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University Dr. Tahar Moulay SAIDA
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Departement of Process Engineering

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Présented by :

KHELLAF Amani Fatima Zohra

and

NAAR Rania Ismahane

Control of physico-chemical quality and pharmacological activity Of some commercial essential oils (Case of pharmacies in saida city)

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Jury :

Mr. M. BOUDINAR	**Maître de Conférences B**	**Saida University**	**President**
Mr. M. ADJDIR	**Professor**	**Saida University**	**Examiner**
Mr. A. BENHELIMA	**Maître de Conférences A**	**Saida University**	**Rapporteur**

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We will be now and always at your service

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I dedicate this work:

To my mother for the love she always gave me, her encouragement and all the help she gave me during my studies, her patience and support. No words of dedication can express my respect, appreciation and love for her. The sacrifices I made for my education and my well-being. You find here, dear Mother, in this humble act, the fruit of many devotions and sacrifices as well as the expression of my deepest gratitude and love. May God grant her health, happiness, prosperity and a long life to fill her old age with joy one day.

To my best uncle Mourad

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To the one who shared with me the most beautiful and the most difficult moments during the execution of this work.

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Amani

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EO : Essential Oil.

ISO : International Organization for Standardization.

BPF : Good Manufacturing Practices.

GLP : Good Laboratory Practice.

AFNOR : French Association for Standardization.

AMM : Marketing Autorisation.

GCMS : Gas Chromatography/Mass Spectrometry.

CNIS : National Center for Computing and Statistics.

pH : Hydrogen Potential.

AI : Acid Value.

PI : Peroxide index.

SI : Saponification index.

EI : Ester index.

A% : Acidity percentage.

TLC : Thin layer chromatography.

Abstract :

The pharmaceutical industry is a strategic economic sector that manages the development, production and marketing of well-monitored, well-controlled pharmaceutical products that comply with national and international standards. The objective of this study is the physico-chemical and microbiological quality control of essential oils sold in pharmacies in the city of Saida. The pharmacological investigation revealed the importance of Aromatherapy in the treatment of the local population. Six essential oils have recorded their importance among the local population; they are coconut oil, peppermint oil, clove oil, almond oil, lavender and castor oil. All analyzes carried out on each of the samples gave results in accordance with the standards of the European Pharmacopoeia as well as the technical file of the product. The identification of the control carried out by CCM shows that the products are generally compliant. These tests therefore showed that the products are of good pharmaceutical quality.

Key words: essential oils, Control quality, pharmacy, physico-chemical, pharmacological.

Résumé :

L'industrie pharmaceutique est un secteur économique stratégique qui gère l'élaboration, la production et la mise sur le marché des produits pharmaceutiques bien surveillés, bien contrôlés et conformes aux normes nationaux et internationales. L'objectif de cette étude est le contrôle de qualité physico-chimique et microbiologique des huiles essentielle vendues dans les pharmacies de la ville de Saida. L'enquête pharmacologique a dévoilé l'importance de l'aromathérapie dans le traitement de la population local. Six huiles essentielles ont enregistrés leurs importance au sein de la population locale, il s'agit de L'huile noix de coco, l'huile de menthe poivrée, l'huile de girofle, l'huile d'amande, l'huile de lavande et l'huile de castor. Toutes les analyses effectuées sur chacun des échantillons, ont donné des résultats conformes aux normes de la pharmacopée Européen ainsi que le dossier technique du produit. L'identification le contrôle réalisé par CCM montre que les produits sont en générale conforme. Ces essais ont montré donc que les produits sont de bonne qualité pharmaceutique.

Mots clés : Huile essentielle, contrôle de qualité, pharmacie, physico-chimique, pharmacologique.

ملخص:
تُعد صناعة الأدوية قطاعًا اقتصاديًا استراتيجيًا يدير تطوير وإنتاج وتسويق المنتجات الصيدلانية الخاضعة للمراقبة الجيدة والمراقبة جيدًا والتي تتوافق مع المعايير الوطنية والدولية. الهدف من هذه الدراسة هو مراقبة الجودة الفيزيائية والكيميائية والميكروبيولوجية للزيوت الأساسية التي تباع في الصيدليات في مدينة صيدا. كشف التحقيق الدوائي عن أهمية العلاج بالروائح في علاج السكان المحليين. سجلت ستة زيوت عطرية أهميتها بين السكان المحليين ، وهي زيت جوز الهند وزيت النعناع وزيت القرنفل وزيت اللوز والخزامى وزيت الخروع. أعطت جميع التحليلات التي تم إجراؤها على كل عينة نتائج وفقًا لمعايير دستور الأدوية الأوروبي بالإضافة إلى الملف الفني للمنتج. يوضح تحديد عنصر التحكم الذي أجرته CCM أن المنتجات متوافقة بشكل عام. لذلك أظهرت هذه الاختبارات أن المنتجات ذات جودة صيدلانية جيدة.

الكلمات المفتاحية: زيت عطري ، ضبط الجودة ، صيدلية ، فيزيائي-كيميائي ، دوائي.

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General introduction

The search for quality in the broad sense is currently a fundamental concern for companies. The pharmaceutical industry is one of those sectors where the quality of the products and of all the activities surrounding these products is paramount. This is why this industry developed well before the implementation of AFNOR and ISO standards, standards of its own. These are Good Manufacturing Practices (BPF), Good Laboratory Practice (GLP).

These standards have been put in place little by little by the pharmacopoeias of different countries, in conjunction with progress in the methods of analysis of pharmaceutical products. Substances and pharmaceutical preparations for human use described in the various pharmacopoeias must be manufactured in accordance with BPF according to which the processes, premises and installations must comply with the standards, the provisions of the manufacturing or marketing authorization, the applicable regulations and, in the case of products intended for export, any mandatory international standards.

The implementation of these measures requires a deliberate company strategy, the awareness, motivation and involvement of all staff.

To meet all these objectives, Algeria and its pharmaceutical structure has laboratories where all pharmaceutical forms are subject to various controls:

physicochemical, microbiological and toxicological ensuring the qualitative component and bioequivalence studies assuming therapeutic efficacy identical to the molecule, essential for marketing of drugs.

Among all the drugs we opted for essential oils sold at pharmacies in the city of Saida.

For this, various questions require answers have been asked; whose essential oils deserve this marketing authorization (AMM)? Does it meet the standards of the European Pharmacopoeia 2008, 6th edition and the European Pharmacopoeia 2011, 7th edition?

To successfully address this issue, we have set the following objectives:

- Demonstration of the quality of essential oils on the physico-chemical level carried out within the laboratory,
- The experimental part was preceded by theoretical reminders developed from a bibliographic research.

Chapter I : Essential oils

I.1. History :

The first recorded use of essential oils was probably by the Egyptians who employed them for a range of purposes including medicinal, cosmetic, in religious ceremonies, and for embalming the dead. About the same time, the Chinese were using herbs and aromatic plants extracts for medicinal purposes and their procedures were, eventually, assimilated into Indian Ayurvedic medicine.

After the fall of the Egyptian empire, the ancient Greeks assumed much of the Egyptian knowledge and essential oils were employed by Hippocrates in aromatherapy. In due course, Greek medicine was taken up by the Romans who were impressed by the power of fragrances and also used them enthusiastically in aromatherapy. Subsequent to the dissolution of the Roman Empire and the resurgence of the Arabian empire, the use of essential oils was continued in the manner of the Romans and during this period an efficient distillation process was developed for their extraction [1].

During the dark ages, the monks in their monasteries persisted with the use of herbs for medicinal purposes and, although they did not recognize the nature of their activity as such, they used the antibacterial and antipesticide properties of many essential oils to cleanse wounds and control infestation. During the renaissance, the holistic approach to medicine, pioneered by Hippocrates, came back into vogue and Paracelsus used herbs (the knowledge of which largely came from folk remedies) to effect a cure for leprosy.

The term aromatherapy was introduced by the French perfumer Gattefosse and appeared to have arisen from a personal experience after burning his hand in the laboratory. He treated his hand with pure lavender oil, which apparently, immediately eased the pain and the burn subsequently healed with no infection and little or no scarring. This technique was subsequently used with great success in treating soldiers wounded in the First World War. Today, essential oils are widely used as perfumes, food flavors and for medicinal purposes, but many of their bactericidal and antipesticide uses have been replaced by antibiotics and synthetic antipesticide and antifungal agents [1].

I.2. Essential oil definition :

Essential oils are aromatic, volatile liquids obtained from plant material through steam distillation and named after the plant from which they are derived. Essential oils can be defined as either products or mixtures of fragrant substances or as mixtures of fragrant and odorless substances [2].

These fragrant substances are chemically pure compounds that are volatile under normal conditions. Essential oils vary greatly, sometimes due to genetic causes, but also because of climate, rainfall, or geographic origin. They are composed principally of lipophilic and highly volatile secondary plant metabolites, principally mono and sesquiterpenes, but other types of compounds such as allyl and isoallyl phenols may also be present. Other substances that have been identified in volatile oils include coumarins, anthraquinones, and alkaloids, which are often distillable, while some diterpenes, fat and other nonvolatile compounds can be obtained from essential oils by methods other than distillation.

The applications of essential oils are diverse, widely used in cosmetics and perfumes, they also have medicinal applications due to their therapeutic properties as well as agro-alimentary uses because of their antimicrobial and antioxidant effects [2].

I.3. Classification :

Table 1 : Essential oils classification [3].

Type	**Plants**
Balsamic	Benzoin, Opopanax, Peru Balsam, Vanilla
Camphorous	Camphor, White Rosemary (Camphor CT), Sage, Common (Dalmatian), Sage, Spanish Spike Lavender.
Cineolic	Cajeput, Eucalyptus Globulus, Eucalyptus Radiata, Niaouli, Ravintsara, Rosemary (Cineole CT), Sage, Spanish
Citrus	Bergamot, Eucalyptus, Lemon, Grapefruit, Lemongrass, Lemon Verbena, Lime, Mandarin, Myrtle, Bitter, Blood, Orange, Sweet, Tangerine, Tea Tree, Yuzu
Coniferous	Cypress, Fir Needle, Siberian, Pinyon, Pine, Scotch, Hemlock, Spruce, Black
Earthy / Rooty	Carrot Seed, Manuka, Spikenard (Nard / Jatamansi), Vetiver
Floral	Geranium, Rose, Jasmine, Lavandin, Lavender, Lavender, Spike, Linden Blossom, Neroli, Petitgrain, Rose, Sage, Clary, Tuberose, Ylang Ylang
Fruity	Chamomile, German, Roman, Tagetes, The Citrus Oils

Green	Geranium, Violet Leaf
Herbaceous	Basil, Bay Laurel, Bergamot Mint, Cannabis, Catnip, Marjoram, Oregano, Sage, Common (Dalmatian)
Medicinal	Camphor, White Tea Tree, Common, Wintergreen
Minty	Cornmint, Peppermint, Chocolate, Spearmint
Mossy	Oakmoss
Peppery	Cubeb, Pepper, Black Pepper, Pink
Resinous	Elemi, Frankincense, Myrrh
Spicy	Allspice, Caraway Seed, Cardamom, Cassia, Cinnamon, Clove Bud, Ginger, Laurel Leaf, Nutmeg
Oody Woody	Cedarwood, Atlas Cedarwood, Virginian, Palo Santo, Rosewood, Sandalwood

Essential oils can be categorized together based on their primary scent characteristics. Some oils can be associated with more than one scent category or fragrance family (Fig. 1), the aroma can vary between one distillation and another for natural reasons [3].

Fig. 1 : Essential oils categories [3].

