented and viscous in appearence. With ultrasound assisted extraction, large amount of extracts could have been obtained in very short time.

Natural extracts can be obtained during many methods, it has been proven that with UAE very good results can be obtained, which is favourable as it is an environmentally friendly method. In addition, the use of natural extracts is very useful for the food, pharmaceutical and cosmetic industries, as they do not present toxicity, unlike other synthetic compounds. For these reasons, the fact that they are beneficial to human physiology and that they can be obtained using green techniques makes plant materials a raw material that is in growing demand. They are becoming more and more popular for their benefits and multiple applications.

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8. APPENDIX

In the experiments of this thesis, a certain amount of plant material has been used for each test. However, all calculations have been made taking into account the dry content of each plant, as this is usually made to assess the results more properly.

$$dried\ plant\ mass\ (g) = \frac{plant\ mass\ (g)\ x\ dry\ content\ (\%)}{100}$$

7.1 Dry content measurements

	Test 1	Test 2	Test 3
Glass mass (g)	52.84	51.86	54.11
Plant + glass mass (g)	54.78	54.24	57.1
Plant mass (g)	1.94	2.38	2.99
After drying mass (g)	54.51	53.91	56.65
Dry plant mass (g)	1.67	2.05	2.54
Dry content (%)	86.08	86.13	84.95
Moisture content (%)	13.92	13.87	15.05

Table 26. Dry content measurement of Lavandula angustifolia (3 parallel measurements).

	Test 1	Test 2	Test 3
Glass mass (g)	52.83	54.09	51.86
Plant + glass mass (g)	55.83	58.66	56.33
Plant mass (g)	3	4.57	4.47
After drying mass (g)	55.53	58.18	55.87
Dry plant mass (g)	2.7	4.09	4.01
Dry content (%)	90	89.50	89.71
Moisture content (%)	10	10.50	10.29

Table 27. Dry content measurement of Thymus vulgaris L. (3 parallel measurements).

	Test 1	Test 2	Test 3
Glass mass (g)	111.1	116.68	112.48
Plant + glass mass (g)	114.25	120.02	115.58
Plant mass (g)	3.15	3.34	3.1
After drying mass (g)	113.94	119.69	115.27
Dry plant mass (g)	2.84	3.01	2.79
Dry content (%)	90.16	90.12	90.00
Moisture content (%)	9.84	9.88	10.00

Table 28. Dry content measurement of Mentha spicata L. (3 parallel measurements).

	Test 1	Test 2	Test 3
Glass mass (g)	54.93	52.15	52.84
Plant + glass mass (g)	56.91	53.77	54.87
Plant mass (g)	1.98	1.62	2.03
After drying mass (g)	56.69	53.61	54.66
Dry plant mass (g)	1.76	1.46	1.82
Dry content (%)	88.89	90.12	89.66
Moisture content (%)	11.11	9.88	10.34

Table 29. Dry content measurement of Mentha piperita (3 parallel measurements).

7.2 Steam distillation - hydrodistillation

	1	2	3
Plant mass (g)	100.08	30.01	30.01
Dried plant mass (g)	85.79	25.73	25.73
Volume distilled water (ml)	1000	1000	1000
Essential oil mass (g)	3.12	1.32	1.02
Essential oil volume (ml)	3.5	1.6	1.15
Density (kg/m3)	892.09	824.38	889.34
Yield (%)	4.079	6.219	4.470

Table 30. Results of Lavandula angustifolia hydrodistillation.

	1	2	3
Plant mass (g)	50.02	50.04	49.99
Dried plant mass (g)	44.89	44.90	44.86
Volume distilled water (ml)	1000	1000	1000
Essential oil mass (g)	0.20	0.42	0.61
Essential oil volume (ml)	0.22	0.52	0.65
Density (kg/m3)	929.09	814.42	934
Yield (%)	0.49	1.16	1.45

Table 31. Results of Thymus vulgaris hydrodistillation.

	1	2	3
Plant mass (g)	50.09	50.17	50.00
Dried plant mass (g)	45.13	45.20	45.05
Volume distilled water (ml)	1000	1000	1000
Essential oil mass (g)	0.22	0.23	0.52
Essential oil volume (ml)	0.34	0.39	0.39
Density (kg/m3)	636.47	587.18	1339.49
Yield (%)	0.75	0.86	0.87

Table 32. Results of Mentha spicata L. hydrodistillation.

