Mestrado em Ciências Aplicadas à Saúde

ÓLEOS ESSENCIAIS USADOS EM DERMOCOSMÉTICOS: REVISÃO SOBRE AS SUAS ATIVIDADES BIOLÓGICAS

Cassandra Miranda Pinto Cunha

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If you can't be a pine on the top of the hill,
Be a scrub in the valley — but be
The best little scrub by the side of the rill;
Be a bush if you can't be a tree.

If you can't be a bush be a bit of the grass,
And some highway happier make;
If you can't be a muskie then just be a bass —
But the liveliest bass in the lake!

We can't all be captains, we've got to be crew,
There's something for all of us here,
There's big work to do, and there's lesser to do,
And the task you must do is the near.

If you can't be a highway then just be a trail,
If you can't be the sun be a star;
It isn't by size that you win or you fail —
Be the best of whatever you are!

Douglas Malloch

ABBREVIATIONS AND ACRONYMS

ATCC – American Type Culture Collection

CFU – Colony forming unit

COSMOS – Cosmetics Organic and Natural Standard

DPPH – 1,1-Diphenyl-2-picrylhydrazyl

EDTA – Ethylenediamine tetraacetic acid

EO – Essential Oil

GC-MS – Gas Chromatography-Mass Spectrometry

HD – Hydrodistillation

IC_{50} – Half-maximal inhibitory concentration

ISO – International Organization for Standardization

MIC – Minimum Inhibitory Concentration

MRSA – Methicillin-resistant Staphylococcus aureus

NATRUE - International Natural and Organic Cosmetics Association

O/W – Oil-in-Water

PASI – Psoriasis Area and Severity Index

UV-B – Ultraviolet-B

VAS –Visual Analog Scale

W/O – Water-in-Oil

Dedication

I dedicate this work to all my ancestors, to my father, my mother and my brothers.

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To my advisor Professor André Araújo who was always attentive to my progress and guided me here.

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RESUMO

Introdução: Atualmente é assinalável a procura pela utilização de constituintes de origem natural nas formulações cosméticas em detrimento dos compostos sintéticos. Diversos estudos avaliam o potencial dos óleos essenciais quando incorporados em diversas formulações cosméticas e estudam as suas atividades biológicas.

Objetivos: Este trabalho pretende elaborar uma revisão da literatura sobre os óleos essenciais testados em formulações dermocosméticas e cujas atividades biológicas foram avaliadas por meio de testes *in vitro* e/ou *in vivo*. Assim, os principais objetivos para este estudo foram: identificar os óleos essenciais que têm sido utilizados em formulações cosméticas; e compilar informações sobre as principais atividades biológicas testadas em formulações cosméticas.

Metodologia: Foi realizada uma pesquisa até ao ano de 2021 nas bases científicas de dados PubMed e Web of Science, com recurso a diferentes termos de pesquisa e foram selecionados e analisados diversos artigos científicos de estudos *in vitro*, *in vivo* em animais e ensaios clínicos envolvendo o desenvolvimento de formulações dermocosméticas contendo óleos essenciais e a análise das suas atividades biológicas.

Resultados: Diversos estudos demonstram que a atividade antimicrobiana (antibacteriana e antifúngica) é aquela que tem maior foco de estudo, principalmente através de testes *in vitro*. Os estudos *in vivo* também foram realizados ou em animais ou em estudos clínicos apresentando diferentes efeitos, como ação repelente, inibição do crescimento dos pêlos e ação contra enxaqueca, entre outros. Em relação ao tipo de formulações, foi evidente de que os cremes são os mais utilizados.

Conclusão: Existe um enorme potencial para o uso de óleos essenciais em futuras formulações na indústria cosmética e farmacêutica, em particular como conservantes, mas perspetiva-se também a exploração das suas outras atividades biológicas.

PALAVRAS-CHAVE

Atividades biológicas, Dermocosméticos, Formulações, Óleos essenciais, Conservantes.

ABSTRACT

Introduction: Currently, the search for the use of constituents of natural origin in cosmetic formulations in detriment of synthetic compounds is remarkable. Several studies assess the potential of essential oils when incorporated into various cosmetic formulations and study their biological activities.

Objectives: This work intends to prepare a literature review on essential oils tested in dermocosmetic formulations and whose biological activities were evaluated through *in vitro* and/or *in vivo* tests. Thus, the main objectives for this study were: to identify the essential oils that have been used in cosmetic formulations; and compile information on the main biological activities tested in cosmetic formulations.

Methodology: A search was carried out until the year 2021 in the scientific databases PubMed and Web of Science, using different search terms, and several scientific articles from *in vitro* and *in vivo* studies in animals and clinical trials involving development were selected and analyzed of dermocosmetic formulations containing essential oils and the analysis of their biological activities.

Results: Several studies demonstrate that the antimicrobial activity (antibacterial and antifungal) is the one that has the greatest focus of study, mainly through *in vitro* tests. *In vivo* studies were also carried out either in animals or in clinical studies showing different effects, such as repellent action, inhibition of hair growth and action against migraine, among others. Regarding the type of formulations, it was evident that creams are the most used.

Conclusion: There is a huge potential for the use of essential oils in future formulations in the cosmetic and pharmaceutical industry, in particular as preservatives, but the exploitation of their other biological activities is also planned.

KEYWORDS

Biological activities, Dermocosmetics, Essential oils, Formulations, Preservatives.

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1. INTRODUCTION

Cosmetics can be defined as preparations designed to improve appearance by direct application to the skin and their purpose is to improve the quality of life and the self-esteem of the people who use them (Dreno et al., 2014). It is important to clarify that there is a huge difference between a cosmetic and a drug, that is related to the product's intended use and sometimes the same product can meet both definitions. According to European Medicines Agency, a drug or medicinal product can be defined as "*any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological function by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis"* (CD, 2001).

While the Regulation European Commission (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 defines cosmetic products as "*any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours*" (European Parliament & Council of the European Union, 2009).

In addition, products intended to be ingested, inhaled, injected or implanted in the human body are not considered cosmetic products (*Cosméticos - INFARMED, I.P.*, n.d.).

1.1. COSMETIC FORMULATIONS

The assessment of whether a product is a cosmetic product has to be made on the basis of a case-by-case assessment, taking into account all characteristics of the product. Thus, cosmetic products can include many formulations that include creams, emulsions, lotions, gels and oils that can be used on the skin. Additionally, it belongs to this group makeup products, soaps, perfumes and toilet waters, bath preparations, depilatory products, deodorants and antiperspirants, hair care products, including, for example, fixing products and cleaning and straightening products; teeth, lips and mouth care

products; nail care products; external intimate hygiene products; and also, tanning, self-tanning, whitening and anti-wrinkle products (European Parliament & Council of the European Union, 2009).

Cosmetics can be subdivided into three physical states: the solids like face powders, talcum powders, face packs, masks, compact powders, the semi-solids such as creams, ointments, wax base creams, pastes, and the liquids like lotions, moisturizers, hair oil, conditioners, shampoos, cleansing milk, mouthwashes, deodorants, liniments, sprays, and others (Kapoor, 2005).

Considering this classification the semi-solid formulations are considered more promising over solid and liquids considering their property to cling to the surface of application for a reasonable duration before they have worn off (Bhowmik, 2012). Semi-solids refer to a composition which, at room temperature has the consistency of a cream, ointment or paste. Semi-solids cosmetics may not be free flowing like a liquid, but they will not have the same structure as a solid. This type of formulation will not have a definite shape it cannot be moulded.