I.3.1. Classification according to chemical composition :

I.3.1.1. Biosynthesis :

Terpenoid and phenylpropanoid derivatives are the main components found in essential oils. In most plants, their essential oils contain terpenoids at around 80%. But the presence of phenylpropanoid derivatives affords the essential oils significant flavor, odor, and piquant.

These two groups of compounds are derived from two different pathways from different primary metabolites [4].

A. Biosynthesis of terpenoids :

The name terpene was first attributed to the compounds with molecular formula $C_{10}H_{16}$ by Kekule, a German chemist, due to its abundance in turpentine oil (Fig. 2).

The derivatives $C_{10}H_{16}O$ and $C_{10}H_{18}O$ were named generically camphor and related to terpenes. Wallach, Kekule's assistant, characterized pinenes, limonene, dipentene, phellandrene, fenchone, terpinolene, and sylvestrene, which at that period were recognized as artifacts for turpentine oil. But nowadays, they are considered as compounds of some essential oils [1].

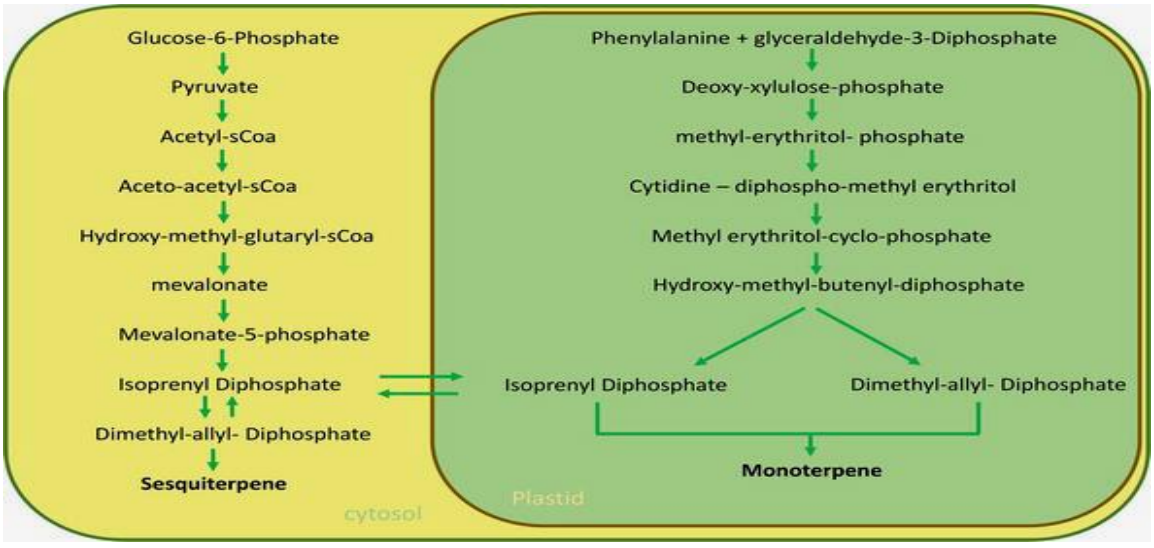

Fig. 2 : Biosynthesis of terpenoids [5, 6].

B. Biosynthesis of phenylpropanoids :

The synthesis of this class of compounds in aromatic plant leads to a wide variety of compounds (Fig. 3).

The enzymatic arsenal involved in the synthesis of volatile compounds in the plant is not well known. Therefore, there are many approaches to explain the synthesis of those groups of

compounds. But from a metabolite point of view, the starting point of these volatile phenols is the phenyl-alanine that is transformed into cinnamic acid by the phenylalanine amino lyase. The cinnamic acid in turn is transformed into para-COUMARIC acid. This latter compound depending on the enzymatic ability of the plants can undergo two different ways of synthesis [7].

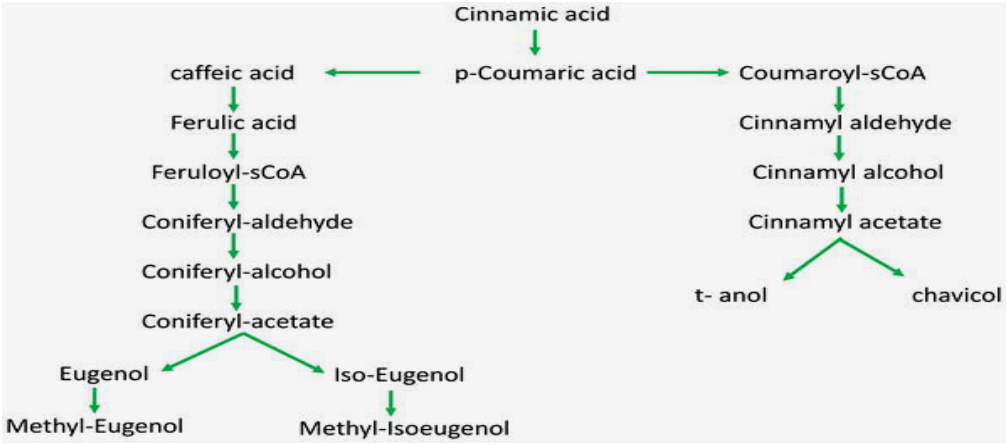

Fig. 3 : Biosynthesis of phenylpropanoids [5].

I.3.2. Calssification according to different uses and effects :

Table 2 : Essentail oils calssification accroding to theirs effects and uses [8].

Type	**Uses and effects**
Basil	➢ Essential oil is used to sharpen concentration and alleviate some of the symptoms of depression. ➢ It may relieve headaches and migraines. ➢ It should be avoided during pregnancy.
Bergamot	➢ Essential oil is said to be useful for the urinary tract and digestive tract. ➢ When combined with eucalyptus oil it may help relieve skin problems, including those caused by stress and chicken pox.
Black pepper	➢ Essential oil is commonly used for stimulating the circulation, muscular aches and pains, and bruises. ➢ Combined with ginger essential oil, it is used to reduce arthritis pain and improve flexibility.
Chamomile	➢ Essential oil can treat eczema.

Citronella	➢ Essential oil is a relative of lemongrass and acts as an insect repellent.
Clove	➢ Essential oil is a topical analgesic, or painkiller, that is commonly used for toothache. ➢ It is also used as an antispasmodic antiemetic, for preventing vomiting and nausea, and as a carminative, preventing gas in the gut. ➢ It has antimicrobial, antioxidant and antifungal properties.
Eucalyptus	➢ Essential oil can help relieve the airways during a cold or flu. ➢ It is often combined with peppermint. ➢ Many people are allergic to eucalyptus, so care should be taken.
Geranium	➢ Essential oil can be used for Trusted Source skin problems, to reduce stress, and as a mosquito repellant.
Jasmine	➢ Essential oil has been described as an aphrodisiac. ➢ While scientific evidence is lacking, research has shown that the odor of jasmine increases beta waves, which are linked to alertness. ➢ As a stimulant, it might increase penile blood flow.
Lavender	➢ Essential oil is used as an antiseptic for minor cuts and burns and to enhance relaxation and sleep. ➢ It is said to relieve headache and migraine symptoms.
Lemon	➢ Essential oil is said to improve mood Trusted Source, and to help relieve the symptoms of stress and depression.
Rosemary	➢ Essential oil may promote hair growth, boost memory, prevent muscle spasms, and support the circulatory and nervous systems.
Sandalwood	➢ Essential oil is believed by some to have aphrodisiac qualities.
Thyme	➢ Essential oil is said to help reduce fatigue, nervousness, and stress.
Tea tree	➢ Essential oil is said to have Trusted Source antimicrobial, antiseptic, and disinfectant qualities. ➢ It is commonly used in shampoos and skin care products, to treat acne, burns, and bites. ➢ It features in mouth rinses but it should never be swallowed, as it is toxic.

Yarrow	➢ Essential oil is used to treat symptoms of cold and flu, and to help reduce joint inflammation.

I.3.3. Essential oil extraction :

Essential oils can be extracted from several plants with different parts by various extraction methods.

The manufacturing of essential oils and the method used for essential oil extraction are normally dependent on botanical material used. State and form of material is another factor used for consideration [9].

Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss in bioactivity and natural characteristics. For severe case, discoloration, off-odor/flavor as well as physical change such as the increased viscosity can occur. Those changes in extracted essential oil must be avoided [9].

I.3.3.1. Distillation methods :

Distillation methods are a group of methods using steam as compound vector or transporter (Fig. 4).

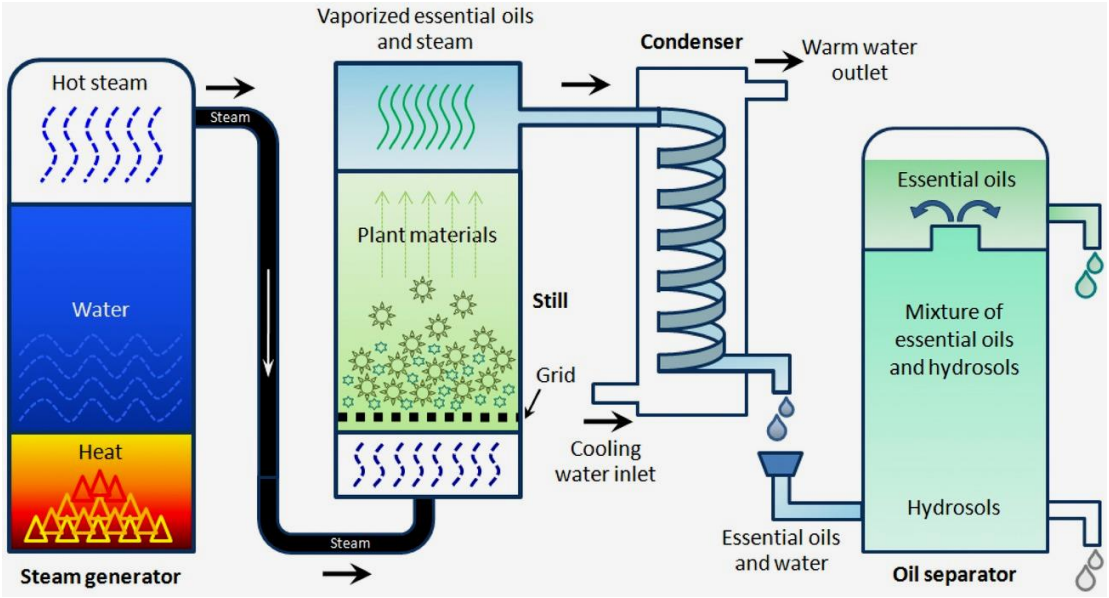

Fig. 4 : Diagrammatic illustration of steam distillation method [9].

In fact, in distillation method, the plant material may be immersed or not in water, and after heating to water boiling point, the impression created in the reactor by steam as well as the high temperature will create the vaporization of these volatile compounds from their stockade cell to the environment of the reactor.

The gas is pouched throughout a cooler. The condensation of the water and volatilized compounds from their vapor to water phase form a mixture that can be separated according to their density. There are two varieties of distillation methods: the hydrodistillation and the so-called steam distillation methods (Fig. 5) [10-12].

