CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Chemical analysis of essential oils from lemon balm (*Melissa officinalis*)

BACHELOR'S THESIS

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Author: Kseniia Zaikova

Supervisor: Ing. Klára Urbanová, Ph.D.

Declaration

I hereby declare that I have done this thesis entitled “Chemical analysis of essential oils from lemon balm (*Melissa officinalis*)” 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.....

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Kseniia Zaikova

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Abstract

The aim of this bachelor thesis was to conduct the chemical analysis of volatile compounds contained in the essential oil of *Melissa Officinalis*. In the literature review part, these methods were described as well as the main information about the plant, it's medicinal benefits and most commonly represented constituents of the plant - citral (and its isomers), caryophyllene and citronellal. In the experimental part of my work, essential oils were obtained from 3 types of lemon balm tea available on the Czech market, extracted using hydro-distillation method and analysed by gas chromatography with mass spectrometry (GC/MS).

Compounds were identified, and results were compared between each other and with literature data. Results had revealed differences in chemical composition among the 3 samples and confirmed the presence of citral, citronellal and caryophyllene and caryophyllene oxide as main constituents. Substantial differences were found in chemical composition of analysed samples in comparison with literature data.

Key words: Lemon balm, GC-MS analysis, essential oil, *Melissa officinalis*, extraction

Contents

List of tables

List of pictures

List of figures

List of the abbreviations used in the thesis

GC-MS – Gas chromatography-mass spectrometry

IUPAC – International Union of Pure and Applied Chemistry

SOD – Superoxide Dismutase

GSH – Glutathione

Rf – Retention Factor

RI – Retention Index

RT – Retention time

MSD - Mass spectrometry detector

KI - Kovats retention index

1. Introduction

Melissa officinalis is medicinal plant of Laminaceae family, which is also known as Lemon Balm, Bee balm, Honey Balm and is indigenous to Southern Europe, the Mediterranean region, Western Asia, and North Africa (Pereira et al. 2009) and is currently being cultivated worldwide.

As of today, it's been used in traditional medicine to treat various illnesses ranging from headache, migraine, to insomnia, anxiety, vertigo and syncope, anaemia, asthma, bronchitis, amenorrhea, heart failure, digestion problems and nausea, arrhythmias, epilepsy, and rheumatisms (Carocho et al. 2015). In addition, the plant is used in a food industry due to its the antimicrobial and antioxidant properties (Moradkhani et al. 2010), and widely consumed as a flavouring used in sauces, in the form of herbal tea, cosmetics and essential oil obtained from Lemon Balm is popular in aromatherapy.

This plant contains volatile compounds, triterpenoids, phenolic acids and flavonoids (Shakeri et al. 2016). Studies demonstrate that compounds from *Melissa Officinalis* exhibit anti-stress (Scholey et al. 2014), anti-viral (Kucera et al. 1965), antioxidant (Dastmalchi et al. 2008), antispasmodic, antimicrobial, antitumoral (De Sousa et al. 2004), antidepressant, anti-inflammatory and spasmolytic properties (Dastmalchi et al. 2008).

The plant contains low amounts of essential oils that include geraniol, citronellal, geranial and neral as citrus aroma compounds, mostly caryophyllene and caryophyllene oxide in varying proportions (Chizzola et al. 2018).

This Bachelor Thesis focuses on analysis of chemical compounds, obtained from lemon balm available on the Czech Market, literary research of health benefits and possibilities of preparation of the extract.

Hydro-distillation is an extraction method, which was used to obtain essential oil from the samples. In this method the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapours in a condenser (Rassem et al. 2016). For analysis of essential oils from lemon balm extracts and following identification of active compounds gas

Chromatography with mass spectrometry were used. Gas chromatography-mass spectrometry (GC-MS) is an analytical method allows the identification of volatile organic compounds in the mixture. chromatography. After identification of compounds, possible differences in a chemical composition of essential oils were compared between samples and with data from scientific articles.

2. Literature review

2.1. Lemon Balm

2.1.1. Botanical overview

Official scientific name of Lemon Balm is *Melissa officinalis*, it is a perennial herbaceous plant and belongs to the Lamiaceae/Labiatae family and typically grown in herb gardens and border fronts. Taxonomical Classification is presented in the Table 1.

Plant grows 30-100 cm tall and adapts to a wide range of soils including poor ones. Sometimes branching with ascending stems to erect leafy stems. The stems on their sides are light green, 4-angled and single-furrowed. These stems are spreading pairs of opposite leaves, gradually becoming smaller as they ascend. The leaves are edible and ovate in shape, wrinkled, squarely stemmed and are up to 9 cm long and 5 cm across. Its flowers are white and yellow, individual flowers are 8-13mm, in loose, small bunches, emerging from the axils of the leaves that appear in late spring to mid-summer. The root system is rhizomatous and fibrous.

Table 1: Taxonomic classification of Lemon Balm

Rank	Scientific Name and Common Name
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	*Melissa* L. – balm
Species	*Melissa officinalis* L. – common balm

Source: USDA Natural resources Conservation Service

2.1.2. Cultivation and propagation

The plant should be cultivated in temperate and subtropical region with full to partial sun, moist to mesic conditions with fertile soil and can survive moderate frost (Singh & Prawal 2015). The seeds will germinate best if they are not covered and are planted in well-drained soil with a pH between 4.5 - 7.8 (Bailey 1949). This plant is easy to cultivate once it becomes established and can be propagated from soft wood cutting and seed.

Early in the autumn, the stolons should be planted to establish the plants before the first frost. In the spring or early autumn, seed should also be sown. Lemon balm seeds are very small and therefore should be sown shallowly, i.e. covered only with a fine layer of soil. The planting density should be 45 000 to 100 000 plants/ha from seed, seedlings, or rooted stolons. Transplanting of cuttings in the field with the spacing of 20-30 cm apart in the row, and 50-60 cm between the plants (Singh & Prawal 2015). In the study conducted by Saglam et al. (2004), it was observed that the 40 cm distance between plant to plant and 20 cm distance between line to line are best for plantation.

Yield and oil content may be increased several times. It may be achieved with the application of nitrogen during the growing season, usually applied after harvest to promote new growth of the shoot. Annually a fertiliser, which contains sulphur, potassium, nitrogen and phosphorus should be applied as well as a regular water supply, because as the water requirement for this plant is high.