Ointments are opaque or translucent, viscous, and have a greasy texture. This type of formulation increases hydration and temperature of the skin because tend not to evaporate when rubbed into the skin. They are used in dry skin areas free from hair because the preparation is difficult to apply. Compared to creams, lotions and solutions, ointments are the least spreadable of the three and the more hydrating.

Creams have moistening and emollient properties and are effective in both dry and weepy/exudative skin conditions. Creams can generally be used on all areas of the body and are chosen for infected and exudative conditions. Creams are emulsions of water-in-oil (W/O) or oil-in-water (O/W). An emulsion is a two-phase system consisting of two immiscible liquids, one of which (dispersing phase) is evenly dispersed as globules in the other (continuous phase). If water is the dispersing agent in the oil, it is called a W/O emulsion (oily creams). W/O emulsions have an occlusive effect by moisturizing the stratum corneum. If oil droplets are dispersed throughout the aqueous phase, the emulsion is called O/W emulsion (vanish creams). It has the advantage of being non-greasy and therefore easily removable from the skin surface (Mayba & Gooderham, 2018).

Lotions are less hydrating of semisolids formulations but they are beneficial when applied to the skin with hair because of their ease of spreading.

Gels are non-greasy and provide a refreshing sensation when applied. These formulations dry on the skin without being occlusive and, for these reasons, are very well accepted (Mayba & Gooderham, 2018).

One of the goals of cosmetic formulations is the protection of the skin against exogenous or endogenous harmful agents, maintaining dermal homeostasis. The protection function of cosmetics goes through sun protection to protect against mosquitos. Cooling and toning are also common functions of cosmetic formulations (Aburjai & Natsheh, 2003).

1.2. COSMETICS BASED ON ESSENTIAL OILS

Medicinal plants have in their composition active principles serving as a basis, for phytotherapy, but also for the preparation of new products in the cosmetics area (Aburjai & Natsheh, 2003). Among the raw materials with the greatest potential for the development of natural products in the cosmetics industry, different types and fractions of medicinal plants and plant extracts stand out, as well as, raw materials of tropical flora, such as natural dyes, fruits, vegetable oils, essential oils (EOs) and resins (Varma, 2010).

The pharmaceutical and therapeutic potential of EOs has been known since ancient times (Dreger & Wielgus, 2014). There are references to the use of cosmetics for tension and fatigue report the millenary use of EOs. Cosmetics are used as cooling agents for the skin and usually contain EOs such as menthol, camphor and eucalyptus and/or menthol derivatives (Aburjai & Natsheh, 2003). There are many formulations, such as oils, gels, creams and lotions that are made from a mixture of different plant species, including EOs, that provide adequate sun protection against harmful ultraviolet rays (Lall et al., 2020). In other cases EOs were extensively employed in all types of soaps, lotions and perfumes as a natural preservative for pharmaceutical and personal care products and as an antifungal (Aburjai & Natsheh, 2003).

Nowadays, EOs are widely subject of intensive scientific research due to their potential as active pharmacological compounds. EOs as well as their single constituents are widely used in cosmetic products due to the offer of a variety of beneficial activities. Several biological activities has been described to the EOs such as analgesic, antiseptic, antimicrobial, carminative, diuretic, spasmolytic to hyperaemic and stimulatory (Sarkic & Stappen, 2018). In fact, one of the main activities that have been demonstrated is the antimicrobial activity (antibacterial and antifungal) of many EOs leading to be considered as potential substitutes for the use of traditionally synthetic preservatives (Dreger & Wielgus, 2014; Varvaresou et al., 2009). Due to the emergence of increasingly resistant microorganisms, the loss of curative effects of many synthetic active ingredients had renewed interest in therapies that use EOs as the antimicrobial activity (Brito et al., 2014).

A large number of cosmetic formulations have been developed based on herbs and herbal oils. There are some regulatory entities such as *Cosmetics Organic and Natural Standard* (COSMOS), to define minimum requirements and globally standardize the rules for the certification of natural and organic cosmetics (*COSMOS-Standard Cosmetics Organic and Natural Standard*, 2020). Also, the International Natural and Organic Cosmetics Association, NATRUE, was created in Europe to promote and standardize the development of natural and organic cosmetics worldwide (*NATRUE Label Criteria: Requirements to Be Met by Natural and Organic Cosmetics NATRUE Criteria*, 2021). The International Organization for Standardization (ISO) created the ISO 16128 standard, which defines techniques and criteria for the development of natural and organic cosmetics (*ISO - ISO 16128-1:2016 - Guidelines on Technical Definitions and Criteria for Natural and Organic Cosmetic Ingredients and Products — Part 1: Definitions for Ingredients*, n.d.).

1.2.1. The essential oil markets

Currently, there is a growing trend for consumers to choose natural cosmetics that meet the same ethical requirements as their lifestyle, due to the awareness of the need for an alliance between health and sustainability and preservation of the environment, but

above all with the belief that these products are safe (Centre for the Promotion of Imports from developing countries (CBI), 2005; Rossi et al., 2007; Vasiljević & Bojović, 2018).

The increasing prevalence of health problems, such as bronchitis, and the need to treat symptoms, such as headaches and cold, in a more natural way, is creating more demand for beneficial EOs in aromatherapy applications. One of the reasons the EOs market is increasing is because, unlike most conventional medicines, EOs have no major side effects. The growing demand for organic products is another factor that influences consumption trends. In 2018 the industry has witnessed a strong increase in demand for 100% vegetable oils, devoid of synthetic fragrances and components of animal origin.

One of the main materials of natural origin for the development of cosmetic formulations are EOs. The global EOs market was estimated at 250 kilotons in 2020 and is expected to grow at a compound annual growth rate of 7.5% from 2020 to 2027. This is related to increasing demand for these compounds in the food, personal care, cosmetics, and aromatherapy markets. Europe accounts for the largest share of the global EOs market (Grand View Research, 2020).

Most consumers demand EOs for various purposes, such as to increase the freshness of the air at home, adding drops of EOs in aroma pots or aroma diffusers, and in an aroma bath adding oils to the water, for example. Young women use EOs to make homemade cosmetics because of their natural content and medicinal benefits (Grand View Research, 2020).

The cosmetics industry is constantly growing and innovating, increasingly improving its products for consumers that led to the study of the potential biological activities that these can possess in cosmetic formulations (Carvalho et al., 2016). Particular attention has focused on the applications of plant EOs as food preservatives (Carvalho et al., 2016; Dhifi et al., 2016). The enormous diversity of this group of natural compounds and wide spectrum of biological properties make them attractive for many industries and new areas of application (Dreger & Wielgus, 2014)

According to the Food Drug Administration, the crude EOs labelled Generally Recognized As Safe comprise lavandin, menthol, rose, sage, oregano, cinnamon, basil, clove, coriander, nutmeg, ginger, and thyme EOs. Similarly, the registered EOs constituents included thymol, carvacrol, eugenol, linalool, carvone, vanillin,

cinnamaldehyde, citral, and limonene, all of which are considered safe under the limitations on the accepted daily intake (*Electronic Code of Federal Regulations (ECFR)*, n.d.; Falleh et al., 2020).