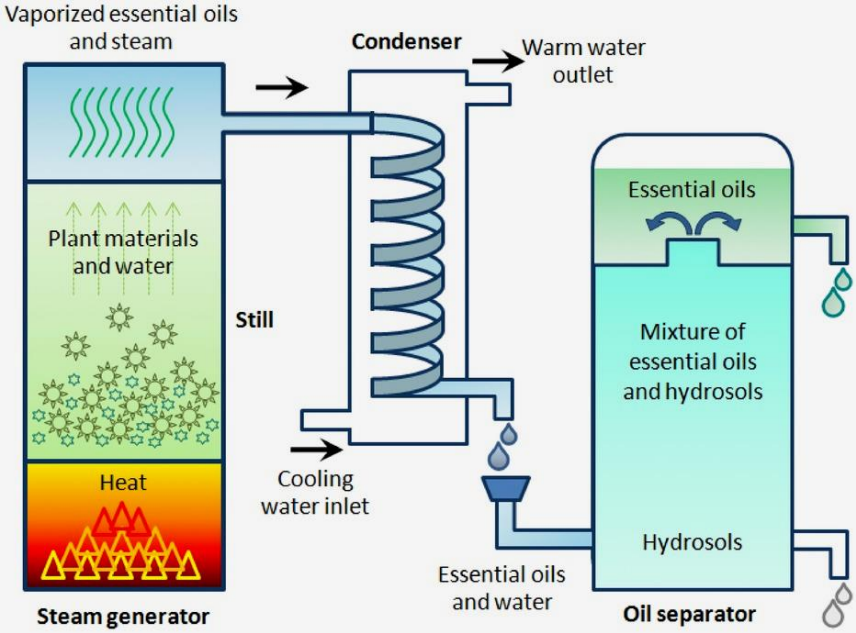

Fig. 5 : Diagrammatic illustration of hydrodistillation method [9].

Hydrodistillation is an essential oil extraction method in which the material is immersed in water, but in the steam distillation, the plant material is not in direct contact with water but will go through, for example, steam flow before entering the cooler [13].

I.3.3.2. Microwave-assisted essential oil extraction :

Microwave-assisted essential oil extraction is a variant of the distillation method where the heating source has been changed from the normal electric heating cap by the microwave. The plus here is the hypothetic increasing in extraction yield: hypothetic because the increase in yield is not as spectacular as tough [14, 15]. It is true that it is better to crush the plant material, but in comparison to the classic distillation method, the essential oil yield is systematically the same.

The principle of this method is based on the change of the polarity of water by the waves and of course the heating that will play the same role as in classic distillation method. This method has in addition the limit of the normal distillation method, the fact that the microwave can lead to chemical stereo switching from one isomer to another [16].

I.3.3.3. Expression :

This method is specific for citrus pericarp. In fact, to avoid the thermal destruction, cold pressing of the citrus fruit rinds as the essential oil is store at that part of the plant has led to a

good quality oil. The limit of this technique is the fact that it is not applicable to other parts of the plant [10-12].

I.3.3.4. Enfleurage :

Enfleurage is an old technique of essential aroma extraction. It is based on the solubilization of the essential oil's component on a greasy wax. When using cold extraction, it can take more than a month, but this can be reduced to a few hours by heating.

The aromatized wax is then called concrete. This later undergoes solubilization in a polar solvent and then partitioned with absolute ethanol. The product obtained after this process is at most a part of the essential oil as present in plant, and it contains many other terpenes that can be solubilized in fats used; that is, while at the end, the product is not called essential oil but absolute [17].

I.3.3.5. Supercritical fluid extraction :

This method is the most modern and sophisticated. It uses gases at their supercritical stage. The gas at supercritical stage is liquid due to the high pressure applied to it. Many solvents can be used as the method brings the solvent at its temperature and pressure above its thermodynamic critical point, but the most used is carbon dioxide (CO_2) for the reason that it needs less pressure to be liquified, it is less reactive than other, it is non inflammable, it is non toxic and available at low cost with high purity, and most importantly it can be removed from the plant material using just the press release [17].

This method is based on the fact that gas at the supercritical state can enter throughout the plant material like a gas and dissolve component like a liquid. After the extraction procedure, the essential oils compounds are mixed with the supercritical fluid (in liquid form). The separation is performed by reducing temperature and increasing the pressure up to room conditions. The principal limit of this method is the complexity of the system [13, 18, 19].

I.4. Pharmacological property of essential oils :

A range of essential oils have been found to have various degrees of antimicrobial activity and are believed to have antiviral, nematicidal, antifungal, insecticidal, and antioxidant properties. Aromatherapy applications include massage, topical applications, and inhalation.

Topical applications, massage oils, and bath and skin care products are absorbed through the skin. Massaging the area where the oil is to be applied can boost circulation and increase absorption. Some argue that areas that are richer in sweat glands and hair follicles, such as the head or the palms of the hand, may absorb the oils more effectively.

Aromatherapy is a complementary therapy. It does not provide a cure for diseases, rashes or illnesses, but it can support conventional treatment of various conditions [8].

I.5. Risks :

Each essential oil has its own chemical makeup and reason for use, so it is important to speak with a trained aromatherapist, nurse, doctor, physical therapist, massage therapist or pharmacist before applying or using an oil for healing purposes.

A trained professional can recommend and teach how to use each product, giving proper instructions on application or dilution [8].

Consumers should also be aware that the U.S. Food and Drug Administration does not monitor aromatherapy products, so it can be difficult to know whether or not a product is pure or if it is contaminated or synthetic.

Some beauty and household products, such as lotions, make-up, and candles contain products that may appear to be essential oils, but they are really synthetic fragrances.

Like medications, essential oils must be treated with respect. It is important to seek professional advice and to follow instructions carefully [8].

I.6. Caution when using essential oils :

Since essential oils cause reactions in the body, not all the oils will benefit everyone. Chemical compounds in essential oils can produce adverse effects when combined with medications. They may reduce the effectiveness of conventional drugs, or they may exacerbate health conditions in the individual.

A person with high blood pressure, for example, should avoid stimulants, such as rosemary. Some compounds, such as fennel, aniseed, and sage act similarly to estrogen, so a person with an estrogen-dependent breast or ovarian tumor should avoid these.

Concentrated products may be poisonous before dilution and should be handled with care. A maximum concentration of (5 %) is recommended [20].

Some oils produce toxins which can cause damage to the liver, kidneys, and nervous system, especially if taken internally. Swallowing essential oils can be hazardous, and fatal in some cases.

Individuals with any of the following conditions should be extra careful when using aromatherapy:

- An allergy, or allergies ;
- Hay fever, a type of allergy ;
- Asthma ;
- Skin conditions such as eczema or psoriasis.

People with the following conditions must be extremely cautious :

- Epilepsy ;
- Hypertension, or high blood pressure.

If the oil is to be mixed with a carrier, the individual should tell the aromatherapist or massage therapist about any nut allergies, because carrier oils are often obtained from nuts and seeds [20].

Aromatherapy can have side effects, but these are normally mild and do not last long. They include :

- Nausea ;
- Headaches ;
- Some allergic reactions.

Use of aromatherapy by pregnant or nursing mothers has not been proven safe by research, so it is not recommended. During the first trimester of pregnancy, aromatherapy may pose a risk to the developing fetus. Women who are breastfeeding should avoid peppermint essential oil, as it may be expressed in breast milk.

Essential oils derived from citrus may make the skin more sensitive to ultraviolet light, increasing the risk of sunburn.

Some oils may affect the function of conventional medicines, so people who are using medications of any type should first check with a qualified pharmacist or doctor.

Finally, when storing Trusted source essential oils, it is important to be aware that light, heat, and oxygen can affect the integrity of the oil. Products should come from a respected and trustworthy source, to be sure of the quality. Following instructions carefully reduces the risk of compromising the user's health.

Aromatherapy can help alleviate some conditions, but it should be used correctly, under the supervision of a qualified practitioner [20].

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Chapter II : Quality control of pharmaceutical products

II.1. Introduction :

The pharmaceutical industry is a sector that includes research, development, manufacturing and marketing of medicines for human and animal medicine. All these activities are carried out with a high level of quality assurance and in a very strict regulatory environment.

Laboratory testing is an integral part of Good Manufacturing Practice, and the requirements for this are described in the *Good Manufacturing Practice* (GMP) regulations.

II.2. Pharmaceutical quality :

II.2.1. Definition :

Quality can be defined as a "set of properties and characteristics of a product or characteristics of a product or service that gives it the ability to satisfy expressed or implicit needs" [1].

Quality is the result of putting a high-performance product on the market, available at a reasonable price, and to which are associated services associated with it [2].

II.2.1.1. The price :

In principle, a company must bring or maintain its cost of sales at a level well below that of a level well lower than the possible selling price, if it wants to keep a margin allowing it to investigate margin to invest and pay its shareholders.

II.2.1.2. Availability:

Ensuring product availability means providing the means to make the product accessible.

II.2.1.3. The service :

The service represents the elements that the customer expects to find with the product that he buys. This can be, depending on the case : [3]

- An assistance ;
- A guaranty ;
- Instructions for use and maintenance ;
- A distribution network ;
- Competence ;
- Integrity ;
- Communication with the client.

II.2.1.4. Quality Assurance :

II.2.1.4.1. Definition :

It can be defined as "the set of pre-established and systematic actions necessary to systematic actions needed to give appropriate confidence that a product or service will satisfy the given requirements related to the safety of the product or service "quality".

Quality assurance is based on simple common sense principles, that are not considered separately, but which constitute a whole "system" when combined in a code or general program [4].

The components of this system consist of a chain of precautions that are put in place from manufacturing to use, these are:

- The rules of good manufacturing practice (B.P.F.),
- Stability studies,
- Quality control of medicines in the laboratory,
- Certification system for pharmaceutical products,
- Rules of good storage and distribution practice,
- Rules of good practice for dispensing and use.

II. 2.1.4.2. Objective of Assurance quality :

The first goal is to ensure compliance with the requirements, and the second goal is to show that it is possible to achieve the first goal by using documents that clearly, precisely, and easily explain all the precautions and quality-enhancing measures used.

These rules can be defined as follows:

- Organize the company or activity according to the requirements of the Quality Assurance program,
- To question the means to accomplish the tasks with optimal quality guarantees of quality,
- Describe the way tasks are accomplished with formalized documents,
- Be able to prove at any time that the tasks have been carried out of quality,
- Describe the way in which tasks are carried out with formalized documents,
- Be able to prove at any time that the tasks have been carried out in accordance with the documents,
- Search for remedies to all technical failures observed in the products,
- To search for remedies to all technical problems observed in the products, in the way of carrying out the tasks or in the organization and take measures to prevent the occurrence of the failure,

In practice, quality assurance is based on the following six rules :

- Write out what you must do (Procedures, Quality Plans, Quality Manual),
- Do what you have written,

- Write what you have done,
- Keep the trace (Traceability, Archiving),
- Check what is done (Audit, Quality Control),
- Progress (Correct, Maintain, Improve, Predict, Organize) [3].

II.2.1.5. Personnel :

The establishment and maintenance of a satisfactory quality assurance system relies on all personnel. In order to have a sufficient number of qualified personnel to carry out the tasks assigned to them. An organizational chart must be established that clearly identifies pharmaceutical responsibilities.

II.2.1.5.1. Personnel hygiene :

Hygiene programs must be established and adapted to the different needs of the company. They must include procedures for health and hygiene of personnel. The prevention of eating, drinking or smoking in the areas used it must be posted and mentioned.

II.2.1.5.2. Formation :

The objective of training is to ensure that personnel are qualified for the tasks assigned to them. This training, which applies to all categories of personnel, must take into account the theoretical aspects and the implementation of good practices.

II.2.1.6. Premises and equipment :

In general, the premises and equipment must be located, designed, constructed, adapted and maintained so as to ensure safety and suitability for the purpose of the work, suitable for the operations to be performed. Their design, layout, arrangement and use must be such as to eliminate the risk of error and to permit cleaning and maintenance to ensure the proper preservation of pharmaceutical products.

II.3. Use of the quality tool : (The 5 M rules)

II.3.1. Workforce (Human Resources) :

- Organizational chart,
- Job descriptions (individual),
- Responsibilities,
- Training,
- Qualification,
- Motivation ...

In summary: Knowledge, Knowing how to do and Knowing how to be.