This herb is very slow to germinate, but with seeds, cuttings or root divisions it can be propagated. The only maintenance, which is needed, is the protection from weeds and cuttings down after it flowers. Best harvesting time just before the flowers open when the volatile oil concentration is at its peak. If the plant is cultivated for essential oil production, it’s advised to cut plants to the ground in spring as the first 5-25% of flowers open (The Herb Society of America 2007).

2.1.3. Postharvest handling

Several studies have shown that drying of the essential oils has considerable influence on the effect of drying temperature, drying time and a different species. Lemon balm is cut and then dried in the shade to preserve the chemical composition of the plant. High amounts of direct sunlight will cause volatile oils to disappear (DAFF 2012). Study conducted by Ghasemi et al. (2013) evaluated that the maximum essential oil content (0.43%) obtained in 48 hrs oven-drying while minimum content (0.03%) obtained from drying under microwave with the power of 500 W. Dried leaves should be dried in a dark place with good air circulation (The Herb Society of America 2007).

2.1.4. Utilisation

The essential oil content in lemon balm ranged from 0.02% to 0.30%, which is quite low compared with other members of the Lamiaceae family (Singh & Prawal 2015), therefore the production cost and price of the essential oil is very high on the market. (Brickell & Zuk 1997)

The herb is used in skin and body care. For example, in a study conducted by Teimouri and Teymouri (2018), it was observed that hydro-alcoholic extract of *Melissa officinalis* that after the application of the water extract of *Melissa officinalis*, 75% of participants improved the skin pore, 65% of participants improved neurological problems and acne infections and 92% recovery of hypersecretion of Sebaceous glands.

Leaves of lemon balm add a lemon-scent flavour to many oils, vinegars, dishes, and herbal liqueurs as well used in to make a tea (fresh or dried leaves). The fresh leaves and flowers are used in salads, fish, meat and bean dishes. Oil is used in perfume, leaves and flowers are also utilized in wine-making (Singh & Prawal 2015).

2.1.5. Medicinal Benefits of Lemon Balm

Melissa officinalis is widely used for therapeutic and non-therapeutic functions that trigger its vital worth. Various mixtures and various medicinal properties of its extract, oil, and leaves have been used in traditional medicine from ancient times.

2.1.5.1. Antimicrobial activity

Citrals (geranial and neral) and citronellal demonstrated antibacterial and antifungal activities of the essential oil obtained from *Melisa officinalis*. In vitro, *Melisa officinalis* essential oil exerted notable antimicrobial effects on gram-negative pathogenic bacteria (Pseudomonas aeruginosa, Salmonella enteritidis, salmonella, E. coli.) The highest activity of the essential oil was observed on E. coli and the multiresistant strain of enterics sonei (Mimica-Dukic et al. 2004).

Essential oil from *Melissa Officinalis* demonstrated antibacterial activity against food-borne pathogens and spoilage bacteria (Hussain et al. 2011).

According to Abdellatif et al. (2014) Essential oil obtained from the leaves of Algerian *Melissa Officinalis* presented high antimicrobial activity against all microorganisms targeted mainly against five human pathogenic bacteria, one yeast Candida albicans and two phytopathogenic fungi tested.

2.1.5.2. Antioxidant activity

Several in vitro and in vivo studies have shown antioxidant activity for essential oil and extracts of *Melisa officinalis* and it is important to note that there is a positive correlation between antioxidant activity potential and amount of phenolic compounds of the extracts (Esmaelli & Rohani 2012). In 2011, clinical trial was conducted to assess the capacity of *Melissa officinalis* infusion in improving oxidative stress in radiology staff. The outcomes demonstrated a significant improvement in plasma levels of SOD, catalase,

and GSH peroxidase as well as reduction of plasma DNA damage, lipid peroxidation and myeloperoxidase activity (Zeraatpishe et al. 2011).

Extract of lemon balm has the ability to scavenge natural free and synthetic radicals. This is important as it indicates that the extract may have the potential to prevent oxidative damage in vivo by preventing free radical-mediated oxidative stress (Dastmalchi et al. 2008).

Melissa officinalis and its phenolic compounds exert antioxidant activity through free-radical scavenging, inhibition of lipid peroxidation, and increasing endogenous antioxidant enzymes. Therefore, the therapeutic effects of the plant might be attributed to its antioxidant activity for the prevention and treatment of oxidative stress-related diseases such as neurodegenerative and cardiovascular illnesses (Bayat et al. 2012).

2.1.5.3. Anti-inflammatory activities

With reference to traditional records, *Melissa officinalis* has been used to treat several inflammatory diseases including joint inflammation and asthma. In the study conducted by Birdane et al. (2007), pre-treatment with aqueous extract of *Melisa officinalis* significantly decreased the nociceptive response in mice and reduced inflammagen-induced paw edema in rats. Anti-inflammatory mechanism of essential oil seems to be related to the presence of citral compound (Bounihi et al. 2013).

2.1.5.4. Antidepressant and antianxiety

In a study by Ibarra et al. (2010) a 15-day treatment was conducted with *Melissa officinalis* extract in male mice reduced anxiety-like and relieve stress-related response during a test. Chronic administration of *Melissa officinalis* was shown to relieve stress-related effects (Cases et al. 2011).

According to the results from Kennedy et al. (2004) the ingestion of single doses of methanol extract or dried leaves has been reported to modulate mood, increase self-ratings of calmness and reduce laboratory-induced stress in healthy volunteers.

2.1.5.5. Antitumoral

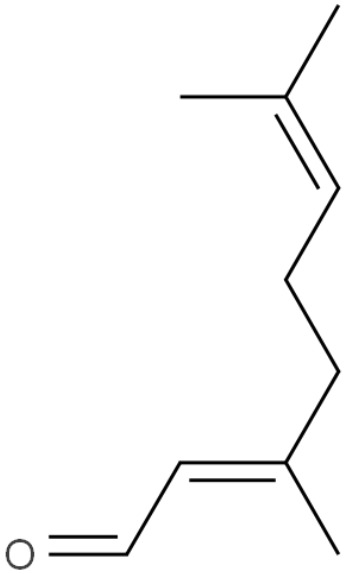
Geraniol, a terpene alcohol found in *Melissa officinalis* essential oil, has been reported to inhibit the proliferation of human colon cancer cells invitro (Carnesecchi et al. 2001) and the growth of pancreatic (Burke et al. 1997), hepatic and skin (Yu et al. 1995) tumours in-vitro and in-vivo.

Research conducted by De Sousa et al. (2004), evaluated the effect of *Melissa Officinalis* essential oil on the viability of five human cancer cell lines and a mouse melanoma cell line and the results have demonstrated that it exhibits antioxidant and tumoricidal activity, which indicates its potential use for cancer prevention and/or treatment.

