universität wien

D I P L O M A R B E I T

„Effects of harvesting and post harvest treatments on yield and quality of essential oils of *Melissa officinalis* and *Solidago puberula*."

zur Erlangung des akademischen Grades
Magistra Pharmaciae (Mag[a].pharm.)
an der Fakultät für Lebenswissenschaften
der Universität Wien

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Studienrichtung:	Pharmazie (A449)
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Wien, im Oktober 2008

Die vorliegende Arbeit wurde in der Zeit von Juni 2007 bis September 2008 unter der Leitung von Univ.-Prof. Mag. Dr. Gerhard Buchbauer und Herrn André Bélanger, Ph.D, Chemistry, am „*Centre de récherche et de développement en horticulture (CRDH)* in Saint-Jean-sur-Richelieu, Québec, angefertigt.

Danksagungen

Die Möglichkeit, neue Methoden zur Extraktion ätherischer Öle und verschiedene Behandlungsmethoden und die anschließende Bewertung des Inhaltsstoffmusters kennen zu lernen, stellte für mich eine neuartige Vorgangsweise im Bereich der analytischen Chemie dar. Die Erstellung der Diplomarbeit entwickelte sich zu einer interessanten Aufgabe.

Das Thema meiner Diplomarbeit erforderte einen 4monatigen Aufenthalt in Saint Jean sur Richelieu, Quebec, Canada. Von Herrn André Bélanger, Ph.D, Chemistry, wurde sowohl das Thema als auch das nötige Grundlagenwissen vermittelt. Auch für die Organisation des Studienaufenthaltes, die Bereitstellung eines Arbeitsplatzes und sein förderndes Interesse an dieser Arbeit möchte ich mich herzlich bedanken.

Ganz herzlich Danke ich dem CRDH für die Bereitstellung der Laborutensilien und die freundliche Aufnahme in Saint Jean.

Herrn Univ.-Prof. Mag. Dr. Gerhard Buchbauer danke ich sehr herzlich für die Durchsicht der Diplomarbeit, die intensive Betreuung in Wien und für die manchmal nicht ganz einfache Formulierung ins Englische.

Frau France Boudreau, technische Mitarbeiterin von Dr. André Bélanger, CRDH, Saint Jean sur Richelieu, Québec, danke ich für die Unterstützung bei den Laborarbeiten und die Mithilfe in der Durchführung der GC-FID-Messungen. Weiters bedanke ich mich für Ihre Geduld bei der Beantwortung der Unmengen von Fragen meinerseits.

Herrn Francis Mainguy danke ich für die Mithilfe bei der Ernte der Pflanzen und für wertvolle Anregungen in zahlreichen Gesprächen.

Vielen Dank auch an meine Kollegen/Innen an der Universität Wien und meinen Freunden für die moralische Unterstützung, vor allem in Zeiten, in denen wenig weiterging.

Mein besonderer Dank gilt auch meinen Eltern und meinen Schwestern für ihr stetiges Interesse und ihre Unterstützung während meiner gesamten Studienzeit.

Acknowledgements

The possibility of becoming acquainted with new methods for the extraction of essential oils and different working methods and the following evaluation of the components represented for me a new procedure within the range of analytic chemistry. The carrying out of the thesis developed to an interesting task.

The study required an educational stay of four month in Saint Jean sur Richelieu, Quebec, Canada. The topic was arranged by Mr. André Bélanger, Ph.D., chemistry and I thank him for imparting the necessary basic knowledge. Also for the organization of the study stay, the allocation of a working place and his promoting interest in this work I would like to thank him cordially.

For the supply of the laboratory equipment and the friendly acceptance at the CRDH I would like to thank.

I thank Mr. Univ.-Prof. Mag. Dr. Gerhard Buchbauer very cordially for the examination of the thesis, the intensive support in Vienna and for his tips how to verbalize difficult facts in English language.

For the support with the laboratory work and the assistance in the execution of the GC/FID measurements I appreciate the help of Mrs. France Boudreau, technical employee of Dr. André Bélanger, CRDH, Saint Jean sur Richelieu, Quebec. Further, I thank her for her patience to answer the enormous amount of my questions.

I thank Mr. Francis Mainguy for the assistance with the harvest and for valuable suggestions in numerous discussions.

Thanks also to my colleagues at the University Vienna and my friends for the moral support, particularly in times, in which success was poor.

My special thanks also apply to my parents and my sisters for their constant interest and their support during my entire study time.

CHAPTER 1:

EVALUATION OF THE ESSENTIAL OILS OF DIFFERENT VARIETIES OF MELISSA OFFICINALIS

CHAPTER 2:

EVALUATION OF THE ESSENTIAL OIL OF SOLIDAGO PUBERULA AT DIFFERENT HARVESTING DATES AND DIFFERENT DRYING STAGES.

Chapter 1

Evaluation of the essential oils of different varieties of *Melissa officinalis*

1. ABSTRACT

The general objective of the study was the evaluation of essential oils from different varieties of *Melissa officinalis* (Lorelei, Quedlinburger, Erfurter Aufrechte, Aufrechte, Stamm NLC, Lemona, and Citronella) and to examine the influences of harvesting time and drying stage of the plant material according to yield and quality of the essential oil. In addition investigations on corresponding hydrosols were carried out.

Samples from the seven varieties were harvested in different stages of growing and were submitted to different extraction methods. The plants were harvested either in their first year of growing or had reached their second year (Citronella and Quedlinburger). The harvest of the plants took place in the time between June and September 2007.

To get some comparable results samples grown in the greenhouse were also analyzed. The extraction of oil was done by the use of different methods, like DW, DS or WSD.

Analysis was done by gas chromatography (GC/FID). Results from the GC were compared and supplemented using the Kovats index database.

The analysis of seven different varieties of *Melissa* showed that the most powerful detected components were monoterpene aldehydes (citronellal, geranial and neral). Further important components were (E)-β-ocimene, (Z)-β-ocimene, borneol, α-terpineol and from group of sesquiterpenes β-caryophyllene and caryophyllene oxide could be found. Within the seven varieties higher changes of yield and composition of components could be observed.

Comparing the different extraction methods higher yields and a higher number of components could be observed using WSD. The oil obtained with this method showed a good quality, because the number of undesired secondary products is infinitely small.

In addition investigations on plants at different growing stages were carried out. It was found that different harvesting dates significantly affected the yield of

components. Especially fluctuation of main components (neral, geranial and citronellal) was higher than for all other components.

Within the study, analysis from different drying stages of the plant material was applied. It was found that changes in plant moisture did affect the yield of oil. The seven varieties showed higher yields of oil after drying. Regarding main components it can be concluded that dried plants are likely to get the same yield as fresh plants.

1. ZUSAMMENFASSUNG

Generelles Ziel der Diplomarbeit war die Evaluierung von ätherischen Ölen von verschiedenen Varietäten von *Melissa officinalis* (Lorelei, Quedlinburger, Erfurter Aufrechte, Aufrechte Stamm NLC, Lemona, Citronella) und die Prüfung des Einflusses auf den Gehalt und die Qualität des ätherischen Öls in Abhängigkeit vom Erntezeitpunkt und vom Trocknungszustand der Pflanzen. Zusätzlich wurden Untersuchungen an verschiedenen Hydrosolen durchgeführt.

Proben der sieben Varietäten in unterschiedlichen Vegetationsstadien wurden geerntet und verschiedenen Extraktionsmethoden unterworfen. Die Pflanzen befanden sich dabei entweder im ersten Wuchsjahr oder hatten bereits ihr zweites Jahr erreicht (*Citronella* and *Quedlinburger*). Die Ernte der Pflanzen fand in der Zeit von Juni bis September 2007 statt.

Um vergleichende Ergebnisse zu erhalten wurden auch Analysen an Pflanzen durchgeführt, die im Glashaus gezogen wurden.

Die Extraktion erfolgte unter zu Hilfenahme verschiedener Methoden wie, Hydrodestillation, DS oder Wasserdampfdestillation.

Die Analyse des Öls erfolgte mittels Gaschromatographie (GC/FID). Die Ergebnisse wurden anschliessend mit Hilfe der Kovats-Index Datenbank verglichen und ergänzt.

Die Analyse des Öls von *Melissa officinalis* ergab einen hohen Gehalt an Monoterpenen mit den Hauptkomponenten Citronellal, Geranial und Neral. Weitere wichtige Vertreter waren (E)-β-Ocimen, (Z)-β-Ocimen, Borneol, α-Terpineol und von der Gruppe der Sesquiterpene konnten β-Caryophyllen and Caryophylleneoxid nachgewiesen werden. Es zeigte sich, dass die verschiedenen Melissesorten unterschiedlichen Gehalt und unterschiedliche Zusammensetzung aufwiesen.

Die Wasserdampfdestillation erwies sich als die beste Methode bezüglich Gehalt und Zusammensetzung. Das Öl erzielte eine gute Qualität weil die Zahl an unerwünschten sekundären Pflanzenprodukten relativ gering gehalten werden konnte.

Zusätzlich wurden die Pflanzen in unterschiedlichen Vegetationsstadien geerntet und der Gehalt des Öls untersucht. Die unterschiedlichen Erntezeitpunkte trugen signifikant zu einer Erhöhung oder Erniedrigung des Gehalts bei. Besonders die Schwankungen von Neral, Geranial und Citronellal waren höher als bei allen anderen Komponenten. Grund dafür ist einerseits die längere Wachstumsphase der Pflanzen und andererseits die günstigen klimatischen Bedingungen, wodurch sich die Zusammensetzung des Öls deutlich verändern kann.

Zusammenhänge von unterschiedlichen Trocknungsgraden der Pflanzen auf den Gehalt des Öls konnten aufgezeigt werden. Eine größere Menge an Öl konnte aus trockenen Pflanzen gewonnen werden. Aufgrund der Studie kann angenommen werden, dass der Trocknungsprozess zwar den Gehalt des Öls merklich beeinflusst, aber der Gehalt der einzelnen Komponenten nur geringe Schwankungen aufweist.

2. INTRODUCTION

2.1 Melissa officinalis

Melissa officinalis is used for a long time because of its mild sedative, spasmolytic and also because of its antimicrobial and antioxidative properties. Therapeutically important contents are amongst others essential oils, flavonoids and tannins. Their application in different tea mixtures and their use in pharmacy are not only in Europe very common [1]. Furthermore, the relative new indication area of *Melissa officinalis,* the treatment of Herpes simplex, shows that the investigation of new therapeutical possibilities of simple herbs is still not completed.

For example, the composition of lemon balm oil has been the topic of many existing investigations, showing different components and different yields of the essential oil.

In 2005 the Groupe PAM – ACW, Suisse, reported the yield and components of different varieties of *Melissa officinalis* at two different locations [2]. The same varieties were now planted at a location in Canada, were examined and the results reported in this thesis.

Finally, the oil content and oil composition of Melissa oil and the influence of plant species, time of harvesting, different pretreatments (for example drying and freezing) and different extraction methods were studied. Furthermore the chemical composition of the hydrosols of the different varieties was examined and compared with each another. Hydrosols are the by-products of DS and are often used because of their high therapeutical value.

2.1.1 History

Lemon Balm is one of the plants mentioned frequently in history. The first botanical description of the plant was done by Theophrastus of Ephesus (327-287 B.C.) in his *"Historia Plantarum"*.

Between 50 and 80 AD the Roman scholar Plinius (23-79 AD) noted that bees preferred lemon balm to other plants and it was also used to feed bees for a long time [3]. Many associations referring to bees are found in several European languages. The Latin species name *Melissa* comes from the Greek *melisso-phyllon* "bee-leaf". Also the Hungarian *méhfü* "bee-grass", the Bulgarian *matitsa* "bee-queen" or the Dutch *bijenkruid* "bee-herb" refers to the feeding quality of *Melissa* [4]. The first therapeutical use is situated into his time. In Pliny´s work "*Historia naturalis"* we can find examples of the usefulness of *Melissa* in medicine. Rheumatism, inflammations, diarrhoea, splenetic diseases and "cloudy eyes" are only a few indications mentioned there.

The Greek Physician Dioscorides (50-100 AD) applied the plant on bites, (e.g. scorpion and dog) and then dropped some more lemon balm into wine for the patient to drink. It was reputed to be a reliable for the stings of beasts and scorpions.

But the real supporters of lemon balm were the Arabs. They realized that lemon balm was good for heart disorders, as well as for lifting the spirits. The most important names in this connection are certainly Rhazes and Avicenna [3].

In Europe, in the north of the Alps, Hildegard of Bingen (1098-1179), abbess of Benedictines, wrote in her work "Physica", "*Melissa officinalis* unites the forces of 15 other herbs" and described its various applications such as an antiphlogistic and antispasmodic medicine. [5].

It was also esteemed by Paracelsus (1493-1541), who believed that lemon balm can completely revivify a man. Furthermore, it was used to proceed from a disordered state of the nervous system.

Formerly a spirit of Balm, combined with lemon-peel, nutmeg and angelica root, enjoyed a great reputation under the name of Carmelite water, being deemed highly useful against nervous headache and neuralgic affections [6].

In the American Edition of "Pereiara´s Materia Medica", balm tea is noted for inducing sweating in fevers and regulating menstruation.
However, the effects of balm are similar to, though milder than, those of other labiate plants. The mildness of its operation arises from the small portion of volatile oil which the plant contains [3].

2.1.2 General information

Melissa officinalis belongs to the family of the Lamiaceae. *Melissa ssp.* is native to Southern Europe and North Africa. The seedling may be originated in the eastern parts of the Mediterranean, the regions of the Black Sea and Minor Asia. In the middle age, it was introduced to the Mediterranean and far beyond the Alps. Now it grows wild in the Mediterranean area and in the western parts of Asia and it is cultivated throughout the world [7].

Melissa officinalis is a herbacous plant with a strong, agreeable odor, reminiscent of lemon. It rather attracts attention through its scent rather than its appearance. It`s an evergreen plant, 30 to 60 cm in height that builds many branches. The leaves become up to 8 cm long and 5 cm wide and they are arranged in opposing pairs on square stems. They are ovate or sometimes have a cordate form. Sometimes they are hairy. The leaf edge is strongly toothed. The upper surface is strongly dark-green, the lower surface light green. Hairs are only on the top side, on the lower surface only the venation is hairy. Venation steps out on the lower

surface strongly. The blossoms are small, of white or light rose color. The flowering time is from July through September. [3]

Observed in the microscope *Melissa officinalis* shows the following criteria:

- Diazytical stomate on the lower surface
- Single-cell short hairs along the nerves
- Upside with numerous conical papillae
- Long, single-cell hairs on top

2.1.3 Applications and effects

The plant was used for many different purposes such as a medicine, an addition in cosmetic, for cooking and as an ornamental. The traditional use of Lemon balm is as a sedative and spasmolytic. It is also used because of its antiviral, antibacterial and antidepressive effects. In folk medicine lemon balm is recommended also for nervous complaints, hysteria and melancholia, chronic bronchial catarrh, toothache, headache and an external for rheumatism, nerve pain and stiff necks [8].

2.1.3.1 Legal requirements for the quality of the drugs

Pharmacopoeias insist on the dried leaves and the essential oil from *Melissa officinalis* with a minimum content of 0.02-0.8% of essential oil and at least 4% of phenolic carbonic acids.

Further quality requirements from the Ph.Eur. (Pharmacopoea Europaea) are loss on drying (max. 12.0 %), ash (max. 12.0 %), foreign constituents (max. 3.0%) and the content of residual humidity (max. 12 %).

Oil of lemon balm is very expensive and is therefore sometimes adulterated with *Cymbopogon flexuosus* (Lemongrass) or citrus pile oil [1].

2.1.4 Cultivation and harvesting

Melissa officinalis can be cultivated from seeds or from cuttings. Cold climate may affect the plant growth negatively. Lemon balm grows best in a sunny and warm climate. According to Guenther [9] the plant needs a deep and shaded soil of medium consistency. In light and dry soils the leaves turn yellow and the yield of oil diminishes.

It flourishes on nutrient-rich soils favourably on loamy sand with an optimal pH-value of 6 to 7.

The leaves from *Melissa ssp.* should be harvested on a clear and dry day and should be dried carefully – it has a tendency to get black when it is dried too quickly.

2.1.5 Chemical composition of Melissa oil

Major components that can be found are monoterpene hydrocarbons, flavonoids and tannins.

Main components of the oil are the monoterpene aldehydes citronellal and citral (neral and geranial), which are also the smell carriers of the oil. All together, these aldehydes form the principal part (40-75%). Genuine lemon balm oil contains β-caryophyllene and caryophyllene epoxide; the absence of these substances refers to falsifications [1]. From the group of natural substances of the terpenes the occurence of monoterpene hydrocarbons (myrcene, (Z)-β-ocimene, (E)-β-ocimene, β-pinene), monoterpene alcohols (linalool, nerol, geraniol, and citronellol), monoterpene aldehydes (citronellal, neral, and geranial) and some sesquiterpene hydrocarbons (β-caryophyllene und caryophyllene epoxide) is well-known.

Figure 1: Main components of Melissa oil

Due to the large geographical spreading of *Melissa officinalis* essential oils differ in the composition and in content.

Guenther indicates for an essential oil from Melissa at the beginning of the flowering stage a content of 0.014%. The fresh herb during full bloom gave 0.010% of oil with an odor of citral and citronellal. Distilled oils from France, Russia, Calabria and Oregon pointed a content of 0.014 – 0.13% in Oregon.

The chemical composition varies very strongly between the different planting locations. Dorronsoro (1919) reported that an oil distilled in Sevilla (Spain) contained 42% of aldehydes (bisulfite method), while Albricci reported 31.82% of citral in an oil from Calabria. Chiris (1924) obtained from dried herbs of Melissa oil that had no odor of citral. Burlage examined oil distilled from plants collected in Oregon and found that it contained 4% of phenols and 17% of aldehydes and ketones. For Salgues (1942) the main components are geraniol, linalool, and citronellol (about 34 to 38%) [9].

Another investigation from Suisse progressed at two different sites and with 11 varieties of *Melissa officinalis*, revealed a higher yield in deeper altitudes and in milder climate. Furthermore upright varieties gave more yields on essential oil and antioxydants. The profile of the oil changed in the course of the season. The content of monoterpenes is higher in autumn than in spring [2].

3. MATERIAL AND METHODS

3.1 Plants

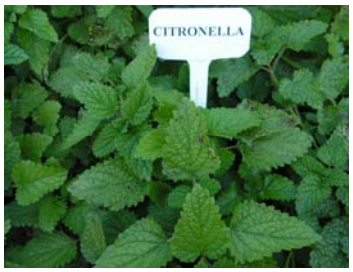
In this study 7 different varieties of *Melissa officinalis,* all of them in the first year of growing and 2 varieties from the second year, were compared according to their yield and composition of essential oil.
All of them are originated to European countries. Varieties 1 to 6 were introduced from Germany, while variety 7 was developed from Agroscope ACW/DSP in Switzerland.

	VARIETY	BREEDER	COUNTRY
1	Citronella	N.L.Chrestensen	Germany
2	Quedlinburger	N.L.Chrestensen	Germany
3	Erfurter Aufrechte	N.L.Chrestensen	Germany
4	Stamm NLC	N.L.Chrestensen	Germany
5	Lemona	Pharmasaat	Germany
6	Aufrechte Typ	Pharmasaat	Germany
7	Lorelei	Agroscope ACW/DSP	Switzerland

The habitus from *Melissa officinalis* is genetically fixed. Thus, there are varieties which grow upright and others, which are characterized by a down-lying status. After the first harvest, all following buildups are upright. Generally one can differentiate between upright and reclined varieties, which are developed from the group sorts *"Melissa Erfurter Aufrechte"* and *"Melissa Quedlinburger Niederliegende"* [11]. There might be some difficulties to distinguish between the different varieties after the first cutting. In the first year of growing the different varieties can be distinguished between their height, their growth and their color.

The following pictures have been taken from the processed plants at the time of harvesting.

1. Melissa Citronella

Height (cm): 50-60 Height (cm): 5-18 Height (cm): 10-25

Growth: upright

Color: dark green

2. Melissa Quedlinburger



Height (cm): 50-60cm Height (cm): 15-30cm Heigth (cm): 8-15cm

Growth: upright

Color: light green

3. Melissa Erfurter Aufrechte


Height (cm): 10-30 Height (cm): 20-30

Growth: diagonal

Color: light green

4. Stamm NLC

Height (cm): 15-25 Height (cm): 20-30

Growth: diagonal

Color: light green

5. Melissa Lemona

Height (cm): 15-30 Height (cm): 8-10

Growth: reclined

Color: dark green

6. Melissa Aufrechte

Height (cm): 10-25 Height (cm): 8-20

Growth: reclined

Color: dark green

7. Melissa Lorelei

Height (cm): 20-25 Height (cm): 25-30

Growth: upright

Color: light green

3.2 Location and soil properties

The project was accomplished at the Agriculture and Agri-Food Canada experimental farm at Acadie (45°18`N, 73°20`W), Quebec, Canada. Acadie is situated 50km from Montreal (Québec) at an altitude of 47m.

Soil properties at Acadie shows an optimal pH-value (6.9) and an optimal K^+-ion (243kg/ha) and PO_4^{3-} (249 kg/ha) value. Soil is from deep and medium consistency. The experimental site is located in a sunny area and shows sun exposure the whole day [12].

3.3 Climatic conditions

The climate of Canada can be divided due to the size of the country into different zones. General characteristics of the Canadian climate are long snow-rich winters and short hot summers. The coastal regions and the South of the country are affected by the Maritime climate and are therefore more moderate. In the provinces of Québec and Ontario the seasons are pronounced more clearly and temperatures can range from -20°C to 40°C. Generally, the summers are very warm with an average temperature of 25°C and an average precipitation of 90mm. Climate during summer 2007 was very dry but was intermitted with some heavy rain showers in July.

3.4 Experimental structure

The project was divided into different sections starting with the seeding of the plants and further transplantation to the site. All plants were cultivated from seeds and were transplanted on the 23th of May 2007, with 0.2m row space at the experimental farm of Acadie. The length of each row was 10m and for each variety two rows with a space of 1m were used. The number of plants for each variety was 100.

3.5 Care and fertilization

Plants were grown without the use of any fertilizer. Beside of weeding on 20th of June 2007 no treatment was carried out.

3.6 Harvesting and treatment of the material

3.6.1 Harvesting

In order to get representative samples, the project was divided into different sections depending on the date of harvesting.
Harvesting took place on different dates at the experimental farm of Acadie near Saint Jean sur Richelieu leaving the first plants of each row.

3.6.1.1 Melissa Quedlinburger and Melissa Citronella

a.) One m^2 of variety 1 and 2, in their second year of growing, were collected on different days at the experimental farm of Acadie. All plants were harvested manually on a dry and sunny day, all of them in their non flowering stage, 10 cm above the soil surface. They were stored in plastic bags and were brought to the Horticultural Research and Development Center (HRDC) of Saint-Jean-sur Richelieu. There the plant material was weighed, and divided into two equal parts. One part was stored in the cold storage chamber at -2°C to +10°C until analysis.

The second part was used for drying. The plant material was submitted either to hydro-distillation or DS whereby two parallel beginnings were accomplished.

b.) The second sampling of Melissa *Citronella* and *Melissa Quedlinburger* (second year) took place on the 25th of July in the morning. At this time plants had all reached their flowering stage. The top 25-30cm of the plants of one row were cut manually, on a dry and sunny day. The whole plant material (from about $7m^2$) was divided into two equal parts. One part was dried in the greenhouse for 2 days. The whole plant material (fresh or dry) was submitted to steam distillation (20 l) for 4 hours. A small amount of plants was brought to DW in order to get a better comparison between the different methods.

c.) The third samples of *Melissa Citronella* and *Melissa Quedlinburger* were taken on the 14th of August 2007 after inflorescence. Only a small amount (~100-150g) of this plant material was analyzed using DW.

3.6.1.2 Seven varieties

a.) Two plants of each variety in their first year of growing were harvested at the experimental farm on the 14th of August 2007. All plants were cut 2cm above the soil surface. The plant material was brought to the HRDC and stored in the cold storage chamber until analysis. Two different methods (DW and DS) were used to analyse the samples.

b.) Furthermore, plants from each variety grown in the greenhouse of the HRDC were sampled. The seeding took place at the 16th of April 2007. The plants were cut 3-5 cm above the soil surface and brought immediately, after cutting into 3 pieces, to DW. Plants from the green house were not dried.

c.) The second and third samples of the different varieties were taken on the 4th and 5th of September and on the 18th of September. For the samples at the beginning of September 20 plants were taken for each post harvest treatment (fresh and dry) and the plant material was either stored in the cold storage chamber or dried in the greenhouse of the experimental farm of Acadie. The entire

plant material was used for DS (20L). The rest of the plants in the two rows were cut at the 18th of September, except 10 plants at the beginning of each row, which were left for further growth monitoring. Plant height (cm) and amplitude (cm) was measured before every cutting from soil level to highest point using plants with 2 different hights. Green herb yield (t/ha): The weight of 20 plants was taken after cutting the plants manually 2 cm above the surface and transformed to t/ha. Hydrolate: Hydrolate was analyzed from *Melissa Citronella* and *Melissa Lorelei.*

3.6.2 Drying

For drying different methods were used. From the first samples which were collected on the 26th of June fresh plants of approximately 640g were dried in the oven at 32°C. The plants were kept in the oven at room temperature for another 15 hours.

The fresh plants from the second sampling at Acadie were dried in the greenhouse of the HRDC for 48 hours and then stored in the cold storage chamber until analysis.

20 plants from each variety, harvested at the different dates in September, were dried in the greenhouse of the experimental farm of Acadie for approximately two days, whereas the drying time, depending on plot yield, differed for each variety. The plants from the third sampling obtained from one row were also dried in the greenhouse for 4 days.

3.7 Isolation of the essential oil

To obtain the oil from the plant material three different types of steam distillation were used. Steam distillation is a special separation method for temperature sensitive substances, like essential oils. By using steam the boiling points of the volatile components depress and the oil evaporates at lower temperatures.

The leaves were separated from the main stems by hand and a sample of fresh plants or of dried plants was taken from each part. The material was submitted

either to DW or DS or WSD (5 l and 20 l). The duration of each method was 4 hours.

3.7.1 Hydrodistillation or distillation in water (DW)

Using hydrodistillation (DW) the plant material is immersed in an appropriate volume of boiling water (in the study were used 4000ml in a 5000ml balloon) – the steam evaporates the essential oil and condense in the following water – cooled condenser and is then collected in the following recuperator with hexane as collecting solvent. The obtained liquid contains hydrosol and oil. The oil on the top can easily be skimed off.

The method of DW has benefits and drawbacks. A large amount of energy is needed for heating the water that is containing the material. But it is said that DW produces a finer product, as hot water is cooler than steam [13].

3.7.2 Hydrodiffusion or direct steam distillation (DS) (5l)

Using hydrodiffusion (DS) the plant material is not immersed into hot water. The steam is produced in a 5000ml glass balloon placed under the filled tube and is led through the plant material. The steam evaporates the essential oil that becomes liquid again in the following condenser and is collected in the recuperator, which is filled with distilled water and hexane as a collecting solvent. In the recuperator the liquid separates into two products, the aqueous hydrosol and the oleous essential oil. The hydrosol can then be collected in a glass beaker. Hydrosol is the by–product of DS of aromatic plants. Compared with the essential oil it contains also water-soluble components of the plant and shows therefore a different quality. It does not contain any tannic acids and bitter substances, which can lead to skin irritations. Hydrosols often find application in cosmetic, medicine and are also used as smell and taste materials for cooking.

3.7.3 Water-cum-steam distillation (WSD)

The main principle of steam distillation with 20 l (WSD) of water is the same as for the 5 l DS using hot steam that is produced in a separate boiler that evaporates the essential oil. The system is designed for a larger amount of plant material and is mainly used for producing essential oils in a higher quantity.

3.8 Analysis of essential oils and hydrosol

3.8.1 Essential oil

For analysis of the oil, the combined oil-hexane fraction was dried over anhydrous sodium sulphate and diluted with a factor 10 by adding of hexane.
One ml of the combined fraction was put into a vial. The remaining 9 ml were concentrated using a rotavapor and the rest of the hexane was removed under a nitrogen stream. This step was necessary for the calculation of the yield of oil.
Because of the high volatility of the components a volume of 0.02µl (from the evaporated 9ml), a volume of 1.00µl (from the 1ml) and a volume of 0.02µl (from the samples dried over nitrogen) was injected for comparison. This was necessary to see if any components got lost during the process.

3.8.2 Hydrosol

To attain the components of the hydrosols liquid-liquid extraction was used. Using this method the components in the aqueous hydrosol are transferred to an organic solvent which is immiscible with water.

The hydrolate was extracted three times with 50ml of dichloromethane, filtered and dried over sodium sulphate and rinsed two times with 20ml of dichloromethane. The solvent was removed using a rotavapor and then completely dried under a nitrogen stream.

3.8.3 Gaschromatography (GC/FID)

A gaschromatograph (GC) equipped with a flame ionisation detector (FID) was used for analysis. In gas chromatography, the sample is vaporized and injected onto chromatographic columns and then separated into many components (Fig. 2). The elution is brought about by the flow of an inert gaseous mobile phase. The carrier gas (such as helium, argon and nitrogen) serves as the mobile phase that elutes the components of a mixture from a column containing an immobilized stationary phase. Gas chromatographic separation occurs because of differences in the positions of adsorption equilibria between the gaseous components of the sample and the stationary phases.

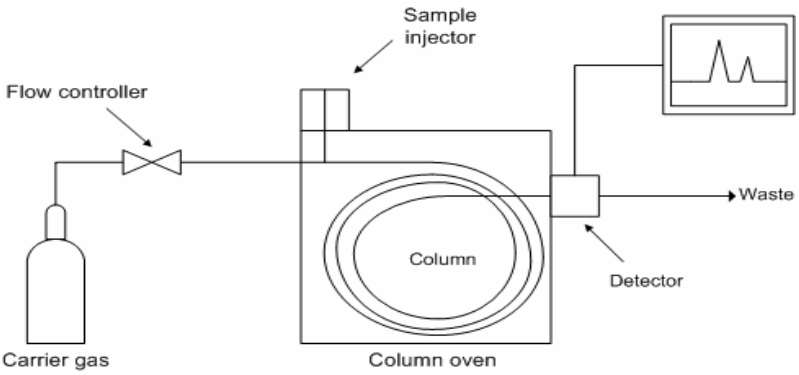


Figure 2: Diagram of a gas chromatograph [14]

The separated components leave the column in certain period of time (dependent on the molecular weight) and pass the detector [14]. An FID consists of a hydrogen/air flame and a collector plate. The effluent from the GC column passes through the flame (Fig. 3), which breaks down organic molecules and produces ions. The ions are collected on a biased electrode and produce an electrical signal. The FID is extremely sensitive with a large dynamic range; its only disadvantage is that it destroys the sample [15].

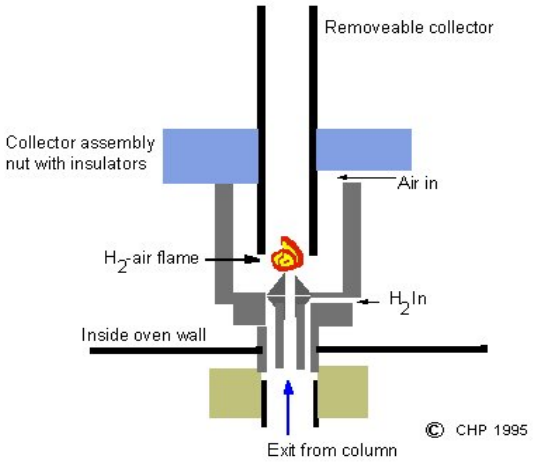


Figure 3: Schematic FID [15]

3.8.3.1 GC-Analysis

The GC analysis was carried out on a GC Varian 3400, equipped with a non polar SPB – 1 column (crossed linked dimethylsiloxan) and a polar Supelcowax-column. (30 m x 0.25mm, film thickness 0.25µm). Helium was used as a carrier gas with a flow rate of 1.5ml/min. Temperature was programmed from 40°C – 160°C at 2°C/min. and from 160°C to 240°C at 20°C/min. and was held for 16 min. The injector and detector temperatures were 230°C and 250°C, respectively. To attain a constant result, mixtures of C8-C30 n-alcanes and of 27 standards were injected every two weeks as a reference. Identification of the components was achieved by comparison of their retention times with those of authentic standards and essential oils of known composition. Unknown components were identified using the Kovats Index (KI) database. Each component has a specific KI on a polar and apolar column depending on which kind of column is used. This combination makes it possible to identify the components of a hydrolate analyzed by GC.

