universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Contribution to the knowledge of the essential oils of *Origanum* species grown in Spain

Verfasser

Martin Babeluk

angestrebter akademischer Grad

Magister der Pharmazie (Mag.pharm.)

Wien, 2013

Studienkennzahl lt. Studienblatt:	A 449
Studienrichtung lt. Studienblatt:	Diplomstudium Pharmazie
Betreuerin / Betreuer:	Univ.-Prof.Mag.Dr.Dr.h.c. Brigitte Kopp

Index

The experimental part of this work was performed in the Faculty of Pharmacognosy of the Univertsity of Barcelona in corporation with the Department of Pharmacognosy of the University of Vienna during a period from October till Jaunary of 2012/2013. It was part of the Erasmus/Sokartes program.

At the beginning of this work I want to thank everybody who made it possible to study abroad and supported me accomplishing this study.

First I want to thank my coordinater Univ.-Prof.Mag.Dr.Dr.h.c. Brigitte Kopp of the Department of Pharmacognosy of the University of Vienna how enabled me to study in Barcelona.

I owe special thanks to my supervisors in Barcelona, Prof.Dr. Salvador Cañigueral and Prof.Dr. Roser Vila for their openness, kind assistence and help, without this work wouldn't have been possible.

Also, I want to thank my colleague Enric Gibert for the warm working climate and the technical support.

Furthermore I want to thank Prof. Segundo Rios and Vanessa Martinez-Francés (CIBIO, Univerity of Alacant) as Jesus Burillo (CITA, Zaragoza) for providing the samples of plant material and the samples of essential oils.

Furthermore I'm thankful for my parents how always supported me and my study at the University of Vienna as my decision to live and work for one semester in Barcelona.

Dankeschön! ***Thank you!*** ***Muchisimas gracias!***

1 Introduction

Oregano is one of the most important spices in the world and used for flavoring meat and sausage products, salads, stews, and soups[2] but also for its therapeutical properties since the antiquity[1]. Also today these herbs and their essential oil are used widely in medicines, food or cosmetic technology and are cultivated in many countries.[2] Over 60 different species are used under this name, mainly from the genus *Origanum* (Lamiaceae) and *Lippia* (Verbenaceae). In Europe mainly *Origanum sp*. is used[2], because of that this work focuses on that genus.

Many *Origanum* species were used traditionally for centuries as a treatment for e.g. gastro-intestinal disturbances like hyperacidity, gastritis, ulcer and spasms.[3] Also pharmacological activities such as antifungal, antibacterial, antiparasitic, antioxidant, analgesic, anti-inflammatory and antispasmodic characteristics[2], and even antimalarial[4] and inhibitory potential against growing of food spoiling yeast[5] and were observed in some of them. This is mainly due to their essential oils, rich in oxygenated monoterpenes such as thymol and carvacrol or linalool, terpinene-4-ol and sabinene hydrate.[2] In addition, there is an increasing interest in oregano, especially in cultivating alternative crops and utilizing them in functional foods.[2]

Many species, such as *Origanum onites* L., *Origanum vulgare* L., *Origanum heracleoticum* L. or *Origanum x majoricum* Cambess. have already been subjected to former investigations thanks to their interest as spice, for their antiseptic abilities and to their potential for economic cultivation. But still, due to their variability, the composition of the essential oil can vary within one species greatly, and this influences the aromatic and pharmacological characteristics[2]. Thus, more investigation about the compositions is required. In addition, easy, fast and cost-effective analytical methods can help in quality control, especially of the plant material.

Although many efforts have been made to investigate many species of this genus, there are still chemically and pharmacologically little described ones. Therefore chemical exploration of those that are lesser known, like *Origanum virens* Hoffmans & Link, or so far not chemical investigated, such as *Origanum paui* Martínez, mark the first step to faciliate scientific and economical progress.

2 Objectives

The general objective of this work was to contribute on the investigation of the chemical composition of *Origanum* species.

The specific objectives were:

1.) To investigate the composition of the essential oils of the so far uninvestigated species *Origanum paui* Martínez;

2.) To determine the qualitative and quantitative composition of the essential oils of different *Origanum* samples grown in Spain;

3.) To compare the chromatographic fingerprints of these *Origanum* samples by HPTLC.

3 Essential oils

Plants which contain essential oils have been used in medicine, nutrition and cosmetics for thousands of years and the invention of distillation, around 1000 years ago, made it possible to use them in pure form.[6]

The European Pharmacopeia defines essential oils as “Odorous product, usually of complex composition, obtained from a botanically defined plant raw material by steam distillation, dry distillation, or a suitable mechanical process without heating. Essential oils are usually separated from the aqueous phase by a physical process that does not significantly affect their composition.”[7]

They can be found mainly in leaves, flowers, fruits, roots, rhizomes and wood, lesser in stipes and barks and can be distributed in all parts of the plant or only concentrated in very few organs or tissues. Their typical characteristics are that they are (apart from few exceptions) at room temperature liquid, that they have a lower density than water, a high refractive power, intensive smell and taste and a very bad solubility in water but high solubility in lipophilic solvents. They are normally defined by their chromatographic spectrum obtained by gas chromatography.[6]

3.1 Chemistry

Essential oils are complex mixtures of substances. Most of them are terpenoid, beneath them phenylpropane derivates, simple phenols and their ethers, straight hydrocarbons and their derivates, short hydrocarbon acids and few molecules containing nitrogen or sulfur can be found. The terpenes can be found as hydrocarbons, alcohols, aldehydes, acids, esters, ketones and oxides and can be differentiated after their number of carbon atoms:

Monoterpenes with 10 Carbon atoms are derived from 2,6-dimethyloctane or geraniol and can be acyclic, mono- or bicyclic. Over 150 different derivates are already described.

Sesquiterpenes with 15 Carbon atoms are derived from 2,6,10-Trimethyl-n-dodecane or farnesol and can be acyclic, mono-, bi-, or tricyclic. With over 1000 different sesquiterpenes identified they build the largest group among the terpenes in essential oils.

Diterpens with 20 Carbon atoms have a boiling point over 300ºC and are therefore not as common as their smaller siblings, though they can be found in essential oil obtained by cold extraction and pressing.

Apart of the group of the terpenes, phenylpropanic derivates are also of major importance as components of some essential oils. They can be found as aldehydes, phenols and phenolethers that are derived from cinnamic acid, as cumarines are.[6]

3.2 Production

The method for obtaining the essential oil depends on the kind and amount of oil and on the part of the plant they are extracted from. Additional economic parameters can be important especially when a big mass is wanted to be obtained industrially. The most common way is the steam distillation, where either the plant material is mixed with water or then distilled (hydro distillation) or externally produced water steam is passing through the plant material to evaporate the essential oils. Because of the high yield this is the most common way to obtain thermical stabile oils. Apart of that dry distillation can be done with stems or barks and doesn't involve additional water in the distillation process. There is also the possibility of mechanical process, when the oil is pressed out of the plant or the enfleurage with animal fat. The constituents of the essential oils can also be obtained by extraction with lipophilic solvent or supercritical gas (mostly CO_2).[6]

4 The genus *Origanum*

With oregano a flavor and aroma (in form of the dried drug or the essential oil) is ment that is used all over the world. Over 60 different species are used under this name, mainly from the genus *Origanum* (Lamiaceae) and *Lippia* (Verbenaceae). In Europe mainly *Origanum sp*. is used and cultivated, but also in rural forms especially in Mediterranean countries widely common.[2]

The genus *Origanum* consists of more or less 40 species, and is divided in three groups and ten sections. While *Origanum vulgare* L. and *Origanum heracleoticum* L. (synonym *Origanum* L. *vulgare* subsp. *viridulum* (Martrin-Donos) Nyman) belong to group C (calyces with 5 (sub)equal teeth) and to the section *Origanum*, *Origanum virens* Hoffmans & Link (synonym *Origanum vulgare* subsp. *virens* (Hoffmanns. & Link) Ietsw.) belongs to group A (2 or 1-lipped, rather large 4-12 mm long calzces; bracts rather large, 4-25 mm long, membranous, usually purple, sometimes yellowish-green, more or less glabrous) and the section *Brevifilamentum*. *Origanum x majoricum* Cambess. is a sterile hybrid of *O*. *majorana* L. and *O*. *vulgare* L. ssp. *virens* Ietsw.[2, 16]

The essential oil which influences the aroma and pharmacological activities mostly can differ in quantity and composition between the species, but they can be distinguished by the major amounts of thymol and carvacrol (with large quantities of γ-terpinene and p-cymene) or linalool, terpinene-4-ol and sabinene hydrate. The two commercially most used species because of their high essential oil contents are *O. vulgare* L. *subsp. hirum* Letsw. (Greek Oregano) and pot majoram, which is derived form *O. heracleoticum* L. and *O. onites* L.[2] In the monography of the European Pharmacopeia only *O. vulgare* L. *subsp. hirum* Letsw. and *O. onites* L. are listed,[7] because of their rich essential oil yield and high amount of carvacrol and thymol. Also *O. heracleoticum* L. and *O. virens* Hoffmans & Link are described as rich in carvacrol and/or thymol, although the composition of the essential oil of *O. virens* Hoffmans & Link can vary much.[19,18,14,13] Also *O*. *vulgare* L. shows heterogenity in the constituents of its essential oils,[17] although the main constituents are normally thymol,

carvacrol, p-cymene, γ-terpinene and thmyol and carvacrol ether. *O*. *majorana* L. as part of the group B (2 or 1-lipped rather small calyces, 1,3-3,5 mm long; bracts rather small 1-5 mm leaf-like in texture and color, more or less hairy) and the section *Majorana* is rich in *cis*- and *trans*-sabinene hydrate and also sabinene and α-thujene. *Origanum x majoricum* Cambess., as a hybrid of those two species of two different compositions of volatile oils, may also show a mix of these substances.[2]

5 Gas Chromatography

Gas chromatography is an analytical technique invented by Martin and Synge published in 1952. As every chromatographic system it is used to separate different substances mixed together by using their specific solubility in two phases. Due to different physical or chemical characteristics of the phases, one mobile and one stationary, this can be achieved.[11]

In the gas chromatography the mobile phase is a gaseous one, passing through a column where the stationary phase, a very viscous liquid, is applied on the inner surface. The sample needed to be separated into its components enters the system via an injection system where it gets mixed with the mobile phase, usually helium, argon, nitrogen or hydrogen. It passes the heated column which used to be a capillary one with lengths of 25 m, 60 m up to 200 m where the substances separate. Due to different distribution between the two phases the substances leave the column at different times, called retention times. The leaving substances are then characterized by a detector.[8]

This allows for volatile substance mixtures like Essential Oils a very high separation quality and makes it the most powerful technique in this analytical sector.[8]

5.1 Retention indices

The retention time, defined as the time between the injection of the sample and the summit of the detected peak, can be used for identification efforts of the detected substances. Those times are heavily influenced by many parameters though, such as e.g. injection technique, unsteadiness in the temperature or the gasflow, the kind of the used equipment etc. Therefore relative retention times are used for identification as they are more reproducible. Mostly a mix of n-alkanes, known as Kovats Indices is used.

But, the retention time of two different substances can be equal at a specific chromatographic system. Therefore the same chromatography should be done twice, normally on a polar and on an apolar column, and furthermore other techniques, such as GC-MS or ^{13}C-NMR should be done to validate the results.[8]

5.2 Stationary phase

The choice of the stationary phase defines the outcome of a chromatography fundamentally. There are a variety of columns coated with different stationary phases that differ much in polarity and other chemical characteristics as the maximum temperature they can be used at. Silicone polymers carrying different amounts of polar groups are most common. They normally consist of a Silicone backbone carrying methyl groups. The polarity is defined by the number and kind of groups that replace methyl.

Beside these systems another type of much used phases is the polyethylenglycols (PEGs). A very stabile sol-gel technology is used to coat the columns with the PEGs and allow them to be used at temperatures up to 320ºC. The Carbowax line is one of the most common ones and is also used in this work.[9]

5.3 Split/Splitless Injector

The most common injection technique is by a microsyringe entering a heated liner in which a very small amount of the liquid injected is evaporated and also split it into two parts. This split makes it possible to let only 0.1-10% of the injected liquid enter the column and alter the dimension of the peaks.[9]

5.4 Detectors

A big variety of detectors were invented and used for GC, although the Flame Ionization Detector, FID, is one of the most used and known systems thanks to its high sensitivity and universal applicability.[9]

In the **Flame Ionization Detector**, a small oxygen-hydrogen flame burns the substances which leave the column, producing ions. The amount of the ions is recognized as current by a collector electrode, constituting the amount of substance that has left the column. This makes possible to register the time and amount of the leaving substances. Except few substances the FID can detect therefore nearly all organic compounds.[9]

5.5 Gas Chromatography-Mass Spectrometry

The GC-MS proved itself in recent years as one of the most potent systems for investigation of essential oil compounds. It is very effective at identifying small volatile substances by the MS after the substances were split up by the GC. If the calibration permits, a quantification of the substances can also be made with this technique, but at least a prediction about the proportions is able to be done.[9]

5.6 Mass Spectrometry (MS)

In the MS the substances are identified by ionizing and fragmenting them and then detecting the produced ions. The first two steps can be done by leading the substances through a beam of electrons. The collision ionizes the molecules

which then mostly fragment in a specific way. The molecular Ion M^+ represents the not fragmented ionized molecule.

A big variety of detectors are used for selecting and detecting the fragments, such as quadrupol, iontrap, or time of flight. However, ion trap has been used in recent years more often for analyzing complex mixtures, such as essential oils are.

In a mass spectrum the intensity of the fragments are shown sorted by mass/load-ratio. The spectra of terpenes, as most of the compounds of essential oils are, do have many similarities; they differ in the proportional intensity of their peaks though. While monoterpenes can be identified easily with nowadays databases, sesquiterpenes could make more difficulties as they are lesser studied and additional examination can be necessary.[8]

6 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC is a method based on the same theoretical concept as thin layer chromatography.[10] The stationary phase is usually a silica gel, coated on a carrier as glass. The mobile phase, normally a mixture of different liquids, is put into a glass chamber where the plate is placed to soak it up. Therefore a TLC includes the steps of sample application, development, visualization or detection of the separated analytes and eventually quantification. With development the actual chromatographic process, happening while the mobile phase is running up the plate, is meant. As the majority of TLCs are done with industrially produced silica plates the choice of the mobile phase is vital for the outcome of the chromatography. But usually previous described and recommended mixtures are used and apart of that there are principles published to choose a mobile phase. Often, the choice is done by trial and error due to the fact that TLC is very simple, fast and cheap to do.[9]

The diffence between HPTLC and TLC is the usage of plates with a thinner layer of a silica gel of smaller particle size (5-7 µm) immobilized to augment the quality of separation and sensibility. Also can be machines used to automatize the operations to enhance the reproducibility of the chromatograms. The sample application is done semi- or automatically, the development automatically (under monitored and preset conditions), as is the derivatization, done by dipping or spraying the derivatizing agents on the plate. The visualization by daylight, long or short-wave UV light, is done in a scanner.[10]

6.1 Sample Application

The sample application determines the quality of the chromatography vitally, which is a reason why the HPTLC has a higher reproducibility than TLC. Basically three points are necessary: the correct and precise position of all samples, the exact application of sample volume and that the plate is not damaged during the application.

