THE ACTIVE COMPONENTS OF LAVENDER USE FOR ANXIETY AND INSOMNIA

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1. ABSTRACT/RESUM

- Lavender essential oil has been used in folk medicine since ancient times as an anxiolytic, mood stabilizer, sedative, analgesic, anticonvulsive and a wide range of neurological disorders, therefore a recent increase in the popularity of alternative medicine and natural products has renewed interest in lavender as a potential natural remedy when normal medicine cannot be employed.

Consequently, this memory briefly describes not only the oil composition of lavender, taking into account several factors such as genotype, seasons and extraction method, but also the therapeutical effects of the principal components found in this valuable substance like sedative, antimicrobial and antinoceptive effects, the mode of action of each one and the commercial products containing the oil. In order to achieve these objectives, a deep research had been carried out from several databases, web pages and books, with the aim to achieve a solid scientific base.

In conclusion, this memory demonstrates that the factors studied (genotype, seasons and extraction method) have a direct impact on the chemical configuration of the essential oil of lavender. In addition, clinical evidence demonstrates the efficacy of lavender's oil against sleeping and anxiety disorders, it's antibacterial and antifungal activity and finally it's antinoceptive properties. Evidence that can be seen through wide range of commercial products found nowadays in the market in order to treat the disorders mentioned above.

- L'oli essencial de lavanda ha estat utilitzat en la medicina tradicional des de l'antiguitat com a ansiolític, estabilitzador de l'estat d'ànim, sedant, analgèsic, anticonvulsiu i altres desordres neuronals, motiu pel qual un recent augment en la popularitat de la medicina alternativa i de productes naturals ha renovat l'interès per la lavanda com a potencial remei natural quan la medicina tradicional no pot ser utilitzada.

Conseqüentment, aquesta memòria descriu breument no només la composició de l'oli essencial de la lavanda, tenint en compte variïs factors com el genotip, les estacions de l'any i el mètode d'extracció, sinó també els efectes terapèutics dels principals components que es poden trobar en aquesta apreciada substància com seria sedatius, antisèptic i antinoceptiu, el mecanisme d'acció de cadascun d'ells i els productes comercials que contenen l'oli. Per tal d'aconseguir aquests objectius, es va fer una recerca profunda a partir de bases de dades, pàgines web i llibres amb l'objectiu d'aconseguir una base científica sòlida.

Per concloure, aquesta memòria demostra que els factors estudiats (genotip, estacions de l'any i mètode d'extracció) afecten la configuració química de l'oli essencial de lavanda. Adicionalment, l'evidència clínica demostra l'eficàcia de l'oli en desordres de la son i l'ansietat, el poder antibacterià i antifúngic i finalment les propietats antinoceptives. Tot això es pot veure en el gran ventall de productes comercials que es poden trobar al mercat avui en dia destinats a tractar els desordres mencionats anteriorment.

2. THE INTEGRATION OF THE THREE KNOWLEDGE AREAS:

Plant Phisiology Unity, Pharmacognosy and Phytotherapy Unity and Therapeutical Pharmacology Unity.

The main aim of this project consists in linking and integrating the different knowledge areas with the knowledge acquired throughout the degree in pharmacy. This project is monitored by the Plant Physiology Unity, which mainly focuses on the area of Natural Products and Plant Biotechnology. On the other hand, other areas such as Pharmacognosy and Phytotherapy and Therapeutical Pharmacology will also be considered.

As mentioned before, the main field of study of this paper is Plant Physiology. Therefore, this project contains a brief description and characterization of the taxonomy and botanic of lavender.
In the secondary area of Pharmacognosy and Phytotherapy, essential oil is widely detailed, focused not only on how it is synthesized and produced, but also the different composition between the main species and the huge variety of factors, which affect this chemical composition.
Finally, the pharmacological activity in anxiety and insomnia is related to the compounds found in the oil and the mechanisms and evidence of this one are demonstrated with the analysis of several clinical studies, which corresponds to the Therapeutical and Pharmacology Department.

In conclusion, the integration of all areas of knowledge above mentioned will allow the achievement of the main objective of the project, which consists in understanding the essential oil of lavender, not only the chemical composition, synthesis and factors that affect this configuration, but also the therapeutical effects related to anxiety and insomnia of the compounds that are found in a major proportion and commercial products for this disorders.

3. INTRODUCTION

A recent increase in the popularity of alternative medicine and natural products has renewed interest in lavender and their essential oils as potential natural remedies. The term lavender is considered to come from the latin "lavando", part of the verb "lavare" (to bathe), as the romans employed it to perfume their baths. Lavender is traditionally alleged to have a variety of therapeutic and curative properties, ranging from inducing relaxation to treating parasitic infections, burns, insect's bites and spasms.

There is growing evidence suggesting that lavender oil is an effective remedy to treat several neurological disorders when normal pharmacology cannot be employed, therefore lavender's oil is considered to have a significant clinical potential in its own or as adjuvant therapy in different disorders. Several animal and human investigations suggest anxiolytic, mood stabilizer, sedative, analgesic, anticonvulsive and neuroprotective properties. However many research has to still be done, as several factors such as skin type, quality and the size of area being treated, may affect the level and rate of lavender absorption.

Consequently, this project is useful to increase knowledge of lavenders pharmacological effects and improve the future experimental and clinical research plans.

3.1. BOTANICAL DESCRIPTION

3.1.1. The genus *Lavandula*

Lavandula belongs to the *Lamiaceae* (Labiatae) family and is member of the subfamily *Nepetoideae.* Although it's evident classification, *Lavandula* genus is a really divergent and rather mixed group. However what's common about all of them, is that many species are highly aromatic due to the presence of glands that cover all the plant and produce the essential oil.

Scientific classification	
Kingdom	*Plantae*
Division	*Magnoliophyta*
Class	*Magnoliopsida*
Order	*Lamiales*
Family	*Lamiacea*
Subfamily	*Nepetoidae*
Tribe	*Lavanduleae*
Genus	*Lavandula*

TABLE 1 – Scientific classification of the genus *Lavandula* [63].

This genus offers a big assortment, as you can find from woody shrubs that can reach a metre in high, to annual herbs or perennial woody-based shrubs. Leaves can be entirely or deeply dissected and the flowers (spike) consists of cymes, a branching determinate inflorescence with a flower at the end of each branch in two arrangements: either an opposite decussate arrangement (each pair of flowers forms a right angle with the pair above or below) or an alternate spiral arrangement [1] [2].

The cymes are subjected by bracts, which vary in their size, shape and nervation, which can be diagnostic for many species. They can also be single flowered (usually without bracteoles) or multi flowered (with 3 to 9 flowers per cyme) with bracteoles (normally smalls). Moreover, calyx is bilabiate and varies in the number of nerves, lobe shape, presence of appendage, colour… therefore these characters provide many important information which is used to diagnose both sections and species. Corolla is usually bilabiate, tubular and with five lobes which vary in size, colour, shape and markings [1].

Although the species exhibit a wide variation of morphological characters, it is evident that natural groupings of related taxa can be recognised. The species and intraspecific taxa are arranged according to sections, of which there is six presently recognised. These sections can be distinguished by differences in habit, leaf shape, arrangement of flowers in the verticils, bract, calyx and corolla characters.

However, there is some species whose position is uncertain, like hybrids (which are dealt at the end of each section), but the intersectional hybrids are placed after the sections.

SECTIONAL CLASSIFICATION	
1. Section *Lavandula (*=Section *Spica* Ging) 1. *L. Angustifolia* Mill. Subsp. *Angustifolia* 2. *L. latifolia* Medik	2. Section *Dentatae* Suarez-cerv.& Seone-Camba 3. Section *Stoechas* Ging. 4. Section *Pterostoechas* Ging. 5. Section *Subnudae* Chaytor

TABLE 2 - Sinopsis of sectional classification and taxa recognised. Table addapted [3].

Section number 1, *Lavandula (=Spica* Ging), is the one that contains species with the major linalool proportion, is formed by woody shrubs with simple leaves (generally linear in shape). Even more, Cymes not only have an opposite decussate arrangement, but also 5 to 7 flowers with a maximum of 3 to 9, with bracteoles. Bracts are variable in shape, however they have reticulate veining. Moreover, calyx has a tubular form with a very short stalk, with thirteen or eight nerves. The corolla has an upper lobe larger than the lateral lobes.

3.1.2. Lavandula angustifolia MILLER SUBESP. angustifolia

Shrub 50cm high, with linear-lanceolate grey leaves when young, becoming greener in age. Inflorescence stalk usually unbranched, with a compact spike. Bracts are normally ovate-rhombic and bracteoles really small. Calyx thirteen-nerved and with small circular appendage. Moreover, corolla is bilaterally symmetrical, and is nearly twice the length of the calyx. It contains prominent lobes with a wide range of colours, from blue/mauve to rarely violet/pink [3] [4] [5].

This species produces the best quality oils and can be found in mountainous areas (over 1500m) from SW, South Central Europe (Italy, France and Spain). These origins make this species the hardest in cultivation [3] [4].

3.1.3. Lavandula latifolia Medik

Shrub 50-70cm high, whose leaves are grey and with linear- lanceolate outline. Inflorescence stalk is distinctly branched, usually forming a trident shaped flower spike. Bracts are linear-lanceolate in shape and bracteoles distinct to 4mm long. Moreover, calyx is also thirteen-nerved but with a rotund appendage. Corolla is strongly bilaterally-symetrical from blue to mauve in colour [3] [4] [6].
This species can also be found in SW, South Central Europe, however the altitude is much higher (1200m) and the quality of the oil is much lower [3].

3.2. HISTORICAL REVIEW OF THE USE OF LAVENDER

Historically, lavender dates from ancient times, as the Egyptians used it for cosmetics and for embalming. A clear example of that is Tutankhamen's tomb, which contained jars of unguents with a strong lavender scent [6]

However, the first written record that talks about medicinal properties belongs to Dioscorides, a Greek medicine writer (40-90 $_{AD}$), who was the first to mention the healing properties of lavender. He probably found it in *L.stoechas* and that's the reason why the romans used it to perfume their hair, bodies, beds and communal baths. He also attributed to the plant some laxative and invigorating properties and he highly recommended its use for chest complaints. That's why Galen (129-99AD) included lavender in his list of antidotes for poison and bites. [5]

In the 7th century, the use of lavender spread throughout Europe as a consequence of the Arab conquest. In Germany, a writer, composer, philosopher and visionary called Abbess Hildergard of Bingen (1098-1179) clearly distinguished between two species: *L.spica and L.*vera. [7].

She also determined that lavender was useful for palsy, especially when eated as powder on top of a slice of bread. She also said this genus had a strong odour which was effective not only to clear your eyes but also terrifies malign spirits. In addition, she noticed that, as a consequence of cooking the spike with wine or honey and water, it could be used to relieve pulmonary congestion. At last but not least, in her descriptions, she highly suggested a bath steeped in lavender in order to prepare the nervous system for a peaceful sleep [8] [9].

Important historic figures, started to use lavender in their everyday life. For instance, Queen Elisabeth I of England, drank lavender tea to treat her headaches, therefore she encouraged the development of lavender farms. In addition Charles VI of France, stuffed his cushions with lavender not only to have a deep sleep, but also to repel insects and plagues [6].

Furthermore, John Gerard (1545 – 1612), a botanist and herbalist similarly to Abbess Hildergard, announced that destilated water of *L.vera* or the flowers preserved with sugar, were useful for nervous system disorders such as catalepsies, migraines, epilepsy and fanting. He also suggested that lavender was helpful for "passions of the heart" referring maybe to palpitations and heart issues. In adition, this species could be useful for the shaking associated with Parkinson. In the other hand, *L.stoechas* could be administrated not only for diseases of the chest and headaches, but also for epilepsy, apoplexy, to open all internal organs and provoke diuresis [10].

In the same way, Nicholas Culpeper (1616 – 1654), a English botanist, herbalist, physician and astrologer claimed that lavender was really useful for head and brain pain, just as Hildergard. Nevertheless he added that this genus also gives strength to the stomach and opens the liver and spleen, just as Gerard, but he also adds that it's useful to expel the dead child and the afterbirth (placenta) from the mother's body. He was the only character that attributes this abortive effect to lavender. He also gave lavender some interesting unknown facets, as he declares that gargles relieve toothache and improves voice loss [5].