II.3.2. Raw materials :

- Approval of suppliers and annual survey,
- Follow-up of purchases,

- Reception, labeling, storage, preservation, management,
- Controls,
- Safety data sheets,
- Computerized stock management,
- Weighing recorded,
- Specific management: solvents, reagents, standards, dangerous products...

II.3.3. Materials :

- Purchasing (choice of suppliers),
- Qualification,
- Inventory and coding,
- Operational and technical protocols,
- Maintenance,
- Metrology,
- Controlled cleaning.

II.3.4. Environment :

- Suitable premises,
- Good organization,
- Respect of procedures,
- Avoid confusion and contamination,
- Environmental control (bacteriological and particulate) with validated cleaning procedures validated,
- Strict hygiene and safety rules.

II.3.5. Methods :

- Validated methods (developed in R&D, described in the AMM file),
- Quality documentation : Codified, validated, classified, distributed, archived, revised...
- Procedures, Protocols, Instructions, User guides,...
- Approvals (traceability),
- Permanent control ensured by Quality Audits of conformity,
- Alert process for a good control of the risks with adapted decision making (protection of Public Health) [5].

II.4. Quality control :

Consists of carrying out a series checks and measurements to determine whether the quality objectives are met.

For products, it is often the verification of the compliance with requirements appearing in the file of marketing authorization (AMM) or the pharmacopoeia, the verification being generally followed by a sorting between compliant and non-compliant entities [6].

II.4.1. Quality control of essential oils :

The most important thing is that essential oils sold are without chemical fillers, additives or synthetics. Most companies prove this by sending their oils to independent 3rd party labs, which perform a GC/MS (Gas Chromatography/Mass Spectrometry) test to start, and sometimes a multitude of other chemical tests, as well. The best brands make these test results easily available to the consumer [6].

II.4.1.1. Labeling according to the European Pharmacopoeia :

- Scientific name of the raw material used,
- Latin name (genus, species and godfather) (ex.: Thymus vulgaris L.),
- Part used (depending on the part used fruits, flowers, leaves, ... the compositions are very different),
- Name of EO « Essential Oil » (rose geranium, lemongrass, mandarin...).
- In appropriate cases:
- Type of EO: geographical origin,
- Production method,
- Name and concentration of any added antioxidants,
- Additional processing steps [7].

II.4.1.2. Conservation :

- In a tight and well-filled container,
- Under nitrogen atmosphere at the start if possible,
- Protected from light (in aluminum or stainless steel),
- At a cool temperature or in the fridge,
- Limited shelf life (3 years) to avoid the appearance of resins and peroxides [7].

II.4.2. World market for essential oils :

Essential oil is a fragrant, volatile chemical produced by certain plants that can be extracted as a liquid by distillation of steaming aromatic plants (leaves, flowers, barks, seeds, stems) in the most popular extraction method. Despite the fact that it is termed oil, this material is devoid of fat. The majority of essential oils are available for free. They're available in pharmacies, supermarkets, specialty stores, and online.

In recent years, the global market for essential oils has been in sharp evolution. The main leaders in this market are constantly on the lookout for new fragrances and molecules in order

to diversify their range. Some 3000 essential oils are known, of which about 300 are of commercial importance [8].

The quantities of essential oils produced in the world are very variable. The annual production of certain essential oils exceed 35,000 tons, while that of the others can reach a few kilograms [9]. In 2008, world production of essential oils was dominated by Brazil and India with respective productions of 28.6% and 25.6%. Then follow for a third of the market United States, China and Argentina with production reaching respectively 16.8%, 9.0% and 4.9%. Nine countries with in-house productions between 0.1 and 2% (France: 1%) account for 10% in the world market.

Finally, the last 10% are distributed among the other countries of the world with a production lower than 0.1% [9]. Exports of essential oils exceeded one billion dollars annually between 1998 and 2002, according to an analysis of international market trends. Essential oils are mostly produced in the United States [10]. The prices of essential oils sometimes reach very high levels which exceed 50,000 US$ per liter and in particular for the essences of highly prized plants in the international market. Table 1 gives, according to 2008 data, oils essential products most in demand on the international market [9, 11].

Table 1: Most demanded essential oils in the global market

Essential Oils	**Volume (tonne)**	**Producing Country**
Citronnelle	1 800	Chine, Sri Lanka
Menthe des bois	32 000	Inde, Chine, Argentine
Eucalyptus type cineole	4 000	Inde, Chine, Argentine
Orange	51 000	USA, Brésil, Argentine
Menthe poivrée	2 367	Inde, USA, Chine
Citron	9 200	Argentine, Italie, Espagne
Eucalyptus (type citronellal)	1 000	Chine, Brésil, Inde, Vietnam
Feuille de clou de girofle	1 800	Indonésie, Madagascar
Verveine exotique	1 200	Chine
Menthe verte	1 800	USA, Chine
Bois de cèdre (Chine)	1 650	USA, Chine
Lavandin	1 100	France
Patchouli	1 200	Indonésie, Inde

II.4.2.1. Marketing of essential oils in Algeria:

In Algeria, the marketing of aromatic and medicinal plants is done in the raw state, or in the conditioned state in essential oil. The data presented in this article are based on the statistics of foreign trade in agricultural products and the statistics of the National Center for Computing and Statistics, 'CNIS' of the General Directorate of Customs carried out on the basis of customs declarations and statistical data of foreign trade.

Algeria is absent from top fifteen importing or exporting countries and its share of the world market is almost non-existent. If we consider the averages analyzed for the main exporting and importing countries, Algeria remains at insignificant rates in the world market by 0.0007%. The main suppliers of Algeria are developing countries. If China and India provide Algeria for a long time, Pakistan is became the first supplier since 2013 [12].

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Chapter III : Materials and methods

III.1. Objectif :

Essential oils are natural materials widely used in many fields all over the world and it's become an integral part of everyday life and for that consumers have a right to good quality produce that is safe for consumption, so in our work here we analyze some essentials oils sold at pharmacies in the city of Saida (Fig. 6) to control their composition and pharmacological effect.

III.2. Presentation of the study area :

Algeria is rich in several thousand botanical species belonging to the domain of North African flora consists of part of the Mediterranean flora and is a subset of the flora of Africa; its geographical position, offers a very great ecological and floristic diversity. It is one of the Mediterranean countries with a long medical tradition and traditional know-how. From where Saïda City (in Arabic: ولاية سعيدة / in Berber: Tamnaḍt Saïda) occupies a central position in western Algeria, it is limited:

- In the North, by the city of Mascara,
- In the South, by the city of El-Bayadh,
- To the West, by the city of Sidi Bel-abbes,
- To the East, by the city of Tiaret.

Fig. 6 : Geographical map of the study area

The first part of our practical work consists of an investigation at pharmacies (Saida) through which we tried to have an idea on the number and nature of essential oils (natural products) from aromatic plants sold at pharmacies, this approach allowed us to choose the following essential oils: Lavender oil, almond oil, Peppermint oil, Castor oil, Clove oil and Coconut oil.

III.3. Essential oils physico-chemical analyses:

III.3.1. Physical analyses:

The refractive index, pH and optical rotation are determined by methods that comply with AFNOR standards [1].

III.3.1.1. Refractive index determination:

Refractive index depends on the nature of fatty carbon chains present in the oil and on the temperature.

The refractive index is the ratio between the speeds of light in vacuum and in substance. In practice, the speed of light in air is used instead of that in vacuum; the wavelength chosen is that of the average of **D** lines of sodium (589.6) nm.

The refractive index of a given substance varies with the wavelength of incident light and with temperature. Note the refractive index η^{t}_{D}

Where; "D" or "t" is the temperature in degrees Celsius.

Refractive indices are measured using a refractometer (Fig. 7) at room temperature then reduced to 20°C by the formula [1]:

$$\mathbf{I_{20} = I_t + 0.00045\ (T\text{-}20°C)\ \ ....\ (1)}$$

Where :

I_{20} : reference refractive index at 20°C;

I_t : refractive index at room temperature or measurement;

T: ambient or measurement temperature.

It should be noted that the refractive index of essential oils varies from 0.0004 per degree around 20°C.

Fig. 7 : Refractometer

For the determination of refractive index we falow steps :

- Refractometer is adjusted by measuring the refractive indices of standard products (distilled water, where refractive index is 1.3330 at 20°C);

- For this, it should first calibrated with distilled water. So, we put a few drops of water on the prism and then leave it for 5 min, the time that its temperature equilibrates with that of the device and the laboratory. The refractive index is then read on the Refractometer.
- Before placing it in the instrument, bring the test sample to a temperature approximately equal to that at which the measurement is to be made. And we waited, to take the reading, until the temperature is stable.

III.3.1.2. Density determination :

The density of Essential oils was determined with classic method of measuring:

For this, we measured the weight and volume of oil and perform the calculation according to the following formula [1]:

$$d_a = \frac{\rho a}{\rho eau} \quad \text{..... (2)}$$

$$\rho_a = \frac{m_a}{V_a} \quad \text{..... (3)}$$

Where :

$\mathbf{d_a}$: density

$\mathbf{p_a}$: volumic mass is equal to the weight (m) [g] divided by the volume (v) [ml]

III.3.1.3. pH measurement :

pH is the abbreviation for hydrogen potential, measures the chemical activity of hydrogen ions (H^+) (also commonly called protons) in solution. This measurement was made using a pH meter (Fig. 8).

Fig. 8 : pH-meter

III.3.2. Chemical indices :

III.3.2.1. Acid value (AI) determination :

Acidity is the oil content of free fatty acids resulting from the hydrolysis of triglycerides and conventionally expressed as oleic acid (g/100g of oil).

Acid number corresponds to the number of milligrams of potassium (KOH) necessary to neutralise the free fatty acids in 1g of fat.

The method consists in measuring the free fatty acids with a titrated potassium hydroxide solution.

$$R\text{-}COOH + NaOH \text{ ----------} R\text{-}COONa + H_2O$$

For the determination of acid value we falow steps :

First, we measured 5 g of oil and we dissolved it in 25 ml of an equal volume of ethanol/diethyl ether [1:1] neutralised. For dosing free carboxylic functions, we have used an ethanolic solution of KOH at (0.1 mol/l) and 1 mL of 1% phenolphthalein dissolved in absolute alcohol. During this operation, we see that the light yellow color of the liquid (the color of the essential oil) turns on neutralisation to a pink color.

The acid value is calculated according to the formula [1]:

IA= (56,1 x V x N) / p …… (4)

Where :

V: number of milliliters of titrated ethanolic solution KOH,

N: exact normality of titrated ethanolic solution KOH, or it is the exact concentration, in mol/l,

P: weight of test sample (essential oil) in grams.

III.3.2.2. Peroxide index (PI) determination :

This index gives us the quantity of peroxide present in the sample, the latter being expressed in milliequivalents of active oxygen contained in one gram of product, oxidizing potassium iodide with the release of iodine. Indeed, this index allows us to evaluate the state of freshness of oil.

This peroxide index (PI) estimates the state of oxidation; it is a slow but inevitable mechanism. Indeed, oils can oxidize in the presence of oxygen and certain factors favoring this harmful phenomenon (high temperature, water, enzyme, trace metals Cu, Fe, Co, etc.). This auto-oxidation or aldehyde oxidative rancidity initially leads to the formation of peroxides or hydro peroxides which subsequently decompose into aldehyde carbonyl derivatives and hydro ketones (responsible for the smell) and various oxygenated products (alcohols, acids, etc.).