The KI describes the retention behaviour of a component as equivalent to that of a hypothetical n-paraffin hydrocarbon, usually containing a mixed number of carbon atoms.

By definition the Index I_A of a substance is given by:

$$KI = 100N + 100n \times \frac{\log tR(A) - \log tR(N)}{\log tR(N+n) - \log tR(N)}$$

KI.....Kovats-Index

N.....number of carbon atoms in the alcane before the unknown component

n.....number of carbon atoms in the alcane after the unknown component

A.....unknown component

tR.....adjusted retention time [16]

4. RESULTS

4.1 Melissa Quedlinburger and Melissa Citronella (2nd year)

By using different extraction methods, the execution of two parallel test series and the analysis of different concentrations the content of plant material from different varieties could be worked out well. It turned out that the composition of the oil exhibited a very similar content with the particular parallel attempts. Therefore, an average value of the two attempts was used for the respective evaluation.

The plant material at different growing states was analyzed using the following treatments (DS = Hydrodiffusion; WSD = Water-cum-steam distillation, DW = Hydrodistillation):

Identification		Treatment	Date of Treatment
First sampling (20070626)			
1A, 1B	Quedlinburger fresh	DS	20070627
1C, 1D	Quedlinburger fresh	DW	20070628
1E, 1F	Quedlinburger dry	DS	20070704
1G, 1H	Quedlinburger dry	DW	20070706
2A, 2B	Citronella fresh	DS	20070703
2C, 2D	Citronella fresh	DW	20070629
2E, 2F	Citronella dry	DS	20070705
2G, 2H	Citronella dry	DW	20070709
Second sampling (20070725)			
11A	Quedlinburger flowers	DW	20070806
11B	Quedlinburger flowers fresh	WSD	20070814
11C	Quedlinburger flowers dry	WSD	20070822
12A	Citronella flowers	DW	20070806
12B	Citronella flowers fresh	WSD	20070817
12C	Citronella flowers dry	WSD	20070821
Third sampling (20070814)			
11E	Quedlinburger after flowering	DW	20070824
12E	Citronella after flowering	DW	20070827

4.1.1 Yield of essential oil

A light pale oil was obtained from the aerial parts of *Melissa officinalis* using DS and DW. The content of oil was variable and depended on the different extraction methods and the stage of growing. Furthermore different parameters such as humidity, the amount of fresh plants and the amount of dry plants affected the yield of oil.

The parameters were calculated with the following equations:

$$Humidity(\%) = \frac{amountoffreshplants(g) - amountofdryplants(g)}{amountoffreshplants(g)} \times 100$$

$$weightofdryplants = \frac{freshplantsused(g)}{100} \times \%dryness$$

$$Dryness(\%) = 100 - humidity(\%)$$

The yield of oil was calculated by the formula:

$$Yield(\%) = \frac{amountofoil(g)}{weightofdryplants} \times 100$$

The plant material which was needed for the calculation was put into a paper bag and dried in an oven at 105°C for 24 hours.

4.1.1.1 Melissa Quedlinburger

The yield of the oil ranged from 0.004 to 0.228% (Fig. 4, see chapter 6). Using fresh plants (1A1B; 1C1D) the yield was higher as for dry plants. The highest yield was obtained with DS (1A1B) of the fresh plants in their non flowering stage. Using DW yield of oil was a little bit lower than the one obtained by DS (0.228 and 0.206%). Dry plants yield was higher when DW (0.200%) was used.

The oil rate changed in the three vegetation periods. The content in the stage before flowering [1] reached 0.206%, it decreased to 0.044% in the flowering stage [2] and increased in the last stage [3] to a content of 0.097%.
Only a percentage of 0.004 and 0.006% of oil could be found in the hydrolate (11B1; 11C1). In the pure oil which was received with WSD (20l), a yield of 0.096 and 0.105% could be obtained. Drying had no negative effect on yield of oil.

4.1.1.2 Melissa Citronella

Highest yield of oil (Fig. 5, see chapter 6) was achieved when DW was used. (2C2D; 2G2H). A yield of 0.179% for the fresh plants and a yield of 0.151% for the dry plants were reached. Using DS the yield of oil was 0.153 and 0.149%. Comparing the three vegetation periods the highest yield was obtained in the stage before flowering [1] (0.179%). It decreased to 0.095% in the flowering stage [2] and increased in the stage after blooming [3] to a content of 0.104%. All the results were obtained using DW. A small percentage of 0.007 and 0.005% could be found in the hydrolate. The oil yield for WSD was 0.017% for the fresh and 0.029% for the dry plants.

4.1.2 Chemical analysis of the essential oil extract

In the presented analysis more than 25 components were identified, representing almost 100% of the total oil. The major components were monoterpene aldehydes (neral, geranial and citronellal) and as second important group sesquiterpenes and their oxides, such as β-caryophyllene, E-β-farnesene and caryophyllene oxide occured.

4.1.2.1 Results from the stage before flowering

Two different extraction methods were used and compared with each other. The plants were harvested in their second year of growing and the results were discussed on selected examples. Generally, Melissa oil showed a high number of components. Not all were identified and only components over 1% were

considered in this study. From the group of monoterpenes (E)-β-ocimene, the smell carriers and main components citronellal, neral and geranial and α-terpineol were identified. Sesquiterpenes were represented by α-copaene, geranyl acetate, α-bourbonene, β-bourbonene, β-caryophyllene, α-humulene, E-β-farnesene, germacrane D, α-cadinene and caryophyllene oxide.

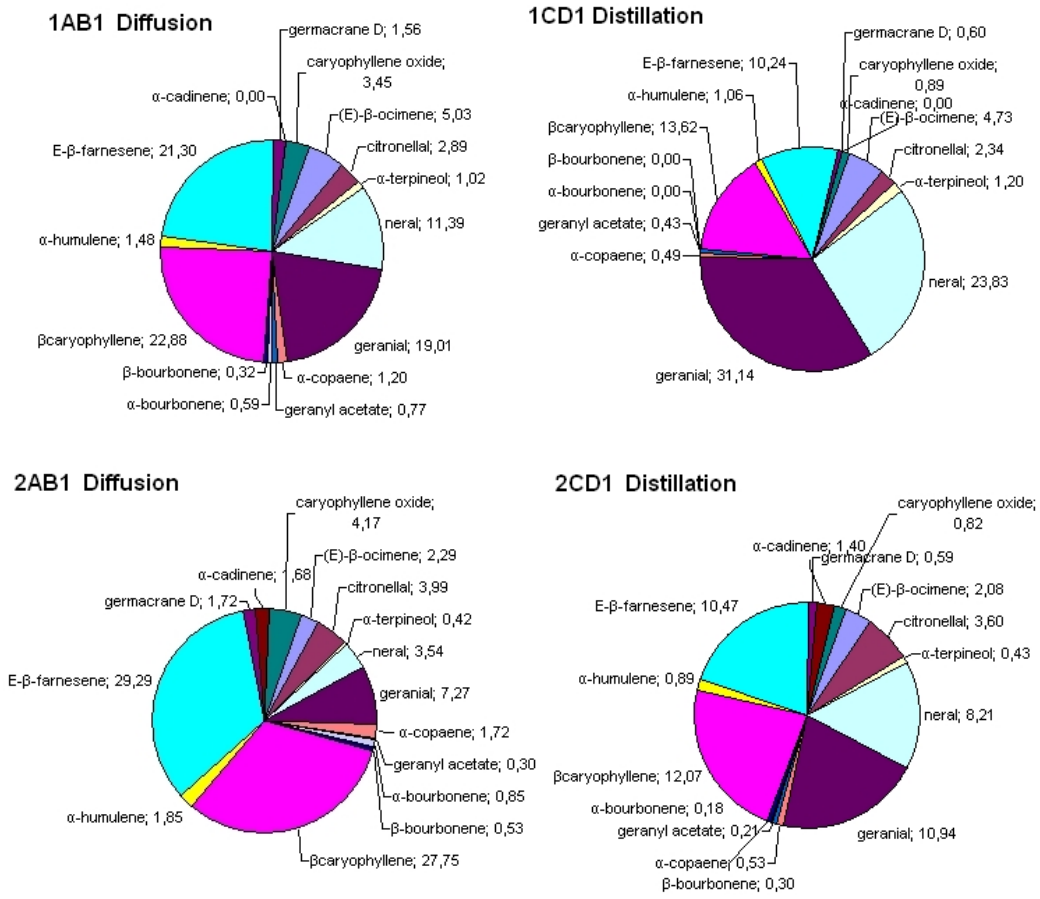


Figure 4a: Main components of *Melissa Citronella* (2AB1, 2CD1) and *Melissa Quedlinburger* (1AB1, 1CD1), 2nd year of growing, harvesting date 20070626, including hexane.

The distribution of the components changed with the two different methods (Fig. 4a). All dominating components belong to the group of monoterpenes, such as geranial and neral and to the group of sesquiterpenes, such as β-caryophyllene and (E)-β-farnesene. Comparing the two methods from the *Melissa Quedlinburger* oil, the yield of sesquiterpenes was higher for the oil obtained by DS contrary to the

distilled oil which indicated a higher yield of monoterpene aldehydes. Furthermore, the variation of the content of neral, geranial, β-caryophyllene and (E)-β-farnesene was higher between DW and DS oil compared to all other components. Representatives from sesquiterpenes α-copaene and caryophyllene oxide, showed a higher concentration in the oil (DS); it was reduced in DW oil by 50%.

The oil from *Melissa Citronella* showed the same distribution of monoterpene aldehydes and sesquiterpenes. Neral and geranial again were represented in a higher amount in distilled oil (8.2 and 10.9%) whereas the content of sesquiterpenes was higher in the oil obtained by DS.

Results from the stage of flowering and from the stage after flowering

The isolation and analysis of the essential oil from the stage of flowering were again carried out by the isolation and analysis methodes described in Chapter 2.7 and Chapter 2.8. According to the instruction of DW only a small amount of plant material was boiled in an appropriate volume of water in a 5 l balloon. Distillation time was 4 hours and the combined oil-hexane fraction was dried over anhydrous sodium sulphate and diluted to a factor 10 by adding hexane.

The remaining plant material was submitted to WSD and analyzed in the GC using the pure oil. Two different drying stages were analyzed. A volume of 0.02µl or 1.0µl from the obtained essential oil was analyzed according to the apparature described in Chapter 2.8.3.1.

4.1.2.2 Melissa Quedlinburger

a) Hydrodistillation (DW)

Main components of essential oil obtained after DW are demonstrated in Appendix 1. As main components again the aldehydes citronellal (8.1%), geranial (18.1%) and neral (13.5%) were found. According to bibliography, the oil contains in addition to citronellal, geranial and neral also small concentrations of (E)-β-ocimene (3.6%), α-terpineol (0.6%) and geranyl acetate (0.4%). From the

sesquiterpenes, β-caryophyllene (17.4%), α-humulene (1.3%), (E)-β-farnesene (13.7%) und germacrane D (0.8%) were represented in a higher amount. Differences between the three vegetation periods were more obvious regarding the main components neral and geranial. They showed a comparable seasonal trend (Fig. 6, see chapter 6) with highest rates in the stage before flowering. At this stage the content of neral amounted to 23.8%. Geranial was represented by 31,1%. In the following growing stages the content of neral and geranial diminished to an amount less than 20%.

b) Water-cum-steam distillation (WSD)

Results obtained by WSD from fresh and dry plants are demonstrated in Fig. 7 (chapter 6). The content of the different drying stages varied between 0.1 and 4.5%. Significant differences between the various components obtained from the different drying stages were observed mainly for (E)-β-ocimene (11.3%/7.7%), neral (10.2%/11.4%), geranial (12.6%/17.1%) and caryophyllene oxide (0.8%/1.4%). Lower differences in rates were observed for all other components.

4.1.2.3 Melissa Citronella

a) Hydrodistillation (DW)

The concentration of main components amounted to 15.3% (neral), 21.7% (geranial), 17.3% (α-humulene) and 12.9% ((E)-β-farnesene) (Fig 5a). In general, all components had their maximum in the flowering stage. For (E)-β-ocimene, citronellal and β-caryophyllene, however, the highest concentration was detected at the end of June (Fig. 8, chapter 6). Two compounds were absent in the oil before flowering and present in the flowering stage (limonene 3.5% and borneol 7.4%).

b) Water-cum-steam distillation (WSD)

The concentrations of the components were mostly dependent on the extraction method, as well as on the moisture content of the plant material. Figure 9 (chapter 6) shows the composition of *Citronella* oil obtained by WSD from fresh and dry plants. Significant differences could be found for components such as (E)-β-ocimene (6.6%/3.7%), geraniol (2.5%/4.7%), (E)-β-farnesene (19.4%/21.7%) and caryophyllene oxide (0.8%/1.8%). During the drying process, when the plant material was stored in the greenhouse, a significant increase of these substances could be observed. (E)-β-ocimene decreased its amount to 3.7%.

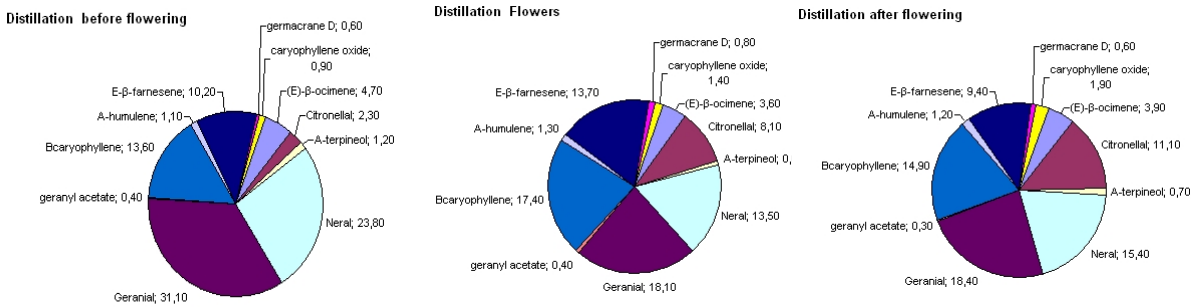


Figure 5a: Volatile components of *Melissa Citronella (*2nd year) obtained from the three different harvesting dates

4.2 SEVEN VARIETIES

4.2.1 Plant height and amplitude

Two characteristic differences to distinguish between the seven varieties are plant height and amplitude. Plant height ranged from 8cm (minimum), observed for variety 5, to a maximum height of 33cm for variety 4. Upright varieties (3, 4 and 7) showed the highest plant height. Nearly all plants reached a height over 20cm and we could confirm the data obtained at previous investigation [2]. Higher plants obtained in Germany [18] for the same varieties can be explained with

different climatic and soil conditions. The amplitude of plants measured from the outside ends ranged from 55cm (*Lemona*) to 85 cm (*Stamm NLC*). As for the height upright varieties showed highest width. A relationship between plant height and amplitude could be found.

4.2.2 Green herb yield (t/ha)

One important aspect for the production of essential oils for industrial purposes is the amount of fresh plants and if there is a relationship between green herb yield and yield of oil. Depending on the different growing stages of the plants and the different dates of harvesting various amounts of plant material could be obtained.
Upright varieties (I, III and IV) showed a good adaptation to climatic conditions (Figure 10, chapter 6).
Generally, the harvest changed in the course of the season. *Melissa Erfurter* and also *Melissa Stamm NLC* nearly doubled their yield within two weeks. Other varieties showed a smaller increase or decrease within two weeks of growing.

4.2.3 Yield of essential oil

A light yellow oil was obtained using DS and DW. The color of the pure oil obtained using WSD was dark- or lightgreen.
The smell of the oil was herbacous-fresh with a lemon taste coming from the main components neral, geranial and citronellal.

4.2.3.1 First sampling

Generally, the highest yield was obtained using DW. Upright varieties showed, except *Melissa Quedlinburger*, highest yield (ranging from 0.04 to 0.29%). *Melissa Lorelei* showed the best yield when DW was used. Lowest yield was obtained by *Melissa Aufrechte* with 0.03%. DS was not the best method concerning the yield of oil. Oil gets lost during the process because a part of the

oil can be found in the corresponding hydrosol. The yield of oil ranged from 0.03 to 0.19%.

Table 4: Yield of essential oil obtained by Hydrodiffusion (DS) and Hydrodistillation (DW) from the first sampling.

	1	2	3	4	5	6	7
DW	0.29	0.04	0.19	0.16	0.13	0.03	0.29
DS	0.08	0.08	0.05	0.19	0.15	0.03	0.04

4.2.3.2 Second and third sampling

Oil rate changed very strongly during the different vegetation periods (Fig. 11, see chapter 6). Drying process showed a positive effect on the content, although the amount of used plant material was less. The influence of the amount of used plant material on the content was higher for the samples at the beginning of October. Generally yield of oil obtained at the beginning of October was higher than the yield of oil from the third sampling (except *Melissa Citronella* and *Melissa Aufrechte* dry). Yield of oil ranged from 0.03% to 0.24%. The highest yield was obtained for dry plants of *Melissa Citronella* from the date of third sampling (0.24%). High yield (more than 0.1%) was also obtained for *Melissa Stamm NLC* and *Melissa Lemona* dry. This was surprising because of the small amount of plants used for WSD.

Small amount of oil was obtained for *Melissa Lorelei*. Because of the less amount of oil a transfer of the oil obtained from the fresh plants from the second sampling to the small vial to get the correct weight was impossible. The loss was larger than the obtained weight.

4.2.4 Chemical analysis of the essential oil extract

This part shows the comparison of the chemical composition from the different varieties depending on different harvesting times and extraction methods. Influence of drying was also considered in some cases.

All substances were identified with the use of the Eso-databank on two different columns - a non polar SPB-1 and a polar DB-WAX column. In some cases identification was not possible because of a missing reference on the wax-side or an overlay of several components on the different sides. Unidentified components were described by their Kovats indices from the SPB1 and/or DB-WAX side.

Analysis of the oil proved to be difficult. Sometimes, components were very unstable and decomposed into different products. Thus, different results for the two injected volumes were obtained. More stable results were obtained from the more diluted 1ml and are therefore considered in this thesis. The various components with a content under 0.1% are only considered in a few cases.

In the presented analysis more than 20 components were identified, representing almost 100% of the total oil. Major components were monoterpene aldehydes such as citronellal, neral and geranial; monoterpenes such as β-pinene, myrcene, (E)-β-ocimene and camphor, monoterpene alcohols like linalool, borneol, terpinen-4-ol and geraniol. In a smaller amount of approximately 1.5% monoterpene esters (bornyl acetate and geranyl acetate) were found. One important group were sesquiterpenes with β-caryophyllene as major component.

4.2.4.1 Oil obtained from samples grown in the greenhouse

Samples from the greenhouse were taken because the influence of different climates and site properties is eliminated. Therefore, one could compare the influence of different growing conditions according to the composition of the oil with the plants grown on the field. A small amount of plant material was submitted to DW and analyzed with the gaschromatographic method described in chapter 2.8.3.1.

Table 5 shows the composition of the different varieties grown in the greenhouse. Components less than 0.1% are not considered in the following table.

All varieties showed nearly the same composition of oil with neral and geranial as main components. They were found in an amount of >30% in all varieties. Further components were citronellal, β-caryophyllene, α-terpineol and borneol. Those

substances were present in an amount of more than one percent. In an amount less than one percent one could find sabinene and β-pinene, myrcene, (E)-β-ocimene, linalool, terpinen-4-ol and geranyl acetate. Sabinene was missing in variety 4 and 7. Variations in content of main components between the different varieties could be observed for citronellal and geranial.

One example for the instability of components can be seen from geranial obtained from Melissa Quedlinburger and Melissa Lemona. Geranial decomposed into two different components with a KI of 1247 and 1249 on the SPB1 side. Unfortunately the identification of those substances was not possible because of a missing reference on the wax side.

Table 5: Chemical composition from the 7 varieties grown in the greenhouse.

Variety	1	2	3	4	5	6	7
sabinene	0,33	0,40	0,31		0,40	0,28	
β-pinene	0,17	0,75	0,69	0,87	0,05	0,47	0,93
myrcene	0,15	0,16	0,14	0,13	0,14	0,15	0,14
(E)-β-ocimene	0,11	0,08	0,12	0,13	0,04	0,18	0,14
linalool	0,35	0,32	0,39	0,44	0,31	0,36	0,43
1121	0,26	0,42	0,41	0,35	0,26	0,40	0,41
citronellal	0,77	3,20	3,47	2,99	0,54	3,87	4,72
borneol	0,87	0,99	0,89	0,82	0,98	0,85	0,64
terpinen-4-ol	0,11	0,14	0,13	0,11	0,14	0,11	0,09
α-terpineol	1,33	1,50	1,35	1,28	1,48	1,34	1,02
neral	31,23	32,99	31,74	28,82	33,16	30,95	26,03
geranial	61,40	15,68	55,45	61,18	19,30	57,20	62,15
1247		40,69			0,92		
1249					39,44		
1299	0,23	0,28	0,23	0,21	0,26	0,25	0,25
geranyl acetate	0,39	0,29	0,25	0,51	0,37	0,28	0,30
βcaryophyllene	1,35	1,28	1,76	1,62	1,20	1,57	2,04

4.2.4.2 Oil from different varieties at different harvesting dates

The composition of the essential oil obtained from the different varieties and extraction methods indicated differences in chemical composition and content of oil. Harvesting date as well as effect of drying was considered in the discussion.

Main components that can be found in all seven varieties in different concentrations were neral, geranial and citronellal. Those monoterpene aldehydes are responsible for the lemony smell of *Melissa ssp.* They occur in all plants in an amount of >20%, whereas citronellal shows larger fluctuations compared to neral and geranial. Further groups that occured in the different varieties were monoterpene hydrocarbons and sesquiterpenes. Distribution of monoterpenes and sesquiterpenes in the analyzed species is shown with the respective discussion.

A) CITRONELLA

1) First sampling

Oil from the 14th of August was distilled using DW and DS. Figure 12 (chapter 6) summarizes the composition of oil extracted by the two different methods (see also Appendix 2 and Appendix 3) for detailed information of extracted oil of *Melissa Citronella*). All dominating components were neral and geranial. They were present with DS and DW and the amount of both was higher for DW (DS: 36.7 and 51.6%; DW: 29.3 and 45.6%). Citronellal existed also in the DS and DW oil (1.2 and 1.7%). Content of monoterpene hydrocarbons was higher for DS. They attained a total amount of 2.32 (DS) and 2.45% (DW). Camphor, terpinolene and β-pinene were absent in the DW oil. From the group of monoterpene alcohols borneol, terpinen-4-ol and α-terpineol were present in both oils. Content was again higher for DS.

One monoterpene ester (geranyl acetate) (DS:1.3%; DW: 2.5%) was as well present as were sesquiterpene hydrocarbons, such as β-caryophyllene, α-humulene, (E)-β-farnesene and germacrane D. 10.7% of β-caryophyllene was obtained from the DW extracted oil (DS:2.8%).

Qualitative analysis from the different analyzed volumes can be found in the corresponding Appendices. An injected volume of 0.02µl from the concentrated pure oil and a volume of 1.0µl from the undiluted 1ml were compared with each other. An increase of nearly all monoterpenes can be noticed. The content of sesquiterpenes decreased.

2) Second and third sampling

In this paragraph the composition of the pure oil obtained by water-cum-steam distillation (WSD) is shown. Compared to DW and DS, WSD is a more sensitive method and dangers of chemical changes of components are minimal.
The composition of the pure oil is in one case comparable with the corresponding hydrosol. Changes that occured in the course of the season are as well considered as were effects of drying. Main components found in the pure oil obtained by WSD (20l) can be seen in Table 6.
Monoterpene aldehydes were again main components, representing more than 70% of the total oil. Main components were neral and geranial with a concentration of more than 30%. Concentration of citronellal (samples on the 4th of September) was with ~ 9.0% lower than the value from neral and geranial. Further important components (concentration >1%) were β-pinene or sabinene, (E)-β-ocimene, borneol and α-terpineol from the group of monoterpenes and geranyl acetate and β-caryophyllene as representatives from sesquiterpenes. All other components in the table are an example for the complex composition of Melissa oil and are only considered in a few cases.

a) Effects of harvesting time

Effects of seasonal changes are discussed only by components attaining a concentration >1.0%. Comparing the results obtained at the different harvesting dates no great differences can be seen, although there are also some outliers. One interesting aspect was that the fluctuation of the main components neral, geranial and citronellal was higher than for all other components. Thus, an amount of 9.1% citronellal was attained at the first sampling (beginning of September), but it changed within two weeks to a content of 22.9%. For neral and geranial one could observe a diminution in content at the two different crops. The difference in concentration was not as high, thus the content varied between 5 and 7% (neral: 30.43 and 25.3; geranial: 34.6 and 27.3%). All other varieties changed their amount slightly during the two different plant stages. Results can be seen in Table 6.

Table 6: Chemical composition of *Melissa Citronella* at different harvesting dates and different drying stages

Name	4C	4E	4D	4F
α-pinene	0,05	0,14	0,20	0,04
β-pinene+sabinene	2,51	2,80	5,26	2,65
β-pinene+sabinene	1,26	1,19	-	0,89
myrcene	0,81	1,04	0,93	0,78
α-terpinene	-	-	0,13	0,05
limonene	0,07	-	-	0,04
(Z)-β-ocimene	0,24	0,27	0,22	0,19
(E)-β-ocimene	2,02	2,24	1,90	1,56
terpinolene	0,18	0,17	0,32	0,27
1081	0,11	-	0,31	0,23
linalool	0,81	0,69	0,90	0,82
1093	0,13	0,20	0,09	0,16
camphor	0,42	0,48	0,32	0,40
1118	0,67	0,69	0,76	0,73
camphene hydrate	0,67	0,64	0,72	0,74
citronellal	9,07	22,97	11,13	21,67
borneol	2,64	2,29	2,63	2,61
terpinen-4-ol	0,10	-	0,29	0,13
α-terpineol	3,46	3,14	3,35	3,48
nerol	-	1,08	-	-
neral	30,43	25,30	27,95	24,13
geraniol	2,14	1,62	0,21	0,12
geranial	34,66	27,33	35,74	30,63
1245	0,35	0,84	0,30	0,76
bornyl acetate	0,11	-	0,08	0,10
1299	0,39	0,39	0,28	0,38
geranyl acetate	1,82	0,78	1,24	1,28
β-caryophyllene	4,37	3,73	3,86	4,68
α-humulene	0,19	-	0,14	0,17
(E)-β- farnesene	0,18	-	-	-

4C sampling 04092007 fresh plants 4D sampling 04092007 dry plants

4E sampling 18092007 fresh plants 4F sampling 18092007 dry plants

b) Effects of drying

The influence of drying on yield and components is discussed only by selected examples, at the main components citronellal, neral and geranial. From the group of monoterpene hydrocarbons are compared β-pinene or sabinene, (E)-β-ocimene and as representatives of the alcohols borneol and α-terpineol. Furthermore β-caryophyllene from the group of sesquiterpenes is used.

As can be seen in Table 6 the content of all components was subject to small fluctuations. One can see a small increase from citronellal for the first sampling (9.0 and 11.1%) and geranial for both samplings. Neral showed a reduced content.

Furthermore, the drying process had no great influence on β-caryophyllene. Concentration of β-pinene decreased during the drying process.

B) QUEDLINBURGER

1) First sampling

Oil from *Melissa Quedlinburger* from the samples at the beginning of August shows the following composition (Figure 12, see chapter 6). Four main components can be found in the oil obtained by both methods. Monoterpene aldehydes represented the main part of the oil, reaching a content of about 85.0%. From the sesquiterpenes we can find β-caryophyllene as main component. Values from citronellal did not differ a lot for both methods. Showing a content of 8.9 or 9.2%, citronellal was the aldehyde reaching the smallest concentration. Neral and geranial were present in a concentration higher than 30.0% for neral or 40.0% for geranial. The content from neral was higher after DW (DW: 32.2%; DS: 27.3%). Geranial attained the highest concentration in oil obtained by DS (47.3%). For the oil obtained by DW a content of 43.9% could be found. For the hydrodistilled oil a remarkable high content of geraniol (3.2%) as an intermediate product was found. The second important group - concerning the concentration of components – was the group of sesquiterpene hydrocarbons. The dominating component was β-caryophyllene with 3.3% for distilled oil and a high content of 6.3% for oil obtained by DS. All other sesquiterpenes (α-humulene, (E)-β-farnesene, germacrane-D and β-caryophyllene) were represented in a concentration < 1.0% and were not present in both oils. As monoterpene alcohols linalool (0.4 and 0.3%), borneol (1.0 and 1.6%), α-terpineol (1.6 and 2.1%), nerol (0.9%) and geraniol (3.2%) were found. Geranyl acetate as representative from monoterpene esters attained a content of 1.0 (DS) and 1.6% (DW). Monoterpene hydrocarbons did not come to a content higher than 1.0%. They arrived at a total amount of 2.4 (DS) and 1.9% (DW). Detailed information about composition of oil can be found in Appendix 4.

2) Second and third sampling

Table 7: Chemical composition of *Melissa Quedlinburger* at different harvesting dates and different drying stages

	5C	5E	5D	5F
β-pinene + sabinene	0,50	0,02	-	0,71
n.i.	0,36	0,34	0,28	1,30
n.i.	0,50	1,00	0,47	-
myrcene	0,47	0,48	0,22	0,57
α-terpinene	-	0,03	-	-
(Z)-β-ocimene	0,14	0,12	-	0,16
n.i.	-	0,11	-	0,20
(E)-β-ocimene	1,18	0,92	0,43	1,27
terpinolene	0,16	0,17	0,12	0,23
linalool	0,39	0,35	0,26	0,45
1093	0,20	0,25	0,08	0,13
camphor	0,39	0,40	0,18	0,25
1118	0,62	0,60	0,43	0,64
camphene hydrate	0,52	0,40	0,34	0,49
citronellal	38,51	52,63	29,15	53,24
borneol	1,64	1,23	1,32	1,34
terpinen-4-ol	-	0,07	-	0,08
α-terpineol	2,30	1,61	1,81	1,72
nerol	0,47	-	-	-
neral	19,89	16,21	19,99	13,19
geraniol	0,68	0,48	-	0,06
geranial	23,81	15,83	33,12	18,23
1245	1,69	2,32	-	1,69
1299	0,33	0,34	0,41	0,30
geranyl acetate	0,81	0,49	1,54	0,45
β-caryophyllene	4,43	3,05	5,92	2,77
α-humulene	-	0,13	0,28	0,10
(E)-β-farnesene	-	0,10	0,26	0,09
germacrane D	-	0,05	0,18	-
1492	-	-	0,09	-
caryophyllene oxide	-	0,06	0,43	0,05
			Components after t = 60min: 2,07%	

5C sampling 04092007 fresh plants
5D sampling 04092007 dry plants
5E sampling 18092007 fresh plants
5F sampling 18092007 dry plants
n.i. not identified

Distillation of *Melissa Quedlinburger* (Table 7) showed very little differences to other varieties. An enrichment of monoterpene aldehydes in a concentration of more than 80% could be achieved for both harvesting dates. Citronellal was thereby present in the highest concentration. Monoterpene alcohols (linalool, borneol, terpinen-4-ol, α-terpineol, nerol and geraniol) yielded in a concentration of 5.5 and 3.7%. Concentration of monoterpene hydrocarbons was not very

representative. One main component ((E)-β-ocimene) in a concentration higher than 1.0% could be found. From the group of sesquiterpene hydrocarbons β-caryophyllene (4.43 and 3.05%) was the main component. All other representatives could be found only in the oil from the second sampling.

a) Effects of harvesting time

A characteristic of *Melissa Quedlinburger* oil is the rapid increase of citronellal within 2 weeks (38.5 to 52.6%) and the decrease of the two further aldehydes over approximately 8.0% for the benefit of citronellal. (neral: 19.9 to 16.2%; geranial: 23.8 to 15.8%). In comparison to the values from the beginning of September we obtained lower concentrations after two weeks of growing for all components. Results from (E)-β-ocimene (1.2/0.9%), borneol (1.6/1.2%), α-terpineol (2.3/1.6), 1245 (1.7/2.3%) and β-caryophyllene (4.4/3.1%) and all other components can be seen in Table 7.

b) Effects of drying

As can be seen in Table 7 drying furnished (in most of the cases) no positive effect on the yield of components. From geranial one can see an increase for both samplings showing in one case a difference of more than 10.0%. For the other components, the variation between fresh and dry plants was not as significant, showing differences between 0.1 and 1.5%. Mentionable differences could be observed for citronellal from the samples at the beginning of September showing a decrease from 38.5 to 29.2%.