Some years later, a American physician called Thomas Palmer (1696) said that "lavender heats the heart" referring that the genus is useful for the weakness, the slow pulse, when the body and the air breathed are cold. In palmer's world, in the same way as the authors above, he concludes that lavender improves palpitations and the passion in the beating of the heart [5] [9].

As a result of all the previous effects, a English empiric doctor called William Salomon (1644 – 1713), not only attributed lavender all of the mentioned responses, but also for snake bites, dog's madness and other poisonous creatures only using dried seeds or leaves [7].

Maud Grieve (1858-1941) commonly known as "Mrs Grieve", lived in Buckinghamshire, where she had a large herbal garden during world war l, with the only aim to illustrate people how to harvest, dry and prepare medicinal supplies. From this moment, she started to publish herbal remedies in which lavender took most of the leadership [5] [7].

Finally, during the Victorian era, Queen Victoria of England started to use lavender in her perfume, which made lavender one of the most popular scents of that time. Street sellers in London started to sell dried lavender in sachet bags for wardrobes and bed sheets, making London the centre of lavender oil production [6].

Nowadays, lavender and it's derivatives are mainly manufactured for a different purpose, as a consequence of a general reversion to the use of dried lavender. All essential oils, not only lavender's, have been put into the front-line as a cure-all for mind, body and spirit, a concept which was created in 1928 by a pharmacist named René Maurice [4].

4. OBJECTIVES

The main objective of this paper is to understand the essential oil of the genus *Lavandula*, not only the composition and chemical configuration of various species of the genus and the factors, which have a direct impact on it, but also the therapeutical effects related to anxiety and insomnia and the commercial products which contain this valuable substance.

In order to achieve this objective, it will be necessary to expand and widen the knowledge acquired during the degree about the different databases, in order to achieve the necessary information related to the title's project.

In addition, to create and write this paper, this project will be directed under three different study areas: Plant Physiology, Pjarmacognosy and Phytotherapy and Therapeutical Pharmacology. The discipline of Plant Physiology will be featured among the rest, for this project has been conducted under the direction of the Plant Physiology Unity of the UB. All the areas of study will be combined and described as described in the previous section (" The integration of the three knowledge areas").

Plus, with the objective to achieve a solid scientific base, a deep bibliographic research will be carried out from reliable fonts (such as books, wide databases and others) in order to document the information given.

Finally, during the research of information and bibliography, it will be crucial to check, relate and interpret the fonts consulted with the purpose to obtain scientific evidence of the concepts addressed.

5. METHODS AND MATERIALS

To elaborate this paper, a bibliographic research was needed with the purpose to find all the information related with the theme of the project and achieve the objectives previously established. Undoubtedly, in order to carry out this research, databases and alternative researches were done before and throughout the elaboration of the project.

The databases consulted were the following:

- PubMed (http://www.ncbi.nlm.nih.gov/pubmed): platform created by NCBI (National Centre for Biotechnology Information) which UB (Universitat de Barcelona) has free access to several databases and full magazines.
- Scopus (http://www.scopus.com/): is the largest abstract and citation database of peer-reviewed literature.
- CRAI (http://crai.ub.edu/): *Centre de Recursos per a l'Aprenentatge i la Investigació de la UB*.

Research criteria consisted in identifying and selecting the most relevant articles, in a chronological order so that the concepts detailed in the project displayed a temporary evolution.

In addition, the book *Lavander, The genus Lavandula* edited by Maria Lis –Balchin (Edited by: Taylor and Francis), played and important role throughout this work, as it was used as a guide during the whole elaboration of the project.

Finally, after searching and going through all the above mentioned, the content of this paper was carefully summarised, organised and displayed with the aid of a tutor in order to achieve a logical planning of all the issues contained.

Last but not least, bibliographical references were managed and added through Mendeley (https://www.mendeley.com), an online software used to keep track of any documental consultations and their authors, year of publishing etc.

6. RESULTS AND DISCUSSIONS

6.1. SYNTHESIS - ESSENTIAL OIL OF LAVENDER

Generally speaking, the essential oil of lavender has been used in alternative medicine for many centuries. Therefore, the phytochemistry of *Lavandula* is well known nowadays. All species that form the *Lavandula* genus and hybrids are highly aromatic plants, whose glands, placed on flowers and leaves, produce a complex variety of essential oils which, not only genes determine this mixture, but also the environmental factors.

To describe and evaluate the compounds of this genus and the therapeutic effects, three species from Section 1 were selected for their important role in the aroma- therapeutic, perfume and cosmetic industry. These are the following: *L. angustifolia, L. latifolia* and *L.hybrida (L. angustifolia x L. latifolia)* commonly known as lavandins, which produce lavender oil, spike oil and lavandin oil respectively. These essential oils are the most seeked-after lavender oils in the cosmetic and aroma-therapeutic industries.
Per contra, other species such as *L. stoechas* and *L.dentata*, have also been used in medicinal practices for their anti-spasmolytic properties, even though they don't play a relevant role in the aim of this project [11] [12].

These valuable substances are produced in very different proportions. For instance, *L. angustifolia* produces relatively low quantities (40 kg per hectare) while *L. latifolia* generates 10kg more (50 kg per hectare) [13].

Given these points, in 1920s *L. hybrida* started to be commercially cultivated. The reason behind its creation was that the oil produced, inherited the characteristics of the essential oils of both its parents (*L.angustifolia* and *L. latifolia)*.

The primary compounds of *Lavandula's* oil are terpenes, whose biosynthesis will be briefly described in order to understand the different compositions of the species.

6.1.1. Biosynthesis of IPP (isopentenyl pyrophosphate)

Lavandula's essential oils are primarily composed monoterpens, although trace levels of sesquiterpenes can also be present. These terpenic substances play diverse physiological, metabolic and structural functions, which are easily produced in large amounts and provide a range of commercially useful products. In spite of the economic significance of terpenes and their many essential functions, little is known about the metabolism and its regulation in plants, as the production and location take place in many different ways and for many other purposes. However, terpenes are produced by a common biosynthetic pathway with control mechanisms that en-sure appropriate levels of those.

All terpenes derive from the union of isoprene molecules (C_5H_8), which follow what is called the **isoprene rule** or **C_5 rule** (Wallach 1914). These principle explains that most terpenes are constructed by several isoprene units $(C_5H_8)_n$, were "n" is the number of linked molecules

of isoprene ; This rule provides the first concept of structural relationship among terpenic natural products [14].
Plants have two metabolic pathways, which serve as the basis for the biosynthesis of molecules used in processes of terpene biosynthesis: mevalonate acid pathway and DXP/MEP pathway.

6.1.1.1. Mevalonate acid pathway or mevalonate- dependent (MEV)

In this pathway, seen in Figure 2, HMG-CoA reductase converts HMG-CoA into mevalonic acid (MVA) in the cytoplasm. This reaction is the key controlling step in plant isoprenoid biosynthesis [15].

The pathway starts with the production of acetoacetyl-CoA from two molecules of acetyl-CoA. A third acetyl-Coa is then condensed and finally forms de HMG-CoA [16]. Both reactions, have not been deeply studied in plants. However it is believed that both of them operate in the same way, as they need Fe and quinone factors to accomplish it [17].
The NAPDH-dependent HMG-CoA reductase, catalyses two reduction steps which forms mevalonate, that undergoes two sequenced phosphorylations from two separate soluble kinases, mevalonate kinase and phosphomevalonate kinase, that form mevalonate -5-pyrophosphate using each of them one molecule of ATP. Finally, formation of IPP is catalysed by mevalonate-5-pyrophosphate decarboxylase who performs a decarboxilate elimination and releases a CO_2 molecule[15][16].

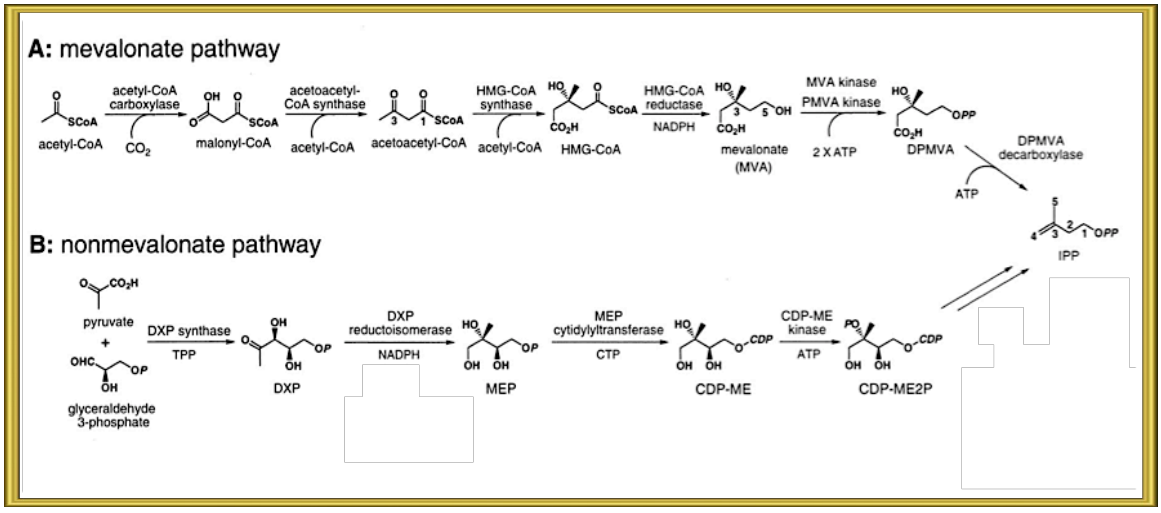

FIGURE 1 - Schematic view of mevalonate and non mevalonate pathways for IPP biosynthesis [64].
MVA: mevalonate; MVA kinase: Mevalonate kinase; PMVA kinase: Phosphomevalonate kinase; DMPVA descarboxylase: Mevalonate-5-pyrophosphate descarboxylase; IPP: Isopentenyl pyrophosphate; TPP: Tiamine pyrophosphate; DXP: 2-deoxy-D-xylulose 5- phosphate; DXP synthase: 1-deoxy-D-xylulose-5-phosphate synthase; DXP reductoisomerse: 1-deoxy-Dxylulose 5-phosphate reductoisomerase; MEP: 2-C-methyl-D-erythritol 4-phosphate; MEP cytidylyltransferase: 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; CDP-ME: 4-diphosphocytidyl-2-C-methyl-Derythriol; CDP-ME2P : 4-diphosphocytidyl-2-C-methyl-Derythriol-2-phosphate.

6.1.1.2. MEP/DXP pathway or non-mevalonate pathway

This pathway was recently discovered. It is described as an 8-reaction pathway, which takes place in plant's plastids, whereas MEV lasts 6.

The non-mevalonate pathway starts with a condensation of pyruvate and glyceraldehyde 3-phospate catalysed by 1-deoxy-D-xylulose-5-phosphate synthase (DXPS) to produce 1-deoxy-D-xylulose 5-phosphate (DXP). However, this first reaction uses thiamine pyrophosphate as a cofactor.

The next reaction consists in a reduction of DXS to 2-C-methyl-D-erythritol 4-phosphate (MEP) by DXP- reductoisomerase (DRI or DXR) in the presence of NADPH and Mn^{2+}. The further biosynthetic step consists in the CTP dependent conversion of MEP into 4-diphosphocyti-dyl-2-C-methyl-D-erythritol (CDP-ME) by MEP cytidylallyltransferase. The two following reactions are described together; first of all, a ATP dependent reaction promoted by CDP-ME kinase converts CDP-ME into CDP-ME2P. The conversion of CDP-ME2P into IPP is still a unexplored area [16] [18].

For many years it was assumed that the MEV was the only existing pathway in order to obtain the main molecule for terpene synthesis. When MEP route was discovered, many investigators established some theories about the final destination of this molecule, which depended on the type of plant studied. Some of them said that the MEV pathway provides precursors for the synthesis of sterols, brassinosteroids, ubiquinone and sesquiterpenes, while the MEP pathway was associated with the synthesis of isoprene, monoterpenes, diterpenes, carotenoids, abcisic acid and others. However, as a consequence of the exchange of metabolites between plastids and cytoplasm, thousands of isoprenoids are synthesised from both pathways, therefore many research groups are working on cellular engineering strategies [19].