There are many cosmetic preparations based on botanicals and natural products which contain bioactive phytochemicals for an instant application on the skin, hair, and eye care (Kapoor, 2005). The development of formulations based on natural products is the restriction of ingredients hindering the process of developing formulations, especially the stabilization and performance of products (Moretti & Aubertin, 2016).

Corazza *et al.*, (2009) developed a study to evaluate the prevalence of herbal compound usage in a dermatological out-patient population. They conclude that herbal preparations were widely used by the study participants. The main reasons are related to the perceived safety of botanical products about synthetic preparations, curiosity about alternative medicines, and seeking an alternative treatment when conventional therapies fail.

1.2.2. Essential oils as cosmetic ingredients

The total EO content of plants is generally very low but after isolation, these oils are highly concentrated and are widely used mainly for external application. EOs are odorous principles stored in special plant cells-glands, glandular hairs, oil ducts, or resin ducts-situated in any part of a plant or its exudations. They are complex natural substances, organic volatile compounds of low molecular weight (below 300) obtained through different techniques such as distillation and pressing of seeds, rhizomes, fruits, flowers, barks, and leaves (Bassolé & Juliani, 2012; Falleh et al., 2020). They have a characteristic odour, are liquid and colourless at room temperature, insoluble in water but soluble in alcohol and ether. Besides that, they have a refractive index and a very high optical activity (Dhifi et al., 2016). EOs mainly consists of lipophilic, small, and non-polar molecules and they are very complex because contain between 20 to 60 different components in very different concentrations and it is the quantity of these components that will define the main biological properties of the oils (Bakkali et al., 2008).

1.2.3. Chemical composition

Most EOs consist of complex mixtures of chemical compounds, and it is often the unique chemical combination that is responsible for any therapeutic activity. The composition can vary according to the season, time of day, growing conditions and genetic make-up of the plant. In general, the chemical composition of EOs is relatively complex, however, the chemical characterization of most EOs has two to three main components at a reasonably high concentration (20–70%) compared to other components present in trace amounts (Swamy et al., 2016).

All chemical constituents present in EOs are formed by five or six major biosynthetic pathways. There are two distinct groups in the constitution of EOs: the main group is composed of terpenes and terpenoids, and the other by aromatic and aliphatic constituents with low molecular weight and belonging to different chemical classes like alcohols, ethers, aldehydes, ketones, esters, amines, amides, phenols, and others (Bowles, 2003).

The terpenes molecules comprise one of the most important groups of active compounds in plants with over 20.000 known structures. These compounds are obtained from the mevalonic acid pathway and the non-terpenic compounds derive from the phenylpropanoid pathway (Bowles, 2003; Dhifi et al., 2016). Terpenes have two (monoterpenes - 10 carbon atoms being classified as a hydrocarbon and oxygenated) or three (sesquiterpenes - 15 carbon atoms) isoprene units in their constitution (figure 1). About 90% of the constitution of EOs are monoterpenes. The designation of terpenoids comes when functional groups are added to the constitution of terpenes (Bowles, 2003). Aliphatic molecules are those that have carbon molecules in linear chains while aromatic compounds are derived from phenylpropane and constitute a ring structure (Bowles, 2003). These natural compounds of EOs have been suggested as promising enhancers of transdermal penetration for hydrophilic and lipophilic drugs (Herman & Herman, 2015).

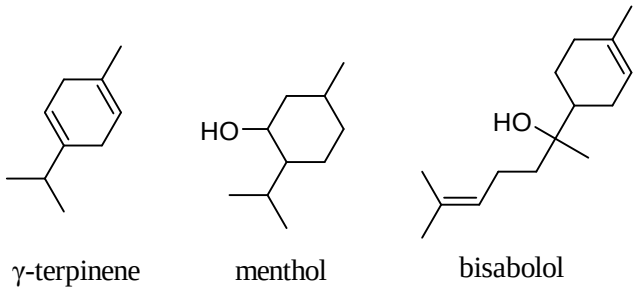


Figure 1 - Chemical structure of monoterpene hydrocarbon (γ-terpinene), monoterpene oxygenated (menthol) and sesquiterpene (bisabolol).

EOs appear to have a good safety profile, however, toxicity can sometimes occur. Their toxicity depends on their compound's concentration and chemical structure. EOs can provoke allergenic reactions. Cytotoxicity, skin irritation and allergic reaction are associated with the fact that these compounds can disrupt the skin's corneocytes and lipid bilayers (Dreger & Wielgus, 2014; Herman & Herman, 2015). There is evidence of contact allergy or allergic contact dermatitis associated with 79 very heterogeneous EOs (De Groot & Schmidt, 2016).

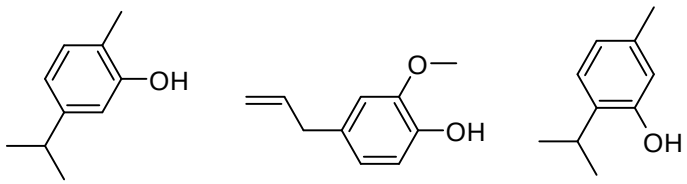
1.3. BIOLOGICAL ACTIVITIES

The factors determining the activity of EOs are related to their composition and the presence of certain functional groups (Swamy et al., 2016). Depending on the physical-chemical constitution of each EOs, a different biological activity can be found.

EOs have shown several scientifically proven cosmetic properties, highlighting their use in cosmetic preparations as natural preservatives, due to their antimicrobial properties. They are used alone or in combination with other preservatives showing antimicrobial activity (Dreger & Wielgus, 2014; Sharmeen et al., 2021). Cosmetic products with an antimicrobial effect can be designated as preparations with the ability to provide protection to the consumer against the presence of antimicrobial compounds (Dreger & Wielgus, 2014). They may be present in the formulations of many different types of cosmetics, like skincare and maintenance products, repairing agents, sunscreens, after-sun, cosmetic textiles, shampoos, soaps, deodorants, depilatories, hair maintenance

(conditioning and shine), mouthwashes, and many others (Carvalho et al., 2016). Preservative components are required in formulations with higher amounts of water, especially in O/W emulsions. Thus, the role of EOs used as natural preservative agents is to prevent contamination by bacteria and fungi in cosmetic products especially in the water phase of the emulsion (where proliferation and growth of microorganisms occur) (Kaczmarczyk et al., 2015). EOs achieve a more effective performance when in combination with synthetic preservatives, as well as chelating and solubilizing agents (Dreger & Wielgus, 2014). To be considered a good preservative agent, an EO must have essential properties such as safety and lack of toxicity, high activity at low concentrations and against a large spectrum of microorganisms, lack of a perceptible fragrance, colour and taste (Kaczmarczyk et al., 2015).

There are a lot of several EOs that demonstrate antimicrobial activities and have been proposed as natural preservatives: tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), lemon grass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), calamint (*Calamintha officinalis*), lavender (*Lavandula officinalis*) and many others (Dreger & Wielgus, 2014). Phenolic compounds are common EO compounds and they are characterized by the hydroxyl group attached to a benzene ring. These compounds are associated with increased antibacterial activity due to the presence of the hydroxyl group in the phenolic structure that is responsible for the disruption of the cytoplasmic membrane, the driving force of protons, active transport, electron flow, and coagulation of cell contents (Sienkiewicz et al., 2012). Examples of these phenolic compounds are carvacrol, eugenol and thymol (figure 2).



carvacrol eugenol thymol

Figure 2 - Chemical structure of phenolic compounds: carvacrol, eugenol and thymol.