C) ERFURTER AUFRECHTE

1) First sampling

The oil extracted from the samples from the 14th of August from *Erfurter Aufrechte* using DW and DS showed differences in chemical composition (Figure

12). A higher amount of components using DW was found. Using GC/FID more than 25 components could be identified in the two samples.

The most dominating components were neral and geranial. They were found with DS and DW with a higher content for DW. (DS: 30.5 and 46.8%; DW: 20.7 and 40.1%). Citronellal was also found in the oil obtained by DW and DS (9.7 and 12%). The content of monoterpene hydrocarbons was higher for DW. They come to a total amount of 2.9% (DS) and 1.9% (DW). Sabinene, β-pinene, α-phellandrene, terpinolene, camphor and two unidentified components (KI 1093 and 1120) could only be found in the oil from DW. The monoterpene, alcohols borneol (0.8 and 1.2%), α-terpineol (1.2 and 1.7%) and linalool (0.6 and 0.5%) were present in both oils. The content was again higher for DS. Terpinen-4-ol was absent in the oil from DS. Monoterpene esters were represented by geranyl acetate (DS: 1.1%; DW: 2.4%), sesquiterpene hydrocarbons by β-caryophyllene (DS: 4.3; DW: 14.8%), α-humulene, (E)-β-farnesene and germacrane D. A small amount of 0.4% of caryophyllene oxide was obtained from the DW extracted oil (DS: 0.2%).

2) Second and third sampling

Table 8 shows the various components obtained by WSD from the plant material from two different harvesting dates and two different drying stages.

Around 80% of the oil monoterpene aldehydes (citronellal, neral and geranial) were main components. Contrary to the oil from *Melissa Citronella*, citronellal (samples from 4th of September) was with about 43.1% over the value from neral (17.9%) and geranial (18.3%). Further important (> 1%) were (E)-β-ocimene, borneol and α-terpineol from the group of monoterpenes and β-caryophyllene as representative from sesquiterpenes. One unidentified component (KI 1245) can be found in a concentration higher than 2%. All other components listed in the table are an example for the complex composition of Melissa oil and are only considered in a few cases.

a) Effects of harvesting time

Effects of harvesting time are discussed only by components attaining a concentration higher than 1.0%. One interesting point that can be adressed is the increase of citronellal and the decrease of neral and geranial within two weeks of growing. Citronellal was present in the oil (18th of September) with more than 50%, neral decreased in the course of the season to 10% and geranial come down to 13%.

Table 8: Chemical composition of *Melissa Erfurter Aufrechte* at different harvesting dates and different drying stages

Name	6C	6E dry	6D	6F dry
β-pinene + sabinene	0,53	0,52	0,40	0,18
β-pinene+ sabinene	0,44	0,35	-	0,87
n.i.	-	0,59	-	-
myrcene	0,53	0,64	0,17	0,37
α -phellandrene	0,15	-	-	-
α -terpinene	0,19	-	-	-
(Z)-β-ocimene	0,18	0,28	-	0,12
1035	0,06	0,11	-	0,24
(E)-β-ocimene	1,44	2,23	0,57	0,95
terpinolene	0,17	0,19	0,15	0,24
linalool	0,55	0,73	0,44	0,84
1093	0,26	0,29	0,12	0,22
camphor	0,42	0,51	0,22	0,31
1118	0,68	0,69	0,53	0,68
camphene hydrate	0,42	0,41	0,33	0,39
citronellal	43,12	53,19	34,41	56,43
borneol	1,44	1,25	1,29	1,05
terpinen-4-ol	0,07	-	-	0,09
α-terpineol	1,91	1,73	1,78	1,39
nerol	-	0,46	-	-
neral	17,88	9,98	14,49	11,57
1210/1748	-	4,87	1,94	-
geraniol	1,03	0,62	-	0,10
geranial	18,25	13,02	21,82	15,87
1245	2,11	2,31	6,49	2,22
1299	0,38	0,29	0,40	0,30
geranyl acetate	0,52	0,29	0,22	-
n.i.	-	-	1,39	-
β-caryophyllene	6,52	4,05	8,88	0,34
α-humulene	0,27	0,16	0,41	4,06
(E)-β-farnesene	0,27	0,16	0,46	0,15
n.i.	-	-	0,19	0,16
n.i.	-	-	0,10	-
caryophyllene oxide	0,10	-	0,57	-
			Components after t_R=60min: 2%	

6C sampling 04092007 fresh plants 6D sampling 04092007 dry plants

6E sampling 18092007 fresh plants 6F sampling 18092007 dry plants

n.i. not identified

b) Effects of drying

As can be seen in Table 8 changes of components during the drying process were more obvious for components reaching a higher concentration. Compontents showing a difference in content of more than 2% were chosen and compared. Citronellal had the highest yield and differences between the different drying stages were most significant. Content diminished from 43.1 to 34.4% (first sampling) and 53.2% to 56.4% (second sampling). As an effect of drying the content of neral diminished from 17.9 to 14.5% at the first sampling and increased from 9.9 to 11.5% in the middle of September. All other main components were positive affected by the process of drying, comparing in particular geranial, one unidentified component (KI-SPB1 1245), β-caryophyllene and α-humulene.

D) STAMM NLC

1) First sampling

Composition of oil from *Melissa Stamm NLC* can be seen in Figure 12. Main components were again the monoterpene aldehydes neral, geranial and citronellal. Content of neral was higher for DS (23.7 and 32.4%), whereas the content of citronellal (9.4 and 3.4%) and geranial (43.9 and 7.7/42.6%) was higher for DW. Classification of geranial could not be done on the SPB1 side. Geranial decomposed into two different components (KI: 1241 and 1245). On the column geranial could be found at a KI of 1702 and a concentration of 47.7%. Geranial as important intermediate product during synthesis was present in the hydrodistilled oil in a concentration of 0.5%. Monoterpene hydrocarbons could be detected only in traces (DS: 2.8%; DW: 1.3%) from them (E)-β-ocimene could be found from hydrodistilled oil (> 1 %). Myrcene, (Z)-β-ocimene and terpinolene were absent in the oil from DS. (Detailed information see Appendix 5).

For monoterpene alcohols a percentage of 4.3 and 2.9 were reached. We got a relative high amount for borneol (1.4 and 0.9%) and α-terpineol (1.9 and 1.3%).

Geranyl acetate (1.9 and 1.3%) as only ester was as present as were sesquiterpene hydrocarbons. Main representatives were β-caryophyllene, α-humulene, (E)-β-farnesene, one unidentified component (KI SPB1:1492) and germacrane D.

2) Second and third sampling

The composition of the oil obtained by WSD can be seen in Table 9. Plants from two different harvesting dates and different drying states were used. Running time for each extraction was 4 hours. Components, which form also the main part of the oil, were (similar to all other varieties) neral, geranial and citronellal. The obtained oil from both samplings contained nearly 80% of monoterpene aldehydes. Distribution of the content was different to the one from other varieties and was with 33.4% (2nd sampling) or 48.4% (3rd sampling) higher for citronellal. Content of neral and geranial came between 20 and 25%. Further mentionable components were β-pinene or sabinene, (E)-β-ocimene, borneol and α-terpineol. The sesquiterpenes were represented by β-caryophyllene.

a) Effects of harvesting time

Content of monoterpene aldehydes was affected most of all by the different harvesting dates. Although a time horizon of only two weeks was between the two samplings one could observe an increase of citronellal from 33.4 to 48.4%. The yield of neral and geranial showed significant differences during the two weeks. A decrease from 20.9 to 15.5% (neral) and 24.9 to 15.0% (geranial) was recorded. For all other components only small changes were found.

b) Effects of drying

The distillates from the two harvests and the two drying stages are listed in Table 9. Remarkable are the relative small fluctuations which arise between fresh and dry plants.

Table 9: Chemical composition of *Melissa Stamm NLC* at different harvesting dates and different drying stages

Name	7C	7E	7D	7F
830	-	-	0,14	0,22
camphene	-	-	0,09	0,07
β-pinene + sabinene	0,91	1,31	0,84	1,37
β-pinene + sabinene	1,07	1,05	1,29	1,28
myrcene	0,57	0,65	0,50	0,62
α-phellandrene	0,13	0,13	-	-
α-terpinene	-	0,08	0,08	-
(Z)-β-ocimene	0,37	0,41	0,24	0,31
1035	-	0,11	0,12	0,30
(E)-β-ocimene	3,12	3,38	2,07	2,52
terpinolene	0,19	0,17	0,24	0,28
1081	-	0,06	0,16	0,10
linalool	0,93	0,95	0,99	1,27
1093	0,21	0,26	0,12	0,19
camphor	0,40	0,43	0,27	0,34
1118	0,57	0,52	0,61	0,57
camphene hydrate	0,55	0,49	0,58	0,61
citronellal	33,43	48,36	34,77	45,70
borneol	1,75	1,45	1,77	1,54
terpinen-4-ol	-	0,06	0,13	0,09
α-terpineol	2,38	1,95	2,38	2,03
nerol	0,41	-	-	-
neral	20,94	15,52	20,04	15,05
geraniol	0,89	0,58	0,16	0,08
geranial	24,04	14,97	26,98	18,68
1244	1,24	1,84	1,05	1,43
1257	-	0,10	-	0,07
1299	0,24	0,27	0,21	0,25
geranyl acetate	0,78	0,32	0,71	0,44
β-caryophyllene	4,48	4,04	3,33	3,85
α-humulene	0,18	0,16	-	0,15
(E)-β-farnesene	0,24	0,18	-	0,16

7C sampling 04092007 fresh plants 7D sampling 04092007 dry plants

7E sampling 18092007 fresh plants 7F sampling 18092007 dry plants

E) LEMONA

1) First sampling

In chromatograms from *Melissa Lemona* different groups of components can be seen (Figure 12). Main group was again the one of monoterpene aldehydes with neral, geranial and citonellal as main representatives. Content of neral and citronellal showed about the same content for DS and DW, whereas geranial decomposed in one case into two different products on the SPB1 side, but

verification on the column (wax side) gave clearer contents of geranial with 50.5%. Detailed results can be seen in Appendix 6. In the front section of the chromatogram monoterpene hydrocarbons with a total amount of 1.6 (DS) and 1.7% (DW) can be found. In both oils sabinene and β-pinene, (E)-β-ocimene, one unidentified component (KI 1118) and camphene hydrate are presented. A larger group with 3.1 and 4.4% formed monoterpene alcohols. The main part from this group was characterized by the presence of linalool (DS: 0.5%; DW: 0.3%), borneol (DS: 1.0%; DW: 1.7%), terpinen-4-ol (DS: 0.1%) and α-terpineol (DS: 1.4%; DW: 2.3%). One monoterpene ester (geranyl acetate) was present in both oils. Main sesquiterpenes were β-caryophyllene (DS: 3.1; DW: 7.4%) and α-humulene (DS: 0.2; DW: 0.3%).

2) Second and third sampling

WSD resulted in various components listed in Table 10. Regarding the different parts of the obtained chromatograms monoterpene aldehydes form the principal part of the oil. Rates from citronellal, neral and geranial were ~ 75%. Concentration increased in the following order: citronellal (14.2/18.1%) < neral (~28.5%) < geranial (~33.0%). β-pinene, (E)-β-ocimene, borneol, α-terpineol, geraniol, geranyl acetate were the second important group of monoterpenes; β-caryophyllene, as representative from sesquiterpenes came to a content of 4.9 or 4.2%.

a) Effects of harvesting time

During the two weeks of growing a number of changes occured in the distilled oil mainly of citronellal, geranial and neral. Contrary to all other varieties there could be observed only a small increase of citronellal (4.04%) and a decrease of geranial from 2.3%. Neral showed about the same concentration in both growing stages. All other components did not change their amount a lot in the course of the season. Results can be seen in Table 10.

b) Effects of drying

Comparing the distillates of *Melissa Lemona* it is noticeable that the drying process exerts only a small influence on the content of the individual substances. The majority of the components are affected negatively by the drying process. Significantly higher differences are reached for citronellal, neral, geranial and β-caryophyllene. While for citronellal, neral and β-caryophyllene the values are relative constant and maximum fluctuations of 2% occur, geranial shows large dispersions. The content of Geranial is affected positively by the drying process and shows concentration differences of approximately 4% for the first harvest and more than 7% for the second harvest date.

Table 10: Chemical composition of *Melissa Lemona* at different harvesting dates and different drying stages

Name	8C	8E	8D	8F
α-pinene	-	0,04	-	0,04
β-pinene + sabinene	1,15	0,03	-	0,03
β-pinene + sabinene	0,25	1,18	2,22	0,95
969	-	0,21	-	-
myrcene	0,99	0,75	0,97	0,37
α-phellandrene	0,06	-	-	-
α-terpinene	0,07	-	-	0,02
limonene	0,06	0,05	-	0,02
(Z)-β-ocimene	0,18	0,13	0,14	0,06
1035	-	0,03	-	0,06
(E)-β-ocimene	1,42	0,99	1,23	0,52
terpinolene	0,24	0,20	0,33	0,21
camphor	0,12	0,10	0,17	0,10
linalool	0,80	0,34	0,84	0,57
1093	0,18	0,18	-	0,08
camphor	0,49	0,48	0,36	0,24
	0,84	0,83	0,89	0,70
camphene hydrate	0,76	0,65	0,73	0,51
citronellal	14,17	18,13	12,33	17,11
borneol	2,88	2,63	2,56	1,91
terpinen-4-ol	-	0,12	-	0,14
α-terpineol	3,81	3,27	3,47	2,54
neral	28,46	28,31	28,69	26,99
1223	-	0,17	-	-
geraniol	1,46	1,15	0,23	-
geranial	34,13	32,47	38,16	39,97
1245	0,67	0,79	0,42	-
bornyl acetate	0,12	0,12	-	0,11
1299	0,41	0,42	0,29	0,43
geranyl acetate	1,18	1,47	1,54	1,46
β-caryophyllene	4,97	4,15	4,29	3,99
αhumulene	0,19	0,16	-	0,16
caryophyllene oxide	-	0,06	-	0,20

8C sampling 04092007 fresh plants

8D sampling 04092007 dry plants

8E sampling 18092007 fresh plants

8F sampling 18092007 dry plants

F) AUFRECHTE

1) First sampling

Chromatographic results from *Melissa Aufrechte* can be divided into two main parts; monoterpenes and sesquiterpenes. They can be divided according to their oxidation stage into hydrocarbons, aldehydes and alcohols.
The main part of the oil is made up of monoterpene aldehydes (geranial, neral and citronellal) (DS: 88.3%; DW: 72.4%). The content of geranial and neral was higher for the oil obtained by DW. All three components are responsible for the smell of the oil. In the first section of Figure 12 monoterpene hydrocarbons with a total amount of 2.6 (DS) and 1.8% (DW) can be found. Quantitative outstanding representatives were β-pinene, myrcene and (E)-β-ocimene. They are present in both oils. Generally monoterpene hydrocarbons were more present in the oil obtained by DW. Monoterpene alcohols were represented by linalool (DS: 0.6%; DW: 0.4%), borneol (DS: 0.9%; DW: 1.6%), terpinen-4-ol (DS: 0.1%) and α-terpineol (DS: 1.4%; DW: 2.2%). Geranyl acetate, as the only representative of esters, comes to an amount of 0.6% for the distilled oil and a content of 1.3% for the oil obtained by DS.
The oil from DS contained sesquiterpenes belonging according to their oxidation stage to hydrocarbons and alcohols. Main components were β-caryophyllene (11.4%) and (E)-β-farnesene (1.1%). Remarkable for DW oil was the high number of components after a KI of 1660, with one unidentified component showing a concentration higher than 1.1%. Sesquiterpenes obtained during distillation were not as well worked out as components obtained by DS. This can be seen at the lower concentration of β-caryophyllene (3.7%) and the lower number of higher sesquiterpene alcohols. For detailed information of chemical composition see Appendix 7.

2) Second and third sampling

The distribution of components obtained from *Melissa Aufrechte* can be seen in Table 11. Contrary to other varieties all three main components (citronellal, neral

and geranial) were present in the oil in equal parts, showing all together a total amount of 75.9 (9C) and 80.76% (9E). Additionally, oil from *Melissa Aufrechte* showed higher concentrations of (E)-β-ocimene, borneol, α-terpineol and one unidentified component (KI SPB1: 1245). Only two sesquiterpenes could be found in the oil, from these β-caryophyllene represented the main part (6.3 (9C) and 3.5% (9E)). All other components whose presence is well known in Melissa oil are not discussed in detail.

Table 11: Chemical composition of *Melissa Aufrechte* at different harvesting dates and different drying stages.

Name	9C	9E	9D	9F
sabinene+β-pinene	0.42	0.6	0.42	0.8
sabinene+β-pinene	0.77	0.66	0.69	0.65
myrcene	0.88	0.66	0.71	0.61
(Z)-β-ocimene	0.22	0.14	0.14	0.14
1035	-	0.05	0.08	0.16
(E)-β-ocimene	1.87	1.08	1.16	1.17
terpinolene	0.22	0.21	0.29	0.3
1081	0.11	0.07	0.16	0.15
linalool	0.64	0.6	0.64	0.83
1093	0.21	0.19	0.13	0.15
camphor	0.45	0.44	0.31	0.32
1119	0.79	0.79	0.85	0.81
camphene hydrate	0.73	0.65	0.65	0.66
citronellal	26.44	26.16	19.7	31.02
borneol	2.73	2.52	2.51	2.08
terpinen-4-ol	-	0.09	0.09	0.13
α-terpineol	3.6	3.27	3.23	2.69
nerol	0.52	-	-	-
neral	21.72	24.94	24.14	21.62
1223	-	0.07	-	-
geraniol	0.92	1.13	0.22	0.12
geranial	27.75	29.66	35.18	29.45
1245	1.23	1.09	0.6	1.04
1257	-	0.12	0.09	-
1299	0.41	0.4	0.36	0.31
geranyl acetate	0.83	0.83	1.33	0.65
β-caryophyllene	6.3	3.46	5.43	3.52
α-humulene	0.24	0.14	0.21	0.13

9C sampling 04092007 fresh plants 9D sampling 04092007 dry plants

9E sampling 18092007 fresh plants 9F sampling 18092007 dry plants

a) Effects of harvesting time

Melissa Aufrechte is a good example showing small changes of secondary plant metabolites within a short period of growing. Main components citronellal, neral and geranial were present in concentrations of more than 20.0%. Fluctuation of concentration of citronellal was compared with concentration of neral and geranial very small (the difference between the two harvests was 0.28%). Values from neral (21.7 and 24.9%) and geranial (27.8 and 29.7%) differed between 1.9 and 3.2%. A higher decline within two weeks showed (E)-β-ocimene (1.87 and 1.08%) and β-caryophyllene (6.3 and 3.5%).

b) Effects of drying

The influence of drying on yield and components is discussed only by selected examples; at the main components citronellal, neral and geranial. Furthermore β-caryophyllene from the group of sesquiterpenes is used. It was found that changes in moisture content of the plants significantly affected yield of citronellal and geranial. The content of citronellal decreased at the first sampling from 26.4 to 19.7% whereas for the second sampling an increase from 26.1 to 31% could be observed. Neral decreased its amount at the beginning of September showing an increase after two weeks of growing. Also geranial decreased its amount from 27.7% to 35.2%. Content of geranial from the second sampling and content of β-caryophyllene were not significantly affected by the process of drying.

G) LORELEI

1) First sampling

The oil from the variety *Lorelei* was obtained using two different methods (DS and DW). The composition of the oil showed nearly the same percentage as for all other varieties and can be seen in Figure 12 (see also Appendix 8 for detailed information of chemical composition). Four main components, citronellal, neral,

geranial and β-caryophyllene, can be found in both oils. One interesting variation can be seen for neral. All other varieties showed nearly the same concentration for both methods. In the oil from *Melissa Lorelei*, neral was present in an amount of 32.8% for distilled oil, contrary to a small amount of 2.0% in the oil from DS. This means that the variation of the neral content changes by 16th times. Aldehydes were found in different concentrations in both oils. The content in distilled oil was with 87.1% higher than in oil obtained by DS (55.1%). Only three components got an amount higher than 1%. Two monoterpene alcohols, borneol (DS: 1.0%; DW: 1.5%) and α-terpineol (DS: 1.4%; DW: 2.0%); from sesquiterpene hydrocarbons one can find β-caryophyllene (DS: 4.0%; DW: 11.6%). All other components were present in a concentration less than 1%. Interesting is also the presence of higher alcanes that occured after 60min.

2) Second and third sampling

Table 12: Chemical composition of *Melissa Lorelei* at different harvesting dates and different drying stages

Name	10C		10E	10D	10F
(E)-β-ocimene	0.22	β-pinene + sabinene	0.12	2.22	0.22
citronellal	6.32	β-pinene + sabinene	0.73		0.20
borneol	0.18	myrcene	0.36	0.97	0.26
α-terpineol	0.40	α-phellandrene	0.04		
1189	0.25	(Z)-β-ocimene	0.11	0.14	
neral	1.17	1035	0.11		0.15
1210	1.06	(E)-β-ocimene	0.84	1.23	0.69
1230	1.55	terpinolene	0.16	0.33	0.12
geranial	2.76	1081	0.03	0.17	
1244	2.50	linalool	0.49	0.84	0.49
1247	0.80	1093	0.24		0.16
1285	1.01	camphor	0.39	0.36	0.27
1300	1.22	1119	0.64	0.89	0.52
1316	0.86	camphene hydrate	0.34	0.73	0.27
1325	0.30	citronellal	53.79	12.33	53.61
1327	1.71	borneol	1.07	2.56	0.92
1356	0.51	terpinen-4-ol	0.07		
α-copaene	1.09	α-terpineol	1.46	3.47	1.30
geranyl acetate	2.43				
1374	0.22	neral	15.62	28.69	10.00
1388	0.23				
β-caryophyllene	63.09	geraniol	0.58	0.23	
α-humulene	2.85	geranial	14.60	38.16	16.52
1435	0.37	1245	2.62	0.42	2.92
1444	0.45	1257	0.09		
(E)-β-farnesene	3.18	1299	0.32	0.29	0.38
germacrane D	1.08	α-copaene	0.40	1.54	0.65
1492	0.54	β-caryophyllene	4.04	4.29	7.78
1498	0.45	α-humulene	0.17		0.36
caryophyllene oxide	0.70	(E)-β-farnesene	0.13		0.30
1571	0.42	germacrane D	0.04		0.16
1803	0.08	caryophyllene oxide	0.08		0.54
					1,01% after
1867	0.05				$t_{R=}$60min.

10C sampling 04092007 fresh plants 10D sampling 04092007 dry plants

10E sampling 18092007 fresh plants 10F sampling 18092007 dry plants

In this paragraph the composition of the pure oil obtained by WSD is shown (Table 12). Normally WSD is a more sensitive method and a danger of chemical

changes of components is minimal. It´s surprising that chemical composition from *Melissa Lorelei* obtained from the samples on 4th of September was totally different to all other varieties. Main component was β-caryophyllene (63.1%). Monoterpene aldehydes presented the second important group with citronellal (6.3%) as main component. The content of neral and geranial (1.2 and 2.8%, respectively) was manifold under the quantity of other varieties. Remarkable is also the presence of various unidentified components between a KI, on SPB1 side, of 1210 and 1356. Also the presence of various sesquiterpenes (with over 1.0%) showed a difference to other varieties. More representative results furnished the samples from the middle of September and the results from dry plants. Results from fresh plants at the beginning of September might be connected with an overheating of the system and as a fact hydrolysis or changes of volatile components occurred.

Concerning the concentration of components from sample 10E there can be seen a closer connection to other varieties. Regarding the results from the 18th of September two main groups were be found. Monoterpenes (95.0%) form the main part of the oil. Sesquiterpenes were present in an amount of about 5% with β-caryophyllene as main component. Monoterpene aldehydes were represented by citronellal (53.8%), neral (15.6%) and geranial (14.6%). Hydrocarbons and alcohols made the remaining part of monoterpenes, with borneol (1.1%) and α-terpineol (1.5%) as outstanding components.

a) Effects of harvesting time

Differences in concentration of the main components can only be shown by comparing results from dry plants. They might be affected by the drying process and should be regarded more critically. Monoterpene hydrocarbons were decreased almost completely within the growing period of two weeks with a decrease of β-pinene or sabinene from 2.22% to 0.22%. The variation of aldehydes during two weeks of growing was higher than for all other components. Citronellal increased from 12.33 to 53.61%. Neral and geranial were more than halved showing a diminution from 28.7 to 10.0% (neral) and 38.2 to 16.5%

(geranial). Percentage of linalool, borneol and α-terpineol as representatives from monoterpene alcohols diminished to an amount of around 1.0%. As main representative from sesquiterpenes β-caryophyllene increased from 4.3 to 7.8%. Sesquiterpenes with a KI (SPB1) higher than 1428 were absent at the beginning of September.

b) Effects of drying

Because of missing reference data from the first sampling (beginning of September), results can only be compared from the second sampling (middle of September). The drying process did not affect as much the yield of components. Mentionable results are the changes in neral showing a decrease from 15.6 to 10% and geranial which increased its amount from 14.6 to 16.5%. All other components stayed almost constant.

4.3 Hydrolate

Chromatograms of hydrolate were dominated by three main peaks. At the beginning (t_r=10.4) β-pinene or sabinene (an exact differentiation could not be made) were main representatives. In the last part neral and geranial were the all dominating components (Fig. 13 and Fig. 14). The main part of citronellal, borneol, geranyl acetate and β-caryophyllene was found in the corresponding oil. Thus e.g. the content of citronellal was nine times higher in the oil than in the hydrolate.

5. DISCUSSION AND CONCLUSION

The main question of the study was whether harvesting date, drying process and extraction method can have an effect on yield and quality of essential oils. Furthermore, different varieties from *Melissa* were compared according to their yield and composition of oil.

The analysis of seven different varieties of *Melissa* showed that the most powerful detected components were monoterpene aldehydes (citronellal, geranial and neral). Further important components were (E)-β-ocimene, (Z)-β-ocimene, borneol, α-terpineol and from the group of sesquiterpenes β-caryophyllene and caryophyllene oxide could be found. Within the seven varieties higher changes of yield and composition of components could be observed.

5.1 Melissa Quedlinburger and Melissa Citronella

In the present study, the content of oil and components showed a closer dependence on the extraction method used. Comparing the results from DW and DS, there seems to be a relationship between extraction method and the number of monoterpenes and sesquiterpenes. The distilled oil showed in both cases a higher amount of monoterpenes, whereas the oil obtained by DS revealed more sesquiterpenes. Contrary to all other methods, WSD showed a very high concentration of (E)-β-ocimene and citronellal. The increase or decrease of substances during the vegetation period is in good agreement with seasonal dynamic as reported by PAM-ACW [2].

Differences between the three vegetation periods were more obvious regarding the main components neral and geranial. They showed a comparable seasonal trend with highest rates in the stage before flowering. At this stage the content of neral amounted to 23.8%. Geranial was represented by 31.1%. In the following growing stages the content of neral and geranial diminished to an amount less than 20%. The oil from *Melissa Citronella* was characterized by the absence of borneol and limonene in the stage before flowering.

Highest concentrations of neral, geranial, caryophyllene oxide and (E)-β-farnesene were detected in dry plants.

5.2 Seven Varieties

5.2.1 Green herb yield

Upright varieties (1, 3 and 4) showed a good adaptation on climatic conditions. The productivity from *Melissa Lorelei* was about 10t/ha.
Generally, the harvest changed in the course of the season. *Melissa Erfurter* and also *Melissa Stamm NLC* almost doubled their yield within two weeks. Other varieties showed a smaller increase or decrease within two weeks of growing.

5.2.2 Yield of oil

The yield of oil was strongly affected by group sort, harvesting time and extraction method. DW was the best method concerning the yield of oil.
Upright varieties (except *Melissa Quedlinburger)* showed highest yield, ranging from 0.04 to 0.29%. *Melissa Lorelei* furnished the best yield when DW was used. The lowest yield showed *Melissa Aufrechte* with 0.03%.
DS was not the best method concerning the yield of oil because the oil gets lost during the process because a part of the oil can be found in the corresponding hydrosol. Yield of oil ranged from 0.03 to 0.19%.

5.2.3 Second and third sampling

The oil rate changed rapidly during the different vegetation periods. The drying process showed a positive effect onto the content, although the amount of used plant material was less. The influence of the amount of used plant material on the

content was higher for the samples at the beginning of October. Generally, it can be said that the yield of oil obtained at the beginning of October was higher than the yield of oil from the third sampling (except *Melissa Citronella* and *Melissa Aufrechte* dry). The yield of oil ranged from 0.03% to 0.24%. The highest yield for the dry plants of *Melissa Citronella* was obtained from the third sampling (0.24%). High yield (> 0.1%) was also obtained for *Melissa Stamm NLC* and *Melissa Lemona* dry. A small amount of oil was attributed to *Melissa Lorelei*.

5.2.4 Chemical composition

The analysis of seven different varieties of *Melissa* showed that the most powerful detected components were monoterpene aldehydes (citronellal, geranial and neral). Further important components were (E)-β-ocimene, (Z)-β-ocimene, borneol, α-terpineol and from the sesquiterpenes, β-caryophyllene and caryophyllene oxide could be found. Within the seven varieties higher changes of yield and composition of components could be observed.

Comparing the different extraction methods higher yields and a higher number of components could be observed using WSD. The oil obtained with this method showed a good quality, because the number of undesired secondary products is infinitely small.

Additionally, investigations on plants at different growing stages were carried out. It was found that different harvesting dates significantly affected the yield of components. Especially fluctuation of main components (neral, geranial and citronellal) was higher than for all other components.

Within the study, an analysis from the different drying stages of the plant material was carried out. It was found that changes in plant moisture did affect the yield of oil. The seven varieties showed higher yields of oil after drying. Regarding main components it can be concluded that dried plants are likely to get the same yield as fresh plants.