6.1.2. From IPP to terpenes

Once we obtain IPP (product from both pathways), an IPP isomerase produces Dimethylallyldiphosphate (DMAPP) from it. This step is necessary because IPP itself is insufficiently reactive to initiate the condensation to higher terpenoids [14]. The action of various prenyl transferases generates terpenoids of higher order generating the following branch points: geranyl pyrophosphate (GPP; C_{10}), farnesyl pyrophosphate (FPP; C15) and geranyl geranyl pyrophosphate (GGPP; C20).

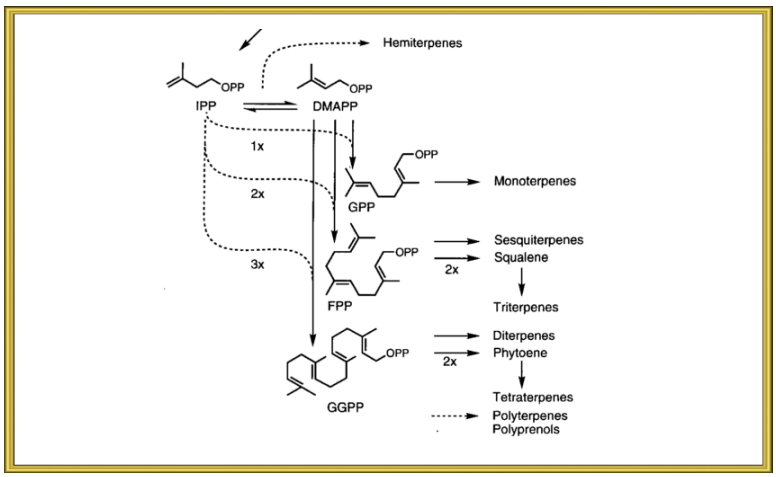

FIGURE 2 - Terpene skeleton byosinthesis by prenyl transferases. 1x, 2x, 3x indicates the number of IPP units added [14].
IPP: Isopentenyl diphosphate; DMAPP: Dimethylallyldiphosphate; GPP: Geranyl pyrophosphate; FPP: Farnesyl pyrophosphate; GGPP: Geranyl geranyl pyrophosphate.

The diversity of higher terpenes arises from the capability of diphosphate synthase enzymes (PDPSs) to condense two IPP or one IPP with DMAPP in head-to-tail o non-head-to tail orientations [20] [21].

The head-to-tail coupling of two IPP units to geranyl disphosphate (GPP) or cisoid isomer neryl diphosphate (NPP; C_{10}) is catalysed by geranyl diphosphate synthase (GPPs) and neryl diphosphate synthase (NPPs) respectively. On the other hand, the non-head-to-tail condensation to lavandulyl diphosphate (LPP; C_{10}) and chrysanthemyl diphosphate (CPP; C_{10}) is catalysed by lavandulyl diphosphate synthase (LPPs) and chrysanthemyl diphosphate synthase (CPPs) respectively. Once all this C_{10} molecules are formed, monoterpenes synthase (mTPSs; that were first described in *Nicotiana tabacum* and *Mentha spicata*[22]) transform and elaborate the different variety of monoterpenes [20].

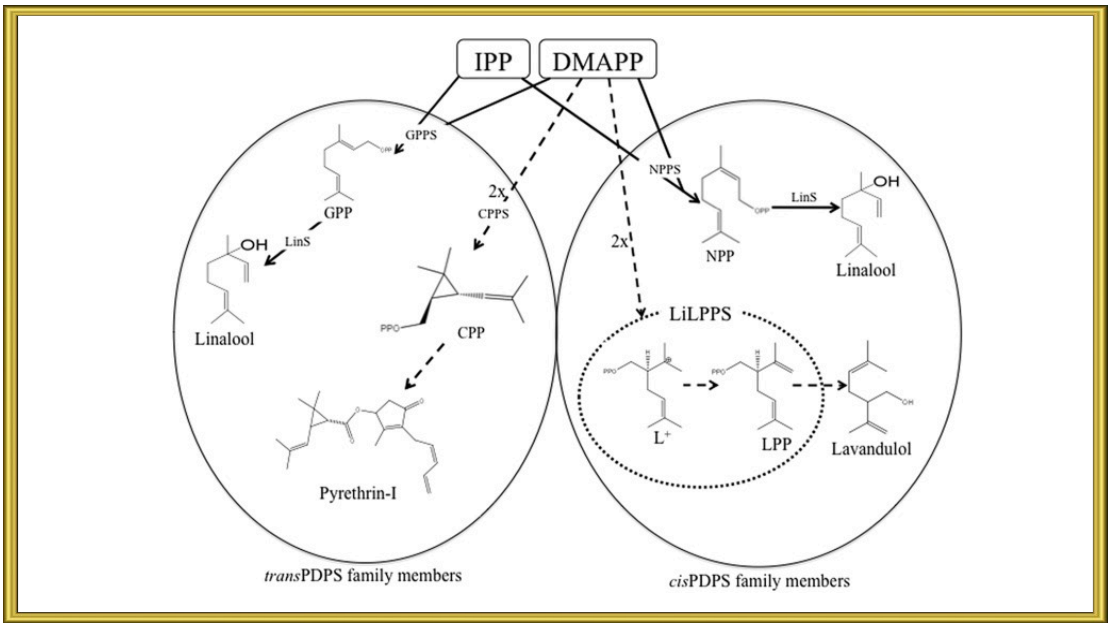

FIGURE 3 - Schematic representation of IPP and DMAPP condesation catalysed by the PDPs family [20].
IPP: Isopentenyl pyrophosphate; DMAPP: Dimethylallyldiphosphate; GPPS: Geranyl diphosphate synthase; GPP: Geranyl pyrophosphate; CPPS: Chrysanthemyl diphosphate synthase; CPP: Chrysanthemyl diphosphate; NPPS: Neryl disphosphate synthase; NPP: Neryl diphosphate; LPPS: Lavandulyl diphosphate synthase: LPP: Lavandulyl diphosphate.

Thus, monoterpenes that deriver from GPP and NPP and the enzymes involved are found in nature and are widely distributed. These C10 terpenoids, such as linalool, 1,8-cineole and so forth, are known as " regular monoterpenes". On the contrary, the irregular monoterpenes derived from LPP and CPP such as lavandulol are encountered in less proportion in nature.

6.2. COMPOSITION - ESSENTIAL OIL OF LAVANDER

6.2.1. Factors that affect composition

As mentioned before, the oils produced by the genus are rich in monoterpenes, however It is important to note, that the chemical composition of the essential oils from different *lavandula* species, hybrids and cultivars, show not only interspecific but also intraspecific differences, which may sometimes be due to climatic, geographical or seasonal differences or due simply to the amount of watering or fertilisation used.

There is also, however, a further point to be considered which is the variation due to genotypes (which can occur in plants growing in close proximity or in considerable distance), plus, another significant aspect is the method used in the oil extraction.

6.2.1.1. Genotype

In 1995 Boelens established the major components of lavender, spike lavender and lavandin oil (Table 3). He affirmed that lavender oil was mainly constituted by linalyl acetate, linalool, cis-Ocimene and lavandulyl acetate, those of spike lavender oil were linalool, 1,8-cineol, camphor, borneol and α- and β-pinene and finally lavandin oil was composed by linalool, linalyl acetate, camphor, 1,8- cineol and borneol [23].

Essential oil	Percentage of essential oil in		
	Lavender oil	Lavandin oil	Spike lavender oil
Linalyl acetate	12-54	19-26	0-1.5
Linalool	10-50	20-23	26-44
Cis-and/or tans-Ocimene	1.0-17	1.0-3.0	0-0.3
Lavandulyl and acetate	0.1-14	0.5-0.8	0.2-1.5
1,8-cineole	2,1-3.0	10	25-36
Camphor	0-0.2	12	5.3-14.3
α- and β-pinene	0.02-0.3	0.6-0.9	1.6-3.6
Borneol	1.0-4.0	2.9-3.7	0.8-4.9
Caryophyllene and or its oxide	3.0-8.0	2.7-6.0	0.1-0.3
Myrcene	0.4-1.3	1.2-1.5	0.2-0.4
Farnesene	Trace	1.1	0.2-0.3
Germacrene D	0.2-0.9	1.0-1.2	___
Camphene	0.1-0.2	0.3-0.6	0.2-1.8
Limonene	0.2-0.4	0.9-1.5	1.0-2.2

TABLE 3 – Comparison of the major constituents (those which make up > 1% of the total oil). The species compared are: *Lavandula angustifolia,* which produces lavender oil, *Lavandula hybrida,* which produces lavandin oil and finally *Lavandula latifolia*, which produces Spike lavender. Percentatges in red show the components that appear in major proportion [23].

According to Boelens, what's characteristic about lavender oil, is the tremendous amount of linalyl acetate, linalool, cis or trans ocimene and lavandulyl acetate whereas spike lavender is rich in 1,8-cineole, camphor, α- and β-pinene and borneol. As a consequence of that, lavandin oil has earned his oil profile from both lavender and spike lavender, and that's the reason why the composition contains a high percentatge of linalyl acetate, linalool, 1,8- cineol, camphor and borneol [23].

However, *L. angustifolia* is reported to produce the highest quality oil, while *lavandin* is considered lower due to the high quantity of camphor and low linalyl [13].
Spike lavender oil is distinguished from *angustifolia* and latifolia not only on the different amount of 1,8-cineole, camphor and α- and β-pinene, but also by the presence of camphene and limonene.

6.2.1.2. Seasons

Evidence that demonstrates the effect of season on the oil composition can be found in an article of a research group of the Jaen University. In particular they focused on the variations in the chemical composition of spike lavender cultivated in southern Spain oil due to seasonal variations. They studied the composition six essential oil samples (extracted by steam distillation) of twigs of spike lavender (*Lavandula latifola* Med.*)*, harvested in three different locations of southern Spain during full flowering and fruiting phonological stages.

First of all, it is important to note that the oil yield during flowering period was considered higher (2.5- 7 times) than those during fruiting period, due to the fact that the oil- containing flowers and seeds fall in that phonological stage. The examination allowed the identification of 56 constituents, which determined that the analysed oils belong to the chemotype linalool/1,8-cineole/camphor.

Evidently, making reference to Table 4, 1,8-cineole reduced its presence from flowering to fruiting; this approach is similar to camphor's case, except for the population LI-3. On the other hand, linalool had the opposite behaviour, as the presence increases except for LI-3 population. For the rest of constituents, a general decreasing on the percentage levels where observed [24].

	Percentage composition					
	Full flowering (summer)			Fruiting (fall)		
Compound	LI-1a	LI-2a	LI-3a	LI-1b	LI-2b	LI-3b
α-pinene	1.9	1.8	1.2	0.9	1.0	0.6
camphene	0.6	0.5	0.5	0.4	0.4	0.5
sabinene	0.8	0.8	0.6	0.4	0.5	0.3
β-pinene	2.4	2.6	1.7	1.1	1.4	0.8
myrcene	0.8	0.8	0.8	0.4	0.4	0.3
limonene	0.2	0.2	0.9	0.2	0.2	0.2
1,8-cineole	34.9	33.9	29.1	28.0	30.8	28.6
linalool	27.2	32.4	32.1	43.1	40.8	30.5
camphor	14.0	11.2	14.9	11.8	10.8	23.2
borneol	1.5	1.3	3.6	0.9	1.1	2.0
terpinen-4-ol	0.4	0.4	0.4	0.3	0.4	0.5
α-terpineol	1.4	1.6	1.4	0.9	1.2	0.8
β-caryophyllene	1.0	1.9	1.1	0.6	0.8	0.5
(E)-α-bisabolene	2.3	1.3	1.9	1.9	0.7	0.5

TABLE 4 - Percentage composition of the main and characteristic components of Spike lavender obtained from *Lavandula* latifolia harvested in summer and fall seasons.LI-1a and LI-1b: harvested in "Garganta de Hornos"; LI-2a and LI-2b: harvested in "Rio Madera"; LI-3a and LI-3b: harvested in "El Leganillo" [24].

This point was also sustained by the work from the University of Belgrade, both Biology and Pharmacy faculties, who proved seasonal variations in the composition of the essential oils by GC and GC/MS, obtained from the same genotype of *Lavandula angustifolia* cultivated in Belgrade. They collected 21 samples each month during the vegetation cycle and were separated into three clades: I, II, III depending on the oil composition [25] .

