CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Chemical analysis of oregano (*Origanum vulgare*) essential oil

BACHELOR'S THESIS

Prague 2022

Author: Nela Kotrčová

Supervisor: Ing. Klára Urbanová, Ph.D.

Declaration

I hereby declare that I have done this thesis entitled “Chemical analysis of oregano *(Origanum vulgare)*” essential oil independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague date

.....

Nela Kotrčová

Acknowledgements

I would like to express my gratitude to my supervisor Ing. Klára Urbanová, Ph.D. who was always willing to help, advise me, and support my ideas and opinions. She always answered all my questions and provided me with feedback during the process of writing this thesis.

I would like to also thank to Faculty of Tropical AgriSciences for the opportunity to work in laboratories, it was a great experience for me.

Abstract

The aim of this bachelor thesis was to perform a chemical analysis of volatile compounds contained in *Origanum vulgare* essential oil. In the literature review, there were described the main information about oregano, its most important bioactive compounds (carvacrol, thymol, linalool, p-cymene, γ-terpinene) as well as its medicinal benefits, and the methods of oil obtaining and analysing.

In the experimental part of this work, essential oils were obtained from 3 types of dried oregano leaves available on the Czech market, extracted using hydro-distillation method. One sample of commercially produced oregano essential oil, which was used for this research was bought on the internet. Those samples were analysed by gas chromatography with mass spectrometry (GC/MS).

Compounds were identified, and results were compared between each other and with literature data. The results confirmed the presence of carvacrol, thymol, linalool, p-cymene, γ-terpinene as the main constituents of oregano essential oil. Results had revealed minor differences in chemical composition among the 4 samples. In comparison with literature data, there were found substantial differences in chemical composition of analysed samples.

Key words: Oregano, GC/MS analysis, essential oil, *Origanum vulgare*, extraction

Contents

1. Introduction 1

2. Literature Review 2

2.1. Oregano 2

2.1.1. Botanical overview 2

2.1.2. Cultivation and propagation 3

2.1.3. Post-harvest handling 3

2.1.4. Utilisation 4

2.1.5. Medicinal benefits 4

2.1.5.1. Antimicrobial activities 5

2.1.5.2. Antioxidant 5

2.1.5.3. Anti-inflammatory and anticancerous activity 5

2.1.5.4. Cardiovascular diseases 6

2.1.5.5. Effect on metabolic syndrome and obesity 6

2.1.5.6. Antiproliferative activity 6

2.2. Bioactive compounds 8

2.2.1. Carvacrol 8

2.2.2. Thymol 9

2.2.3. Linalool 10

2.2.4. p-Cymene 11

2.2.5. γ-Terpinene 12

2.3. Chromatography 13

2.3.1. Gas chromatography 14

2.4. Mass spectrometry 15

2.5. Extraction methods 16

2.5.1. Hydro-distillation 16

2.6. Scientific studies 18

3. Objectives 22

3.1. Main objective 22

3.2. Specific objectives 22

4. Methods and materials 23

4.1. Samples 23

4.2. Extraction and GC/MS analysis 24

5. Results 27

6. Discussion 32

7. Conclusions 34

8. References 35

List of tables

Table 1: Taxonomic classification of oregano 2
Table 2: The composition (area %) of *Origanum vulgare* L. essential oil . 19
Table 3: Main compounds, expressed as percentage of chromatographic area of *L. officinalis* and *O. vulgare* essential oils. 20
Table 4: Chemical composition of *Origanum vulgare* essential oil. 21
Table 5: Identification of bioactive compounds (Chemical composition) . 28

List of figures

Figure 1: Structure of carvacrol molecule 8
Figure 2: Molecular structure of thymol 9
Figure 3: Molecular structure of linalool 10
Figure 4: Molecular structure of p-cymene 11
Figure 5: Molecular structure of γ-terpinene 12
Figure 6: Structure of gas chromatograph 14
Figure 7: General scheme of a mass spectrometer 15
Figure 8: Hydro-distillation Clevenger apparatus system 18
Figure 9: Sample 1 23
Figure 10: Sample 2 23
Figure 11: Sample 3 23
Figure 12: Sample 4 24
Figure 13: Hydro-distillation extraction of essential oil from the samples n. 2 and n. 3 25
Figure 14: Extracted samples of essential oregano oil 26
Figure 15: Chromatograms (from right to left: sample 1, sample 2) 29
Figure 16: Chromatograms (from right to left: sample 3, sample 4) 30
Figure 17: Mass Spectrum of molecule carvacrol 31

List of the abbreviations used in the thesis

GC- Gas chromatography

GLC- Gas-liquid chromatography

GC/MS- Gas chromatography/Mass spectrometry

GSC- Gas-solid chromatography

KI- Kovats retention index

MS- Mass spectrometry

Rf- Retention factor

RT- Retention time

1. Introduction

Origanum vulgare also known as oregano, or wild marjoram is an aromatic medicinal herb belonging to *Lamiaceae* family. It is one of the best-known herb natives in the Mediterranean region and western Eurasia (Pezzani et al. 2017). Oregano is cultivated worldwide; it requires a lot of sunlight relatively dry soil and free-draining compost (BBC Gardeners' World Magazine 2022).

In recent years, the popularity of this herb has been increased. Oregano is mainly used as a flavouring herb in many world cuisines. Dried leaves and inflorescences that are together extremely rich in antioxidative properties are used as human and animal food. Oregano essential oil contains high amounts of carvacrol, thymol, γ-terpinene, and p-cymene. Those substances are used in medicine and have anti-inflammatory, analgesic, antiarthritic, antiallergic, anticarcinogenic, antidiabetic, cardioprotective, gastroprotective, hepatoprotective, and neuroprotective properties. Oregano is also well known for inhibiting microbial and fungal toxin production (Skoufogianni et al. 2019).

This Bachelor thesis is focused on the chemical analysis of volatile compounds of oregano essential oil. The oil was obtained from four different samples. Three of them were from dried oregano which is available on the market in the Czech Republic. Those samples were compared with the one sample obtained from commercially produced essential oregano oil. Moreover the method which was used to obtain essential oil from dried oregano for the research is called hydro-distillation. Dried leaves of oregano mixed with water are heated until boiling. This process creates steam which carries molecules of essential oil. Finally, the steam is cooled with water in the condenser and turns into a liquid essential oil (Li et al. 2014).

Results from the samples were obtained by gas chromatography-mass spectrometry (GC/MS). It is an analytical method for the identification of volatile organic compounds in a sample. After the identification, the results were compared with each other, and with data from other scientific articles.

2. Literature Review

2.1. Oregano

2.1.1. Botanical overview

It is recognised at least 61 species of 17 genera belonging to six families that are mentioned under the name oregano. Oregano, scientifically titled *Origanum vulgare* belongs to the Lamiaceae family of the order Lamiales (Skoufogianni et al. 2019).

This herb´s appearance is characteristic to Mediterranean types. In general, oregano grows in 20-90 cm in height. Dark green leaves are egg-shaped and hairy, 10-40 mm long and 5-25 mm wide with smooth toothed edges (Skoufogianni et al. 2019). Every leave is situated on the opposite side of the woody stem. The many-flowered inflorescence is grouped into short dense lateral or terminal spikes (Kintzios 2012). The corolla has a typical white to pale purplish colour, and each of them has two lips (Skoufogianni et al. 2019). In areas with moderate climates, the flowering period lasts from late June to August. Each flower creates four small-seed structures when it becomes mature. Oregano has been developed by humans over centuries for its unique flavours ranging from spicy to sweet. As a result, new subspecies began to emerge (Pacao 2020). The essential oil is findable in small glands in the foliage. This gives the plant its characteristic taste and aroma.

Table 1: Taxonomic classification of oregano

Classification	
Kingdom	*Plantae* - Plants
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Magnoliopsida* - Dicotyledons
Subclass	*Asteridae*
Order	*Lamiales*
Family	*Lamiaceae* / Labiatae - Mint family
Genus	*Origanum* L. - origanum
Species	*Origanum vulgare* L. - oregano

Source: USDA Natural resources Conservation Service (2022)

2.1.2. Cultivation and propagation

In nature, oregano grows mainly in higher altitudes. It is tolerant to cold during winter and dry soil during summer. Oregano can be cultivated as a perennial or an annual herb. When it is grown as a perennial, it requires enough sun-light and is recommended to divide roots every three years for achieving better taste. Oregano could be grown both in the ground and in pots (Peter 2001).

For commercial purposes, oregano is being cultivated naturally even today because it is relatively easy. However, to avoid exploitation, there is an effort to cultivate oregano in domestic areas. Oregano is cultivated in two ways: seeds or cutting. Seeds should be sown in a seed box during spring. After germination, when seedlings reach 7 cm tallness, they are put outside to the ground 30 cm apart. Cuttings are made from full-grown herbs in spring when leaves are firm enough to prevent wilting.

In commercial production, ploughing of the soil and fertilisation with ammonium phosphate during the winter months is sufficient for oregano cultivation. Under domestic conditions, pest management is usually reduced to a regular manual weeding. Irrigation is required only while planting and a few other times during the first year. In the following years, plants are developing an efficient root system and thus no further irrigation is usually needed.

The yield of raw oregano ranges from 2.5 to 3.5 t/ha. The essential oil obtained from oregano leaves ranges from 0.5 to 1.5 % of dry weight. The average lifespan of oregano is five to six years. The first harvest comes in the first year, and after every following year it happens two times. The best timing for harvesting depends on climate, plant´s life stage and the frequency of irrigation. For the highest amount of essential oil, the best time is when at least 50 % of plants in the field have started flowering (Peter 2001).