The hydrophobicity of the EOs gives them the ability to disrupt lipids in bacterial cell membranes, breaking their structures (Dhifi et al., 2016). However, Gram-negative bacteria are more resistant to EOs than Gram-positive bacteria. That fact is due to the outer membrane surrounding the cell wall of Gram-negative bacteria that restricts the diffusion of hydrophobic EOs compounds, so they are generally more active against Gram-positive bacteria (Cimanga et al., 2002; Nazzaro et al., 2013).

EOs are used as an antibacterial agent (Edris, 2007), antifungal and insecticidal properties (Bakkali et al., 2008). EOs can be used as antibacterial agents against some respiratory tract pathogens. A clinical study demonstrated that a mouthwash containing EOs showed comparable antiplaque and antigenivitis with the activity of chlorhexidine. EOs are antiviral proprieties and some studies reported antiviral activity against HSV-1 and HSV-2 in vitro (Edris, 2007). Some research reveals that EOs are effective also in cardiovascular and nervous system disorders; they have also been shown to be able to reduce cholesterol levels, decrease and regulate glucose levels and have antioxidant properties (Chrysargyris et al., 2020), due to phenolic components, monoterpenes alcohols, ketones, aldehydes and hydrocarbons (Bakkali et al., 2008; Edris, 2007; Sienkiewicz et al., 2012). In recent years, some research reveals that EOs are effective in anti-tumoral therapy such as lemon balm (*Melissa officinalis)* and tea tree (*Melaleuca alternifolia*), among others (Calcabrini et al., 2004; Cavalieri et al., 2004; de Sousa et al., 2004). This ability of EOs is very important as it can help decrease the impact of age-related chronic diseases (Chrysargyris et al., 2020). The EOs of oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) and tea tree (*Melaleuca alternifolia)* bergamot (*Cymbopogon martinii*) and lavender (*Lavandula angustifolia*) are examples of the most important EOs with antioxidant properties (Baratta et al., 1998; Caldefie-Chézet et al., 2006; Kıvrak, 2018; P. K. Mishra et al., 2016; Yanishlieva et al., 2006).

EOs also have anti-inflammatory activity and can be used in diseases such as rheumatism, allergies or arthritis. This ability is associated with its antioxidant properties and their interactions with signalling cascades involving cytokines and regulatory transcription factors and on the expression of pro-inflammatory genes (Dhifi et al., 2016). The EO of *Melaleuca alternifolia* was reported to have a considerable anti-inflammatory activity (Caldefie-Chézet et al., 2006; Hart et al., 2000; Yoon et al., 2000).

In dermatology, EOs are useful due to their antibacterial, antimycotic, anti-inflammatory, astringent, and regenerative properties to treat skin diseases such as psoriasis, dandruff, eczema, seborrheic dermatitis, and cellulite, besides that its anti-inflammatory properties, due to flavonoids, can help to heal wounds (Sienkiewicz et al., 2012). EOs can also be used in cosmetics as cooling agents, providing a refreshing, long-lasting sensation on the skin through skin formulation and hair care products. When EOs are incorporated into a hair product, the aim is to provide shine and conditioning effects, stimulate healthy growth, repel fleas and lice and even control dandruff. They can also be used for permanent waving systems, providing an improvement of texture and a pleasant aroma (Aburjai & Natsheh, 2003).

The following chapters include the Objectives, Methods, Results, the Discussion, and the Conclusions and future perspectives sections. Chapter 4 is the results section and is divided into three subchapters, formulations with EOs tested *in vitro*, *in vivo* in animals, and clinical studies. Chapter 5 is the discussion of the results and chapter 6 is about the conclusions and future perspectives of the present work.

2. OBJECTIVES

This work intends to perform a literature review about the EOs that were tested in dermocosmetic formulations and whose biological activities were evaluated through *in vitro* and/or *in vivo* studies either in animal models and in clinical studies.

Thus, the main objectives foreseen for this study were:

- Identify EOs that have been used in cosmetic formulations based on the analysis of scientific articles that meet the inclusion and exclusion criteria, further described;
- Compile information about the main biological activities tested in cosmetic formulations. The information is represented in the form of tables to improve comprehension distinguishing the types of studies: *in vitro*, *in vivo* and clinical studies. In these tables, relevant aspects of the research that allow concluding the proposed objectives were designated: the biological activity under study, the type of formulation, the studied EO, the test performed and the main results of the research.

3. METHODS

Within the scope of this research project, an investigation was carried out in the PubMed and Web of Science databases.

The investigation included several search terms, among them, cosmetic, dermatological, essential oil, formulation and skin, having been found and analysed several scientific articles from *in vitro*, *in vivo* studies in animals and clinical studies involving dermocosmetic formulations containing EOs.

Initially, it were defined the inclusion criteria that covered articles that comprise the development of a formulation, with the inclusion of EOs and evaluation of its biological activities, without any time period restriction. Articles that do not meet the cumulative criteria were not included. An initial search of articles published until the beginning of the year 2021 was carried out. The exclusion criteria corresponded to articles that do not mention the development of a dermocosmetic formulation or the biological activity evaluation and also articles that included encapsulation techniques of EOs. The process of filtering the results was reading the title and the summary in order to evaluate if the EO was incorporated in any vehicle.

4. RESULTS

4.1. FORMULATIONS WITH ESSENTIAL OILS TESTED *IN VITRO*

This subsection refers to the *in vitro* studies that evaluated antimicrobial and antioxidant activities. The main results are represented in Table 1 and there are present eleven articles present between the year of 2002 to the year of 2019. The subsection is subdivided into the two different bioactivities that were studied, antimicrobial (antibacterial and antifungal) and antioxidant.

Studied EOs are into the formulation alone or in synergy with others EOs. Some studies compared the antimicrobial activity of the EOs with preservatives that are on the market. Different concentrations of the EOs were tested. Due to the difference in characteristics of EOs, formulations and tests performed in the *in vitro* assays, the next table resume some of the principal aspects and the better results are distinguished.

4.1.1. Antimicrobial activity

Cosmetics, like any product containing water and organic/inorganic compounds, in order to ensure consumer safety and, consequently, increase their shelf life, require preservation against the most frequent microorganisms found in cosmetics such as *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Burkholderia cepacia*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Enterobacter gergoviae*, and *Serratia marcescens* (Neza & Centini, 2016).

To evaluate the antimicrobial activity that the EOs promote to the formulation, different assays were performed: agar-well diffusion method, challenge test, comparison of the number of colonies, disc method and effectiveness testing (e.g., plate count method; ten-fold dilutions method). The specific strains recommended to be used in these tests can be obtained from official cell culture collections, such as the American Type Culture Collection (ATCC). The commonly tested strains are potentially pathogenic representatives of Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), mould (*Aspergillus niger)* and yeast (*Candida albicans*) (Halla et al., 2018).