6. FIGURES

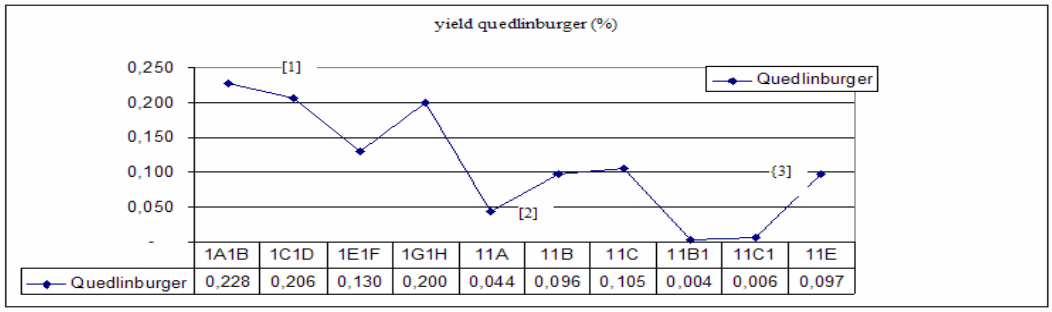


Figure 4: Yield of oil from *Melissa Quedlinburger* obtained by DW (1C1D, 1G1H, 11A, 11E), DS (1A1B, 1E1F) and WSD (11B, 11C) at different harvesting dates: [1] before flowering, [2] flowers, [3] after flowering

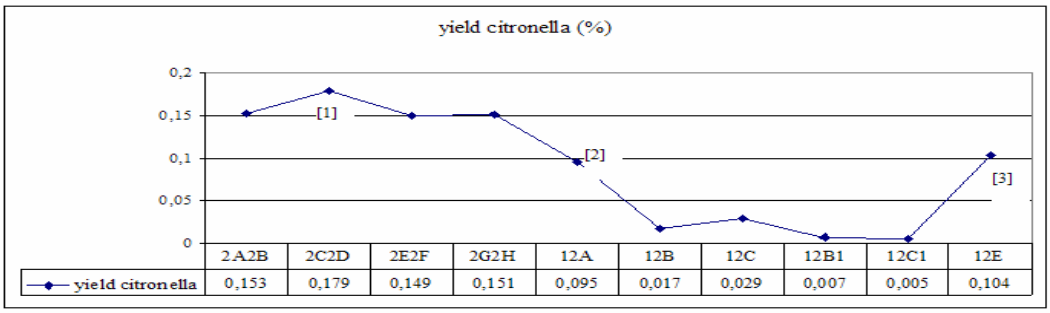


Figure 5: Yield of oil from *Melissa Citronella* obtained by DW (2C2D, 2G2H, 12A, 12E), DS (2A2B, 2E2F) and WSD (12B, 12C) at different harvesting dates: [1] before flowering, [2] flowers, [3] after flowering

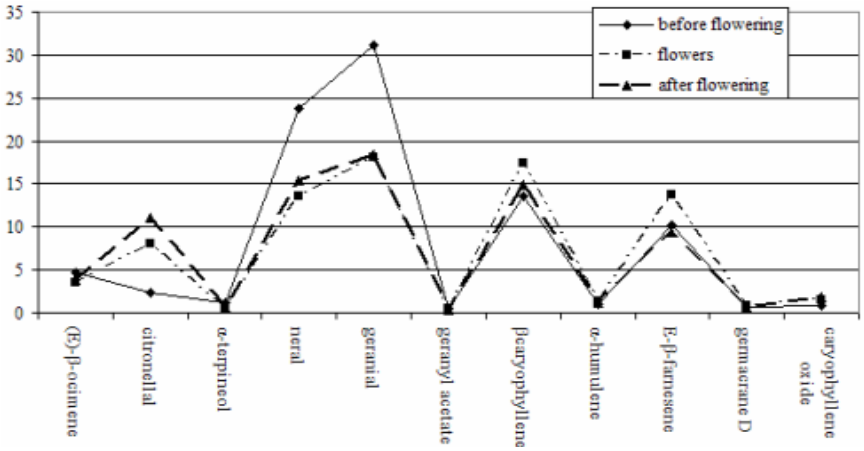


Figure 6: *Melissa Quedlinburger* fresh plants, list of main components obtained by DW at different harvesting dates (2007/06/28-2007/08/06-2007/08/24)

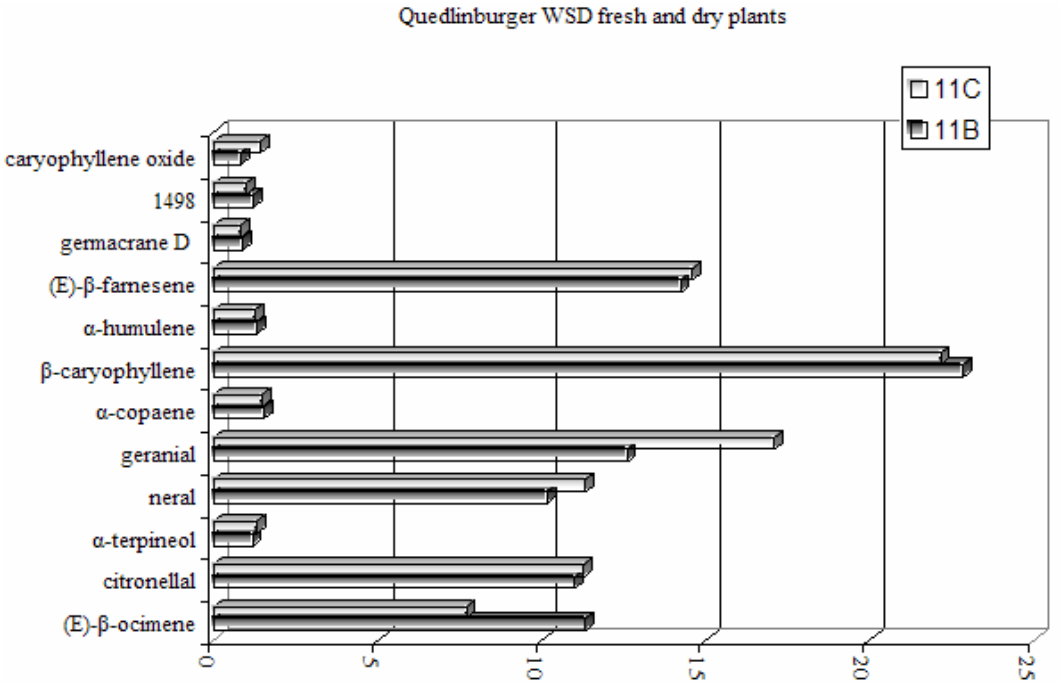


Figure 7: *Melissa Quedlinburger* fresh (11B) and dry (11C) plants, WSD 20070814 and 20070822

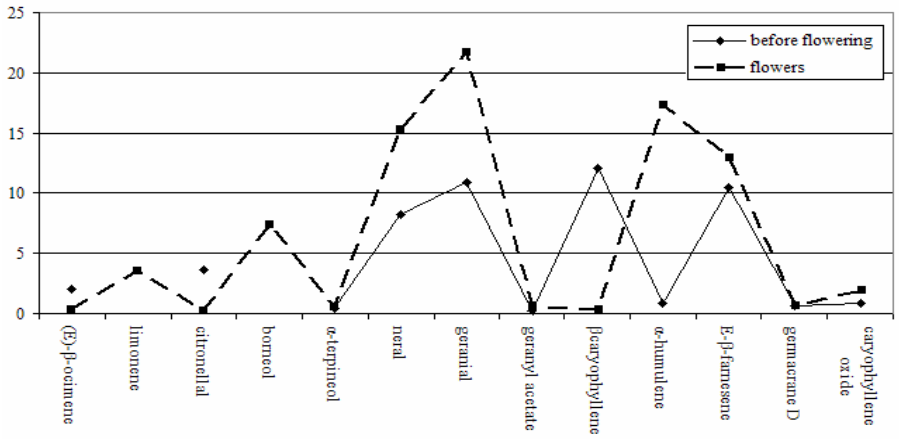


Figure 8: *Melissa Citronella* fresh plants, list of main components obtained by DW at different harvesting dates (2007/06/29-2007/08/06-2007/08/27)

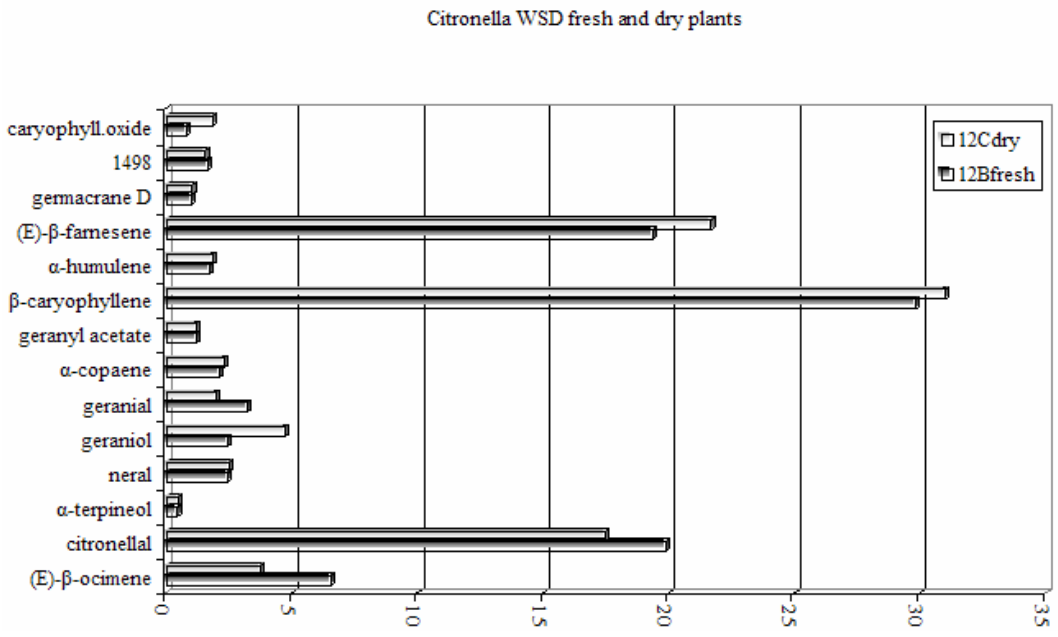
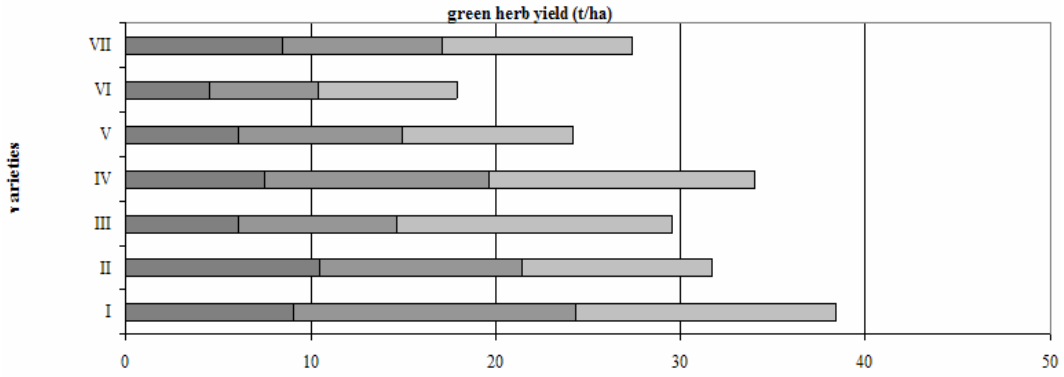
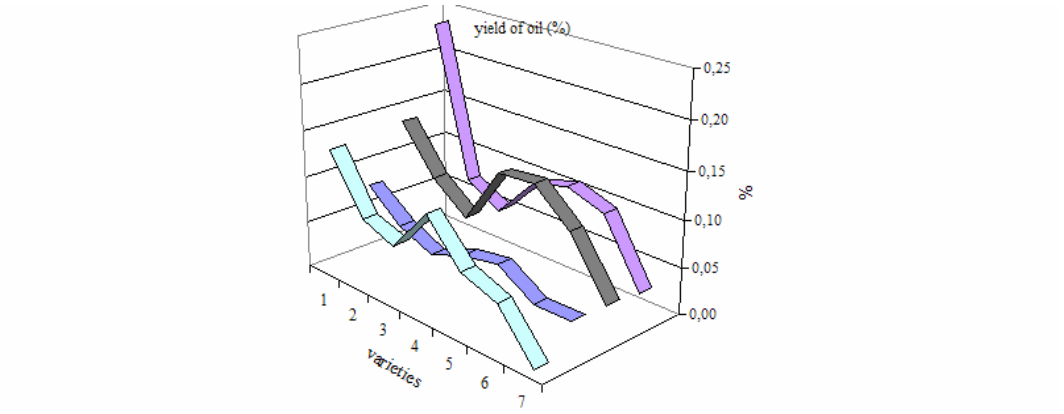


Figure 9: *Melissa Citronella* fresh (12B) and dry (12C) plants, WSD 20070817 and 20070821



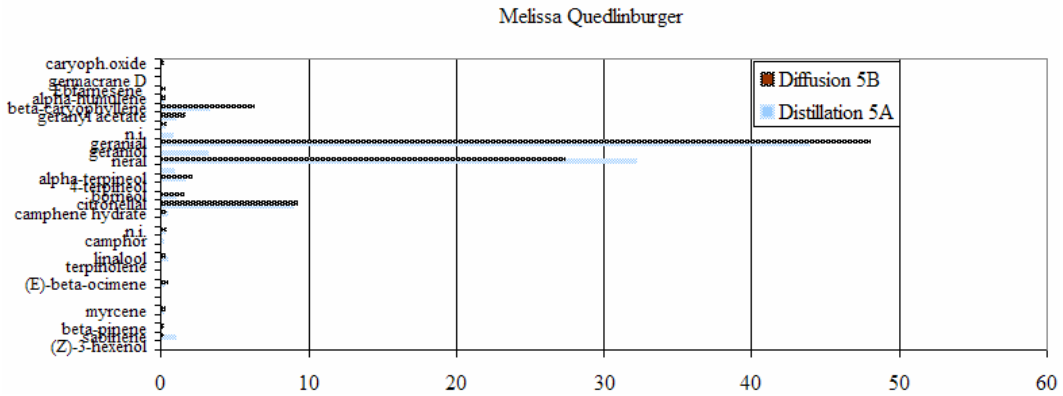
	I	II	III	IV	V	VI	VII
20070918	14	10,2	15	14,4	9,3	7,5	10,3
20070904	15,3	11	8,5	12,1	8,8	5,9	8,6
20070814	9,1	10,5	6,1	7,5	6,1	4,5	8,5

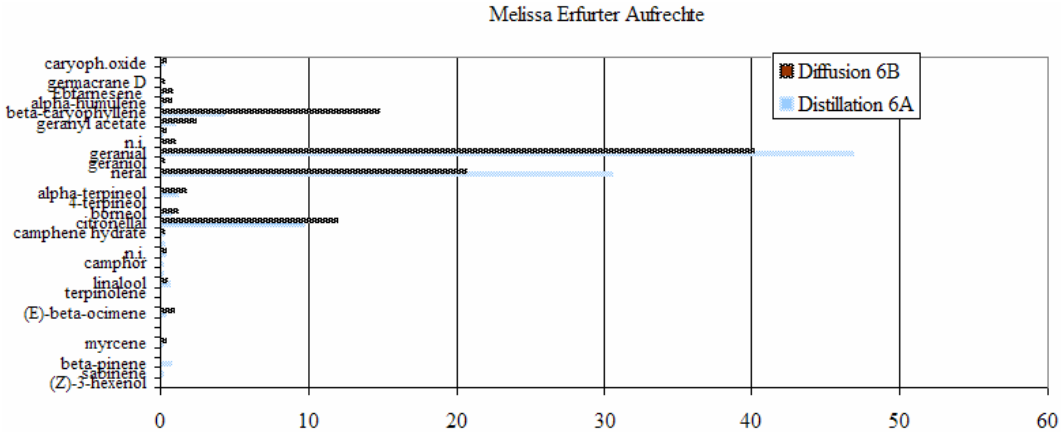
Figure 10: Green herb yield (t/ha) of the 7 varieties from different harvesting dates from August to September 2007.

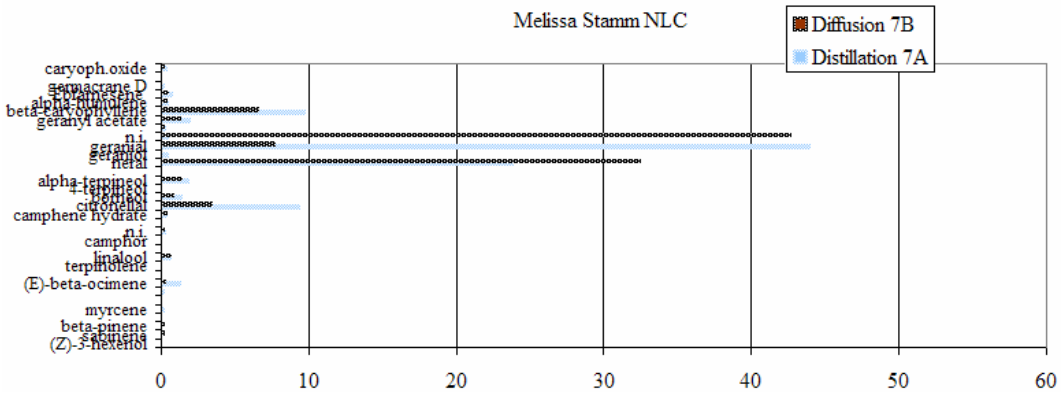


	1	2	3	4	5	6	7
2nd sampling	0,13	0,07	0,06	0,11	0,06	0,05	0,00
3rd sampling	0,08	0,05	0,03	0,05	0,06	0,03	0,03
2nd sampling dry	0,14	0,09	0,06	0,12	0,12	0,09	0,03
3rd sampling dry	0,24	0,07	0,05	0,10	0,11	0,09	0,03

Figure 11: Yield of oil obtained by WSD (20l) from the second and third sampling, fresh and dry plants, 7 varieties.


Melissa Quedlinburger
Diffusion 5B
Distillation 5A
caryoph.oxide
germacrane D
t-bfarnesene
alpha-humulene
beta-caryophyllene
geranyl acetate
n.i.
geranial
geraniol
neral
alpha-terpineol
4-terpineol
borneol
citronellal
camphene hydrate
n.i.
camphor
linalool
terpinolene
(E)-beta-ocimene
myrcene
beta-pinene
sabinene
(Z)-3-hexenol
0
10
20
30
40
50
60


Melissa Erfurter Aufrechte
Diffusion 6B
Distillation 6A
caryoph.oxide
germacrane D
t-bfarnesene
alpha-humulene
beta-caryophyllene
geranyl acetate
n.i.
geranial
geraniol
neral
alpha-terpineol
4-terpineol
borneol
citronellal
camphene hydrate
n.i.
camphor
linalool
terpinolene
(E)-beta-ocimene
myrcene
beta-pinene
sabinene
(Z)-3-hexenol
0
10
20
30
40
50
60


Melissa Stamm NLC
Diffusion 7B
Distillation 7A
caryoph.oxide
germacrane D
t-bfarnesene
alpha-humulene
beta-caryophyllene
geranyl acetate
n.i.
geranial
geraniol
neral
alpha-terpineol
4-terpineol
borneol
citronellal
camphene hydrate
n.i.
camphor
linalool
terpinolene
(E)-beta-ocimene
myrcene
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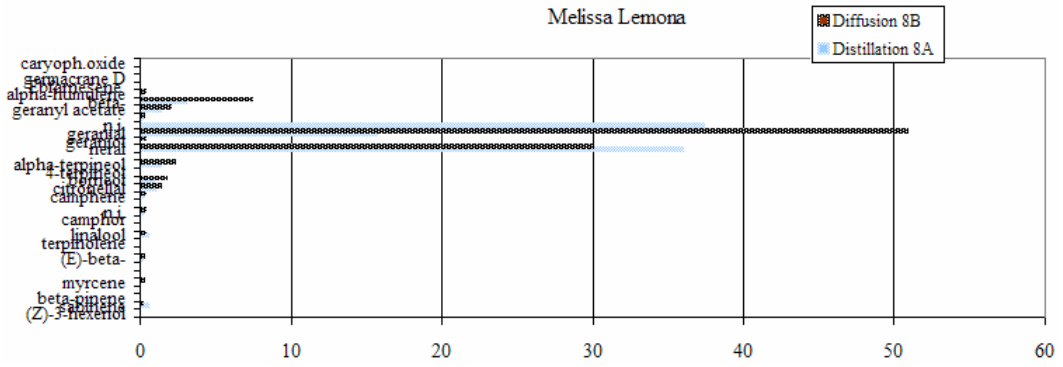
sabinene
(Z)-3-hexenol
0
10
20
30
40
50
60


Melissa Lemona
Diffusion 8B
Distillation 8A
caryoph.oxide
germacrane D
alpha-humulene
geranyl acetate
n.i.
geranial
neral
alpha-terpineol
citronellal
camphene
n.i.
camphor
linalool
terpinolene
(E)-beta-
myrcene
beta-pinene
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0
10
20
30
40
50
60

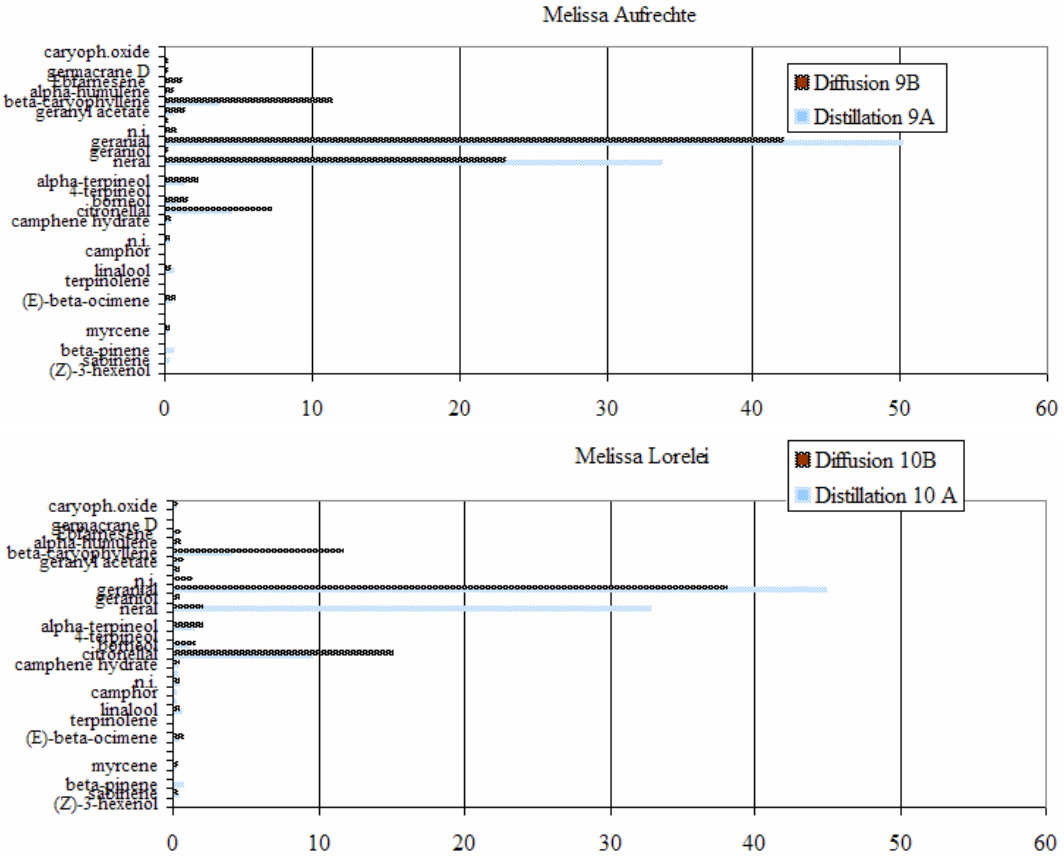

Figure 12: Composition of essential oil obtained by DW and DS at the beginning of August 2007, 7 varieties

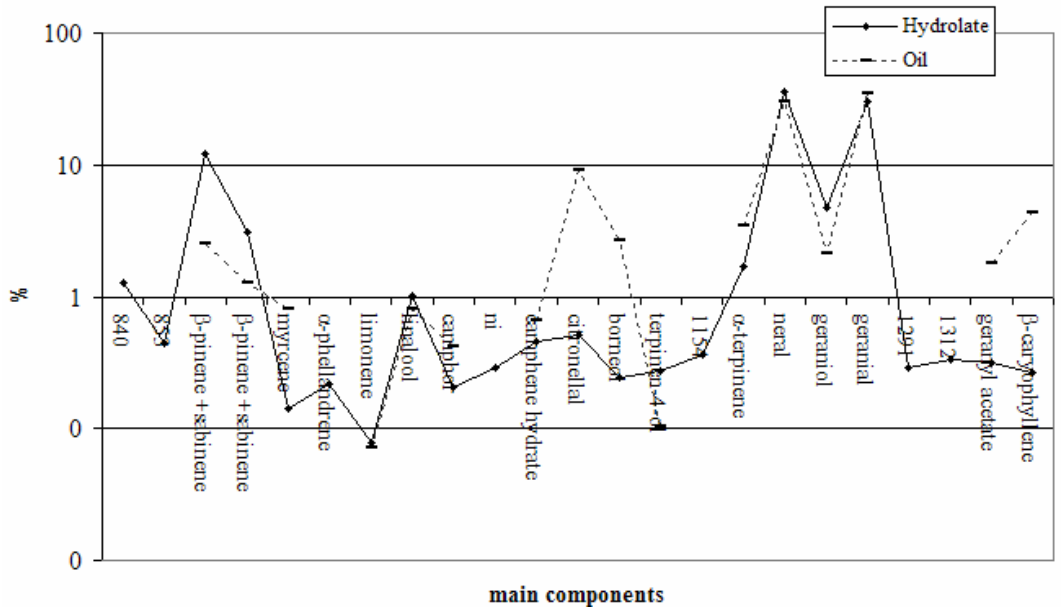

Figure 13: *Melissa Citronella* fresh plants, composition hydrolate-oil, harvesting date: 20070904

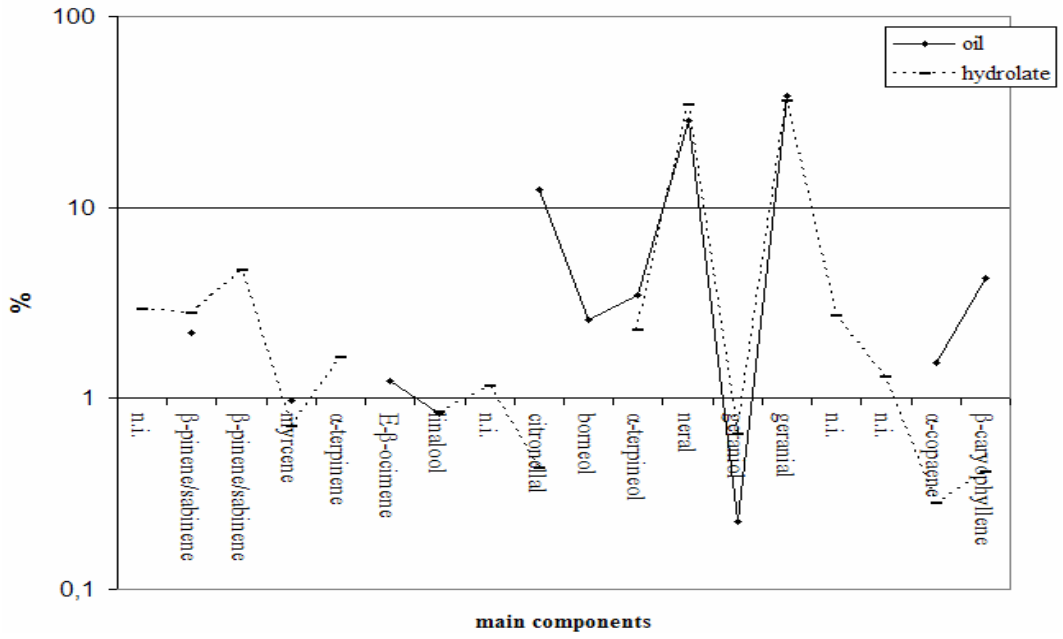

Figure 14: *Melissa Lorelei* fresh plants, composition hydrolate-oil, harvesting date: 20070904

7. APPENDIX

Appendix 1. Volatile components of *Melissa Quedlinburger (*2nd year) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14

Title:	Melissa 11A1-070906			Melissa 11A2-070906	
Identification :	Distillation 070806			Distillation 070806	
Volume:			1µl		0.02µl
	Ret.Time	**KI**	**conc**	**KI**	**conc**
Name	**min**	**SPB1**	**%**	**SPB1**	**%**
			Hexane 12,4%		Hexane 88.0%
	4,835	825	1,483	822	0,343
	5,231	839	1,002	835	0,287
	5,614	851	1,136	847	0,354
	5,766	856	0,207	853	0,067
β-pinene	10,473	967	0,873	963	0,327
myrcene				979	0,062
α-terpinene				998	0,059
(Z)-β-ocimene	13,979	1029	0,339	1024	0,132
(E)-β-ocimene	14,599	1040	3,115	1035	1,213
linalool	17,453	1084	0,507	1080	0,142
				1113	0,074
	19,702	1118	0,291	1115	0,096
	19,840	1120	0,399	1125	1,751
citronellal	20,537	1132	7,088	1137	0,075
borneol	21,215	1142	0,352		
α-terpineol	22,378	1160	0,511	1155	0,114
neral	25,983	1211	11,854	1202	1,858
geraniol	27,684	1238	0,870	1230	0,111
geranial	28,030	1243	15,848	1234	2,232
	28,158	1245	0,428		
α-copaene	35,765	1360	0,573	1354	0,059
geranyl acetate	36,123	1365	0,322		
β-bourbonene	36,743	1374	0,390		
β-caryophyllene	38,288	1397	15,244	1389	1,444
α-humulene	40,178	1428	1,177	1421	0,090
	41,181	1445	0,385	1449	0,812
(E)-β-farnesene	41,970	1458	12,009		
	43,246	1478	0,595		
germacrane D	43,396	1480	0,731		
	43,855	1487	0,367		
	44,179	1492	0,428	1492	0,107
	44,606	1498	1,895		
caryophyllene oxide	47,135	1543	1,252		
	50,827	1604	1,404		
t-cadinol	50,998	1607	0,342	1607	0,074

51,481	1616	2,556
52,909	1642	0,298
63,567	1840	0,914
65,247	1872	0,460

Appendix 2: Volatile components of *Melissa Citronella* (oil from DW) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14

Title	Citronella4A1070821				Citronella4A2070821			
Identification :	Distillation070814				Distillation 070814			
injected volume	1.0µl				0.02µl			
			%Hex: 93,260				**%Hex: 43,600**	
Name	**Ret.Time min**	**KI SPB1**	**conc. %**	**conc. % plus solvent**	**Time min**	**KI SPB1**	**conc. %**	**conc. % plus solvent**
(Z)-3-hexenol	5,298	842	0,009	0,129	5,271	841	0,107	0,19
sabinene	10,410	966	0,047	0,703	10,382	965	1,112	1,972
β-pinene	10,489	967	0,024	0,351				
975	10,933	975	0,003	0,047	10,913	975	0,052	0,093
myrcene	11,393	983	0,011	0,170	11,376	983	0,197	0,349
(Z)-β-ocimene					13,973	1029	0,056	0,099
(E)-β-ocimene	14,594	1040	0,027	0,407	14,579	1040	0,466	0,826
1056					15,567	1056	0,029	0,051
terpinolene	16,782	1074	0,006	0,083	16,757	1074	0,081	0,144
linalool	17,456	1084	0,036	0,531	17,444	1084	0,502	0,89
1093	18,111	1093	0,003	0,046	18,095	1093	0,050	0,089
camphor	19,062	1107	0,007	0,109	19,050	1107	0,099	0,176
n.i.	19,709	1118	0,019	0,277	19,694	1118	0,232	0,411
camphene hydrate	19,901	1121	0,020	0,295	19,887	1121	0,242	0,43
citronellal	20,467	1131	0,078	1,164	20,458	1130	0,991	1,757
borneol	21,197	1142	0,075	1,116	21,190	1142	0,898	1,592
terpinen-4-ol	22,024	1154	0,005	0,080	21,843	1152	0,029	0,051
1154					22,016	1154	0,067	0,119
α-terpineol	22,364	1159	0,112	1,655	22,360	1159	1,253	2,222
neral	26,027	1212	2,474	36,710	26,064	1212	21,259	37,694
geranial	28,083	1244	3,478	51,606	28,108	1244	26,582	47,132
n.i.	31,939	1299	0,014	0,202	31,937	1299	0,101	0,18
geranyl acetate	35,865	1362	0,088	1,311	35,865	1362	0,539	0,956
β-caryophyllene	38,124	1395	0,187	2,777	38,125	1395	1,394	2,472
α-humulene	40,138	1428	0,009	0,140	40,139	1428	0,059	0,104
caryoph.oxide	47,104	1543	0,006	0,091				