2.2. Bioactive Compounds

2.2.1. Citral

3,7-dimethylocta-2,6-dienal (according to IUPAC)



Picture 1: Structure of Citral molecule

Source: https://www.chemspider.com/

Citral is a mixture of two isomeric acyclic monoterpene aldehydes: geranial (trans-citral, citral A) and neural (cis-citral, citral B) (Saddiq & Khayyat 2010). It has a molecular formula $C_{10}H_{16}O$, molecular weight 152.237 g/mol and is a clear yellow colored liquid, which is insoluble in water and has a lemon-like odor (CAMEO Chemicals version 2.7.1. 2019).

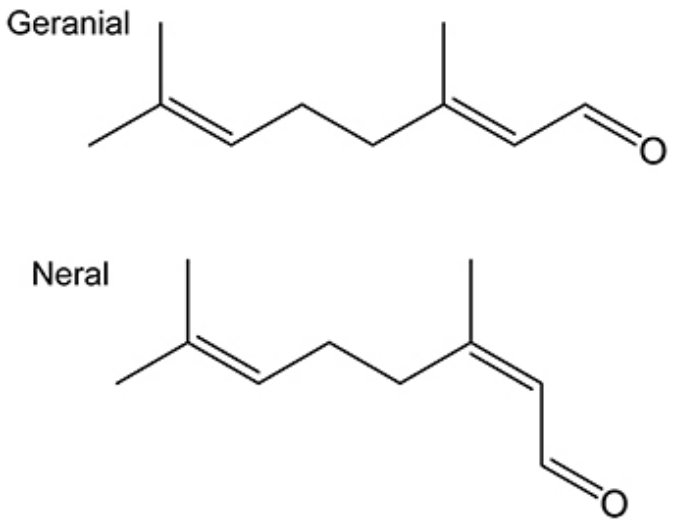
Because of its strong aroma, citral is utilised as a preservative in addition to its other uses in the cosmetic industries, soap and food. The compound showed antimicrobial activity against fungi as well as Gram-positive and Gram-negative bacteria (Onawunmi 1989).

Scientific papers report that citral is used in the production of vitamin A and has a wide spectrum of therapeutic activities, such as anti-inflammatory, anticancer and antimicrobial properties. On the contrary, citral is the monoterpene, which means that it is chemically unstable, and is subjected to oxidation on exposure to air and the high volatility of citral decreases its pharmacological efficacy (Bhalla et al. 2013).

As a natural acyclic monoterpene, citral was found in a wide variety of plants (Grayson 1988) and this compound and its epoxide can act as fungicidal and bactericidal agents (Marcus et al. 2013).

Isomers can only be distinguished based on gas chromatography properties (different Kovats retention index). Geranial's and Neral's chemical structure is presented in the picture 2.

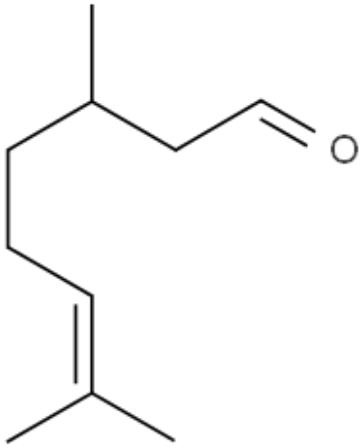
Picture 2: Structure of Geranial and Neral (Isomers of Citral)



Source: Mellström et al. 2016

2.2.2. Citronellal

3,7-dimethyloct-6-enal (according to IUPAC)



Picture 3: Structure of Citronellal molecule

Source: https://www.chemspider.com/

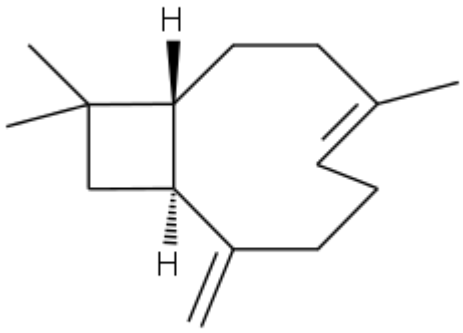
Citronellal has a formula $C_{10}H_{18}O$, molecular weight 154.253 g/mol and is a monoterpene, predominantly formed by the secondary metabolism of plants.

Citronellal is found in herbs and spices, has a strong lemon-citronella-rose odor moderately soluble in water and occurs in many essential oils, particularly from eucalyptus (National Center for Biotechnology Information).

It is found in more than 50 other essential oils and because of its lemon-like aroma is used as a flavor additive in food industry, fragrances and as an ingredient in insect repellents. Citronellal along with citral, geranial, linalool, and citronellol is one of the most important terpenes. It is a versatile reagent (it has a chiral center, an isolated double bond, and an aldehyde function) which is biodegradable, obtainable from renewable sources, and can be used in different processes. Citronellal is an attractive compound in organic synthesis, because it can be easily obtained from natural sources. These synthetic aspects match with several green chemistry principles and make citronellal an excellent tool for the implementation of cleaner and environmentally-friendly processes (Lenardao et al. 2007). Research shows that citronellal has strong antifungal qualities (Nakahara et al. 2013) as well as anti-microbial, antiviral and an anti-cancer (Upadhyay 2010). However, the intensity disappears quickly as it is a very volatile compound.

2.2.3. Caryophyllene (β-caryophyllene)

(1R,4E,9S)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene (according to IUPAC)



Picture 4: Structure of β-caryophyllene molecule

Source: https://www.chemspider.com/

β-caryophyllene (caryophyllene or beta-Caryophyllene) has a formula of C15H24 and a molecular weight of 204.357 g/mol.

Beta-Caryophyllene is a natural bicyclic sesquiterpene and is a constituent of many essential oils (e.g rosemary, *Cannabis sativa*, rosemary, hops), can be found in food and has a sweet and dry taste. This compound is found as a mixture with ring-opened isomer α-humulene (obsolete name: α-caryophyllene), and isocaryophyllene (the cis double bond isomer) (Wishart et al. 2018).

This compound many medicinal and therapeutic benefits. According to Yoo and Jwa (2019), β-caryophyllene exhibits the antimicrobial activity against periodontopathogens and may improve periodontal health. Tchekalarova et al. (2018) stated that β-caryophyllene dose-dependently improved spatial memory performance.