It is the number of micrograms of peroxide contained in one gram of product and oxidizing potassium iodide with the release of iodine. For the determination of this index we falow steps:

In a glass bottle, 2g of oil were disolved with 10 ml chloroform by stirring. After this, 15 ml of pure acetic acid and 1ml of saturated potassium iodide solution (1g/mL) were added. In the dark, the glass bottle was shaked for 5 min. Then, 25 ml of distilled water and a few drops of starch solution (1g/100ml) (iodine indicator released) were added until color disappears. Finally, the released iodine is titrated with a 0.01N solution of sodium thiosulfate ($Na_2S_2O_3$). A blank test is carried out under the same conditions

Peroxide index (PI) expressed in (mg of O_2/g), is given by the following formula [1]:

$$\mathbf{PI = (V_0 - V_1) / P \times 8000 \ldots\ldots\ldots (5)}$$

Where :

V_0: volume of thiosulfate used for the sample (mL);

V_1: volume of thiosulfate used for the blank test (mL);

P: weight of the test sample (g).

III.3.2.3. Saponification index (SI) determination :

Saponification index represents the number of milligrams of potassium hydroxide (KOH) necessary to transform the fatty acids and glycerides of one gram of fat into soap.

Saponification index value allows us to estimate the lengths of the carbon chains of the fatty acids constituting the oil on the one hand, and to calculate the average molecular masses of the fatty acids and triglycerides which contain the oil.

For the determination of saponification index we falow steps :

In a flask 6g of oil was added to 75ml of potassium hydroxide (KOH) ethanolic solution 0.5N. We heat the mixture with reflux heating assembly (Fig. 9).

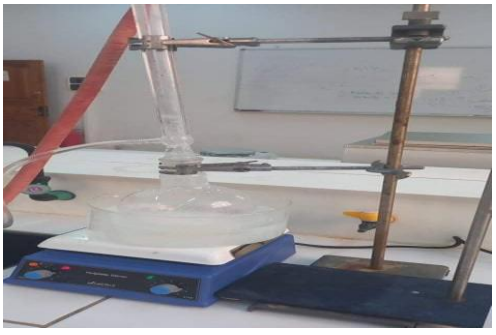

Fig. 9 : Reflux heating instrument

After one hour, to be sure that the saponification is complete, we stop the heating then 4 to 5 drops of phenophthalein are added. Then the excess potassium is carried out with

hydrochloric acid (0.5N) until discolored. A blank test is carried out under the same conditions.

The saponification index is given by the following relationship [1]:

$$SI = \{(v_0 - v_1) \times 56,1 \times N\} / P \quad \ldots\ldots\ldots (6)$$

where :

V_0: Volume of HCl solution used for the blank test,

V_1: Volume of HCl solution used for the sample,

N: Normality of HCl solution of the sample,

P: Weight in g of the sample.

III.3.2.4. Ester index (EI) determination :

Ester Index is the mass in milligrams of potash required for the hot saponification of esters contained in one gram of fatty substance. It is calculated from the Acid Index (AI) and the Saponification Index (SI). It allows to evaluate a possible hydrolysis of triglycerides.

The Ester Index is calculated according to the following equation [1]:

$$EI = SI - AI \quad \ldots\ldots\ldots (7)$$

where :

SI: Saponification Index,

AI: Acid Index.

III.3.2.5. Acidity percentage (A%) :

It corresponds to the percentage content of fatty acid present in the oil and represents an important parameter in the evaluation of its quality. This dosage tells us about the degree of deterioration of the oil and to estimate the level of free fatty acids in the oil expressed in oleic acid.

For the determination of Acidity percentage we falow steps :

First, 0.5 to 1g of fat was added to 10 ml ethanol. This one were titrated with ethanolic sodium hydroxide solution (0.1 N) after 4 to 5 drops of phenophthalein are added.

Acidity percentage is calculated according to the following equation [1]:

$$A\% = \frac{V \times C \times M}{10 \times E} \quad \ldots\ldots\ldots (8)$$

where :

V : volume of NaOH solution used (ml) ;

C : exact concentration of NaOH solution used (mol/L) ;

E : mass of the sample (g) ;

M : molar mass molaire of oleic acid (g /mole) (282,4 g/mole).

III.4. Thin layer chromatography (TLC) :

The technique presented here is thin layer chromatography (TLC). It uses a stationary phase deposited on an aluminum plate. The mobile phase is carried by capillary action towards the top of the plate [2].

In this work, the TLC was used for the separation and the highlighting of the compounds present in essential oils.

At first, we introduce into our tank (chromatographic system), the chosen eluent. The latter can be a solvent or it can be a mixture of several solvents.

At the start of the test, a deposit line was drawn approximately 1 cm from the edge of the plate; on which we used a micropipette (0.5µl) of each sample (essential oils) was deposited and then dried quickly. Once it is ready, place the plate in the chromatographic tank containing the eluent system and cover it.

When the solvent front is approximately 1 cm from the upper end, stop the test by removing the plate and then air drying it. Used iodine vapors as a means of revealing stains. The positions of the colored spots (spots) must be noted by circling them just at the end of the chromatography because certain products disappear with time. Finally calculate the frontal ratio (Rf) for each spot by the following relationship:

$$Rf = \frac{\text{Distance parcourue par le constituant}}{\text{Distance parcourue par l'éluant}} \quad \text{..... (9)}$$

III.5. Antimicrobial activity :

The agar diffusion method is used to test the antimicrobial activity. The culture medium used is Mueller-Hinton agar for bacteria and for yeasts (4 mm thickness). The bacterial and yeast strains tested are; *E. coli* (CECT 515), *B. subtilis* (CECT 498), *P. aerogenes* (CECT 110), *S. aureus* (CECT 240) and *C. albicans* (CECT 1394).

Microbial suspensions of 10^8 cells/ml were prepared from young cultures in a sterile saline solution (0.85%), having a turbidity close to that of McFarland 0.5 [3].

The seeding of the 1 mL inoculum is carried out on the surface. The disks (Wathman paper and sterilized) are placed on the surface of the Petri dish. Each disc is impregnated with a (10 µL) of essential oils. To allow a good diffusion of the essential oils, the Petri dishes are left to rest for 30 min at room temperature before being incubated at 37°C for 24 hours. The dishes

are incubated in an oven at 37°C for 12 to 18 hours for the bacteria and at 25°C for 48 hours for the yeasts. In results, the absence of microbial growth results in a translucent halo around the disk, identical to sterile agar, the diameter of which is measured and expressed in millimeters [4].

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Chapter VI : Results and discussions

IV.1. Introduction :

The physico-chemical properties such as Rotatory power, Refractive index, Acid index and Density etc., constitute a means of verification and control of the quality of essential oils. These physico-chemical properties are determined according to a precise protocol and comply with standards laid down by the French Association for Standardization (A.F.N.O.R) and the International Organization for Standardization (I.S.O).

IV.2. Pharmacological investigation :

A pharmacological survey was made near some pharmacies located in the city of Saida.

This investigation will undoubtedly allow us to have an idea of all the essential oils existing and sold at the level of pharmacies as pharmaceutical products as well as the sales threshold for each product (essential oil).

IV.2.1. First pharmacy :

The survey made at the first pharmacy allowed us to collect some information on the number, type and rate of sale for each essential oil (Fig. 10).

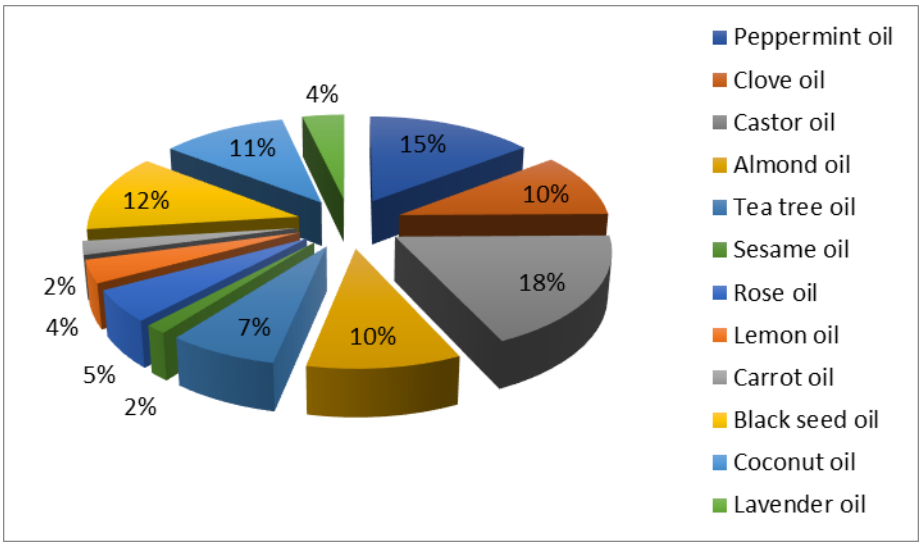

Fig. 10 : Presentation of number of essential oils sales at the 1st pharmacy.

From these results it is clear that the most popular oils salt at this pharmacy clearly are castor oil with 18% and peppermint oil with 15 %, also the least sold oils are carrot oil and sesame oil with 2% of sales. Most people use oils as dermatological, healing and anti-inflammatory products.

IV.2.2. Second pharmacy :

The same objectives led us to the second pharmacy. Then different information is obtained and is illustrated in Fig. 11.

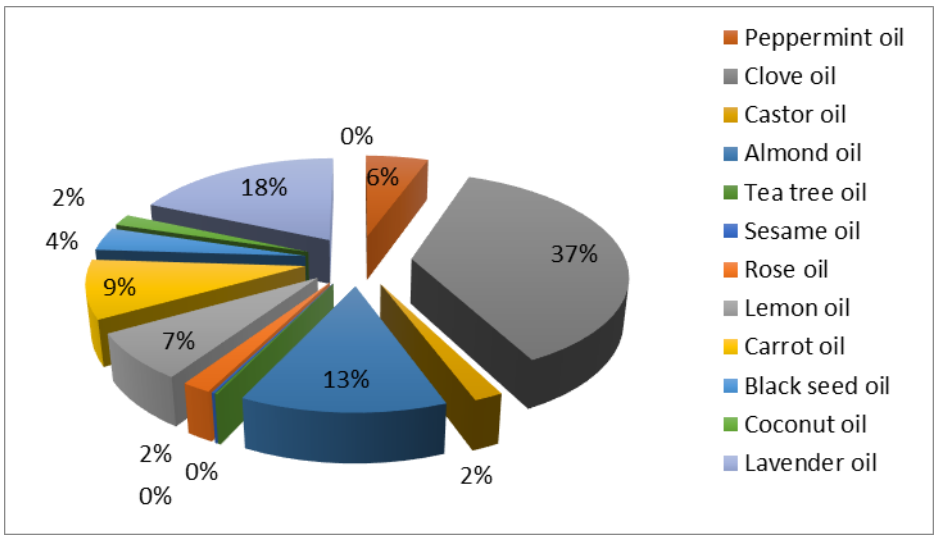

Fig. 11 : Presentation of number of essential oils sales at the 2nd pharmacy.

In this pharmacy, we observe that Clove oil is also popular at the second pharmacy with 37% of sales and its net clear that is a higher number of sales than the first pharmacy and in second lavender oil has 18% of the sales. Here it should be noted that people use oils for dermatology, anti-inflammatory, antimicrobial, antioxidant, hypertension and abdominal purposes. It should be noted that these oils find other uses such as cosmetic uses.

IV.2.3. Third pharmacy :

We continued our visit to the various pharmacies. This time, the following pharmacy provided us with information which is translated in Fig. 12.

