Chapter

Essential Oils from Medicinal Plants: Extraction Techniques, Biochemical Characterization, and Technical Analysis

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Abstract

Essential oils, called volatile oils or ethereal oils, are natural metabolic secretions widespread in the most varied organs of the plant. Some of these natural products are extremely potent and precise as action due to their complex molecular substances, in which mono- and sesquiterpenes constituents predominate, and contain aromatic compounds. The technological process of obtaining volatile oil intervenes decisively in its composition and its quality. In this chapter, the general overview of essential oils, their chemistry, extraction methods, and analyses are described. In detail, the chemical composition of essential oils is influenced by biotic, abiotic, and genetic factors, which are discussed in this chapter. In addition, extraction of EOs is one of the most effort-requiring and time-consuming processes. In this chapter, different methods used for the extraction are discussed. Furthermore, chemical structures in essential oil have been provided in detail. This chapter also discusses some of the developments in chromatography for essential oils analysis starting from gas chromatography to coupled techniques.

Keywords: essential oil, extraction, composition, terpenoids, biomolecules, analyses

1. Introduction

Several plant species have been recognized to possess volatile chemical compounds since antiquity, which can be extracted as "essential oils" using an appropriate lipid solvent. Despite only constitute a small portion of a plant, essential oils provide aromatic plants their distinctive qualities, which are employed in the food, fragrance, and pharmaceutical industries [1]. Today, it is evident that essential oils are mostly composed of highly complex, volatile organic compounds which are insoluble in water, mainly composed of monoterpenes and sesquiterpenes, representing one of the four main biological classes of natural compounds alongside polyphenols, alkaloids, and glycosides [2]. According to the French pharmacopoeia, essential oils or volatile oils are "products of generally quite complex composition containing the volatile principles contained in plants and more or less modified during preparation" [3].

These oils are separated from the various plant sources using different techniques. Even though it appears to be very simple to isolate such oils, the composition of the oil may vary considerably according to the method of extraction used. In 1998, AFNOR through its AFNOR NF T 75-006 standard defined an essential oil as a "Product obtained from a plant raw material, either by steam entrainment, either by mechanical processes from the citrus epicarp, or by dry distillation" [3]. Once the oils are obtained, the fundamental contribution of the organic chemistry to the industry resides in their characterization. In fact, the identification and determination of the components of the essential oil using a variety of techniques. We therefore thought that it was very appropriate to want to update the scientific knowledge regarding their indications, chemistry and toxicology thus making it possible to establish the links between these three concepts. We believe it's important to highlight to recent scientific studies regarding the utilization of essential oils. The aim of this chapter is to describe (i) the different extraction method, (ii) the chemical composition of essential oils, and (iii) the analytical techniques employed for the isolation and identification of phytoconstituents.

2. Techniques for obtaining essential oils

According to the definition provided by AFNOR, essential oils can be extracted either by cold expression (case of Hesperides) or by distillation which is available in hydro-distillation or steam entrainment. These three methods can be used in a continuous or discontinuous system, at atmospheric pressure, in overpressure or in depression [4]. These methods cannot be detailed without describing the processes taking place during the distillation and/or steam training:

2.1 The phenomena occurring during extraction

- First there is the actual extraction or hydro-diffusion step which consists of release of volatile compounds in the aqueous medium. This release is due to a phenomenon physical increase in the internal pressure of plant matter which has swollen by passive or osmotic water absorption, but also to a chemical phenomenon exerted by water [4, 5].
- Then comes the co-distillation of water and volatile elements [5, 6].
- Finally, the separation of the essential oil from the condensates involving the coalescence and settling [7].

2.1.1 Hydro-diffusion

This is under the influence of the osmotic exchanges that take place between the substrate plant and water phase, but also under the influence of physical forces [8].

Physical forces: When the plant mass is in a medium saturated with water, it follows that various hydraulic pressures build up. A plant particle submerged in water undergoes total hydraulic pressure, the components of which are as follows [9, 10]:

- Osmotic pressure
- Matrix pressure: between the particles and the water adsorbed on their surface

- Static pressure: exerted on the plant membranes, due to the conditions operative, it is zero at atmospheric pressure

- Gravity.

In addition to these different forces, there are different types of migration. Migrations of volatile compounds within the plant substrate

- Capillary diffusivity due to the porosity of the plant mass.

- Molecular diffusivity: the components of essential oils can migrate by simple molecular diffusion through plant tissues.

2.1.2 Co-distillation

At this stage, the gasoline is passed from the surface of the plant particle to the aqueous medium where it disperses. The entrainment of organic molecules during distillation is governed by two physical laws [11, 12].

- Dalton's law: the pressure of the vapor mixture is equal to the sum of the tension's vapors of the various constituents: PT = TH + TE.

- Raoul's law: the ratio of the quantities of products distilled simultaneously is function of the voltage and vapor densities at the distillation temperature chosen.

2.1.3 Coalescence and settling

$$\text{Mole H/Mole E} = \text{TH}$$

Isolation of volatile compounds largely depends on their solubility in water so that the distillate can be more or less rich in polar constituents, we then distinguish [13, 14]:

- part of the distilled oil is dissolved in water, this portion is of the order of 1%, rarely more than 2% and for some phenolic derivatives polar more than 5%.

- Another part is emulsified in water at the level of 10%.

- The last fraction is emulsified with water and organic molecules thirds playing the role of surfactants, it can exceed 10%.

2.2 Methods and equipment for obtaining essential oils

a. Extraction by entrainment with water vapor:

The vegetable mass is subjected to a stream of steam (without prior maceration), the vapor saturated with volatile components is condensed and then decanted.

b. Extraction by hydro-distillation:

The plant material is immersed in water, the whole is brought to the boil under pressure most often atmospheric.

c. Hydro-distillation under pressure:

It is strongly recommended for essential oils that are difficult to distill and/or with thermolabile compounds. Indeed, volatile compounds of high molecular mass like those of sandalwood, ginger and vetiver, cannot be pressure distilled atmospheric at acceptable temperature avoiding their degradation.

d. The heat pump system:

Based on the single or double effect still, the heat from the condenser is used to contribute to the formation of the vapor that will pass through the raw material. This is above all to save energy (60%) and cooling water (90%) [15].

e. Turbo-distillation:

This is an accelerated batch hydro-distillation, it is done under pressure atmospheric the only difference with conventional distillation is the presence of a turbine which shreds the plant material and agitates it. The latter increases the surface contact between the steam and the substrate and thus increases the yields energy and production. This device can be equipped with enrichment system vapors, most often it is a reflux system.

f. Microwave assisted distillation:

This is a laboratory process that has never been able to for technical reasons find its place in the industry. This is a particularly interesting technique for the gain of time it provides and by its performance.

g. Cold expression

Cold expression is reserved for the extraction of volatile compounds in the pericarps of Hesperides. This is a mechanical treatment that involves tearing the pericarps rich in secretory cells. The released gasoline is collected by a stream of water and receives all the usual product of entrainment with water vapor, hence the name of essential oil (AFNOR).

3. Biochemistry of essential oils

In the world of essential oils, terpenoids are by far the group of products most important natural, next to phenylpropanoids (C6-C3), C6-C1. The term terpene referred to the whole group in ancient literature but today it is limited to the designation of monoterpenoid hydrocarbons [16, 17]. Terpenoids are defined as substances composed of units of isoprene (2-methylbutadiene). This is not often present in essential oils and is not a synthetic intermediate either. But the 2-methylbutadiene backbone is easily recognizable in the structure of terpenoids. We distinguish between monoterpenes, and sesquiterpenes.