Table 1 – *In vitro* studies of antimicrobial and antioxidant activity of the formulations

Activity	***Type of formulation***	***Essential oil and concentration***	***Experimental assay***	***Results***	***Reference***
Antimicrobial activity	Cream	*Lavandula angustifolia* (0.5%, w/w)	Challenge test Gram-positive bacteria: - *Staphylococcus aureus* Gram-negative bacteria - *Escherichia coli* - *Pseudomonas aeruginosa* Fungi: - *Candida albicans* - *Aspergillus brasiliensis*	- It was confirmed the efficacy of the preservative (the formulation fulfilling the A type criteria)	(Bogdan et al., 2019)
	Hydrogel, oleogel and bigel	*Bidens tripartita* (100 mg/g)	Agar-well diffusion method Fungi: - *Candida glabrata 7771* - *Candida krusei ATCC 6258* - *Candida albicans 7562* - *Candida albicans 7389-3* - *Candida tropicalis 7389-2* - *Candida krusei 7525* - *Candida parapsilosis ATCC 22019* - *Candida parapsilosis 7448*	- The hydrogel-based formulation exhibited antifungal activity against all tested Candida strains (mean of 9.3 mm) except for the clinical strain of *Candida parapsilosis* - Bigel and oleogel have shown the lowest activity with a mean of diameters of 8.0 mm and 6.3 mm, respectively	(Tomczykowa et al., 2018)
	Hydrophilic ointment base, macrogol blend ointment base, macrogol cream base, simple ointment base, and white petrolatum base	*Cymbopogon martini* (0.5, 1, and 2% for antibacterial activity); 1, 1.5 and 2% for antifungal activity)	Agar-well diffusion method Gram-positive bacteria: - *Staphylococcus aureus* - *Streptococcus pyogenes* Gram-negative bacteria: - *Pseudomonas aeruginosa* - *Klebsiella pneumonia* Fungi: - *Candida albicans* - *Aspergillus niger*	- Hydrophilic ointment base with the EO showed the highest antimicrobial with inhibition zone diameter ranging from 4.0 to 33.3 mm and antifungal activity with the inhibition zone diameter ranging from 21.0 mm to greater than 140 mm compared to the other formulations tested (macrogol blend ointment base; macrogol cream base; simple ointment base and white petrolatum base)	(Gemeda et al., 2018)

			- *Microsporum canis* - *Trichophyton mentagrophytes* - *Trichophyton verrucosum* - *Trichophyton rubrum*		
	Cream	Lavender (0.1%)	Number of microbe colonies	- No colonies developed in the EO formulation - Protection was achieved against the development of microorganisms for 3 months	(Andrys et al., 2018)
	Cream	Rosemary, lemon (1 and 2%)	Plate method Gram-positive bacteria: - *Staphylococcus aureus* ATCC 6538 Gram-negative bacteria: - *Pseudomonas aeruginosa* ATCC 15442 Fungi: - *Candida albicans* ATCC 10231	- All emulsions contain less than 10 CFU/g, which proves their high microbial quality	(Kaczmarczyk et al., 2015)
	Gel Malacalm®	*Citrus aurantium*, *Lavandula officinalis*, *Origanum vulgare*, *Origanum majorana*, *Mentha piperita*, *Helichrysum italicum* var. i*talicum* (0.1-10%)	Microdilution test Fungi: - *Malassezia pachydermatis* (clinical isolate)	- All active EOs were at the same concentration both fungistatic and fungicidal, except for *Helichrysum italicum* that did not show any antifungal effect - *Origanum vulgare* showed the lowest MIC, being active at 0.8% - The overall MIC value of Malacalm® was 0.3%	(Nardoni et al., 2014)
	Cream	*Lavandula officinallis, Melaleuca alternifolia, Cinnamomum zeylanicum* (2.5%)	Effectiveness testing (plate count method; tenfold dilutions method) Gram-positive bacteria: - *Staphylococcus aureus* ATCC 29213 Gram-negative bacteria: - *Pseudomonas aeruginosa* ATCC 27853 - *Escherichia coli* ATCC 25922 Fungi: - *Candida albicans* ATCC 14053	- The inhibition zone varied between 8-44 mm for EOs, 7-9 mm for extracts and 8-9 mm for methylparaben - *Cinnamomum zeylanicum* against gram-positive bacteria has shown an inhibition diameter of 44 mm, against gram-negative bacteria, the inhibition diameter of *Cinnamomum zeylanicum* was 24 mm and 32 mm for *Pseudomonas aeruginosa* and *Escherichia coli*, respectively	(Herman et al., 2013)

				- All tested creams with EOs and extracts at 2.5 % concentration exhibited antimicrobial activity stronger than methylparaben	
	Cream and shampoo	*Calamintha officinalis* (1.0 and 2.0, v/v)	Microbial challenge test: single or mixed cultures Gram-positive bacteria: - *Staphylococcus aureus (ATCC 6538P)* - *Staphylococcus epidermidis (ATCC 12228)* Multiresistant Gram-positive bacteria: - *Methicillin-resistant Staphylococcus aureus (MRSA, clinical isolate,)* Gram-negative bacteria: - *Escherichia coli (ATCC 25922)* - *Pseudomonas aeruginosa (ATCC 9027)* - *Pseudomonas aeruginosa (cream isolate)* - *Burkholderia cepacia (ATCC 25416)* Fungi: - *Candida albicans (ATCC 10231)* - *Candida albicans (clinical isolate)* - *Aspergillus niger (ATCC 16404)*	- There were no differences in the antimicrobial activity of the EO between single strains and mixed cultures - *Calamintha officinalis* EO at 2.0% concentration reduced the microbial inoculum. - At 1.0% reduce the bacterial inoculum by a factor of 10^3 within 7 days, without the increase until the 28th day - At 2.0% reduction the bacterial inoculum by a factor of 10^3 within 14 days of challenge with no increase up to the 28th day) being ineffective as regards for Staphylococcus aureus standard and MRSA strain - Shampoo formulation reduces bacterial inoculum by a factor of 10^3 in 14 days, with no increase until the 28th day	(Nostro et al., 2004)
	Cream	*Artemisia afra, Pteronia incana Lavandula officinalis, Rosmarinus officinalis* (0.5, 1.0 and 1.5, v/w)	Challenge test Gram-positive bacteria - *Staphylococcus aureus* ATCC 2592 Gram-negative bacteria - *Escherichia coli* ATCC 35218 - *Pseudomonas aeruginosa* (environmental isolate) - *Pseudomonas aeruginosa* ATCC 27853 - *Ralstonia pickettii*. Fungi - *Aspergillus niger* ATCC 16404	- *Artemisia afra* EO was the most efficient in reducing the load of artificial contaminations in the aqueous cream formulation within 2-7 days	(Muyima et al., 2002)

			- *Candida albicans* ATCC 10231		
	Sodium laurate monostearin cream, macrogol cream, macrogol blend ointment, simple ointment, white petrolatum alone	*Ocimum gratissimum* (2%)	Agar diffusion assay Gram-positive bacteria - *Staphylococcus aureus* NCTC 657 - *Staphylococcus aureus* (wound isolate) Gram-negative bacteria - *Pseudomonas aeruginosa* ATCC 10145 - *Pseudomonas aeruginosa* (wound isolate) - *Proteus* spp. (wound isolate)	- From the formulations, the higher antibacterial activity was observed with macrogol blend ointment formulation containing the EO	(Orafidiya, 2002)
Antioxidant activity	Ointment	*Lavandula aspic* L. (4%)	DPPH and superoxide oxygen radicals inhibition	- DPPH assay: IC_{50} EO (5166.66 µg/mL) vs. IC_{50} ascorbic acid (20.73 µg/mL) - Superoxide anion: IC_{50} EO (16483.33 µg/mL) vs. IC_{50} ascorbic acid (0.32 µg/mL) - The EO antioxidant activity was lower than that of the ascorbic acid through both methods	(Ben Djemaa et al., 2016)

ATCC, American Type Culture Collection; CFU, colony forming unit; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EO, essential oil; IC_{50}, half-maximal inhibitory concentration; MIC, Minimum Inhibitory Concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*.