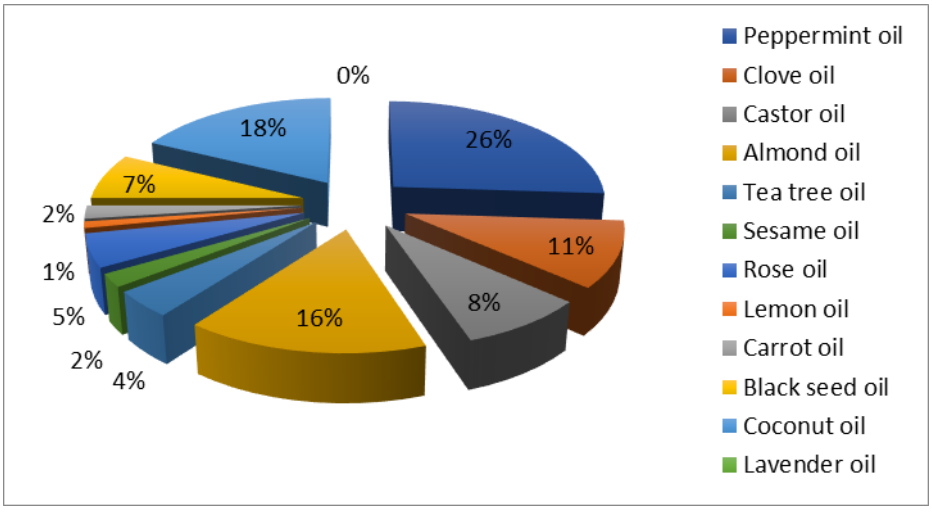

Fig. 12 : Presentation of number of essential oils sales at the 3rd pharmacy.

Illustrated results for current pharmacy investigation shows that peppermint oil is the most sold oil with 26% followed by almond oil with 16% of sales. The most questions asked to the pharmacies oriented and guided us to the reason for which these essential oils are used. They find for dermatology and anti-inflammatory value.

IV.2.4. Fourth pharmacy :

We head to another pharmacy. Another survey was established for more information and for the same reasons. The personnel interviewed provided us with data which are presented in Fig. 13.

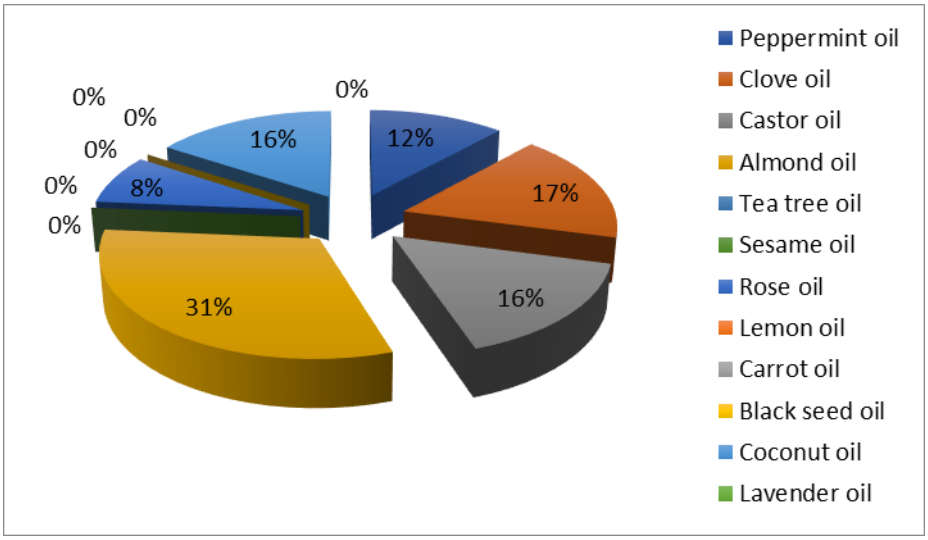

Fig. 13 : Presentation of number of essential oils sales at the 4st pharmacy.

In the fourth pharmacy we notice that almond oil has the best percentage with 31% of sales, but likewise we notice that castor oil, clove oil, coconut oil and peppermint oil also have an interesting percentage such as 17%, 16%, 16% and 12% of sales. In this time the reason of use of essential oils is for dermatology and antioxidant purposes.

IV.2.5. Fifth pharmacy :

For the same reasons and objectives, the fifth pharmacy was our next destination. All the results collected following this interview are illustrated in Fig. 14.

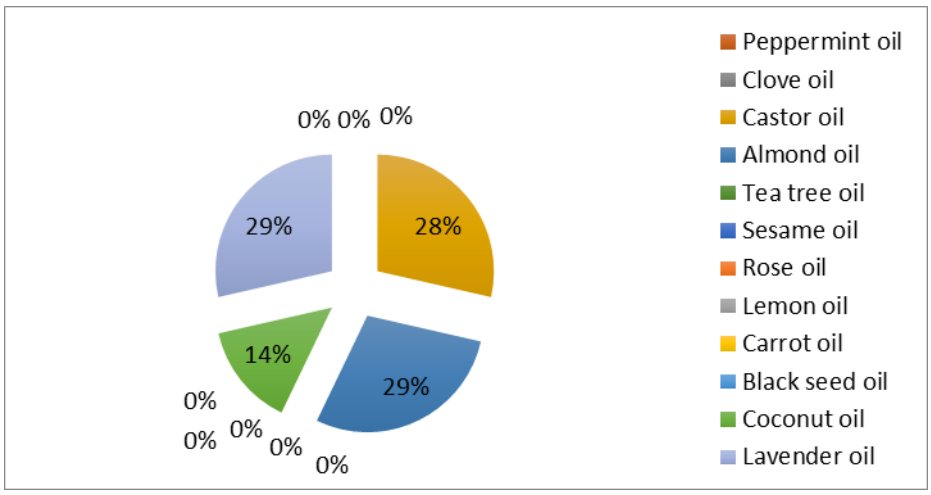

Fig. 14 : Presentation of number of essential oils sales at the 5th pharmacy.

At the fifth pharmacy we did not see much of essential oils for sale, all that we found was castor oil, lavender oil and almond oil with 29% of sales, and coconut oil with 14% of sales, we have been told that unavailability of other essential oil is because of their low rotation.

The reason behind using those essential oils was for dermatology, antioxidant and antimicrobial benefits.

After this investigation we chose 6 essential oils which were the most sold :

- Peppermint oil
- Almond oil
- Lavender oil
- Coconut oil
- Castor oil
- Clove oil

In parallel, another interrogation was stored on randomly selected patients. The set of questions asked serves other purposes. In total 74 patients were investigated whose 45 women and 29 men (Fig. 15).

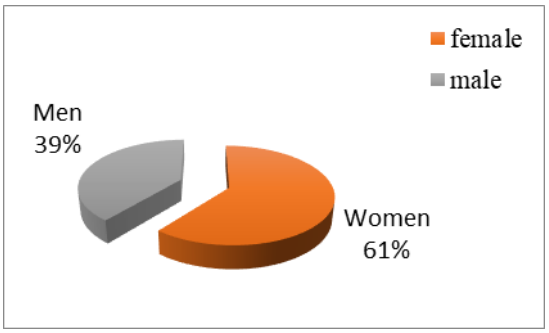

Fig. 15 : Patients questioned about EO

We notice that the number of women is twice the number of men. This reflects the belief and importance of traditional medicine towards women more than men.

At the same time, the patients questioned were asked what they prefer for treatment; medicines, essential oils or others. The responses obtained are shown in Fig. 16.

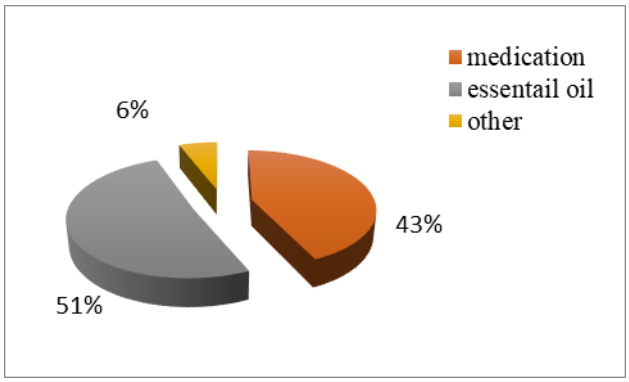

Fig. 16 : represents the different product that patients prefer to use for treatment.

Other such as: cosmetics

From this figure it is clear that the major population questioned prefers to be treated with essential oils 51% against 43% for drugs. This can be explained by the threshold importance and the great confidence that traditional medicine occupies in the daily life of the inhabitants of Saida city.

IV.3. Physico-chemical analyses of essential oils :

IV.3.1. Physical analyses :

3.1.1. Refractive index determination :

The refractive index is a qualitative identification parameter and a way to verify the degree of purity of essential oils. Each substance has its specific refractive index.

We used a refractometer to know the refractive index; the results are illustrated in the following Fig. 17.

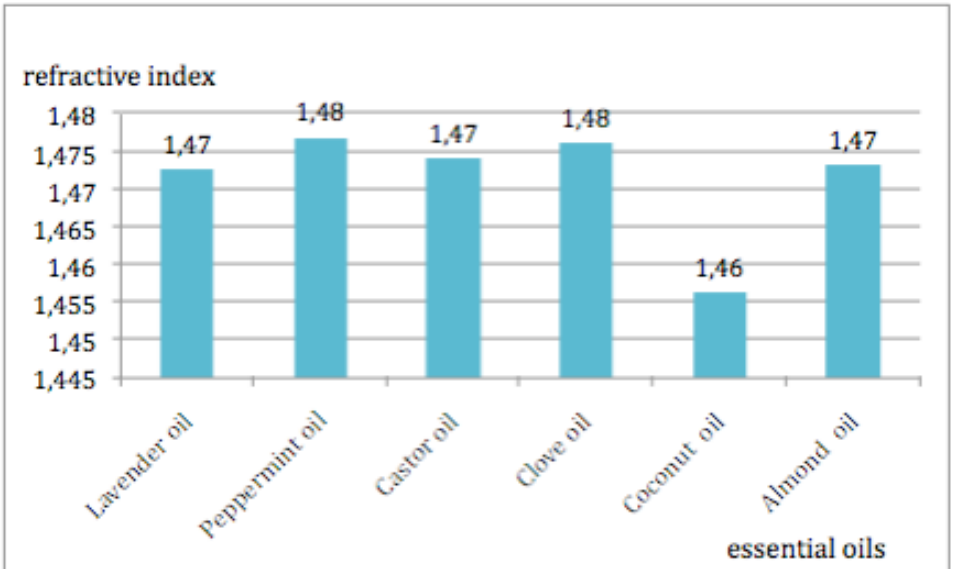

Fig. 17 : Refractive index results.

The refractive index values are between 1.4769 and 1.4562, of which mint oil recorded the highest refractive index 1.4769 and coconut oil the lowest 1.4562

The low refractive indices of these essential oils indicate their refraction of light, which could favor their use in cosmetic products [1, 2]. Refractive indices of these essential oils are higher than that of water at 20 ºC (1.3356). This shows their richness in components that deflect polarized light [3].

According to BOUKHATEM *et al.*, [1] the refractive index varies essentially with the content of monoterpenes and oxygenated derivatives. A high content of monoterpenes will give a high refractive index.

IV.3.1.2. Density determination :

The relative density is a very important criterion for evaluating the quality of an essential oil in different fields (cosmetics, pharmacy, food industry, etc.) (Fig. 18).