2.1.3. Post-harvest handling

After the harvest, leaves and stem tips of oregano are dried. For the small amount of the plant material, the best way is put the material on the drying sheets to the shade to avoid the direct sunlight. The shade will preserve the green colour and the characteristic aroma.

In commercial production, the harvested material is dried under natural conditions as it was mentioned above, dried in the oven, or freeze-drying. Moisture content of fresh oregano is 75 %. It is required to reach 7-12 % of moisture content by drying. In the oven, the process of drying is done at 30-35 °C and lasts approximately 24 hours (Peter 2001). The freeze-drying also called lyophilisation is the method when herbs are firstly being frozen at -20°C and dried by sublimation of ice in a vacuum afterward. The advantage of this method is stability of the plant material properties. However, the cost is much higher than the other methods mentioned (Skoufogianni et al. 2019). Studies show that those drying methods do not significantly affect water-holding capacity, odour, or the taste of dried oregano. Finally, the dried material is stored in airtight containers to prevent any external impacts and conserve the quality (Peter 2001).

2.1.4. Utilisation

Oregano essential oil has many uses in the food industry thanks to its original taste, aroma, and a high number of vitamins. It also contains many anti-bacterial components. Thus, it can be used as a natural preservative, for example during meat drying process (Hernández et al. 2016) or for post-harvest fruits and vegetables.

It has also significant importance in skin-care cosmetics. Oregano essential oil has highly calming properties and soothes skin conditions such as rosacea and hyperpigmentation. Carvacrol, which is the main compound in oregano essential oil, speeds up the healing process and keep the skin healthy and good-looking (Han & Parker 2017).

2.1.5. Medicinal benefits

The utilisation of oregano essential oil in traditional medicine is important to mention as well since it has many positive effects (e.g., antioxidative, antiproliferative, anti-inflammatory, antifungal, antibacterial) on human's health.

Antimicrobial activities

Oregano essential oil has both antifungal and antibacterial properties. Phenols which are represented by e.g., carvacrol and thymol, are believed to be the most potent antimicrobials, followed by alcohols, ketones, ethers, and hydrocarbons.

2.1.5.1.

Antifungal activities have an application in the food industry as a bio preservative to fight with yeasts and moulds. There is a study which demonstrated therapeutic potency of oregano essential oil. Furthermore, the oil was used on experimentally induced dermatophytosis (a disease of skin) in rats (Leyva-López et al. 2017).

Antioxidant

2.1.5.2. As an antioxidant, the oregano essential oil can serve as a prevention against an oxidative stress due to high content in phenolic compounds (Babili et al. 2011). In general, an oxidative stress is an imbalance between the production of reactive oxygen species (free radicals), which are produced as a by-product of oxygenation and metabolism, and the body's ability to rapidly break down and detoxify reactive intermediates. In humans, it is responsible for many diseases, such as Alzheimer's disease, Parkinson's disease, chronic-inflammation, arthritis, some types of cancer, diabetes, and atherosclerosis (Leyva-López et al. 2017).

2.1.5.3.

Anti-inflammatory and anticancerous activity

Inflammation is a biological response by which human body's white blood cells and other immunity reactors protect the body from infection from outside invaders, such as e.g., bacteria and viruses.

During an inflammation process, there is an increase of inflammatory mediators such as cytokines, prostaglandins, enzymes, nitric oxide, and reactive oxygen species. If it happens that the process is not controlled, the pro-inflammatory mediators will start overproducing. It might trigger pathological processes related to diseases such as arthritis, atherosclerosis, and cancer. Preventing the production of these mediators is therefore an essential goal in the treatment of inflammatory diseases.

There are some studies which show that oregano could be used as anti-inflammatory agents and could be used in preparations for the prevention or treatment of inflammation-related diseases. However, given that oregano essential oil can have a toxic

effect on cells, several in vivo and clinical studies are needed before it can be used as an alternative treatment for inflammation (Leyva-López et al. 2017).

According to Han & Parker (2017), there is evidence, that carvacrol as the main part of oregano essential oil has anticancerous properties. It has a potential to be used in skin-care products with anti-inflammatory and anticancerous properties.

Cardiovascular diseases

2.1.5.4. In general, cardiovascular diseases cover disorders of the heart and blood vessels. Those disorders can be chronic (caused by smoking, diabetes), or caused by atherosclerosis, which is the most common cause. The consequences of these disorders can be, for example heart attacks and strokes (WHO 2021).

Atherosclerosis is usually a result of an abnormal inflammatory response. It is an increase of plasma cholesterol and triglyceride levels. Dantas et al. (2015) proved that carvacrol can lower total plasma cholesterol and triglyceride levels, thanks to experiments conducted in normotensive rats. Nevertheless, there is still needed to do more research (Leyva-López et al. 2017).

Effect on metabolic syndrome and obesity

2.1.5.5. Metabolic syndrome is basically a medical term for a combination of diabetes (type 2), high blood pressure (hypertension) and obesity. Obesity is one of the most common health problems worldwide, and it is also one of the most dangerous heart attack risk factors.

Some studies have suggested that essential oils from *Lamiaceae* family plants may play a key role in reversal of some factors of metabolic syndrome. It was shown that oregano essential oil, which contains carvacrol and thymol as the main substances, was successful in reducing high levels of hypoglycaemia (α-amylase and α-glucosidase) as the main root of metabolic syndrome (Leyva-López et al. 2017).

2.1.5.6.

Antiproliferative activity

Generally, essential oils could exhibit antiproliferative effects. It means that substances from essential oils can inhibit growth of some cancer cells.

According to Begnini et al. (2014) oregano essential oil can inhibit cell proliferation in human breast adenocarcinoma and human colon adenocarcinoma. Active compounds are terpinen-4-ol, thymol, γ-terpinene and carvacrol (Leyva-López et al. 2017).

2.2. Bioactive compounds

2.2.1. Carvacrol

5-Isopropyl-2-methylphenol (according to IUPAC)

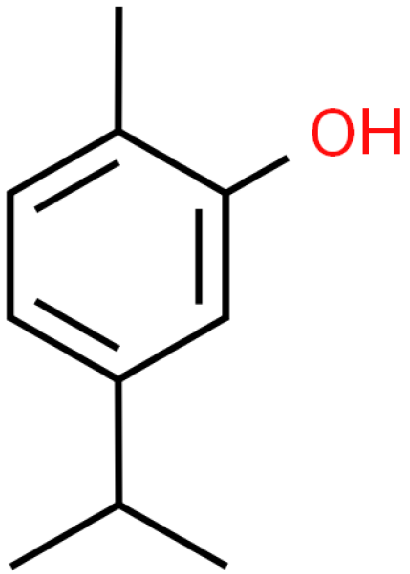

Figure 1: Structure of carvacrol molecule

Source: ChemSpider (2022)

Carvacrol is a phenol which has monoterpenoid nature. It contains methyl and isopropyl functions in para position to each other on a phenol ring. The structure is very similar to thymol. The only difference between them is the position of hydroxyl group (Can Baser 2008). The molecular formula of carvacrol is $C_{10}H_{14}O$ and its molecular weight is 150.2176 g/mol (NIST 2022). This volatile compound can be found in aromatic plants and essential oils. It looks like colourless to pale yellow slightly viscous oil, the smell reminds cool and herb spicy incense (ChemicalBook 2022).

Thanks to anti-microbial attributes, carvacrol is abundantly used in food industry as a preservative. It can destroy or inhibit the growth of noxious bacteria (for example, *Salmonella enterica, Escherichia coli*) that commonly form in untreated food and prolong shelf-life. According to Can Baser (2008), carvacrol was tested as a fungicide, and it was found out that it is more effective in comparison with commercial fungicides.

In many studies, carvacrol exhibited strong antitumor activities. It effectively inhibits or slows cancer-cells proliferation. Significant effects were showed in lung cancer, colorectal cancer, human oral squamous cell carcinoma, tongue cancer, prostate cancer, gastric cancer, breast cancer, and many others (Sampaio et al. 2021).

2.2.2. Thymol

5-Methyl-2-(propan-2-yl) benzenol (according to IUPAC)

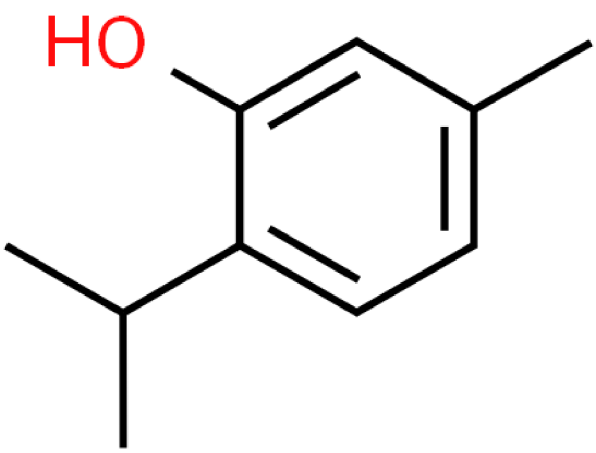

Figure 2: Molecular structure of thymol

Source: ChemSpider (2022)

Thymol is a monoterpene phenolic derivative of cymene. Its molecular formula is $C_{10}H_{14}OH$, and molecular weight is 150.2176 g/mol (NIST 2022). It is a white crystalline substance with a pleasant odour (herbal, thyme, medicated, earthy) and strong antiseptic properties (Pherobase 2022).

Thymol with carvacrol is now known to kill bacteria and fungi. Those substances are together used in food industry as a natural preservatives and healthy flavourings. Thymol is also used as insecticide, to produce commercial repellents and finds useful application in the eradication of the parasite causing varroasis in bee colonies (Marchese et al. 2016).