Research conducted on mice shows the potential of beta-caryophyllene as a treatment for anxiety and depression, the effects of compound on mouse subjects related to conditions of stress and anxiety were tested. The authors emphasize that this study, for the first time, demonstrates that beta-caryophyllene is effective at producing antidepressive and anxiolytic effects (Bahi et al. 2014).

2.3. Chromatography

Chromatography is a technique for separating and/or identifying organic and inorganic compounds in a mixture and solvents in order to be analysed and studied, works with low concentrations and tiny samples. The basic principle is that components in a mixture have different tendencies to adsorb onto a surface or dissolve in a solvent (UC Davis LibreTexts Library 2018).

Chromatography includes 3 components: stationary phase (doesnt move, soild or liquid), a mobile phase (moves and composed of a gaseous or liquid component) and separated molecules (Coskun 2016). Firstly, the mobile phase moves through the stationary phase collecting compounds to be tested, then continues to travel through the stationary phase, taking the compounds with it. During different stages in the stationary phase various components of the compound will be absorbed and each liquid inside the sample undergoes absorption withing different time scale in solid or liquid phase. Secondly, while the components of the mobile phase move through the stationary phase they separate. When different components of the compound stop to move and separate from the other components, the results of any chromatography are received.

Retention factor-Rf is a quantitative indication of how far a particular compound travels in a particular solvent. For example, if the unknown compound is close or the same as the Rf value for the known compound is an indicator that the two compounds are similar or identical.

There are different types of chromatography, which may be divided into 2 groups:

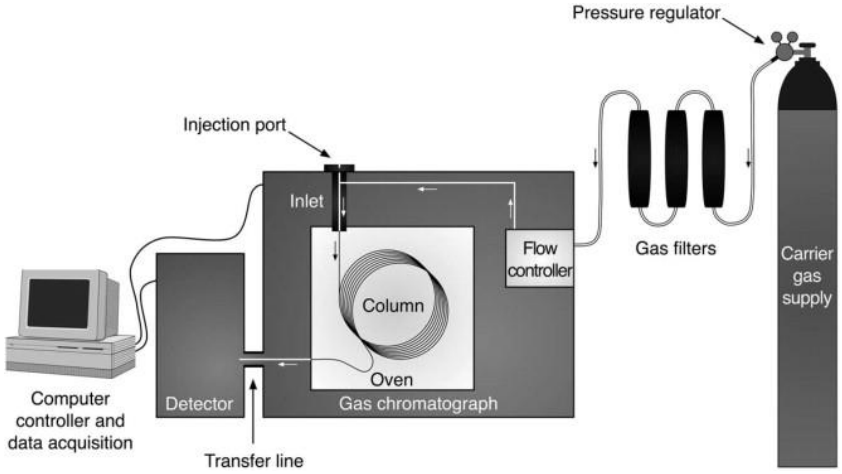
- According to the shape of chromatography (stationary phase)
    1) Paper chromatography (paper serves as a stationary phase and separates and identifies mixtures by colour)
    2) Thin layer chromatography (thin layer of silica gel, alumina or cellulose absorbed on an inert substrate)
    3) Column chromatography (the stationary phase is packed in a column)
- According to the physical state of mobile phase
    1) Gas chromatography (mobile phase is gas-e.g He)
    2) Liquid chromatography (mobile phase is liquid)

2.3.1. Gas chromatography

A typical gas chromatograph consists of an injection port, a column, carrier gas flow control equipment, ovens and heaters for maintaining temperatures of the injection port and the column, an integrator chart recorder and a detector (UC Davis LibreTexts Library 2018).

Gas chromatography (GC) is a technique, which is used to analyse volatile substances in the gas phase by separating complex mixtures based on differences of polarity and vapor pressure/boiling point. First, a tiny sample of the mixture of substances being analysed is placed using a small syringe and injected into the machine via an injection port into the inlet (alternative name: injector), it's done by the autosampler, which introduces a sample automatically into the inlets. Then components of the mixture are heated and instantly vaporize. The vaporized components are then carried by an inert gas (usually helium or nitrogen), which is the mobile phase, passes through the inlet, and sweeps the sample onto the column, where the stationary phase is. Column is a is a thin glass or metal tube usually filled with a liquid that has a high boiling point and is enclosed in a temperature-controlled oven. Separation takes place as the mixture travels through the column. Then separated components of the analysed sample exit the column, enter the detector and move along it (provides an electronic signal equivalent to the number of eluting analytes), identifies components and prints a peak on a final chart, that has a sequence of peaks which correlates with all the substances in the mixture (Stauffer et al. 2008).

Picture 5: Structure of Gas Chromatograph



Source: Fire Debris Analysis 2008 (34)

2.4. Mass Spectrometry

Mass spectrometry is a tool, which is used for identifying molecules in a mixture, analyse a purified protein, study the protein content or detect impurities in a sample of a sample of cells.

A mass spectrometer produces charged particles (ions) from the chemical substances, followed by using an electric and magnetic field to measure the mass (weight) of the charged particles.

Mass spectrometers can be divided into three parts: the ionisation source, the analyser, and the detector, all under workstation control complete with software packages to assist in data acquisition (Naylor & Babcock 2010).

The process is mainly divided in 4 steps: Ionization, Acceleration and deflection, detection.

1. Ionization.

This is a stage, where molecules in a sample are vaporized, followed by an electron beam bombarding the vapours in order to transform the vapours to ions (knocking one or more electrons off to give a positive ion). As mass spectroscopy measures the mass of charged particles, meaning that solely ions are to be detected and neutral molecules are not to be seen.

2. Acceleration

During the acceleration the positive ions accelerate towards negative plates at a speed dependent on their mass (lighter molecules move quicker than heavier ones) in order for all of them to have the same kinetic energy.

3. Deflection

Ions of different mass travel through the spectrometer at different speeds and deflected by a magnetic field according to:

- their charges (number of positive charges on the ion meaning how many electrons were knocked off during the first stage, the more the ion is charged, the more it gets deflected)
- their masses (the lighter the ions, the more they are deflected)

4. Detection

The beam of ions of increasing mass in turn pass through the machine, reach the detector and provide an output in the form of a mass spectrum (Ashcroft 2004).

2.5. Extraction methods

Essential oils are the products extracted from natural plants by physical means using different conventional methods such as dry distillation, distillation, and cold press. However, these extraction techniques may generate the degradation of some unsaturated compounds and the loss of some components by hydrolysis or by thermal effects (Li et al. 2014).

The traditional technologies related to essential oil processing (for example: steam distillation, cohobation, hydro-distillation, solvent extraction, enfleurage and maceration) are of great importance and are still being widely used worldwide. Hydro-distillation is typically used for isolation of essential oils from the medicinal and aromatic plants (Rassem et al. 2016).