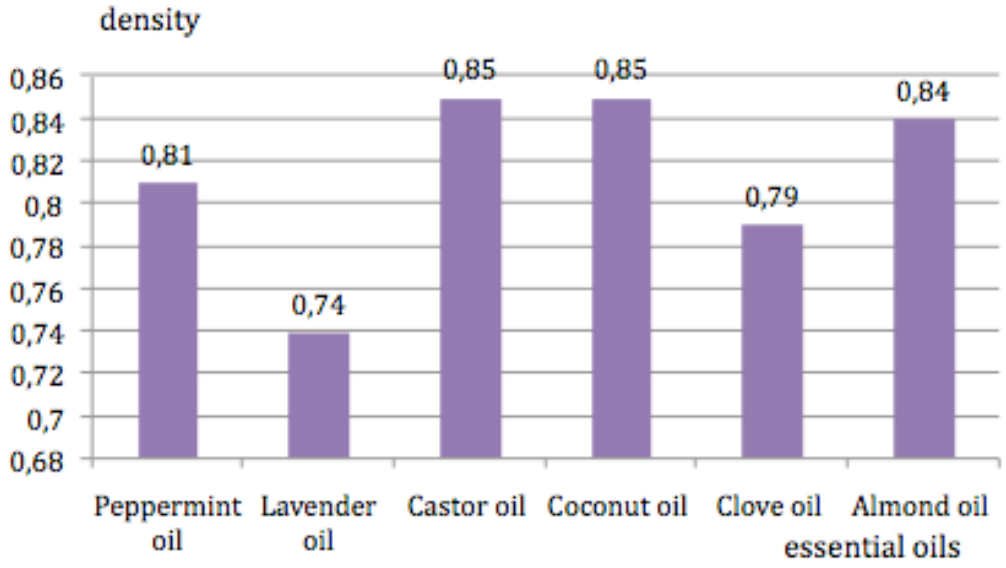

Fig. 18 : Denstity results.

Tests concerning density measurement directed us towards acceptable values. They are all less than 1. The highest density value was recorded in castor oil and coconut oil (0.85). However, the lowest value was reported for lavender oil (0.74).

It can easily provide insight into the naturalness of products as well as attempts at fraud and tampering. The density of an oil increases with the average length of fatty acid chains [4].

IV.3.1.3. PH determination :

The determination of pH value directs us to the type and quality of our oils, whether they are acidic or basic. The different results recorded were translated in the form of a histogram. Fig. 19.

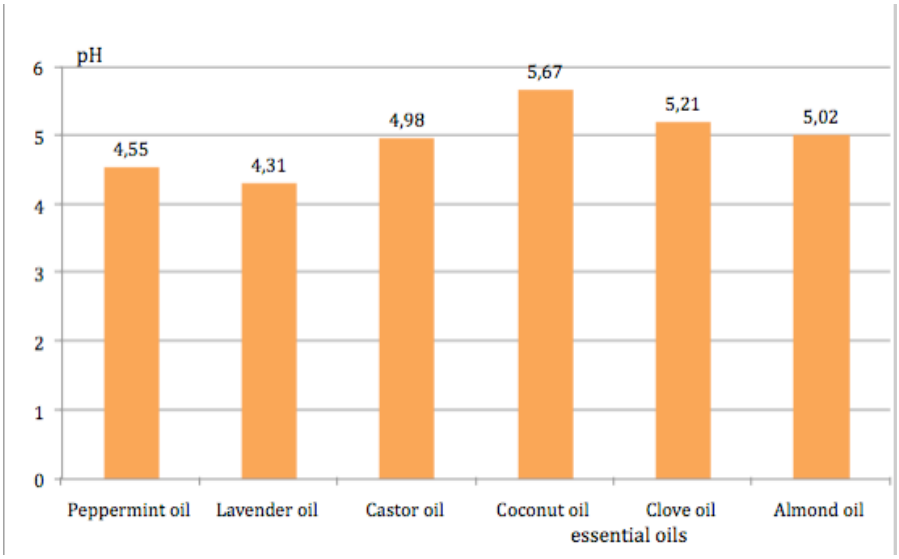

Fig. 19 : PH results.

From the results it is very clear that our oils have an acidic appearance with values below 6. Coconut oil recorded the lowest content of $[H^+]$ (5.67) however lavender oil showed the higher content of $[H^+]$ (4.31).

pH plays a decisive role during chemical and biochemical reactions and can influence the stabilizing properties of an essential oil. Our oils have an acid appearance with values below 6, these values can lead to a good stabilizing character against microorganisms which will allow the oils to play a role as a food preservative.

IV.3.2. Chemical indices :

IV.3.2.1. Acid index (AI) determination :

Like the other indexes, the acidity index plays a key role in determining the quality of oils. The results obtained from this test are shown in Fig. 20.

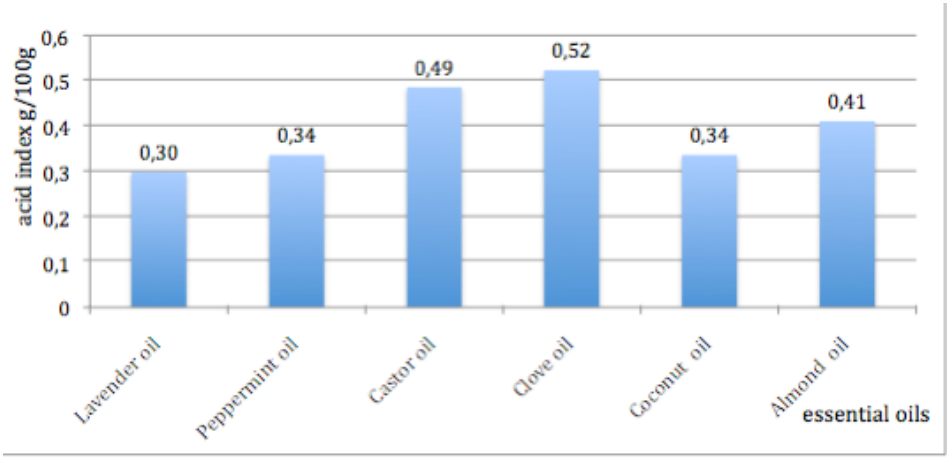

Fig. 20 : Acid index results.

Acid number gives an idea of the level of free acids present in the essential oil. It can also inform us about the susceptibility of the oil to undergo alterations, in particular oxidation. In our study, this index, certainly within the standards, remains relatively low. According to the results of fig. 20, our essential oils presented values lower than 0.53 mg KOH/1g of EO. This value obtained is much lower than those recommended by the AFNOR standard [5], which are around 4.5 to 6.5 mg KOH/1g of EO. The difference noted for the value of the acid index of our oils compared to the values promulgated by AFNOR standard, can be explained by the method of conservation of the plant, its storage duration, chemical composition of its EO, possible phytochemical reactions during hydrodistillation, etc. These are factors among others that can influence the behavior and characteristics of EO.

IV.3.2.2. Peroxide index (IP) determination :

The second analysis listed in the chemical indices is the peroxide index. The various results recorded are shown in Fig. 21.

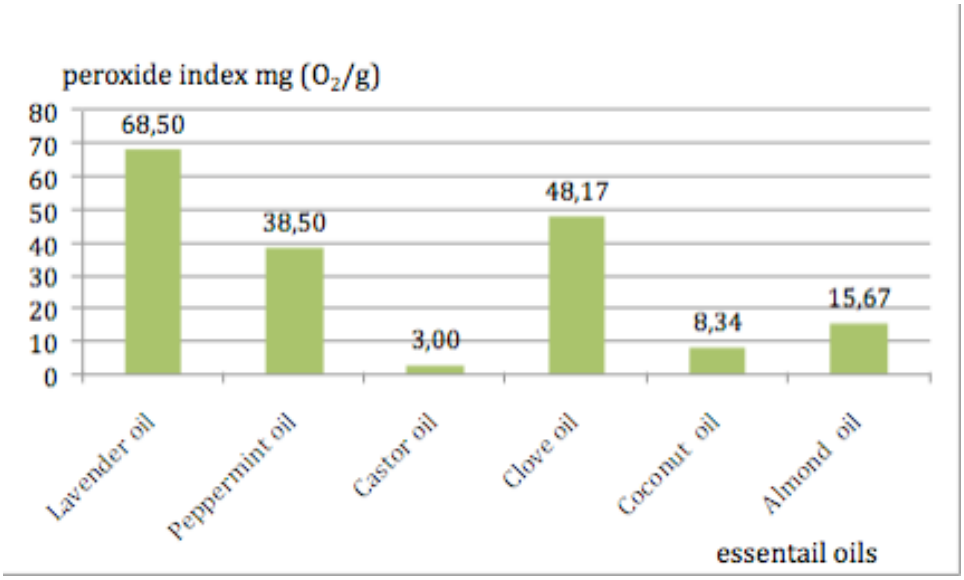

Fig. 21 : Peroxide index results.

Peroxide Index reveals the first degrees of oxidation of the oil characterized by the presence of peroxides or hydro-peroxides, the latter which evolve through the other more stable forms materialized in volatile and non-volatile products. This very useful index informs us of the storage conditions, extraction methods and helps us to appreciate the first stages of an oxidative deterioration of the product [6, 7].

It should be noted that the highest peroxide index values correspond to the samples (lavender oil, clove oil, mint oil) with values of 68.50, 48.17 and 38.50, respectively.

Oil oxidation begins after the fruits are picked from the tree, and continues during storage and processing. Fats can oxidize in the presence of oxygen and certain contributing factors (high

temperature, water, enzyme, trace metals: Cu, Fe...). Indeed, good hygiene and manufacturing practices will have a positive impact on the content of peroxides just after extraction.

IV.3.2.3. Saponification index determination :

The saponification index is measured using the titration method. The results obtained are shown in Fig. 22.

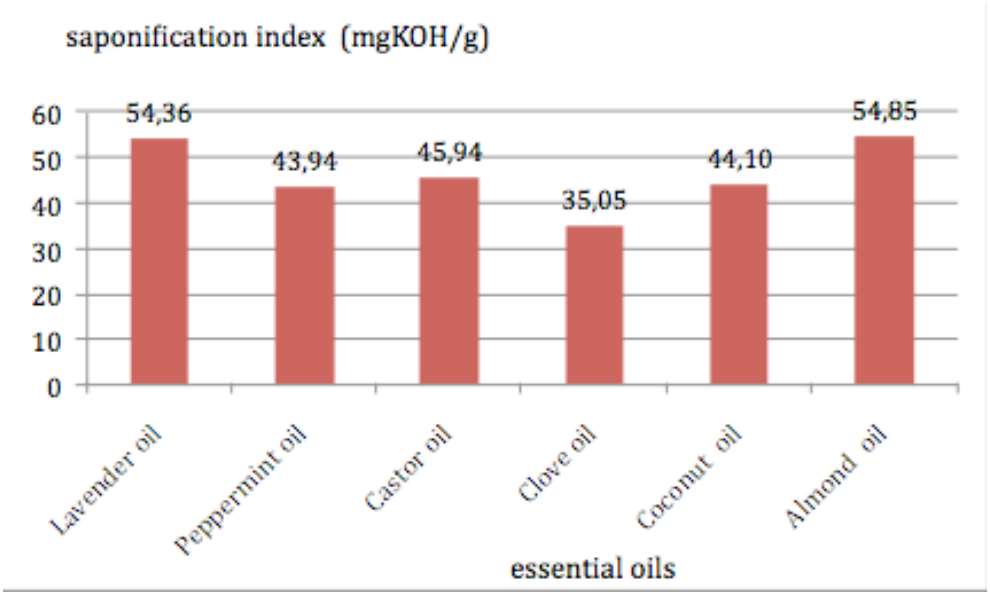

Fig. 22 : Saponification index results.

Saponification Index is by definition the quantity of potash expressed in mg necessary to transform the free and bound fatty acids contained in 1g of fatty substances into soaps. The determination of this index is important, because it makes it possible to characterize the molecular weight and the average length of the fatty chains to which it is inversely proportional (the greater the molecular weight PM of the average length of fatty acids, the higher the index of saponification is low).

It should be noted that the highest saponification index values correspond to the samples (Almond oil and Lavender oil) with values of (54,85 and 54,36) respectively.

We can deduce that almond and lavender oils are pure oils rich in long-chain and unsaturated fatty acids.

IV.3.2.4. Ester Index (EI) determination :

One of the important indices belonging to the physico-chemical indices is the ester index. The results obtained are shown in Fig. 23.