2.2.3. Linalool

3,7-Dimethylocta-1,6-dien-3-ol (according to IUPAC)

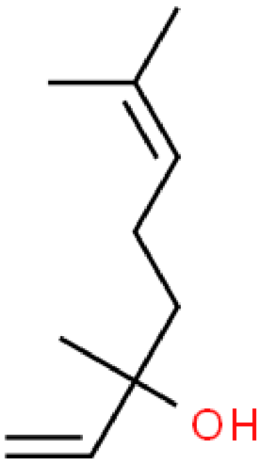

Figure 3: Molecular structure of linalool

Source: ChemSpider (2022)

Linalool is a monoterpene compound and one of the major components of aromatic essential oils. It is colourless to very pale-yellow liquid (Letizia et al. 2003). Linalool has its specific smell reminding muscat, lavender or parsley. The molecular formula of linalool is $C_{10}H_{18}O$, and the molecular weight is 154.2493 g/mol (NIST 2022).

This volatile compound is mainly used in cosmetics as a fragrance material for its fresh, floral smell and anti-inflammatory properties against several bacteria and fungi (Peana et al. 2002). Cosmetic products containing linalool include for example soaps, detergents, shampoos, lotions, and many skin-care compounds. Linalool as a terpene is also used in repellents (Skincare Lab 2022).

2.2.4. p-Cymene

1-Methyl-4-(propan-2-yl) benzene (according to IUPAC)

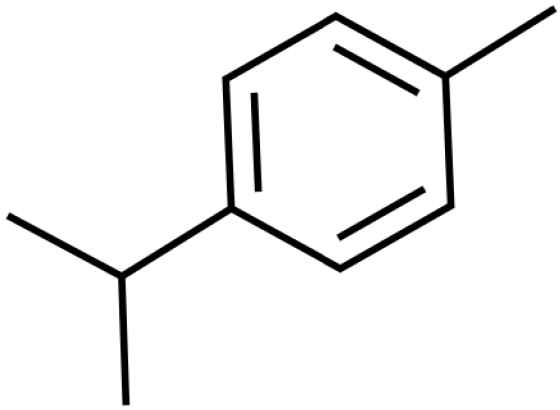

Figure 4: Molecular structure of p-cymene

Source: ChemSpider (2022)

p-Cymene is an organic compound classified as an aromatic alkylbenzene related to a monoterpene it has lemon, fruity, fuel-like odour (Pherobase 2022). Its molecular formula is $C_{10}H_{14,}$ and molecular weight 134.22 g/mol. p-Cymene is a common constituent in many essential oils (cumin, thyme), plant metabolite, and a human urinary metabolite (PubChem 2022). There are two less common geometric isomers related to p-cymene (m-cymene and o-cymene) (NIST 2022).

2.2.5. γ-Terpinene

1-methyl-4-(propan-2-yl) cyclohexa-1,4-diene (according to IUPAC)

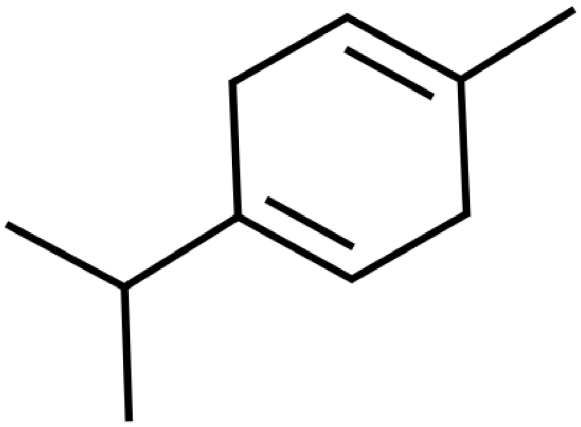

Figure 5: Molecular structure of γ-terpinene

Source: ChemSpider (2022)

Gamma-terpinene is a natural compound and one of three isomeric monoterpenes differing in the positions of their two double bonds (alpha, beta). It acts as an antioxidant, a plant metabolite, a volatile oil component, and a human xenobiotic metabolite (PubChem 2022). Its molecular formula is $C_{10}H_{16,}$ and molecular weight is 136.24 g/mol (NIST 2022).

2.3. Chromatography

The first chromatography process was done by Russian scientist and botanist M. S. Tswett at the beginning of 20th century (Poole 2010). Since that time, new chromatography techniques have been developed and nowadays, it is an important tool used in science.

The main principle of chromatography is a physical separation of inorganic compounds in a substance for subsequent analysis. Components are being separated and distributed between two phases: stationary phase and mobile phase. The stationary phase is not movable (components could be solid or liquid), whereas the mobile phase (components could be liquid or gas) can move (Poole 2010). These two phases differ from each other by some basic physicochemical property, e.g., polarity. Along with the moving mobile phase, the sample is also carried through the system. The separated components of the sample (analytes) interact to varying degrees with the stationary and mobile phases. The analytes that are more attracted to the stationary phase move more slowly and are retained longer than the analytes that are less attracted to the stationary phase. Based on this principle, the components of the mixture are partitioned.

The useful tool for comparing results from chromatogram is a retention factor (Rf). Generally, it is the ratio of the distance the spot moved above the origin to the distance the solvent front moved above the origin in a particular material. If the unknown composite is close or the same as the Rf value for the already known composite is an indicator that the two compounds are similar or identical (Coskun 2016).

Chromatography itself has many different types in many different categories. It could be according to the physicochemical principle of division, the state of the mobile phase, the stationary phase arrangement, or according to the purpose.

Division according to the state of mobile phase:

- Liquid chromatography: the mobile phase is liquid (e.g., Hexane)
- Gas chromatography: the mobile phase is gas (e.g., Helium)

2.3.1. Gas chromatography

Gas chromatography (GC) is a method for the separation and analysis of gas, liquid, and solid substances with boiling point up to approximately 400 °C. The sample must be thermally stable at the temperature required for vaporization. There are two types of gas chromatography: gas-solid chromatography (GSC), and gas-liquid chromatography (GLC). GSC is when the solid absorbent serves as the stationary phase whereas in GLC, the stationary phase is a liquid spread on inert support or coated as a thin film onto the wall of a capillary column (Poole 2010). The mobile phase in GC is an inert carrier gas (e. g., He) which is being passed through a column under high pressure. Then the sample is vaporized and enters a gaseous mobile phase when it is ready for analyzation (Coskun 2016). The flow rate of the inert carrier gas is controlled to ensure reproducible retention times and to minimise detector drift and noise. The components contained in the sample are dispersed between mobile phase, and stationary phase on the solid or liquid support based on relative solubility in the liquid phase and relative vapor pressures. Finally, the carrier gas and sample pass through a detector which measures the quantity of the sample, and generates an electrical system automatically integrates the peak area, performs calculations, and prints out a report with quantitative results and retention times.

Basic components of a typical gas chromatograph are carrier gas, flow control, sample inlet and sampling devices, columns, controlled temperature zones (ovens), detectors, and data systems (MacNair & Miller 1998).

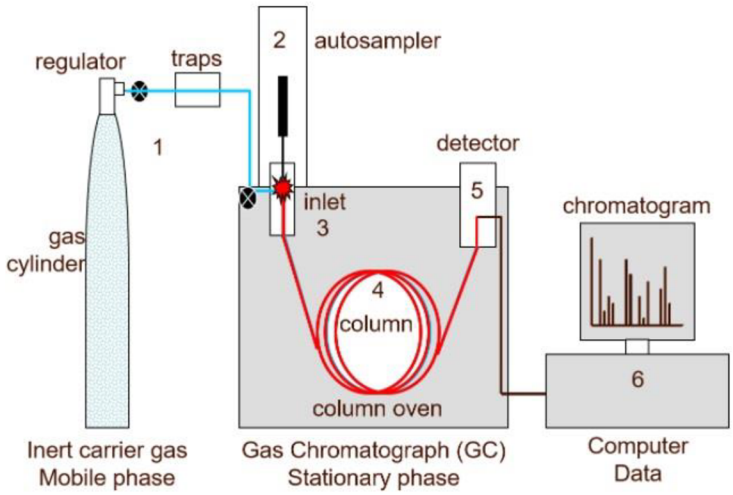

Figure 6: Structure of gas chromatograph

Source: Turner (2022)

2.4. Mass spectrometry

Mass spectrometry (MS) is one of the most information-rich detectors for recognising of identities of a sample. The individual compounds rinse from the GC column, they enter the electron ionization (MS) detector. In this part, the compounds are blasted with a stream of electrons causing them to break up into fragments. What is measured in MS are individual ions in kilograms per Coulomb (MacNair & Miller 1998).

There are many types of mass spectrometers, but they have common characteristics. It is consisted of an ion source, a mass analyser, and a detector which are operated under high vacuum conditions.

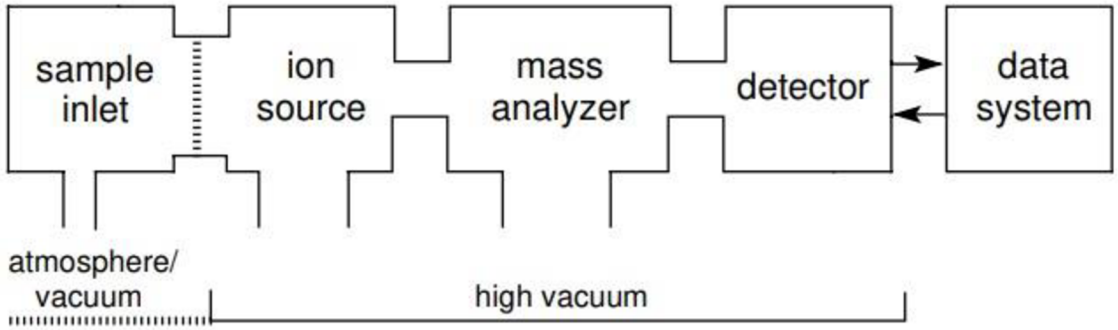

Figure 7: General scheme of a mass spectrometer

Source: Gross (2004)

The process of mass spectrometry is divided in three steps: ionization, acceleration and deflection, and detection.