There are many different methods of distillation in the production of essential oils, and the choice of the technique depends on the following aspects; water solubility and volatility of the essential oil, sensitivity of the essential oil to the action of water and heat.

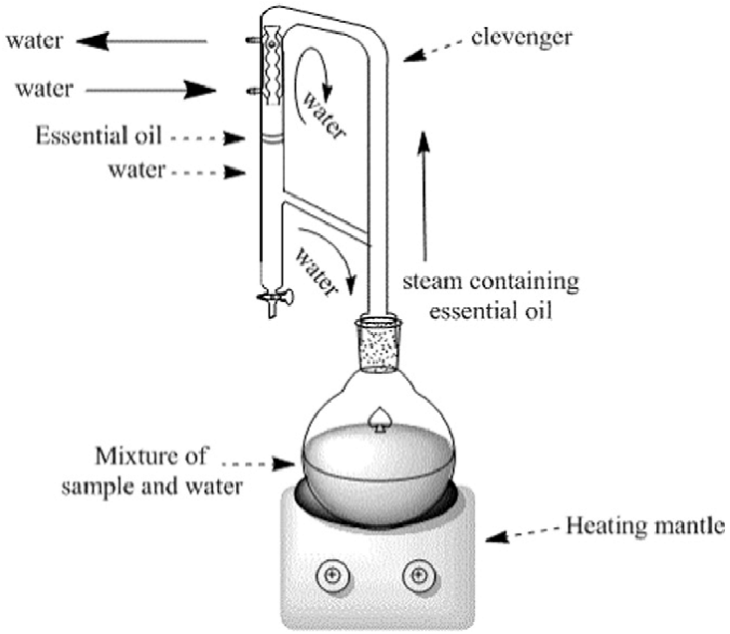
2.5.1. Hydro-distillation

Essential oils are natural products, which contain a large number of volatile molecules. There are several techniques of producing Essential Oils, depending on the physical and constituent properties of the originating source.

The conventional method for the extraction of essential oils is hydro-distillation and it is a form of steam distillation, recommended by the French Pharmacopoeia for the extraction of Essential oils from dried spices and the quality control of Essential oils in the laboratory. Obtained essential oils are different from the original essence due to the long treatment duration (Li et al. 2014).

It is a technique, where the essential oils are evaporated by heating a mixture of water or different solvent with plant materials directly immersed in water (flowers, leaves, stems, twigs, leaves, herbs, spices, wood and roots) until boiling under atmospheric pressure in an alembic, where the heat allows the release of odorous molecules in plant cells. Then these volatile compounds and water form a mixture, evaporate together at the same pressure and then it is condensed and separated in a Florentine flask. The required time of this extraction technique depends on the plant material being processed. The structure is composed of the condenser and a decanter to gather the condensate and to separate essential oils from water due to their immiscibility and density difference (Li et al. 2014; Rassem et al. 2016).

Picture 6: Hydro-distillation extraction



Source: Samadi et al. 2016

2.6. Scientific Articles

Chemical analysis of volatile substances contained in lemon balm oil by GC-MS has been the subject of many studies. Although mere analysis of volatiles in extracts was not the specific aim of the following studies, they were selected for comparison with results of own research done in the framework of my bachelor thesis.

In the paper written by De Sousa et al. (2004), analysis of lemon balm leaves was conducted to evaluate the potential antioxidant and antitumoral activity. Leaves were harvested at 0900 h, dried at 40˚C until complete dehydration had been achieved, and the essential oil was obtained by hydrodistillation in a Clevenger-type apparatus. A Shimadzu QP5050A gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument was used to analyse the oil.

It was observed that harvesting at 0900 h and drying the leaves at 40 ˚C resulted in the best chemical composition (Blank et al 2002). The chemical composition of the essential oil of *Melissa officinalis* is presented in the table 2.

Table 2: Composition of the essential oil of *Melissa officinalis* leaves harvested at 0900 h and oven dried at 40 ˚C.

Table 1 Composition of the essential oil of *Melissa officinalis* leaves harvested at 0900h and oven dried at 40°C.

Retention time (min)	Component	(%)	RRI exp.*	RRI lit.**
4.891	6-Methyl-5-hepten-2-one	1.10	982	985
7.795	Linalool	0.83	1099	1098
9.227	–	0.49	1141	–
9.914	*Cis*-Chrysanthenol	1.81	1161	1162
10.563	Isomenthol	2.91	1180	1182
13.084	Neral	39.28	1241	1240
14.343	Geranial	47.32	1270	1270
16.066	Methyl geranate	0.50	1322	1323
16.622	–	0.70	1336	–
17.576	–	0.44	1356	–
18.105	–	1.00	1372	–
18.508	Geranyl acetate	1.51	1383	1383
20.160	(E)-Caryophyllene	0.94	1424	1418
26.707	Caryophyllene oxide	1.17	1584	1581

*RRI, relative retention index calculated applying Van den Dool & Kratz (1963). **Comparison of the relative retention time with those reported by Adams (1995).

Source: De Sousa et al. 2004

Esmaelli and Rohani (2012) examined in vitro antioxidant activities and essential oil of lemon balm (*Melissa officinalis*) from Iranian plant in Sanandaj (Northwest of Iran). Chemical composition of the essential oil from the aerial parts (200g) of *Melisa Officinalis* obtained by hydro-distillation of 3.5 h, using a Clevenger-type apparatus and followed by GC-MS analysis. GC-MS analysis of the volatile oil of the main components were cedrane (14.1 %) 2,2,8,8-tetramethyl-5-nonaanone (12.6 %), hexadecanal (7.4 %), transsabinene hydrate (7.8 %), E-citral (7.0 %), Z-citral (6.1 %) and hexadecanoic acid (5.7 %). The results of GC-MS analysis of chemical composition of the essential oil are presented in Table 3, with the percentages composition.