3.1 Terpenoids

3.1.1 Monoterpenoids

Geranyl pyrophosphate is the precursor of monoterpenoids it is formed by two five-carbon unit. Heterolysis of the bond between a pyrophosphate oxygen and distal carbon gives a cation (carbocation) of geranyl, the one that allows different pathways of monoterpenes biosynthesis. During the various biosynthesis described in the literature, other cations according to reactions under enzymatic control, which allows each plant according to its genetic material to produce specific terpenoids in kind or in quantity. Due to the high reactivity of the different cations observed and the diversity enzymatic, monoterpenes whether acyclic, monocyclic, or bicyclic exhibit a multiplicity of functionalization (**Figure 1**). We can then differentiate:

Aldehydes: Often acyclic such as geranial and citronellal

- Alcohols: Acyclic such as geraniol, linalool, and citronellol; monocyclic like menthol and α-terpineol, bicyclic like borneol and the fenchol
- Acyclic ketones like tagetone, monocyclic like menthone and carvone, bicyclics like fenchone, camphor, and thujone
- Esters especially of lynalyl acetates, citronellyl, menthyl, etc.
- Ethers such as eucalyptol (1.8-cineole)
- Peroxides such as ascaridole
- Phenols like thymol and carvacrol.

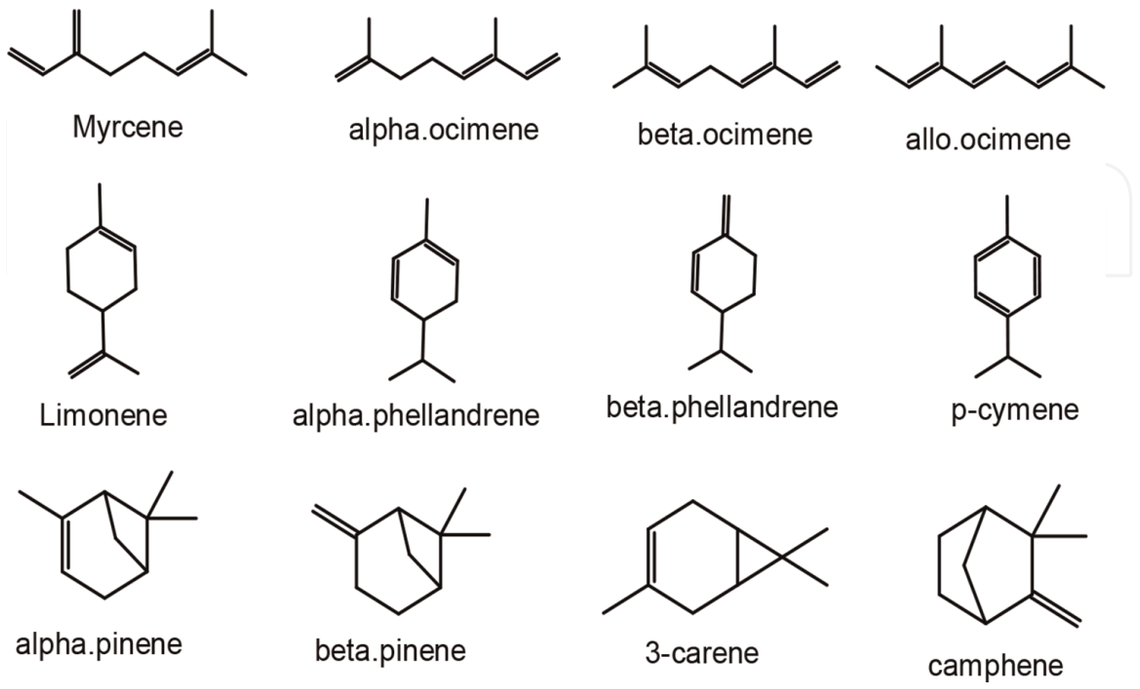

Figure 1.
structures of some terpenoids.

Many of these products can be artifacts formed from dehydration of alcohol. Their presence in essential oil could well be due to the process extraction. Thus, the para-cymene being among the most stable can be an artifact obtained by various reactions (cyclization and/or isomerization and/or oxidation) from a number important of products.

From this brief description of the nature of monoterpenoids, we can cite the most common plants whose essential oils contain these products. Myrcene is a very common compound in hops, among others, and in some spices. Ocimene and alloocimene and their isomers are present in almost all essential oils, the most common isomer of β-ocimene is limonene.

Citronellol is a dihydrogeraniol quite common in nature in various forms enantiomeric rose, geranium, and lemongrass have the richest rates. The characteristic smell of roses is due to a mixture of geraniol, nerol, citronellol, and 2-phenylethanol. It should be noted that in essential oils there are also esters of these alcohols (**Figures 2** and **3**).

3.1.2 Sesquiterpenoids

Sesquiterpenoids contain 15 carbon atoms, which gives a high boiling point and therefore lower volatility. So, they will be a little less numerous in an essential oil and they will only rarely be responsible of its smell. In the same way as the geraniol, precursor of all the monoterpenoids, the farnesol is that of all sesquiterpenoids. Condensation between a pyrophosphate isopentenyl and geranyl pyrophosphate leads to farnesyl pyrophosphate (**Figure 4**).

Dehydration of farnesol gives a farnesyl cation which plays a role in formation of sesquiterpenes, similar to the role of the cation of geranyl for the synthesis of

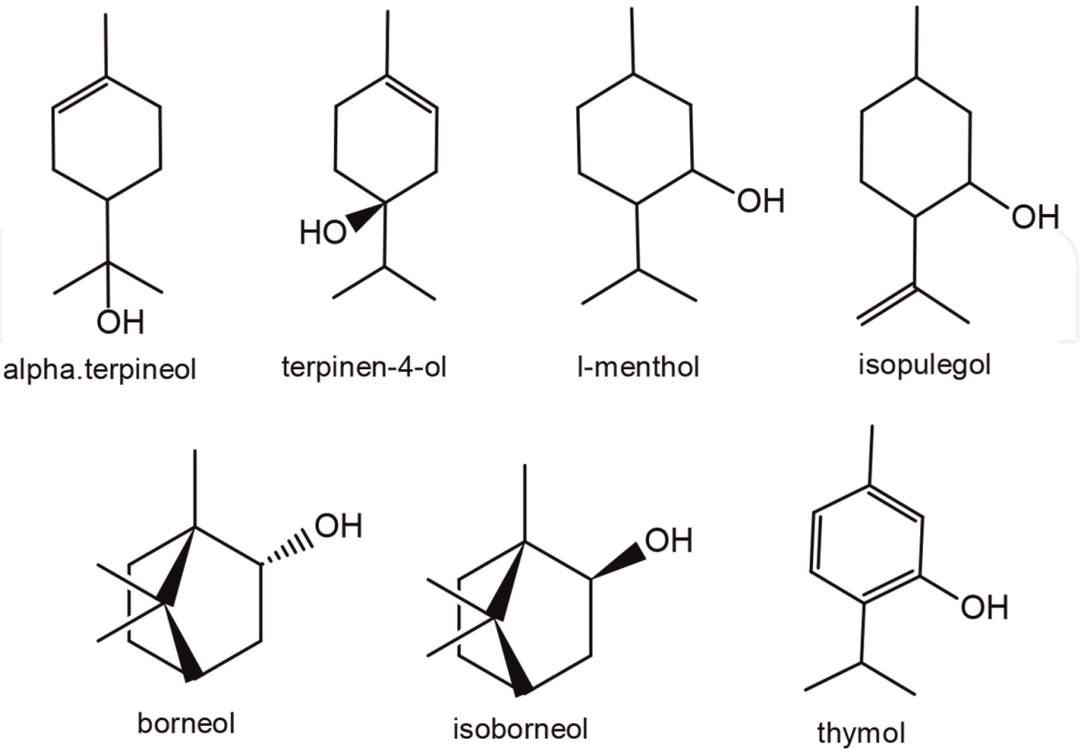

Figure 2.
Examples of cyclic alcohol-functional monoterpenoids.