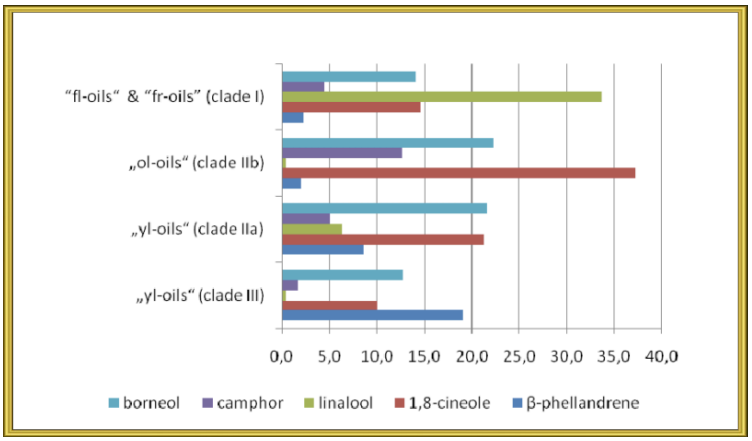

FIGURE 4 – Dominant components of the essential oil of the basic clades of *Lavandula angustifolia* cultivated in Belgrade. Clade I (fl-oils & fr-oils) = flowers and fruits. Clade IIa (ol-oils) = Young leaves from May, before flowering. Clade IIb (yl -oils) = old leaves (August-April). Clade III (yl-oils) = Young leaves from April [25].

Clade I corresponds to fruits and flowers and is rich in linalool (linalool chemotype). Clade II is divided into clade IIa, which contains young leaves before flowering season that has large amounts of 1,8-cineole and borneol (1,8-cineole chemotype), and clade IIb, that is formed by old leaves and is rich in 1,8-cineole. Finally clade III, that is formed by the young leaves from April, is β-phellandrene dominant.

To sum up, the results of this part of the study indicate that the origin of the plant is a very important factor that has a direct impact on the quality of the essential oil.

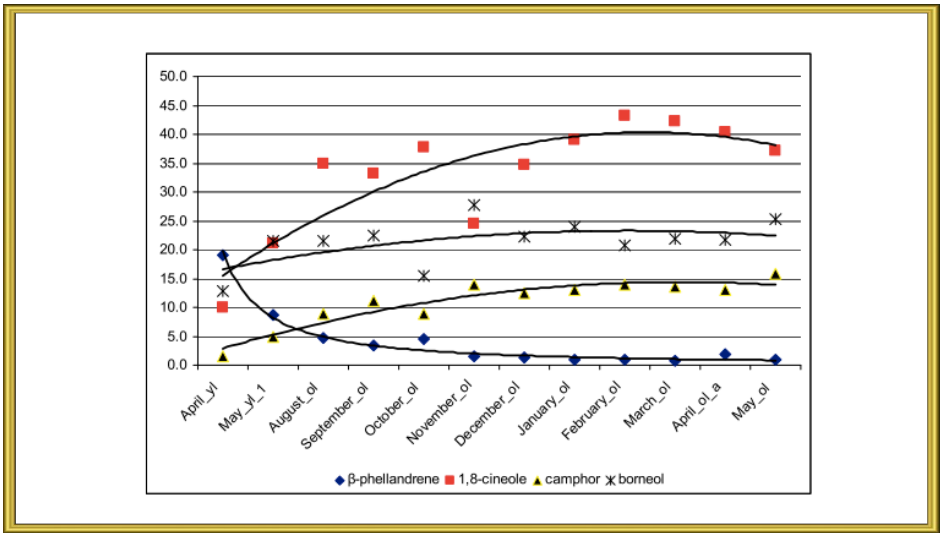

FIGURE 5 – Seasonal variations from April to May of the concentration of the dominant components of the essential oils of *Lavandula angustifolia* cultivated in Belgrade [25].

Figure 5 clearly shows that seasons play an important role in the oil composition. It can be observed that borneol and camphor have a more or less increase, whereas 1,8-cineole and β-phellandrene show opposite trends in seasonal changes.

So this project from the University of Belgrade demonstrated that the final composition of the oil depended not only on the part of the plant studied, but also of the stage of development which the sample of oil is studied (the season) [25].

6.2.1.3. Extraction method – steam distillation

Various extraction methods are used in the manufacture and extraction of essential oils, and the method employed is normally dependant on the type of botanical material is being used. Therefore, not only the method used but also the time, have a direct impact on the composition of the oil.

In the *Lavandula* genus, the oil is secreted in small globular glands in many parts of the plant such as: calyx, corolla, leaves, stalks and branches. However, the richest part in essential oil is the fresh flowering tops, between the hairy calices. These ones have to be ripe enough in order to avoid traces, which may not only affect the oil composition but also contribute to a "green" note in the oil.

Steam distillation is the conventional technique used to extract lavender's essential oil. Steam produced by a steam inlet is forced to pass the plant material, which is also supported on a perforated grid. Steam, which contains de essential oil, passes through a cooling system, which makes it condense. The condensed product contains the essential oil plus hydrosols, which can be separated due to the difference of densities. Steam can be readily controlled, and there is no thermal decomposition of oil constituents therefore, it is the most widely accepted process for large-scale oil production, however an industrial equipment is used instead [3] [4] [26] [27].

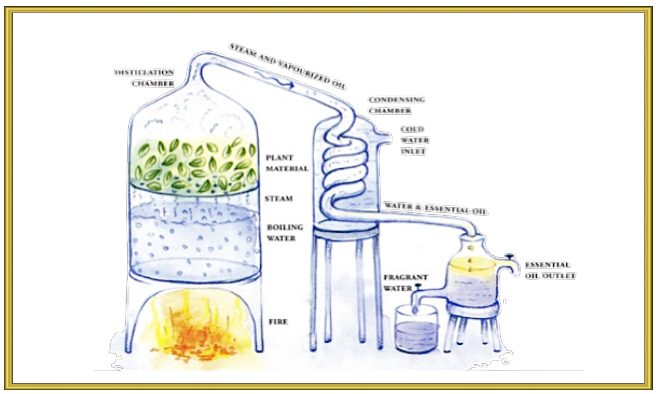

FIGURE 6 – Representation of a traditional still shows how steam passes plant material carrying volatile compounds which condenses and forms the fragant water (hydrolat) and the essential oil [4].

The basis of steam distillation:

It all starts with the first contact of heat with the lavender's oil glands, which burst. The oil spilled forms numerous homogeneous patches on the herb surface. This oil is in contact with water forming an oil-water interface from the steam condensed on the herb. In the boiling point, steam particles condense and transfer their latent heat in but, as oil and water are immiscible, they condense on the oil-water interface and consequently, surrounds the surface patch and the oil can be vaporized. As lavender's herb surface is hairy, it enlarges the area where the steam is condensed, but it will continuously be giving up heat to vaporize oil, which directly affects to the extraction time [28].

It is important to note, that steam distillation works because water and oil are immiscible, so they boil independent of each other. Following the principles of steam distillation, if the addition of both pure vapour (total vapour pressure) pressures exceeds atmospheric pressure, boiling will begin. As a consequence of that, the boiling point is slightly less than the normal boiling point of water; therefore the oil, which firstly had an extremely high boiling point, will vaporize under very mild conditions.

Evidence that demonstrates the effect of the time employed on the extraction on the oil composition can be found in a study carried on in United States in 2013, which consisted on evaluating the effect of distillation time (DT) on lavender essential oil yield and composition. Dried lavender flowers from *L.angustifolia* were used, plus steam distillation at different DT, which were performed in 3 replicates (time was measured from the first drop of oil visible until the end of DT, point which heating was turned off and removed) [29].

DT (min)	EO yield	Cineole	Fenchol	Camphor	Linalool Acetate
1.5	0.50 e	34.52 a	2.89 a	6.57 f	15.0 c
3	0.97 e	26.21 b	2.66 b	7.78 cde	18.6 c
3.75	1.18 e	25.13 b	2.63 b	8.20 cd	19.4 c
7.5	2.02 d	15.57 c	2.28 c	9.06 ab	25.8 b
15	3.44 c	12.13 c	2.11 c	9.22 a	30.1 b
30	4.77 b	8.15 d	1.88 d	8.39 bc	35.4 a
60	6.14 a	7.61 d	1.84 d	7.66 de	36.5 a
90	6.25 a	7.12 d	1.80 d	7.64 de	37.0 a
120	6.26 a	7.45 d	1.83 d	7.49 e	36.1 a
150	6.64 a	6.37 d	1.74 d	7.20 ef	37.8 a
180	6.83 a	6.61 d	1.76 d	7.37 e	37.5 a
240	6.78 a	6.49 d	1.75 d	7.29 e	37.3 a

TABLE 5 - Mean essential oil yield (wt/wt %) and concentration of cineole, fenchol, camphor and linalool acetate from 12 distillation times (DT). Within each column, means followed by the same letter are not significantly different at the 5% level of significance [29].

Results showed that DT had an effect on essential oil yield, and on the concentrations of fenchol, linalool acetate, camphor and cineole. The essential oil yield was low at 1,5 to 3 min DT, increased at 7,5 min DT and again a 15 and 30 min reaching the maximum at 60 min DT. The concentration of cineole and fenchol had the maximum at 1,5 min DT, whereas camphor and linalool acetate at 7,5 – 15 min DT. So, DT can be used as a simple tool for obtaining lavender oil with variable chemical profiles, therefore DT clearly has a direct impact on yield and composition.

Moreover, in 2013 a work from Avignon and Torino, performed a comparative study of the ability of a number of different methods to extract the essential oil from lavandin flowers in order to find the most advantageous in term of extraction kinetics, essential oil quality and quantity. The extraction of the essential oils was performed using eight different methods three classical and five innovative: hydrodistillation (HD), turbodistillation (THD), steam distillation (SD), ultrasound +steam distillation (US-SD), microwave: ISMH, EV in situ (MSD), MHG, MSDf [30].

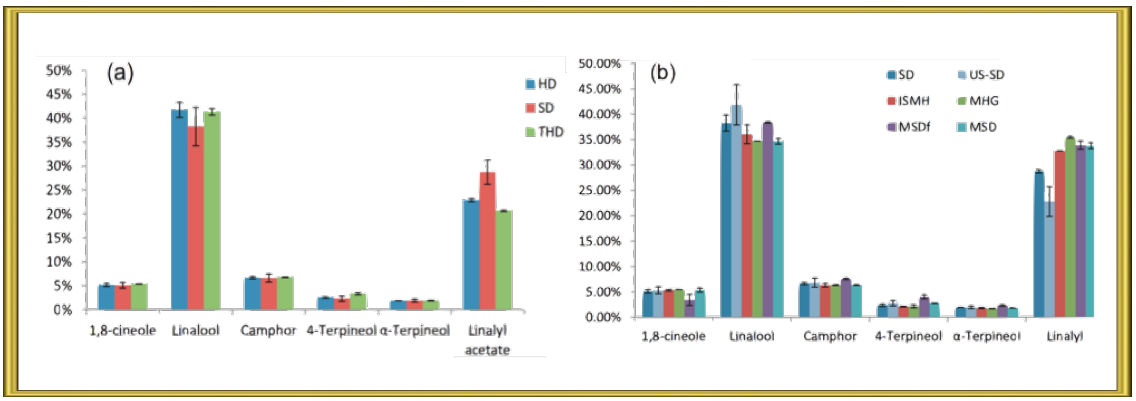

FIGURE 7 – Means of the compounds of essential oil of lavandin dried flowers, obtained by: (a) conventional techniques: hydrodistillation (HD); turbodistillation (THD); steam distillation (SD); and (b) ultrasound + steam distillation (US-SD); microwave: ISMH; EV in situ (MSD); MHG ; MSDf [30].

Results showed that the major components appeared using all the different methods employed, however quantities varied more or less according to the extraction technique used. Figure 7 (a) shows that essential oils obtained from HD and SD have similar composition except linalool and linalyl acetate. This could be as a consequence of linalyl acetate

degradation to linalool. However in SD, as lavandin has no direct contact with water, degradation is less marked, therefore it was thought that water could be the initiator of the formation of linalool. In Figure 7 (b), the essential oil obtained via microwave was compared to SD, therefore it can be seen that the quantity of linalyl acetate obtained in SD had higher values than microwave extractions. As parallel, the values of linalool from microwave extractions are lower than the ones from HD, therefore the degradation of linalyl acetate mentioned before appeared to be limited with microwave because lavandin had no contact with water [30].