Bogdan *et al.*, (2019) evaluated the antimicrobial preservative effect of a new cosmetic product containing lavender EO in a plastic container-plastic jar polypropylene homopolymer and compared it against the glass receptacle used as a control. The tested product was hand and body cream. The microbiological properties against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungi (*Candida albicans* and *Aspergillus brasiliensis*) by using the challenge test according to SR EN ISO 1190:2012. The challenge test confirmed the efficacy of the preservative, the formulation fulfilling the A type criteria.

In a study conducted by Tomczykowa *et al.*, (2018) it was tested the *Bidens tripartite* EO, known as bur marigold, was for the treatment of candidiasis incorporated in novel gel formulations (hydrogel, oleogel and bigel, a mixture of hydrogel and oleogel). These three formulations were prepared with the EO in their constitution, and they were also prepared without the introduction of this (control group or placebo). It is known that hydrogel and oleogel contain different ingredients in their composition (e.g., in the hydrogel was used sodium alginate as a gelling agent while in the oleogel was used Aerosil® 200 as a gelling agent), which can be a determining factor in the results. The EO extracted of the aerial parts of the plant was analysed by gas chromatography-mass spectrometry (GC-MS) to evaluate the major components which are p-cymene (4.82%), β-linalool (2.85%), (Z)-cymene (2.60%) and α-fellandrene (1.10%). Physical-chemical analysis of all formulations was also performed. The firmness, compression of the formulations increases with the introduction of the EO. Adhesiveness increases in the case of oleogel and bigel and is maintained in the case of the hydrogel. Previously to the antifungal evaluation, to exclude the potential of toxic effects of *Bidens tripartite* EO on human dermal fibroblasts, methyl thiazolyl tetrazolium and single-cell gel electrophoresis assays were performed for the first time. These studies proved the safety of the *Bidens tripartite* EO in the tested concentrations that were further used to prepare the formulations. Antifungal activity of the EO against Candida spp. yeasts reference (*Candida krusei* ATCC 6528, *Candida parapsilosis* ATCC 22019) and clinical strains of Candida spp. were used (*Candida krusei*, *Candida parapsilosis*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*) and it was measured (and compared to the EO in formulations) by the growth inhibition diameter. Approximately 50 µg of the sample was applied to the wells. EO activity at the corresponding concentrations in DMSO (50 µL)

was also analysed on the same plates. Solutions of ketoconazole and clotrimazole in DMSO and commercially available products with Nizoral® and Clotrimazole® were used as positive controls. The antifungal activity of the hydrogel supplemented with 100 mg/g *Bidens tripartite* EO concentration was demonstrated against all tested Candida strains, except for *Candida parapsilosis* (clinical strain). The highest activity was observed against *Candida tropicalis* and *Candida krusei* (3.7 mm difference compared to hydrogel without *Bidens tripartite* EO). With the bigel formulation the growth inhibition zone diameters were observed in *Candida glabrata* (mean of diameters 8.0 mm), *Candida tropicalis*, and both *Candida parapsilosis* strains (mean 7.7 mm). Finally, with the oleogel minimum antifungal activity (mean 7 mm) was demonstrated only for the clinical *Candida krusei* strain, and for individual measurements of *Candida parapsilosis* ATCC 22019, *Candida tropicalis* and *Candida glabrata* (mean of diameters 6.3 mm). Among all the tested gel formulations which were combined with *Bidens tripartite* EO, the hydrogel-based formulation appears to be the preferred combination that exhibited antifungal activity against all tested Candida strains (Tomczykowa et al., 2018).

A study conducted by Gemeda *et al.*, (2018) it was evaluated and developed safe and effective topical antimicrobial formulations using *Cymbopogon martini* EO. This EO, known as palm rose was extracted using hydrodistillation (HD) aerial part *Cymbopogon martini* and topical formulations were prepared in five different semisolid bases ointments with and without EO (hydrophilic ointment base, macrogol blend ointment base, macrogol cream base, simple ointment base and white petrolatum base). Then, it was investigated the *in vitro* antimicrobial activity on EO and topical formulations. The following bacterial and fungal strains were used in the study: *Klebsiella pneumoniae* (ATCC 13883 and clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853 and clinical isolate), *Staphylococcus aureus* (ATCC 25923 and clinical isolate), *Streptococcus pyogenes* (ATCC 19615 and clinical isolate), *Aspergillus niger* (ATCC 10535 and clinical isolate), *Candida albicans* (clinical isolates), *Microsporum canis* (clinical isolate), *Trichophyton mentagrophytes* (ATCC 18748 and clinical isolate), *Trichophyton rubrum* (ATCC 28188), and *Trichophyton verrucosum* (clinical isolate). Minimum inhibitory concentrations (MIC) of *Cymbopogon martini* EO were determined using an agar dilution assay with concentrations of EO ranging from 0.25% (v/v) to 4% (v/v) and the results demonstrated the susceptibility of fungi *vs.* bacteria. It was also determined the activity

of the raw EOs, before its incorporation. The highest growth inhibitory activity of the EO was exhibited at 4% concentration against *Streptococcus pyogenes* clinical isolate, while the least was the concentration of 0.25% against *Pseudomonas aeruginosa* clinical strains. The antimicrobial assay of the formulation performed was the agar well diffusion technique which consists of placing about 0.1 mL of the formulation in each well and after incubation at 25°C for 7 days (for fungi) and 37°C for 24 h (for bacteria), measured from the diameter of the inhibition zones. As is presented in Table 1, different concentrations of the EO were used, in the range of 0.5 and 2% for testing the antimicrobial activity. Among topical formulations, the highest antimicrobial activity was recorded in hydrophilic ointment followed by macrogol blend ointment. Interestingly, in several test parameters, these are the formulations with the best characteristics. The formulation of hydrophilic ointment and ointment with macrogol mixture show good release with a pH value of 5.8 and hydrophilic ointment at 6.2 and spreadability values of 14.7 and 10.5, respectively. The antimicrobial activity of EO was higher in fungal pathogen compared to bacteria. Gram-positive bacteria were more sensitive than Gram-negative bacteria. In conclusion, topical formulations of *Cymbopogon martini* EO can be alternative topical agents with safe broad-spectrum activity for the treatment of skin disorders. Further studies should focus on shelf-life study and clinical study of the product (Gemeda et al., 2018).

Another study conducted by Andrys *et al.*, (2018) evaluated the microbial evolution of a cosmetic emulsion. First, it was prepared an emulsion O/W with the lavender EO incorporated at the concentration of 0.1%. The objective was to compare the preservative activity of this EO with a commercial preservative containing dehydroacetic acid and benzyl alcohol. After three months of the preparation of emulsions, the microbial evaluation was performed at room temperature and high temperature (45ºC) for 3 months. This study also evaluated the storage conditions and the formulation without any preservative changed its colour and odour under extreme temperature conditions and with exposure to light. Results of the microbial activity were expressed by the number of microbe colonies that were formed and showed that lavender EO can be used as a preservative in cosmetic emulsion, protecting it against the development of microorganisms for a period of at least 3 months. The formulation without any preservative (positive control) developed 23 microbe colonies at 37ºC, and 5 at 22ºC. The

results indicated that this EO can be used as an ingredient to prevent ageing processes, as well as a natural preservative. In addition, the synthesis of procollagen by fibroblasts treated with lavender EO was observed. The results demonstrate that the use of 0.01, 0.001 and 0.0001% EOs isolated from *in vitro* plants stimulate human skin fibroblast cells to the production of procollagen. It was further performed that lavender EO used in the concentration of 0.1% in a cosmetic emulsion is characterised by preservative effect for the period of 3 months.