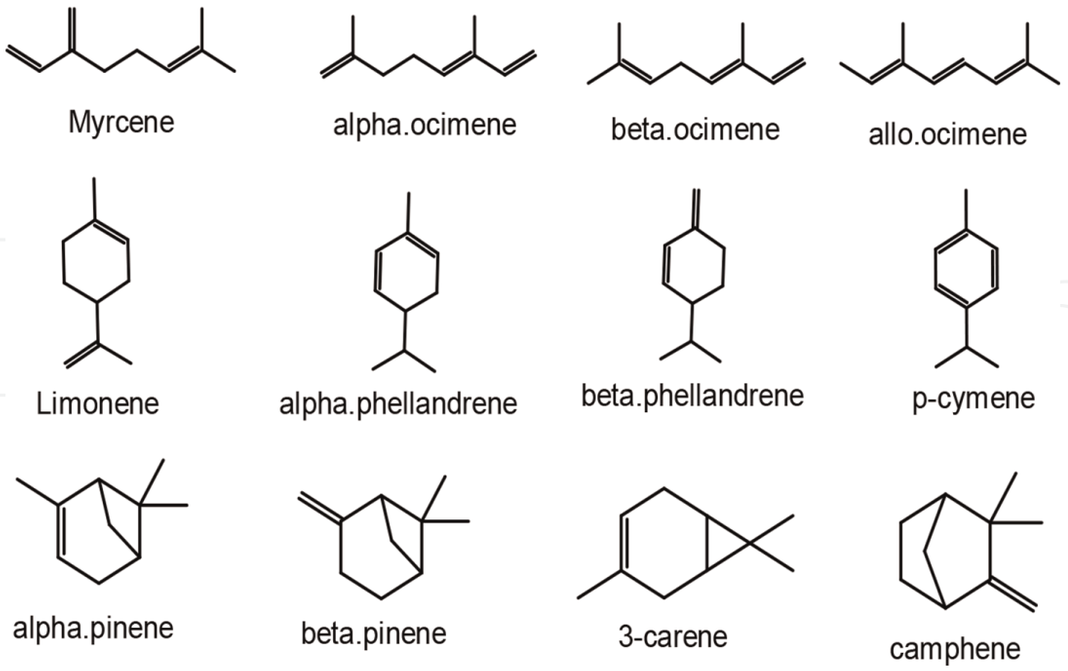

Figure 3.
Commonly encountered monoterpenoids.

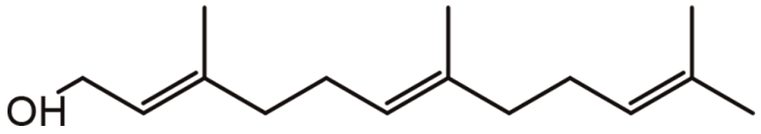

Figure 4.
Structure of farnesol.

monoterpenoids. A synthesis from farnesyl pyrophosphate allows a number greater potential of cyclic structures compared to a synthesis from the geranyl pyrophosphate. This is explained by the presence of three double bonds on the farnesyl molecule as well as by a greater variation in structure because there is a number significant rearrangements, oxidation of eliminations possible [18].

3.2 Shikimic acid derivatives: (phenylpropanoides, C6-C1, C6-C3)

Shikimic acid is a synthetic intermediate for plants, a precursor of lignin flavonoids [16]. Lignin is a structural constituent of plants, main component of wood. Flavonoids import to plants as agents' antioxidants and protection against ultraviolet radiation, they also give their colors to plants. With regard to the products found in essential oils, the key metabolite from shikimic acid is chorismic acid which can borrow several biosynthetic pathways. But the path that interests us the most is that of pre-phenic acid obtained by Claisen-type peri-cyclic rearrangement of the acid chorismic. This is the pathway which leads via phenylpyruvate to alanine and tyrosine to using amino transferase among others. This rearrangement is under the influence of chorismate-mutase. Phenylalanine allows us after reduction and elimination of nitrogen by phenyl-ammonia-lyase to go to cinnamic acid which, by hydroxylation of the nucleus aromatic, gives us o-coumaric acid and p-coumaric acid. From these. The latter are caffeic acid, then ferulic acid and then methylene caffeic acid (**Figure 5**).

Thus, in an essential oil we can find:

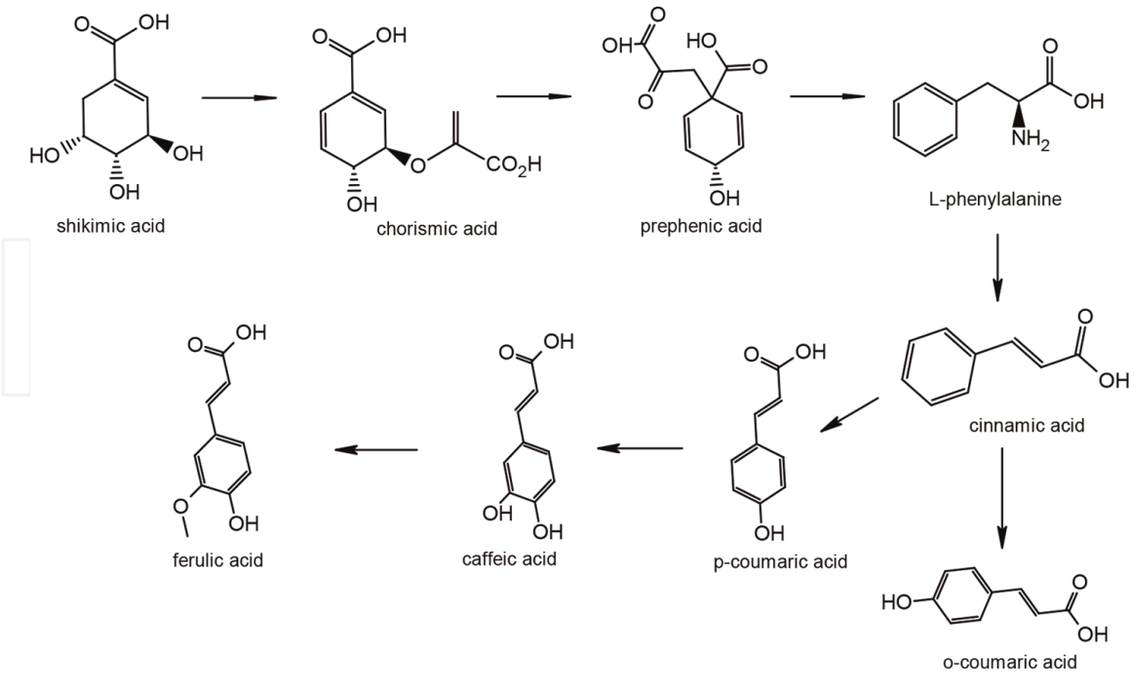

Figure 5.
Shikimic acid derivatives.

3.2.1 Benzoic acid derivatives

These are mainly C6-C1 phenol acids obtained by hydroxylation of the acid benzoic which itself comes from the aromatization of shikimic acid but without the addition of the three carbons of phosphoenol-pyruvate [19]. These derivatives also exist in free form than in combination with the ester or heterosides. In general, there are two categories: the pure benzyl acid-alcohols and the aldehydes corresponding to them and obtained by oxidative cleavage of the side chain (**Figure 6**).

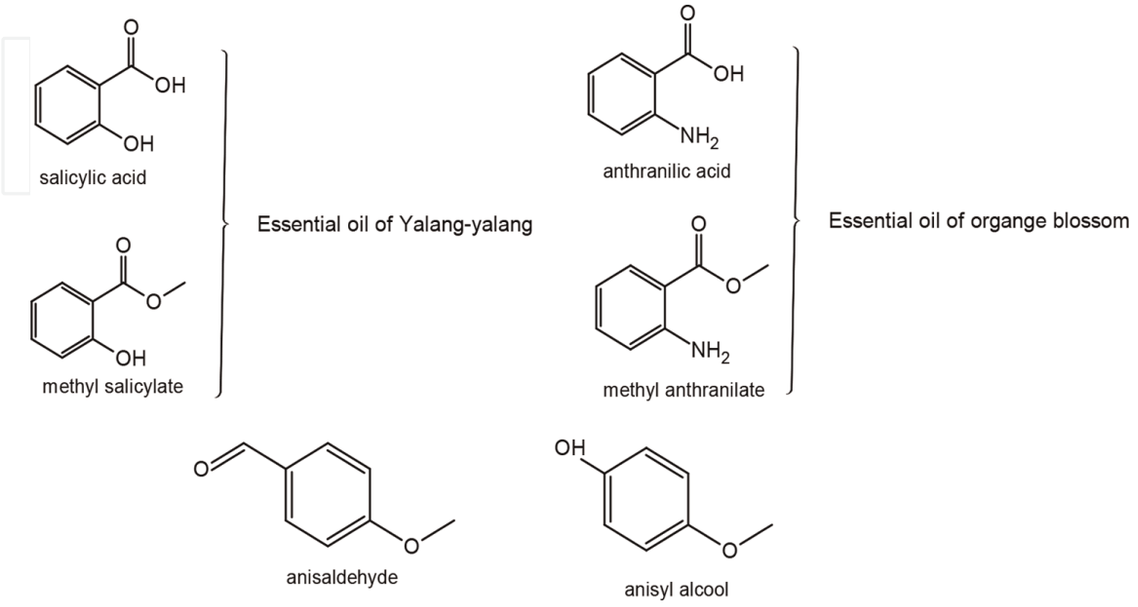

Figure 6.
Benzoic acid derivatives.