	1	2	3
Plant mass (g)	50.02	50.16	49.99
Dried plant mass (g)	44.80	44.92	44.77
Volume distilled water (ml)	1000	1000	1000
Essential oil mass (g)	0.66	0.60	0.67
Essential oil volume (ml)	0.74	0.78	0.81
Density (kg/m3)	885.41	768.97	830,37
Yield (%)	1.65	1.74	1.81

Table 33. Results of Mentha piperita L. hydrodistillation.

7.3 SOXHLET extraction

Lavandula Angustifolia

- **N- pentane**

N-pentane	1	2	3
Plant mass (g)	16.01	15.02	14.88
Dried plant mass (g)	13.84	12.98	12.86
Flask mass (g)	121.66	118.99	124.52
Flask + extract mass (g)	122.99	120.21	125.79
Extract mass (g)	1.33	1.22	1.27
Yield (%)	9.59	9.42	9.85
Average Yield (%)	9.62		
Standard deviation	0.21		

Table 34. Results of Lavandula angustifolia Soxhlet extraction- N-pentane.

- **96% EtOH**

96% EtOH	1	2	3
Plant mass (g)	15.68	16.84	16.37
Dried plant mass (g)	13.55	14.55	14.15
Flask mass (g)	134.58	111.01	130.90
Flask + extract mass (g)	139.81	116.54	136.04
Extract mass (g)	5.23	5.54	5.15
Yield (%)	38.62	38.05	36.39
Average Yield (%)	37.69		
Standard deviation	1.16		

Table 35. Results of Lavandula angustifolia Soxhlet extraction- 96%EtOH.

Thymus Vulgaris L.

- **N-pentane**

N-pentane	1	2	3
Plant mass (g)	23.39	22.34	24.10
Dried plant mass (g)	20.99	20.04	21.63
Flask mass (g)	127.14	128.52	102.56
Flask + extract mass (g)	128.28	129.53	103.74
Extract mass (g)	1.14	1.00	1.18
Yield (%)	5.44	5.01	5.44
Average Yield (%)	5.30		
Standard deviation	0.25		

Table 36. Results of Thymus vulgaris L. Soxhlet extraction- N-pentane.

- **96% EtOH**

96% EtOH	1	2	3
Plant mass (g)	23.22	23.48	23.48
Dried plant mass (g)	20.84	21.07	21.07
Flask mass (g)	120.90	115.13	126.41
Flask + extract mass (g)	127.79	121.75	133.29
Extract mass (g)	6.89	6.62	6.88
Yield (%)	33.05	31.42	32.67
Average Yield (%)	32.38		
Standard deviation	0.85		

Table 37. Results of Thymus vulgaris L. Soxhlet extraction- 96%EtOH.

Mentha spicata L.

- **N-pentane**

• N-pentane	1	2	3
Plant mass (g)	17.82	17.87	17.44
Dried plant mass (g)	16.05	16.10	15.72
Flask mass (g)	113.98	104.46	115.87
Flask + extract mass (g)	114.30	104.84	116.22
Extract mass (g)	0.33	0.38	0.35
Yield (%)	2.04	2.35	2.25
Average Yield (%)	2.21		
Standard deviation	0.16		

Table 38. Results of Mentha spicata L. Soxhlet extraction- N-pentane.

- **96% EtOH**

96% EtOH	1	2	3
Plant mass (g)	18.71	19.51	19.74
Dried plant mass (g)	16.86	17.57	17.78
Flask mass (g)	134.58	132.74	130.89
Flask + extract mass (g)	138.38	136.94	135.00
Extract mass (g)	3.80	4.20	4.11
Yield (%)	22.55	23.88	23.14
Average Yield (%)	23.19		
Standard deviation	0.67		

Table 39. Results of Mentha spicata L. Soxhlet extraction- 96% EtOH.

Mentha piperita L.

- **N-pentane**

• N-pentane	1	2	3
Plant mass (g)	16.72	17.00	16.96
Dried plant mass (g)	14.86	15.11	15.07
Flask mass (g)	123.65	127.13	102.55
Flask + extract mass (g)	124.14	127.76	103.08
Extract mass (g)	0.49	0.63	0.53
Yield (%)	3.29	4.14	3.52
Average Yield (%)	3.65		
Standard deviation	0.44		

Table 40. Results of Mentha piperita L. Soxhlet extraction- N-pentane.