6.3. PHARMACOLOGICAL ACTIVITY - ESSENTIAL OIL OF LAVANDER

As it has been stated, lavender oil has an important historical backgroung as a natural remedy for many pathologies and disorders, making it one of the most appreciated oils in phytotherapy and aromatherapy. The present section covers the biological and therapeutical properties of lavender found in various databases.
The biological activity of the essential oil of lavender is well known, as it has been investigated in several species including human beings, *in vitro* and *in vivo*. As early as 1920, several authors have reported on the sedative action of the aroma of lavender, therefore this project will analyse and comment various papers following a chronological order.

6.3.1. SEDATIVE AND CALMING PROPERTIES

6.3.1.1. Mode of action

In 1921, Macht and Ting published an article, which demonstrated the sedative action of various aromatic drugs and fumes. The first part of the experiment consisted on training 60 adult albino rats in a circular maze, which consisted of a series of concentric circular runways with communicating passages and *cul-de-sacs*, through which the animal was taught the way to the centre of the apparatus where food was placed. As a consequence of that, when their normal running time was constant and the number of errors were minimum, rats were treated with the drugs and the behaviour was again observed. The substances investigated were: tincture of valerian, asafetida, musk, lavender and extract of violets and oil of roses. The administration was with a glass funnel, where raw cotton with a few drops was placed on the neck of the apparatus. Control experiments were done in the same way, instead they had no drug or were impregnated with water and ethyl alcohol [31].

The results observed showed that the effect of violet perfume was not uniform, and the rest of odoriferous substances such as valerian and asafoetida, had a distinct sedative effect on the behaviour of rats. However, in the other preparation studied (such as lavender), the results were doubtful, as the amount of drug inhaled was much lower. As a consequence of that, the data obtained could not be related to the absorption of drugs into blood, instead it was more reasonable to assume that the sedative effect obtained was associated with the action of the substances divided into small particles on the olfactory organs. So the conclusion was that lavender produced some reflexes through the olfactory lobes, which played an important role on the sedative effects of this oil [31].

The sedative action of lavender was finally confirmed with the work of Buchbauer between 1991 and 1993, who like Macht and Ting, studied the effect of vapour [32] [33]. He found that 78 per cent in the motility of mice resulted from inhalation of ambient lavender oil for and hour, with similar effects for linalool (73%) and linalyl acetate (63%). However he investigated a more psychological action rather than pharmacological [34] [35].

Moreover, Lis-Balchin promoted a chain of experiments and investigations about the pharmacological effects of lavender. In 1996, her research group reported that lavender had a spasmolytic activity on guinea-pig ileum in vitro and in 1997, not only they observed spasmolytic action on rat uterus, but also a decrease on the tone of skeletal muscle in vitro [36] [37] [38].

Finally, in 1999, Lis-Balchin and her research group established that linalool and linalyl acetate were the oil compounds responsible for the spasmolytic action in guinea-pig ileum. They observed that a concentration of *Lavandula* essential oil produced a significant inhibition of the contraction and size of the smooth muscle induced by exogenous acetylcoline. This showed that the oil was not affecting either the nerve conduction or the release of acetylcoline, but it was acting directly on the smooth muscle, therefore, the oil had a postsynaptic effect [36] [39] .

In addition they saw that the concentration of *Lavandula* oil, which reduced the contraction of smooth muscle to acetylcholine, had a similar effect on the contraction due to exogenous histamine (Figure 8). If the essential oil had contained an atropine-like compound (reversible antagonist of the muscarinic receptors), the response to histamine would be unaffected (as histamine acts of H1 receptors and increases calcium levels): as this did not occur, it was concluded that the oil did not contain an atropine-like compound and that the smooth muscle was relaxing by some other mechanism [39] .

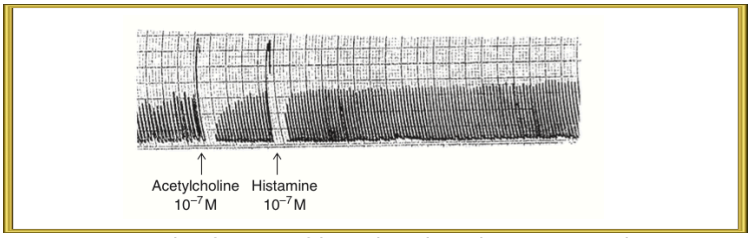

FIGURE 8 - Mode of action of lavender oil on the guinea-pig ileum *in vitro* effect of exogenous acetylcholine and histamine, indicating a similar effect and thus a postsinaptic activity. Arrows indicate addition of both substances [39].

Having reached this point, the inhibition of the electrically stimulated contractions of guinea-pig ileum appeared to be postsynaptic and not atropine like.

Moreover, they saw that the relaxation of the intestinal smooth muscle due to the adrenoreceptor activation, increased cAMP levels as a result of the stimulation of adenylate cyclase. cAMP is metabolized by phosphodiesterases, so they thought that the essential oil contained a substance which was capable to stimulate adenylate cyclase. So, when they experimented with various phosphodiesterase inhibitors such as trenquinsin, the spasmolytic

activity due to lavender's oil (linalool and linalyl acetate) was potentiated from 17.1 to 34,6 per cent [39].

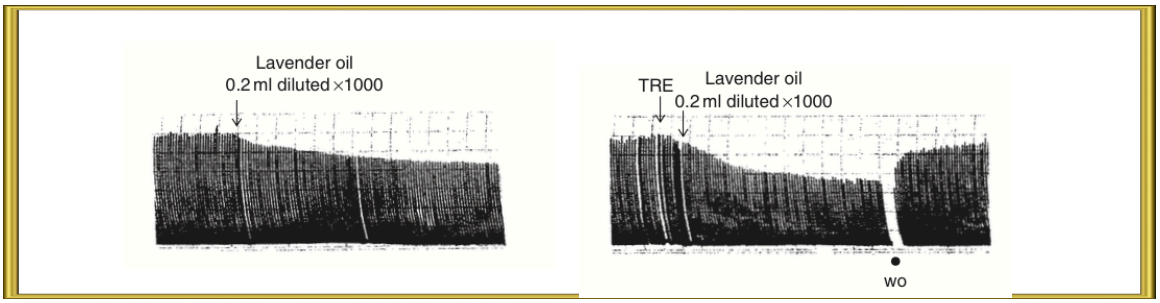

FIGURE 9 - Mode of action of lavender oil on the guinea-pig ileum *in vitro*: the enhancement of the spasmolysis following the application of trenquensin (TRE), a phosphodiesterase inhibitor, suggesting that cAMP is involved.

Intestinal smooth muscle contraction involves the influx of calcium ions through L-type channels, therefore if these channels were blocked, a spasmolityc effect on the intestinal smooth muscle would appear. As a consequence of that, it is therefore possible that the essential oils with a spasmolytic activity may contain components capable of blocking calcium channels.

6.3.1.2. Psychological mechanisms

The pharmacological mechanisms explained above do not involve any perception of lavender odour, therefore psychological effects have to be considered, as the compounds that form the odour can access to the body and acting directly on the brain through blood stream and absorption through lungs or olfactory mucosa. For instance, linalool and linanyl acetate, which are the main terpenoic components of lavender oil, due to the lipophilic character, have steric interaction with cell membranes that produces the suppression of electrical activity, which suggests a light sedative or anaesthetic effect.

This section examines the scientific research, to see if there is a basis to this effect, how it affects the brain and psychological states and whether these effects can be seen in practice. More specifically, psychological responses to odour may involve an interaction of an individual's experience, the particular situation and the individual's state of mind. This combination makes both interpretation and prediction of results difficult.

In 1999, an author named Aoshima, analysed the effect of various perfumes of lavender on GABAa receptors (expressed by mRNA in *Xenopus* oocytes from rat brain). They measured electrical responses of GABAa receptors, and concluded that the essential oil of lavender potentiated the GABAa receptor-response, as well as benzodiazepines, by increasing the affinity of GABA to the receptors (at low concentration of GABA, as high concentrations desensitization the GABAa receptors). The result is a anxiolytic, anticonvulsant and sedative effect as the aroma can penetrate skin and/or lung, reach blood and get through the blood-brain barrier (linalool and linalyl acetate were detected 20 min after administration) [40].

There have been several studies of the brain's responses to odours. these have used the changing electrical activity, picked up by electrodes, in response to lavender odours as a measure of brain activity (EEG). This type of measure has the advantage of being objective rather than subjective, although the interpretation of results may be complex. This changes in the frequency bands of EEG to lavender oil were examined by Diego in 1998, who confirmed an increase in frontal alpha and beat-2 waves, suggesting an increase of drowsiness. However he did not report a change in the theta waves, while other authors did. However the mechanism in which EEG patterns change is not really determined, as many individual factors influence, especially in real-life applications.

6.3.1.3. Evidence of sedative and calming activity

Quantitative human studies on the effect of inhaled essential oils on stress, anxiety and sleep disturbances have been published since 1990 to nowadays, which contrast the mode of action seen above. Therefore, some of the most important studies will be briefly analysed in order to confirm this sedative and anxiolytic effect described.

- **Sleep, mood, anxiety and relaxation**

In 1998, Diego (from the University of Miami), carried out a study on which they examined aromatherapy effects of rosemary and lavender on relaxation, anxiety, mood, EEG activity and math computations. 40 subjects were seated on a special massage chair with a piece of cotton 3 inches from their nose for a period of three minutes (three drops). Subjects were instructed to breathe normally through their noses quietly and with their eyes closed [41].
The people studied had to respond to a questionnaire before and after the inhalation which evaluated the different parameters (Table 6) [41].

Measure	Lavender N =20		Rosemary N= 20	
	Pre aromatherapy	Post aromatherapy	Pre aromatherapy	Post aromatherapy
State anxiety	34,32	31,21	33,30	26,30
Depressed mood	2,66	1,16	1,45	1,74
Tense-relaxed	6,10	7,68	7,00	8,70
Drowsy-alert	6,16	5,95	6,00	7,30

TABLE 6 – Comparison of means obtained from the results of the questionnaire, of the effects of the two treatment groups before and after aromatherapty, lavender and rosemary on anxiety, depression, tension and

Lavender group reported feeling more relaxed, more sleepy and with slight drowsiness (as Buchbauer [33]). Rosemary on the other hand, considered an alerting aroma, produced the contrary effect.

In order to evaluate the brain activity, EEG was recorded for a three-minute period before, during and after the aromatherapy session with the eyes closed. They also submitted participants to math computations in order to evaluate the alertness effects attributed to rosemary [41].

The results supported previous findings on lavender's effect: the lavender group revealed an increased frontal alpha power, suggesting drowsiness, while the rosemary group had a decreased alpha power, suggesting alertness. The math computation results showed that both groups, lavender and rosemary, performed the computations faster after the aromatherapy session, but the lavender group had more accuracy, therefore it was thought that the lavender group was more relaxed and so they were more able to concentrate [41].
This research indicates that the aromas can effect in a psychological or physiological way, however, further research has to be made in order to understand the mechanisms of these effects.

- **Sleep deprivation in hospitalized patients**

Sleep deprivation in hospitalized patients is common and can have serious detrimental effects on health and illness recovery; that's why a group of nurses in Baltimore (2014), carried out a pilot study in an intensive medical care unit (IMCU). They saw that sleep disruption was very common not only in patients with a higher disease severity but also in patients who die. Therefore they studied the effectiveness of lavender scent on vital signs (blood pressure, heart beat, respiratory rate and oxygen saturation) and sleep quality [42].

Fifty patients were selected, 25 of them (control group) received usual care, while the treatment group received usual care and 3ml of pure essential oil of lavender in a small glass within 1m of the patient from 10 $_{PM}$ until 6 $_{AM}$. Aromatherapy was maintained throughout the night and vital signs were measured at the start of the intervention, then at 4-hour intervals throughout the night, and at the end of the intervention. At 6 $_{AM}$ patients were asked to complete the Richard Campbell Sleep Questionnaire [42].