1. *Ionization*

Firstly, the analyte molecules must be ionized to be attracted (or repelled) by appropriate magnetic or electric fields. The ionization source is heated (under vacuum), so most samples will easily vaporise and subsequently ionize. Ionization is usually performed by impacting a high energy electron beam.

2. *Acceleration and deflection*

In this stage, positive ions accelerate towards negative plates. To reach the same kinetic energy, positive ions move at speed which is dependent on their mass. Havier molecules are moving slower than the lighter ones.

Ions which are traveling through the spectrometer at different speeds and deflection are divided according to their mass (lighter/heavier) and charges (positive/negative).

3. Detection

The detection is the final part of MS in which the ions are sensed. in the form of a mass spectrum

A mass spectrum is a two-dimensional representation of signal intensity versus mass/charge number of ion (m/z). Received signals are called peaks which directly reflect the abundance of ionic species of that respective m/z ratio (Gross 2004).

2.5. Extraction methods

Extraction as such means in the pharmaceutical point of view the separation of the tissue parts of plant or animal from the medicinal substances by using selective solvents. In general, to obtain medicinal ingredients from plant material, there are five steps needed to be done: size reduction, extraction, filtration, concentration, and drying (Handa et al. 2008).

Essential oils are products extracted from natural plants by physical means only. Every method has different advantages and disadvantages. The quality of extracted essential oil depends on thermal effects, degradation of some unsaturated compounds, process duration, loss of important components by hydrolysis, and the type and quality of raw plant material.

Methods, which are mostly used for extraction worldwide are water distillation, water and steam distillation, cohobation, maceration and enfleurage. The popular technique for used for isolation of essential oils from the medicinal and aromatic plants is called hydro-distillation (Li et al. 2014).

2.5.1. Hydro-distillation

Hydro-distillation is a method for the extraction and quality control of essential oils from dried spices recommended by the French Pharmacopoeia. The plant material is immersed directly into the distilled water and then heated until boiling under atmospheric

pressure. The generated heat allows the release of fragrant molecules in plant cells. The mixture of the plant material and distilled water form an azeotropic mixture, which can be evaporated and after condensed. In the Florentine flask due to their immiscibility and density difference, the azeotropic mixture can be separated into the wanted essential oil, and the rest (Li et al. 2014).

Hydro-distillation of plant material is based on three physicochemical processes:

1. *Hydro-diffusion*

It is a diffusion of essential oils and hot water through plant membranes. The heated water enables dissolving volatile oil in the water within the glands. It leads to osmosis through the swollen membranes, and the oil finally reaches the outer surface, where it is vaporised by passing steam.

2. *Hydrolysis*

In this context, the hydrolysis is defined as a chemical reaction between water and certain constituents of essential oils (esters). Those constituents react with water to form acids and alcohols due to high temperature. If the amount of water is large, it may lead to large amounts alcohol and acid, and results in a decreased yield of essential oil, which is a disadvantage of hydro-distillation.

3. *Decomposition by heat*

Essential oils containing volatile compounds, which are unstable in high temperatures, may affect the final yield and quality of essential oil. Thus, it is necessary not to exceed the recommended temperatures and do the process under normal atmospheric pressure (Handa et al. 2008).

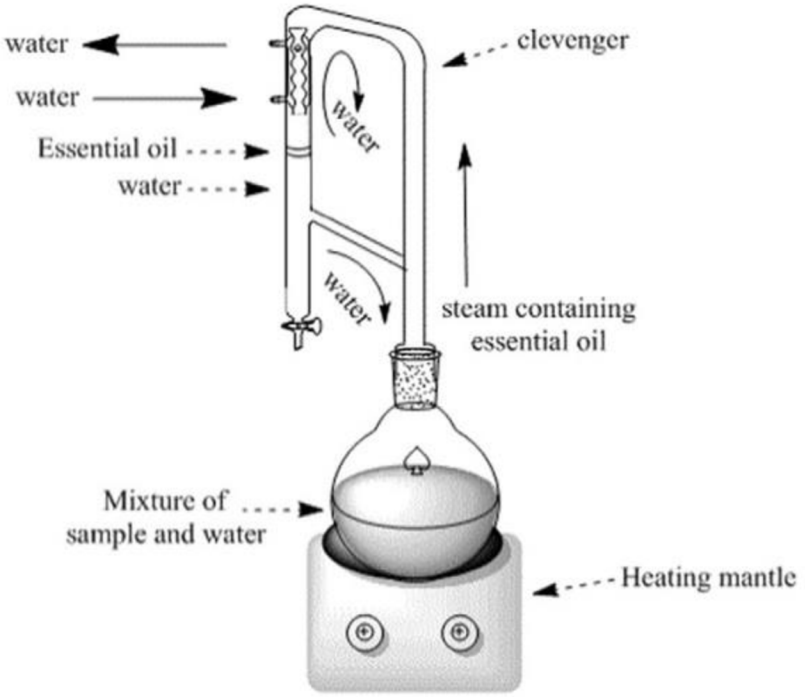

Figure 8: Hydro-distillation Clevenger apparatus system

Source: Samadi et al. (2016)

2.6. Scientific studies

There are many already done studies which are focused on chemical analysis of volatile substances in oregano essential oil by GC/MS. Those studies were selected to compare the own results from my bachelor thesis.

The study done by Kulisic et al. (2004) was focused on different methods for testing antioxidative activity of oregano essential oil. The plant material of *Origanum vulgare* L. (flowered tops and stalks) was air-dried in room temperature for 10 days. A hundred grams of the dried plant material were hydro-distilled in modified Clevenger-type apparatus for three hours.

The analysis of the volatile compounds were done by a Hewlett-Packard GC/MS system. The testing antioxidative activity was done by three methods and it was shown that the single method is insufficient, thus it is necessary to use all three of them. Furthermore the proven antioxidants in this paper were thymol, carvacrol, and oxygen containing compounds. The chemical composition of the essential oil of *Origanum*

vulgare L. is presented in the table 2. It shows that the most abundant components are thymol (35.0 %), carvacrol (32.0 %), and γ-terpinene (10.5 %).

Table 2: The composition (area %) of *Origanum vulgare L.* essential oil

			In total oil	In fraction
Hydrocarbons CH fraction (CH)				
1.	α-Thujene	1031	1.4	5.2
2.	β-pinene	1102	–	0.7
3.	Myrcene	1149	–	6.1
4.	α-Terpinene	1161	3.6	10.4
5.	γ-Terpinene	1231	10.5	31.0
6.	*p*-Cymene	1247	9.1	22.1
7.	Terpinolene	1262	–	0.9
8.	Alloocimene[b]	1351	–	0.3
9.	α-Copaene	1466	–	0.4
10.	β-Burbonene	1496	–	0.3
11.	*trans*-Caryophyllene	1578	2.4	9.1
12.	Aromadendrene	1583	–	0.4
13.	α-Humulene	1638	–	1.5
14.	Ledene	1644	–	0.3
15.	β-Bisabolene	1694	1.4	2.0
16.	δ-Cadinene	1729	0.5	3.8
17.	α-Muurolene	1735	–	0.2
				Total 95.8
Oxygen containing compounds fraction (CHO)				
18.	1-Octen-3-ol	1411	1.0	0.8
19.	Borneol	1653	1.0	1.0
20.	Thymol	2115	35.0	47.3
21.	Carvacrol	2140	32.0	46.4
			Total 97.9	Total 95.5

Source: Kulisic et al. (2004)

Martucci et al. (2014) have done the research focused on oregano (*Origanum vulgare*) and lavender (*Lavandula officinalis*) essential oils as antioxidant and antimicrobial additives of biogenic gelatine films. The oregano essential oil was obtained by hydro-distillation using a Clevenger type apparatus from fully formed, freshly dried oregano leaves collected in Mar del Plata in Argentina. The analysis of oils was done by GC/MS using an Agilent gas chromatograph model 7890 A equipped with an auto-sampler ALS and coupled to an Agilent single quadrupole mass spectrometer (MS) model 5975C.

Table 3 reveals that the oregano essential oil' s most abundant components are carvacrol (26.70 %), p-cymene (15.20 %), and γ-terpinene (15.10 %).

Table 3: Main compounds, expressed as percentage of the chromatographic area of *L. officinalis* and *O. vulgare* essential oils.