Table 3: Results of GC-MS analysis of chemical composition of the essential oil of *Melissa officinalis.*

Table 1. Percentage composition of the essential oil of lemon balm *M. officinalis*

Compound	RI [a]	(%)
4-Hydroxy-4-methyl-2-pentanone	980	2.8
Hepten-2-one	985	0.3
Decane	999	0.5
Linalool	1098	0.2
Citronellal	1153	1.3
3-Octyne	1200	1.0
Z-Citral	1316	6.1
E-Citral	1341	7.0
Decanol acetae	1355	0.6
trans- Sabinene hydrate	1360	7.8
3,7-Dimethyl-2,6-octadien-1-ol	1400	5.7
Decanol acetate	1409	0.6
(*E*)-Caryophyllene	1418	1.2
Aroma dendrene	1440	1.9
Bakerol	1440	1.3
Cedrane	1480	14.1
Caryophyllene	1580	5.0
Hexadecanal	1973	7.4
Eicosane	2000	4.6
Octadecanoic acid	2010	3.3
4-Hydroxy-4-methyl- 2- pentanone	2015	2.8
6-Methyl-5-hepten-2-one	2020	0.3
Geranyl acetate	2025	1.2
2,2,8,8-Tetramethyl-5-nonaanone	2030	12.6

[a] Retention indices as determined on a DB-5 column using the homologous series of n-alkane

Source: Esmaelli & Rohani (2012)

3. Objectives

3.1. Main objective

To analyse volatile compounds extracted from lemon balm teas available on the Czech Market.

3.2. Specific objectives

1. Literature review focused on properties, health and chemical composition of essential oil of lemon balm (*Melissa officinalis*).

2. Preparing and chemical analysis of three extracts of lemon balm samples and comparison between each other and with literature data.

4. Materials and methods

4.1. Samples

The analysis was performed on three samples of lemon balm tea, commonly available in the Czech Republic.

The following lemon balm teas were used for analysis:

Sample 1. Meduňka sypaná bio bylinný čaj 50 g (Producer- Sonnentor**)**

Sample 2 .Čaj Dukát Melisa Medunka čaj tradiční 20 x 2,5g (Producer-Dukat)

Sample 3. Dr.Max Čaj meduňka n.s.20x1.5g (Producer-Dr. Max)

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

Picture 7: Sample n. 1. Picture 8: Sample n. 2. Picture 9: Sample n. 3.

Source (picture 7 and 8): Author of the Thesis.

Source (picture 9): https://www.drmax.cz/max-caj-medunka

4.2. Extraction and GC-MS analysis

All samples were prepared in the same way. 40 g of the dry sample was mixed with 500 ml of distilled water in the flask.

Flask with plant material mixed with distilled water was placed in the heating nest on a Clevenger distillation apparatus. After bringing water to boiling, 4 hours and then the obtained essential oil, which was collected in a vial. Essential oils were dissolved in hexane prior to measurement. Concentration of samples were 1 µl/ml.

Picture 10: Hydro-distillation extraction of essential oil from the sample n.1

Source: Author of the Thesis

Each sample was analysed three times. Overall, there were nine measurements. 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.

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 for splitless mode. 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 MSD 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 s.

Data was obtained through MassHunter Workstation Software Qualitative Analysis Version B.07.00. Mass spectra were found and identification of the volatile compounds was done by comparison of their mass spectra against mass spectra covered by NIST 2.2 library. Confirmation of the accuracy of identification was done by comparison of KI (Kovats retention index). To search for the KI, internet database pherobase.com was used, source of the Kovats Indexes was Adams (2007) results measured on a DB-5 column. Not all substances could be verified by comparison of KI, because some retention indexes were not available.

5. Results

During the GC-MS analysis 71 compounds were together identified in all 3 samples and are presented in the table 4. Sample 1 contained 59 compounds, Sample 2-55 compounds and sample 3-58 compounds. Most represented compounds among tested samples are citronellal, neral, geranial, anethole, methyl citronellate, caryophyllene and caryophyllene oxide.

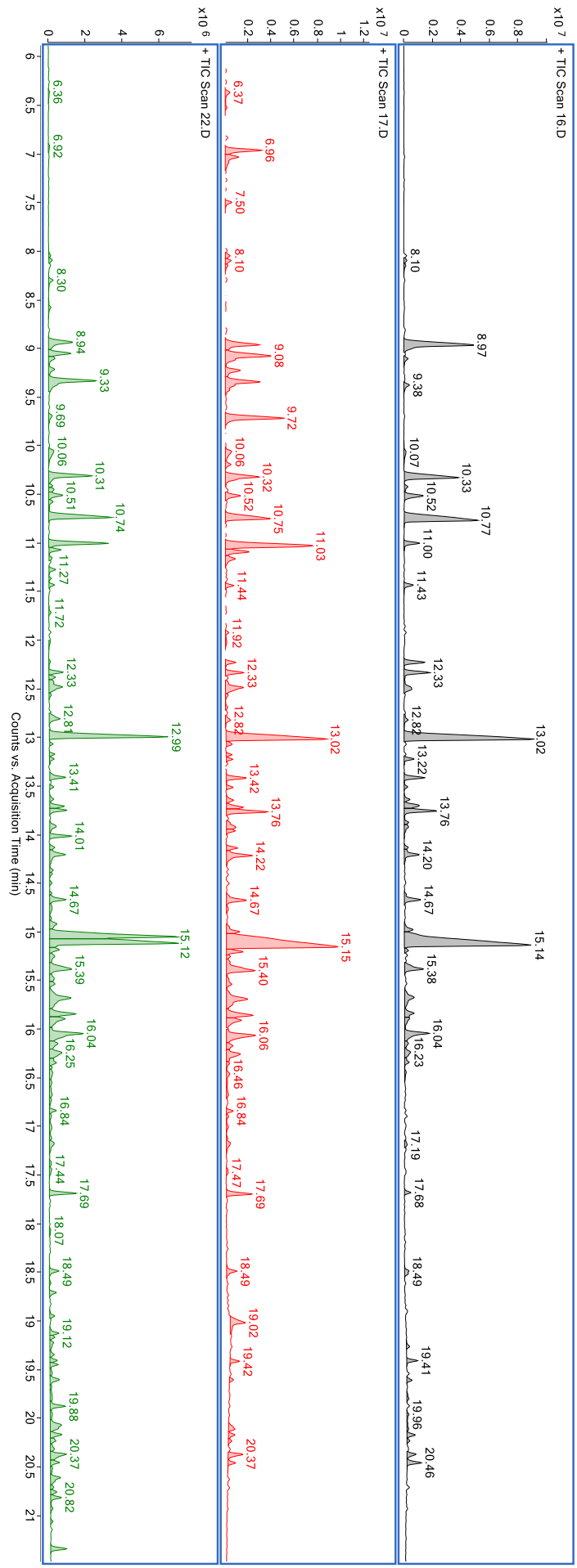
Caryophyllene oxide accounted for the largest proportion 2 samples (22.83%-Sonnentor, 18.68%-Dukat) and 2nd largest- 9.31% in the Dr.Max lemon balm tea. The percentage of Neral (Citral Z) and Geranial (Citral E) - (the most commonly presented compounds in the essential of *Melissa Officinalis)* accounts for 6.07%-sample 1, 2.32%-sample 2, 2.62%-sample 3 for Neral, and 10.40%, 5.00% and 3.93% for Geranial respectively.