The results showed that vital parameters followed similar trends in both groups. However the treated group experimented a decrease in blood pressure whereas the control group increased. This pattern was clearly pronounced from 10 $_{PM}$ to 4 $_{AM}$ [42].

Item	Control (N=25)	Intervention (N=25)
Deep/light sleep	41,44 (32,50)	52,60 (34,09)
Ease of falling sleep	36,92 (30,83)	47,76 (34,41)
Awakenings	46,36 (34,61)	46,24 (35,47)
Ease to return to sleep	36,20 (33,22)	49.48 (37,22)
Quality of sleep	39,56 (32,52)	45,16 (38,99)
Overall sleep score	40,10 (23,42)	(48,25 (32,09)

TABLE 7 – Comparison of scores from the two treatment groups, for Richard Campbell Sleep Questionnaire. Adapted table [42].

In Table 7, patients who had aromatherapy reported a higher quality of sleep than patients in the control groups; however the difference between the two groups was not significant. This finding may be related to the size of the sample and the fact that most of subjects were women (as sleep is affected by sex-related factors). Another limitation is that the quantity inhaled is unknown and the questionnaire can only be formulated after sleep time, so sleep was only measured once [42].

- **Anxiety in waiting room for plastic surgeon**

Cosmetic plastic surgery improves the appearance of people with congenital malformations, disfiguring wounds, burn injuries… and is growing the number of people who chose this

option in order to correct this aesthetic problems, which can cause serial psychological damage. However, this type of interventions are attached to a pre-operative anxiety and tension.

Scent as an environmental feature has been studied extensively in order to avoid this unpleasant sensation before undergoing any type or surgery; therefore a research group studied the influence of ambient scent and music on patients. Eight scents were included: vanilla, lavender, mint, lemon grass, rose, magnolia, orange and mango, some of them with relaxing effects (vanilla and lavender) and some with the opposite effect (mint). They randomly selected 21 participants, who were asked to smell the oils from the bottle and evaluate how pleasant and relaxing on a 5-point Likert-scale they found the smells. Between scents, they had to smell their own skin in order to neutralize. The results on Figure 10 show that the scent that corresponds to lavender was the most pleasant and relaxing from all the scents studied [43].

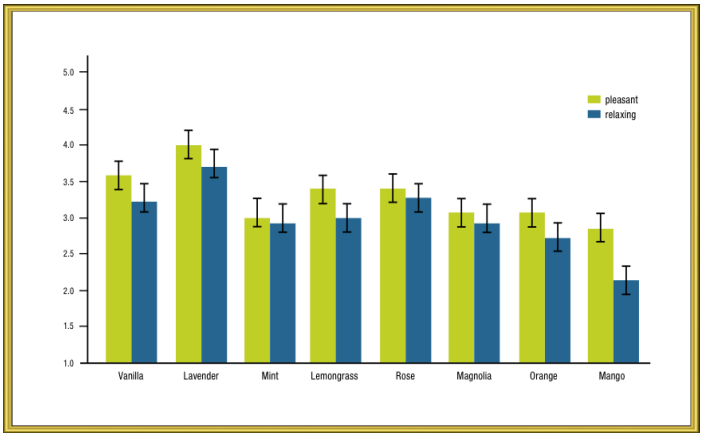

FIGURE 10 – Comparison of the means pleasantness and relaxation ratings of the diferent scents studied [43].

- **Dental anxiety**

An extremely recent study from India (May 2015) studied the possible deal with lavender fragrance on dental patient anxiety. Fear for dentists and dentistry is a common and potentially distressing problem, both for public and dental practitioners. Dental anxiety is managed with conscious sedation or general anaesthesia, but this not only carries some risk, but also interactions with other medications and allergies. Therefore aromatherapy is an alternative medical approach, which includes absorbed or ingested essential oil for prophylactic treatment. So the goal of the study was to learn about the effect of lavender essential oil on anxiety levels of patients awaiting dental procedures in a dental office [44].
They used a candle warmer with essential oil diluted (1:1) with water and placed it in the waiting room, whereas the other group normal water was used. After 15 minutes, patients were asked to fill a questionnaire consisting of five psychometric questions, which the total score goes from 5 to 25. Table 8 contains results after applying t-student [44].

Characteristic	*Lavender* group	Control group	*P* value*
Mean anxiety scores	11.74±4.10	15.40±4.18	0.001
Age groups (years)			
15-24	15.56±4.16	14.00±3.57	0.411
25-34	12.07±3.79	14.20±3.29	0.145
+35	10.23±3.43	16.43±4.52	0.002
P value**	0.002	0.144	-
Sex			
Male	10.538±3.20	14.142±4.38	0.014
Female	13.041±4.62	17.000±3.35	0.030
P value**	0.029	0.015	-

TABLE 8 – Comparison between means of age groups and sex of the anxiety scores from the results of a questionnaire between lavender and control group [44].

They observed that patients exposed to lavender had less anxiety respect the control group and, as the age increased, anxiety scores significantly decreased in this group. Moreover, females showed a pronounced decrease in anxiety levels than males.

To conclude, the study demonstrated that lavender scent in patients could cause a decrease of dental anxiety below the level of phobia [44].

- **Anxiety, stress and depression in pregnancy**

Studies show that anxiety and stress in pregnancy can result in foetal growth retardation, preterm delivery, increased nausea and vomiting, preeclampsia, antenatal depression and anxiety and other negative effects.

A research group from Iran, tested the effect of lavender cream and footbath on anxiety, stress and depression in pregnant women, as many studies demonstrate that lavender is harmless, with no reported adverse events in non-pregnant women [45].

They recruited 141 pregnant women who were divided into three groups (N=47 each). One group applied lavender cream and footbath, the second group only cream, and the last one the placebo cream. All participants were instructed verbally and written on how they had to apply the cream and the footbath, even though participants and investigators didn't known what contained the tubes that were handled to patients. Anxiety, stress and depression were assessed using a self-administered questionnaire at three time points: baseline, 4^{th} and 8^{th} week after intervention [45].

Variables	Lavender & footbath n=47* Mean (SD)	Lavender n=47 Mean (SD)	Placebo n=47† Mean (SD)	Difference between groups P
Anxiety (0-21)				
Baseline	4.12 (2.89)	5.31 (3.11)	5.02 (2.71)	0.11
4th week follow-up	3.47 (3.56)	3.27 (3.32)	5.44 (3.62)	0.002
8th week follow-up	2.52 (2.29)	2.76 (3.14)	4.30 (2.520	0.003
Stress (0-21)				
Baseline	5.93 (3.25)	6.29 (3.50)	7.21 (3.63)	0.18
4th week follow-up	5.84 (3.54)	6.34 (3.93)	8.57 (3.74)	0.006
8th week follow-up	5.28 (3.34)	5.82 (3.56)	8.74 (2.94)	< 0.001
Depression (0-21)				
Baseline	3.68 (2.82)	3.91 (3.48)	4.80 (3.16)	0.19
4th week follow-up	3.86 (3.36)	4.08 (3.38)	5.73 (4.10)	0.10
8th week follow-up	2.15 (2.32)	3.12 (2.84)	5.79 (3.18)	< 0.001

TABLE 9 - Comparison of anxiety, stress and depressions scores at diferent time points(baseline, 4th week and 8th week after treatment) in the three study groups (Oral lavender plus footbath, oral lavender or placebo). Table adapted [45].

These findings showed that the level of anxiety, stress and depression in both lavender and foot- bath and lavender alone groups were lower than the placebo group in both 4th and 8th weeks. Adding foot-bath did not significantly improve effectiveness compared to the use of lavender cream alone.

Therefore the results of the study showed that anxiety, stress and depression in pregnant women could be improved using lavender cream with or without foot-bath.

- **Anxiety disorder; double-blind comparison to placebo and paroxetine**

The generalized anxiety disorder (GAD) is defined by excessive anxiety and worry for at least 6 months. Patients at least have three of the six anxiety symptoms: restlessness, fatigue, difficulty concentrating, irritability, muscle tension and sleep disturbance. Strongest evidence of clinical efficacy in the treatment of GAD has been with selective serotonin reuptake inhibitors, benzodiazepines… however this drugs have been ranked as a secondary choice, due to the unfavourable tolerability profile.

Therefore in 2014 a research group from Germany and Austria, wanted to demonstrate the effectiveness of Silexan in front of placebo and paroxetine on GAD [46].
Silexan is an active substance manufactured by Dr Williams Schwabe in Germany, prepared from the fresh flowering tops of *Lavandula angustifolia* by steam distillation for oral use. The main constituents of this product are linalool and linalyl acetate, which account for about 70% of the ingredients [47].

536 patients were randomized into 4 groups: Silexan 160mg, Silexan 80mg, Paroxetine 20mg, Placebo. Subjects were instructed to swallow one unchewede capsule per day during 10 weeks. They measured anxiety with HAMA (Hamilton Anxiety Scale) before and after the treatment in order to compare effectiveness of Silexan [46].

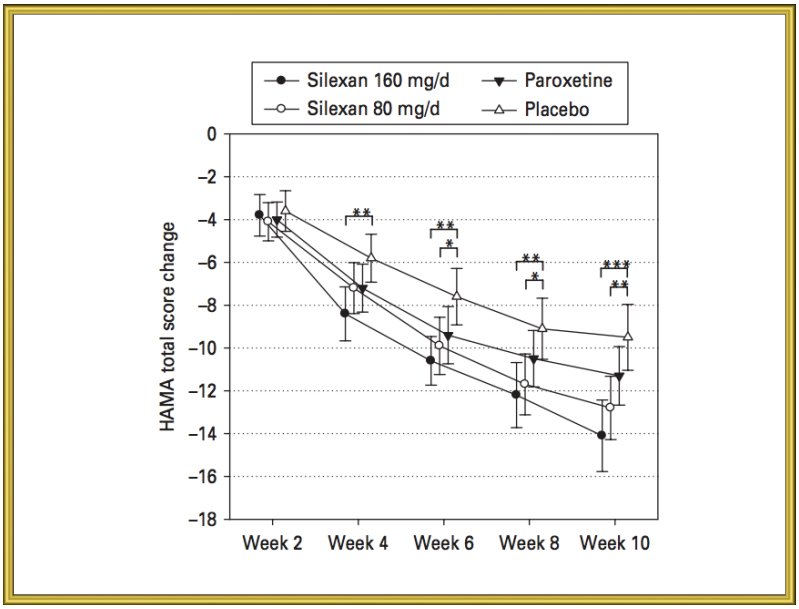

FIGURE 11 - HAMA(Hamilton Anxiety Scale) total score change for the diferent study groups(Silexan 160mg/d, Silexan 80mg/d, paroxetine or placebo); two-sided t-tests: *P<0,05, **P<0,01, ***p<0,001 [46].

The study groups showed a monotonic decrease of HAMA total score that was most pronounce in the Silexan treatment groups, followed by paroxetine and placebo (Figure 11). Therefore the differences between the different slopes tended to increase over time.

Silexan 160mg was significantly inferior to placebo after 4-week treatment while Silexan 80mg became significant at week 6, however both of them remained significant until the end of treatment. For paroxetine a borderline significant difference to placebo was observed after 6 weeks.

In conclusion, the trial demonstrated that Silexan had not only, a superior efficacy over placebo but also the same effectiveness as paroxetine (one of the drugs currently recommended as first-line treatment in GAD), this was because the three treatment groups had approximately the same percentage of patients that responded to the treatment [46] .
In this experimental setting, Silexan demonstrated convincing anxiolytic efficacy, therefore this product could be a tolerated option in the treatment of GAD.

6.3.2. ANTIMICROBIAL PROPERTIES

Traditionally, lavender and his extracts have been used in foods, beverages and pharmaceutical/cosmetic products for their antimicrobial and antioxidant properties, however there are relatively a few reports on the antimicrobial properties of lavender, due mainly to this modest activity.

6.3.2.1. Mode of action

- Antibacterial mode of action:

Even though the mechanistic process is poorly understood, investigations of the mode of action of terpenoids against bacteria were held by Knobloch, who suggested that the site of action appeared to be at the phospholipid bilayer in 1986. He established that terpenes caused disorders in protein translocation, phosphorylation steps and electron transport (which phospholipids catalyse). However the activity in whole cells was studied in 1988, and

appeared to be more complex, as they had to consider not only the structure of the terpene but also the chemical structure of the cell wall [3].