The samples can be put on the plate by contact application (e.g. with a capillary or a microliter syringe) or by spray-on application. While the contact application is used mainly in TLC because it's so easy fast and cheap, spray-on application can improve the outcome significantly. This has different reasons as for example the security of not harming the plate or the possibility of applying the samples in very thin bands and in higher amounts.

For automatic application in HPTLC several instruments are invented, working automatically or semi-automatically when the syringe is loaded with the sample manually. But in either way all parameters are controlled by the computer and many can be programmed.[10]

6.2 Development

The development is the step where the real chromatography is happening. There are many different kinds of chambers where normally the mobile phase is put in and the plate dipped in it and mostly different chambers produce different results. Apart of the type of the chamber many parameters are influencing the outcome such as the saturation of the gaseous phase in the chamber with the volatile mobile phase, the exact position of the plate etc. To monitor and standardize most of these factors automatic chambers are used in HPTLC. The plate is placed in the device and is dipped in the mobile phase automatically after the computer controlled saturation of the chamber. The migration is monitored and after the run the plate can be dried to prevent diffusion or inhomogeneous separation.[10]

6.3 Derivatization

The derivatization is often necessary for making the separated substances visible. Mainly the plate can be derivatized through a gas phase or a liquid phase, applied by spraying or dipping. While few substances are available for the first of the listed solutions spraying and dipping offer a huge variety of derivatizing agents. Spraying has its advantages at the small volumes of reagent needed and the simplicity and quickness. Dipping can derivatize the plate very homogeneously and has a higher reproducibility, especially when a simple machine, dipping the plates in the reagent automatically, is used. Most of the derivatization steps need heating after or before applying the reagent. Therefore mostly ovens and plate heaters are used.[10]

6.4 Visualization

In TLC and HPTLC the visualization is mainly done only by light with different wavelengths. The spots may be visible at different wavelengths; therefore three different modes are commonly used: the evaluation under daylight (or white light), long UV light (356 nm) and short-wave UV (254 nm). The interpretation can be done directly with the plate or pictures can be taken with scanners to enhance the comparability of different plates.[10]

7 Plant material

For this study plant material from different regions were gathered. The botanical identification of the samples which didn't come from Zaragoza was made by Segundo Rios and Vanessa Martinez Frances from the Universitat d'Alacant. Jesus Burillo identified all other drugs. Table 1 views all samples investigated.

Sample	Species	Origin	Growing site	Collection date
M12082	*Origanum heracleoticum* L.	CMC	EBT-JB - Alacant	15.08.2012
M12080	*Origanum paui* Martínez	UTC	Ulea (Murcia)	25.07.2012
M12081	*Origanum paui* Martínez	UTC	EBT-JB - Alacant	31.07.2012
M12079	*Origanum paui* Martínez	ALTC	EBT-JB - Alacant	31.07.2012
M12027	*Origanum virens* Hoffmans & Link	EC	CITA - Zaragoza	2011
M12117	*Origanum virens* Hoffmans & Link	EC	CITA - Zaragoza	2012
M12077	*Origanum virens* Hoffmans & Link	AS	EBT-JB - Alacant	31.07.2012
M12120	*Origanum virens* Hoffmans & Link EO	EC	CITA - Zaragoza	2012
M12029	*Origanum vulgare* L.	EC	CITA - Zaragoza	2011
M12118	*Origanum vulgare* L.	EC	CITA - Zaragoza	2012
M12078	*Origanum vulgare* L.	ANTC	EBT-JB - Alacant	31.07.2012
M12121	*Origanum vulgare* L. EO	EC	CITA - Zaragoza	2012
M12028	*Origanum x majoricum* Cambess.	EC	CITA - Zaragoza	2011
M12119	*Origanum x majoricum* Cambess.	EC	CITA - Zaragoza	2012

Table 1: List of the investigated plant material/ essential oils

EO: Essential oil

EBT-JB: Estació Biològica Torretes – Jardí botànic; Institut Universitari de Biodiversitat CIBIO, Universitat d'Alcant

CITA*:* Centro de Investigación y Tecnología Agroalimentaria de Aragón

EC: Experimental culture

UTC: Traditional culture in Ulea (Murcia, Spain)

ALTC: Traditional culture in Almoardí (Alacant, Spain)

ANTC: Traditional culture in Andalusia

CMC: Comercial mediterranean culture

AS: Agrestal grown in Andalusia

8 Methods

The essential oils, gained by hydro distillation, were investigated with GC-FID and GC-MS in two different columns. The compounds were identified by their retention indices calculated with a mixture of n-alcanes as an internal standard and comparison of their mass spectra with a databank. Their relative proportion was determined with the relative area of their peaks without additional correction factors.

8.1 Isolation Procedure

Hydro distillation was done with raw plant material to gain the pure Essential oil. Therefore a distillation apparatus with a condenser assembly from the type Clevenger[11] as described in the PhEur.[12] The materials were distillated for 3 hours, then their volume determined and finally bottled in vials. At one sample, M12082 *O. heracleoticum*, 1 ml n-Hexane[a] was added into the pear-shaped swelling of the condenser assembly to improve the recovery of oil.

8.2 Gas chromatography

For the GC a Hewlett-Packard gas chromatograph, model 6890 aligned with an FID was used. The separation was achieved with two fused silica capillary columns with different stationary phases: Supelcowax™ 10 (60 m x 0.2 mm i.d. x 0,25 µm film thickness) and Equity™- 1 (60 m x 0.25 mm i.d. x 0.25 µm film thickness). The device was programmed and controlled by HP ChemStation™ program with which the data acquisition and analysis was also done.

At both columns all samples were once pure injected and once as a 1:50 n-Pentane dilution mixed with a 1:20 dilution of a mixture of alcans[b] in n-heptan to verify the retention indices.

8.2.1 Analytical conditions

- Carrier gas: Helium (1.0 ml/min)
- Split ratio: 1:50
- Injector temperature: 250°C
- Oven temperature programme: 2min isothermal at 60°C, subsequently at 2°C/min up to 220°C and then held isothermally for 10 min; a post run was made for 10 min at 25ºC (as seen in Figure 1)
- Detector temperature: 250°C
- Injection Volume: When the pure samples were injected, the syringe was washed with sample, then only air was drawn up with the syringe and

[a] Pancreac Quimica Sau, n-Hexane 95%(UV-IR-HPLC) PAI-ACS, LOT 0000166154, Nº CE: 203-777-6

[b] Supelco, Alcans Mezcla, LOT LB18862, SP40000164

injected so that the real injection volume of the samples are as small as possible. When the diluted samples with the Alcan Mixture were used 0.2 uL sample and 0.8 uL Alcan Mixture was injected.

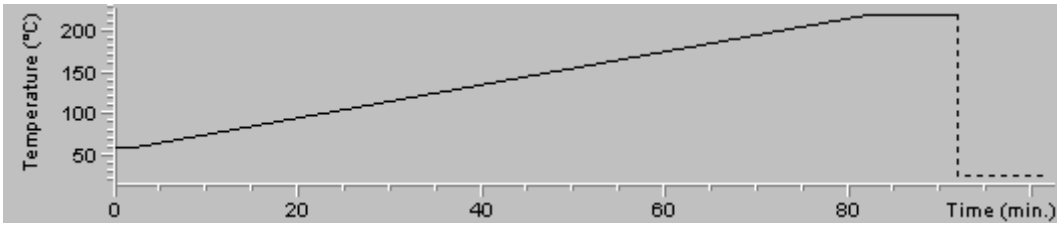


Figure 1: Oven temperature program[c]

8.3 GC-MS

For the GC-MS a Hewlett-Packard 5890 gas chromatograph coupled to a mass selective detector Hewlett-Packard 5971 A was used during the same analytical conditions as in the GC described above. Only pure samples were analysed, and as in the GC only a minimum of them was injected. Again all samples were dissected with both columns. The interface temperature was held at 290ºC and an ionisation voltage of 70eV was selected. The device was monitored with the MS ChemStation™ software.

8.4 Identification and quantification

The identification of the constituents of the essential oils was based on comparing the peaks of the GC-MS with the Wiley6.l library (Wiley MS Data library (6th edn)) and comparing the retention indices with a database built up by information gained from www.pherobase.com and by investigations made in the department of Pharamacognosy of the University of Barcelona.

While the relative quantity of the detected substances was received by averaging the relative peak areas of the chromatograms of the pure samples of both columns, the retention indices were calculated with in dependency of the added alcanes.

The retention indices were calculated with the following formula:

$$\mathbf{RI_A = RI(n) + 100^* [Rt(A) - Rt(n)]/[Rt(n+1) - Rt(n)]}$$

RI_A = Retention index of substance A

RI(n) = Retention index of reference (arbitrary given as full hundreds)

Rt(A) = Retention time of peak A

Rt(n) = Retention time of the first reference peak before peak A

Rt(n+1) = Retention time of the first reference peak after peak A

[c] HP ChemStation™ Program

The mixture of alcanes consists of equal amounts of 16 n-aclanes ranging between n-octane and n-eneicosane. The spectra of this mixture can be seen below in Figure 2 and Figure 3 in the two different coloumns.

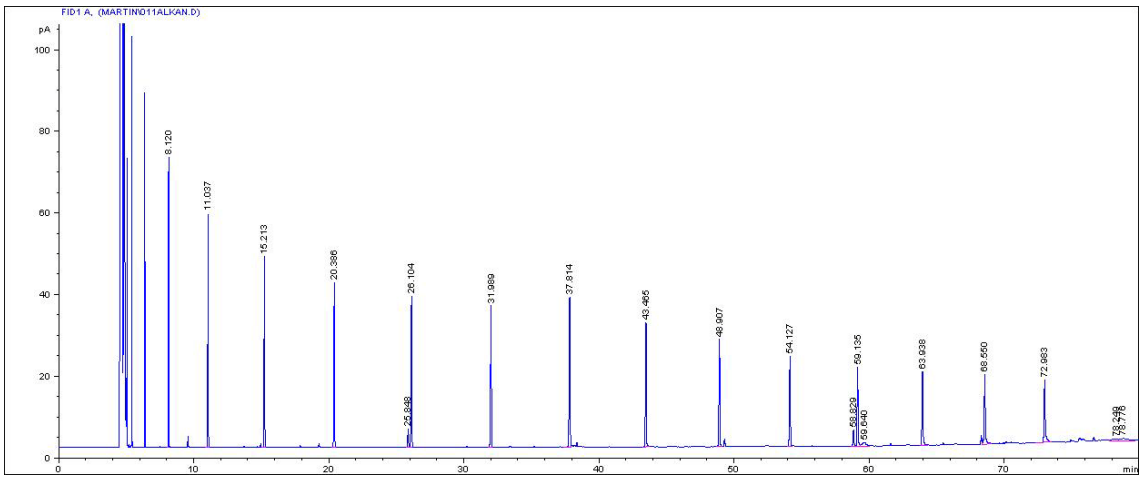


Figure 2: The chromatogram of the n-alcanes separated by the Supelcowax™ 10 coloumn

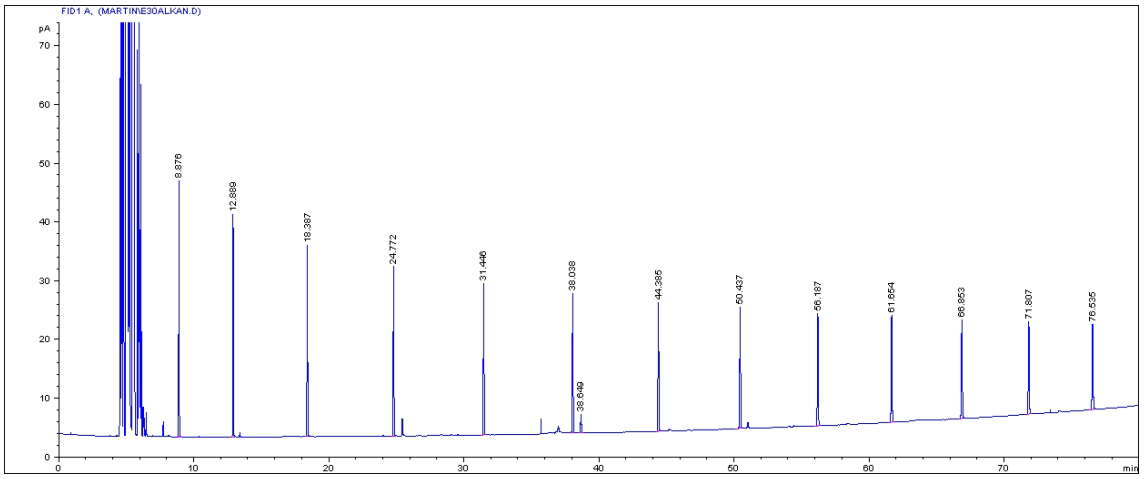


Figure 3 The chromatogram of the n-alcanes separated by the Equity™ - 1 coloumn

8.5 HPTLC

For the HPTLC essential oils diluted 1:50 in toluene and methanolic extracts were used.

For the methanolic extract a small amount of raw plant material was pulverized and tossed through a mesh with a mesh opening of 0.355 µm. 0.5 g of this powder was mixed with 5 mL of methanol and sonicated for 10 min, then centrifuged and filtered.

Two different methods were investigated with the methanolic extracts method 1 was designed to show mainly the volatile essential oils, while method 2 focuses on the flavonoid spectrum. The diluted essential oils were analyzed only with method 1.