Appendix 3: Volatile components of *Melissa Citronella* (oil from DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14

Title	Citronella4B1070822				Citronella4B2070821			
Identification :	Diffusion 070814				Diffusion 070814			
Injected Volume	1µl				0.02µl			
	%Hex: 93,843				%Hex: 73,152			
Name	**Ret.Time min**	**KI SPB1**	**conc. %**	**conc. % plus solvent**	**Time min**	**KI SPB1**	**conc. %**	**conc. % plus solvent**
sabinene + β-pinene	10,431	966	0,020	0,318	10,477	966	0,156	0,582
					10,567	969	0,066	0,247
myrcene	11,385	983	0,017	0,275	11,394	983	0,148	0,552
					13,986	1029	0,058	0,215
(E)-β-ocimene	14,583	1040	0,060	0,971	14,591	1040	0,515	1,919
terpinolene					16,789	1074	0,044	0,163
linalool	17,457	1084	0,025	0,408	17,463	1084	0,173	0,646
camphor					19,068	1107	0,049	0,184
	19,705	1118	0,023	0,371	19,715	1118	0,145	0,542
camphene hydrate	19,895	1121	0,026	0,424	19,905	1121	0,163	0,607
citronellal	20,465	1131	0,102	1,654	20,473	1131	0,630	2,347
borneol	21,193	1142	0,109	1,776	21,201	1142	0,642	2,392
α-terpineol	22,364	1159	0,149	2,413	22,370	1159	0,856	3,188
neral	26,002	1211	1,802	29,269	25,927	1210	8,332	31,035
geraniol	27,711	1238	0,039	0,635	27,611	1237	0,201	0,748
geranial	28,073	1244	2,809	45,618	27,956	1242	11,715	43,633
					28,141	1245	0,042	0,157
	31,937	1299	0,022	0,361	31,941	1299	0,087	0,324
geranyl acetate	35,886	1362	0,154	2,503	35,872	1362	0,484	1,801
β-caryophyllene	38,183	1396	0,661	10,739	38,137	1395	2,206	8,215
α-humulene	40,146	1428	0,030	0,49	40,148	1428	0,089	0,332
(E)-β-farnesene	41,806	1455	0,017	0,271	41,809	1455	0,047	0,173
germacrane D	43,360	1480	0,015	0,24				
caryophyllene oxide	47,104	1543	0,022	0,35				
	53,386	1651	0,018	0,299				
	63,529	1840	0,020	0,329				
	64,958	1868	0,017	0,284				

Appendix 4: Volatile components of *Melissa Quedlinburger* (oil from DW and DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14

Title	Quedlin5A1-070822			Quedlin5A3-070827		Quedlin5B1-070823		
Identification :	Distillation 070815			Distillation 070815		Diffusion 070815		
injected volume	0.05µl			0.02 µl		1.00 µl		
	Ret.Time	**KI**	**conc**	**Temps**	**conc**	**Temps**	**conc**	conc %
Name	**min**	**SPB1**	**%**	**min**	**%**	**min**	**%**	plus solvent
(Z)-3-hexenol				5,323	0,057			
α-phellandrene				11,841	0,378			
sabinene				10,397	0,980	10,431	0,192	0,223
β-pinene + sabinene	10,463	967	0,978	10,464	1,405	10,535	0,166	0,193
975				10,918	0,109			
myrcene	11,386	983	0,152	11,380	0,378	11,375	0,230	0,267
(Z)-β-ocimene				13,975	0,090	13,970	0,047	
(E)-β-ocimene	14,593	1040	0,269	14,583	0,678	14,575	0,413	0,479
terpinolene				16,766	0,169	16,761	0,080	
linalool	17,469	1084	0,416	17,447	0,729	17,447	0,234	0,271
α-campholenal	18,111	1093	0,120	18,087	0,263			
camphor	19,097	1108	0,145	19,071	0,279	19,052	0,083	
1119	19,728	1119	0,274	19,699	0,463	19,691	0,290	0,336
camphene hydrate	19,910	1122	0,462	19,887	0,717	19,883	0,318	0,369
citronellal	20,453	1130	8,920	20,494	13,805	20,500	7,959	9,243
borneol	21,212	1142	1,018	21,191	1,513	21,185	1,351	1,569
terpinen-4-ol				22,019	0,130			
α-terpineol	22,375	1160	1,617	22,359	2,141	22,353	1,799	2,09
nerol	25,701	1206	0,907					
neral	25,842	1208	32,153	25,968	32,445	26,021	23,512	27,303
geraniol	27,533	1236	3,168	27,645	2,458			
geranial	27,848	1240	43,851	27,984	37,058	28,121	41,278	47,934
1244	28,109	1244	0,808	28,132	0,858	28,178	0,072	
						28,931	0,041	
1299	31,929	1299	0,290	31,929	0,250	31,925	0,307	0,356
geranyl acetate	35,859	1361	0,964	35,858	0,555	35,866	1,401	1,627
β-caryophyllene	38,102	1394	3,309	38,110	2,434	38,153	5,459	6,339
α-humulene	40,130	1428	0,148	40,135	0,092	40,131	0,255	0,296
						41,103	0,047	
						41,792	0,218	0,254
						43,345	0,117	0,136

Appendix 5: Volatile components of *Melissa StammNLC* (oil from DW and DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14, injection volume 0.02 and 1µl

Title:	StammNLC7A1				StammNLC7A2		StammNLC7B1		StammNLC7B2	
Identificatio	Distillation 070816				Distillation 070816		Diffusion 070816		Diffusion 070816	
Injected Volume:	1.0µl				0.02µl		1.0µl		0.02µl	
			%Hex: 93,924		**%Hex:48,016**		**%Hex: 22,51**		**%Hex: 31,372**	
	Ret.Time	**KI**	**conc**	**conc %**	**conc**	**conc%**	**conc**	**conc%**	**conc**	**conc%**
Name	**min**	**SPB1**	**%**	**plus solvent**	**%**	**plus solvent**	**%**	**plus solvent**	**%**	**plus solvent**
sabinene	10,454	967	0,006	0,103	0,102	0,196	0,146	0,188	0,412	0,600
β-pinene	10,500	967	0,008	0,135	0,155	0,299	0,156	0,201	0,380	0,554
975									0,129	0,188
myrcene	11,381	983	0,014	0,230	0,241	0,464				
(Z)-β-ocimene	13,972	1029	0,009	0,142	0,159	0,306			0,070	0,103
(E)-β-ocimene	14,576	1040	0,082	1,342	1,493	2,871	0,215	0,278	0,629	0,917
terpinolene	16,773	1074	0,006	0,093	0,087	0,168				
linalool	17,443	1084	0,037	0,612	0,536	1,031	0,468	0,603	0,801	1,167
1093									0,149	0,217
camphor	19,062	1107	0,005	0,086	0,070	0,135	0,064	0,083	0,132	0,192
1118	19,703	1118	0,019	0,316	0,262	0,504	0,119	0,153	0,197	0,288
camphene hydrate	19,895	1121	0,020	0,325	0,262	0,505	0,253	0,327	0,386	0,562
citronellal	20,481	1131	0,569	9,362	7,397	14,229	2,642	3,410	4,027	5,868
borneol	21,191	1142	0,083	1,371	1,029	1,979	0,667	0,860	0,893	1,302
1155							0,063	0,081	0,087	0,126
α-terpineol	22,357	1159	0,113	1,863	1,346	2,589	1,041	1,344	1,363	1,985
neral	25,949	1210	1,444	23,770	12,766	24,559	25,130	32,434	23,535	34,294

geraniol	27,668	1238	0,029	0,469	0,229	0,441			1,556	2,268
geranial	28,027	1243	2,668	43,904	20,647	39,719	5,931	7,655	29,379	42,809
1245	28,144	1245	0,028	0,462	0,345	0,664	32,980	42,566	0,211	0,307
1299	31,927	1299	0,017	0,284	0,116	0,224	0,139	0,179	0,088	0,128
geranyl acetate	35,869	1362	0,118	1,935	0,607	1,169	1,022	1,319	0,511	0,745
1368	36,288	1368	0,009	0,146						
β-caryophyllene	38,157	1395	0,593	9,760	3,786	7,283	5,063	6,534	3,336	4,862
α-humulene	40,137	1428	0,029	0,473	0,153	0,295	0,255	0,328	0,135	0,197
(E)-β-farnesene	41,799	1455	0,044	0,729	0,194	0,373	0,349	0,450	0,149	0,217
germacrane D	43,353	1480	0,014	0,223			0,094	0,122		
1492	44,136	1492	0,008	0,135						
caryophyllene oxide	47,098	1543	0,022	0,358			0,152	0,196		
							0,328	0,423		
							0,137	0,177		

Appendix 6: Volatile components of *Melissa Lemona* (oil from DW and DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14, injection volume 0.02 and 1µl

Title	Lemona8A1				Lemona8A2		Lemona8B1		Lemona8B2	
Identification	Distillation 070817				Distillation 070817		Diffusion 070817		Diffusion 070817	
Injected Volume	1µl				0.02 µl		1µl		0.02 µl	
			%Hex: 86,128		**%Hex: 87,071**		**%Hex:95,699**		**%Hex:27,062**	
	Ret.Time	**KI**	**conc**	**conc%**	**conc**	**conc%**	**conc**	**conc%**	**conc**	**conc%**
Name	**Min**	**Spb1**	**%**	**plus solvent**	**%**	**plus solvent**	**%**	**plus solvent**	**%**	**plus solvent**
sabinene/β-pinene	10,390	965	0,073	0,523	0,201	1,553	0,009	0,219	0,387	0,531
	10,917	975	0,007	0,049						
myrcene	11,382	983	0,019	0,137	0,061	0,475	0,011	0,249	0,494	0,677
									0,050	0,069
									0,071	0,098
(E)-β- ocimene	14,589	1040	0,023	0,163	0,067	0,515	0,012	0,283	0,558	0,765
terpinolene	16,759	1074	0,011	0,080			0,004	0,089	0,130	0,178
linalool	17,450	1084	0,074	0,537	0,130	1,004	0,014	0,336	0,455	0,624
1093	18,099	1093	0,006	0,045						
camphor	19,052	1107	0,013	0,096			0,003	0,077	0,108	0,149
1118	19,698	1118	0,035	0,254	0,061	0,471	0,015	0,354	0,442	0,605
camphene hydrate	19,889	1121	0,036	0,262	0,057	0,439	0,018	0,414	0,487	0,667
citronellal	20,465	1131	0,136	0,983	0,215	1,659	0,060	1,401	1,664	2,282
borneol	21,197	1142	0,133	0,958	0,176	1,365	0,075	1,742	1,908	2,616
terpinen-4-ol	22,020	1154	0,017	0,121						
α-terpineol	22,371	1160	0,199	1,438	0,273	2,113	0,099	2,310	2,400	3,291
1179	23,742	1179	0,006	0,045	0,105	0,814				
neral	26,202	1214	4,985	35,936	4,882	37,760	1,292	30,029	23,377	32,051
geraniol					0,304	2,349	0,014	0,328	0,210	0,288
geranial	28,022	1243	2,184	15,742	5,980	46,252	2,184	50,765	34,414	47,183
1248	28,324	1248	5,174	37,297			0,005	0,125	0,107	0,146

									0,062	0,085
1299	31,960	1299	0,026	0,187			0,013	0,293	0,190	0,260
geranyl acetate	35,901	1362	0,187	1,348	0,106	0,822	0,089	2,073	1,022	1,401
β-caryophyllene	38,173	1395	0,426	3,074	0,311	2,409	0,320	7,436	4,166	5,711
α-humulene	40,157	1428	0,022	0,155			0,015	0,342	0,159	0,218
caryoph.oxide	47,117	1543	0,013	0,094			0,015	0,358		
							0,014	0,332		
							0,010	0,236		
							0,006	0,131		

Appendix 7: Volatile components of *Melissa Aufrechte* (oil from DW and DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14, injection volume 0.02 and 1µl

Title	Aufrechte9A1-070905				Aufrechte9A2-070823		Aufrechte9B1-070905		Aufrechte9B2-070823	
Identification	Distillation 070821				Distillation 070821		Diffusion 070821		Diffusion 070821	
Injected Volume	1µl				0.02µl		1µl		0.02µl	
			%Hex: 91,636		%Hex: 83,936		%Hex: 97,109		%Hex: 91,286	
Name	**Ret.Time Min**	**KI SPB1**	**conc %**	**conc % plus solvent**	**conc %**	**conc % plus solvent**	**conc %**	**conc % plus solvent**	**conc %**	**conc % plus solvent**
sabinene	10,403	966	0,028	0,337	0,114	0,711	0,003	0,120	0,043	0,494
β-pinene	10,470	967	0,051	0,606	0,218	1,357			0,029	0,328
	10,911	975	0,007	0,086	0,029	0,181				0,541
myrcene	11,382	983	0,013	0,153	0,055	0,343	0,008	0,284	0,047	0,158
(Z)-β-ocimene					0,017	0,107			0,014	0,633
(E)-β-ocimene	14,587	1040	0,032	0,388	0,142	0,882	0,021	0,715	0,116	0,488
terpinolene	16,768	1074	0,007	0,088	0,026	0,162				0,546
linalool	17,449	1084	0,049	0,582	0,161	1,003	0,011	0,388	0,055	10,321
1093	18,097	1093	0,008	0,098	0,032	0,197				2,145
camphor	19,070	1108	0,011	0,137	0,039	0,242				2,887
1118	19,701	1118	0,026	0,310	0,080	0,497	0,010	0,334	0,043	0,164
camphene hydrate	19,894	1121	0,033	0,392	0,099	0,614	0,011	0,379	0,048	25,449
citronellal	20,483	1131	0,381	4,555	1,149	7,153	0,209	7,215	0,899	0,253
borneol	21,200	1142	0,072	0,861	0,203	1,266	0,045	1,558	0,187	41,908
terpinen-4-ol	22,021	1154	0,010	0,117	0,020	0,127				0,866
α-terpineol	22,369	1159	0,114	1,368	0,311	1,937	0,063	2,178	0,252	0,184
nerol							0,004	0,132	0,014	0,351
neral	26,088	1213	2,818	33,695	5,563	34,632	0,669	23,150	2,217	0,973
geraniol					0,338	2,106	0,007	0,240	0,022	8,710
geranial	28,176	1245	4,190	50,099	6,811	42,398	1,215	42,015	3,652	0,376
bornyl acetate					0,066	0,412	0,004	0,155	0,016	0,670

1299	31,954	1299	0,017	0,206	0,028	0,176	0,010	0,360	0,031
geranyl acetate	35,885	1362	0,048	0,578	0,057	0,356	0,039	1,334	0,085
β-caryophyllene	38,162	1395	0,309	3,692	0,474	2,949	0,330	11,399	0,759
α-humulene	40,159	1428	0,016	0,186	0,019	0,116	0,016	0,565	0,033
(E)-β-farnesene	41,822	1456	0,008	0,098			0,032	1,109	0,058
germacrane D							0,005	0,184	
α-cadinene							0,006	0,200	
caryoph.oxide	47,124	1543	0,011	0,132					
t-cadinene							0,008	0,269	
(Z,E)-farnesol							0,012	0,401	

Appendix 8: Volatile components of *Melissa Lorelei* (oil from DW and DS) in order to their Kovats indices (KI) using a non polar column, harvesting date 2007/08/14, injection volume 0.02 and 1µl

Title	Lorelei10A1				Lorelei10A2		Lorelei10B1		Lorelei10B2	
Identification	Distillation070822				Distillation070822		Diffusion 070822		Diffusion 070822	
Injected Volume	1µl				0.02µl		1µl		0.02µl	
			%Hex: 90,579		**%Hex: 71,210**		**%Hex: 60,415**		**%Hex: 13,039**	
Name	**Ret.Time min**	**KI SPB1**	**conc %**	**conc. % plus solvent**	**conc %**	**conc. % plus solvent**	**conc %**	**conc. % plus solvent**	**conc %**	**conc. % plus solvent**
(Z)-3-hexenol	5,300	841	0,008	0,085						
sabinene	10,417	966	0,039	0,414	0,252	0,874	0,107	0,271	0,199	0,229
β-pinene	10,487	967	0,070	0,748	0,493	1,714	0,040	0,101	0,323	0,372
975	10,931	975	0,008	0,087	0,060	0,207				
myrcene	11,399	983	0,014	0,153	0,106	0,367	0,107	0,271	0,410	0,471
limonene									0,066	0,076
(Z)-β-ocimene									0,139	0,159
(E)-β-ocimene	14,602	1040	0,035	0,368	0,258	0,895	0,283	0,714	1,130	1,299
terpinolene	16,779	1074	0,011	0,112	0,063	0,218			0,122	0,141
linalool	17,467	1084	0,057	0,610	0,293	1,017	0,149	0,377	0,509	0,585
1093	18,106	1093	0,015	0,155	0,091	0,317			0,118	0,135
camphor	19,083	1108	0,019	0,198	0,102	0,354	0,057	0,143	0,187	0,215
1118	19,713	1118	0,034	0,364	0,168	0,582	0,157	0,396	0,495	0,569
1121	19,856	1121	0,025	0,270	0,121	0,419	0,141	0,356	0,461	0,530
1121	19,901	1121	0,032	0,337	0,147	0,510				
citronellal	20,538	1132	0,898	9,532	4,166	14,470	5,976	15,097	18,435	21,199
borneol	21,218	1142	0,092	0,974	0,380	1,320	0,577	1,458	1,795	2,065
terpinen-4-ol	22,037	1155	0,012	0,129					0,067	0,077
α-terpineol	22,383	1160	0,136	1,444	0,570	1,978	0,807	2,038	2,318	2,666
nerol					0,221	0,768				
neral	26,078	1212	3,087	32,762	9,105	31,627	9,180	23,191	20,184	23,210

geraniol					0,662	2,298	0,153	0,387		
geranial	28,147	1245	4,226	44,852	10,374	36,033	15,027	37,961	29,876	34,355
1246	28,211	1246	0,010	0,102	0,200	0,696	0,520	1,314	1,146	1,318
1299	31,961	1299	0,022	0,236	0,046	0,158	0,166	0,420	0,307	0,353
geranyl acetate	35,891	1362	0,034	0,359	0,053	0,184	0,273	0,690	0,446	0,512
1388	37,650	1388	0,009	0,091			0,053	0,133		
β-caryophyllene	38,170	1395	0,376	3,991	0,862	2,994	4,598	11,615	7,451	8,568
α-humulene	40,167	1428	0,019	0,206			0,214	0,540	0,298	0,342
(E)-β-farnesene	41,828	1455	0,015	0,160			0,218	0,550	0,260	0,299
caryoph.oxide	47,129	1542	0,019	0,198			0,108	0,273	0,068	0,079
1839	63,553	1839	0,059	0,625			0,284	0,717		
1872	65,247	1872	0,041	0,436						

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Chapter 2

Evaluation of the essential oil of *Solidago puberula* at different harvesting dates and different drying stages.

1. Abstract

In the study Solidago oil was extracted by three different methods, hydrodistillation (DW), hydrodiffusion (DS) and water-cum-steam distillation (WSD), and compared according to the yield and composition of the essential oil. Plant material was harvested at three different dates in the time between the beginning of August and the middle of September. One part of the plants was analysed after drying.

The contents of monoterpene hydrocarbons, sesquiterpene hydrocarbons and sesquiterpene alcoholes were detected by GC/FID and compared and supplemented with the use of the Kovats index database.

During ontogenesis it became obvious that highest yield of oil was reached in the first two plant stages after extracting the oil by DW. This large difference in yield was probably due to the fact that the last sampling took place a few days before extraction and that harvesting took place on a rainy day. Also the plant stage could be one reason for the less amount of essential oil. On selected components the influence of harvesting time was discussed, e.g. α-pinene, β-pinene, myrcene and limonene. Contrary to all other previous investigations [9, 10] one did not get constant results during the different stages. Content of all components after DW and diffusion was higher in the first and the last plant stage. Only α-pinene obtained by DS attained a higher concentration in the flowering stage.

Contrary to all expectations drying had a positive effect as well on concentration as on yield. Thus, it was possible to increase the amount of oil in the last two plant stages. The concentration of selected components (α-pinene, β-pinene, myrcene and limonene) was not very constant during the different stages. In the first stage nearly all components decreased their content after drying. In the following plant stages one could see an increase of nearly all components after drying. Furthermore, results obtained by the different extraction methods showed variations after drying.

2. INTRODUCTION

2.1 Solidago puberula

The second plant worked on in the study is *Solidago puberula.* Solidago is used since times because of its diuretic, astringent and diaphoretic qualities. Therapeutically important contents are triterpene saponins, flavonoids, essential oils, phenol glycosides and acidic polysaccharides with indication areas like urinary stones, renal gravel, cramps and flushing therapy [1]. Pharmacological proved effects exist at the moment only for flavonoids, phenolglycosides, triterpene saponins and caffeic acid derivatives. For other components, for example essential oils only few investigations were carried out.

Comparative studies of the European species *Solidago virgaurea* with the North American species *Solidago canadensis L.* and *Solidago gigantea Ait.* resulted in higher yield and an other composition of the contents also concerning the essential oils. Differences in therapeutic effects are to be assumed [2].
Concerning the composition and the content of the essential oils only few specific investigations were carried out so far. The harvesting time, different pre-operative methods and also climatic conditions play an important role regarding the quality and the yield of the oil. For *Solidago puberula* which is domestic in the north of Quebec only few investigations concerning these questions can be found. In the present study these questions are drawn up.

2.1.1 History

Solidago virgaurea is used since 700 years as a medicinal plant. The genus name solidago, derived from the latin verb solida ("whole") and ago ("to make") refers to its early use as a wound healing drug. The name of species virgaurea dated

from the Latin verb virga (rod) und aureus (golden) and describes form and colour of the whole inflorescens.
Hieronymus Bock (1498-1554) mentioned the use of Solidago from the old Germanic people as a wound healing. Also Martin Luther esteemed the plant as a good remedy for his numerous diseases. However one can find in old writings, starting from the middle age, the use as medicinal plant.
First mentioned in Spain, Arnaud de Villeneuve (1238-1311), reported from a man, who had a bladder stone, that after eating this herb for nine days, he lost a handful urinary gravel.

Kroeber (1934) refers to Solidago or "Heidnisch Wundkraut" as a drug for "bad healing wounds", parulis, disease of the kidney and complaints of the upper respiratory system [3].

Goldenrods have their fix place as a diuretic because of their draining attributes. Only in the last century the German medicine Rademacher referred to the great importance of the goldenrods as a kidney cure [3].

Many herb books report on Solidago as cures. Nevertheless it lost its meaning as a wound healing drug and finds now application as diuretics, diaphoretics and for the treatment of urinary tract disorders.

2.1.2 General Information

Solidago ssp. belongs to the plant family *Asteraceae*. The common name Goldenrod embraces a genus of plants with more than 130 species. Generally native to North America, also a few species are native to Europe, Asia, Northern Africa and South America. Medicinal goldenrods occur in Bulgaria, Hungary, Poland and other Eastern European countries.

Solidago ssp. is a perennial herb that builds in the first year a basal rosette with oblong or ovate leaves. The leaves are strongly toothed or have a smooth edge.

However, hair-growth, leaf edge and leaf shape vary very strongly between the different varieties.
The upper surface is dark-green the lower surface is light green. The average plant height is 1.2m [2]. The flowering is very typical for Asteraceae. One can find two kinds of flowers on *Solidago*. The flowers at the inner portion of the head have a symmetrical set of petals (corolla) and these are the disc flowers; the flowers at the margin have an asymmetrical corolla or set of petals which are modified so that they are strap-shaped and are called ray flowers.
Flowering time is from August to October [4].

2.1.3 Application and Effects

Folk medicine knows goldenrods already for years as means against haemorrhoids, anuresis and kidney stones. Rademacher used the herb also with nephritis, arthritis, with asthma and with gastric affection. The opinion of Rademacher were represented and developed further by Bohn, Duché, and Leclerc et al.. Thus goldenrod was recommended also during kidney contraction (Bright kidney illness), to lithiasis, as astringens and external with ulcerating wounds.

In vitro antimycotic effects against Candida and Cryptococcus were focused [3].

Now the plant is mainly used as diuretics, antiphlogistic and because of its antispasmodic effects. Main indication areas are urinary stones, renal gravel, cramps and flushing therapy. Furthermore the plant shows antimicrobial, anti-inflammatory and antiexsudative effects [1].

In aromatherapy oil of *Solidago puberula* finds appliance for indication areas like varices, coronary spasms, adenitis, renal insufficiency, neuritis, sciata and insomnia [5].

2.1.4 Cultivation and Harvesting

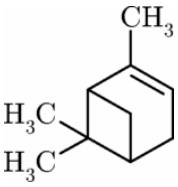
Solidago ssp. can be cultivated from seeds. The wild plants can also be excavated. Plants and also seeds from some varieties are available. Unfortunately, no further literature concerning cultivation of *Solidago ssp.* could be found.

2.1.5 Chemical composition of Solidago oil

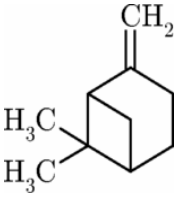
Existing investigations on several varieties [6, 7, 8, 10] report the occurence of monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes, benzoic acid and salicylic acid esters. The main terpene hydrocarbons found in most of the species are α- and β-pinene, limonene, myrcene, β-phellandrene among the monoterpenes and germacrane-D and β-caryophyllene among the sesquiterpenes.

Another investigation on *Solidago chilensis* shows pumiloxide (15.3%), an unusual labdane diterpene, as major component, followed by γ-cadinene (5.6%), limonene (4.1%), caryophyllene oxide (3.6%), isospathulenol (3.2%) and β-elemene (3.1%).

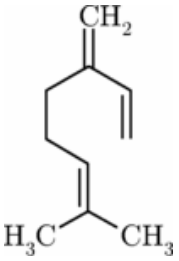
Main components of solidago oil



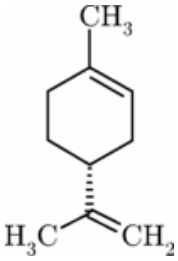
α-pinene



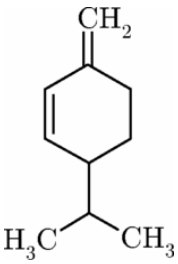
β-pinene



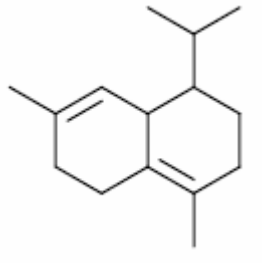
myrcene



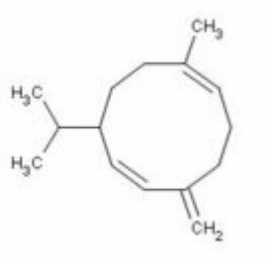
limonene



β-phellandrene



cadinene



germacrane D

In one investigation on *Solidago puberula* from Laboratoire Laseve (Quebec) the following compounds were found: α-pinene, camphene, sabinene, β-pinene, myrcene, α-phellandrene, δ-3-carene, para-cymene, limonene, β-phellandrene, (*Z*)-β-ocimene, trans-β-ocimene, terpinolene, linalool, borneol, terpinen-4-ol, α-terpineol, bornyl acetate, α-copaene, β-cubebene, β-elemene, β-caryophyllene, α-humulene, γ-muurolene, germacrene D and A, α-muurolene, γ-cadinene, δ-cadinene, spathulenol, caryophyllene oxide, t-cadinol, t-murolool, α-murolool, α-cadinol and farnesol.

According to Holland, Johnson and Sorrels, goldenrod should be harvested when most of the plants are in bloom, since the oil yield in relation to the quality is highest at that stage of growth. The freshly cut goldenrod should be distilled at once; certainly not later than two or three days after cutting to obtain the highest yield [9].

More current studies from Poland showed only few differences in yield and the composition of the oil during the development period 0.32% before flowering and 0.37% during the other stages [10].

3. MATERIAL AND METHODS

3.1 Plants

In the present study the plant material of *Solidago puberula* at different stages of growing before flowering (1), at full flowering (2) and after flowering (3) was investigated.

Many of the goldenrods have characteristic patterns to be distinguished. From the 32 species growing in Canada, *Solidago puberula* is native to the mountain regions in the northern part of Quebec and to the most parts of the Laurentides. *Solidago puberula* prefers sandy soil and it grows mostly in rocky woods, at sand barrens and open prairies. Average plant height is 20-100cm. Stems are finely roughened at least in the area of the flowers. Basic sheets are blunt and spatulated, sheets on the stems are oblong-oval [11].

3.2 Harvesting and treatment of the plant material

3.2.1 Harvesting

The aerial parts of *Solidago puberula* growing wild were collected on different days and on two different sites in Grondines (Quebec). Grondines is situated about 90km to Quebec City and has an altitude of 71m. Both fields were opened to prairie landscape with sandy soil. Plant material was identified and harvested with the help from Francis Mainguy from Aliksir.

Dates of sampling Grondines 2007

Site	**Number of samplings**	1st sample, before flowering, buds	2nd sample flowering stage	3rd sample after blooming
Grondines	3	2007-08-02	2007-08-17	2007-09-15

Growth state of the plants and date of harvesting	
	20070802 Grondines before blooming, all plants developed first buds
	20070817 Grondines flowering stage, all plants were in full bloom
	20070915 Grondines after blooming

3.2.2 Drying

One half of the fresh plants from each sampling was dried in the greenhouse of the HRDC and then stored in the cold storage chamber until analysis. Average drying time was 2 days.

3.3 Isolation and analysis of the essential oil

Essential oil was obtained using DW and DS (5L) and one isolation was done in the 20L steam distillation apparatus. Running time for the methods was one, two or four hours.

Three different concentrations and volumes (0.02µl of the more concentrated 9ml, 1µl taken from the 1ml and 1µl of a 1:5 diluted sample) of oil were analysed by GC-FID on two different colums with different polarity (a non polar SPB-1 column and a polar Supelcowax-column; 30 m x 0.25 mm, film thickness 0.25µm). Helium was used as a carrier gas. Identification of volatile compounds was made with the Kovats Index database.

Kovats Indices (KI) can be calculated with the following formula resulting in a specific value on the two different columns:

$$KI = 100N + 100n \times \frac{\log tR(A) - \log tR(N)}{\log tR(N+n) - \log tR(N)}$$

KI.....Kovats-Index

N.....number of carbon atoms in the alkane before the unknown component

n.....number of carbon atoms in the alkane after the unknown component

A.....unknown component

tR.....adjusted retention time

4. RESULTS

The plant material at different growing states was analysed using the following methods:

Table 1: Treatment and date of treatment of plant material during the different growing states. (treatments: DW = Hydrodistillation, DS = Hydrodiffusion, WSD = water-cum-steam distillation).