3.2.2 Ferulic acid derivatives

Ferulic acid itself is a derivative of benzoic acid, reducing its chain lateral leads to a very important family of essential oil components [20]. The key components are eugenol found in the essential oils of camphor tree, cinnamon, jasmine, basil among others, and isoeugenol present in essences of cassia, cloves, nutmeg. Eugenol methyl ether (methyl eugenol) is very common in nature, but however, raises questions about the toxicological safety of the use of oils essential oil comprising this compound, we can cite for example the essential oil of *Melaleuca alternifolia* which contains 98% methyleugenol (**Figure 7**).

3.2.3 Cinnamic acid derivatives

These are C6-C3 compounds most often designated by the term "Phenylpropanes" are the most numerous metabolites of shikimic acid and are universally distributed free or combined (esters, amides, glucosides). The pattern C6-C3 can polymerize to give lignin or cyclize to give coumarins or further lengthen its side chain to end up with flavonoids.

Cinnamic acid is obtained from chorismate via a phenylalanine which undergoes for this a stereospecific elimination of ammonia this reaction requires a phenyl ammonia-lyase. Most often the cinnamic acids encountered are esters or aldehydes. Cinnamic acid is present without modification in the essential oils of Cassia and Styrax. The corresponding aldehyde is in the essences of camphor, Cinnamyl alcohol and its esters in Daffodils and Lilacs. Lactonization of o-coumaric acid gives the nucleus of coumarins such as bergapten, oxygenated derivatives of the essential oils of Bergamot and Petitgrain. Still from cinnamic acid one can obtain estragol (methylchavicol) and by methylation of the phenol ring and reduction of the carboxylic acid function to alcohol function and elimination of the latter one obtains anethol. Estragol is naturally present in sage, rosemary, basil, and honeysuckle. Dillol is present in Apiaceae (fennel, anise, coriander) in lavender and Ylang-Ylang [20] (**Figure 8**).

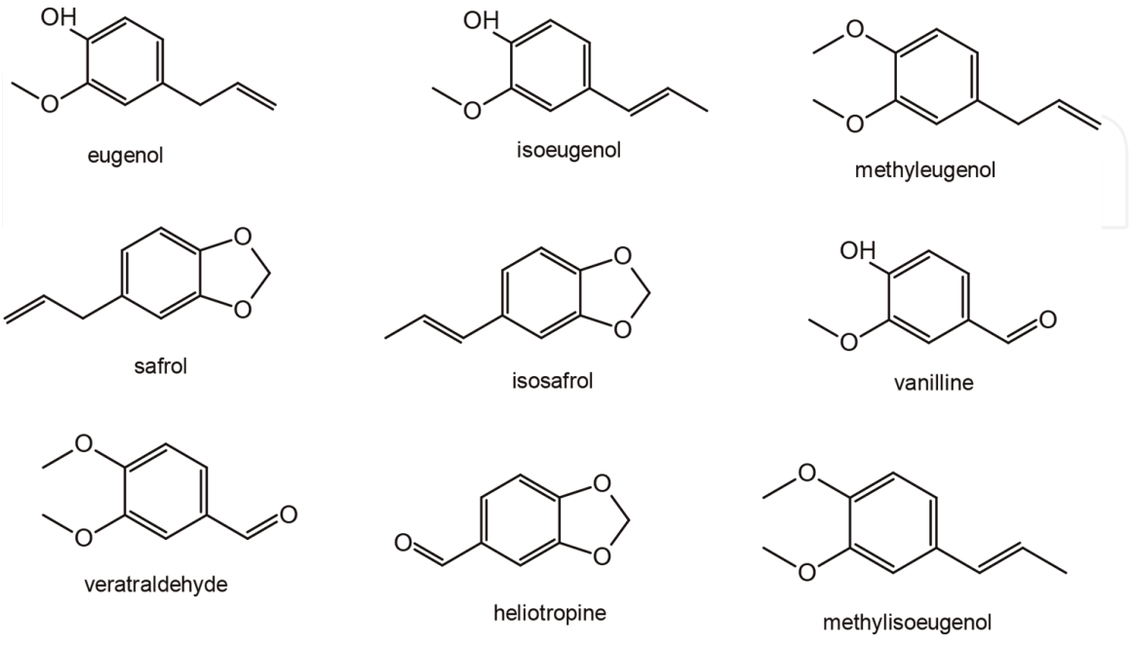

Figure 7.
Derivatives of ferulic acid.

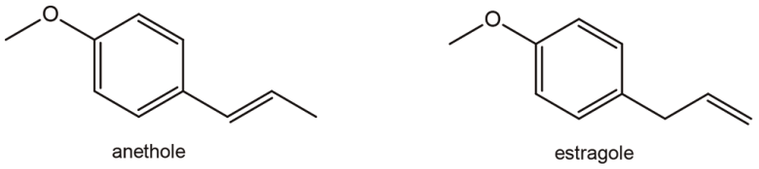

Figure 8.
Structures of anethole and estragole.

4. Oil analysis techniques essential

For the analysis of essential oils, or at least for their chemical analysis, of many techniques have emerged during the second half of the previous century. We can then distinguish the techniques of separation of chemical components from the techniques of detection by proposing the following classification:

- Chromatographic separation techniques: CCM, HPLC, GIC, SFC
- Analysis techniques without separation/fragmentation: UV, IR, MS
- Coupling techniques: CPG-MS, CPG-UV, HPLC-CPG, HPLC-MS, CPG-IRTF, SFC-CPG

4.1 Chromatographic separation techniques

4.1.1 TLC or thin layer chromatography

This is the first and most widely used chromatographic technique, it provides simple information about the physicochemical characteristics of the components of a mixed. Many pharmacopoeias advocate the use of this technique given its simplicity for the characterization of essential oils in routine testing.

The foundations of TLC applied to essential oils were established by Stahl in 1969 [21, 22] and by Geiss in 1987 [20] who studied a significant number of metabolites secondary aromatic plants. Then in 2003, Shema and Fried [21] published the "CCM Handbook." Other approaches to CCM gave rise to the high CCM performance followed by overpressure TLC and phase rotation chromatography (RPC) which are forced flow techniques.

However, this technique should be indicated for the rapid determination of the different pathways and/or chemical families present in a given essential oil.

4.1.2 Gas chromatography

This is an analytical chemistry technique that separates compounds volatile or volatilizable without degradation (non-thermolabile). His power of separation exceeds that of all other techniques, at least for essential oils.

4.1.2.1 Principle and apparatus

This chromatographic method makes it possible to separate the compounds either by partition or by adsorption. This is a differential migration of the constituents of the

mixture to be analyzed at through a chosen substrate. The best illustration of the evolution of CPG (**Figure 9**) applied to essential oils is found in the many works carried out for the determination of oil components essential of rue Ruta graveolens. Thus in 1961, only eight components were identified by Bruno during the first CPG of this oil; then in 1964, this number rose to 20 with the use of a Perkin-Elmer type chromatograph equipped with a thermal conductivity. Then with the introduction of programming systems temperature we went to 80 components and using a capillary column at high resolution with a flame ionization detector 150 components are obtained (1981) [23, 24].

4.1.2.2 The fast and ultra-fast GC

It was necessary, as part of a routine examination, to develop these two variants of the CPG. So, to significantly accelerate a CPG we act at the following levels.

- The dimensions of the column are reduced: the internal diameter and the length.
- The coatings are reduced: a thinner stationary phase.
- The flow of carrier gas is increased.
- Temperature transitions are accelerated during cycles.