- Antifungal mode of action:

In many investigations, the antifungal action of lavender has been more effective in the inhibition of conidial germination than of radial growth.
Conidial germination is the first vital step in the sequence of events which leads to the establishment of a germ-tube and subsequent hypha. It all starts with hydratation followed by the action of lytic enzymes. This makes the conidial cell wall break, and permits the emergence of the germ-tube initial, and therefore there is a hypha extension. Mc. Ewan in 1994 established that the components of lavender volatile oil produce a disruption in these events, which produces an unsuccessful conidial germination [3] [48].

6.3.2.2. Evidence of antimicrobial activity

- **Antibacterial activity**

In one of the larger studies in this field, Deans and Ritchie in 1987 studied the antibacterial activity of fifty volatile oils at four different concentrations against a range of twenty-five genera of bacteria. They soaked discs with herb extracts and placed them on a surface of agar seeded with yeast. They punched the agar creating wells of 10μl to control the volume of oil. In the case of lavender, after measuring the inhibition zone, it was found to be most effective to the faecal indicator organism *Enterococcus faecalis*. In contrast, with *Klebsiella pneumoniae,* its presence resulted to enhance the growth [49].

- **Antibacterial and antifungal activity**

The results from above were obtained using the oil with all the compounds, that's why in 1998 Lis - Balchin and her research group got deeper into the matter, due to the large variety of "lavenders" found in the market, which differ not only in name, but also in chemical composition. They established the bioactivity of 105 commercial essential oils against 25 species of bacteria, 20 strains of *Listeria monocytogenes* and three filamentous fungi [50].

Essential oil	Linalol (%)	Linalyl acetate (%)	Antibacterial activity:[a] No. affected	Antifungal activity[b] *Aspergillus niger*	*Aspergillus ochraceus*	*Fusarium culmorum*
Lavender 1	29.7	42.8	19	82	90	79
Lavender Bul	51.9	9.5	23	84	29	8
Spike lavender	43.1	4.0	19	93	58	31
Lavender Fr 1	26.1	47.9	16	93	58	31
Lavender Fr 2	29.1	43.2	13	57	44	77
Lavandin	28.7	39.4	17	93	86	69
Lavender Bul[c]	2.3	79.8	22	74	84	89

TABLE 10 - Correlation between high linalol or linalyl acetate content of commercial essential oil of the genus *Lavandula* and bioactivity. Fr= Frenche. Bul= Bulgarian. [a]antibacterial activity tested against 25 diferrent bacteria. [b]antifungal activity calculated in Table 1 from paper[50]. [c]extracted by supercritical carbon dioxide .Table adapted [50].

Results showed similarities with the results of Deans and Ritchie, but the biological action was in fact very variable, but not correlated with the major oil components.

They also saw that spike lavender was equally as active against the 25 bacteria as the first lavender sample, but the linalool and linalyl acetate content were widely different.

The two Bulgarian lavender oils obtained by different means (steam distillation vs supercritical carbon dioxide), showed a high and similar antibacterial activity but different antifungal activity, which suggested that different oil components may be responsible for the activity against different microorganisms.

Therefore Lis-Balchin's study concluded that the components of the essential oil, which showed variable though frequently high effectiveness, were linalool, linalyl acetate, 1,8-cineole and many monoterpene hydrocarbons. This point was also found in a study of Pattnaik in 1997, who found that linalool could inhibit 17 of 18 bacteria and 10 of 12 fungi [50] [51].

Name on bottle	Linalool	Linalyl acetate	Anti-*Listeria* activity*
A Lavandin	28·7	39·4	1
B Lavender (French)	26·1	47·9	4
C Spike	43·1	4·0	12
D Lavender (Bulgarian) CO_2	2·3	79·8	18
E Lavender (Bulgarian)	51·9	9·5	11
F Lavender (French)	29·1	43·2	0
G Lavender	29·7	42·8	1

TABLE 11 - Anti- *Listeria monocytogenes* action of diferent lavender samples related to the chemical composition of linalool and linalyl acetate. *number of strains inhibited [50].

Results were really spread, when studying anti-listeria activity, as they were really different between samples, ranging from 0 to 18 strains (out of 20). The highest activity was found for the Bulgarian lavender (extracted by supercritical CO_2), which had the highest linalyl acetate concentration. However other lavenders and lavandin which had more proportion of linalyl acetate, didn't show effectiveness.

Spike lavender and the other Bulgarian lavender sample were lower in activity but had high linalool contents.

So this study indicated a considerable variation in the activity of the "same" volatile oil against the 20 strains, which totally coincided with Aureli's work.
This indicates the importance of analysing each bath of commercial oils, as the name given on the sample container may differ with the real chemical composition. The adulteration of essential oils with synthetic components may rise the proportion of enantiomers for a large number of components than in pure botanical samples: This can greatly influence the bioactivity.

Although the *in vitro* activity of lavender shows antimicrobial activity, no *in vivo* study has been intensively investigated, as the clinical trials are both limited and inconclusive. Ferley in 1989, investigated the potential of essential oils in preventing infection in chronic bronchitis

patients, administering a prophylactic dose of 20 drops of an oil mixture (mint, clove, thyme, cinnamon and lavender), three times a day for five months. The overall number of supervening infections and their characteristics were not modified by the treatment, but the frequency of relapse was significantly lowered in the treated group [48] [52].

6.3.3. ANTINOCEPTIVE PROPERTIES

6.3.3.1. Mode of action

In 2008 a research group from Brazil, studied the involvement of glutamate receptors on the antinoceptive effect of linalool, confirming the theory of Elisabetsky [53] [54].

Glutamate Receptors play an important role in pain; therefore they injected mice 20μl of glutamate intraplantarly and they stayed under observation for 15 minutes, and measured the amount of times the animals spent linking or biting the injected paw. Mice were pre-treated with linalool intraperitoneal (i.p.), oral (p.o.), intrathecal (i.t.) and intraplantarly) [54].

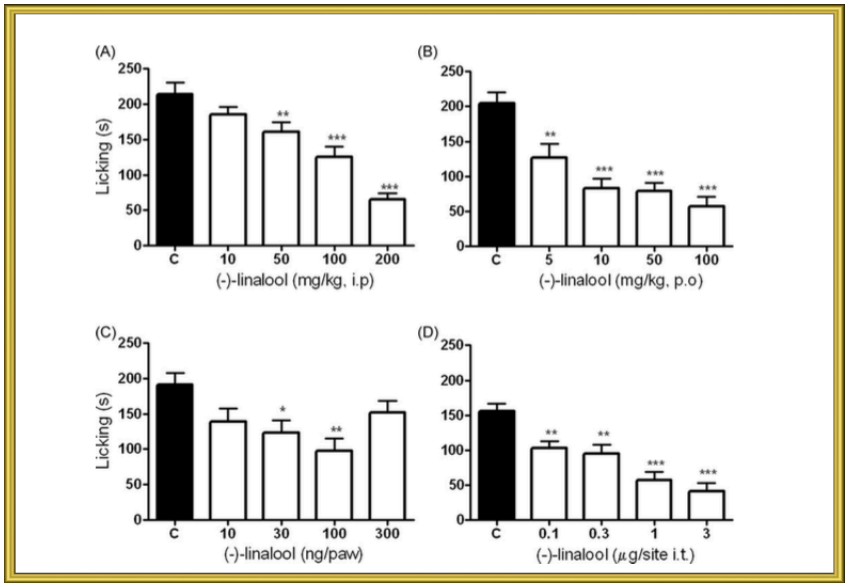

FIGURE 12 - Effect of linalool administered intraperitoneally (A), orally (B), intraplantarly (C) and intrathecally (D) against liking induced by intraplantar injection of glutamate in mica. Each column represents the mean of six to eight animals. C represents the control animal (injected with saline) and asterisks denote the significance levels *P<0,05, **P<0,01 and ***P<0,001 compared to the control groups (ANOVA) [54].

Results showed that A, B and D caused a significant and dose-dependent inhibition of glutamate-induced nociception, while intraplantar treatment with linalool partially inhibited pain.

However, a second experiment was done in order to detect which receptor involved. Glutamate receptor are divided into two major classes:

- Ionotropic receptors: NMDA (N-methyl-D-aspartate), AMPA (α –amino-3-hydroxy-5-methyl-4-isoxazolepropionate) and kainate receptor.
- Metabotropic receptors: coupled to GTP-binding proteins which modulate the production of second messengers [55] .

Once they confirmed the effect of linalool on pain, they carried out a second experiment, using ionotropic and metabotropic agonists of excitatory aminoacids (EAAs) receptors, administered intrathecally to mice, which previously received linalool i.p. [54].

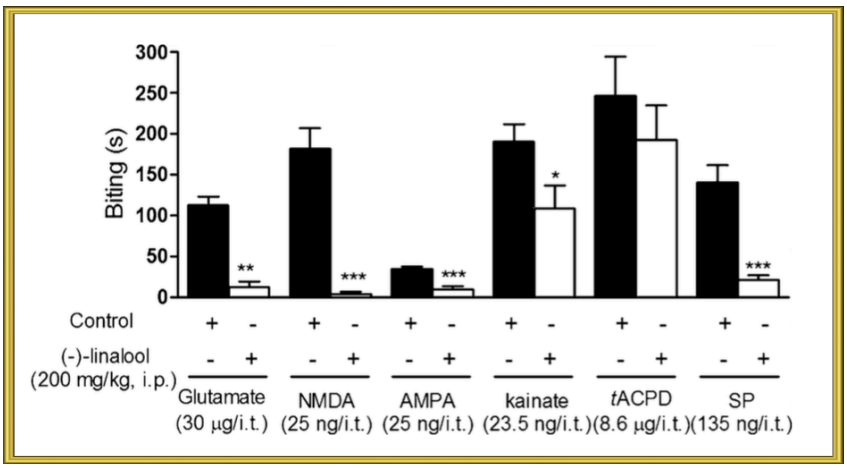

FIGURE 13 - Effect of linalool i.p. on the bitting reponse caused by intrathecal injection of glutamate, NMDA,AMPA, Kainate, trans-ACPD and Substance P in mice. Each column represents the means of six to eight animals and asterisks denote the significance levels: *P<0,05, **P<0,01 and ***P<0,001 when compared to control group values (ANOVA) [54].

Researchers evaluated the biting behaviour and from the figure below concluded, that linalool seemed to be related with ionotropic glutamate receptors. Concretely, linalool produced a significantly attenuated biting response induced by glutamate, and NMDA and, to a lesser extent, AMPA and kainite.

In summary, the antinoceptive effect of linalool was mediated by the interaction with ionotropic glutaminergic receptors, via NMDA receptors.

6.3.3.2. Evidence of antinoceptive activity

- **Dysmenorrhea and amount of menstrual bleeding**

Dysmenorrhea is one of the most commonly reported symptoms by adolescents and adult women in gynaecologic departments. Many of these women suffer from a number of physical and emotional symptoms such as: nausea, abdominal pain, weakness…

A research group from Iran investigated the effect of inhaled lavender oil on dysmenorrhea and other menstrual symptomatology. 96 female students aged between 18 and 28 years old were divided into two groups: the intervention group, which used lavender oil (*L. angustifolia*) diluted in sesame oil in a 2:1 ratio, and the placebo group, which only used sesame oil. Students were instructed on how to apply the oil: 3 drops on their palms, rub them together and keep their hands at a distance of 7-10cm from the nose and inhale for 5 minutes. This treatment was administered 1 hour after experiencing dysmenorrhea. They were asked to use the treatment every 6 hours for the first three days [56].

In order to evaluate the efficacy of lavender oil, a questionnaire had to be answered before and after the treatment. Results are shown on Table 12 [56].