8.5.1 Method 1

Reference substances:	2 µl of carvacrol diluted in 2 ml of toluene
	1 mg of thymol diluted in 1 ml of toluene
Stationary phase:	HPTLC Si 60 F_{254}
Mobile phase:	Dichlormethane 100%
Development:	saturated chamber
	Developing distance 70 mm from lower edge
	Relative humidity 33%
Derivatization reagent:	Anisaldehyde reagent
	Preparation: to 170 ml of methanol ice-cold slowly 20 mL of acetic acid and 10 ml of sulfuric acid was added and mixed well. The mixture was cooled to room temperature and then 1 ml of anisaldehyde was added
	Reagent use: the plate was dipped into the reagent (time 0, speed 5) and heated at 100ºC for 6 min

8.5.2 Method 2

Reference substances:	1 mg of rutin diluted in 1 ml of methanol
	1 mg of rosmarinic acid diluted in 1 ml of methanol
Stationary phase:	HPTLC Si 60 F_{254}
Mobile phase:	Ethyl acetate, formic acid, water 15:1:1
Development:	saturated chamber
	Developing distance 70 mm from lower edge
	Relative humidity 33%
Derivatization reagent:	Natural products and PEG reagent
	Preparation: 1 g of NP was dissolved in 200 ml of ethyl acetate. 10 g of PEG was dissolved in 200 ml of DCM

Reagent use: the plate was heated at 100ºC for 3 min, dipped while still hot into the NP reagent (time 0, speed 5) and dried in a stream of cold air. Afterwards, it was dipped in PEG reagent (time 0, speed 5) and dried in a stream of cold air.

All steps were automatized, except the sample application which was done semi-automatically.

Before the plates were used every plate was put into a development chamber with methanol in it to clean the plate and prevent alteration of the chromatogram.

9 Results

9.1 Essential oils composition

9.1.1 *Origanum paui* Martínez

Three samples of *Origanum paui* Martínez were investigated (Table 1). The content of essential oil in the samples, determined by hydrodistillation according to Ph.Eur., ranged from 0.8 to 3.2%. The substances identified in the essential oil are listed in Table 2. The percentages of the different types of constituents are shown in Figure 4.

Identified compound	M12079	M12080	M12081	Molecule type *
Terpinen-4-ol	19,9	10,4	14,8	OM
cis-Sabinene hydrate	19,8	35,5	28,9	OM
γ-Terpinene	8,2	2,9	6,2	MH
α-Terpineol	6,6	5,8	5,7	OM
Linalool	4,7	6,1	2,8	OM
α-Terpinene	4,6	1,2	3,5	MH
Sabinene	3,9	1,5	4,3	MH
trans-Sabinene hydrate	3,3	4,1	3,4	OM
β-Caryophyllene	3,0	6,0	4,3	SH
Linalylacetate	2,7	4,4	3,7	OM
cis-β-Ocimene	2,1	1,0		MH
Terpinolene	1,8	0,7	1,4	MH
β-Bisabolene	1,5	4,1	2,2	SH
1-Terpineol	1,4	0,9	0,9	OM
Myrcene	1,4	0,6	1,2	MH
p-Cymene	1,4	0,7	0,9	MH
Limonene	1,3	0,6	1,2	OM
Bicyclogermacrene	1,1	2,5	1,8	SH
Germacrene-D	1,1	1,3	1,6	SH

Selinen-4-ol	1,0			OS
Thymol	0,9	1,4	0,8	OM
β-Phellandrene	0,8	0,4	0,9	SH
Hexadecanoic acid	0,5			O
Spathulenol	0,4	0,6	0,4	OS
α-Pinene	0,4	0,1	0,3	MH
Caryophyllene oxide	0,4	0,5	0,3	OS
Geranyl acetate (geranyle + neryle)	0,3	0,4	0,4	OM
α-Thujene	0,3	0,1	0,3	MH
trans-β-Ocimene	0,3			MH
1-Octen-3-ol	0,3	0,2	0,2	O
β-Pinene	0,3	0,4	0,2	MH
Geraniol	0,3	0,4	0,4	OM
δ-Cadinene	0,3	0,2	0,1	SH
Neryl acetate	0,2	0,2	0,2	OM
α-Humulene	0,2	0,3	0,2	SH
Nerol	0,1	0,1	0,1	OM
cis-Dihydrocarvone	0,1	0,2	2,4	OM
β-Bourbonene	0,1	0,1	0,1	SH
Isospathulenol	0,1	0,1	0,1	SH
Camphene	0,1			OM
Carvacrol	0,1	0,1	0,1	OM
β-Sesquiphellandrene	0,1			SH
Aromadendrene	0,1	0,0	0,1	MH
α-Phellandrene	0,1		0,1	SH
Bornylacetate	0,1	0,1		OM
Octen-1-ol acetate	0,1	0,1	0,1	O
trans-Verbenol	0,1			OM
trans-Linalool oxyde	0,1			OM
1,8-Cineole	0,0			OM
1-Octen-3-yl-Acetate		0,1		O
Borneol		0,3		OM
cis-α-Bisabolene		0,1	0,1	MH
trans-beta-Ocimene		0,2	0,4	MH
β-Elemene		0,1	0,1	SH
Total	98,0	97,0	97,0	
Content of essential oil	0,8%	2,3%	3,2%	

*MH: Monoterpene hydrocarbone; OM: oxygenated Monoterpene; SH: Sesquiterpene hydrocarbone; OS: oxygenated Sesquiterpene

Table 2: Identified compounds of the samples of *Origanum paui* Martínez

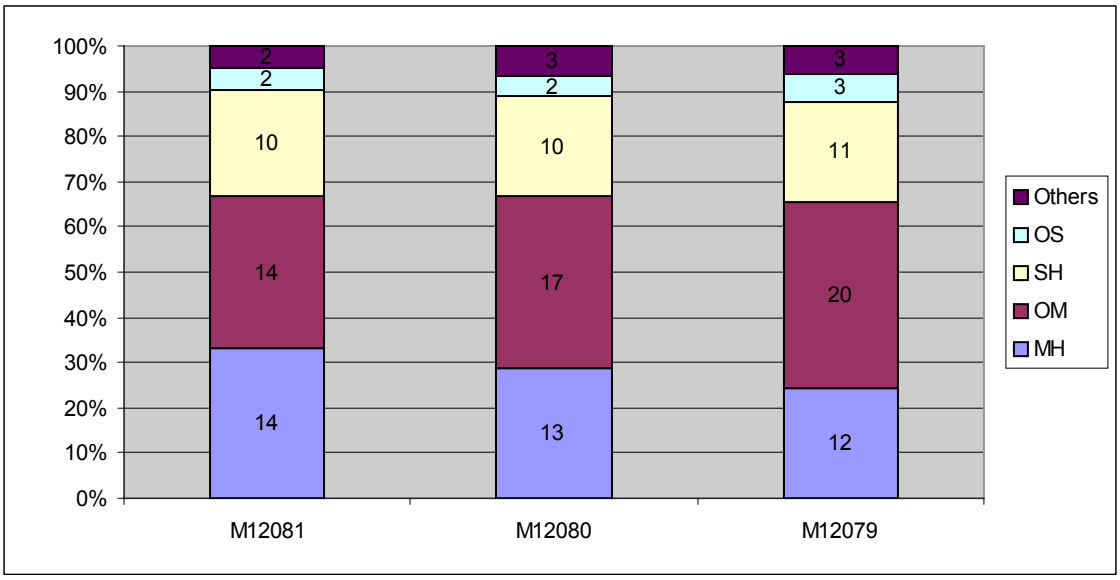


Figure 4: Percentages of the different types of constituents of the essential oil of *Origanum paui* Martínez.

9.1.2 *Origanum virens* Hoffmans & Link

There were three different samples of this species investigated, M12077 was grown wild and collected in the area of Andalusia, Spain, M12027 and M12117 were both from the same origin, Zaragoza, Spain, but M12027 was collected in 2011 and M12117 in 2012. There was also an essential oil from M12117 investigated, that was distillated in Zaragoza in a semi-industrial distillation device (M12120). But this oil and the oil distilled from the plant material after the procedure described in 0 showed no notable difference so it can be seen as the same sample. The substances identified listed in Table 3, the amount of the chemical substance types in Figure 5.

Identified compound	**M12027**	**M12077**	**M12117**	**Molecule type***
γ-Terpinene	**20,17**	**2,64**	**28,76**	MH
α-Terpineol	**0,12**	**27,25**		OM
Thymol	**40,49**	**2,95**	**12,79**	OM
p-Cymene	**10,75**	**6,02**	**6,76**	MH
β-Caryophyllene	**3,97**	**3,52**	**8,54**	SH
Estragole			**3,37**	PP
Thymol-methyl-ether	**3,78**	**1,08**	**2,31**	OM
Carvacrol-methyl-ether	**2,87**	**0,11**	**1,77**	OM
α-Terpinene	**1,66**	**0,55**	**2,95**	MH
β-Bisabolene	**0,97**	**1,83**	**1,53**	SH
Caryophyllene-oxyde	**1,22**	**0,65**	**2,82**	OS
Germacrene D	**1**	**0,57**	**3,09**	SH
cis-β-Ocimene	**0,95**	**0,43**	**3,4**	MH
Myrcene	**0,87**	**0,39**	**2,09**	MH
Carvacrol	**0,47**	**37,95**	**0,26**	OM
Terpinen-4-ol	**1,24**	**0,82**	**0,19**	OM

Spathulenol	**1,05**	**0,81**	**0,44**	OS
cis-α-Bisabolene			**0,77**	SH
α-Humulene	**0,5**	**0,57**	**1,11**	SH
Aromadendrene	**0,7**	**0,52**	**0,11**	MH
δ-Cadinene	**0,49**	**0,35**	**0,5**	SH
Sabinene	**0,09**	**0,44**	**0,57**	MH
Bicyclogermacrene	**0,36**	**0,55**	**0,42**	SH
trans-Sabinene hydrate	**0,26**	**0,37**	**3,15**	OM
trans-β-Ocimene	**0,35**	**0,12**	**3,22**	MH
β-Phellandrene			**0,34**	MH
Borneol	**0,21**	**0,43**		OM
Linalool	**0,32**	**3,47**	**0,19**	OM
E,E-α-Farnesene	**0,31**	**0,16**	**0,74**	SH
α-Pinene	**0,09**		**0,51**	SH
Limonene	**0,28**	**0,2**	**0,34**	OM
β-Elemene			**0,26**	SH
1-Octen-3-ol	**0,05**	**0,46**		O
β-Bourbonene	**0,23**	**0,06**	**0,39**	SH
Selinen-4-ol		**0,37**	**0,08**	OM
γ-Cadinene	**0,23**	**0,18**		SH
α-Thujene	**0,19**	**0,07**	**1,15**	MH
Ledene (viridiflorene)	**0,22**	**0,16**		SH
α-Amorphene	**0,31**	**0,16**	**0,14**	MH
α-Copaene	**0,16**	**0,08**	**0,28**	SH
Carvone	**0,11**	**0,17**		OM
β-Pinene	**0,06**	**0,13**	**0,23**	MH
Viridiflorol		**0,12**		OS
α-Phellandrene	**0,12**	**0,05**	**0,24**	SH
Camphene			**0,11**	MH
Eugenol	**0,11**			PP
β-Cubebene	**0,11**	**0,06**	**0,26**	SH
Terpinolene	**0,09**	**0,07**	**0,11**	MH
Allo-Ocimene			**0,09**	MH
p-Cymene-8-ol	**0,06**	**0,08**	**0,15**	OM
α-Muurolene	**0,1**	**0,06**		SH
cis-Sabinene hydrate	**0,88**	**0,06**	**0,05**	OM
3-carene			**0,06**	MH
Total	98,55	97,05	96,65	
Content of essential oil	4,1%	1,0%	2,4%	

*MH: Monoterpene hydrocarbone; OM: oxygenated Monoterpene; SH: Sesquiterpene hydrocarbone; OS: oxygenated Sesquiterpene

Table 3: Identified compounds of *Origanum virens* Hoffmans & Link

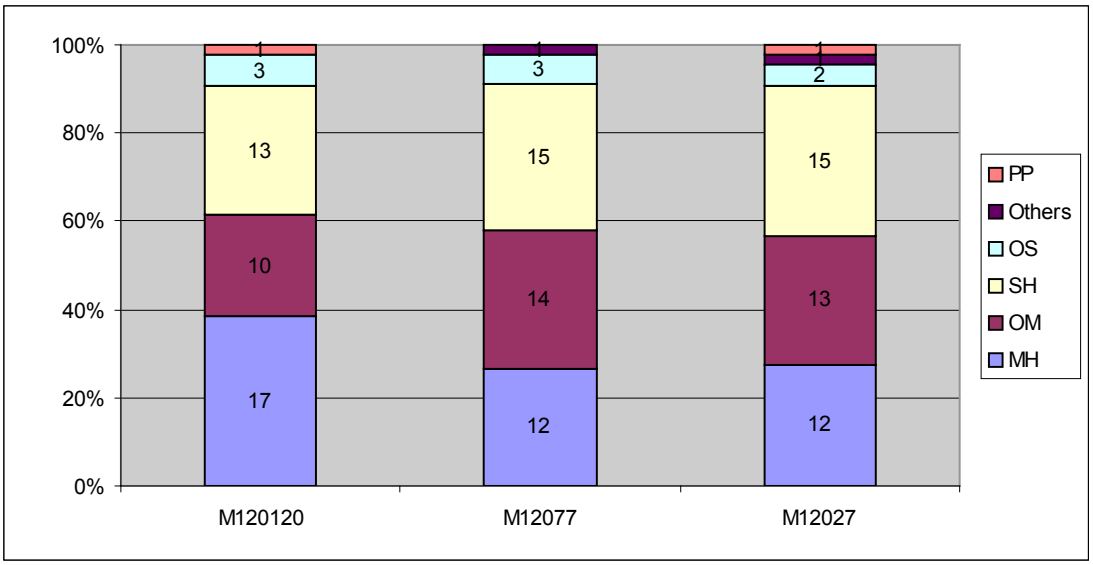


Figure 5: Fractions of the chemical types of the substances of the *Origanum virens* Hoffmans & Link samples

9.1.3 *Origanum x majoricum* Cambess.

Two samples from the same origin were collected, one (M12028) was harvested in 2011 and the other one (M12119) in 2012. Table 4 shows the identified substances of the essential oils and Figure 6 the amounts of the chemical substance groups.