Thus, the separation speed is considerably increased. This CPG technique rapid or ultrafast has been tested on lime essential oil with similar parameters to what was mentioned previously, that is to say a capillary column 5 m long, of 50 μm internal diameter, 0.05 μm coating, and a gas flow rate of 120 cm/min [25]. We arrive at a chromatography carried out in 1990s, i.e., 33 times faster than a traditional GIC, but generally a fast GIC lasts 13 min and instead of 60 for a classic GIC.

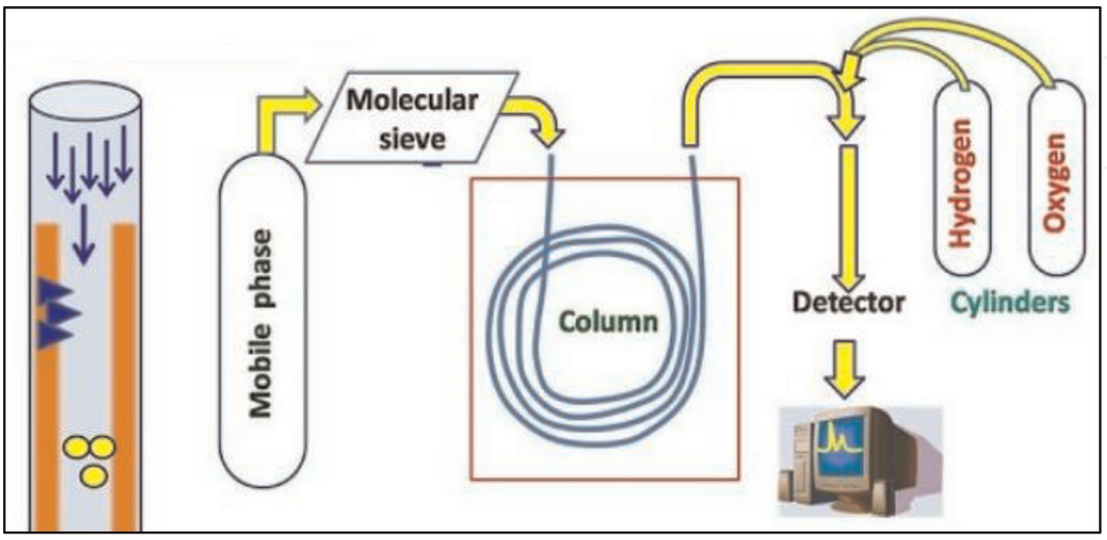

Figure 9.
Apparatus for a GC.

We can try to summarize the differences between the different types of chromatography in the gas phase by the following **Table 1**:

4.1.2.3 Chiral GC

This is an interesting evolution of CPG and consists of an enantioselectivity of the capillary column. The interest is even greater than essential oils are very often rich in mixture, racemic or not (depending on the botanical species and the chemotype), two enantiomers. This chiral chromatography then makes it possible to separate into using various stationary phases such as diamide phases which interact with chemical compounds through hydrogen bonds or as complex phases metal with low thermal stability. But most often we use derivative phases very selective cyclodextrin and used since their invention in particular in the determination of the enantiomeric composition of monoterpenoids and sesquiterpenoids of many essential oils [26].

4.1.2.4 The two-dimensional CPG CPGx CPG

This is a gas phase separation technique of course, in which all the compounds eluted from a first column are, directly after the latter, subjected to separation in a second column of different selectivity (Ecole supérieure de physics and industrial chemistry of Paris). The two columns are connected in series to the by means of a modulator which samples the effluent from the first column and transfers it, with or without concentrating it towards the second column. So the latter must be able to separate the different constituents in a time shorter than the duration of the modulation, and this is why a second column of the fast or ultra-fast CPG type is used.

The elution peak from column 1 is a first detector and each fraction is focused and continuously injected into column 2. In general, the detector1 and valve assembly constitutes a modulator. By joining the chromatograms of each column, one obtains a two-dimensional retention plane. This technique can be complicated by putting a first non-chiral column then a second chiral [27]. In the world of essential oils, this method has proven particularly effective in the study of the EO of Bergamot citrus bergamia in which only the L (−) enantiomers of linalool and linalyl acetate. So, this type of chromatography allows to update the adulterations of this essential oil.

		GIC conventional	GIC fast	GIC ultra-fast
Dimensions of the column	L	30 m	10 m	10–15 m
	Θ	0.25 mm	0.1 mm	0.1 mm
	Coating	0.25 μm	0.1 μm	0.1 μm
Temperature programming		50–350°C 3°C/min	50–350°C 14°C/min	45–325°C 45–200°C/min
Carrier gas Flow (flow)		H2 36 cm/s	H2 57 cm/s	H2 120 cm/s
Injection frequency (sampling or analysis)		10 HZ	20–50 HZ	50–250 HZ

Table 1.
The different types of gas chromatography.

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4.1.3 Liquid chromatography

Due to the preponderant place of CPG in the analysis of essential oils, the liquid chromatography is most often only used for preparatory steps or semi-preparatory. Or at the limit for the individual isolation of a compound for its structural study.

4.1.3.1 HPLC or high-performance liquid chromatography

4.1.3.1.1 Principle and apparatus

The sample to be analyzed is pushed by a mobile phase into a packed column a stationary phase of fine particle size. The flow rate of the mobile phase is high which leads to an increase in pressure in the system. This high flow decreases the time required to separate the components along the stationary phase. The thin particle size of the stationary phase allows better separation of the components. Indeed, for the same volume of stationary phase, the exchange surface increases if the "grains" that compose it are of smaller diameter. The peaks obtained are therefore narrower the resolution is improved (the peaks are well separated, we can therefore differentiate them), the detection threshold is also lower (narrow and high peaks are easier to isolate from the background noise as wide and low peaks). The combination of these attributes speed and high-resolution leads to the term "high performance (**Figure 10**)."

The solvents used are miscible combinations of water and various liquids organic (alcohols, acetonitrile, dichloromethane, etc.).

Often the composition of the mobile phase is changed during analysis, this is the so-called "gradient" or "graduated elution" mode (in opposition to the "isocratic" mode, for which the composition of the mobile phase remains the same throughout the analysis). Through example, on an apolar column, using a water/methanol mixture as phase mobile, the more hydrophobic components are eluted with a high concentration of methanol, while the more hydrophilic components are preferentially eluted with a low methanol concentration. Depending on the nature of the stationary phase, we will start with a high methanol concentration or vice versa [28].

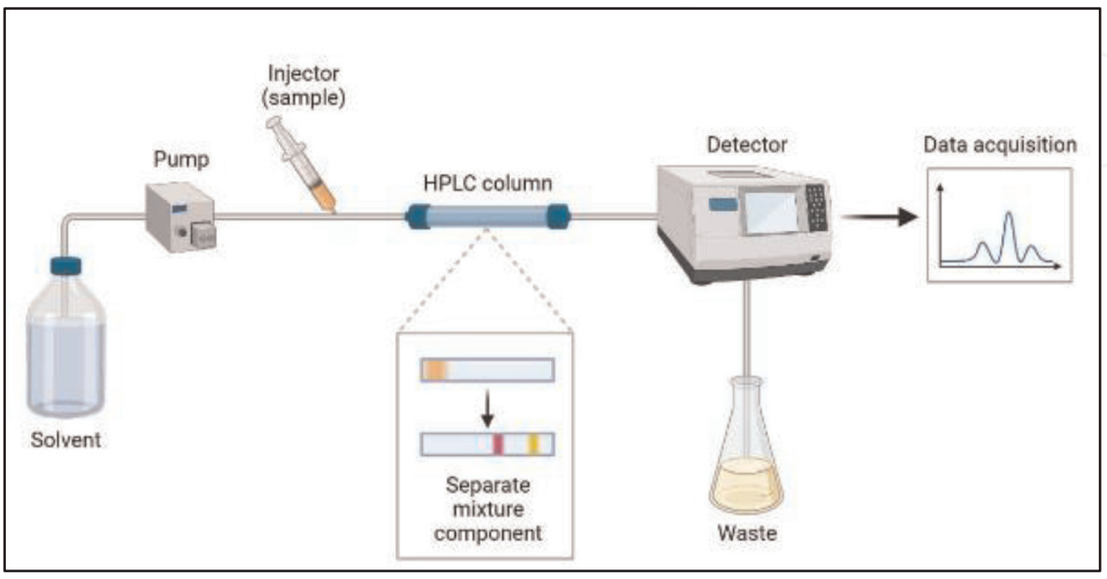

Figure 10.
Assembly for an HPLC.