Identification		Treatment	Date of Treatment
		First sampling 20070802	
1A	Solidago puberula	DW	20070803
1B	Solidago puberula	DS	20070804
1C	Solidago puberula dry	DW	20070808
1D	Solidago puberula dry	DS	20070807
1E	Solidago puberula	DW 2 hours	20070808
		Second sampling 20070817	
2A	Solidago puberula	DW	20070820
2B	Solidago puberula	DS	20070820
2C	Solidago puberula dry	DW	20070823
2D	Solidago puberula dry	DS	20070823
2E	Solidago puberula	WSD (20l)	20070824
2EHY	Solidago puberula Hydrolate	WSD (20l)	20070824
		Third sampling 20070915	
3A	Solidago puberula	DW 1 hour	20070920
3B	Solidago puberula	DS	20070920
3C	Solidago puberula dry	DW	20070927
3D	Solidago dry	DS	20070927

4.1 Yield of essential oil

The yield and colour of the oil differed depending on the extraction method. A light yellow oil was obtained using DW and DS. The colour of the pure oil obtained using steam distillation was bluish-green. The smell of the oil was herbacous, fresh and conifer-like.
The content of the oil was variable and depended on the different extraction methods and the stage of growing (Fig.1). Furthermore, different parameters such as moisture, the amount of fresh plants and the amount of dry plants affected the yield of oil and were considered in the calculation of yield. The yield of the oil ranged from 0.21% to 0.59%.

Highest yield of essential oil was achieved during the first two stages of growing (1 and 2) whereas it decreased after blooming (3) to a content of not more than 0.36%. The reason could be because the isolation of oil took place 4 days after harvesting. Another explanation could also be the different climate conditions during the harvest and different soil properties at the two sites.
After blossoming the oil content differs not significantly between DS and DW. The drying status did not affect the yield of oil either. During the distillation process (3A) some problems were encountered. Due to the large amount of plant material and because of the occurence of saponines some foam formation was observed. To overcome this problem boiling chips or a special distillation support to destroy the foam were used but no improvement was achieved. In the following steps the distillation 3A was stopped after 1 hour. The yield of oil was 0.21%. This was not surprising because according to Guenther (1952) the plant material requires at least 2 hours for completion.

The yield of the oil before blooming ranges from 0.33 to 0.60% (Fig.2). Using DW the yield was higher. During the distillation process the oil circulates in the closed system, whereas for DS a part of the oil may be deposited in the

corresponding hydrosol (open system). The percentage of yield was also higher using the dry plants. Furthermore the total content of oil was obtained after two hours of distillation (1E). For further investigations on Solidago it is not necessary to run the method for 4 hours. Distillation for 2 hours gave a yield of 0.53%.

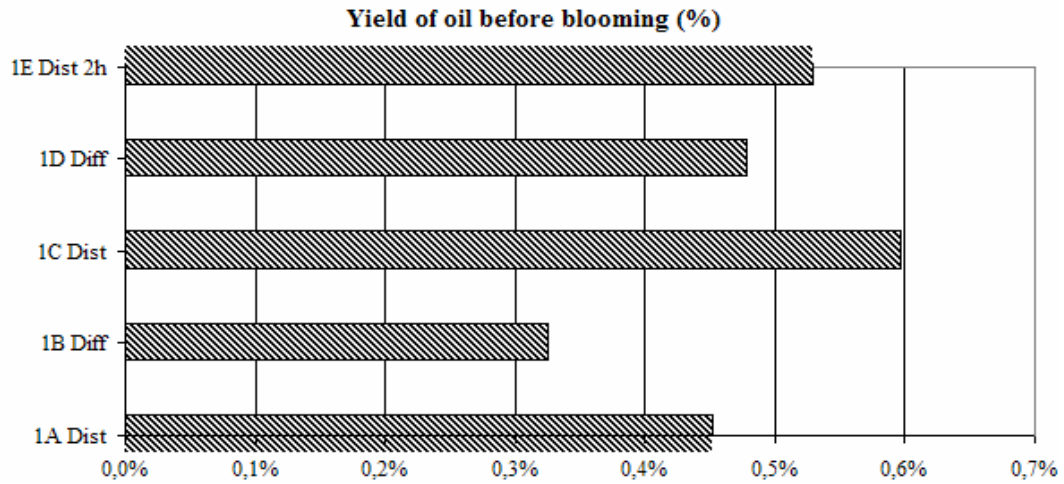


Figure 2: Yield of oil before blooming (%)

Yield of oil during flowering stage from the fresh plant material ranged from 0.43% and 0.59% for DS and DW, respectively (Fig 3). The content of the oil for the dry plants was 0.52% and 0.56%, respectively. Using dry plants the difference in the yield of oil was not that high as for the fresh plants.

Only a percentage of 0.01% of oil can be found in the hydrolate. In the pure oil a yield of 0.57% was obtained.

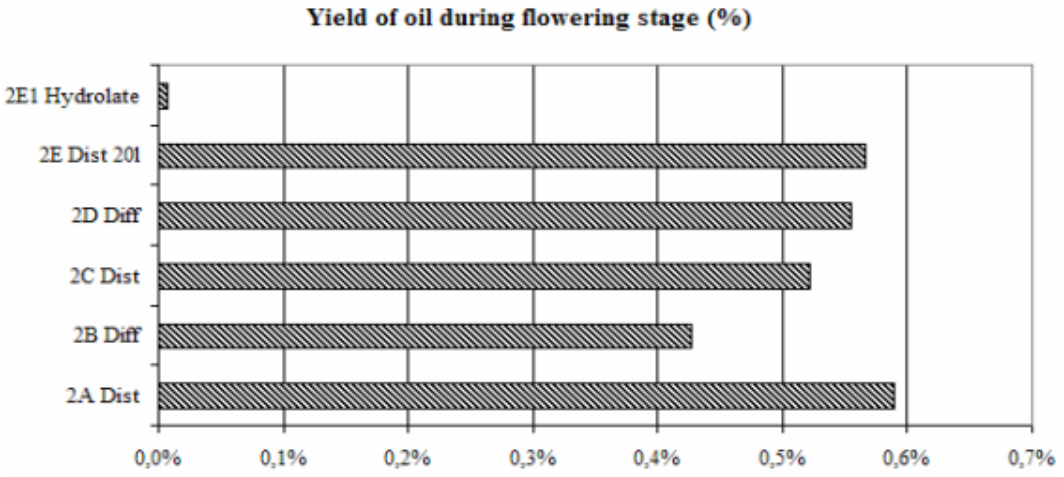


Figure 3: Yield of oil during flowering stage (%)

4.2 Chemical analysis of the essential oil extract

In the analysis 36 components were identified, representing almost 100% of the total oil. The major components were monoterpenes such as α-pinene, camphene, β-pinene, myrcene, α- and β-phellandrene, limonene, *(Z)*-β-ocimene and (*E*)-β-ocimene; monoterpene alcohols (linalool and borneol); sesquiterpenes like α-copaene, β-cubebene, β-caryophyllene, α-muurolene, germacrane D and (*E*)-β-farnesene. Sesquiterpene alcohols (spathulenol, α-and τ-cadinol and farnesol) occured in a smaller amount of approximately 0.3%.

Three major groups of components can be found in Solidago oil (Fig. 4). In all growing stages the most important components in an amount of more than 60.0% were monoterpene hydrocarbons. Their content ranged from 63.4% (DS) and 95.2% (DW) in their bulk forming stage (I) and decreased to a content of 60.6% (DS) and 51.8% (DW) in the flower stage (II). After flowering (III) the content of monoterpene hydrocarbons increased again to 65.2% and 94.6%, respectively.

The second important group were sesquiterpene hydrocarbons. Their amount ranged from 13.3% (DW) to 3.2% (DS). In the course of the growing season the percentage decreased to only 1.6% using DW staying steady in the last growing state, whereas the results using DS were relatively constant. In a small amount of less than one percent sesquiterpene alcohols occured which varied slightly during the season.
The results for the dry plants are not considered in this evaluation, but will be discussed later.

All dominating components that can be found during all plant stages were α- and β-pinene, myrcene and limonene. They occured in all plant stages in an amount of at least more than 10%. Highest yield was generally obtained using DW (with two exceptions). No constant results were obtained during ontogenesis.

4.2.1 α- and β-pinene

4.2.1.1 α-pinene

The component with the highest yield at all plant stages and for all extraction methods was α-pinene (Fig. 5).
Whereas in the first stage (bud formation) (1A-1E) the results obtained for fresh and dry plants were nearly the same (43.4% and 42.1%). The content achieved for DW was almost the double of the one obtained for DS (22.1% and 21.2%). Compared to this, the results for all other stages were very different. It was surprising that the amount received after 2 hours of distillation was with 47.6% higher than after 4 hours (42.1%). This means the distillation process was finished after two hours and the long process of 4 hours distillation is therefore not necessary.
The content of α-pinene which was obtained during flowering stage (2A-2D) was higher for the dry plants, but with 50.9% or 49.5% no significant difference between the two DW and DS could be found. When using fresh plants the difference was higher between DW and DS. A lower amount (26.7 and 35.3% for DW and DS, respectively) was achieved compared to the amount of the dry plants.

After flowering (3A-3D) the content of α-pinene was about the same for the fresh and dry plants using DS. When using DW the content from the fresh plants was higher (38.7%) than the content from the dry plants (23.6%).

4.2.1.2 β-pinene

Using diffusion, the content of β-pinene was higher for the fresh plants (6.9%) and lower (4.3%) for the dry plants. DW gave almost twice the amount of β-pinene than after DS (11.2% and 8.7%). As for α-pinene the content obtained after two hours was higher (10.3%) than after four hours (8.7%). In the flowering stage the percentage increased from 4.8% or 5.5% to 8.4% or 8.3% for the dry plants. In

the last growing stage contents were 14.4% and 8.7% or 12.6% and 13.9% for the fresh and dry plants, respectively.

4.2.2 Myrcene and Limonene

4.2.2.1 Myrcene

The content of myrcene attained 16.8% (distillation) and 14.9% (diffusion) for the fresh plants at the first sampling. It decreased in the course of the season to 8.9% and 7.2% and remained low after flowering (7.6%, diffusion). When using DW a higher amount (13.0%) could be obtained in the last growing stage (3A). The result obtained after two hours was slightly higher than the one after four hours (17.2% compared to 17.1%).
The yield of dry plants was higher in all growing stages. When using DS in the last two growing stages, the yield of dry plants was twice as high compared to fresh plants (7.2%; 14.4% and 7.6%; 15.1%). The increase was not that pronounced when using distillation. In the first stage only a slightly higher amount was extracted.

4.2.2.2 Limonene

Results for limonene are similar to those for myrcene. A decrease in the second stage of growing was observed from 17.9% or 14.3% to 7.9% or 9.5% and a further increase in the last stage of growing to 21.3% or 11.0% for distillation and diffusion, respectively.

The content of limonene obtained from dry plants was in all stages higher as for the fresh plants. It increased from the bulk forming stage from 13.4% or 5.4% to a high content of 17.4% or 13.9% in the flowering stage. The highest results were obtained in the last stage of growing with 23.1% or 18.7%.

Yield obtained after two and after one hour of distillation was surprisingly high. After two hours a content of 13.2% was obtained. Even after one hour yield was more than 20% and thus one of the highest results obtained.

4.3 Components of oil obtained by water-cum-steam distillation (WSD)

The main components found in the pure oil obtained by WSD (20l) are listed in Table 4. Most of the components found in a previous study from Laboratoire Laseve (2006) has been confirmed. Variations from the KI and the amount of contents can be explained by the different climatic conditions, the different sites and the use of different columns during GC analysis. The pure oil was injected because WSD (20l) correlates better with the natural process in the plant. The plant material was treated more carefully, hexane was not used as collecting solvent and so there is no need for the following long procedures (diluting – concentration on the rotovapor and the drying over nitrogen). Therefore, there are minimal dangers of chemical changes in constituents. This was to see if any components got lost during the process of DW and DS with hexane as collecting solvent. In Table 2 the composition of *Solidago puberula* oil obtained by WSD can be seen.

Table 2: Composition of Solidago puberula oil, WSD 070824, analysed with GC/FID on two different columns (SPB1 and WAX)

Nom	Time min	KI SPB1	Conc. %	Time min	KI WAX	Conc. %
n.i.	7.984	914	0.07	4.135	996	0.07
A-pinene	8.670	930	47.72	4.553	1016	47.69
camphene	9.033	938	2.22	5.406	1052	2.21
B -pinene	10.373	965	9.04	6.586	1092	7.98
myrcene	11.507	985	14.72	8.814	1155	14.81
A-phellandrene	11.874	991	0.64	8.591	1149	0.54
A-terpinene	12.598	1003	0.02	9.162	1163	0.02
p-cymene	12.769	1006	0.06	13.146	1249	0.05
1.8-cineole + β-phellandrene	13.120	1013	0.44			
limonene	13.407	1018	15.98	10.083	1184	15.99
(*Z*)-β-ocimene	14.004	1029	0.05	11.812	1221	0.05
(*E*)-β-ocimene	14.618	1040	1.82	12.601	1238	1.82
Γ-terpinene	15.021	1046	0.03	12.084	1227	0.03
terpinolene	16.802	1074	0.16	13.875	1262	0.16
linalool	17.448	1083	0.04	29.765	1534	0.02
camphor	18.915	1104	0.07	26.410	1478	0.02
limonene-1.2-oxide	19.412	1113	0.18	22.991	1421	0.18
bornyl acetate	29.206	1260	0.71	30.762	1551	0.72
eugenol	33.432	1322	0.02	61.233	2119	0.01
α-copaene	35.771	1359	0.05	25.769	1468	0.05
α-bourbonene	36.608	1372	0.11	28.568	1513	0.12
β-bourbonene	36.746	1374	0.21			
β-cubebene	37.821	1389	0.02	30.364	1544	0.02
β-caryophyllene	38.159	1394	0.73	31.521	1564	1.03
geranyl acetate	39.994	1424	0.03	46.382	1826	0.03
α-humulene	40.185	1427	0.57	35.548	1632	0.56
(E)-β-farnesene	41.921	1456	3.05	37.938	1673	3.07
B-farnesene	42.813	1470	0.38	39.202	1694	0.38
germacrane D	43.244	1477	0.13	40.652	1721	0.13
A-muurolene	43.845	1485	0.03			
A-farnesene	44.587	1497	0.10	40.782	1723	0.10
caryophyllene oxide	47.131	1541	0.05			
spathulenol	48.608	1566	0.06			
n.i.	50.785	1602	0.03	15.567	1292	0.07
n.i.	50.984	1605	0.02	20.193	1374	0.03
n.i.	51.206	1609	0.03	24.009	1438	0.01
n.i.	51.429	1614	0.03	24.712	1450	0.03
n.i.	53.264	1647	0.03	25.083	1456	0.02
(*Z*,*E*)-farnesol	53.949	1659	0.06	25.293	1460	0.03
n.i.	54.838	1675	0.02	27.214	1490	0.01

n.i. not identified

In the *Solidago puberula* oil component with almost 50% was α-pinene, followed by limonene (16.0%) and myrcene (14.7%). With less than 10% β-pinene (9.0%), (*E*)-β-farnesene (3.1%), camphene (2.2%) and (*E*)-β-ocimene (1.8%) were found. Furthermore, the pure oil also contained components in an amount less than 1% like α-phellandrene (0.6%), bornyl acetate (0.7%), β-caryophyllene (0.7%) and α-humulene (0.6%). These components changed their amount slightly during the different stages and with the different methods.

Analysis of sesquiterpene alcohols proved to be difficult because of a missing corresponding substance on WAX side. Allocation could not be met for substances between a KI of 1602 and 1647. It could thereby deal with different types of cadinol, muurolene or elemene.

4.4 Hydrolate

The composition of the hydrolate is very different to the one of the pure oil because the major part of components was found in the corresponding essential oil. Thus, the content of e.g. α-pinene lies with around 2.4% nearly the twentyfold under the value of the pure oil. Furthermore, substances as camphor, myrcene, limonene and β-pinene showed other values. Remarkable is also the absence of monoterpene alcohols in the pure oil whereas in the hydrolate most of the sesquiterpenes were missing. Other components that can be found in the pure oil in very small concentrations occured in the hydrolate in larger quantities.

Table 3: Composition of hydrolate, WSD 070824, analysed with GC/FID on two different columns (SPB1 and WAX)

Nom	Temps min	Ki SPB1	conc %	Temps min	Ki WAX	conc %
α-pinene	8.479	925	2.43	4.419	1010	2.09
camphene	9.002	937	0.17	5.375	1050	0.17
β-pinene	10.302	963	0.89	6.520	1090	0.84
myrcene	11.386	983	2.56	8.698	1152	2.53
α-phellandrene	11.851	990	0.17			
benzyl alcohol	13.030	1011	42.83	46.814	1834	42.76
limonene	13.309	1016	3.35	9.937	1181	3.38
(E)-β-ocimene	14.617	1040	0.48	12.559	1237	0.49
linalool	17.493	1084	0.92	29.766	1534	0.74
n.i.	19.838	1120	0.63	35.538	1632	0.70
borneol	21.037	1139	0.37			
terpinen-4-ol	21.960	1153	0.63	32.247	1576	0.62
α –terpineol	22.772	1165	0.49			
n.i.	24.922	1194	1.93			
neral	25.922	1209	0.17	36.406	1647	0.18
n.i.	27.877	1240	0.21			
bornyl acetate	29.232	1260	1.97	30.773	1551	1.94
β-caryophyllene	38.153	1394	0.66	31.511	1564	0.96
α-humulene	40.184	1427	0.68	36.598	1651	0.61
(E)-β-farnesene	41.901	1456	3.42	37.920	1673	4.19
β-farnesene	42.808	1470	0.42	39.193	1694	0.40
germacrane D	43.243	1477	0.27	39.322	1696	0.27
n.i.	44.593	1497	0.49			
caryophyll. oxide	47.133	1542	0.18			
spathulenol	48.619	1567	0.58			
n.i.	50.832	1603	0.47			
n.i.	50.990	1606	0.28			
n.i.	51.219	1610	0.27			
n.i.	51.453	1615	0.67			
(*Z*,*E*)-farnesol	53.964	1660	0.42			
n.i.	54.844	1675	0.2			

n.i. not identified

Table 3 shows the composition of the hydrolate (initial attenuation 1:100000 and a remaining concentration of dichloromethane of 30.776%).

As mentioned above there was a large difference between the composition of hydrolate and essential oil. Main component in the hydrolate with 42.8% was benzyl alcohol, a substance that cannot be found in the essential oil. This

substance showed a very good solubility in dichloromethane and was present in the hydrolate in a remarkable high concentration.

Substances that can be found in a higher concentration in the hydrolate but not in the corresponding oil were monoterpene alcohols, like borneol, terpinen-4-ol and α-terpineol (Fig. 7).

Sesquiterpenes were generally missing in the hydrolate. Only sesquiterpenes that can be found in the hydrolate were β-caryophyllene, that occured nearly in the same content as in the essential oil (0.73% and 0.66%), α-humulene, (*E*)-β-farnesene, β-farnesene, germacrane D and α-farnesene. Concentration of all components was higher in the hydrolate than in the oil. Sesquiterpene alcohols showed with 2.9% higher yield than in the essential oil (0.26%).

4.5 Components of oil in the stage of bud formation

The following results were obtained by DW and DS and were compared with each other. Distillation time varied between 1, 2 or 4 hours. Analysis was done by GC/FID. Unidentified components are described by their Kovats indices from the SPB1 and/or DB-WAX column.

Analysis of essential oils reulted in differences in chemical composition and content of components dependent on the two different extraction methods. Furthermore, drying had an influence on composition and content of oil and qualitative analysis indicated different results in chemical composition. For qualitative analysis an injected volume of 0.02µl from the pure oil and 1µl of a 1:5 diluted sample proved to be ideal to get approximately the same concentration and also the same number of components. Undiluted samples (1ml) resulted in a higher number of components, whereas 1:5 diluted samples showed a lower number of components (Fig. 9). The results are from the fresh plants.

4.5.1 Hydrodistillation after four hours

For further comparison of contents the results of the concentrated solution, with an injected volume of 0.02 µl, were used.

As can be seen in the chromatogram from *Solidago puberula* four major groups (Fig.10) can be found. The first group (until 18.9min) encloses monoterpene hydrocarbons, one monoterpene ether (1.8-cineole $t_{R=}$13.11) and one monoterpene alcohol (linalool t_R=17.49). They form with 95.6% the principal part of the oil. Monoterpene hydrocarbons were represented by α-pinene, camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene and camphor. Detailed information about proportional composition shows Appendix 1. Main part of the first group had α-pinene (43.4%), β-pinene (11.2%), myrcene (16.8%) and limonene (17.9%) (see also Fig. 6 and Fig.7). Monoterpene alcohols were represented by linalool, borneol, terpinen-4-ol and α-terpineol. Their retention times ranged from t_R=21.073 to t_R=22.79min.

Bornyl acetate, as only monoterpene ester, can be found at a retention time t_R=29.216 and in a concentration of 0.7%.

Sesquiterpene hydrocarbons, until 44.59min and one sesquiterpene alcohol (*Z,E*-farnesol, t_R=53,959min) can be found in the last part of the chromatogram. Main sesquiterpene hydrocarbons were β-bourbonene, β-caryophyllene, α-humulene, (*E*)-β-farnesene, β-farnesene and α-farnesene. All together they come to a content of 3.2%.

4.5.2 Hydrodistillation after two hours

Also in the chromatogram obtained after two hours four major groups can be found. Main group was again the one of monoterpene hydrocarbons, containing again 1.8-cineole and linalool, that can be found in the first part of the chromatogram. The second part of the chromatogram was made up of monoterpene alcohols and sesquiterpenes. All substances which were present in the oil obtained after four hours were also detected in the oil after two hours. Monoterpene hydrocarbons attained a total amount of 95%. Sesquiterpene

hydrocarbons, as second important group, reached not more than 1.9%. Noticable was also the presence of some sesquiterpene alcohols (t_R=48.62 to 54.87min) and a group of unidentified components after t_R=60min. Generally, the difference in content of components after two or four hours was not so significant. Detailed information on content of different components can be seen in Appendix 3.

4.5.3 Hydrodiffusion

Chromatogram obtained by DS (Fig.11) contained three major groups, monoterpene hydrocarbons, sesquiterpene hydrocarbons and sesquiterpene alcohols. Contrary to DW monoterpene alcohols (borneol, terpinen-4-ol and α-terpineol) were absent in the oil from DS. The first group (until 19.4min) contained monoterpene hydrocarbons, one monoterpene ether (1.8-cineole $t_{R=}$13.10) and one monoterpene alcohol (linalool t_R=17.48). With 65.1% they made the principal part of the oil. The main part of the first group had α-pinene (22.1%), β-pinene (6.9%), myrcene (14.9%) and limonene (14.3%) (see also Fig.5 and Fig.6). Esters were represented by bornyl acetate (t_R=29.2min; 1.5%) and geranyl acetate (t_R=39.4min; 0.04%).

The second and third group was made up of sesquiterpenes, which can be divided on the basis of their chemical criterion into hydrocarbons and alcohols. Hydrocarbons made approximately 13.3% of the total oil while the group of the alcohols is represented by a percentage of only 1%.

Main sesquiterpene hydrocarbons were α-copaene, α-bourbonene, β-bourbonene, β-caryophyllene, α-humulene, (*E*)-β-farnesene, β-farnesene, germacrane D, α-muurolene and α-farnesene. Principal part of this group was made up of (*E*)-β-farnesene (7.9%), β-caryophyllene (1.5%) and α-humulene (1.3%).

The main part from the group of sesquiterpene alcohols was constituted of (*Z,E*)-farnesol (0.3%) and spathulenol (0.2%). All other components were present in an amount below 0.1%. To the second group belongs also caryophyllene oxyde (t_R=47.12) with a content of 0.17% (Appendix 2).

4.5.4 Dry plants

The influence of drying on yield and components is discussed only by selected examples at the main components α- and β-pinene, myrcene and limonene. The group of monoterpene hydrocarbons are further compared: camphene, (*E*)-β-ocimene and as representatives of the esters bornyl acetate. Further β-caryophyllene, (*E*)-β-farnesene and α-humulene, from group of the sesquiterpenes, as oxidized component caryophyllene oxide and as representative of sesquiterpene alcohols (*Z*,*E*)-farnesol is used.

Table 4: Effects of drying presented on selected components

	1A Diffusion	1D Diffusion dry	1C Distillation	1E Distillation dry
α-pinene	22.1	21.2	43.4	42.1
camphene	1.3	1.0	2.0	2.0
β-pinene	6.9	4.3	11.2	8.7
myrcene	14.9	8.7	16.8	17.1
limonene	14.3	17.9	5.4	13.4
(*E*)-β-ocimene	2.6	0.9	2.7	1.9
bornyl acetate	1.5	0.2	0.7	0.6
β-caryophyllene	1.5	0.2	0.4	0.5
α-humulene	1.3	0.2	0.4	0.4
(*E*)-β-farnesene	7.9	1.0	1.9	2.3
caryophyllene oxide	0.2			
(*Z*,*E*)-farnesol	0.3	0.9	0.2	0.0

As can be seen in Table 4 the content of α-pinene and camphene were subject to a small fluctuation. The drying process had no great influence on myrcene, bornyl acetate, β-caryophyllene, α-humulene and (*E*)-β-farnesene. All of them were results from the oil obtained by DW. Values differed between 0.1-0.4%. Concentration of β-pinene decreased during the drying process.

Comparing the results from DS a decrease of content after drying can be seen. A decrease can be seen for most of the components whereas variation lied between 1.1 to 2.6%. Only with myrcene and (*E*)-β-farnesene two substances were found

whose concentration showed a difference of more than 6%. Limonene was for both methods the only component showing an increase in the oil obtained from dry plants. Values differed in content between 3.6 (DW) and 8.0%.
Components of oil in the flowering stage

Extraction of essential oil was done again using the described three methods. Results obtained by water-cum-steam distillation (WSD) are already mentioned in chapter 4.2.1. Extraction time for all methods was four hours.

4.5.5 Hydrodistillation

As can be seen in the chromatogram (Fig.12) obtained by DW from the plants in their flowering stage again four major groups can be found. The first group ranged until 19.8 min. and contained monoterpene hydrocarbons. They form the principal part of the oil (52.0%). Contrary to the oil obtained in the bulk forming stage the content of hexane is in proportion to the content of monoterpene hydrocarbons very high (t_R=1.8min; 45.8%). At the beginning, until the elution of α-pinene, one can therefore notice the occurence of hexyl alcohol, hexenal and one unidentified component. Monoterpene hydrocarbons were represented by α-pinene, camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, p-cymene limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene and the monoterpene ketone camphor. Detailed information about proportional composition shows Appendix 3. Main part of the first group were α-pinene (26.7%), β-pinene (4.8%), myrcene (8.9%) and limonene (7.9%) (see also Fig. 5 and Fig. 6).
Monoterpene alcohols were represented by linalool (t_R = 17.46min), borneol, terpinen-4-ol and α-terpineol. Their retention times ranged from t_R=21.065 to t_R=22.76min.
Bornyl acetate, as the only monoterpene ester, can be found at a retention time t_R=29.21 and in a concentration of 0.3%.

Sesquiterpene hydrocarbons (until 44.596min) and sesquiterpene alcohols (represented by (*Z, E*)-farnesol, different cadinols and spathulenol) can be found

in the last part of the chromatogram. Main sesquiterpene hydrocarbons were α- and β-bourbonene, β-caryophyllene, α-humulene, (*E*)-β-farnesene, β-farnesene, germacrane D and α-farnesene. Appreciable concentrations from the last three groups attained only β-caryophyllene (0.2%), α-humulene (0.2%) and (*E*)-β-farnesene (0.8%). Detailed information on the content of different components can be seen in Appendix 4.

4.5.6 Hydrodiffusion

Information on the content of monoterpenes and sesquiterpenes shows the chromatogram Fig. 13. As can be seen monoterpenes and sesquiterpenes are represented by hydrocarbons and alcohols. The number of components obtained by DS was higher than the number of components obtained by distillation. Again monoterpene hydrocarbons were quantitatively the main group coming to 60.8% (content of hexane was 35.7%). Monoterpene alcohols were presented by α-terpineol. Borneol and terpinen-4-ol were not present in the oil obtained by DS. Sesquiterpene hydrocarbons showed an amount of 2.9%. In addition to components present in the oil after distillation one can find α-copaene, β-cubebene, one unidentified component and α-muurolene. Highest concentrations attained β-caryophyllene (0.4%), (*E*)-β-farnesene (1.6%) and β-farnesene (0.15%). Traces of sesquiterpene alcohols could be found in the oil. Main components were spathulenol (0.03%) and (*Z*,*E*)-farnesol (0.05%). Comparative results can be seen in Appendix 5.

4.5.7 Dry plants

Table 5: Oil obtained from dry plants by two different methods

	2B Diffusion	2D Diffusion dry	2A Distillation	2C Distillation dry
α-pinene	35.3	49.5	26.7	50.9
camphene	1.6	2.0	1.2	2.2
β-pinene	5.5	8.2	4.8	8.4
myrcene	7.2	14.4	8.9	12.4
limonene	9.5	13.9	7.9	17.4
(*E*)-β-ocimene	0.8	1.5	1.2	1.8
bornyl acetate	0.4	0.5	0.3	0.6
β-caryophyllene	0.4	0.7	0.2	0.5
α-humulene	0.3	0.5	0.2	0.5
(*E*)-β-farnesene	1.6	2.4	0.8	1.6
caryophyllene oxide	-	-	-	-
(*Z*,*E*)-farnesol	0.05	0.1	0.1	0.1

Drying of plants in their flowering stage showed a positive effect on the concentration of all components. Differences ranged from 0.1% to about 14%. Highest variation can be seen for monoterpene hydrocarbons like α-pinene, β-pinene, myrcene and limonene. Almost a doubling can be observed in the hydrodistilled oil for α-pinene, β-pinene, myrcene, limonene and bornyl acetate and in the oil from DS for components like myrcene. Fluctuation was not as significant for all oils but generally one can say that an increase of concentration caused by drying can be observed

4.6 Components of oil after flowering

4.6.1 Hydrodistillation after one hour

The composition of the oil after flowering can be seen in chromatogram Fig. 14 showing no great differences in main groups and main components compared with bulk forming stage and flowering stage. First part of the chromatogram revealed a higher number of monoterpenes, which were not all included because of their small concentration (initial ratio of the chromatogram: 1:30.000). The main group were monoterpene hydrocarbons, representing more than 95% of the oil. The quantitative composition did not differ a lot in the course of the season. Until t_R=19.29 monoterpene hydrocarbons were represented by α-pinene, camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, p-cymene, β-phellandrene, limonene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, terpinolene, the monoterpene ether 1.8-cineole and the monoterpene ketone camphor. Main components were discussed at the beginning of the work (Fig.5 and 6). Monoterpene alcohols were also present in a higher number, but are, because of the small concentration, not all considered. Components present in the mentioned initial ratio were borneol (0.2%), terpinen-4-ol (0.2%) and α-terpineol (0.1%). One higher peak can be seen at a time of 29.2min, representing bornyl acetate. Bornyl acetate (1.2%) forms at the same time the change between monoterpenes and sesquiterpenes. Sesquiterpenes exist in an amount of 2.2%. Main representatives are β-caryophyllene (0.3%), α-humulene (0.2%), (*E*)-β-farnesene (0.7%), β-farnesene (0.1%), caryophyllene oxide (0.1%) and spathulenol (0.1%). Detailed information is found in Appendix 6.

4.6.2 Hydrodiffusion

The chromatogram (Fig. 15) of *Solidago puberula* after flowering shows three main groups. Contrary to results obtained by DW the group of monoterpene alcohols was not presented in the DW oil. The principal part of the oil are monoterpene hydrocarbons with more than 65% of the total. The group of monoterpene hydrocarbons did not show great differences in composition and the main part was formed by α-pinene (33.5%), camphene (1.7%), β-pinene (8.7%) myrcene (7.6%), limonene (11.0%) and (*E*)-β-ocimene (1.3%). Representing monoterpene ethers one can find 1.8-cineole (t_R=13.1min) and linalool as representatives of alcohols (t_R=17.46min). The second part of the chromatogram can be divided into sesquiterpene hydrocarbons and alcohols. Only one component- (*E*)-β-farnesene (t_R=41.84; 1.5%) got an amount over one percent. All other components were presented in an amount less than 0.4%. The amount of the whole group, including (*E*)-β-farnesene, was 3.1% (Appendix 7).