Identified compound	M12028	M12119	Molecule type*
Terpinen-4-ol	19,82	20,66	MH
γ-Terpinene	15,14	13,03	MH
cis-Sabinene hydrate	11,64	7,43	OM
Thymol	14,08	0,83	SH
α-Terpinene	4,21	5,96	OS
Sabinene	3,62	5,9	MH
β-Caryophyllene	1,79	6,1	OS
1-Octen-3-ol		3,79	O
Linalool	1,3	6,18	OM
trans-Sabinen-hydrate	2,97	4,23	SH
α-Terpineol	2,66	2,73	P
cis-Ocimene		2,1	MH
p-Cymene	3,12	0,96	OM
Terpinolene	1,58	2,1	SH
Thymol-methyl-ether	1,81		O
trans-β-Ocimene	0,17	3,07	MH
cis-β-Ocimene	1,54		OM
Myrcene	1,2	1,79	OM
Germacrene D	1,09	1,7	OM
Bicyclogermacrene	1,89	0,54	SH

Limonene	0,94	1,48	MH
β-Phellandrene	0,75	0,87	MH
Carvacrol-methyl-ether	0,74		MH
1-Terpineol		0,69	OM
Borneol		0,69	OM
Caryophyllene oxide	0,18	1,01	SH
β-Bisabolene	0,58	0,56	OM
Spathulenol	0,73	0,39	OS
α-Thujene	0,56	0,5	MH
E,E-α-Farnesene	0,47	0,51	OM
α-Pinene	0,19	0,78	SH
α-Humulene	0,2	0,64	OS
β-Pinene	0,16	0,51	MH
δ-Cadinene	0,1	0,48	SH
Carvacrol	0,26		MH
Hexadecanoic acid	0,25		OM
cis-α-Bisabolene		0,2	SH
β-Elemene		0,15	SH
α-Copaene		0,13	SH
α-Phellandrene	0,12	0,13	OM
β-Bourbonene		0,12	SH
Aromadendrene	0,09		SH
Bornylacetate	0,11	0,06	MH
Eugenol	0,11	0,05	SH
Camphene		0,07	MH
Ledene (viridiflorene)	0,07		MH
Viridiflorol	0,06		MH
Isocaryophyllene oxide	0,06		SH
Linalool oxyde		0,06	OM
Total	96,36	99,14	
Content of essential oil	0,8%	1%	

*MH: Monoterpene hydrocarbone; OM: oxygenated Monoterpene; SH: Sesquiterpene hydrocarbone; OS: oxygenated Sesquiterpene

Table 4: Identified compounds of the samples of *Origanum x majoricum* Cambess.

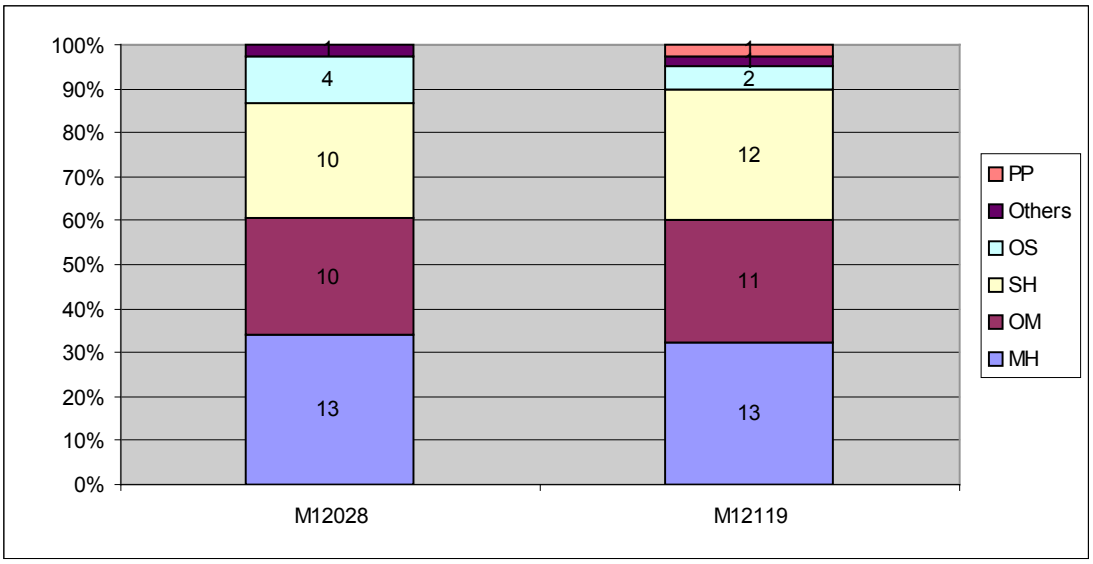


Figure 6: Fractions of the chemical types of the substances of the *Origanum x majoricum* Cambess. samples

9.1.4 *Origanum vulgare* L.

In total there were three different samples of *O. vulgare* L., while two came from Zaragoza and were collected in 2011 (M12029) and 2012 (M12118). From M12118 there was like with *O. virens* Hoffmans & Link a semi-industrial essential oil available but as in 9.1.2 there was no noteworthy difference between that oil and the oil distilled as described in 8.1. The third plant sample (M12078) was from the Andalusia area in Spain, grown in a traditional culture. Table 5 shows the identified substances, a comparation of the chemical types of the substances is shown in Figure 7.

Identified compound	M12029	M12078	M12121	Molecule type*
Caryophyllene-oxyde	32,6	7,4	5,7	OS
β-Caryophyllene	1,7	17,9	12,9	SH
Germacrene D	0,4	14,5	9,0	SH
trans-β-Ocimene		1,9	21,3	MH
Terpinen-4-ol	8,7	1,3	7,4	OM
p-Cymene	7,1	1,2	4,0	MH
γ-Terpinene	2,9	0,6	4,8	MH
E,E-α-Farnesene	0,3	4,8	2,6	SH
cis-β-Ocimene	0,6	2,4	3,8	MH
Spathulenole	1,2	5,3	0,2	OS
Thymol	5,4	0,5	0,7	OM
Sabinene	0,8	3,1	2,2	MH
β-Bourbonene	2,5	2,2	0,7	SH
β-Bisabolene	1,0	2,7	1,2	SH
trans-Sabinene hydrate	2,6		2,0	OM
α-Humulene	0,3	2,5	1,7	SH
Hexadecanoic acid	2,7		1,4	O

cis-Calamenene	3,5	0,2		SH
T-Cadinol		3,4		OS
α-Terpinene	0,9	0,2	1,7	MH
δ-Cadinene	0,2	2,0	0,5	SH
cis-Sabinene hydrate	0,8		1,8	OM
Bicyclogermacrene		2,3	0,1	SH
T-Muurolol		2,4		OS
1-Octen-3-ol	0,8	0,5	0,7	O
endo-Bourbonanol		1,9		OS
Myrcene		0,4	1,4	MH
Aromadendrene	0,3	1,2	0,2	MH
α-Cadinol		1,6		OS
α-Terpineol	0,6	0,6	0,4	OM
α-Phellandrene	1,3		0,1	SJ
Linalool	0,5	0,5	0,4	OM
Carvacrol	0,3	0,9	0,1	OM
Humulene epoxide II		1,3		OS
cis-α-Bisabolene			1,3	SH
β-Pinene	0,3	0,3	0,6	MH
Terpinolene	0,7		0,6	MH
α-Copaene	0,6		0,5	SH
β-Cubebene	0,4	0,5	0,2	SH
Limonene	0,3	0,2	0,5	OM
Viridiflorol	0,3	0,6	0,1	OS
α-Calacorene	0,2	0,7		SH
α-Pinene	0,1		0,7	MH
α-Thujene	0,1		0,6	MH
Estragole			0,7	PP
α-Muurolene		0,5	0,1	SH
γ-Cadinene		0,5		SH
1,8-Cineole		0,5		OM
α-Calacorene		0,5		SH
β-Copaene			0,5	SH
Calarene (b-gurjunene)		0,4		SH
β-Phellandrene	0,2		0,2	SH
p-Cymen-8-ol	0,2		0,1	OM
Carvacrol-methyl-ether			0,3	OM
β-Elemene			0,3	SH
α-Copaene		0,3		SH
Thymol-methyl-ether	0,2		0,1	OM
1,5-epoxy-Salvial-4(14)-ene	0,3			SH
1-Terpineol			0,2	OM
3-Octanol	0,2			O
Borneol			0,2	OM
Camphor			0,2	MH
Selinen-4-ol			0,2	OS
β-Sesquiphellandrene			0,1	SH
trans-Linalool oxyde			0,1	OM

Zingiberene			0,1	SH
Total	84,3	92,9	97,4	
Content of essential oil	0,3%	0,2%	0,4%	

*MH: Monoterpene hydrocarbone; OM: oxygenated Monoterpene; SH: Sesquiterpene hydrocarbone; OS: oxygenated Sesquiterpene

Table 5: Identified compounds of the samples of *Origanum vulgare* L.

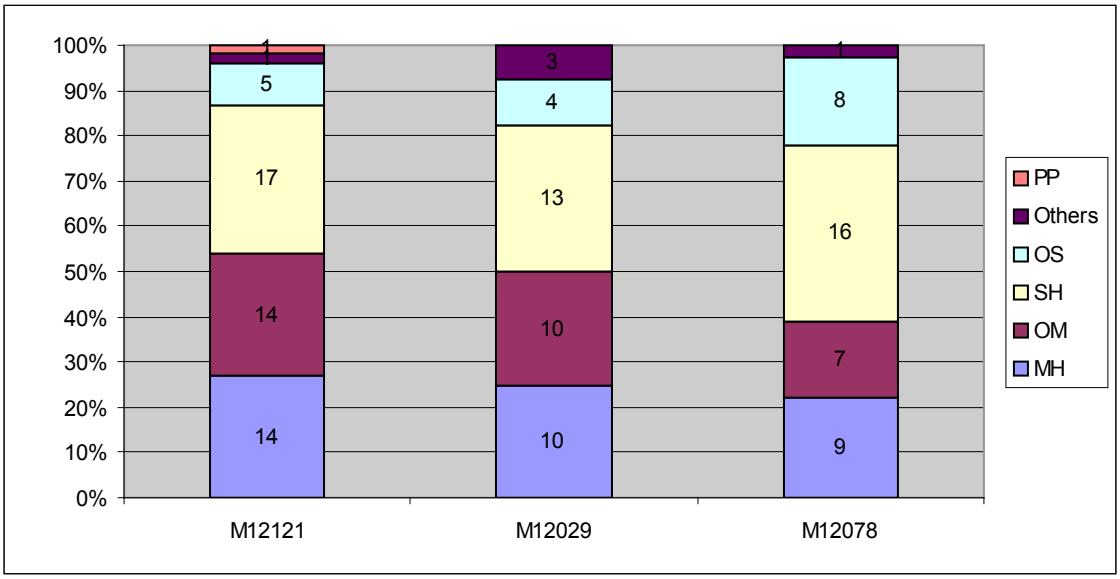


Figure 7: Fractions of the chemical types of the substances of the *Origanum vulgare* L. samples

9.1.5 *Origanum heracleoticum* L.

There was only one sample, M12082, of this species investigated, grown in Andalusia, in a mediterranean culture. Table 6 shows the investigated substances and Figure 8 the chemical types of the substances. There was not enough plant material to determine the amount of the essential oil.

Identified compound	M12082	Molecule type*
Carvacrol	**68,48**	OM
p-Cymene	**17,26**	MH
γ-Terpinene	**1,97**	MH
1-Octen-3-ol	**0,99**	O
Caryophyllene-oxyde	**0,96**	OS
Sabinene	**0,92**	MH
Myrcene	**0,83**	MH
Carvacrol-methyl-ether	**0,71**	OM
β-Caryophyllene	**0,65**	SH
α-Thujene	**0,64**	MH
β-Bisabolene	**0,56**	SH

Thymol-methyl-ether	**0,55**	OM
α-Pinene	**0,54**	MH
trans-Sabinene hydrate	**0,53**	OM
Borneol	**0,49**	OM
α-Terpinene	**0,45**	MH
Terpinen-4-ol	**0,37**	OM
Limonene	**0,35**	OM
Linalyl acetate	**0,3**	OM
Camphene	**0,25**	MH
p-Cymen-8-ol	**0,19**	OM
3-Octanone (=methyl-amyl-cetone)	**0,17**	MH
cis-Sabinene hydrate	**0,16**	OM
β-Pinene	**0,15**	MH
Thymol	**0,14**	OM
Humulene epoxide II	**0,11**	OS
β-Phellandrene	**0,11**	MH
α-Phellandrene	**0,09**	SH
Nerol	**0,09**	OM
Spathulenol	**0,09**	OS
α-Humulene	**0,09**	SH
Bornylacetate	**0,07**	OM
Terpinolene	**0,07**	MH
Carvone	**0,06**	OM
3-carene	**0,06**	MH
3-Octanol	**0,05**	O
cis-Dihydrocarvone	**0,05**	OM
cis-β-Ocimene	**0,05**	MH
α-Terpineol	**0,03**	OM
Germacrene D	**0,03**	SH
Geraniol	**0,02**	OM
β-Bourbonene	**0,02**	SH
Total	**99,7**	

*MH: Monoterpene hydrocarbone; OM: oxygenated Monoterpene; SH: Sesquiterpene hydrocarbone; OS: oxygenated Sesquiterpene

Table 6: Identified compounds of M12082 *Origanum heracleoticum* L.