4.1.3.1.2 HPLC and essential oils

The use of HPLC in the field of essential oils has been abandoned in favor of CPG with convincing results. However, HPLC analysis has a number of advantages, especially when looking for thermolabile compounds that is to say difficult to be analyzed by CPG. The main limitation of this technique lies in the methodology analysis of terpenoids that require a retention factor in an interval narrow. Hence the most common use of silica gel column, n-pentane as phase mobile, low temperature (−15°C in general) and a UV detection system at 220 nm. Under these conditions, the majority of mono and sesquiterpenoid hydrocarbons can be separated and analyzed [28].

Other chromatographic conditions were tested for the analysis of HE in acting on:

- The nature of the stationary phase (theory of complexation chromatography film)
- The temperature of the chromatography
- Setting up an acetonitrile-water gradient in the column

But despite everything, the separating power of HPLC is only remarkable for sesquiterpenoids and diterpenoids, with CPG doing better for monoterpenoids. He is to note however that researchers have succeeded in an enantiomeric separation by HPLC sesquiterpenes from an essential oil using a chiral stationary phase (chiralcel®) [28].

4.1.4 Supercritical fluid chromatography

The CFS uses as mobile phase a fluid or mixture of fluid brought to a point called critical by pressure and temperature control, i.e., at the point where the substance chemical, here the mobile phase, is in a hybrid state between a gas and a liquid. The diffusion coefficient of the fluid is then twice as important while the viscosity is two times less than the corresponding liquid, with a greater density than gas obviously. Most often carbon dioxide (CO_2) is used as the mobile phase, but since its polarity is low (comparable to hexane), it is incorporated in a small quantity polar solvent (methanol, ethanol, water) to be able to elute compounds endowed with a certain polarity [29].

This method also makes it possible to use a range of detection systems that are wider than that of HPLC. Apart from the use of this type of chromatography as a technique analysis, it is interesting to note that it can also be used for the extraction of oils essential. Indeed, several industries use it, most often when it is an EO with thermolabile compounds. We can then collect the majority of the components odorous, polar or non-polar, while avoiding the disadvantages of hydro-distillation or hydro-diffusion. Drawbacks such as hydrolysis reactions, solubilization of a non-negligible part of volatile products in water or the thermodegradation of these. There is no thermodegradation because, for the example of CO_2, the supercritical state is at 31.1°C and 74 bars. The use of this technique for the analysis of essential oils gives rise to a particular enthusiasm among researchers, especially over the past 10 years. We can then cite the establishment of a study protocol for the essential oil of Salvia angustifolia by CFSC [30].

4.1.5 Counter-current chromatography

Also called centrifugal partition chromatography, it consists of a liquid-liquid chromatography using no solid support but two non-liquid miscible (made from two or more solvents). The two phases (mobile and stationary) are liquids which prevents irreversible adsorption phenomena of the mobile phase.

This technique has two variants: 1. HSCCC: high speed counter current chromatography 2. DCCC: drop counter current chromatography.

4.1.5.1 HSCCC

Also called—rotation locular counter current chromatography‖ and developed by Rikakikai, she uses a device made up of sixteen glass tubes, communicating with each other and arranged concentrically. Inside these tubes is the stationary phase which, by rotation and centrifugal force remains fixed in the tubes. It is crossed by a phase mobile under pressure (**Figure 11**).

This is a technique successfully used for the analysis of natural products such as EO [31, 32].

4.1.5.2 Countercurrent droplet chromatography:

Moderately effective, it allows the separation of essential oils into fractions and rarely in pure compounds. Developed by Tanimura in 1970, it consists of a set of 300–600 glass tubes which are connected to each other with tubular connectors in Teflon and filled with the stationary phase (liquid). This is crossed by droplets mobile phase. However, since each compound of the essential oil has a partition coefficient which is specific to it (for a given couple of solvents), there is separation as the passages in the different tubes. It should be noted that for the fragmentation of oils essential you need a water-free solvent system [33] (**Figure 12**).

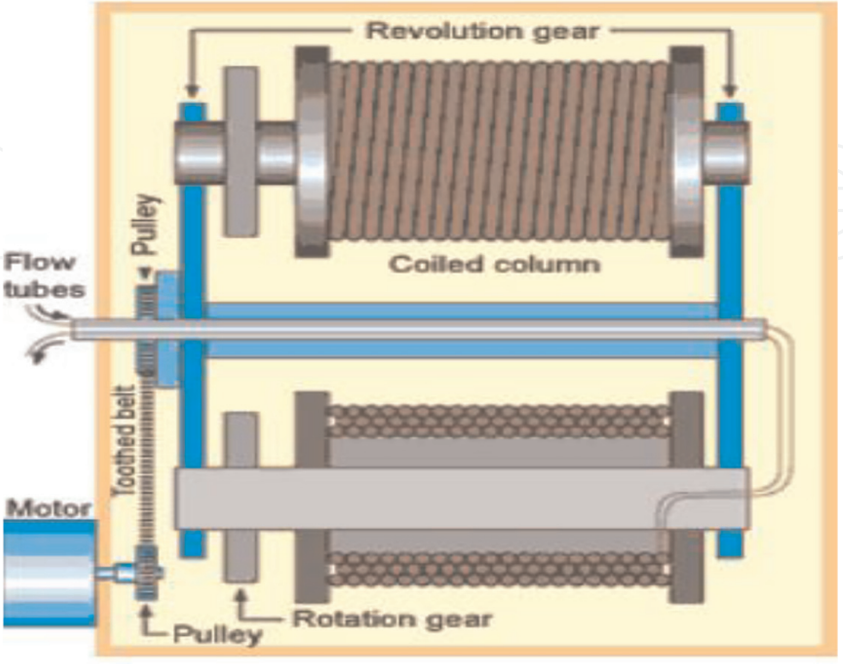

Figure 11.
Composition of an HSCCC device.

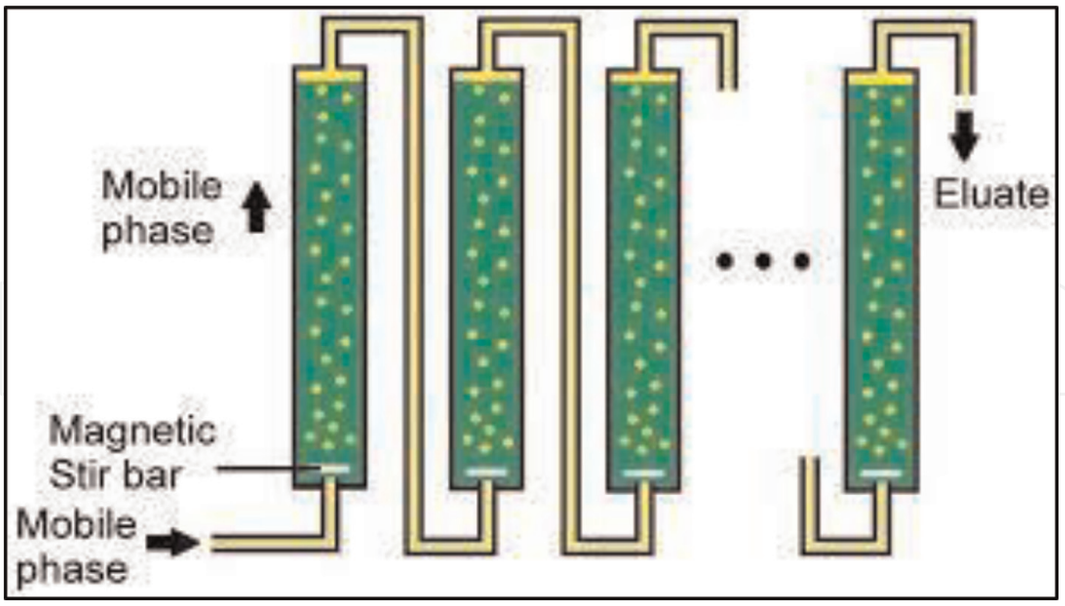

Figure 12.
Migration of the mobile phase of a DCCC device.