Caryophyllene, citronellal, Methyl citronellate and anethole also constituted a significant amount in all 3 samples. As for Citronellal, the largest proportion is found in the sample 1-8.63%, sample 2 contained 3.34% and sample 3-1.91%. Methyl citronellate accounted for the similar amount in all samples- around 2%, sample 1-2.04%, sample 2-2.01%, sample 3- 2.69%. The highest amount of Caryophyllene is found in the sample 1-17.42%, while sample 2-6.63%, and sample 3-3.90%. Anethole is accounts for 1.34%-sample 1, 5.43-sample 2 and 2.96% in a sample 3.

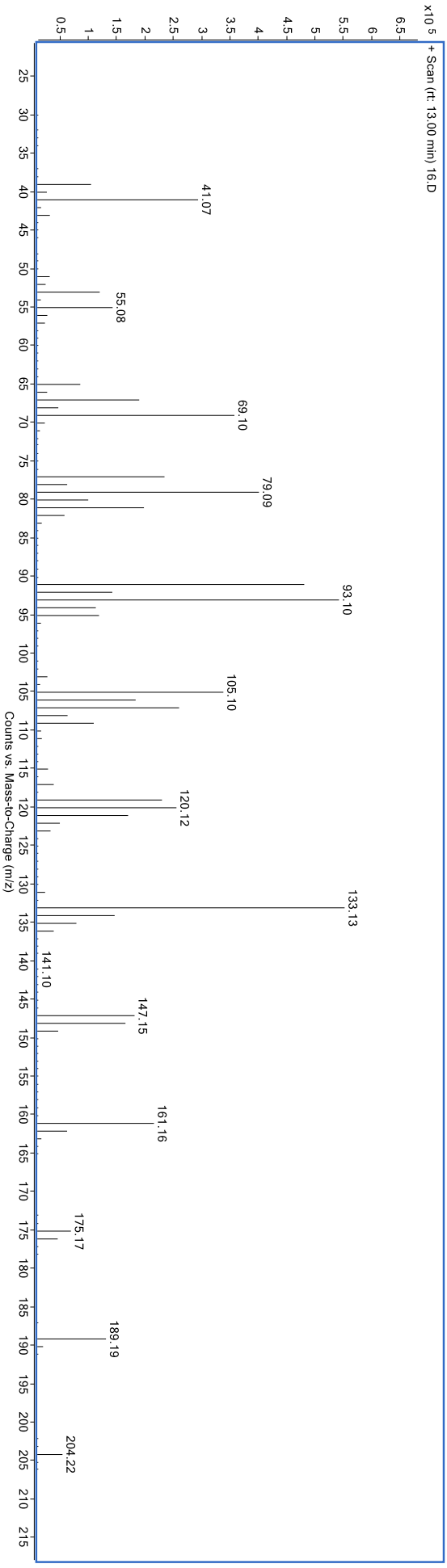
Table 4 shows all identified compounds in 3 samples with Retention Time, KI calculated and found in the literature and the represented sum of the area is shown in percentage (%). Graph 1 represents the chromatograms of all 3 samples studied and Graph 2 shows the mass spectrum of the molecule caryophyllene.

It's important to notice that in the essential oil obtained from the Dr. Max lemon balm tea contained 13.04% of 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (also known as kodaflex txib) and is not presented in other 2 samples. It is a plasticizer commonly used in vinyl flooring, surface coatings, water-based painting and other vinyl-based products. According to Astill et al. (1972) Kodaflex TXIB may be an indirect food additive because of its use in food packaging, TXIB was tested on the guiniea pigs and was slightly irritating to the skin when the skin was exposed uncovered (U.S. Consumer Product Safety Commission 2011), in addition, it may cause eye

irritation, damage to organs through prolonged or repeated exposure and Hazardous to the aquatic environment (European Chemicals Agency, ECHA C&L Inventory 2018).



Graph 1:Chromatograms (from right to left: sample 1, sample 2, sample 3)



Graph 2: Mass Spectrum of Molelucle caryophyllene

Table 4: Identification of bioactive compounds (Chemical composition)
Source: Author of the Thesis

Compound	RT	KI lit	KI calc.	% (average)		
				sample 1	sample 2	sample 3
2-Pentylfuran	6.37	992	994	0.06	0.43	0.17
2-(2-Pentenyl)furan	6.52	1003	1003	0.02	0.18	x
2-Ethylhexanol	6.93	1032	1028	x	x	0.17
p-Cymene	6.96	1026	1030	x	1.66	x
D-Limonene	7.03	1031	1035	0.12	1.73	x
γ-Terpinene	7.5	1062	1064	x	0.81	x
Rosefuran	8.05	1095	1099	0.24	0.38	0.22
Linalool	8.1	1098	1102	0.30	0.38	0.35
cis-Rose oxide	8.3	1111	1115	0.13	1.02	1.18
Citronellal	8.96	1153	1158	8.63	3.34	1.91
Isomenthone	9.06	1164	1164	0.15	3.45	1.47
Isoneral	9.11	1165	1168	0.48	x	2.02
cis-p-Menthan-3-one	9.22	1178	1175	0.03	1.98	0.60
Menthol	9.34	1173	1182	0.14	3.76	3.46
Isogeranial	9.38	1184	1185	0.66	x	x
Estragole	9.7	1195	1206	0.12	3.23	1.57
Citronellol	10.06	1228	1231	0.54	0.66	0.76
Thymol methyl ether	10.19	1235	1240	x	1.25	x
Neral (Z-Citral)	10.32	1240	1250	6.07	2.32	2.62
Carvone	10.42	1242	1257	0.40	x	0.59
Geraniol	10.45	1255	1258	x	x	0.46
Methyl citronellate	10.52	1261	1263	2.04	2.01	2.69
Piperitone	10.58	1282	1267	x	x	1.81
Geranial (E-Citral)	10.75	1270	1280	10.40	5.00	3.93
Anethole	11.01	1301	1297	1.34	5.43	2.96
Isomenthol acetate	11.08	1305	1301	x	1.34	0.75
Carvacrol	11.12	1298	1307	0.10	0.75	0.33
Mesitaldehyde	11.28	1323	1315	0.05	x	0.47
2,4-Decadienal	11.37	1314	1322	0.03	x	0.17
Methyl geranate	11.44	1323	1328	0.81	0.37	0.30
α-Terpinyl acetate	11.87	1354	1359	0.10	x	x
α-Copaene	11.92	1376	1362	0.19	0.19	0.81
Geranyl acetate	12.23	1383	1385	1.70	0.78	x
β-Damascenone	12.38	1393	1396	x	x	0.96
β-Bourbonene	12.49	1384	1405	1.30	1.28	0.84
(E)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene	12.57	1430	1410	0.15	0.19	x
trans-α-Copaene	12.75	1423	1424	0.13	x	x
7,8-Dihydro-3,4-dehydro-β-ionone	12.82	1424	1430	0.56	3.54	3.96
Caryophyllene	13	1428	1445	17.42	6.63	3.90
α-Guaiene	13.07	1439	1450	0.16	0.33	3.55