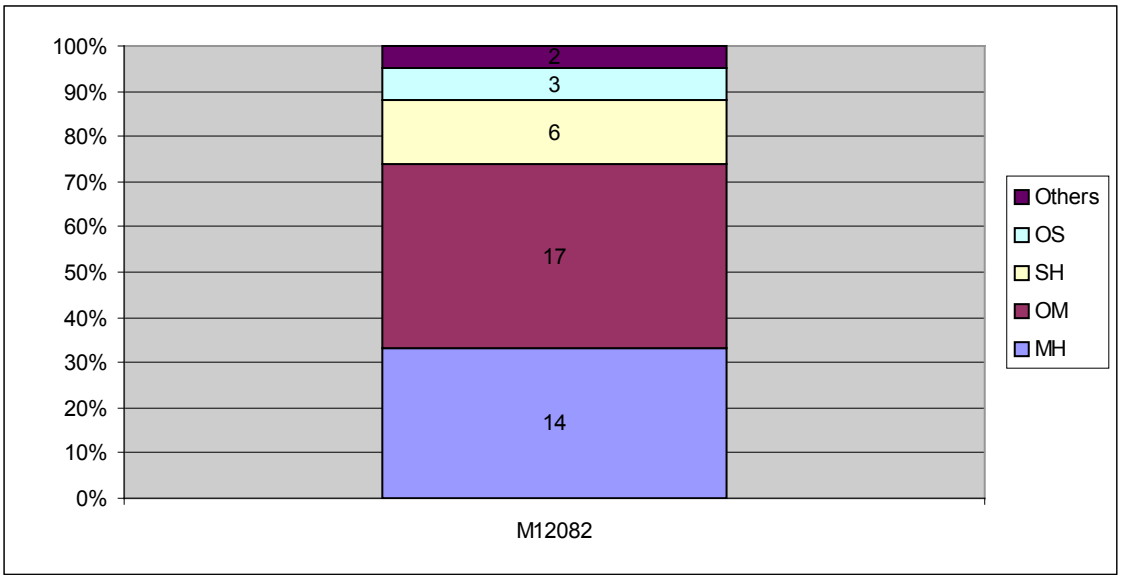
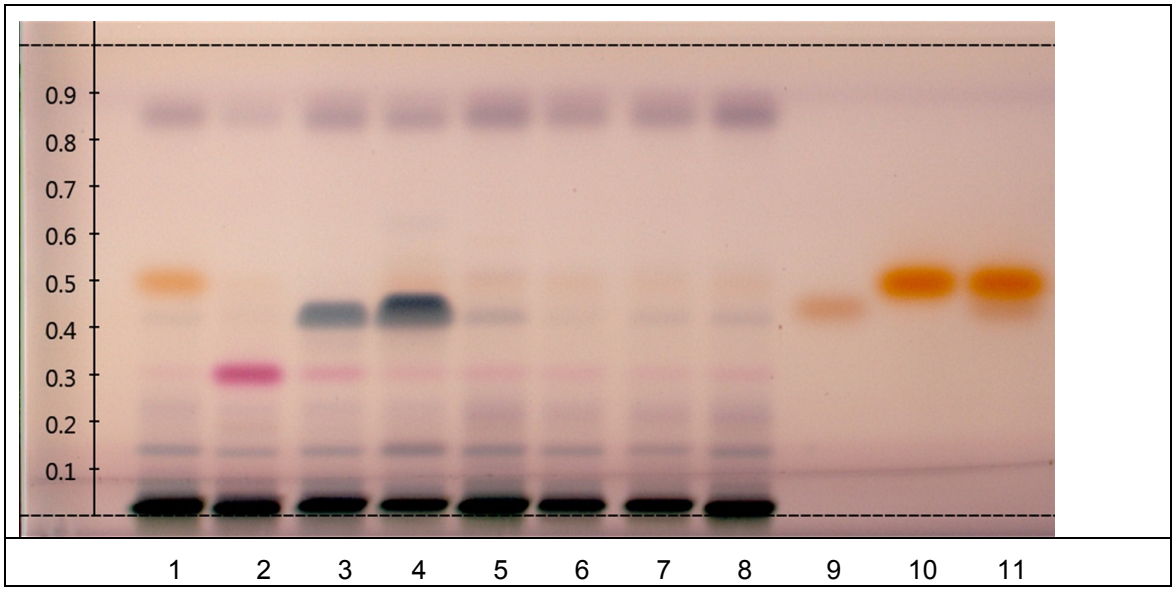


Figure 8: Fractions of the chemical types of the substances of the *Origanum heracleoticum* L. samples

9.2 Comparison of Origanum species with HPTLC

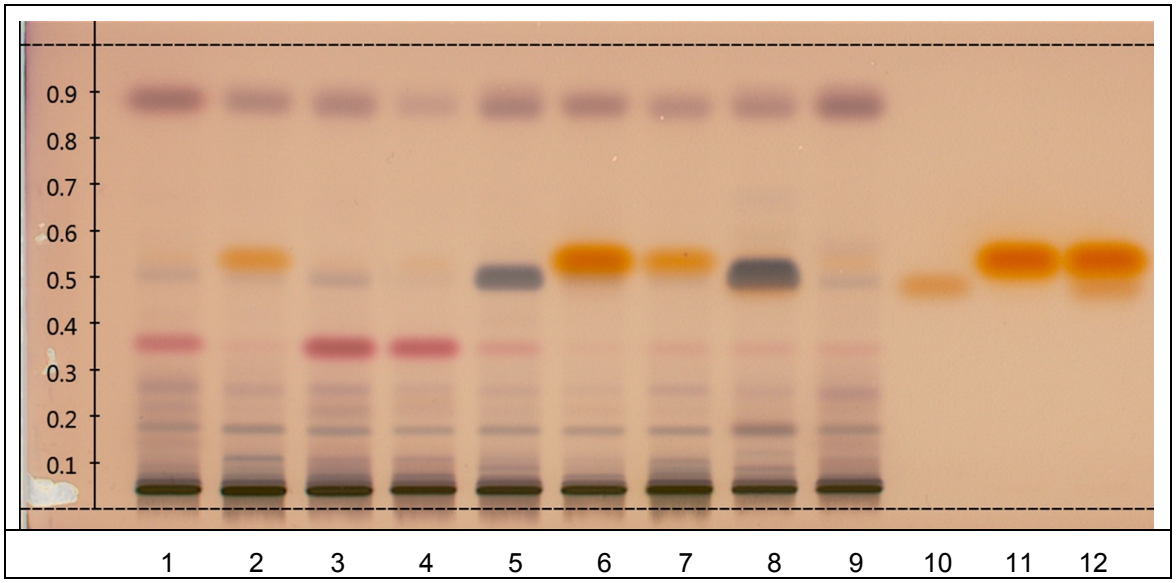
There were three different analysis made to focus on diffent chemical aspects. First, the methanolic extracts of the plant material were analysed with an HPTLC method that focuses on the essential oils. Results are shown in Figures 9 and 10. The same method was then used to analyse the essential oils obtained by distillation, giving the chromatograms shown in Figures 11 and 12. Finally, the methanolic extracts were analysed with a method targeting the polyphenol profile. These results are shown in Figures 13 and 14.



Track	*Sample*	*Application*
1	M12028 O. x majoricum	4 µL
2	M12029 O. vulgare	4 µL
3	M12078 O. vulgare	4 µL
4	M12077 O. virens	4 µL
5	M12079 O. paui	4 µL
6	M12080 O. paui	4 µL
7	M12081 O. paui	4 µL
8	M12082 O. heracleoticum	4 µL

Track	*Sample*	*Application*
9	Reference Carvacrol	1 µL
10	Reference Thymol	1 µL
11	Reference Carvacrol & Thymol	1 uL & 1 µL

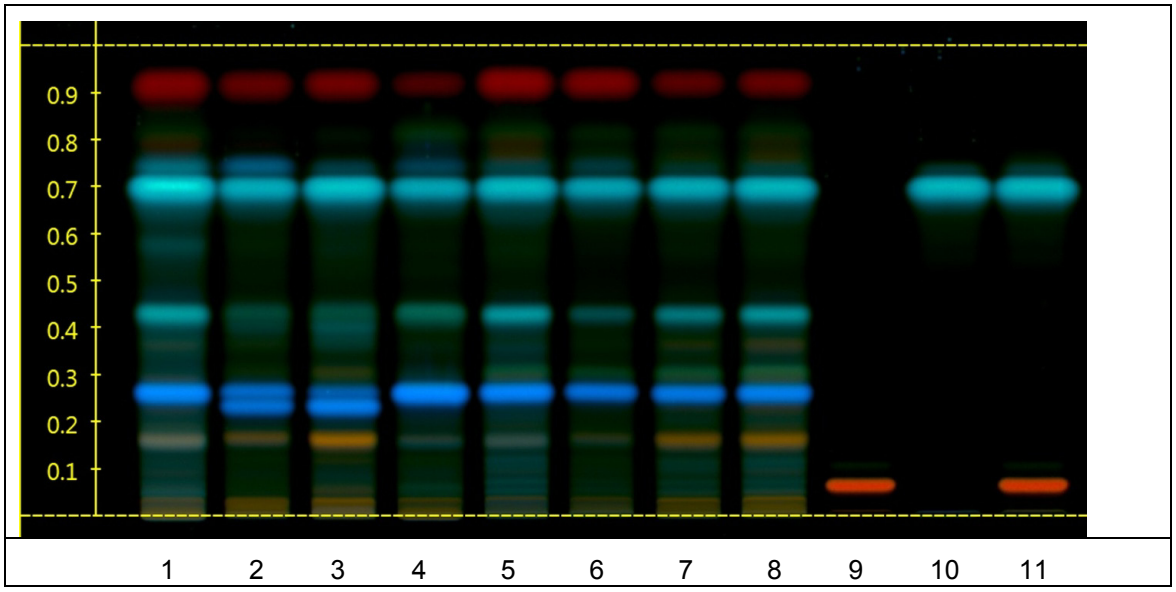
Figure 9: The HPTLC of the methanolic extracts of the plant material separated by method 1 seen under daylight



Track	*Sample*	*Application*
1	M12119 O. x majoricum	4 µL
2	M12028 O. x majoricum	4 µL
3	M12121 O. vulgare	4 µL
4	M12029 O. vulgare	4 µL
5	M12078 O. vulgare	4 µL
6	M12117 O. virens	4 µL
7	M12027 O. virens	4 µL
8	M12077 O. virens	4 µL

Track	*Sample*	*Application*
9	M12082 O. heracleoticum	1 µL
10	Reference Carvacrol	1 µL
11	Reference Thymol	1 µL
12	Reference Carvacrol & Thymol	1 uL & 1 µL

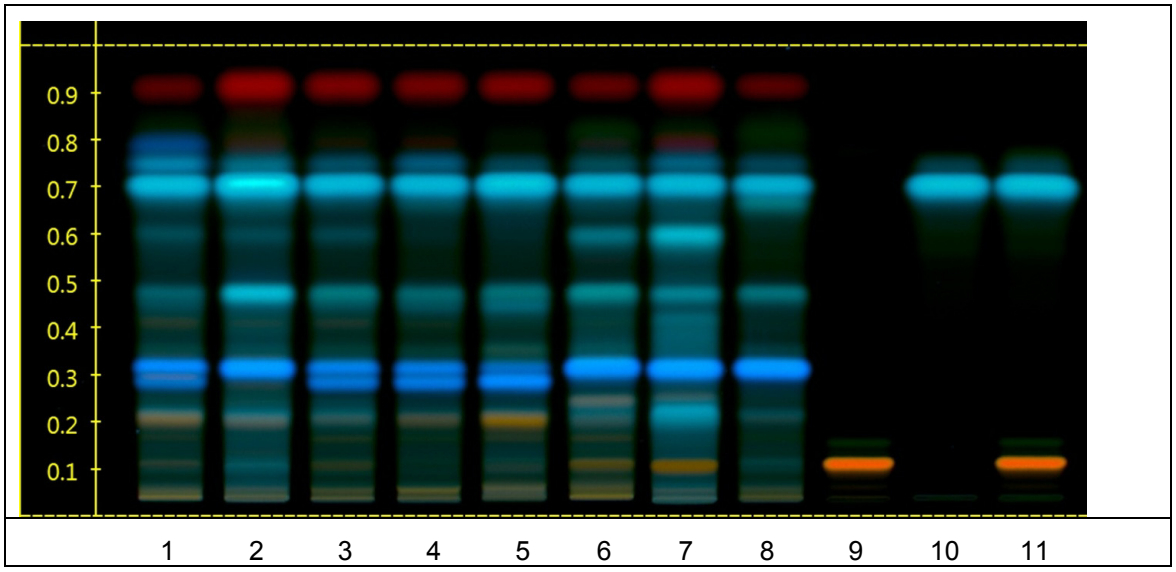
Figure 10: The HPTLC of the methanolic extracts of the plant material separated by method 1 seen under daylight



Track	*Sample*	*Application*
1	M12028 O. x majoricum	4 µL
2	M12029 O. vulgare	4 µL
3	M12078 O. vulgare	4 µL
4	M12077 O. virens	4 µL
5	M12079 O. paui	4 µL
6	M12080 O. paui	4 µL
7	M12081 O. paui	4 µL
8	M12082 O. heracleoticum	4 µL

Track	*Sample*	*Application*
9	Reference Rutin	4 µL
10	Reference Rosmarinic Acid	4 µL
11	Reference Rutin & Rosmarinic Acid	4 uL & 4 µL

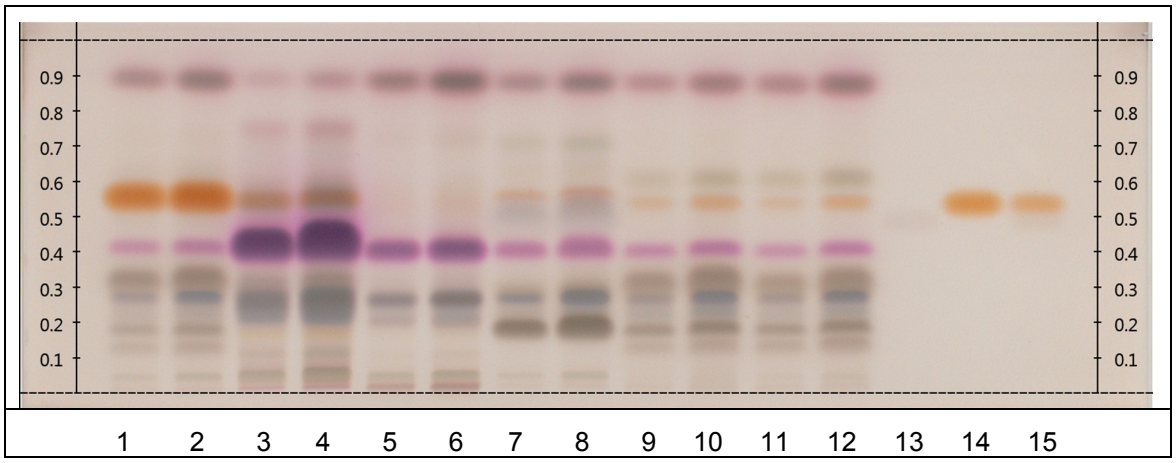
Figure 11: The HPTLC of the methanolic extracts of the plant material separated by method 2 seen under 366nm



Track	*Sample*	*Application*
1	M12119 O. x majoricum	4 µL
2	M12028 O. x majoricum	4 µL
3	M12121 O. vulgare	4 µL
4	M12029 O. vulgare	4 µL
5	M12078 O. vulgare	4 µL
6	M12117 O. virens	4 µL
7	M12027 O. virens	4 µL
8	M12077 O. virens	4 µL

Track	*Sample*	*Application*
9	Reference Rutin	4 µL
10	Reference Rosmarinic Acid	4 µL
11	Reference Rutin & Rosmarinic Acid	4 uL & 4 µL