4.2 Analysis techniques without fragmentation

4.2.1 UV spectroscopy

4.2.1.1 Principle and apparatus

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrometry is a spectroscopy technique involving photons whose wavelengths are in the range of ultraviolet (200–400 nm), visible, and up to near infrared (750−1400 nm). Subjected to radiation in this range of wavelengths, the molecules undergo an electronic transition. This technique is complementary to spectroscopy fluorescence in the sense that fluorescence involves transitions from the excited state down to the ground state while absorption spectroscopy deals with transitions between ground state and excited state.

4.2.1.2 Application to essential oils

The use of this technique for the analysis of essential oils is quite limited since it is not possible to obtain information on a chemical compound individually. But nevertheless, for the research of furanocoumarins, responsible for photo dermatoses in hesperidia essential oils, this is a method to privilege. It is recommended by the European Pharmacopoeia for the analysis of oil essential lime.

4.2.2 Infrared spectroscopy

4.2.2.1 Principle and apparatus

Infrared spectroscopy exploits the fact that molecules have frequencies specific for which they rotate or vibrate in correspondence with levels discrete energy (vibratory modes). These resonant frequencies are determined by the forms surfaces of potential

energy, atomic masses and by vibronic coupling associate. For a vibrational mode in a molecule to be active in the infrared, it must be associated with changes in the permanent dipole. In particular, in the approximations of Born-Oppenheimer and harmonic, when the molecular Hamiltonian corresponding to the electronic ground state can be approximated by a harmonic oscillator at the neighborhood of the equilibrium molecular geometry, the resonance frequencies are determined by the normal modes corresponding to the potential energy surface of the state fundamental molecular electronics. However, the resonant frequencies can be in a first approach related to the strength of the bond, and to the atomic masses of termination. So, the frequency of the vibrations can be associated with a particular bond. Diatomic molecules have only one bond, which can be stretched. Molecules the more complex ones have a lot of bonds, and the vibrations can be conjugated, which leads to infrared absorptions at characteristic frequencies which can be related to chemical groups. For example, the atoms of a CH2 group, which we find commonly found in organic compounds can vibrate in six different ways: symmetrical and antisymmetric stretching, scissoring, rocking (rocking), wagging, and twisting [34].

4.2.2.2 Application to essential oils

Despite the low sensitivity and selectivity of this method in the case of mixtures with many components, in 1971 two hundred essential oils were analyzed for the publication of "Infrared analysis of essential oils" which is the reference of the genre in this domain [35]. On its own, conventional infrared spectroscopy does not allow measurements quantitative. For classic IR, new techniques such as spectroscopy attenuated reflection infrared or NIR-FT Raman spectroscopy, open a new method of analysis of essential oils since it is possible to identify the components of the oils essential using spectrographic references of pure chemical compounds. The definite advantage of these techniques lies in the ease of quality control of EO with the bonus of being able to quantify and analyze the components of an EO In-Situ, that is to say on living plant material (without prior isolation).

4.2.3 Mass spectrometry

4.2.3.1 Principle and apparatus

Mass spectrometry (MS) is a physical technique analysis to detect and identify molecules of interest by measuring their mass and characterize their chemical structure. Its principle lies in the gas phase separation of charged molecules (ions) in function of their mass/charge ratio (m/z).The mass spectrometer is often coupled with a chromatography system in gas phase, and this association, of a separating method and of an identification, allows the study of complex mixtures in trace amounts (a few nanograms of mixture).

The principle of mass spectrometry is as follows:

An organic compound introduced into the mass spectrometer is ionized by electronic bombardment at 70 eV. The ion thus obtained, called the molecular ion, allows the determination of the molar mass of the compound. There may be breaks in the chemical bonds within the molecular ion, forming thus characteristic fragment ions since this possible dissociation does not take place at the chance but according to well-defined mechanisms.

These fragment ions are then separated according to their mass/charge ratio by the application of a magnetic and/or electric field, then collected by a detector. All these fragment ions constitute the mass spectrum, the reading of which allows identification of the molecular structure [36].

4.2.3.2 Application to essential oils

This is the flagship technique for the determination of the molecular structures of isolated compounds. An essential oil mass spectrum always shows ions molecules corresponding to terpenoids with an m/z ratio of 136, 148, 152, and 154. And by focusing techniques without prior separation, the metastable ions are observed compounds such as anethol, fenchone, borneol, and cineol. And a variation is by the direct introduction of a part of the plant with EO (0.1–0.2 mg) which releases by heating the classically detected volatile compounds [37].

4.2.4 ^{13}C NMR spectroscopy

4.2.4.1 Principle and apparatus

NMR spectroscopy is based on the detection of the resonance phenomenon magnetic which occurs when atomic nuclei of non-zero spin are placed in a generally uniform external magnetic field and that they are excited by radio-frequency radiation tuned to the energy differences between different possible states of nuclear spin.

The resonant frequency $\nu 0$ is a first approximation directly proportional to the applied field B0: $\nu 0 = \gamma B0$, where $\gamma = 2\pi\gamma$ is the gyromagnetic ratio.

The fact that each isotope has a unique gyromagnetic ratio allows the NMR technique to be able to be tuned to a element. Just adjust the frequency of excitation and observation on the target nucleus.

The resonance frequency of nuclei also depends on their environment, the spins being in interaction with it. These interactions are called internal interactions by opposition to the external interactions of the spins with the external magnetic field and the radiofrequency radiation. These intra- or intermolecular interactions can be magnetic as is the case for chemical shift and dipole couplings, still or electric, which is the case with the dipole interaction. Interpretation and measurement of these interactions provide valuable information on:

- the nature and number of atoms close to the nuclei studied
- chemical bond
- molecular conformation
- interatomic distances
- molecular mobility

4.2.4.2 NMR and direct analysis of essential oils

In general, it allows the determination of the molecular structures of isolated compounds; but nevertheless, for the study of EOs and complex mixtures it presents a

certain number of advantages in the presence of low volatile or unstable compounds thermodynamically. It has been established since the 1980s that it is possible to determine the constituents of an essential oil by comparing with spectra of pure products [23] (**Figure 13**).

Celery, or rather its essential oil, is extremely rich in limonene, hence a flagrant concordance between the two spectra. With an adequate computer system and a good database (bank of spectra), it is nowadays very easy to determine the composition of an EO by this NMR [38].

4.3 Analysis techniques with coupling

The advantage of a chain coupling of a chromatographic interface with a spectrometer is the ability to analyze the individual spectrum of a compound.

4.3.1 GC and mass spectrometry

This is the most widely used technique for EO analysis due in large part to the ease of handling efficient separation and detection systems, with a relatively low cost [39]. The first CPG-mass analysis dates from 1963.

For the analysis of essential oils, the most common equipment consists of a CPG capillary with an electron ionization quadrupole mass spectrometer. It is very easy to find mass spectrum databases due to very frequent use and worldwide of this technique.

For example, NIST/EPA/NIH 2005. WILEY REGISTERY 2006 and MASS FINDER 2007.