Table continuation:

Geranylacetone	13.18	1455	1458	0.12	0.28	0.31
cis-β-Farnesene	13.22	1458	1461	0.72	0.58	0.66
Humulene	13.42	1454	1477	1.56	0.88	0.68
γ-Muurolene	13.52	1477	1484	0.27	0.38	0.67
β-Ionone	13.71	1485	1499	1.55	1.54	0.90
Germacrene D	13.76	1480	1503	2.71	1.98	0.83
α-Farnesene	13.88	1508	1513	0.37	x	x
Epicubebol	13.92	1494	1517	0.59	0.76	1.15
β-Himachalene	13.96	1500	1520	x	0.54	x
Butylhydroxytoluene	14.01	1514	1524	x	x	1.02
γ-Cadinene	14.14	1513	1535	0.31	1.15	1.01
δ-Cadinene	14.21	1524	1540	1.62	1.88	1.19
Caryophyllenyl alcohol	14.92	1575	1599	x	x	5.04
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	15.05	1587	1610	x	x	13.04
Caryophyllene oxide	15.14	1606	1616	22.83	18.68	9.31
Isoaromadendrene epoxide	15.25	1579	1573	2.90	0.24	0.59
Calarene epoxide	15.3		1635	0.16	x	1.31
Humulene oxide	15.4		1640	2.22	2.73	1.89
Cubenol	15.54	1642	1653	0.28	0.56	1.48
epi-Cadinol	15.68		1665	1.94	2.72	3.01
tau-Muurolol	15.84	1645	1668	1.16	2.13	2.19
Aromadendrene oxide II	15.9	1678	1684	0.79	1.39	2.52
7-Isopropyl-4,10-dimethylenecyclodec-5-enol	16.25	1694	1715	1.00	1.46	1.31
Eremophilone	16.84	1756	1769	0.13	0.76	0.34
Murolan-3,9(11)-diene-10-peroxy	17.17		1800	0.37	0.55	x
14-Hydroxy-δ-cadinene	17.47	1811	1830	0.23	0.20	0.77
Phytone	17.69	1849	1849	0.47	1.19	0.73
Methyl palmitate	18.5	1927	1927	0.63	0.64	0.43
Palmitic acid	19.1	1984	1990	0.26	1.03	0.68
Retinol	20		2100	0.21	0.72	1.01
Phytol	20.37	2122	2122	0.03	0.58	0.94

6. Discussion

Presence of neral (Z-citral) and geranial (E-citral) was stated as the major constituents of *Melissa Officinalis* (De Sousa et al. 2004; Esmaelli & Rohani 2012) and was confirmed during the GC-MS analysis of 3 essential oils obtained from lemon balm tea in this work. To compare, the essential oil from oil of lemon from Iranian plant in Sanandaj contained 6.1% Z-citral and 7.0% E-citral (Esmaelli & Rohani 2012) and is close to the content of 3 samples used in this work: (neral sample n.1 - 6.07%, sample n.2 - 2.32%, sample n.3 - 2.62%; geranial is presented in higher amount, than neral: sample n. 1 - 10.40%, sample n.2 - 5.00%, sample n.3 - 3.93%).

On the contrary, in the research done by De Sousa et al. (2004) lemon balm leaves dried at 40 ˚C consisted of 39.28% (neral) and 47.32% (geranial), Esmaelli and Rohani (2012) concluded that Cedrane accounted for 14.1% and is the mostly represented among all compounds in a studied sample, but it was not detected in the samples used in this work. It is also important to note that while caryophyllene (sample n.1 - 17.42%, sample n.2 - 6.6%3, sample n.3 - 3.90%) and caryophyllene oxide (22.83%, 18.68% and 9.31%, respectively) accounted for a big proportion in all 3 samples studied in this bachelor thesis, it comprised only a small amount in both studies (De Sousa et al. 2004; Esmaelli & Rohani 2012).

Citronellal has also confirmed to be a major compound of *Melissa Officinalis* as it was presented in all 3 samples and in studies by (De Sousa et al. 2004; Esmaelli & Rohani 2012; Chizzola et al. 2018).

There are considerable differences between the results of this study and literature data found and many potential factors, which could have influenced this. For example, cultivation, processing, storage, sample size, and geographic origin of the sample.

7. Conclusions

The main objective of this Bachelor Thesis was to conduct the chemical analysis of volatile compounds extracted from lemon balm teas available on the Czech Market. In the literature review *Melissa officinalis* was described, it's cultivation, propagation, utilisation and medicinal benefits as well as most commonly represented constituents of the plant - Citral (and its isomers), caryophyllene and citronellal. In the experimental part of the thesis, the content (in percentage) of volatile compounds presented in essential oils, obtained from 3 samples of lemon balm tea available on the Czech Market, was identified and measured by GC-MS. In total, 71 compounds were identified. Thus, the main objective and first specific objective of the work was met.

Second specific objective of the thesis was to compare the results of GC-MS analysis between samples and with literature data. GC-MS analysis showed substantial differences in chemical composition among the 3 samples of essential oil. According to the results, the sample 1-Sonnentor contained the highest number of compounds with medicinal benefits among 3 samples, while the sample 3-Dr. Max, contained "Kodaflex TXIB", the plasticizer used in a food packaging and may cause damage to health if consumed in a big amount. As for the comparison with the literature data, GC-MS analysis results has confirmed the presence of citral, citronellal and caryophyllene and caryophyllene oxide as main constituents. Anethole, humulene oxide, ethyl citronellate and caryophyllene were also represented in different amounts, and it corresponds with the findings from the literature review. This confirms that all main and specific objectives of the Bachelor Thesis were met.

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