Components(%)			
Lavander (Lavandula officinalis)		Oregano (Origanum vulgare)	
Linalool	53.50	Carvacrol	26.70
Camphor	8.40	p-Cimene	15.20
Terpinen-4-ol	7.60	γ–Terpinene	15.10
1,8- cineol	6.80	Terpinene	7.50
Borneol	4.70	α– Pinene	5.60
Linalyl acetate	4.20	Iso borneol	3.80
Lavandulyl acetate	0.80	Terpinolene	3.40
Hexilacetate	0.60	β- Myrcene	3.40
1-octen-3-ol	0.55	α– Thujene	3.40
3- octanone	0.40	α- Terpineol	2.30
Myrcene	0.30	Methyl carvacrol	2.20
		Caryophyllene	1.40
		Sabinene	1.40
		Endo borneol	1.20
		Thymol	1.10
		Terpinen-4-ol	1.10
		β- Phellandrene	0.60
		Camphene	0.40
		1,8- cineol	0.30
Total	87.85	Total	96.1

Source: Martucci et al. (2014)

The study described by Netopilová et al. (2021) was aimed at evaluating in vitro antimicrobial interactions between *Origanum vulgare* and *Thymus vulgaris* essential oils against different strains of *Staphylococcus aureus* in both liquid and vapor phases using the broth volatilisation checkerboard method. The chemical analysis of essential oils was performed by GC/MS on a dual-column (HP-5MS and DB-HeawyWAX) gas chromatograph. The samples of oregano essential oil were done by hydro-distillation from dried oregano leaves in 1 l distilled water using a Clevenger-type apparatus. The results of GC/MS analysis from this study are shown in Table 4. The most significant proportions of oregano essential oil consisted of carvacrol (77.92 %, 82.60 %), p-cymene (8.25 %, 5.63 %), and γ-terpinene (4.52 %, 3.33 %).

Table 4: Chemical composition of Origanum vulgare essential oil.

	[1] RI		Component	[2] C	[3] RF	[4] Column				[5] Identification	
						HP-5MS		DB-H.WAX		HP-5MS	DB-H.WAX
	Obs.	Lit.				(%)	c	(%)	c		
1	922 [a]	924	α-Thujene	Monoterpene hydrocarbon (MH)	0.765	1.17	0.19	0.77	0.13	GC/MS, RI	GC/MS
2	929 [a]	932	α-Pinene	MH	0.765	0.67	0.11	0.42	0.07	GC/MS, RI, Std	GC/MS
3	945 [a]	946	Camphene	MH	0.765	0.18	0.03	0.12	0.02	GC/MS, RI, Std	GC/MS
4	973 [a]	974	β-Pinene	MH	0.765	0.16	0.03	0.10	0.02	GC/MS, RI, Std	GC/MS
5	991 [a]	988	β-Myrcene	MH	0.765	1.87	0.31	1.23	0.21	GC/MS, RI	GC/MS
6	1005 [a]	1002	α-Phellandrene	MH	0.765	0.14	0.02	0.08	0.01	GC/MS, RI	GC/MS
7	1009 [a]	1008	3-Carene	MH	0.765	0.08	0.01	0.06	0.01	GC/MS, RI, Std	GC/MS
8	1017 [a]	1014	α-Terpinene	MH	0.765	0.85	0.14	0.63	0.11	GC/MS, RI, Std	GC/MS
9	1028 [a]	1025	*p*-Cymene	MH	0.698	8.25	1.24	5.63	0.88	GC/MS, RI, Std	GC/MS
10	1061 [a]	1054	γ-Terpinene	MH	0.765	4.52	0.74	3.33	0.57	GC/MS, RI, Std	GC/MS
11	1078 [a]	1068	*trans*-Sabinene hydrate	Oxygenated monoterpene (MO)	0.869	0.30	0.06	0.11	0.02	GC/MS, RI	GC/MS
12	1110 [a]	1095	Linalool	MO	0.869	0.11	0.02	-	-	GC/MS, RI, Std	-
13	1185 [a]	1165	Borneol	MO	0.869	0.06	0.01	0.58	0.11	GC/MS, RI, Std	GC/MS
14	1190 [a]	1174	Terpinen-4-ol	MO	0.869	0.64	0.12	0.36	0.07	GC/MS, RI	GC/MS
15	1302 [a]	1289	Thymol	MO	0.808	0.26	0.04	0.47	0.08	GC/MS, RI, Std	GC/MS
16	1314 [a]	1298	Carvacrol	MO	0.808	77.92	13.52	82.60	15.01	GC/MS, RI, Std	GC/MS
17	1430 [a]	1418	β-Caryophyllene	Sesquiterpene hydrocarbon (SH)	0.751	1.89	0.30	1.53	0.26	GC/MS, RI, Std	GC/MS
18	1466 [a]	1452	Humulene	SH	0.751	0.26	0.04	0.18	0.03	GC/MS, RI	GC/MS
19	1517 [a]	1505	β-Bisabolene	SH	0.751	0.45	0.07	0.35	0.06	GC/MS, RI	GC/MS
20	1181 [b]	1185 [c]	D-Limonene	MH	0.765	-	-	0.15	0.03	-	GC/MS, RI
21	1190 [b]	1195 [d]	β-Phellandrene	MH	0.765	-	-	0.15	0.03	-	GC/MS, RI
22	1438 [b]	1445 [e]	1-Octen-3-ol	Others (O)	0.748	-	-	0.22	0.04	-	GC/MS, RI
23	1450 [b]	1450 [f]	*cis*-Sabinene hydrate	MO	0.869	-	-	0.27	0.05	-	GC/MS, RI
24	1579 [b]	1583 [g]	Carvacrol methyl ether	O	0.798	-	-	0.36	0.06	-	GC/MS, RI
25	1848 [b]	1868 [h]	Carvacrol acetate	O	0.901	-	-	0.06	0.01	-	GC/MS, RI
26	1957 [b]	1953 [d]	Caryophyllene oxide	Oxygenated sesquiterpene	0.830	-	-	0.14	0.03	-	GC/MS, RI
			Chemical classes								
			Monoterpene hydrocarbons			17.89				12.67	
			Oxygenated monoterpenes			79.29				84.39	
			Sesquiterpene hydrocarbons			2.60				2.06	
			Oxygenated sesquiterpenes			-				0.14	
			Others			-				0.64	
			Total identified (%)			**99.78**				**99.90**	

Source: Netopilová et al. (2021)

3. Objectives

3.1. Main objective

The main aim is to compare the chemical composition of commercially available oregano essential oil and essential oils obtained from the original plant material.

3.2. Specific objectives

1. Literature review focused on properties, health, and chemical composition of essential oil of oregano *(Origanum vulgare)*.

2. Preparation and chemical analysis of three extracts of dried oregano samples, and one sample of commercial oregano essential oil, and comparison between each other and with literature data.

4. Methods and materials

4.1. Samples

The chemical analysis was demonstrated on three samples of dried oregano and one sample of oregano essential oil. Those samples were bought on the common Czech market. Each sample of dried oregano was selected from different price category. Two samples of dried oregano were regular dried oregano, and one sample, the most expensive one, was labelled organic. The origin of the dried samples is from Europe but not specified, and the origin of the essential oil is from Greece.

The following samples of dried oregano were used for the analysis:

Sample 1: Oregano drcené 10 g (producer: K-Classic, price: 4.90 CZK)

Sample 2: Oregano drhnuté 8 g (producer: Kotányi, price: 19.90 CZK)

Sample 3: Oregáno sušené Bio 7 g (producer: It´s Natural, price: 22.90 CZK)

In the following methodology part, the names are represented as samples by the numbers.

Figure 9: Sample 1

Figure 10: Sample 2

Figure 11: Sample 3

Source: Author of the thesis

The following sample of oregano essential oil was used for the analysis:

Sample 4: Phytos oregano esenciální olej 10 ml (producer: Natur, price: 169 CZK, country of origin: Greece)

Figure 12: Sample 4

Source: Natur (2022)

The producer Natur declares that the process used for obtaining this oregano essential oil (sample 4) was hydro-distillation, and the amount of carvacrol is 82 %.

4.2. Extraction and GC/MS analysis

Three samples of dried oregano were prepared. It was picked 50 grams from each sample, then the samples were separately put into 1 l of distilled water into the glass flask. The sample was then distilled using a Clevenger distillation apparatus. The process lasted four hours. After, the obtained essential oil was transferred to a 2 ml vial. Before analysis the essential oils were dissolved in hexane (PENTA s.r.o.). Concentration of samples were 5 µl/ml.

The fourth sample of oregano essential oil was dissolved in hexane in the same concentration as the previous samples.

Figure 13: Hydro-distillation extraction of essential oil from the samples n. 2 and n. 3

Source: Author of the thesis

GS/MC analysis was performed on Agilent Technologies 5977A MSD equipped with a 38 HP-5 column (5%-phenyl)-methylpolysiloxane, 30 m length, 250 µm internal diameter, 0.25 µm film thickness was employed. Each sample was analysed three times. In total, there were 12 measurements.

The injections were performed using an autosampler immediately after extraction. Injection volume was 1 µl. The inlet GC injection port temperature was maintained at 220° C, the split mode was set on 1:10. The optimized GC oven temperature program was 70 °C (2 min) to 280 °C at 10 °C/min (final temperature held for 10 min). Carrier gas helium was used at a flow rate of 1 mL/min. The MS detector transfer line temperature was maintained at 250 °C with the electron energy of 70 eV. Mass spectra were acquired in the mass range from m/z 30 to 600, using a scan time of 1 sec.

Data was obtained through MassHunter Workstation Software Qualitative Analysis Version B.07.00. An identification of the volatile compounds was performed by comparison of their mass spectra with the mass spectra contained in the NIST 2.2 library. Kovats retention index (KI) was used for comparing and confirmation of correct identification. Internet database pherobase.com was used to search for KIs, the source of KIs are the results of the DB-5 column measurements according to Adams (2007).