In another study, once again, it was focused on evaluating the potential antibacterial (*Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442) and antifungal (*Candida albicans* ATCC 10231) activity of EOs in cosmetic products. In this case, the stability of different creams and several combinations of EOs were evaluated. The formulations are emulsions O/W and the preservative action capacity of rosemary and lemon EO was evaluated. The emulsion consisted of 76.3% water, 8% glycerine, 0.7% potassium hydroxide (aqueous phase) and 15% stearin (oil phase). Nine emulsions were prepared with different options of preservatives (1% rosemary oil; 2% rosemary oil; 1% rosemary oil and 1.5% vitamin E; 2% rosemary oil and 1.5% vitamin E; 1% lemon oil; 2% lemon oil; 1% lemon oil and 1.5% vitamin E; 2% lemon oil and 1.5% vitamin E; 1% lemon oil and 0.5% Phenonip®, that is the mixture of phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben and isobutylparaben). Six emulsions were subject to microbial evaluation. It was excluded two formulations that were considered unstable at the preliminary stability evaluation. The most stable emulsion was the one with the citrus oil (2%) and vitamin E (1.5%) and the most unstable compositions were the ones with rosemary oil (2%), vitamin E (1.5%) and citrus oil (1%). They comprise rosemary or lemon oil with vitamin E or Phenonip®. The results of the microbiological test show that the evaluated emulsions contain less than 10 colony forming units (CFU)/g, which proves their high microbial quality and *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* are not present. To achieve these results is important the use of raw materials that meet quality standards, carrying out the production process with good pharmaceutical practices and then, demonstrate antimicrobial and antifungal effectiveness of natural preservatives (Kaczmarczyk et al., 2015).

In another study the aim was to evaluate the antifungal activity of a solution gel Malacalm®, a commercially available mixture proposed as adjuvant treatment in dermatological disorders, that has incorporated six different EOs in various concentrations namely, *Citrus aurantium* 1%, *Lavandula officinalis* 1%, *Origanum vulgare* 0.5%, *Origanum majorana* 0.5%, *Mentha piperita* 0.5% and *Helichrysum italicum var. italicum* 0.5%. To understand the activity of each compound present in the EOs, major active compounds were isolated and evaluated. As it was expected monoterpenes were the major compounds found in the EOs. Thymol, γ-terpinene and ρ-cymene and carvacrol were the major compounds found in *Origanum vulgare* (oregano). In the *Origanum majorana* (marjoram), the major compounds found were thymol, γ-terpinene and ρ-cymene, terpinen-4-ol and trans-sabinene hydrate. In *Mentha piperita* (peppermint) it was found in high amount menthol, menthofuran and menthone. Linalool was the major compound of two species *Lavandula officinalis* (lavender) and *Citrus aurantium* (bergamot). Limonene and β-pinene are also major compounds of *Citrus aurantium.* In *Helichrysum italicum* (immortelle) the major constituent was α-pinene. The presence of these compounds normally indicates antibacterial activity. In this study, it was also evaluated the MIC values of the compounds most represented in Malacalm® against *Malassezia pachydermatis*. Thymol and carvacrol showed the lowest MIC values, 0.025% and 0.05% respectively while ρ-cymene, 1,8-cineole, limonene and menthol presented a MIC value of 1%. With higher MIC values there were linalool (2.5%), menthone (5%), α-pinene (5%), β-pinene (>10%), γ-terpinene (>10%), trans-sabinene hydrate (>10%) and menthofurane (>10%). The microdilution test assay was performed in concentrations from 0.1% to 10%. The results revealed that *Origanum vulgare* showed the lowest MIC, being active at 0.8% followed by *Mentha piperita* (1%), *Origanum majorana* (1.3%), *Citrus aurantium* (2%), and *Lavandula officinalis* (4%). *Helichrysum italicum* did not yield any antimycotic effect up to 10%, that is, it needs to be more concentrated to have equivalent activity. The MIC value for Malacalm® was very low (0.3%) when compared to MICs of each EO compound, ranging from 0.8% to up to 10%. This finding could be explained by some synergistic effects among different EOs. All active EOs were at the same concentration both fungistatic and fungicidal, except for *Helichrysum italicum* that did not show any antifungal effect (Nardoni et al., 2014).

A study conducted by Herman *et al.*, (2013) compared the antimicrobial activity of EOs (*Lavandula officinalis*, *Melaleuca alternifolia*, *Cinnamomum zeylanicum*), extracts (*Matricaria chamomilla*, *Aloe vera*, *Calendula officinalis*) and methylparaben that were incorporated into the formulations. The EOs and the extracts were used in a concentration of 2.5% and methylparaben at 0.4%. They were tested against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 14053. Then, it was investigated the *in vitro* antimicrobial activity by the disc-diffusion test. EOs demonstrated higher inhibitory activity against bacteria than extracts and methylparaben. The inhibition zone varied between 8-44 mm for EOs, 7-9 mm for extracts and 8-9 mm for methylparaben. *Cinnamomum zeylanicum* EO, known as cinnamon, showed very promising results for both bacteria and fungi. Then, the *in vitro* tests an antimicrobial effectiveness testing was performed to the cream formulation containing EOs, extracts and methylparaben. Among all tested compounds, cinnamon EO was the most potent inhibitor of microorganism growth. This EO completely inhibited *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* growth after 28 days of incubation. *Melaleuca alternifolia* EO totally inhibited *Escherichia coli* growth. For all tested creams with EOs, extracts or methylparaben, the weakest antimicrobial activity was demonstrated against *Pseudomonas aeruginosa*. Also, should be highlighted that all tested creams with EOs and extracts at 2.5% concentration exhibited antimicrobial activity stronger than methylparaben.