4.6.3 Dry plants

Table 6: Effects of drying presented on selected components

	3B Diffusion	3D Diffusion dry	3A Distillation	3C Distillation dry
α-pinene	33.5	36.3	38.7	23.6
camphene	1.7	2.1	2.5	1.6
β-pinene	8.7	13.9	14.4	12.6
Myrcene	7.6	15.1	13	16.4
Limonene	11.0	18.7	21.3	23.1
(*E*)-β-ocimene	1.3	2.3	2.7	3.4
bornyl acetate	0.4	1.0	1.2	1.8
β-caryophyllene	0.4	1.0	0.3	1.3
α-humulene	0.3	0.8	0.2	1.1
(*E*)-β-farnesene	1.5	3.7	0.7	4.0
caryophyllene oxide	0.1	0.2	0.1	0.2
(*Z*,*E*)-farnesol	0.1	0.2	0.1	0.2

For plants after flowering the influence of drying was analysed too. Results can be seen in Table 6. The components of dried plants analysed with DS increased their entire amount. Fluctuation varied between the different components. The main components α-pinene, β-pinene, myrcene and limonene showed a higher fluctuation than other components. Variations differed between 3% and 7%. All other components showed only a small fluctuation. It is interesting that by DW the concentration of α-pinene, camphene and β-pinene was reduced after drying. Comparing all other results from DW one can see an increase of the contents after drying. Most of sesquiterpenes showed a variation between 0.1% and 1.0%. Only with (*E*)-β-farnesene one can find a substance whose concentration showed a difference of more than 3.0%.

5. DISCUSSION AND CONCLUSION

The use of essential oils and their corresponding hydrolates presents a very important part of modern therapy. Therefore, production of essential oils in larger quantities and high quality becomes more and more important.
Isolation and analysis of essential oils is often connected with problems, which can occur meanwhile. The choice of the correct distillation method as well as the flowering stage of the plants play thereby an important role. It is also known that environmental conditions influence the production of secondary plant metabolites. Furthermore, the influence of drying the plant material was discussed in the thesis and it was shown that the preparation of the material is crucial for the amount of oil.

5.1 Methods

For isolation of the essential oil three different methods were used, which exhibit all their assets and drawbacks. Furthermore, the quantity of the used plant material and the quantity of oil resulting from it are some important aspects that should be considered.

5.1.1 Distillation in water (DW)

Using distillation in water the plant material is completely immersed in an appropriate volume of water. One important requirement in this method is that the relation between plant material and water should be kept constant. It is more efficient to use a smaller amount of material. Further, the prevention of an overheating should be ensured. Investigations showed that the best results were obtained when the plant material is in the form of a fine powder [12].
Disadvantages of this method are more frequent than for other processes (energy efficient, release of volatile oil is incomplete, only useful when charges are relatively small, hydrolysis of sensitive components) [12].

5.1.2 Diffusion or direct steam distillation (DS)

One advantage using DS is certainly the higher energy efficiency and higher consistency and reproducibility of results [12]. DS is therefore the mostly applied method for the production of essential oils. One important aspect for the whole project is that the used plant material is packed in the tube loosely to avoid a loss of essential oil. Two products were obtained using DS: essential oils and the corresponding hydrosols. DS is a non closed system and more water soluble components can be found in the hydrosol. Therefore, a smaller amount of components and smaller concentrations of all substances can be found in the oil obtained by DS.

5.1.3 Water cum steam distillation (WSD)

One interesting aspect for the production of essential oils is certainly the relation between used plant material and amount of obtained oil. WSD allows the production in higher quantities and for therapeutical use. The “non-use” of hexane as solvent also represents an advantage of this method.

5.1.4 Comparison of the three methods

One problem that occurred during all extraction methods is for sure the use of hexane as collecting solvent and the various resulting dilution factors after drying over nitrogen. WSD represented therefore a good alternative method to obtain non diluted oil. Comparing the different methods composition of oil obtained by the different methods did not vary a lot. Main components were in all cases α-pinene, β-pinene, limonene and myrcene. Differences can be seen in the number of obtained components. One good criterion to differentiate between distilled oil and oil obtained by DS was the presence of monoterpene alcohols. They were present in a higher amount during all plant stages in distilled oil, but couldn’t be detected in the oil obtained by DS. Generally, a higher amount of components was obtained by distilled oils. Concerning concentration of components no relation

between the various methods could be observed. In conclusion, it was found that distillation for two hours is quite suitable to obtain essential oils in high yield and high concentration.

5.2 Gaschromatographic analysis

Gaschromatography represents one of the best and precise methods for analysing substances according to their absorption on different stationary phases with various polarities. Further combination with FID enables exact analysis results. One problem that occurred during analysis was certainly the overlapping of substances either on the SPB1-side or on the Wax-side. Therefore, an exact assignment of substances, like β-pinene or sabinene, 1.8-cineole or α-phellandrene, and the numerous substances with a KI (SPB1) between 1603 and 1615, was not possible. Verification with GC/MS of those substances could not be accomplished.

5.3 Effects of harvesting time

Various concentrations and variations of components during plant ontogenesis were subject to many previous investigations. Investigations on different *Solidago* species showed that highest yield and quality were obtained when most of the plants were in full bloom [9].

Yield of oil showed drastic differences during ontogenesis. In the first two stages yield of essential oil was significantly higher than in the last stage. This large difference in yield was probably due to the fact that the last sampling took place a few days before extraction and that harvesting took place on a rainy day. Also the plant stage could be one reason for the lesser amount of essential oil. On selected components the influence of harvesting time was discussed, e.g. α-pinene, β-pinene, myrcene and limonene. Contrary to all other previous investigations [9, 10] one did not get constant results during the different stages. Content of all components after distillation and diffusion was higher in the first and the last plant stage. Only α-pinene obtained by DS attained a higher concentration in the flowering stage. However, the observed results and differences are only results

from one series of experiments and may be more generalized with a larger volume of data.

5.4 Effects of drying

Contrary to all expectations drying had a positive effect as well on concentration as on yield. Thus, it was possible to increase the amount of oil in the last two plant stages. The concentration of selected components (α-pinene, β-pinene, myrcene and limonene) was not very constant during the different stages. In the first stage nearly all components decreased their content after drying. In the following plant stages one could see an increase of nearly all components after drying. Furthermore, results obtained by the different extraction methods showed variations after drying.

6. FIGURES

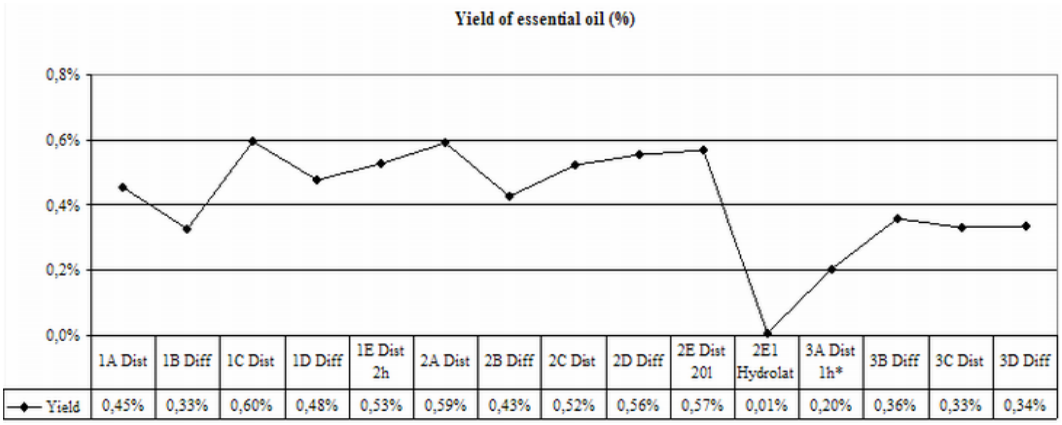


Figure 1: Yield of essential oil (%) obtained from all plant stages and different extraction methods.

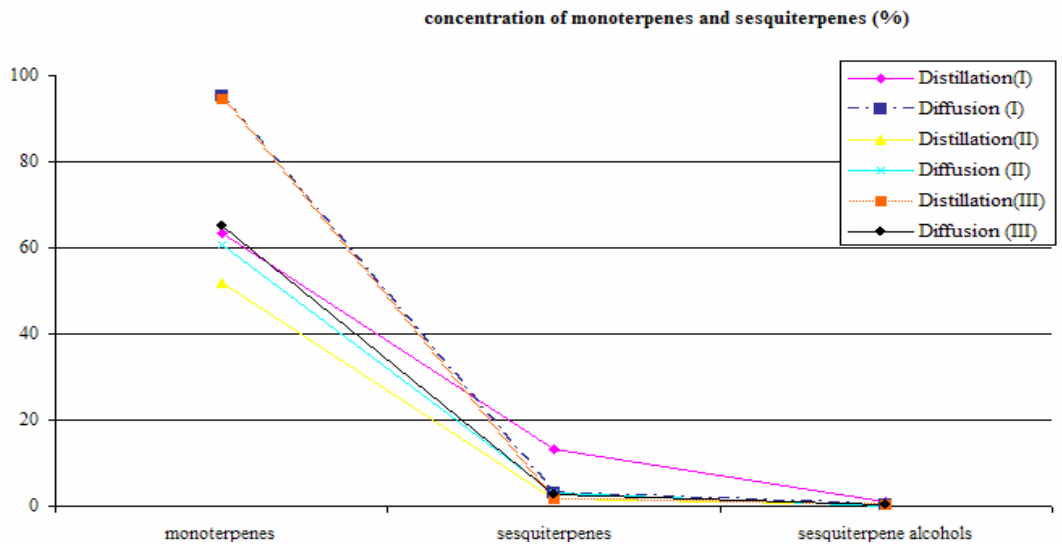
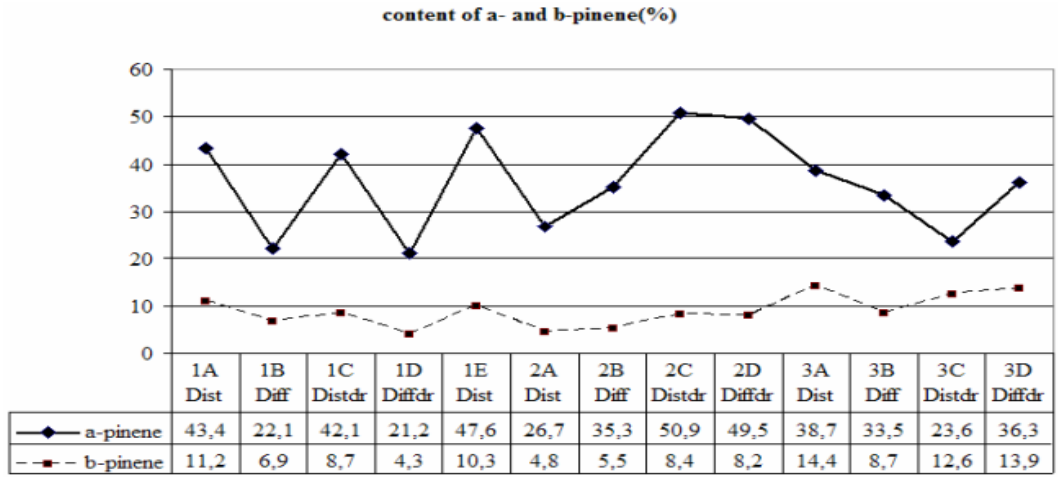
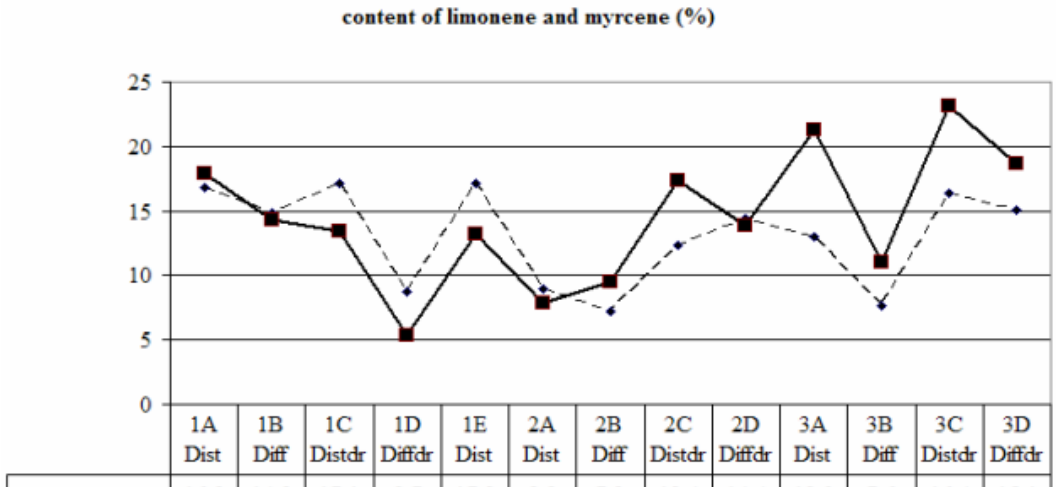


Figure 4: Concentration of monoterpenes and sesquiterpenes (%) for diffusion and distillation at the three plant stages.



	1A Dist	1B Diff	1C Distdr	1D Diffdr	1E Dist	2A Dist	2B Diff	2C Distdr	2D Diffdr	3A Dist	3B Diff	3C Distdr	3D Diffdr
a-pinene	43,4	22,1	42,1	21,2	47,6	26,7	35,3	50,9	49,5	38,7	33,5	23,6	36,3
b-pinene	11,2	6,9	8,7	4,3	10,3	4,8	5,5	8,4	8,2	14,4	8,7	12,6	13,9

Figure 5: Content of α- and β-pinene for the different plant stages and different extraction methods.



	1A Dist	1B Diff	1C Distdr	1D Diffdr	1E Dist	2A Dist	2B Diff	2C Distdr	2D Diffdr	3A Dist	3B Diff	3C Distdr	3D Diffdr
myrcene	16,8	14,9	17,1	8,7	17,2	8,9	7,2	12,4	14,4	13,0	7,6	16,4	15,1
limonene	17,9	14,3	13,4	5,4	13,2	7,9	9,5	17,4	13,9	21,3	11,0	23,1	18,7

Figure 6: Content of myrcene and limonene for the different plant stages and different extraction methods.

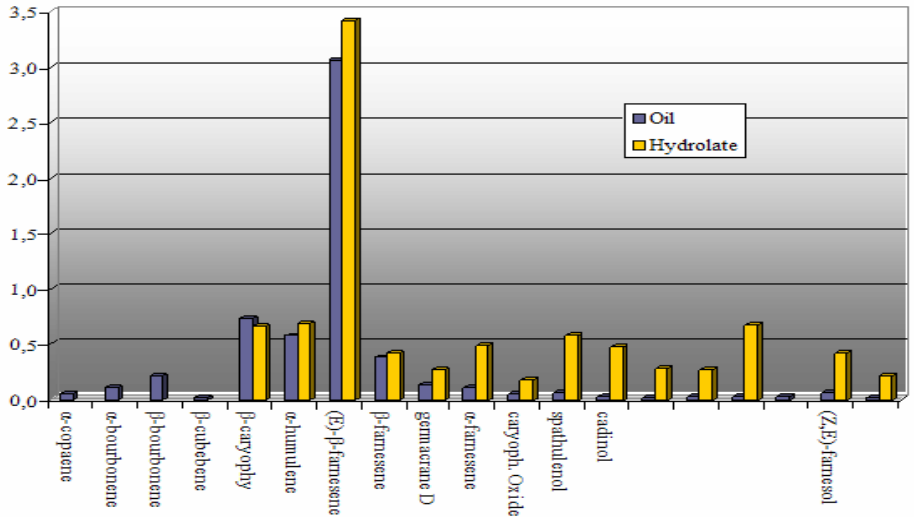


Figure 7: Distribution of monoterpene hydrocarbons and alcohols in oil and hydrolate

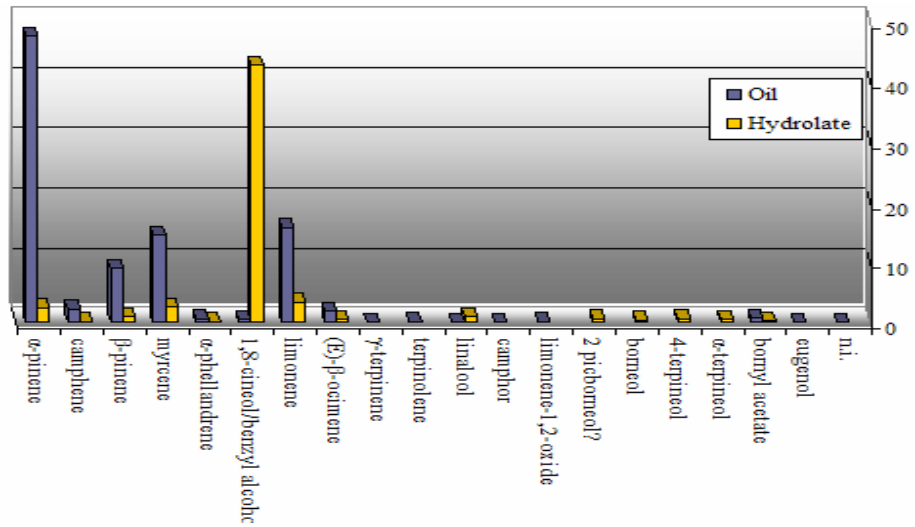


Figure 8: Distribution of sesquiterpenes in oil and hydrolate

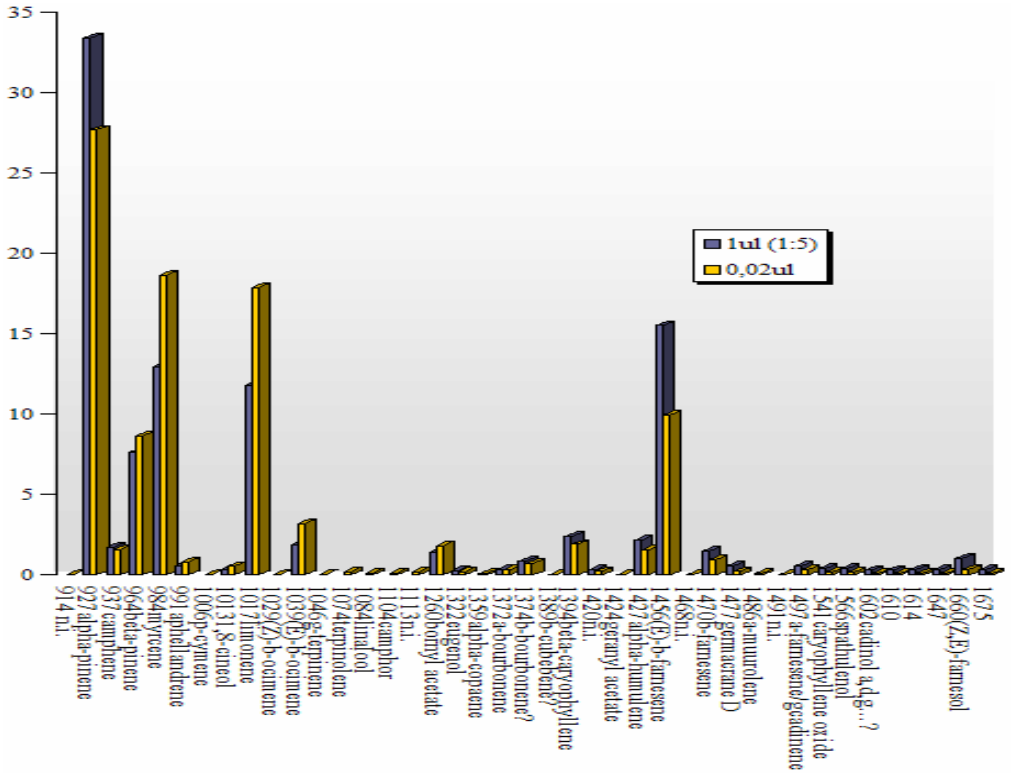


Figure 9: Components of essential oil from 0.02 and 1 µl obtained by hydrodiffusion (1A)