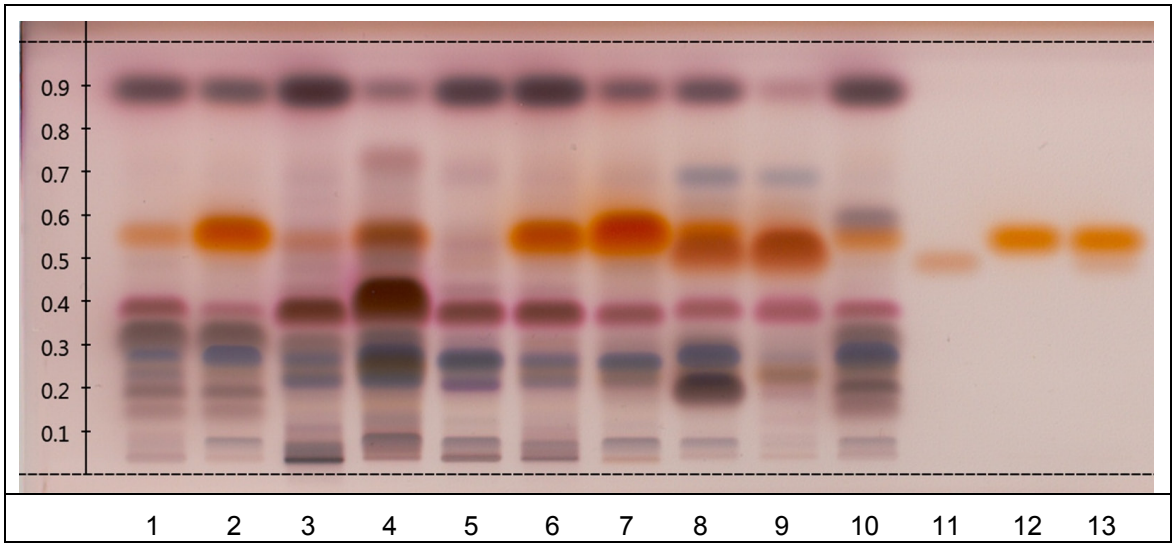
Figure 12: The HPTLC of the methanolic extracts of the plant material separated by method 2 seen under 366nm



Track	*Sample*	*Application*
1	M12119 O. x majoricum	2 µL
2	M12119 O. x majoricum	4 µL
3	M12029 O. vulgare	2 µL
4	M12029 O. vulgare	4 µL
5	M12078 O. vulgare	2 µL
6	M12078 O. vulgare	4 µL
7	M12077 O. virens	2 µL
8	M12077 O. virens	4 µL

Track	*Sample*	*Application*
9	M12079 O. paui	2 µL
10	M12079 O. paui	4 µL
11	M12081 O. paui	2 µL
12	M12081 O. paui	4 µL
13	Reference Carvacrol	1 µL
14	Reference Thymol	1 µL
15	Reference Carvacrol & Thymol	1 uL & 1 µL

Figure 13: The HPTLC of the diluted essential oils separated by method 1 seen under daylight



Track	*Sample*	*Application*
1	M12119 O. x majoricum	4 µL
2	M12028 O. x majoricum	4 µL
3	M12121 O. vulgare	4 µL
4	M12029 O. vulgare	4 µL
5	M12078 O. vulgare	4 µL
6	M12120 O. virens	4 µL
7	M12027 O. virens	4 µL
8	M12077 O. virens	4 µL

Track	*Sample*	*Application*
9	M12082 O. heracleoticum	4 µL
10	M12080 O. paui	4 µL
11	Reference Carvacrol	1 µL
12	Reference Thymol	1 µL
13	Reference Carvacrol & Thymol	1 uL & 1 µL

Figure 14: The HPTLC of the diluted essential oils separated by method 1 seen under daylight

10 Discussion

For the comparation of the compounds of the essential oils the 15 compounds with the biggest percentage were compared in diagrams.

10.1 *O. paui* Martínez

The analysis of the essential oils of the three samples of this species (Table 2) revealed a pretty homogenous composition of the essential oils. *Cis*-sabinene hydrate (19,8-35,5%), terpinen-4-ol (10,4-19,9%), α-terpineol (5,8-6,6%), γ-terpinene (2,9-8,2%), linalool (2,8-6,1%), β-caryophyllene (3,0-6,0%), linalylacetate (2,7-4,4%) and *trans*-sabinene hydrate (3,3-4,1%) were observed as the main constituents. The comparison of the composition of the essential oils (Figure 15) shows that all of them have a very similar pattern.

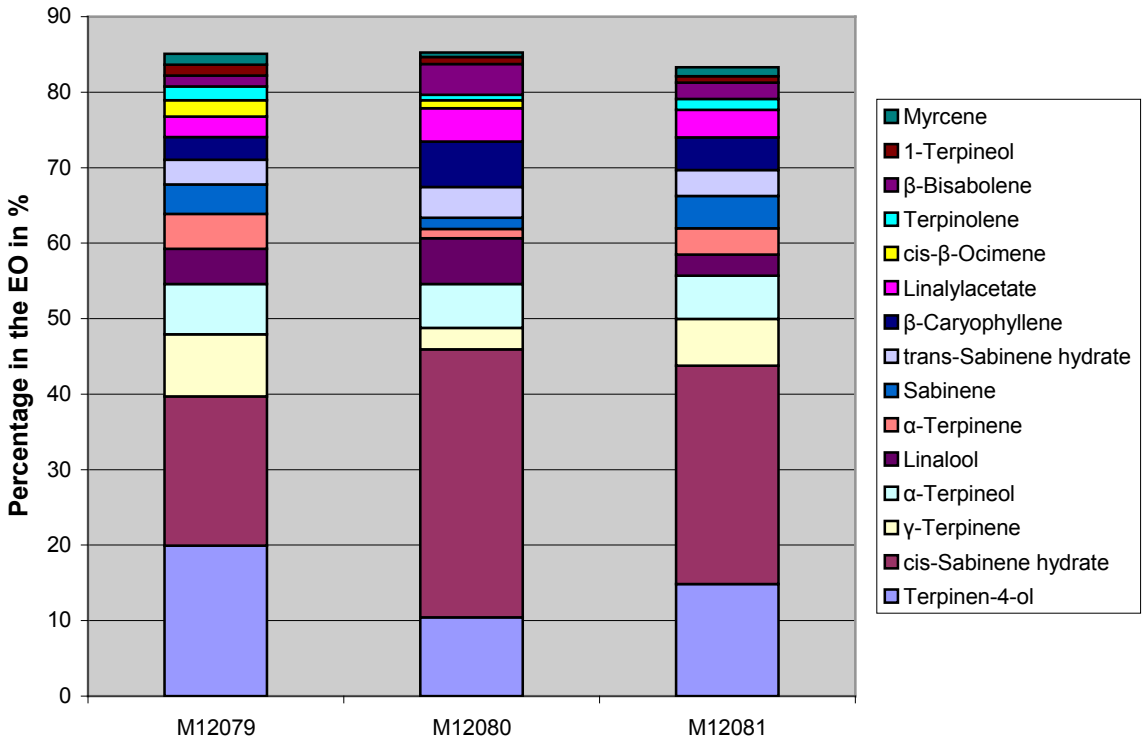


Figure 15: Proportions of the 15 compounds with the biggest quota in *Origanum paui* Martínez

10.2 *Origanum virens* Hoffmans & Link

Essential oils of three samples of this species were investigated. Results are shown in Table 3. The major constituents were in the essential oil of one sample carvacrol (38,0%), α-terpineol (27,3%), p-cymene (6,0%), β-caryophyllene (3,5%), linalool (3,5%) and thymol (3,0%) (M12077). The essential oils of the other two samples (M12027 and M12117) consisted mainly thymol (40,5% and 12,8%), γ-terpinene (20,2% and 28,8%), p-cymene (10,8% and 6,8%), β-caryophyllene (4,0% and 8,5%), thymol-methyl-ether (3,8% and 2,3%), carvacrol-methyl-ether (2,9% and 1,8%) and α-terpinene (1,7% and

3,0%). As it can be seen in Figure 16, where the compopsitions found are compared, the essential oils of *O. virens* Hoffmans & Link show two different patterns. One is rich in carvacol (M12027 and M12117) and one is rich in thymol (M12077). This confirms the few previously published data about this species, where two types are also described: one rich in carvacrol (68,1%), γ-terpinene (9,9%) and p-cymene (4,5%)[13], and another with thymol (19,4-58,0%), γ-Terpinene (10,4-20,5%), β-Caryophyllene (5,3-9,1%), thymol-methyl-ether (1,8-7,1%), bisabolene (2,0-45,0%) and carvacrol-methy-ether (1,19-4,4%)[14] as major constituents.

A forth sample investigated (M12120) is an essential oil obtained by steam distillation in a pilot plant in CITA (Zaragoza), from the plant material of sample M12117. The essential oil obtained by hydrodistillation in our laboratory presented a similar composition to that of M12121, showing a little influence of the distillation process.

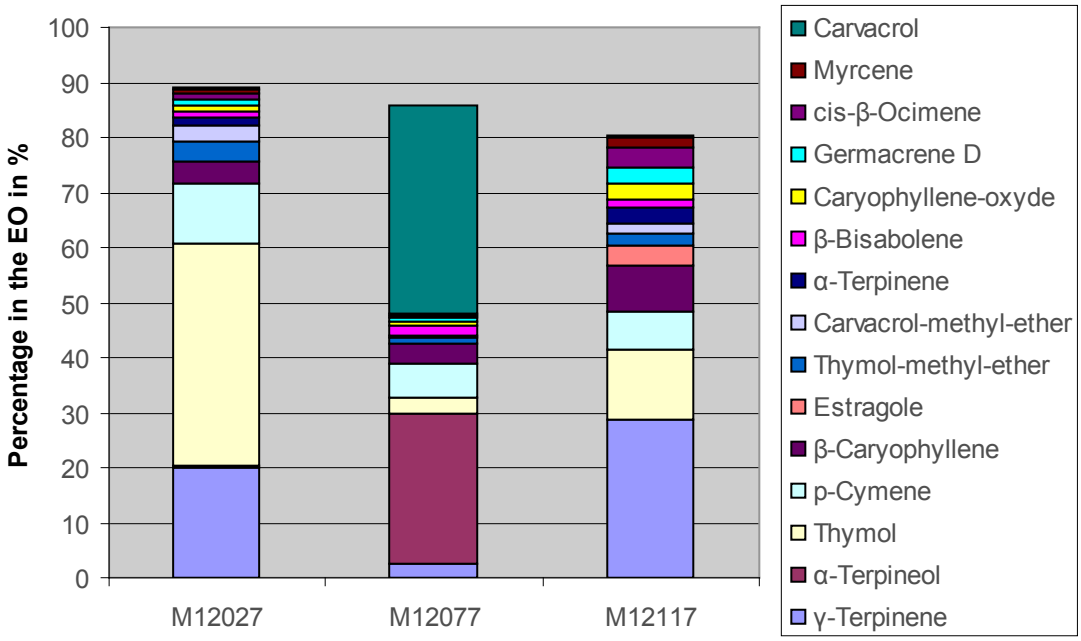


Figure 16: Proportions of the 15 compounds with the biggest quota in *Origanum virens* Hoffmans & Link samples

10.3 *Origanum vulgare* L.

The results obtained in the investigation of the three samples of this species (Table 5) confirm the high chemical heterogenity of the essential oils of *Origanum vulgare* L. As it can be seen in Figure 17 the compositions are rather different. While one sample (M12029) consisted mainly of caryophyllene-oxyde (32,6%), terpinen-4-ol (8,7%), p-cymene (7,1%), thymol (5,4%), cis-calamenene (3,5%) and γ-terpinene (2,9%) the two others (M12121 an M12078) contained β-caryophyllene (17,9% and 12,9%), Germacrene D (14,5% and 9,0%), caryophyllene-oxyde (7,4% and 5,7%) spathulenole (5,3% and 0,2%), E,E- α-farnesene (4,8% and 2,6%), trans- β-ocimene (1,9% and 21,3%) and terpinene-4-ol (1,3% and 7,4%). Although two samples (M12029 and M12118) come from the same origin (Zaragoza), they show minor similarities. The composition of

the third one (M12078), collected in another part of spain, is relatively close to one of the former samples (M12118). This chemical diversity was alrady seen in former investigations.[15]

A forth sample investigated (M12121) is an essential oil obtained by steam distillation in a pilot plant in CITA (Zaragoza), from the plant material of sample M12118. The essential oil obtained by hydrodistillation in our laboratory presented a similar composition to that of M12121, showing a little influence of the distillation process.

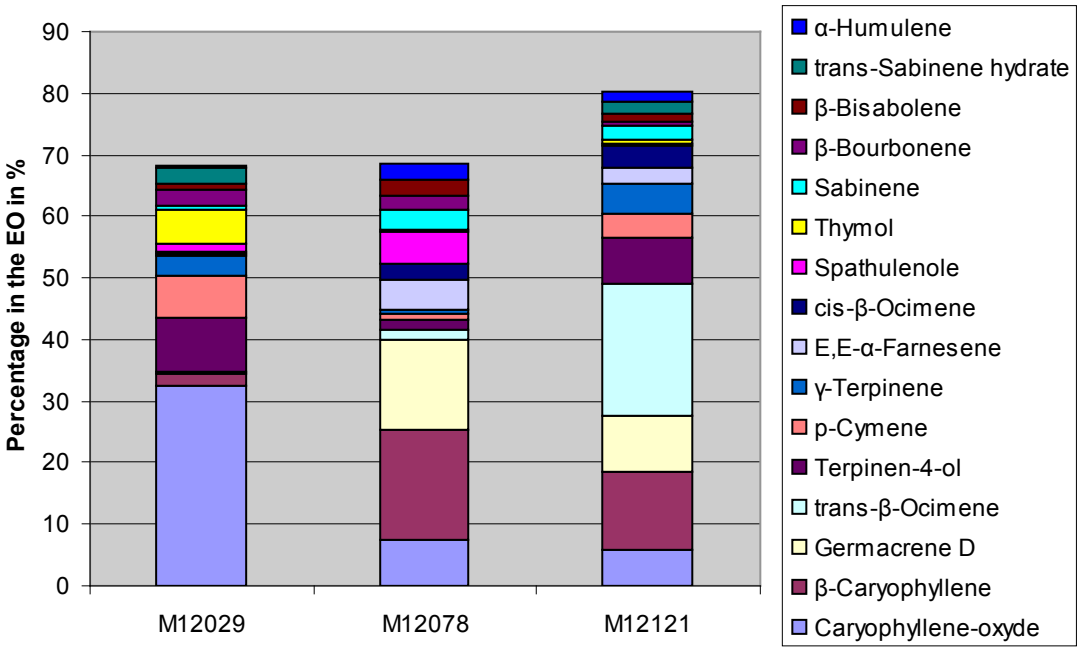


Figure 17: Proportions of the 15 compounds with the biggest quota in *Origanum vulgare* L. samples

10.4 *Origanum x majoricum* Cambess.