- **96% EtOH**

• 96% EtOH	1	2	3
Plant mass (g)	17.64	17.62	18.63
Dried plant mass (g)	15.68	15.66	16.56
Flask mass (g)	121.66	128.51	107.85
Flask + extract mass (g)	125.88	132.47	111.89
Extract mass (g)	4.22	3.96	4.05
Yield (%)	26.90	25.28	24.43
Average Yield (%)	25.54		
Standard deviation	1.25		

Table 41. Results of Mentha piperita L. Soxhlet extraction- 96% EtOH.

7.4 Ultrasonic Assisted Extraction

Ratio (m/v) = 1:15, Tª = 40ºC, T = 10 min, Pulse & Amp = 100

Lavandula Angustifolia L.

UAE 1 - STEPWISE + 2x10 min fresh solvent

M plant= 15.05 g
M dried plant = 13.01 g
V solvent 96%EtOH = 225 ml

Stepwise method	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	114.86	107.89	122.24	
Extract + flask mass (g)	117.49	108.37	122.47	
Extract mass (g)	2.64	0.48	0.23	3.35
Yield (g/100g dm)	20.28	3.70	1.79	25.77
RESIDUE				
Dish mass (g)	102.09			
Dish + residue dry mass (g)	112.62			
Dry residue mass (g)	10.53			
MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$	
	13.01 g	13.88 g	6.72%	

Table 42. Results of Lavandula angustifolia UAE 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	21	35
1	70	35	40
2	65	42	42
3	65	42	23
4	65	42	25
5	64	44	27
6	63	45	28
7	63	40	15
8	63	39	16
9	63	39	17
10	64	39	18

Table 43. Results of Lavandula angustifolia UAE 1 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	22	42
1	70	33	40
2	71	40	40
3	67	44	39
4	64	44	31
5	63	44	30
6	61	45	27
7	63	45	25
8	69	41	19
9	63	41	20
10	64	41	20

Table 44. Results of Lavandula angustifolia UAE 1 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	22	38
1	66	31	38
2	65	37	37
3	61	41	36
4	66	44	36
5	64	41	22
6	64	41	23
7	61	42	24
8	65	43	25
9	60	44	26
10	61	44	27

Table 45. Results of Lavandula angustifolia UAE 1 – Step 3.

UAE 2 - STEPWISE + 2x10 min fresh solvent

M plant= 15.02 g
M dried plant = 12.98 g
V solvent 70 %EtOH = 225 ml (158 ml pure ETOH, 67ml distilled water)

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	114.84	98.73	107.86	
Extract + flask mass (g)	118.90	99.49	108.10	
Extract mass (g)	4.06	0.76	0.24	5.06
Yield (g/100g dm)	31.28	5.83	1.86	38.96
RESIDUE				
Dish mass (g)	116.71			
Dish + residue dry mass (g)	125.42			
Dry residue mass (g)	8.71			

MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$
	12.98 g	13.77 g	6.06 %

Table 46. Results of Lavandula angustifolia UAE 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	28	42
1	67	35	40
2	66	40	40
3	70	42	39
4	63	44	39
5	69	41	21
6	70	42	22
7	69	41	23
8	73	42	24
9	69	42	25
10	68	42	27

Table 47. Results of Lavandula angustifolia UAE 2 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	21	42
1	77	33	40
2	78	39	39
3	82	42	39
4	73	44	39
5	65	40	30
6	72	42	30
7	67	41	31
8	69	42	31
9	66	43	32
10	64	44	32

Table 48. Results of Lavandula angustifolia UAE 2 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	28	46
1	74	36	44
2	76	40	43
3	70	44	43
4	67	38	32
5	68	40	32
6	68	43	32
7	74	40	27
8	73	42	27
9	74	41	24
10	78	42	25

Table 49. Data Lavandula angustifolia UAE 2 – Step 3.

Thymus Vulgaris L.

UAE 1 - STEPWISE + 2x10 min fresh solvent

M plant= 15.05 g

M dried plant = 13.51 g

V solvent 96%EtOH = 225 ml

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	132.76	104.49	114.69	
Extract + flask mass (g)	134.28	104.91	114.99	
Extract mass (g)	1.52	0.42	0.29	2.24
Yield (g/100g dm)	11.29	3.14	2.18	16.6
RESIDUE				
Dish mass (g)	102.09			
Dish + residue dry mass (g)	113.25			
Dry residue mass (g)	11.16			

MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$
	13.51 g	13.4 g	0.79 %

Table 50. Results of Thymus vulgaris L. UAE 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	27	42
1	65	36	41
2	63	42	40
3	62	41	23
4	63	41	23
5	63	42	23
6	62	42	22
7	62	42	21
8	69	40	18
9	66	40	20
10	68	41	21

Table 51. Data Thymus vulgaris L. UAE 1 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	25	41
1	64	34	38
2	62	39	38
3	56	42	38
4	71	41	21
5	66	42	21
6	64	39	20
7	65	40	21
8	65	40	22
9	63	40	23
10	63	42	24

Table 52. Data Thymus vulgaris L. UAE 1 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	25	42
1	63	35	41
2	63	40	40
3	60	44	40
4	67	39	20
5	63	39	22
6	59	40	23
7	59	40	24
8	64	37	15
9	62	37	17
10	62	37	18

Table 53. Data Thymus vulgaris L. UAE 1 – Step 3.

UAE 2 - STEPWISE + 2x10 min fresh solvent

M plant= 15.74 g

M dried plant = 14.12 g

V solvent 70 %EtOH = 225 ml (158 ml pure ETOH, 67ml distilled water)

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	123.67	135.46	113.64	
Extract + flask mass (g)	127.03	136.18	113.92	
Extract mass (g)	3.36	0.72	0.28	4.36
Yield (g/100g dm)	23.79	5.12	1.95	30.85
RESIDUE				
Dish mass (g)	101.01			
Dish + residue dry mass (g)	110.67			
Dry residue mass (g)	9.66			
MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$	
	14.12 g	14.02 g	0.76 %	

Table 54. Results of Thymus vulgaris UAE 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	28	42
1	71	34	40
2	70	39	38
3	70	42	38
4	68	41	19
5	70	42	20
6	67	42	20
7	71	40	18
8	67	40	19.5
9	69	40	20
10	69	40	21

Table 55. Results of Thymus vulgaris UAE 2 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	26	42
1	70	35	42
2	72	39	41
3	70	42	40
4	76	40	18
5	71	41	20
6	71	40	22
7	70	40	23
8	73	41	23.5
9	70	42	25
10	71	42	26

Table 56. Results of Thymus vulgaris UAE 2 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	28	43
1	72	35	42
2	73	40	40
3	72	42	40
4	68	40	14.5
5	69	40	16
6	70	41	17
7	69	41	18.5
8	68	41	20
9	68	42	21
10	69	42	22

Table 57. Results of Thymus vulgaris UAE 2 – Step 3.

Mentha Spicata L.

UAE 1 - STEPWISE + 2x10 min fresh solvent

M plant= 15.14 g

M dried plant = 13.64 g

V solvent 96%EtOH = 225 ml

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	117.22	124.51	122.24	
Extract + flask mass (g)	118.22	124.90	122.45	
Extract mass (g)	1.00	0.38	0.21	1.59
Yield (g/100g dm)	7.35	2.80	1.53	11.68
RESIDUE				
Dish mass (g)	101.00			
Dish + residue dry mass (g)	112.55			
Dry residue mass (g)	11.55			

Table 58. Results of Mentha spicata L. UAE 1.

MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$
	13.64 g	13.14 g	3.64 %

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	23	38
1	68	34	37
2	65	40	36
3	62	44	36
4	61	39	20
5	63	40	20.5
6	64	41	22
7	67	40	22
8	63	41	24
9	63	42	25
10	61	42	27

Table 59. Data of Mentha spicata L. UAE 1 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	22	36
1	69	33	35
2	69	38	34
3	66	42	33.5
4	63	39	19
5	64	39	20
6	65	39	20
7	64	39	21
8	63	39	22
9	68	40	23
10	63	40	24

Table 60. Data of Mentha spicata L. UAE 1 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	23	37
1	68	32	35
2	72	38	34
3	65	43	34
4	64	40	17
5	64	39	18
6	64	39	18.5
7	63	39	19
8	64	39	20
9	64	39	22
10	61	39	22

Table 61. Data of Mentha spicata L. UAE 1 – Step 3.