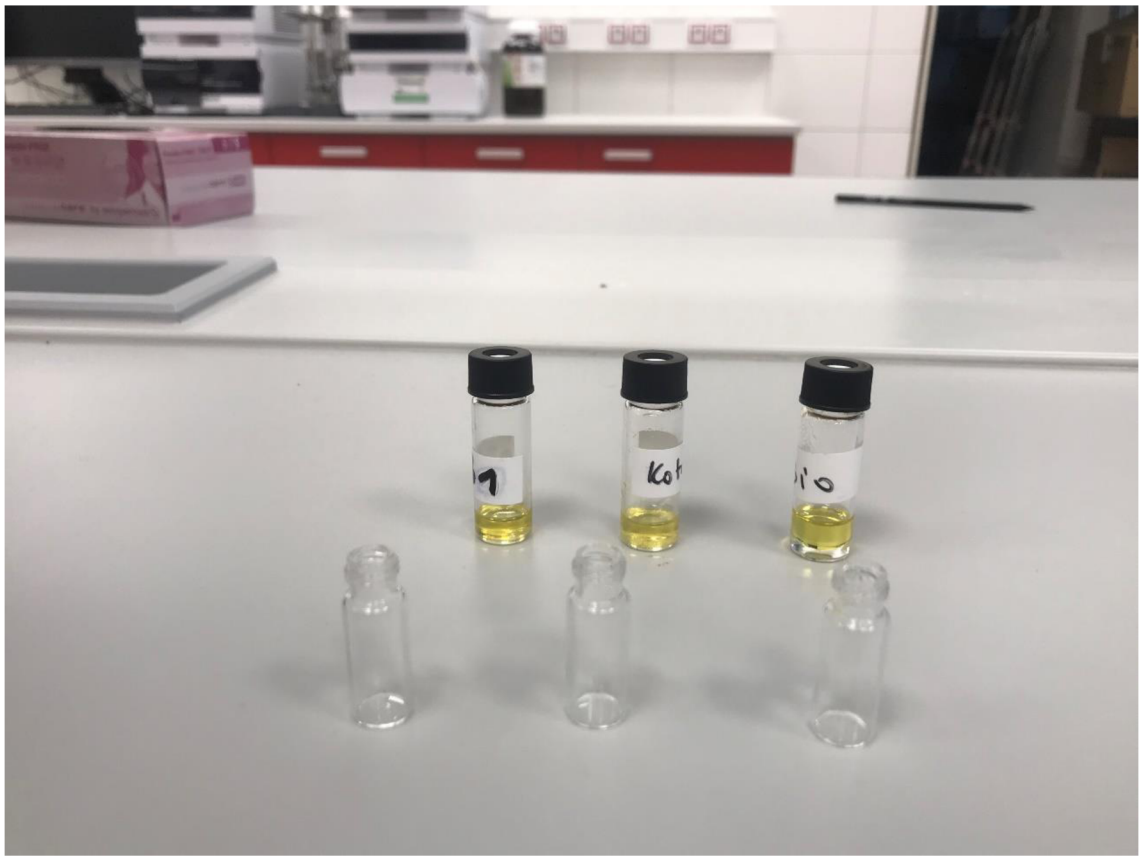

Figure 14: Extracted samples of essential oregano oil

Source: Author of the thesis

5. Results

The results of GC/MS are presented in Table 4. In all four samples together, there were identified 32 compounds. Samples (sample 1, sample 2, sample 3) made from dried leaves of oregano by hydro-distillation contained much more compounds, than the sample taken from commercially produced essential oregano oil (sample 4). Sample 1 contained 28 compounds. Sample 2 contained 25 compounds. Sample 3 contained 29 compounds. Sample 4 contained only 11 compounds. Table 4 also shows Retention Time (RT) of samples, KI which were calculated and found in the literature, and the represented sum of the area is shown in percentage (%).

Carvacrol contains the largest proportion area in all four samples. Of all samples, the most of carvacrol contains the sample 2 made from Kotányi (86.62 %). The second largest proportion of carvacrol contains sample 4 (83.24 %- Phytos oregano esenciální olej). The third was sample 1 (81.75 %- K- Classic). The lowest proportion of carvacrol contained the sample 3 (79.01 %- It´s Natural).

In all four samples, there were found significant amounts of p-cymene, and linalool. As for p-cymene, the largest proportion was found in sample 3 (4.05 %), then in sample 4 (3.32 %), sample 1 contained 3.04 %, and sample 2- 1.37 %. For linalool, the largest proportion accounted for sample 3 (6.57 %), 5.06 % for sample 2, 2.59 % for sample 1, and 0.27 % for sample 4.

γ-Terpinene, which is also covers significant proportion amount in samples, was found in sample 1 (3.30 %), sample 2 (0.67 %), and sample 3 (3.07 %). γ-Terpinene was not found in sample 4.

Thymol was the most significantly found in sample 4 (4.82 %). In the rest of samples, the amount of thymol was not so substantial; sample 1 (0.78 %), sample 2 (0.97 %), sample 3 (0.68 %).

Significantly higher amount of caryophyllene, in comparison with other samples, was examined in sample 4 (6.09 %).

Table 5: Identification of bioactive compounds (Chemical composition)

Compound name	RT	RI calc.	RI lit.	Sample 1 (% avg.)	Sample 2 (% avg.)	Sample 3 (% avg.)	Sample 4 (% avg.)
α-Thujene	5.53	917	931	0.34	0.05	0.25	x
α-Pinene	5.66	923	939	0.32	0.04	0.36	0.47
Camphene	5.92	944	953	0.15	x	0.14	x
1-Octen-3-ol	6.36	974	980	0.13	0.06	0.12	x
β-Myrcene	6.47	982	991	0.73	0.12	0.67	0.44
α-Phellandrene	6.77	1002	1005	0.12	x	0.12	x
3-Carene	6.86	1008	1018	x	x	0.05	x
α-Terpinene	6.96	1015	1018	0.87	0.23	0.87	x
p-Cymene	7.25	1025	1026	3.04	1.37	4.05	3.32
γ-Terpinene	7.63	1059	1062	3.30	0.67	3.07	x
cis-Sabinene hydrate	7.86	1073	1070	0.11	0.07	0.11	x
Terpinolene	8.11	1091	1088	0.13	0.10	0.15	x
Linalool	8.29	1103	1098	2.59	5.06	6.57	0.27
cis-β-Terpineol	8.72	1132	1144	x	0.05	0.05	x
Camphor	9.09	1157	1143	x	x	0.04	0.17
endo-Borneol	9.47	1183	1165	0.95	0.94	0.75	x
Terpinen-4-ol	9.6	1191	1177	0.89	0.70	0.81	x
L-α-Terpineol	9.82	1206	1189	0.17	0.17	0.19	0.43
Isothymol methyl ether	10.48	1252	1244	0.25	0.06	0.13	x
Carvone	10.68	1260	1242	0.14	0.18	0.15	x
Thymol	11.39	1315	1290	0.78	0.97	0.68	4.82
Carvacrol	11.64	1333	1298	81.75	86.62	79.01	83.24
Caryophyllene	13.11	1444	1454	0.59	0.40	0.44	6.09
Aromandendrene	13.36	1464	1439	0.09	x	0.08	x
Humulene	13.56	1475	1455	x	x	x	0.56
β-Bisabolene	14.1	1522	1509	1.04	0.87	0.54	x
γ-Cadinene	14.3	1538	1513	0.08	0.08	0.05	x
Spathulenol	15.18	1611	1582	0.20	0.19	0.14	x
Caryophyllene oxide	15.24	1616	1581	0.46	0.47	0.27	0.19
Isoaromadendrene epoxide	15.37	1628	1612	0.20	x	x	x
Cadinol	15.88	1672	1653	0.40	0.37	0.17	x
Bulnesol	16.05	1688	1666	0.17	0.14	x	x