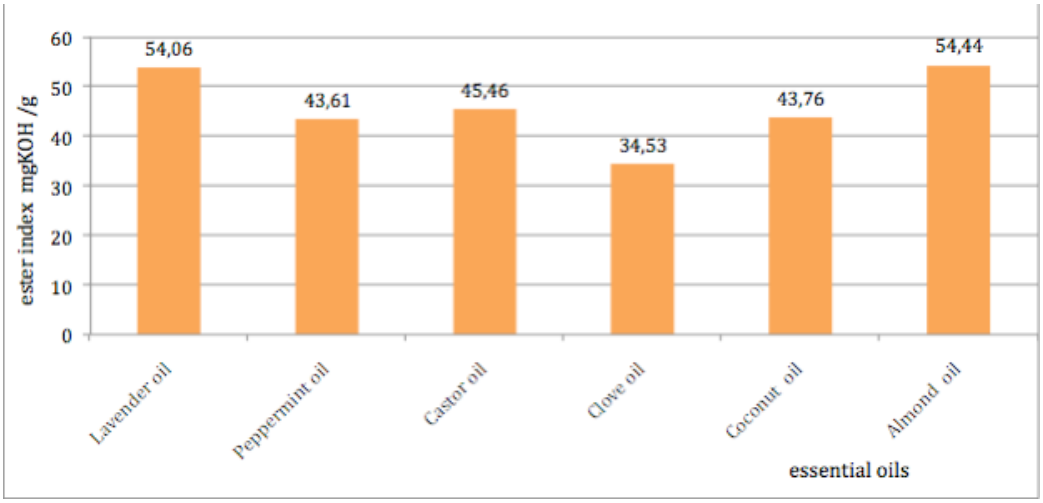

Fig. 23 : Ester index results.

On the other hand, and given that in terms of values, the Ester Index is the difference between Saponification Index and that of Acid, we conclude that as much the Saponification Index is high and the lower the Acid Index, the Ester Index is important.

It is clear that Lavender oil and almond oil have a similar Ester index which is about 54 mg KOH/g.

IV.3.2.5. Percentage of acidity (A%) determination :

The last parameter measured is the percentage of acidity. The results obtained are shown in Fig. 24.

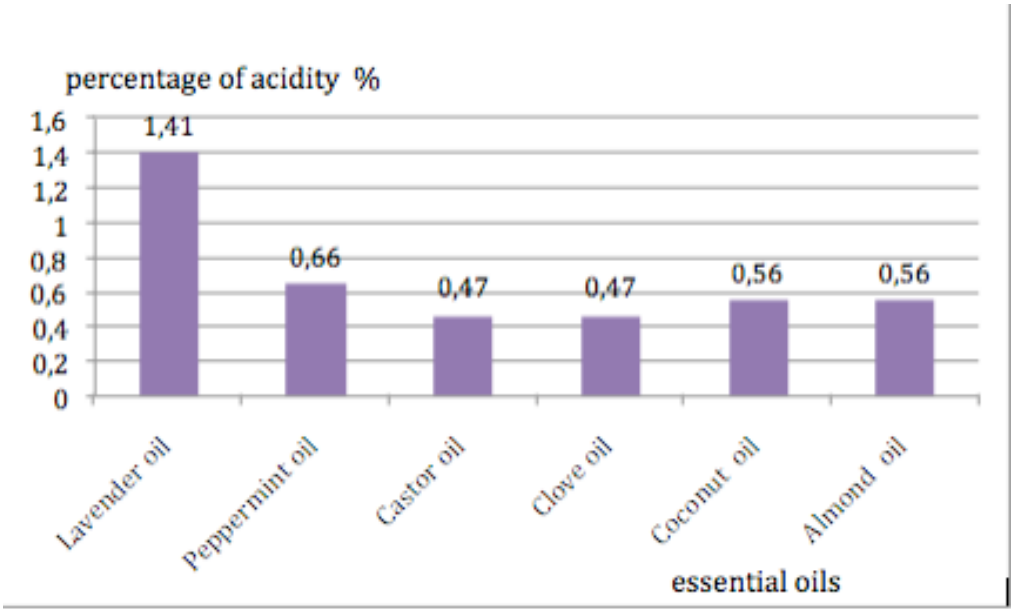

Fig. 24 : Percentage of acidity index results.

As we see peppemint oil, castor oil, clove oil, coconut oil and almond oil have an average value of acidity percentage under 1 mg KOH/g .

IV.4. The antimicrobial activity :

The antibacterial test makes it possible to qualitatively judge the antibacterial activity of our essential oils from the measurement of the diameters of the zones of inhibition learned around the discs which contain the different essential oils.

Unfortunately in our case the antimicrobial activity was negative (Fig. 25) compared to the selected strain, so we left the work open for another bacterial strain.

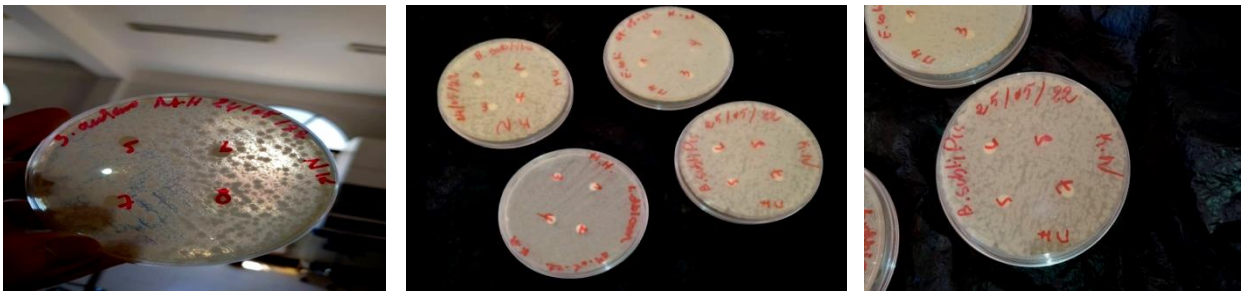

Fig. 25 : Antimicrobial activity results.

Among the six oils we found in the packaging of lavander oil that it has an antibacterial activity so after we did the test, we did not find any inhibition which mean that the lavender oil has no antibacterial activity.

IV.5. Thin-layer chromotography :

We performed thin layer chromatography on our essential oil samples. The resulting chromatogram is shown in Fig. 26 and Fig. 27.

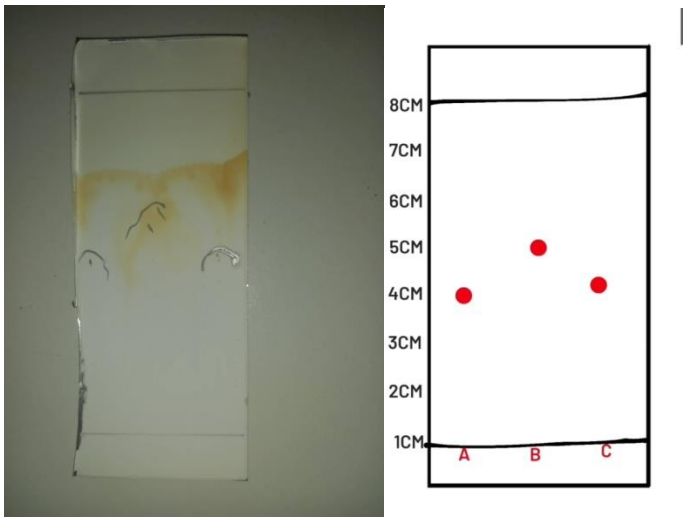

Fig. 26 : Thin-layer chromatography of (lavender , castor and almond) oils .

A: Lavender oil, B: Castor oil, C: Almond oil

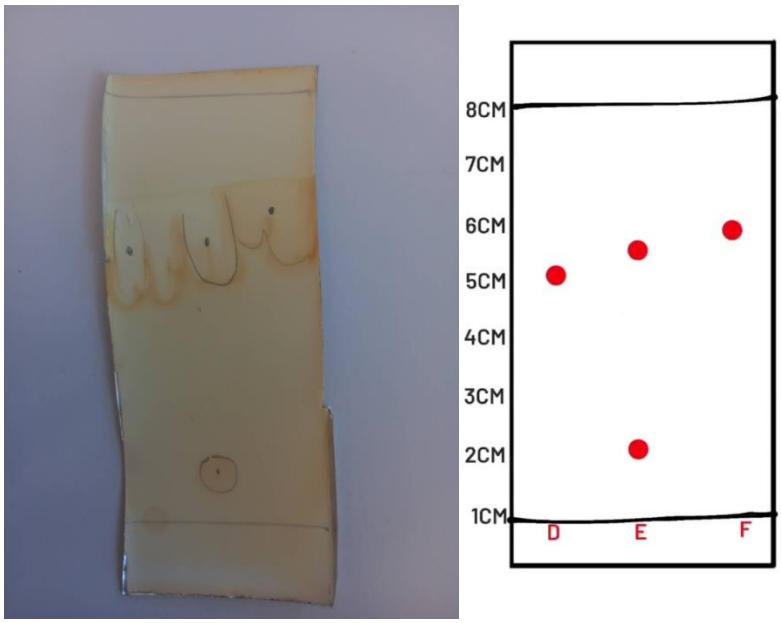

Fig. 27 : Thin-layer chromatography of (clove, peppermint and coconut) oils .
D: Clove oil, E: peppermint oil, F: Coconut oil

According to the British Pharmacopoeia 2008, the spots present in the chromatograms obtained with the solution to be examined must be identical, comparing the coloration, position, dimensions and retardation factors (RF), respectively of the spots. By visual evaluation, the chromatogram obtained with Lavender Oil, Castor Oil, Almond Oil, Clove Oil and Coconut Oil revealed a single spot.

According to [8], a pure substance gives only one spot after revelation in thin layer chromatography. A chemical species is identified in a mixture by comparing its level of migration with that of a known chemical species or reference species, in the event of an identical level of migration, the mixture contains the reference chemical species in question because two species identical chemicals have the same migration rate because they interact in the same way with the fixed phase and the mobile phase of the chromatography.

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Conclusion

Pharmaceutical production is subject to a high level of quality assurance according to the rules of good manufacturing practice (BPF) and good distribution practice (BPD) in order to guarantee a sufficient and constant quantity of drugs from their manufacture to the end of distribution.

It is up to each pharmaceutical company to acquire the technical and human resources necessary to ensure the effective application of these standards. These means are based exclusively on the implementation of a quality assurance system and the goodwill and seriousness of the company's personnel.

The objective of the work that we carried out was precisely to check according to the references the level of application of the rules of control and the level of the quality of the pharmaceutical products (essential oils) manufactured by the Algerian company. A series of physico-chemical tests were carried out in order to control and analyze essential oils by several methods.

At the beginning, the pharmacological investigation made near some pharmacies at the level of the city of Saida, provided us with some information on the types of essential oils existing and sold. So, six essential oils recorded the highest sales thresholds; these include peppermint oil, almond oil, lavender oil, coconut oil, castor oil and clove oil.

It is very clear that women dominate the use of essential oils (61%) more than men (39%).

The results obtained following the physico-chemical analyzes allow us to certify the good quality of the company and the rigor in the application of BPF and BPD. These physico-chemical control results comply with the standards of the European Pharmacopoeia 2008 and British Pharmacopoeia 2008.

Finally, it should be remembered that ensuring the quality of medicines has a cost but "health is priceless" because a counterfeit or inferior medicine will be, whatever its price, always too expensive taking into account the risk for the public health.

In terms of perspective, however, it is interesting to complete the analysis with other studies such as in vivo bioavailability studies which make it possible to precisely determine the therapeutic efficacy of its principles.