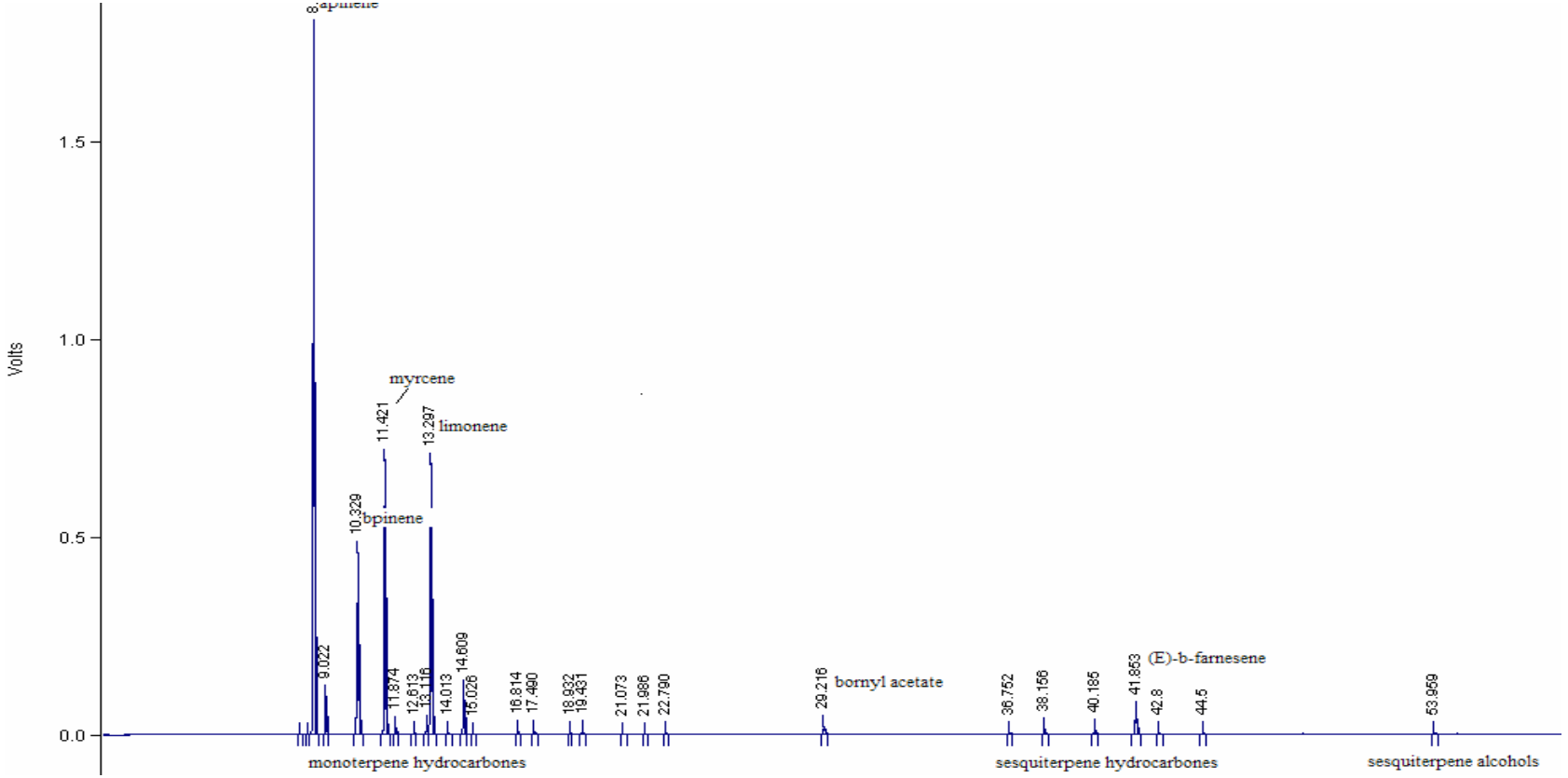


Figure 10: Hydrodistillation (1C2) *Solidago puberula*

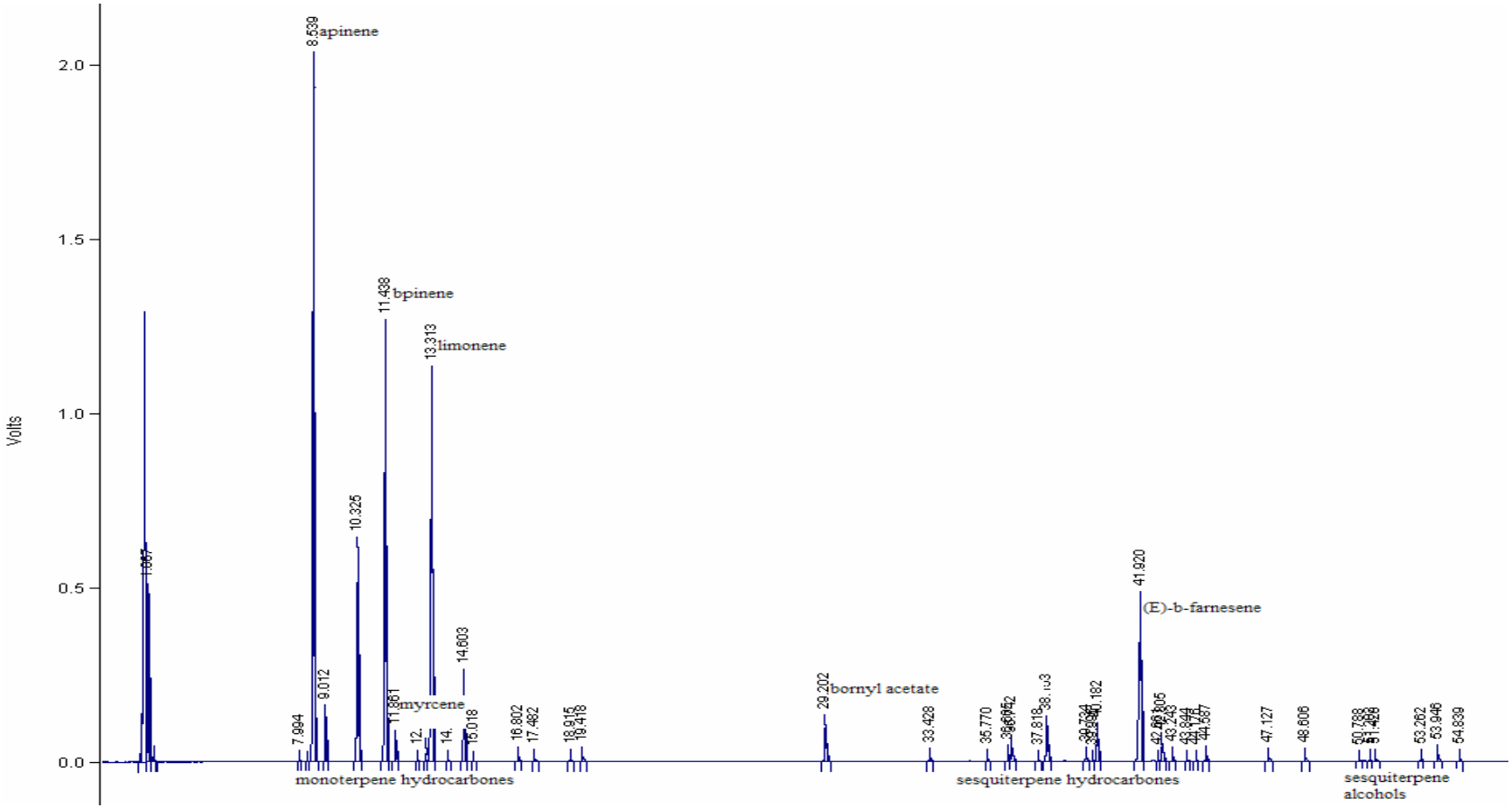


Figure 11: Hydrodiffusion (1A2) *Solidago puberula*

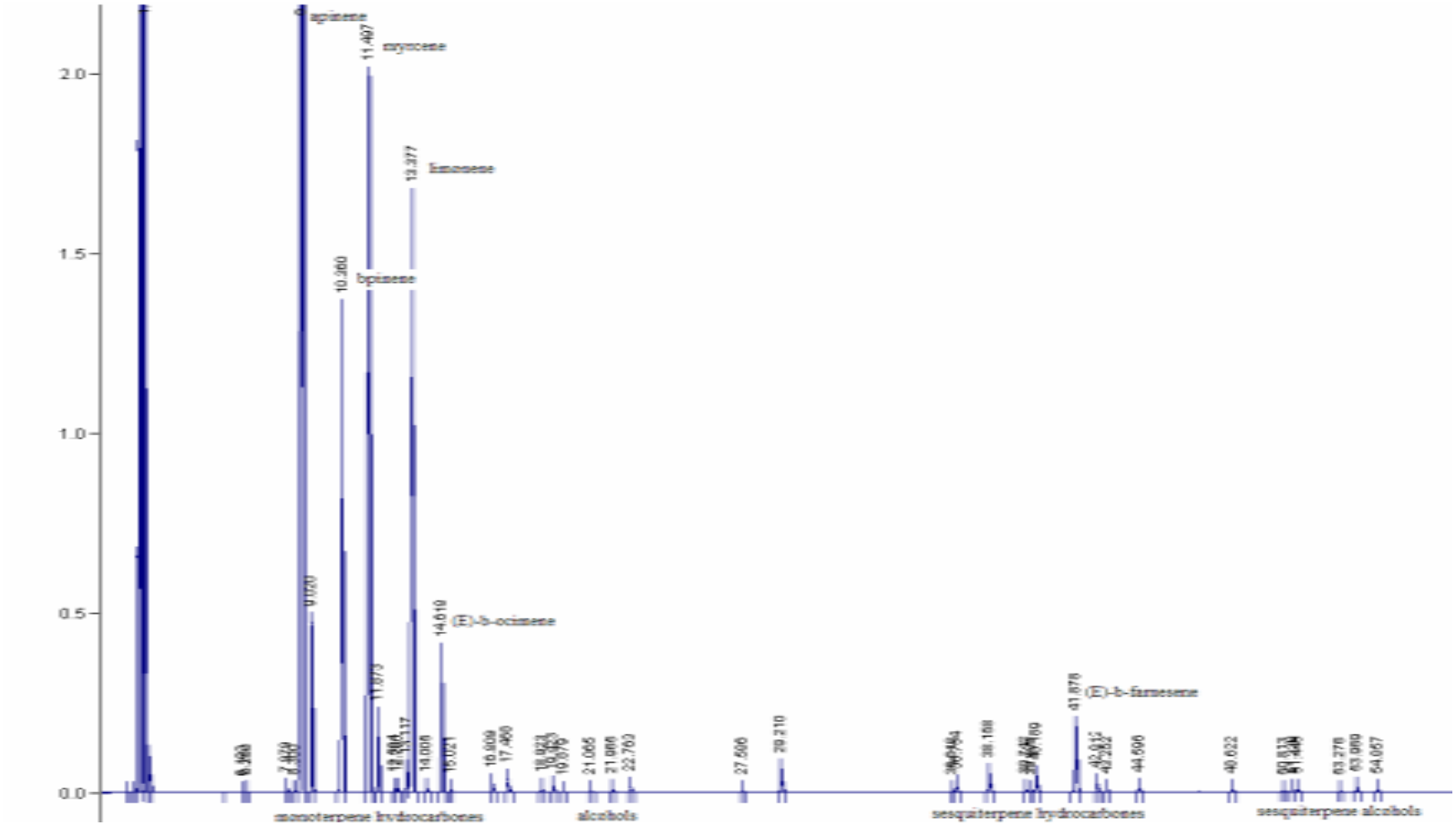


Figure 12: DW flowers (2A2) *Solidago puberula*

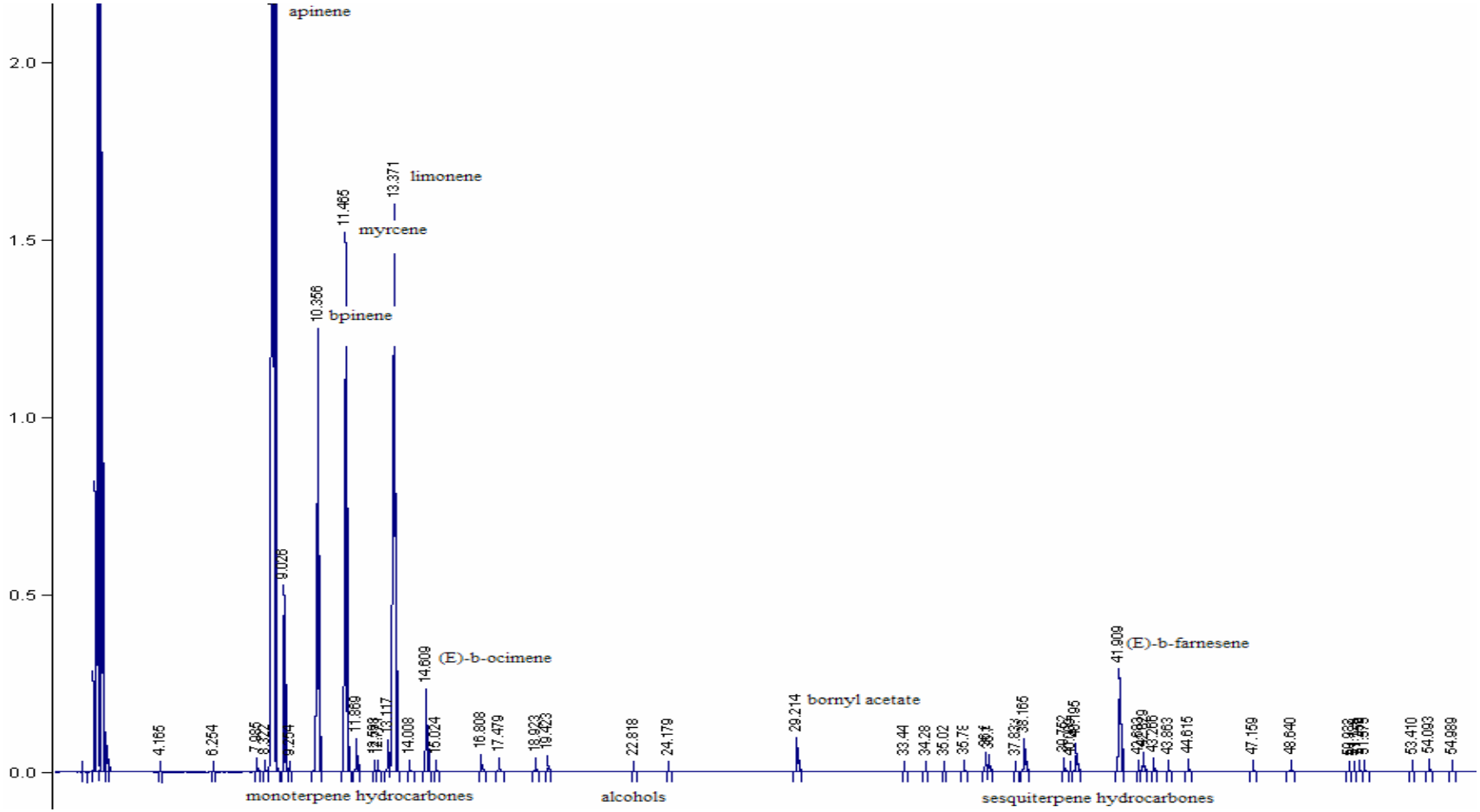


Figure 13: Hydrodiffusion flowers (2B2) *Solidago puberula*

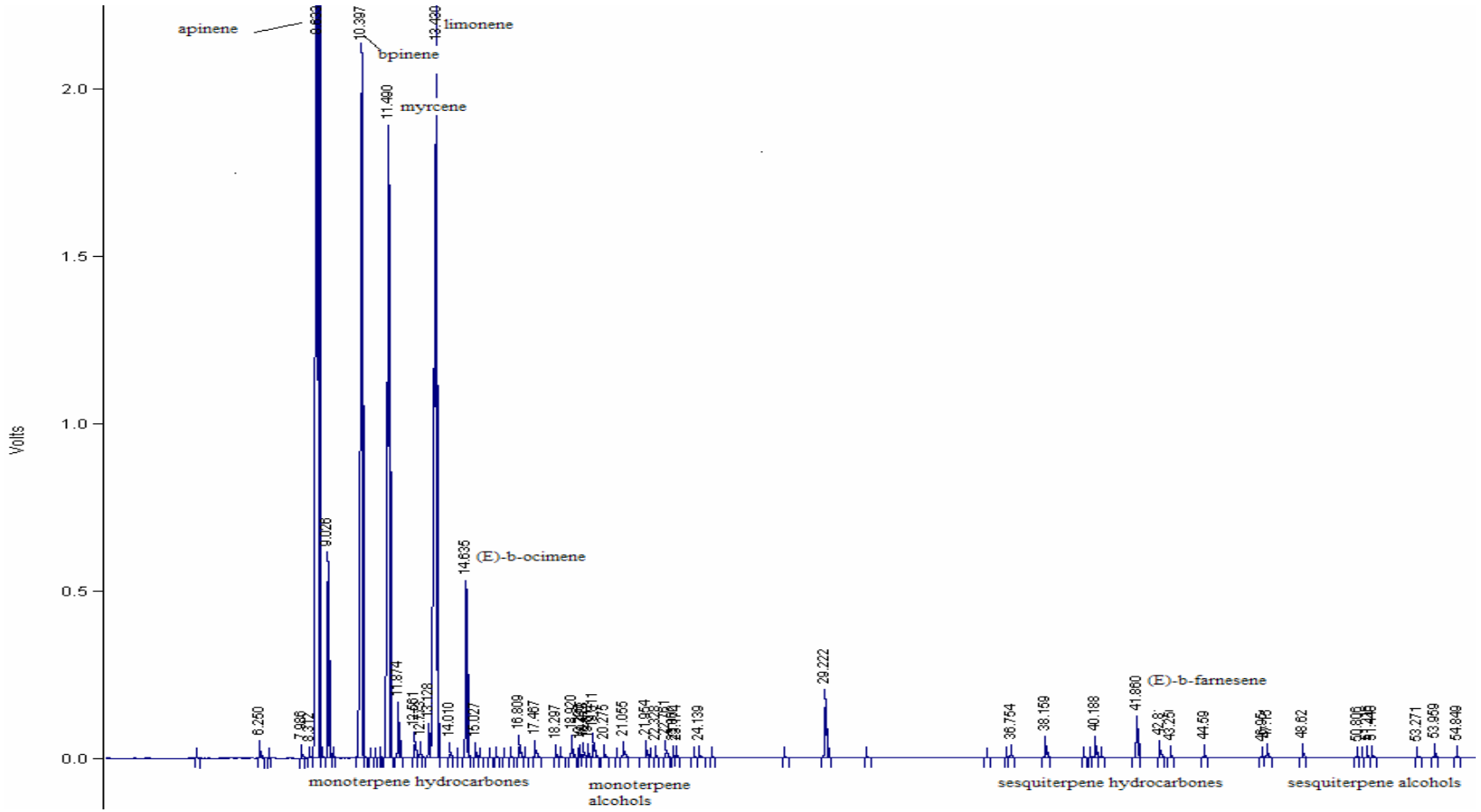


Figure 14: DW (3A2) *Solidago puberula*

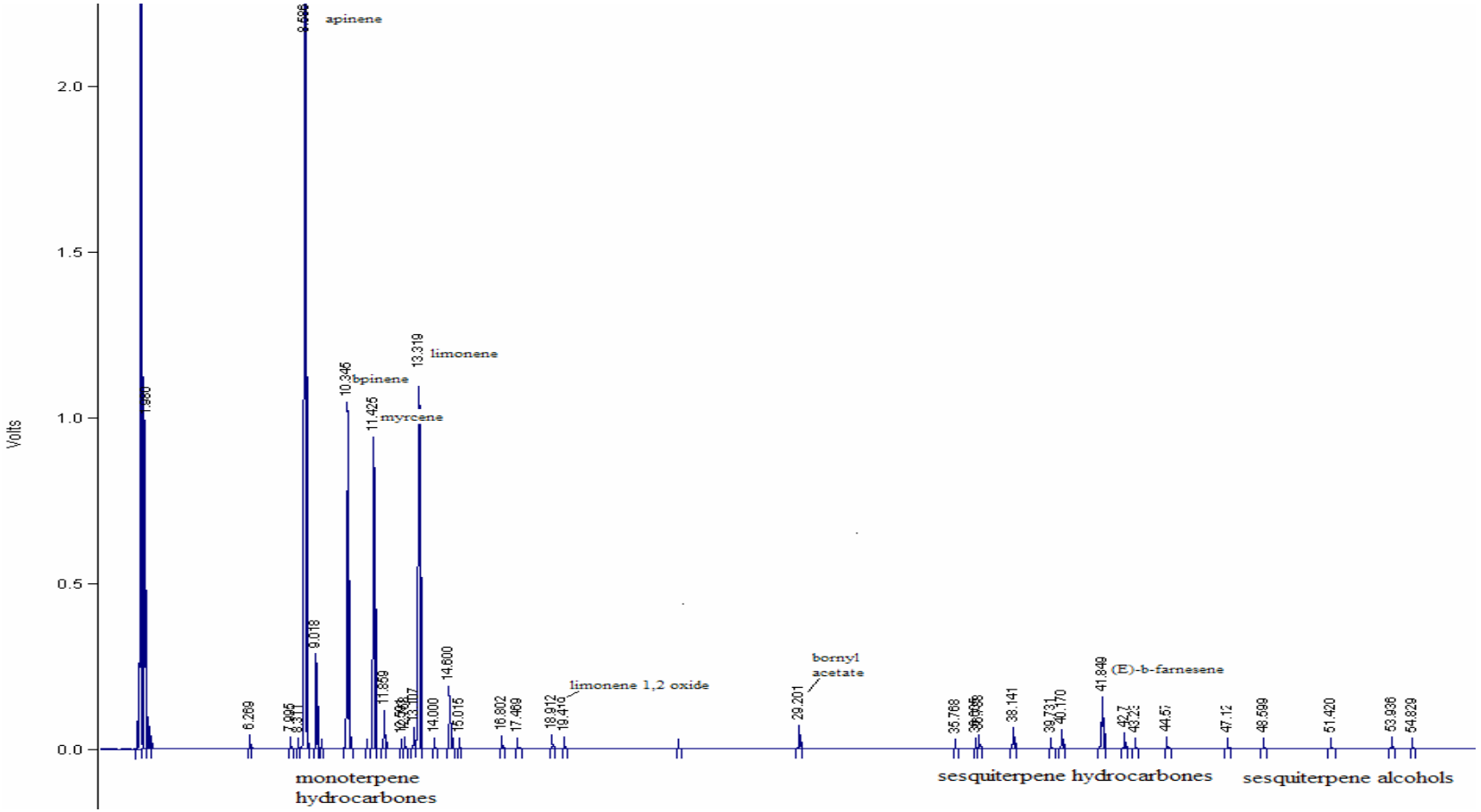


Figure 15: Hydrodiffusion (3B2) *Solidago puberula*

7. APPENDIX

Appendix 1. Composition of *Solidago puberula* obtained by DW in the bulk forming stage harvested on the 2nd of August obtained from two different injected volumes

Title:	Solidago 1A2-070914			Soldiago 1A1-070914	
Identification :	DW 070803			DW 070803	
Volume:			0.02µl		1µl
Nom	**Temps min**	**KI SPB1**	**conc %**	**KI SPB1**	**conc %**
				914	0.061
α-pinene	8.545	927	43.382	935	39.820
camphene	9.022	937	2.023	941	1.791
β-pinene	10.329	964	11.156	968	8.764
myrcene	11.421	983	16.762	988	11.902
α-phellandrene	11.874	991	0.387	993	0.296
α-terpinene	12.613	1003	0.088	1003	0.081
1,8-cineole	13.116	1013	0.468	1014	0.202
limonene	13.297	1016	17.879	1022	13.198
(Z)-β-ocimene	14.013	1029	0.085	1030	0.064
(E)-β-ocimene	14.609	1039	2.732	1042	2.026
γ-terpinene	15.026	1046	0.070		
terpinolene	16.814	1074	0.206	1075	0.172
linalool	17.490	1084	0.225	1084	0.205
camphor	18.932	1104	0.082	1105	0.080
n.i.	19.431	1113	0.172	1113	0.180
borneol	21.073	1139	0.069	1139	0.076
terpinen-4-ol	21.986	1153	0.072	1153	0.102
α-terpineol	22.790	1165	0.128	1165	0.171
bornylacetate	29.216	1260	0.668	1261	1.188
				1323	0.177
				1359	0.081
				1372	0.178
β-bourbonene	36.752	1374	0.148	1375	0.528
β-baryophyllene	38.156	1394	0.448	1396	1.442
				1421	0.143
α-humulene	40.185	1427	0.383	1430	1.462
(E)-β-farnesene	41.853	1455	1.931	1461	8.267
	42.810	1470	0.143	1465	0.072
				1472	0.676
				1478	0.417
				1487	0.076
				1492	0.089
α-farnesene/γ-cadinene	44.596	1497	0.116	1499	0.599

				1542	0.188
				1554	0.057
				1555	0.089
				1568	0.599
				1592	0.081
				1605	0.385
				1608	0.162
				1612	0.529
				1617	0.536
				1643	0.120
				1649	0.255
(*Z*,*E*)-farnesol	53.959	1660	0.176	1663	1.321
				1678	0.802
				1839	0.090
				1869	0.056
				1871	0.080
				2174	0.061

Appendix 2. Composition of *Solidago puberula* obtained by DS in the bulk forming stage harvested on the 2nd of August obtained from two different injected volumes

Title:	SolidagoPuberula1B1				Solidago Puberula 1B2	
Identification :	Diffusion 070804				Diffusion 070804	
Volume:			1.0µl (1:5)		0.02µl	
Nom	**Time min**	**KI SPB1**	**concentration %**	**concentration %Hex: 86.527**	**concentration %**	**concentration %Hex: 20.225**
n.i.					0.037	0.029
α-pinene	8.602	928	33.387	4.498	27.693	22.092
camphene	9.023	937	1.764	0.238	1.606	1.281
β-pinene	10.349	964	7.659	1.032	8.675	6.920
myrcene	11.466	984	12.988	1.750	18.644	14.874
α-phellandrene	11.871	991	0.562	0.076	0.794	0.634
p-cymene					0.049	0.039
1.8-cineole + β-phellandre	13.111	1013	0.370	0.050	0.548	0.437
limonene	13.338	1017	11.779	1.587	17.894	14.275
(Z)-β-ocimene					0.073	0.058
(E)-β-ocimene	14.608	1039	1.888	0.254	3.202	2.554
γ-terpinene					0.039	0.031
terpinolene					0.214	0.171
linalool					0.114	0.091
camphor					0.113	0.090
1113					0.218	0.174
bornyl acetate	29.202	1260	1.399	0.189	1.834	1.463
eugenol	33.423	1322	0.245	0.033	0.178	0.142
α-copaene					0.120	0.096
α-bourbonene	36.602	1371	0.378	0.051	0.329	0.263
β-bourbonene?	36.747	1374	0.859	0.116	0.722	0.576
β-cubebene?					0.059	0.047
β-caryophyllene	38.168	1394	2.461	0.332	1.928	1.538
1420	39.737	1420	0.327	0.044	0.239	0.191
geranyl acetate					0.049	0.039
α-humulene	40.198	1428	2.225	0.300	1.569	1.251
(E)-β-farnesene	42.018	1457	15.534	2.093	9.945	7.934
1468					0.056	0.045
β-farnesene	42.828	1470	1.501	0.202	0.953	0.761
germacrane D	43.248	1477	0.570	0.077	0.240	0.191
α-muurolene					0.092	0.073
					0.053	0.042
α-farnesene	44.582	1497	0.568	0.077	0.320	0.256
caryophyllene oxide	47.122	1541	0.417	0.056	0.207	0.165
spathulenol	48.602	1566	0.428	0.058	0.203	0.162
	50.784	1602	0.310	0.042	0.111	0.088

	51.199	1610	0.313	0.042	0.118	0.094
	51.424	1614	0.338	0.045	0.131	0.104
	53.261	1647	0.366	0.049	0.129	0.103
(Z,E)-farnesol	53.958	1660	1.011	0.136	0.375	0.299
	54.831	1675	0.353	0.048	0.128	0.102

Appendix 3. Composition of *Solidago puberula* obtained by DW (2h) in the bulk forming stage obtained from two different injected volumes

Title	Solidago 1F1 070802		Solidago 1F2 070802		
Identification :	DW 070808 2h		DW 070808 2h		
Volume:		1ul (1:5)			0.02ul
	Ki	**conc**	**Temps**	**Ki**	**conc**
Nom	**SPB1**	**%**	**min**	**SPB1**	**%**
n.i.			7.995	914	0.074
n.i.			8.318	922	0.052
α-pinene	928	42.153	8.623	929	48.391
camphene	937	2.186	9.025	938	2.507
β-pinene	964	8.851	10.359	964	10.445
myrcene	984	13.858	11.483	984	17.438
α-phellandrene	991	0.290	11.875	991	0.345
α-terpinene			12.596	1003	0.085
p-cymene			12.770	1006	0.031
1.8-cineole	1013	0.404	13.115	1013	0.454
limonene	1017	11.698	13.348	1017	13.426
(Z)-β-ocimene			14.008	1029	0.079
(E)-β-ocimene	1039	2.696	14.620	1040	3.035
γ-terpinene			15.023	1046	0.053
terpinolene	1074	0.177	16.810	1074	0.177
linalool			17.478	1084	0.126
camphor			18.924	1104	0.088
n.i.			19.426	1113	0.109
borneol			21.041	1139	0.067
terpinen-4-ol			21.967	1153	0.059
α-terpinene			22.772	1165	0.082
bornyl acetate	1260	1.544	29.213	1260	0.692
β-bourbonene	1374	0.494	36.758	1374	0.081
β-caryophyllene	1394	1.231	38.159	1394	0.275
α-humulene	1427	1.361	40.189	1428	0.248
(E)-β-farnesene	1456	6.986	41.867	1455	1.121
β-farnesene	1470	0.716	42.813	1470	0.100
germacrane D	1477	0.376	43.255	1477	0.046
α-farnesene	1497	0.511	44.599	1497	0.065
caryophyllene oxide	1541	0.262			
spathulenol	1566	0.490	48.623	1567	0.042
	1603	0.420			
	1606	0.188			
	1610	0.449	51.220	1610	0.034

	1614	0.612	51.443	1614	0.045
	1648	0.561	53.280	1648	0.036
(Z.E)-farnesol	1660	0.923	53.962	1660	0.058
	1675	0.562	54.857	1676	0.031

Appendix 4. Composition of *Solidago puberula* obtained by DW in the flowering stage. harvested on the 17th of August obtained from two different injected volumes

Titre	Solidago 2A1-070817				Solidago 2A2-070817	
Identification :	DW 070820 4h				DW 070820 4h	
Volume:			1µl		0.02µl	
Nom	**Temps min**	**Ki SPB1**	**conc %**	**conc% %Hex: 30.749**	**conc %**	**conc% %Hex: 45.767**
α-pinene	8.936	929	36.485	25.266	49.212	26.689
camphene	9.266	937	1.877	1.300	2.158	1.171
β-pinene	10.611	964	7.720	5.346	8.864	4.807
myrcene	11.841	985	13.948	9.659	16.473	8.934
α-phellandrene	12.107	991	0.938	0.650	1.059	0.574
α-terpinene	12.676	1003	0.068	0.047	0.066	0.036
p-cymene	12.873	1006	0.069	0.048	0.076	0.041
1.8-cineole	13.117	1013			0.427	0.232
limonene	13.776	1018	13.866	9.602	14.566	7.900
(Z)-β-ocimene	14.112	1029	0.065	0.045	0.068	0.037
(E)-β-ocimene	14.836	1040	2.133	1.477	2.260	1.226
γ-terpinene	15.131	1046	0.046	0.032	0.047	0.026
terpinolene	16.857	1074	0.165	0.114	0.155	0.084
linalool	17.541	1084	0.292	0.202	0.261	0.141
camphor	18.941	1104	0.071	0.049	0.062	0.034
n.i.	19.443	1113	0.126	0.088	0.103	0.056
n.i.	19.879	1120			0.015	0.008
borneol	21.040	1139	0.042	0.029	0.030	0.016
terpinen-4-ol	21.961	1153	0.084	0.058	0.047	0.026
α-terpineol	22.781	1164	0.154	0.107	0.098	0.053
n.i.	27.573	1235	0.050	0.035	0.022	0.012
bornyl acetate	29.331	1260	0.987	0.683	0.478	0.259
α-bourbonene	36.631	1372	0.086	0.060	0.025	0.013
β-bourbonene	36.854	1374	0.655	0.454	0.145	0.078
β-caryophyllene	38.353	1394	1.623	1.124	0.408	0.222
	39.875	1420	0.213	0.148	0.048	0.026
geranyl acetate	40.088	1424	0.062	0.043	0.018	0.010
α-humulene	40.409	1428	1.723	1.193	0.367	0.199
(E)-β-farnesene	42.413	1455	8.133	5.632	1.522	0.826
β-farnesene	42.583	1470	0.037	0.026	0.181	0.098
germacrane D	43.056	1477	1.055	0.731	0.064	0.035
α-farnesene	43.391	1497	0.387	0.268	0.090	0.049
	43.933	1488	0.080	0.055		
	44.250	1493	0.065	0.045		
	44.735	1500	0.592	0.410		
	47.183	1543	0.258	0.179		
	47.558	1550	0.050	0.035		
	47.950	1556	0.160	0.111		

	48.784	1567	0.577	0.399	0.074	0.040
	50.863	1603	0.187	0.129	0.037	0.020
	51.012	1608	0.315	0.218		
	51.119	1610	0.160	0.111	0.058	0.032
	51.432	1614	0.608	0.421	0.058	0.032
	51.670	1620	0.628	0.435		
	53.066	1648	0.115	0.080	0.023	0.012
(Z,E)-farnesol	53.410	1660	0.246	0.171	0.12	0.064
	54.247	1676	1.378	0.954	0.06	0.031
	55.065	1680	0.717	0.497		

Appendix 5. Composition of *Solidago puberula* obtained by DS in the flowering stage. harvested on the 17th of August obtained from two different injected volumes

Title	Solidago2B1-070817			Solidago2B2-070817	
Identification	Diffusion flowers. 070820			Diffusion flowers. 070820	
Volume			1.0µl	0.02µl	
Nom	**Temps min**	**Ki SPB1**	**conc %**	**conc % %Hex: 35.701**	**conc %**
α-pinene	8.651	929	43.1	35.3	54.9
camphene	9.026	938	1.9	1.6	2.4
β-pinene	10.356	964	6.8	5.5	8.5
myrcene	11.465	984	8.7	7.2	11.2
α-phellandrene	11.869	991	0.3	0.2	0.4
α-terpinene	12.598	1003		0.0	0.0
p-cymene	12.771	1006		0.0	0.0
1.8-cineole	13.117	1013	0.4	0.3	0.4
limonene	13.371	1018	12.9	9.5	14.8
(*Z*)-β-ocimene	14.008	1029		0.0	0.0
(*E*)-β-ocimene	14.609	1039	1.2	0.8	1.3
γ-terpinene	15.024	1046		0.0	0.0
terpinolene	16.808	1074		0.1	0.1
linalool	17.479	1084		0.1	0.1
camphor	18.923	1104		0.1	0.1
α-terpinene	22.818	1165		0.0	0.0
bornyl acetate	29.214	1260	1.1	0.4	0.5
α-copaene	35.789	1359		0.0	0.0
α-bourbonene	36.624	1372	0.8	0.1	0.2
β-bourbonene	36.758	1374	0.7	0.1	0.2
β-cubebene	37.833	1389		0.0	0.0
β-caryophyllene	38.165	1394	2.1	0.4	0.5
geranyl acetate	40.009	1425	0.3	0.0	0.0
α-humulene	40.195	1428	2.0	0.3	0.4
(*E*)-β-farnesene	41.909	1456	12.3	1.6	2.4
1470	42.829	1470	1.2	0.1	0.2
1477	43.266	1477	0.6	0.1	0.1
α-muurolene	43.863	1486	0.2	0.0	0.0
α-farnesene	44.615	1498	0.5	0.0	0.1
caryophyllene oxide	47.159	1542	0.3	0.0	0.0
spathulenol	48.640	1567	0.4	0.0	0.0
1605	50.932	1605	0.3	0.0	0.0
1609	51.129	1609	0.3	0.0	0.0
1613	51.350	1613		0.0	0.0
1617	51.575	1617	0.2	0.0	0.0
	53.410	1650	0.3	0.0	0.0
(*Z*,*E*)-farnesol	54.093	1662	0.8	0.0	0.1

1678	54.989	1678	0.3	0.0	0.0

Appendix 6. Composition of *Solidago puberula* obtained by DW in the stage after flowering. harvested on the 15th of September obtained from two different injected volumes

Titre	Solidago3A1-070915				Solidago3A2-070915	
Identification :	DW 070920				DW 070920	
Volume:			1.0µl			0.02µl
Nom	**Temps min**	**Ki SPB1**	**conc % %hex: 5.641**	**conc %**	**Ki SPB1**	**conc %**
	3.682	776	0.099	0.105		
	6.253	870	0.252	0.267	870	0.129
n.i.	8.002	914	0.075	0.080	914	0.058
	8.422	924	0.103	0.109	922	0.043
α-pinene	8.847	934	41.053	43.507	929	38.732
camphene	9.158	941	2.513	2.663	938	2.494
β-pinene	10.553	968	12.275	13.009	965	14.395
myrcene	11.625	987	9.030	9.570	984	12.981
α-phellandrene	11.948	992	0.478	0.506	991	0.661
α-terpinene	12.571	1003	0.237	0.251	1002	0.293
p-cymene	12.810	1008	0.086	0.091	1006	0.120
1.8-cineole	13.173	1015	0.233	0.247	1013	0.512
limonene	13.608	1023	15.143	16.048	1019	21.293
(*Z*)-β-ocimene					1029	0.095
(*E*)-β-ocimene	14.717	1042	1.772	1.878	1040	2.678
γ-terpinene					1046	0.088
terpinolene	16.827	1075	0.168	0.178	1074	0.238
linalool	17.485	1084	0.106	0.113	1083	0.230
					1095	0.073
camphor	18.929	1105	0.180	0.191	1104	0.243
					1111	0.063
					1113	0.097
					1116	0.073
	19.831	1120	0.236	0.251	1119	0.295
n.i.					1127	0.067
borneol	21.059	1140	0.176	0.187	1139	0.200
terpinen-4-ol	21.962	1153	0.140	0.148	1153	0.169
					1158	0.048
α-terpinene	22.770	1165	0.146	0.155	1164	0.147
					1169	0.049
					1170	0.053
					1184	0.060
bornyl acetate	29.305	1262	1.465	1.552	1260	1.158
β-bourbonene	36.763	1375	0.173	0.183	1374	0.079
β-caryophyllene	38.194	1395	0.553	0.586	1394	0.261

	40.003	1425	0.112	0.118		
α-humulene	40.238	1429	0.613	0.650	1428	0.246
(*E*)-β-farnesene?	42.002	1458	1.994	2.113	1455	0.705
	42.862	1472	0.458	0.485	1470	0.148
	43.278	1478	0.224	0.237	1477	0.061
α-farnesene/γ-cadinene	44.621	1499	0.240	0.255	1497	0.068
					1538	0.039
caryophyllene oxide	47.020	1541	0.171	0.182	1542	0.126
	47.195	1544	0.530	0.562		
	47.912	1556	0.102	0.108		
	48.686	1569	0.545	0.578	1567	0.105
	50.079	1591	0.077	0.081		
	50.871	1605	0.227	0.240	1603	0.039
	51.037	1608	0.124	0.131	1610	0.058
	51.287	1613	0.331	0.351	1614	0.048
	51.501	1617	0.321	0.340		
	52.529	1635	0.091	0.097		
	52.980	1643	0.124	0.131		
	53.336	1650	0.261	0.276	1648	0.041
	54.057	1663	0.620	0.657	1660	0.094
	54.913	1678	0.338	0.359	1675	0.048

Appendix 7. Composition of *Solidago puberula* obtained by DW and DS in the stage after flowering. harvested on the 15th of September. Results from fresh and dry plants.

Titre	Solidago 3B2		Solidago3C2 dry		Solidago3D2 dry	
Identification :	Diffusion 070920		DW 070927		Diffusion 070927	
Provenance :			Aliksir 070915		Aliksir 070915	
	conc %	**conc %**	**conc**	**conc**	**conc**	**conc**
Nom	**%Hex: 30.862**		**%Hex: 0.439**	**plus solvent**	**%Hex: 0.136**	**plus solvent**
870	0.099	0.143				
inc.	0.057	0.082			0.055	0.055
921	0.032	0.047				
α-pinene	33.512	48.471	23.607	23.711	36.279	36.329
camphene	1.730	2.502	1.576	1.583	2.118	2.121
β-pinene	8.724	12.619	12.569	12.624	13.872	13.891
myrcene	7.631	11.038	16.404	16.476	15.077	15.097
α-phellandrene	0.679	0.982	1.369	1.375	0.865	0.866
α-terpinene	0.024	0.035	0.360	0.362	0.048	0.048
p-cymene	0.076	0.111	0.281	0.283	0.178	0.178
1.8-cineole	0.308	0.445	0.693	0.696	0.544	0.544
limonene	11.009	15.923	23.142	23.244	18.671	18.697
(*Z*)-β-ocimene	0.036	0.052	0.130	0.131	0.063	0.063
(*E*)-β-ocimene	1.300	1.880	3.419	3.434	2.287	2.290
γ-terpinene	0.032	0.047	0.130	0.130	0.062	0.062
terpinolene	0.099	0.143	0.351	0.353	0.196	0.196
linalool	0.077	0.111	0.552	0.555	0.163	0.163
1095			0.109	0.110		
camphor	0.122	0.176	0.362	0.363	0.264	0.265
1111			0.080	0.081	0.199	0.200
limonene-1,2-oxide	0.065	0.094	0.171	0.172		
1116			0.108	0.108		
1119			0.363	0.365		
1127			0.096	0.096		
1139			0.255	0.256		
1153			0.182	0.183		
1158			0.092	0.092		
1164			0.385	0.387		
1169			0.128	0.129		
1184			0.107	0.107	0.054	0.054
bornyl acetate	0.431	0.623	1.775	1.783	0.994	0.996
α-copaene	0.025	0.036	0.094	0.094	0.085	0.085
α-bourbonene	0.040	0.057	0.129	0.129	0.153	0.153
β-bourbonene	0.156	0.225	0.493	0.496	0.291	0.291
β-caryophyllene	0.423	0.612	1.332	1.338	1.026	1.028
geranyl acetate	0.047	0.068	0.157	0.157	0.136	0.136
α-humulene	0.330	0.478	1.104	1.108	0.785	0.786
(*E*)-β-farnesene	1.461	2.114	4.037	4.054	3.692	3.697

β-farnesene	0.213	0.307	0.890	0.894	0.719	0.720
germacrane D	0.051	0.074	0.170	0.171	0.183	0.184
α-farnesene	0.069	0.100	0.403	0.404	0.182	0.183
1538			0.105	0.106		
caryophyllene oxide	0.064	0.093	0.228	0.229	0.153	0.153
spathulenol	0.051	0.074	0.258	0.259	0.116	0.117
1610			0.463	0.465	0.068	0.068
t-cadinol	0.033	0.048	0.216	0.217	0.176	0.176
(*Z*,*E*)-farnesol	0.099	0.143	0.456	0.458		
1675	0.033	0.048	0.232	0.233		

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