Source: Author of the thesis

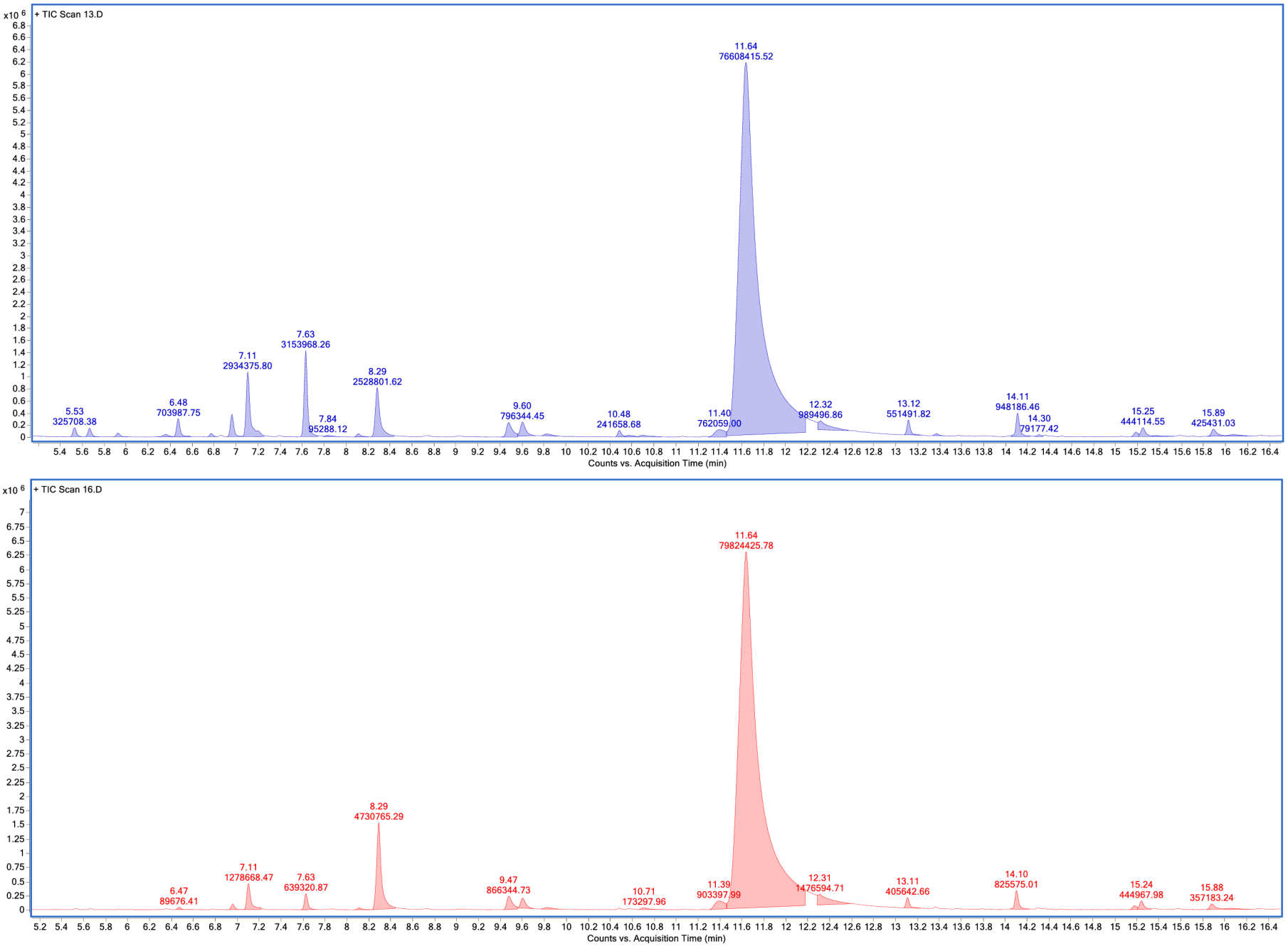

Figure 15: Chromatograms (from right to left: sample 1, sample 2)

Source: Author of the thesis

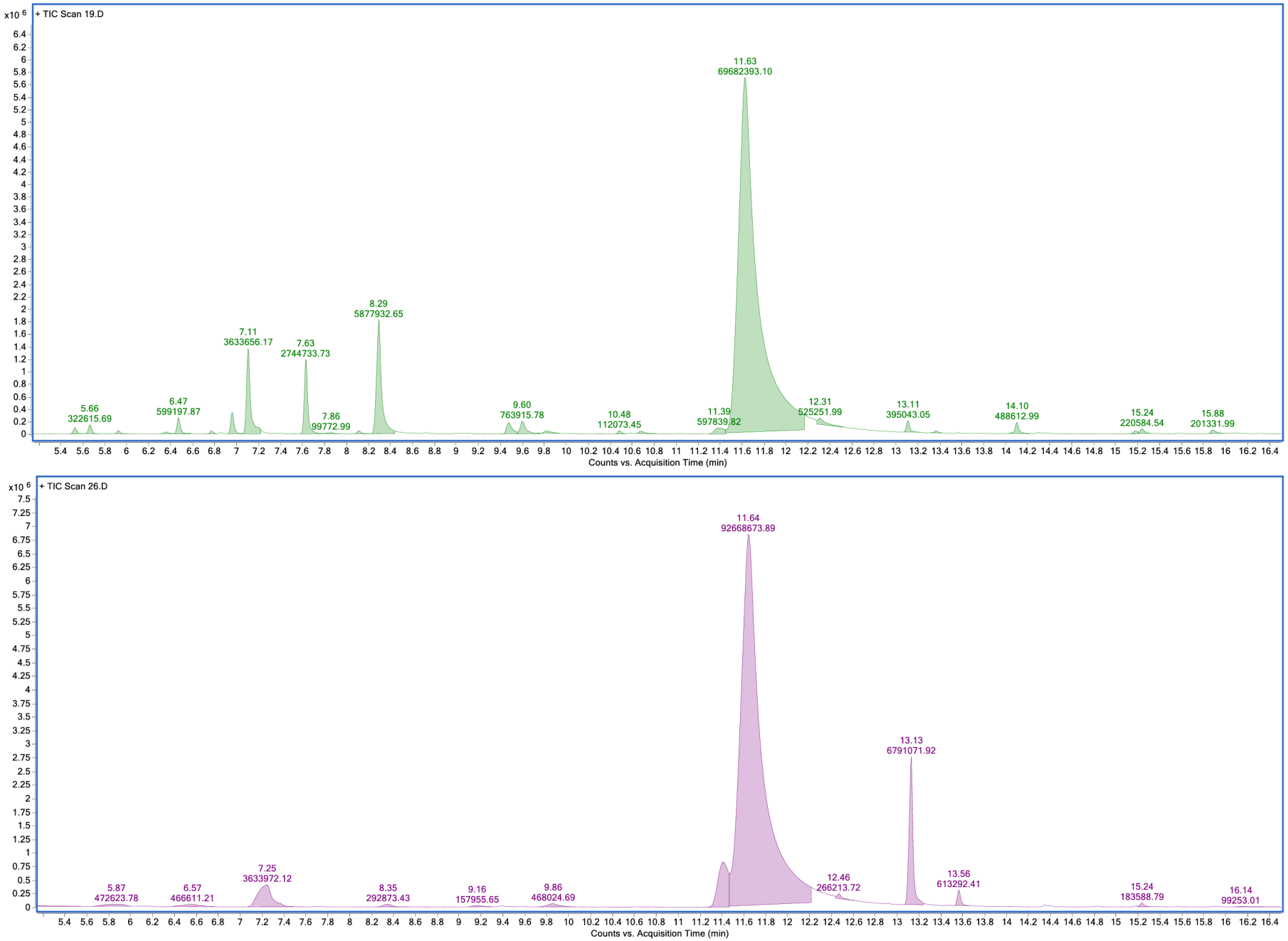

Figure 16: Chromatograms (from right to left: sample 3, sample 4)

Source: Author of the thesis

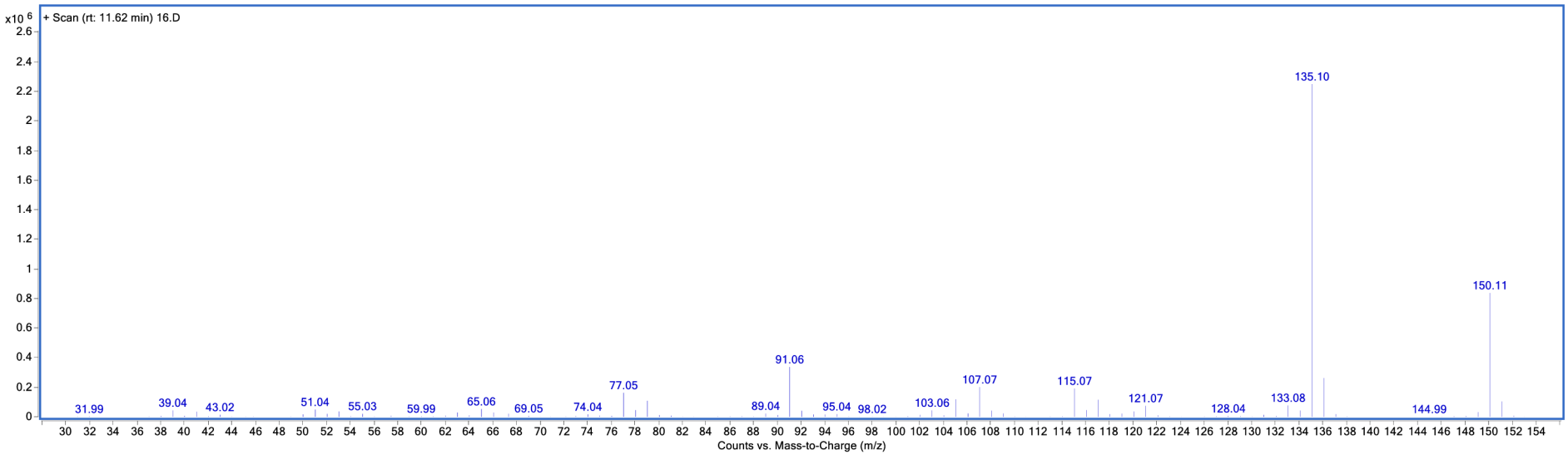

Figure 17: Mass Spectrum of molecule carvacrol

Source: Author of the thesis

6. Discussion

The results were compared with each other and with the relevant scientific literature data. It was confirmed that the presence of carvacrol, thymol, and p-cymene was found out both in scientific studies and in essential oils analysed in this paper.

The research done by Kulisic et al. (2004) showed that during GC/MS the major constituents of *Origanum vulgare* was thymol (35.0 %), carvacrol (32.0 %), and γ-terpinene (10.5 %). In this case, it is interesting that the most abundant compound in the oregano essential oil is thymol, because in the rest of the samples, the most abundant compound is carvacrol. This deviation could be explained by using different parts of plant (flowered tops and stalks) for hydro-distillation, whereas for the rest studies were used especially dried oregano leaves. In addition, it is not recent study thus new technologies could potentially bring different results.

On the contrary, carvacrol as the major oregano essential oil compound was found in the research done by Martucci et al. (2014) and Netopilová et al. (2021). In comparison with my study (carvacrol amounts: 81.75 %, 86. 62 %, 79.01 %, 83.24 %), Martucci et al. (2014) detected significantly lower amount of carvacrol (26.70 %), but higher amounts of p-cymene (15.20 %), and γ-terpinene (15.10 %). Samples of plant material were collected in Mar del Plata in Argentina. The composition of oregano essential oil can be influenced by geographical factor. According to several studies on *Origanum vulgare* done in different parts of the world, geographical factor can influence the chemical composition of essential oil. Different proportions of thymol and carvacrol have been reported as major compounds in this plant species.