The composition found (Table 4) for the essential oils of the two samples analysed, both from the same origin, is very similar (Figure 18). Terpinen-4-ol (19,8% and 20,7%), γ-terpinene (15,1% and 13,0%), cis-sabinene hydrate (11,6% and 7,4%), thymol(14,1% and 0,8%), α-terpinene (4,2% and 6,0%) and sabinene (3,6% and 5,9%) appeared as the major compounds. Apart of the fact that one (M12028) has a much higher amount of thymol than the other (M12119), the major compounds don't differ greatly. Literature data on the composition of the essential oils of this species shows mainly two different types of oils: one with *cis*-sabinene hydrate (24-37%), terpinen-4-ol (6-13%), linalyl acetate (3-8%), *trans*-sabinene hydrate (3-5%), linalool (2-6%), carvacrol (7-18%) and γ-terpinene (4-6%)[16] as major constituents, and another charactresidsed by the presence of *trans*-sabinene hydrate (27,77-36,77%), thymol (17,77-30,77%), α-terpinene (3,13-4,63%), limonene (2,1-3,6%), *cis*-hydrate sabinene (1,43-3,37%), terpinen-4-ol (3.23-5.03%), and carvacrol (trace 3,57%)[17]. The samples analysed in the present work are close to the first type.

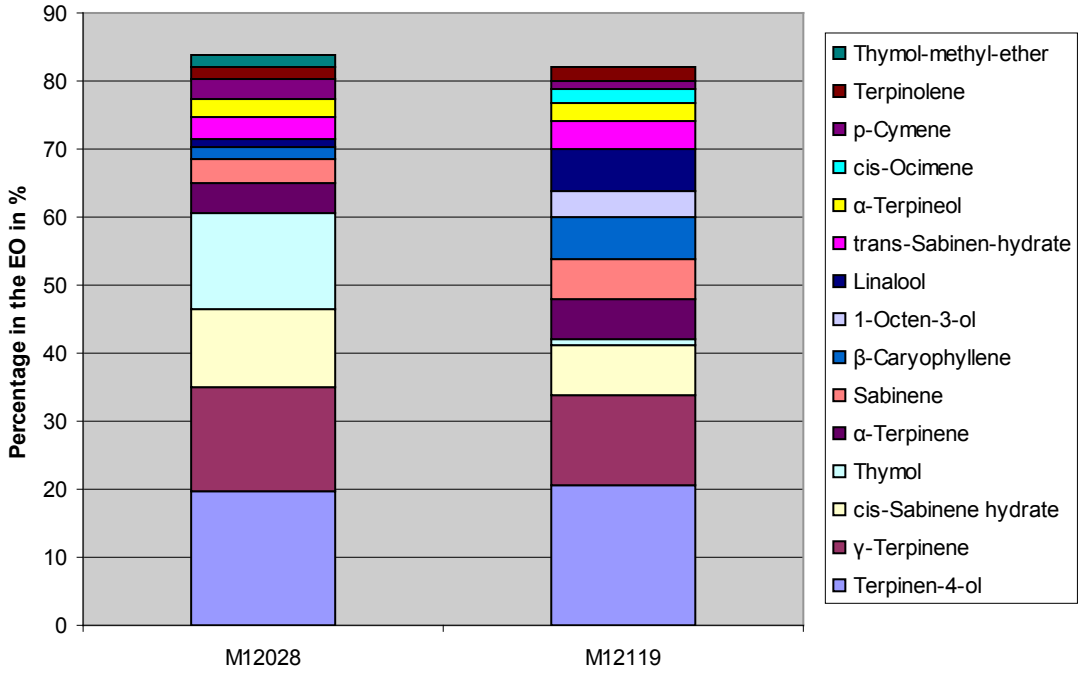


Figure 18: Proportions of the 15 compounds with the biggest quota in *Origanum x majoricum* Cambess. Samples

10.5 *Origanum heracleoticum* L.

There was only one sample available to investigate this species (Table 6). As can be seen in Figure 19, there was one major component, carvacrol, dominating the whole spectrum with a percentage of 68,5%. Followed by only p-cymene (17,3%) and γ-terpinene (2.0%) all the other substances identified could be found in an amount under one percent. The high amount of carvacrol was already described previously (69,0%), also the major importance of p-cymene (10,5%) although the missing of thymol (described as 8,0%-14.8%) is remarkable[18,19]. This is rather interesting because this species is much used comercialy as a spice. [2] Probably, the partition of the phenol content of the essential oil between thymol and carvacrol has minor influence on the antimicrobial propertis of the herbal drug. Even, in the monography of oregano of the European Pharmacopeia, only the minimal content of the sum of carvacrol and thymol is prescribed as requirement.[12]

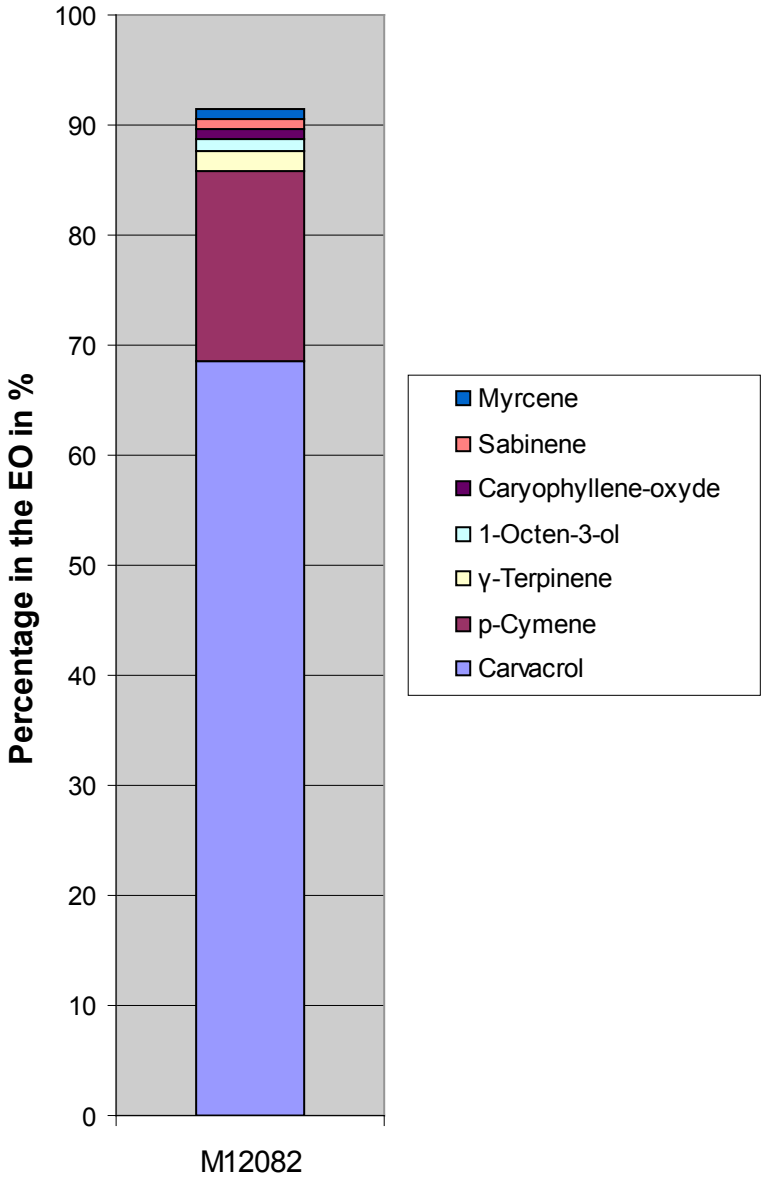


Figure 19: Proportions of the 15 compounds with the biggest quota in M12082 *Origanum heracleoticum* L.

10.6 Differentiation between the species by HPTLC

There were three different analysis made to focus on diffent chemical aspects. First there were methanolic extracts investigated with a method that focuses on the essential oils. The same method was then used to separate the distilled essential oils. Furthermore the methanolic extracts were separated with a method targeting the polyphenol profile.

The analysis of the methanolic extracts focused on the essential oils (Figure 9 and Figure 10) shows fingerprints which are very similar for all of the samples in the upper and lower thirds of the chromatograms. But, between retention indexes 0,3 and 0,7, major differences can be seen. The pink band at 0,37 occurs only in some samples and in different intensities. The differences between one sample of *Origanum virens* Hoffmans & Link (M12077) and the

other two samples (M12117 and M12027) are revealed by this method. The yellowish orange thymol zone does only appear in the samples from Zaragoza (M12117 and M12027). The visual appearance of carvacrol in the fingerprint of M12077 may be covered by the strong blue band. Also the differences between the samples of *Origanum vulgare* L. (in the intensity of the pink bands at 0,37 and the blue bands at 0,49) and the similarities between the *Origanum paui* Martínez and *Origanum x majoricum* Cambess. samples are obvious. *O. heracleoticum* L. doesn't show a band for carvacrol which doesn't fit with the data investigated by GC and GCMS, but it occurs in the HPTLC of the essential oils. The missing of the carvacrol zone could be due to a low content of essential oil in the sample, that unfortunately couldn't be determined because of a lack of enough plant material.

The HPTLC of the essential oils better display the variety of the samples (Figure 13 and Figure 14). GC and GC-MS analysis revealed high amount of carvacrol in the essential oils of one sample of *O. virens* Hoffmans & Link (M12077) and *O. heracleoticum* L., which were also observed by HPTLC, as well as thymol as the major constituent of the essential oils of the other two samples of *O. virens* Hoffmans & Link (M12120 and M12027), one sample of *O. x majoricum* Cambess. (M12028) and *O. vulgare* L. (M12029). Each species can be identified by the characteristic combination of zones especially in the lower half of the chromatogram. In the case of *O. virens* Hoffmans & Link and *O. vulgare* L., two different patters can be observed in this zone of the chromatogram, which show the two different essential oil compositions found within the samples of these species. *O. heracleoticum* L. appears pretty similar to one type of *O. virens* Hoffmans & Link (M12077), both samples being rich in carvacrol, but differ in the intensity of the big grey band at Rf of 0,18 and the blue band at 0,25.

Results on the polyphenol fingerprint of the methanolic extracts are shown in Figure 11 and Figure 12. In general, the chromatograms show three main blue fluorescent zones in every sample. One at the Rf of rosmarinic acid, one at Rf of 0,48 and one at 0,32.

This chromatographic method shows a specific profile for the *O. vulgare* L. samples. They can be identified by a blue fluorescent band at Rf = 0,29. This band occurs also in one of the samples of *O. x majoricum* Cambess. but this sample can be distinguished by another blue fluorescent band at Rf = 0,79.

The chemical differences between the samples of *O. virens* Hoffmans & Link are visible. A blue flourescent band at Rf = 0,61 is observed in the chromatograms of the two samples from Zaragoza (M12120 and M12027), but not in the other one (M12077). This zone also occurs in the samples of *O. x majoricum* Cambess. and of one of *O. vulgare* L. (M12121) but those differ because of the lack of a yellow band at Rf = 0,27.

The chromatograms obtained with this method present very homogenous patterns for *O. paui* Martínez and *O. heracleoticum* L., so it is not helpful for distinguishing these two species.

11 Conclusions

1. The main components of the essential oil of *Origanum paui* Martínez are cis-sabinene hydrate (19,8-35,5%), terpinen-4-ol (10,4-19,9%), α-terpineol (5,8-6,6%), γ-terpinene (2,9-8,2 %), linalool (2,8-6,1%), β-caryophyllene (3,0-6,0%), linalylacetate (2,7-4,4%) and trans-sabinene hydrate (3,3-4,1%).
2. The samples of the *Origanum virens* Hoffmans & Link species showed two different pattern compositions of the essential oils. One sample contained mainly carvacrol (38,0%), α-terpineol (27,3%), p-cymene (6,0%), β-caryophyllene (3,5%), linalool (3,5%) and thymol (3,0%). The other two samples showed as major compounds thymol (40,5% and 12,8%), γ-terpinene (20,2% and 28,8%), p-cymene (10,8% and 6,8%), β-caryophyllene (4,0% and 8,5%), thymol-methyl-ether (3,8% and 2,3%), carvacrol-methyl-ether (2,9% and 1,8%) and α-terpinene (1,7% and 3,0%). This two oil types are consistent with the already published data on this species.
3. The *Origanum vulgare* L. samples differed also in the compositions of their essential oils. While one sample consisted mainly of caryophyllene-oxyde (32,6%), terpinen-4-ol (8,7%), p-cymene (7,1%), thymol (5,4%), cis-calamenene (3,5%) and γ-terpinene (2,9%) the two others contained β-caryophyllene (17,9% and 12,9%), germacrene D (14,5% and 9,0%), caryophyllene-oxyde (7,4% and 5,7%) spathulenole (5,3% and 0,2%), E,E- α-farnesene (4,8% and 2,6%), trans- β-ocimene (1,9% and 21,3%) and terpinene-4-ol (1,3% and 7,4%). This confirms the high phytochemical diversity of this species.
4. The essential oils of the samples of *Origanum x majoricum* Cambess. consisted mainly of terpinen-4-ol (19,8% and 20,7%), γ-terpinene (15,1% and 13,0%), cis-sabinene hydrate (11,6% and 7,4%), thymol(14,1% and 0,8%), α-terpinene (4,2% and 6,0%) and sabinene (3,6% and 5,9%). This composition is rather similar to published data although different contents of cis- and trans-sabinene-hydrate have been observed previously.
5. In the essential oil of *Origanum heracleoticum* L., carvacrol (68,5%), p-cymene (17,3%), γ-terpinene (1,97%), 1-octen-3-ol (1,0%) and caryophyllene-oxyde (0,96%) were identified as the main constituents. Previous investigation also showed high percentages of carvacrol but also of thymol.
6. The analysis of the essential oils by HPTLC shows specific fingerprints for the species analysed and also allows to distinguish the different varieties of *O. vulgare* L. and of *O. virens* Hoffmans & Link.
7. The HPTLC analysis of the methanolic extracts using the method focused on the essential oil constituents shows the differences between the different types of each species, but is less useful than the analysis of the distilled essential oils in order to distinguish between all the species.
8. The HPTLC fingerprints of polyphenol analysis of the methanolic extracts show three main blue fluorescent zones that are common to all samples analysed. The method allows identifying the samples of *O. vulgare* L.,

but the similarity between the other species and/or the variability inside a single one makes the identification of those species difficult.