Symptoms	Control group (%)			Lavender group (%)			OR[a] (95% CI)[b]
	None	Mild	Moderate	None	Mild	Moderate	
Abdominal pain and backache	26	4.2	69.8	31.2	42.7	26	6.8 (2.9, 15.9)
Tiredness	21.9	14.6	63.4	33.3	47.9	18.8	12 (4.6, 31.5)
Vomiting	46.9	46.9	6.2	37.5	41.7	20.8	8.7 (3.2, 23.3)
Nausea	58.3	29.2	12.5	40.6	40.5	18.9	11.5 (4.6, 29)
Weakness	25	37.5	37.5	28.1	52.1	19.8	6.48 (2.6, 15.6)
Diarrhea	49	41.7	9.4	37.5	41.7	20.8	6.6 (2.8, 15.4)
Headache	28.1	65.6	6.2	39.6	43.8	16.7	12.6 (4.9, 32.4)
Mood change	24	65.6	10.4	34.4	42.7	22.9	5.6 (2.3, 13.6)
Faint	24	65	11	33.3	50	16.7	16.2 (4.9, 53.8)
Hot flushing	24	67.7	8.3	32.3	51	16.7	9.1 (3.2, 25.5)
Nasal congestion	31.2	63.5	5.2	38.5	43.8	17.7	8.5 (3.2, 22.1)

[a] For example, the odds of abdominal pain and backache in the control group was 6.8 times that of in the lavender group
[b] If the 95% CI excludes the value of 1 is statistically significant.

TABLE 12 - Percentage of students in each level of symptoms for the lavender group and the control groups. Along with the OR (95% CI) using ordinal logistic regression for each symptom [56].

The study showed that lavender inhalation had a positive effect on the severity of dysmenorrhea. Moreover the findings showed that students in the lavender group experienced lower dysmenorrhea in a noticeable way.

- **Migraine headache**

In 2011 a research group from Iran and Germany studied for the first time a placebo controlled clinical trial in order to evaluate the effects of lavender's essential oil in the management of migraine headache. 47 patients with migraine were divided into cases and controls. The intervention group had to rub 2-3 drops onto their upper lip and inhale its vapour for 15 minutes, whereas the control used liquid paraffin. They were asked to score the severity of their headaches at 30-minute intervals up to a total of two hours according to Visual Analogue Scale (VAS) [57].

Results showed that in the intervention group, there was a significant reduction in the severity of headache in the first to the sixth headache, whereas the control group, the reduction was significant but only in the first to forth attack. In addition from the 129 headache attacks in the intervention group, 92 responded totally or partially to lavender. All responders had a total o partial reduction of headache in the first 2 hours of lavender use. Moreover, 74% of patients had an improvement in their symptoms, whereas the control group only a 58% of patients reported a decrease in the symptomatology [57].

To conclude, the present work reported a significant positive effect of lavender oil on the reduction of headaches severity.

6.4. PRODUCTS CONTAINING LAVANDER

René Maurice Gattefossé, considered nowadays the father of aromatherapy, created this term in 1928. He accidentally discovered the curative effects of the essential oils in his laboratory, as a result of a tremendous explosion. He burned his hand and submerged it in essential oil of lavender, which caused a sensation of relief. However René also saw that the injuries were rapidly cured and healed. As a consequence of that, he studied the effect of essential oils for many years [4].

6.4.1. PRANARÔM INTERNATIONAL

Pharmacist by profession and passionate about essential oils, Dominique Badoux heads **Pranarôm international** since 1991. Thanks to his commitment to develop aromatherapy with scientific rigor, has managed to convert Pranarôm in a leader of this sector. Pranarôm international offers a wide variety of products for different purposes, which contain essential oils. Therefore, some of them, which contain essential oil of the genus *Lavandula,* will be briefly described in this section.

6.4.1.1. PRANARÔM- CHEMOTYPED ESSENTIAL OILS

This laboratory offers a selection of 60 essential oils biologically certified (which proceed from ecological agriculture). These oils are: 100% natural: they do not contain any molecule of semi or total synthesis, 100% pure: not mixed with other vegetal oils or alcohols, and 100% integrate: without decolouration, rectifications peroxidation and elimination of compounds [58].

- **LAVANDA – *Lavandula angustifolia ssp angustifolia***

10 ml

Distillation	Therapeutic properties
Flowering top	➢ Sedative, hypotensive and soothing ➢ Antiseptic and healing ➢ Antispasmodic
Composition	
Terpene alcohols: 28,97% - Linalool **Terpene esters**: 29,75% - Linalyl acetate	

Advice for use

- **Nervous system:** 1 to 3 drops per day (depending on the indications). 3 to 4 drops on the solar plexus, cuffs and sole.
- **Cutaneous infections**: apply on affected zone several times a day.
- **Sleep disorders and stress:** 2 drops on the reverse of the pyjama's collar or pillowcase.

Indications

- **Problems of nervous origin:** spasm, insomnia, anxiety …
- **Infectious dermatitis**: sores, burns, eczemas, mycosis…
- **Allergic dermatitis:** pruritus, mosquito bites…
- **Skin disorders:** Acne, psoriasis, cuperosis, rosacea…

TABLE 13 – Squematic table about Lavanda – Pranarôm International [4] [58] [60] [61] [65].

- **ESPLIEGO – *Lavandula latifolia = Lavandula Spica***

10 ml

Distillation	Therapeutic properties
Flowering top	➢ Antimicrobial ➢ Fungicide ➢ Antiviral and immunostimulant ➢ Analgesic and anti-inflammatory ➢ Antitoxic ➢ Expectorant
Composition	
Terpene alcohols: 42,12% - Linalool **Terpene oxides:** 23,86% - 1,8-cineole **Terpene ketones**: 11,78% - Camphor	

Advice for use

- **Dermatological affections:** 1 to 2 drops applied several times on skin.
- **Respiratory affections:** 3 to 4 drops in vegetal oil → apply on thorax and massage.
- **Poisonous bites:** 3 drops every 5 minutes during half and hour.

Indications

- **Skin disorders:** burns, injuries and acne
- Poisonous bites
- **Respiratory infections**: otitis, sinusitis…
- **Chronic inflammatory affections**: arthritis

TABLE 14 - Squematic table about Espliego – Pranarôm International [4] [58] [60] [61] [66]**.**

- **LAVANDÍN SUPER – *Lavandula Hybrida CT super = Lavandula burnatii CT super***

10 ml

Distillation	Therapeutic properties
Flowering top	➢ Antispasmodic ➢ Antimicrobial ➢ Anti-inflammatory ➢ Healing ➢ Sedative, relaxing and soothing
Composition	
Terpene alcohols: 34,17% - Linalool **Terpene esters**: 36,41% - Linalyl acetate	

Advice for use

- **Pain:** 2 to 3 drops 3 times per day applied externally.
- **Anxiety and sleep disorders:** 3 drops on cuffs, solar plexus or sole or atmospheric diffusion 30 minutes before going to sleep.

Indications

- Anxiety and sleep disorders
- **Skin disorders:** edema, cutaneous abscesses, burns, and injuries.
- Muscle cramps and contractures
- Depressive states, migraines…
- **Mycosis:** nail, feet…

TABLE 15 – Squematic table about Lavandín Super – Pranarôm International [4] [58] [59] [60] [61].

6.4.1.2. PRANARÔM- AROMANOCITS

- **SPRAY SUEÑO Y RELAJACIÓN BIO (Spray sleep and relaxation BIO)**

100 ml

Composition	Effects
Sweet orange Bitter orange Lavandin super Lavanda Mandarine Roman chamomile Bergamota	➢ Relaxing ➢ Sleep ➢ Facilitates sleep ➢ Prevents nightmares and negative thoughts
Other information	
	Pregnant women Children older than 1 year old

Advice for use

- **Atmosphere:** 15 minutes before sleep, pulverise for 4 seconds in all the room.
- **On sheets:** 15 minutes before sleep, pulverise for 4 seconds at a distance of 20cm on the pillowcase, pyjama or a piece of cloth next to the bed.

Both ways can be combined o alternate.

TABLE 11 – Squematic table about Spray sueño y relajación – Pranarôm International [58] [61]**.**

6.4.1.3. PRANARÔM- MECLA PARA DIFUSIÓN (spreading mix)

- **PROVENCE**

30 ml

Composition	Effects
Lavandin Lemon Eucalyptus Rosemary	➢ Relaxing ➢ Sleep ➢ Facilitates sleep ➢ Prevents nightmares and negative thoughts
Other information	
	Forbidden children less than 2 years.

Advice for use

Dilute some drops on a small amount of water inside the diffusor (quantity of drops depends on the model of diffusor used).

Diffusors have ultrasonic waves which produce dispersion of micro particles of the product and produces:

- Purification of the air
- Humidifies the air
- Improves the olfactory characteristics of the environment

TABLE 12 – Squematic table about Provence – Pranarôm International [58] [61]**.**

6.4.1.4. PRANARÔM- OLÉOCAPS

- **OLÉOCAPS 7 – SUEÑO Y ESTRÉS**

30 capsules

Composition	Effects
Vegetal oil of canola Mejorana Lemongrass Lavandín Mandarine	➢ Relaxing ➢ Calming ➢ Mental equilibrium in stress ➢ Sleeping

Other information

+6años

Forbidden pregnant women.
No more than 9 capsules / day.
No addiction

Advice for use

- **Prevention:** 1 to 2 capsules per day before meal or 2 capsules 1 hour before the stressing event.
- **Calming action:** 1 capsule three times a day before meals, for a period of maximum 20 days. Repeat if necessary after a period of 10 days without treatment.
- **Sleep disorders:** 2 capsules 30 minutes before sleep

TABLE 13 – Squematic table about Oléocaps 7 Sueño y estrés – Pranarôm International [58] [61].

6.4.2. WELEDA

In 1920 Dr. Ita Wegman, a chemical Oskar Schmiedel and Dr. Rudolf Steiner, started to investigate about the anthroposophy medicine and created the first medicines, that were formulated with the same ideals as Weleda has nowadays. These medicines were elaborated exclusively from natural substances, which stimulated the forces of the body to beat illness [62].

- **BAÑO RELAX DE WELEDA**

200 ml

Composition	Effects
Lavender oil Limonene* Linalool * Geraniol* *from natural essential oils	➢ Anti stress ➢ Relaxing ➢ Calming

Other information

Do not use more than half an hour.
Ideal water temperature: 37-38ºC.

Advice for use

Shake before use. Put 2 or 3 taps full of product in a full bath (in order to prevent evaporation of the product). It can also be used in the shower applying the product on a sponge.

TABLE 14 – Squematic table about Baño relax – Welleda [62][67].

7. CONCLUSIONS

From a personal standpoint, the elaboration of this project has allowed me to not only improve my knowledge on the many scientific databases available to obtain the necessary information for the development of this paper, but it has also helped me to acknowledge the importance of a reliable bibliographic research in order to ensure a solid scientific base. In addition, I have come to understand the difficulties of writing a scientific article in relation to necesssary documentation and bibliographical research prior to its publication. Furthermore, throughout the entire process of drafting this memory, I was given the chance to apply and combine the main contents of all three areas of knowledge by linking them through a common transversal concept.

As for the content of this paper, I would like to point out the different species which form the genus *Lavandula* and the hybrids present different oil compositions due to several factors such as genotype, seasons and extraction method. For instance, *L. angustifolia* and *L. hybrida* had a major proportion of the monoterpenes linalool and linalyl acetate than *L. latifolia*, which was rich in 1,8-cineole, even though the three of them belong to the same genus. Moreover, flowering stage made the oil richer in monoterpenes than the fruiting stage due to the loss of flowers and seeds during this phonological stage, which demonstrated that seasons had a direct impact on the chemical configuration. Something similar happened with the extraction of the essential oil, as the technique employed and the performance time affected both yield and composition.

As for it's practical uses, lavender oil demonstrated clinical evidence for not only sedative and calming properties but also antimicrobial and antinoceptive effects. Evidence of this lies upon the mode of action of the oil: The sedative effect of linalool and linalyl acetate was due to several mechanisms: an increase of cAMP, blockade of calcium channels, potentiation of GABA-receptor and changes in brain activity (EEG). Plus, the antibacterial activity is due to the effect of linalool, linalyl acetate and 1,8-cineole on the phospholipid bilayer and the antifungal is caused by the inhibition of the conidial germination of fungi. Finally, the antinoceptive effect was due to the interaction of linalool with ionotropic glutaminergic receptors via NMDA receptors.

Clinical evidence was found for each of the uses above stated. Firstly, lavender oil has proven to be effective in cases of several sleep and anxiety disorders, as well as in sleep deprivation in hospitalised patients, in cases of plastic surgeon anxiety, in dental anxiety and finally for depression and anxiety in pregnancy. Secondly, the essential oil inhibited the growth of *Klebsiella pneumonia*, several *Aspergillus* and *Listeria monocytogenes.* Lastly, lavender oil demonstrated to be effective in front dysmenorrhea and bleeding.

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