UAE 2 - STEPWISE + 2x10 min fresh solvent

M plant= 15.46 g

M dried plant = 13.93 g

V solvent 70 %EtOH = 225 ml (158 ml pure ETOH, 67ml distilled water)

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	114.68	98.75	113.56	
Extract + flask mass (g)	127.03	99.54	113.84	
Extract mass (g)	2.91	0.79	0.28	3.98
Yield (g/100g dm)	20.87	5.67	2.01	28.55
RESIDUE				
Dish mass (g)	101.01			
Dish + residue dry mass (g)	110.72			
Dry residue mass (g)	9.71			
MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$	
	13.93 g	13.69 g	1.74 %	

Table 62. Results of Mentha spicata UAE 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	27	38
1	74	35	38.5
2	72	40	38
3	72	43	38
4	70	42	20
5	68	40	20
6	73	41	21
7	69	40	22
8	65	41	23
9	67	41	24
10	68	42	25

Table 63. Data of Mentha spicata UAE 2 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	26	40
1	70	33	35
2	72	37	36
3	71	40	37
4	67	42	37
5	71	40	18
6	73	40	19
7	71	40	20
8	76	40	20
9	67	41	22
10	70	41	23

Table 64. Data of Mentha spicata UAE 2 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	25	39
1	75	32	37
2	71	37	36
3	72	40	35
4	68	42	35
5	77	38	18
6	74	39	19
7	70	40	20
8	78	40	22
9	71	40	22
10	71	40	23

Table 65. Data of Mentha spicata UAE 2 – Step 3.

Mentha piperita L.

UAE 1 - STEPWISE + 2x10 min fresh solvent

M plant= 15.19 g

M dried plant = 13.60 g

V solvent 96%EtOH = 225 ml

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	114.85	135.38	135.86	
Extract + flask mass (g)	116.07	135.86	136.20	
Extract mass (g)	1.22	0.48	0.35	2.04
Yield (g/100g dm)	8.97	3.50	2.53	15.00
RESIDUE				
Dish mass (g)	102.09			
Dish + residue dry mass (g)	113.31			
Dry residue mass (g)	11.22			

MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$
	13.60 g	13.26 g	2.52 %

Table 66. Results of Mentha piperita L. UAE 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	25	48
1	64	39	47
2	60	45	46
3	64	42	17
4	64	42	18.5
5	63	42	20
6	65	39	15
7	63	37	18
8	64	38	19
9	68	38	20.5
10	61	38	21

Table 67. Data of Mentha piperita L. UAE 1 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	24	45
1	67	36	41
2	64	42	40
3	57	45	39
4	62	42	17
5	64	41	18
6	62	40	20.5
7	61	40	21
8	63	41	23
9	61	40	24
10	61	41	25

Table 68. Data of Mentha piperita L. UAE 1 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	20	28
1	70	29	29
2	68	33	29
3	66	38	29
4	69	40	29
5	65	42	29
6	64	43	29
7	63	41	17
8	64	40	20
9	63	40	21
10	64	40	22

Table 69. Data of Mentha piperita L. UAE 1 – Step 3.

UAE 2 - STEPWISE + 2x10 min fresh solvent

M plant= 15,08 g

M dried plant = 13,51 g

V solvent 70 %EtOH = 225 ml (158 ml pure ETOH, 67ml distilled water)

	Step 1	Step 2	Step 3	SUM
EXTRACT				
Flask mass (g)	118.99	113.57	111.00	
Extract + flask mass (g)	121.80	114.35	111.24	
Extract mass (g)	2.81	0.78	0.24	3.82
Yield (g/100g dm)	20.80	5.76	1.77	28.33
RESIDUE				
Dish mass (g)	86.93			
Dish + residue dry mass (g)	96.68			
Dry residue mass (g)	9.75			
MASS BALANCE	IN	OUT	$\lvert ERROR \rvert$	
	13.51 g	13.58 g	0.53 %	

Table 70. Results of Mentha piperita UAE 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	26	41
1	69	35	39
2	67	39	38
3	67	42	38
4	72	41	18
5	69	40	18.5
6	71	40	20
7	70	40	21.5
8	69	41	22.5
9	71	42	24
10	70	42	25

Table 71. Data of Mentha piperita L. UAE 2 – Step 1.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	25	52
1	82	35	50
2	68	41	48
3	67	44	46
4	69	42	21
5	71	42	23
6	69	42	24
7	70	40	16
8	70	39	17,5
9	71	40	19
10	69	40	20

Table 72. Data of Mentha piperita L. UAE 2 – Step 2.

t (min)	P (W)	T(ºC)	Tbath (ºC)
0	0	24	41
1	70	33	40
2	67	36	39
3	66	40	38,5
4	67	43	38
5	71	40	14
6	73	40	14,5
7	74	40	16
8	72	40	18
9	70	40	19
10	68	40	20

Table 73. Data of Mentha piperita L. UAE 2 – Step 3.