The most similar chemical composition with this study reported Netopilová et al. (2021). The values of carvacrol (77.92 %, 82.60 %), p-cymene (8.25 %, 5.63 %), and γ-terpinene (4.52 %, 3.33) of these samples were almost identical. This study is the most recent and it was conducted under the similar conditions as this bachelor thesis.

The results of samples 1, 2, 3, 4 examined in this Bachelor thesis were compared between each other. It was found out that the results of samples 1,2,3 were comparably

similar. Sample 4 did not differ in the main compounds; however, the overall number of compounds was considerably smaller.

It was confirmed that the amount of carvacrol declared by Natur (82 %) was found in the sample 4 (83.24 %).

Even though, the results did not differ significantly, overall, there are considerable differences between the results of this study and the data found in literature. There are several factors, which potentially could have influenced this. The most important factor is probably geographical distribution, which was mentioned in many of the studies focusing on the chemical analysis of *Origanum vulgare*. Other factors can be considered, for instance as time of cultivation, methods of processing, sample size, time of storage as well as place of storage, packaging, and others.

7. Conclusions

The main objective of this Bachelor thesis was to provide the chemical analysis of volatile compounds in oregano essential oil extracted from dried leaves of oregano available on the Czech market, and one commercially produced oregano essential oil. In the experimental part of this thesis, in total, 32 volatile compounds were identified. The content (in percentage) of volatile compounds presented in essential oils, obtained from 4 samples of oregano available on the Czech Market, was measured and identified by GC/MS.

The following main features of *Origanum vulgare* were described in the literature review. It is botanical overview, cultivation, propagation, post-harvest handling, utilization, and medicinal benefits. The next section of literature review focused on the most represented bioactive compounds; carvacrol, thymol, linalool, p-cymene, and γ-terpinene. This fulfilled the first specific objective of the thesis.

The second specific objective was the preparation and chemical analysis of three extracts of dried oregano samples, and one sample of commercial oregano essential oil, and comparison between each other and with literature data. An introduction to gas chromatography, mass spectrometry, and extraction method, which was used in this Bachelor thesis was presented in the last part of the literature review. In the experimental (methods and materials) part, the process of preparation, and chemical analysis of the samples were described. Finally, the results were presented in results part and evaluated and compared with the other literature data in discussion.

GC/MS analysis showed substantial differences in chemical composition among the 4 samples of oregano essential oil. The highest amount of carvacrol as the major compound with medicinal benefits was represented in sample 2 (producer: Kotányi). In terms of comparison with literature data, the results of GC/MS analysis confirmed the presence of carvacrol, thymol, linalool, p-cymene, and γ-terpinene as major constituents.

8. References

Adams RP. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition. Allured Publishing Corporation, Carol Stream, IL.

Babili, F., J. Bouajila, J. Souchard, C. Bertrand, F. Bellvert, I. Fouraste, C. Moulis, and A. Valentin. 2011. Oregano: Chemical Analysis and Evaluation of Its Antimalarial, Antioxidant, and Cytotoxic Activities. Journal of Food Science **76**:C512-C518.

Begnini, K., F. Nedel, R. Lund, P. Carvalho, M. Rodrigues, F. Beira, and F. Del-Pino. 2014. Composition and Antiproliferative Effect of Essential Oil of Origanum vulgare Against Tumor Cell Lines. Journal of Medicinal Food **17**:1129-1133.

Can Baser, K. 2008. Biological and Pharmacological Activities of Carvacrol and Carvacrol Bearing Essential Oils. Current Pharmaceutical Design **14**:3106-3119.

ChemicalBook. 2022. Carvacrol | 499-75-2. Available from https://www.chemicalbook.com/ChemicalProductProperty_EN_CB6134604.htm (accessed February 28, 2022).

Coskun O. 2016. Separation techniques: chromatography. Northern clinics of Istanbul **3**(2): 156.

Dantas, B. et al. 2015. Participation of the TRP channel in the cardiovascular effects induced by carvacrol in normotensive rat. Vascular Pharmacology **67-69**:48-58.

Handa, S., S. Khanuja, G. Longo, and D. Rakesh. 2008. Extraction technologies for medicinal and aromatic plants. Pages 22, 40-44, 1st edition. Earth, Environmental and Marine Sciences and Technologies, Trieste (Italy).

Han, X., and T. Parker. 2017. Anti-inflammatory, tissue remodeling, immunomodulatory, and anticancer activities of oregano (*Origanum vulgare*) essential oil in a human skin disease model. Biochimie Open **4**:73-77.

Hernández, H., A. Fraňková, T. Sýkora, P. Klouček, L. Kouřimská, I. Kučerová, and J. Banout. 2016. The effect of oregano essential oil on microbial load and sensory attributes of dried meat. Journal of the Science of Food and Agriculture **97**:82-87.

BBC Gardeners' World Magazine. 2020. How to grow oregano. Gardener' s World.com. Available from https://www.gardenersworld.com/how-to/grow-plants/how-to-grow-oregano/ (accessed February 14, 2022).

ChemSpider. 2022. ChemSpider. Available from http://www.chemspider.com/Chemical-Structure.html (accessed April 8, 2022).

Kintzios, S. 2012. Oregano. Handbook of Herbs and Spices **2**:417-436.

Letizia, C., J. Cocchiara, J. Lalko, and A. Api. 2003. Fragrance material review on linalool. Food and Chemical Toxicology **41**:943-964.

Leyva-López, N., E. Gutiérrez-Grijalva, G. Vazquez-Olivo, and J. Heredia. 2017. Essential Oils of Oregano: Biological Activity beyond Their Antimicrobial Properties. Molecules **22**:989.

Li, Y., A. Fabiano-Tixier, and F. Chemat. 2014. Essential Oils: From Conventional to Green Extraction. SpringerBriefs in Molecular Science:9-20. Available from https://link.springer.com/chapter/10.1007/978-3-319-08449-7_2.

Skincare Lab. 2022. Linalool in Skin Care - Is It Safe for Skin? Skincarelab.org. Available from https://www.skincarelab.org/linalool-in-skin-care/ (accessed February 28, 2022).

MacNair, H., and J. Miller. 1998. Basic gas chromatography. Pages 14-15, 153-157, 1st edition. Wiley, New York.

Marchese, A., I. Orhan, M. Daglia, R. Barbieri, A. Di Lorenzo, S. Nabavi, O. Gortzi, M. Izadi, and S. Nabavi. 2016. Antibacterial and antifungal activities of thymol: A brief review of the literature. Food Chemistry **210**:402-414.

Martucci, J., L. Gende, L. Neira, and R. Ruseckaite. 2015. Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. Industrial Crops and Products **71**:205-213.

Natur. 2022. Phytos Oregano esenciální olej 10 ml - Natur. Available from https://www.natur-pro.cz/aromaterapie/phytos-oregano-esencialni-olej-10-ml/ (accessed April 8, 2022).

Netopilova, M., M. Houdkova, K. Urbanova, J. Rondevaldova, and L. Kokoska. 2021. Validation of Qualitative Broth Volatilization Checkerboard Method for Testing of

Essential Oils: Dual-Column GC–FID/MS Analysis and In Vitro Combinatory Antimicrobial Effect of Origanum vulgare and Thymus vulgaris against Staphylococcus aureus in Liquid and Vapor Phases. Plants **10**:393.

Pacao, E. 2020. Oregano. StuDocu.com. Available from https://www.studocu.com/id/document/universitas-siliwangi/kesehatan-masyarakat/oregano-plants/8795152 (accessed February 17, 2022).

Peana, A., P. D'Aquila, F. Panin, G. Serra, P. Pippia, and M. Moretti. 2002. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine **9**:721-726.

Pezzani, R., S. Vitalini, and M. Iriti. 2017. Bioactivities of Origanum vulgare L.: an update. Phytochemistry Reviews **16**:1253-1268.

Peter, K. 2001. Handbook of herbs and spices. Chapter 14. CRC; Woodhead Publishing, Boca Raton, etc.; Cambridge, England.

Pherobase: Database of Pheromones and Semiochemicals. 2022. Pherobase: Semiochemicals - Index. Available from https://www.pherobase.com/database/compound/compounds-index.php (accessed April 8, 2022).

Poole, C. 2010. The essence of chromatography, 1st edition. Elsevier, Amsterdam.

PubChem. 2022. gamma-Terpinene. National Library of Medicine. Available from https://pubchem.ncbi.nlm.nih.gov/compound/gamma-Terpinene (accessed March 7, 2022).

PubChem. 2022. p-Cymene. National Library of Medicine. Available from https://pubchem.ncbi.nlm.nih.gov/compound/p-Cymene (accessed March 29, 2022).

Sampaio, L., L. Pina, M. Serafini, D. Tavares, and A. Guimarães. 2021. Antitumor Effects of Carvacrol and Thymol: A Systematic Review. Frontiers in Pharmacology **12**: 702487

Skoufogianni, E., A. Solomou, and N. Danalatos. 2019. Ecology, Cultivation and Utilization of the Aromatic Greek Oregano (Origanum vulgare L.): A Review. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **47(3)**: 545-552.

Turner, D. 2022. Gas Chromatography – How a Gas Chromatography Machine Works, How to Read a Chromatograph and GCxGC. Available from https://www.technologynetworks.com/analysis/articles/gas-chromatography-how-a-gas-chromatography-machine-works-how-to-read-a-chromatograph-and-gcxgc-335168 (accessed April 8, 2022).

USDA Natural Resources Conservation Service. 2022. Origanum vulgare L. Available from https://plants.sc.egov.usda.gov/home/plantProfile?symbol=ORVU (accessed April 8, 2022).

WHO. 2021. World Health Organization. Cardiovascular diseases (CVDs). Available from https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed March 4, 2022)