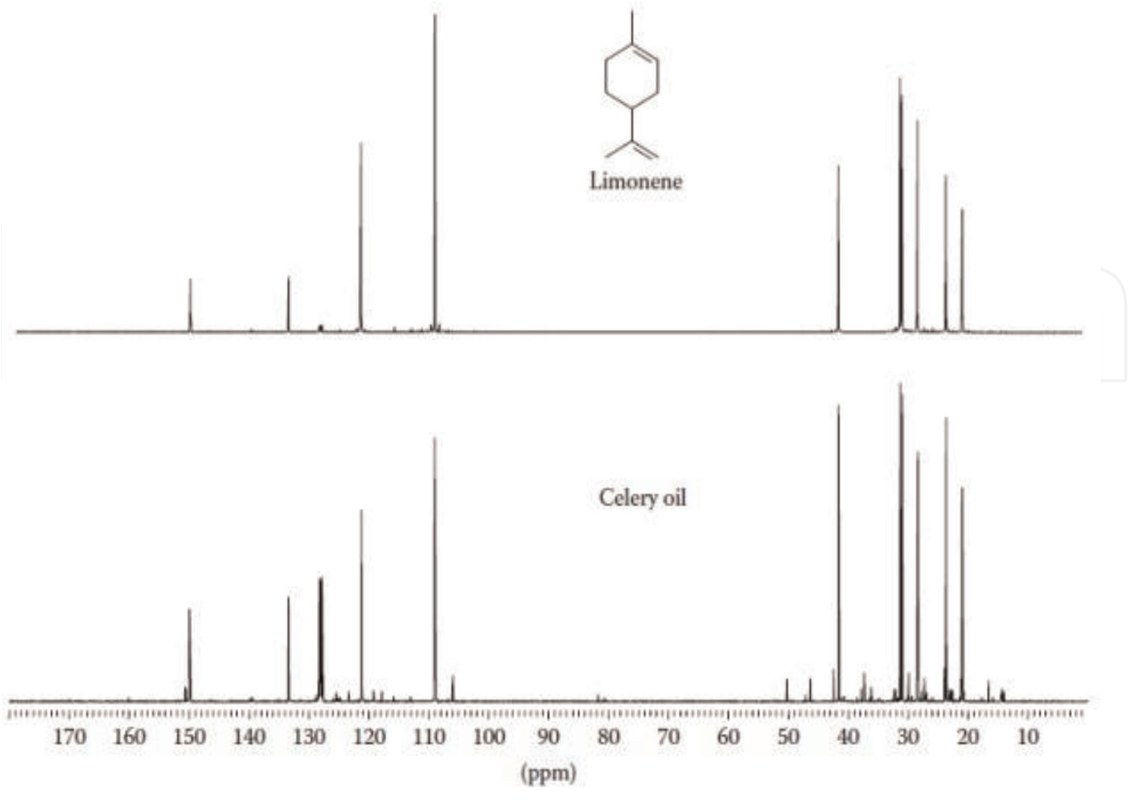

Figure 13.
Similarity between the ^{13}C NMR spectra of celery and limonene.

For the ionization of the compounds of essential oils, we can use two processes, electronic impact or chemical ionization which struggles to ionize alcohols and terpenic esters.

4.3.2 CPG and Fournier transform infrared

This is a complementary method to CPG-mass insofar as by TF infrared spectrometry distinguishes isomers of compounds eluted by GC not observable with mass spectrometry [40].

4.3.3 GPC and ultraviolet spectroscopy

The serial connection of the two devices, allows in the case of the use of a diode array detector, to detect and sometimes identify a certain number terpenoid hydrocarbon [41].

4.3.4 GPC and atomic emission spectrometry

This is only a complementary technique to CPG-Masse and CPG-FTIR.

4.3.5 GPC and isotope ratio mass spectrometry

A very interesting technique in the analysis of essential oils. It consists of the determination of the isotopic composition of the compounds eluted by carrying out combustion of these. We can then calculate the $^{13}C/^{12}C$ ratios: $^{18}O/^{16}O$ and $^{2}H/^{3}H$. This is the most sophisticated technique for judging the authenticity of oils essential.

4.3.6 HPLC-CPG

It is very simply a matter of putting an HPLC column and a CPG clone end to end. We can then have a modulation system to be able to choose the eluted fractions by HPLC and which it is desired to undergo CPG. The main thing is to choose a phase mobile volatilizable HPLC. A good separation of the esters, alcohols, and carbonyls of the compounds is then obtained from EO [42] (**Figure 14**).

4.3.7 HPLC-Mass and HPLC-NMR

The use of these two techniques is not widespread due to the relative HPLC ineffectiveness with respect to essential oils (see paragraph HPLC).

4.3.8 Extraction in supercritical fluid coupled to the CPG

It should be noted here that the supercritical fluid extraction is not a chromatographic technique since it involves extracting from the plant, at a critical point pressure and temperature specific to each chemical compound, which is then directly introduced into the chromatographic column, and which can therefore be identified. This is a fairly common technique in the world of essential oils since a many researchers use it for the analysis of herbal drugs such as Rosemary [41], *Thymus vulgaris* [42] orange, or cedarwood.

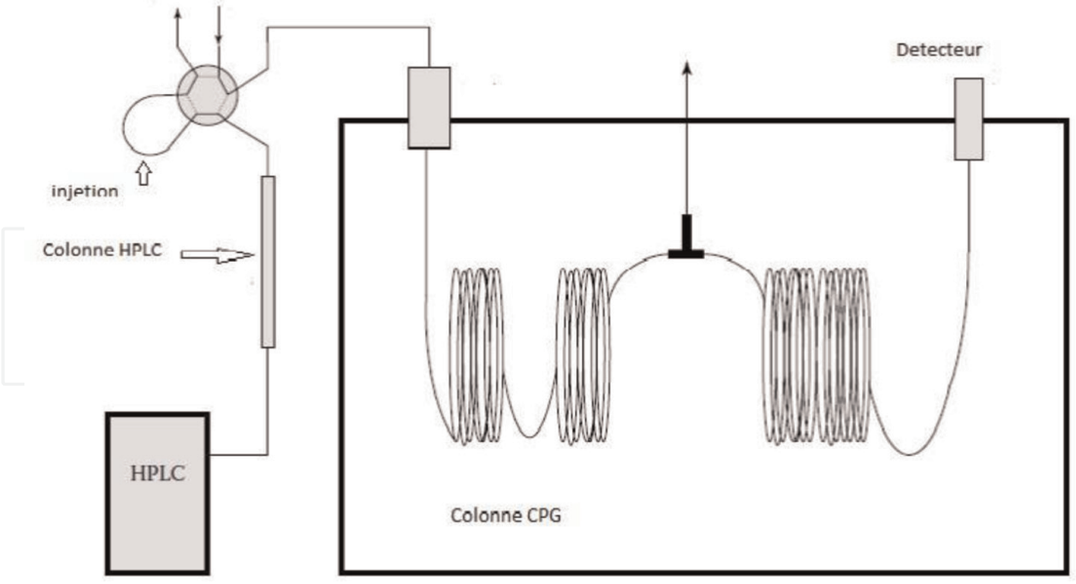

Figure 14.
HPLC-CPG coupling.

4.3.9 Super critical fluid chromatography (SFC) coupled with CPG

To be distinguished from the previous technique by the fact that the substance injected is oil essential and not the plant drug. In general, this is a common technique, used among other things for the analysis of the EO of sweet orange. Three hydrocarbon fractions are then obtained: aldehydes, alcohols, and esters [42].

4.3.10 SFC-Masse and SFC-FTIR

These are techniques based on the separating power of chromatography in supercritical fluid and on the complementarity between infrared spectrometry and mass spectrometry. They were applied during the characterization studies of Hops EO [43].

5. Conclusions

The chromatographic and the spectroscopic techniques fully changed the chemical analysis of the essential oils. The chemical composition of the essential oils was studied with the help of IR-spectroscopy, UV-Vis spectroscopy, gas chromatography, NMR spectroscopy. The enhanced demand for the essential oil in various fields of life provoked us to access the reliable methods for the essential oil analysis, and the techniques used are the GC-MS and GC analyses.

The characterization of the essential oil was carried out by using the gas chromatography. The compounds that are present in the essential oil was confirmed by using the GC and GC-MS analysis. The storage and handling of the essential oil also affect its yield and quality, ad essential oil was deposited in the oil glands that are present in the organization of the plant material. Essential oils are the natural volatile compounds having loveable odor. The essential oils are isolated mostly from the hydro-distillation method which is more suitable for this process and easy to carry. Whole parts of the

plants are used for the extraction of plants. Steam distillation method is expensive than the hydro-distillation, so it is less preferred. Essential oils have good medicinal applications and used in the treatment of different diseases including the infectious diseases, depression, anxiety, act as the antifungal, antimicrobial, anticancer, and wound healing; they are also used in cosmetics and perfume industries. Researchers and industry professionals would surely benefit from this study's information as they choose the best extraction techniques for obtaining the highest yield and quality attributes.

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