12 Summary

Oregano is an aroma, used as a spice in many countries around the world [2] and is also used in traditional medicine for centuries [1]. Mainly plant material from the genus *Origanum* (Lamiaceae) and *Lippia* (Verbenaceae) is used under this name, while *Origanum sp.* is mainly used in Europe.[2] Also, pharmacological activities of species of this genus are already confirmed, such as antifungal, antibacterial, antiparasitic, antioxidant, analgesic, anti-inflammatory and antispasmodic characteristics. [2] While only *Origanum onites* L. and *Origanum vulgare* L. *subsp. hirum* Letsw. are descirbed in the monography of the Ph. Eur. because of their richness in essential oil and the high percentages of carvacrol and thymol[7], other species may have potential for use in pharmacy. Therefore essential oils and methanolic extracts of 5 different *Origanum* species, *Origanum pau*i Martínez., *Origanum heracleoticum* L., *Origanum virens* Hoffmans & Link, *Origanum vulgare* L., and *Origanum x majoricum* Cambess. were studied. The essential oils were obtained by hydro distillation for 3 hours and their composition was observed with GC and GC-MS. Furthermore the essential oils and the methanolic extracts were compared with HPTLC. These methanolic extracts were produced by extracting pulverized plant material with methanol in an ultrasonic bath. The so far not chemically investigated species *O. pau*i showed as major components of its essential oil cis-sabinene hydrate (19,8-35,5%), terpinen-4-ol (10,4-19,9%), α-terpineol (5,8-6,6%), γ-terpinene (2,9-8,2 %), linalool (2,8-6,1%), β-caryophyllene (3,0-6,0%), linalylacetate (2,7-4,4%) and trans-sabinene hydrate (3,3-4,1%). The essential oils of the other species showed compositions which fit with published data. The samples of *O. virens* showed two different chemical patterns of their essential oils (main components carvacrol (38,0%), α-terpineol (27,3%), p-cymene (6,0%), β-caryophyllene (3,5%), linalool (3,5%) and thymol (3,0%); or thymol (40,5% and 12,8%), γ-terpinene (20,2% and 28,8%), p-cymene (10,8% and 6,8%), β-caryophyllene (4,0% and 8,5%), thymol-methyl-ether (3,8% and 2,3%), carvacrol-methyl-ether (2,9% and 1,8%) and α-terpinene (1,7% and 3,0%)). In the essential oils of the samples of *O. vulgare* also two different compositions were observed (caryophyllene-oxyde (32,6%), terpinen-4-ol (8,7%), p-cymene (7,1%), thymol (5,4%), cis-calamenene (3,5%) and γ-terpinene (2,9%); or β-caryophyllene (17,9% and 12,9%), germacrene D (14,5% and 9,0%), caryophyllene-oxyde (7,4% and 5,7%) spathulenole (5,3% and 0,2%), E,E- α-farnesene (4,8% and 2,6%), trans-β-ocimene (1,9% and 21,3%) and terpinene-4-ol (1,3% and 7,4%)). The main constituents of the essential oils of the *O. x majoricum* samples were identified as terpinen-4-ol (19,8% and 20,7%), γ-terpinene (15,1% and 13,0%), cis-sabinene hydrate (11,6% and 7,4%), thymol(14,1% and 0,8%), α-terpinene (4,2% and 6,0%) and sabinene (3,6% and 5,9%) and of the *O. heracleoticum* samples as carvacrol (68,5%), p-cymene (17,3%), γ-terpinene (1,97%), 1-octen-3-ol (1,0%) and caryophyllene-oxyde (0,96%). Therefore none of these samples met the requirements for the percentage of carvacrol and thymol in the essential oil of the Ph. Eur. (minimum 60%)[7] except *O. heracleoticum*. But in the sample of this species the quanitity of the essential oil couldn't be determined because of a lack of plant material.
The HPTLC analysis of the essential oils can be used for distinguishing the different chemical fingerprints of the *O. virens* and the *O. vulgare* samples and

also shows characteristic patterns for each species. The analysis of the methanolic extracts with the same HPTLC method also shows the heterogenities within the samples of these two species but is less useful for distinguishing all the species from each other. The HPTLC fingerprints of polyphenol analysis of the methanolic extracts don't allow to differentiate between the species.

13 Zusammenfassung

Oregano ist ein Aroma, das in vielen Ländern der Welt als Gewürz verwendet wird[2] und auch seit Jahrhunderten in der traditionellen Medizin Verwendung findet.[1] Hauptsächlich Pflanzenmaterial der Genera *Origanum* (Lamiaceae) und *Lippia* (Verbenaceae) wird unter diesem Namen benützt, wobei vor allem *Origanum sp*. in Europa verwendet wird.[2] Des weiteren wurden bereits pharmakologische Aktivitäten von Spezies dieses Genus beobachtet, wie anitfungale, antibakterielle, antiparasitäre, antioxidative, analgetische, entzündungshemmende und spasmolytische Charaktaristika. Während nur *Origanum onites* L. und *Origanum vulgare* L. subsp. *hirtum* letsw. in der Monographie des europäischen Arzneibuches wegen ihres hohen Gehalts an ätherischem Öl und hohen Prozentsatz von Carvacrol und Thymol in eben diesem beschrieben werden[7], haben vielleicht auch andere Spezies Potential zur pharmazeutischen Verwendung. Deshalb wurden die ätherischen Öle sowie die methanolischen Extrakte von 5 verschiedenen *Origanum* Spezies, *Origanum paui* Martínez., *Origanum heracleoticum* L., *Origanum virens* Hoffmans & Link, *Origanum vulgare* L., und *Origanum x majoricum* Cambess. untersucht. Die ätherische Öle wurden durch dreistündige Wasserdampfdestillation gewonnen und deren Komponenten mittels GC und GC-MS ermittelt. Des weiteren wurden die ätherischen Öle und die methanolischen Extrakte mittels HPTLC verglichen. Diese methanolischen Extrakte wurden durch Extraktion von pulverisiertem Pflanzenmaterial mit Methanol in einem Ultraschallbad hergestellt. Als Hauptkomponenten des ätherischen Öls der davor noch nicht chemisch untersuchten Spezies *O. paui* wurden cis-Sabinenhydrat (19,8-35,5%), Terpinen-4-ol (10,4-19,9%), α-Terpineol (5,8-6,6%), γ-Terpinen (2,9-8,2 %), Linalool (2,8-6,1%), β-Caryophyllen (3,0-6,0%), Linalylacetat (2,7-4,4%) und trans-Sabinenhydrat (3,3-4,1%) ermittelt. Die Zusammensetzungen der ätherischen Öle der anderen Spezies stimmten mit bereits veröffentlichten Daten überein. Die Muster von *O. virens* zeigten zwei unterschiedliche Profile ihrer ätherischen Öle (Hauptkomponenten Carvacrol (38,0%), α-Terpineol (27,3%), p-Cymen (6,0%), β-Caryophyllen (3,5%), Linalool (3,5%) und Thymol (3,0%); oder Thymol (40,5% und 12,8%), γ-terpinene (20,2% und 28,8%), p-cymene (10,8% und 6,8%), β-Caryophyllen (4,0% und 8,5%), Thymol-methyl-ether (3,8% und 2,3%), Carvacrol-methyl-ether (2,9% und 1,8%) und α-Terpinen (1,7% und 3,0%)). In den ätherischen Ölen der Muster von *O.* vulgare wurden ebenfalls zwei verschiedene Kompositionen beobachtet (Caryophyllene-oxyd (32,6%), Terpinen-4-ol (8,7%), p-Cymene (7,1%), Thymol (5,4%), cis-Calamenen (3,5%) and γ-Terpinen (2,9%); oder β-Caryophyllen (17,9% und 12,9%), Germacren D (14,5% und 9,0%), Caryophyllen-oxyd (7,4% und 5,7%), Spathulenol (5,3% und

0,2%), E,E- α-Farnesen (4,8% und 2,6%), trans-β-Ocimen (1,9% und 21,3%) und Terpinen-4-ol (1,3% und 7,4%)). Die Hauptkomponenten des ätherischen Öls der Muster von *O. x majoricum* wurden identifiziert als Terpinen-4-ol (19,8% und 20,7%), γ-Terpinen (15,1% und 13,0%), cis-Sabinenhydrat (11,6% und 7,4%), Thymol(14,1% und 0,8%), α-Terpinen (4,2% und 6,0%) and Sabinen (3,6% und 5,9%) sowie bei dem Muster von *O. heracleoticum* als Carvacrol (68,5%), p-Cymen (17,3%), γ-Terpinen (1,97%), 1-Octen-3-ol (1,0%) und Caryophyllen-oxyd (0,96%). Deshalb entsprachen keine Muster der Anforderung an den Carvacrol- und Thymol-Anteil des ätherischen Öls (min. 60%) der Ph. Eur.[7] außer *O. heracleoticum*. Allerdings konnte bei dem Muster dieser Spezies der Gehalt an ätherischem Öl wegen einer zu geringem Menge an Pflanzenmaterial nicht bestimmt werden.
Die Analyse der ätherischen Öle mit HPTLC kann verwendet werden, um die verschiedenen chemischen Fingerabdrücke innerhalb der *O. virens*- und *O. vulgare*-Muster zu unterscheiden und zeigt außerdem charakteristische Profile für die verschiedenen Spezies. Die Analyse der methanolischen Extrakte mit der gleichen HPTLC-Methode kann ebenfalls die Heterogenität innerhalb der oben genannten zwei Spezies zeigen werden, jedoch ist sie weniger hilfreich bei der Unterscheidung zwischen den Spezies. Mit den HPTLC-Fingerabdrücken der methanolischen Extrakte mit einem auf deren polyphenolische Profile gerichteten Verfahren ist keine Differenzierung zwischen den Spezies möglich.

14 References

[1] Bariceric D, Bartal T. The biological/pharmacological activity of the *Origanum* genus. In: Kintzios, S.E, Oregano: The genera *Origanum* and *Lippia*. Taylor and Francis, London, 2002; 177–214

[2] Kintzios S.E, Oregano: The genera *Origanum* and *Lippia*; Taylor & Francis, London 2002

[3] Dogan Y, Baslar S, Ay G, and Mert. H.H. The use of wild edible plants in Western and Central Anatolia (Turkey). *Econ. Bot.* 2004; 58(4): 684–690,

[4] Hussain A, Anwar F, Rasheed S, Poonam N, Janneh O, Satyajit A. Composition, antioxidant and chemotherapeutic properties of the essential oils from two Origanum species growing in Pakistan; Revista Brasileira de Farmacognosia Brazilian Journal of Pharmacognosy 58(4): 684–690; 21(6): 943-952

[5] Souza E, StamfordT, Lima E, Trajano V. Evectiveness of *Origanum vulgare* L. essential oil to inhibit the growth of food spoiling yeasts; Food Control 2007; 18: 409–413;

[6] Wagner H. Pharmazeutische Biologie; 5th Ed, Gustav Fischer Verlag Stuttgart/New York 1993

[7] Council of Europe; European Pharmacopoeia – 7th Edition Volume 1, 01/2011 673

[8] Bandoni A. Los Recursos Vegetales Aromáticos en Latinoamérica, Editorial de la Universidad Nacional de La Plata, Argentina 2000

[9] Miller J, 2nd Ed, Chromatography, John Wiley & Sons, Inc., Hoboken, New Jersey 2005

[10] Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medical Plants, Thieme Medical Publisher, inc., New York 2007

[11] Sandra P, Bicchi C. Capillary Gas Chromatography in Essential Oil Analysis, Huething, Heidelberg 1987

[12] Council of Europe; European Pharmacopoeia – 7th Edition Volume 1, 01/2011

[13] Salgueiro L, Cavaleiro C, Pinto E, Pina-Vaz C, Rodrigues A, Palmeira A, Tavares C, Costa-de-Olivera S, Goncalves M, Martinez-de-Oliveira J. Chemical Composition and Antifungal Activity of the Essential Oil of Origanum virens on candid Species; Planta Med 2003; 69: 871-874

[14] Castilho P, Savluchinske-Feio S, Weinhold T, Gouveia S. Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal; Food Control 2012; 23 552-558

[15] Azizi A, Hadian J, Gholami M, Friedt W, Honermeier B; Correlations between Genetic, Morphological, and Chemical Diversities in a Germplasm Collection of the Medicinal Plant Origanum vulgare L.; CHEMISTRY & BIODIVERSITY 2012; Vol. 9, 2784-2801

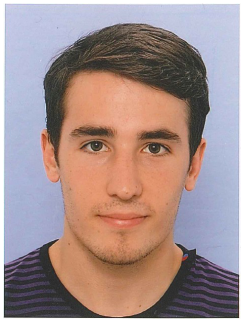
[16] Tabanca N, Özek T, Baser K, Tümen G. Comparison of the Essential Oils of Origanum majorana L. and Origanum x majoricum Cambess. Journal of Essential Oil Research 2004;16:3, 248-252

[17] Dambolena S, Zunino M, Lucini E, Olmedo R, Banchio E, Bima P, Zygadlo J. Total Phenolic Content, Radical Scavenging Properties, and Essential Oil Composition of Origanum Species from Different Populations; J. Agric. Food Chem. 2010; 58, 1115–1120

[18] Čabarkapa IŠkrinjar M, Nemet N , Milovanović I. Effect of *Origanum heracleoticum* L. Essential Oil on Food-borne *Penicillium aurantigriseum* and *Penicilium chrysogenum* Isolates; Proc. Nat. Sci, Matica Srpska Novi Sad 2011; 120, 81—91

[19] Dzamic A, Sokovic M, Ristic M, Grujic-Jovanovic S, Vukojevic J, Marin P. Chemical Composition and Antifungal Activity of Origanum heracleoticum Essential Oil; Chemistry of Natural Compounds 2008; Vol. 44, No. 5, 659-660

Curriculum vitae



Name:	Martin BABELUK
Ausbildung:	Ab WS 2008 Studium Pharmazie an der Univ. Wien
	2003 bis 2008 HTL TGM in Wien, Fachrichtung Elektronik/Biomedizin-Technik
	1999 bis 2003 AHS GRG 23 in Wien
Fortschritt des Studiums	WS 2012/2013 Auslandsaufenthalt in Barcelona
	Abschluss des Zweiten Studienabschnitts am 28.09.2012
	Abschluss des Ersten Studienabschnittes am 29.09.2009
	Beginn des Diplomstudiums Pharmazie im WS 2008
Arbeitserfahrung	09/2011 MedUni Wien, Universitätsklinik für Kardiologie, Forschungsgruppe Tumor Endothelium / Anti-Angiogenic Cancer Therapy; Hauptaufgaben: Zellkultur, PCR, Western Blot, Konfokalmikroskopie
	07/2010 MedUni Wien, Universitätsklinik für Chirurgie, Forschungsgruppe Proteomics; Hauptaufgaben: Gelelektrophorese, Zellkultur, Bradfordtests, Western Blot
	08/2009 Patentanwalt Babeluk, Neuanmeldungsabteilung
	07/2006 Roche Diagnostics Graz, Global Support Systems
	08/2005: K&N Schalter Entwicklungs-Ges. m. b. H., Entwicklungslabor
Sprachliche Kenntnisse:	Englisch und Spanisch: fließend in Wort und Schrift
	Japanisch, und Französisch: Grundkenntnisse
Besondere Kenntnisse:	Programmiersprachen: MS-Office (Word, Excel); C++/C; SQL