A study conducted by Nostro *et al.*, (2004) it was tested the efficiency of *Calamintha officinalis* EO as a preservative in two topical products, a cream and a shampoo. The *Calamintha officinalis* EO from the calamint plant was obtained by HD. Four creams were prepared with different preservative conditions: cream preserved with EO 2.0% (v/w); cream preserved with EO 1.0% (v/w); as a positive control, a cream preserved with a fluid active combination of Phenonip® (0.5%); as a negative control, a cream without any preservative. Similarly, three shampoos were prepared with different preservative systems: shampoo preserved with EO at a concentration of 2.0% (v/v); a shampoo preserved with a fluid active combination of Phenonip® (0.5%) as positive control and shampoo without any preservative as a negative control. To determine the efficacy of *Calamintha officinalis* EO in the formulations, the microbial challenge test

was performed in single and mixed cultures of different strains of bacterial (Gram-positive and Gram-negative) and fungi as showed in Table 1. The preparations with the EO in concentrations of 1.0% and 2.0% (v/v) were placed in sterile containers and separately inoculated with each mixed culture. The 1.0 and 2.0% (v/v) *Calamintha officinalis* EO was assayed for the preservative activity of the cream and the shampoo. The challenge test was performed with each formulation considering a contact time of 0, 2, 7, 14, and 28 days. After these periods, the viability of the inoculated cells and their ability to grow was evaluated by a growth control consisting of cream without EO. Following the standards of the European Pharmacopoeia, it was possible to distinguish formulations with inhibitory capacity for microorganisms: criterion A is considered to be the ability to reduce the bacterial inoculum by a factor of 10^3 within 7 days, without the increase until the 28th day while criterion B is considered the ability to reduce bacterial inoculum by a factor of 10^3 in 14 days, with no increase until the 28th day. The results clearly demonstrated that the *Calamintha officinalis* EO at 2.0% concentration in the cream formulation, reduced the microbial inoculum in 7 days, satisfying the criterion A of the European Pharmacopoeia while in the case of the shampoo formulation, *Calamintha officinalis* EO at the concentration of 2.0% reduced the microbial inoculum in 14 days, satisfying only the criterion B. Relatively to the cream, it should also be highlighted that *Calamintha officinalis* EO in a concentration of 2% reduced Gram-positive bacteria by a factor 10^3 and it reduced even more Gram-negative bacteria by a factor of 10^4. Usually is more difficult to inhibit Gram-negative bacteria because they are very persistent and predominant in the case of cosmetic spoilage multiplying in creams and lotions also in the presence of preservatives. In the unpreserved formulations, there was a major persistence of Gram-negative rather than Gram-positive bacteria. This exception could be explained maybe due to the presence of ethylenediamine tetraacetic acid (EDTA) at the cream formulation that acted as a synergic agent. Analysing the study data, after 28 days the antibacterial activity of the cream formulations at the concentrations of 1 and 2% was identical. The difference lies in the time (in days) it takes to achieve this antibacterial activity. The concentration with a concentration of 2.0% after two days already has less CFU than the formulation with a lower concentration of EO. In relation to the shampoo formulation, even with the presence of surfactants, which exert some antimicrobial activity, the concentration of 2.0% of the *Calamintha officinalis* EO

was insufficient to reduce Gram-positive bacteria, according to requirement criterion A, satisfying the criterion B. For Gram-negative bacteria, the unpreserved shampoo (control) was a good growth substrate whereas the EO-preserved shampoo showed satisfactory preservative effectiveness. Making a comparative analysis of the two formulations developed, it was possible to conclude that the nature of the formulation in which it was incorporated had a considerable effect on its efficacy. The fact that it did not satisfy the strictest criterion (criterion A), which resides in the speed of action of the EO, does not mean that it is directly a disadvantage. Although the shampoo at the same concentration as the cream (2.0%) did not meet the European Pharmacopoeia A criterion, the shampoo showed good preservative efficacy against Gram-negative bacteria, which are very persistent and are typical water (main component of the shampoo) contaminants, that is, they can contaminate the shampoo more easily than the cream. The European Pharmacopoeia recommends the satisfaction of criterion A, but in justified cases, it imposes the satisfaction of criterion B, due, for example, to the organoleptic properties of the formulation. Thus, this study demonstrated that this EO has preservative properties, with no significant differences between standard and wild strains and that is necessary to analyse each cosmetic as a single (Nostro et al., 2004).

EOs can be recommended as candidate natural cosmetic preservatives and the aim of a study conducted by Muyima *et al.,* (2002) was to compare the potential of some new EOs for application as preservatives in natural cosmetics. It was considered four aromatic plants, two south African indigenous plants (*Artemisia afra* and *Pteronia incana*) and two Mediterranean aromatic plants (*Lavandula officinalis* and *Rosmarinus officinalis*). In these EOs, camphor, 1,8-cineole and pinene were found to be the most frequent major compounds identified by GC-MS. Some important compounds appeared exclusively in one particular EO. These were: 3-thujanone, as a major component of *Artemisia afra* EO; p-mentha-8-ene-2-ol, as a major component of *Pteronia incana* EO; and 2-bornanol, as a major component of *Lavandula officinalis* EO. After GC-MS analysis of the EOs, it was tested the antimicrobial activities by the agar diffusion test. Bacterial plates were incubated at 25°C for 48 h, while fungal plates were incubated at 30°C for 96 h, after which zones of inhibition were measured. All the EOs tested showed some microbial reduction abilities, although the reduction varied with the test organism species, the type and the concentration of the EO used, as well as the time of incubation. The antimicrobial

activities of the EOs were tested and except for *Pseudomonas aeruginosa* strains, the EOs displayed remarkable antimicrobial activities against all common test organisms (including bacteria and fungi). The EOs were tested neat and in dilutions of 1:1 and 1:2 EO-ethanol. The undiluted EOs proved to be more effective, followed by 1:1 EO-ethanol dilution. This test was performed on all the four different EOs, and it is noteworthy that in general, they showed remarkable antimicrobial activities against the test organisms. *Artemisia afra* was the most efficient. *Artemisia afra* neat oil for Gram-positive bacteria has a very large diameter inhibition zone: *Staphylococcus aureus* with an inhibition zone of 21.3 mm. The fungi tested, *Candida albicans* had too a high diameter zone of 22.3 mm. The Gram-negative bacteria, *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* (clinical) showed the lowest values both in neat and diluted EOs with values around 6.0 mm of diameter. The Gram-negative bacteria (*Escherichia coli* and *Ralstonia pickettii*) showed higher activity values with the neat EOs with the diameter zone of 17.7 mm and 21.7 mm. After the antimicrobial test, a challenge test was performed on W/O cream. Three different concentrations (0.5, 1.0 and 1.5% (v/w)) were used for each EO. Relative to the Gram-negative bacteria, *Escherichia coli* has progressively decreased over time with all the EOs tested. Gram-positive bacteria *Staphylococcus aureus* showed a remarkable sensitivity towards all the EOs tested. All EOs in the various concentrations had antibacterial activity against *Staphylococcus aureus* after 24 and 48 hours. In general, fungi were highly sensitive to the EOs compared to the bacteria. *Candida albicans* seemed, after 24 hours with a great reduction of microorganisms. The results showed that all EOs showed remarkable preservative capabilities, being the *Artemisia afra* EO the most efficient in reducing the load of artificial contaminations in the cream formulations and could therefore be considered as alternative preservatives against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Ralstonia pickettii*, *Candida albicans* and *Aspergillus niger*. In addition to assuring protection against microbial contamination, since relatively high concentrations of the EOs were used, they could at the same time serve as natural fragrances in cream formulation (Muyima et al., 2002).

One study developed a formulation of a topical antibacterial product containing EO from *Ocimum gratissimum* leaf. The objective of the study was to develop an effective topical antibacterial formulation of the African basil EO that could serve as an antiseptic

agent for the treatment of minor wounds, boils and pimples. The products were evaluated by agar diffusion assay against type strains and clinical isolates: *Staphylococcus aureus* NCTC 6571, *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* wound isolate, *Pseudomonas aeruginosa* wound isolate and *Proteus* spp. wound isolate. Methanolic solutions and liquid paraffin solutions were prepared to compare the activity of the EO. Concentrations of 0.25%, 0.5%, 1.0% and 2% of EO in 50% methanol and 10% and 50% in 80% methanol were prepared. These methanolic solutions of the EO exhibited higher antibacterial effects compared to liquid paraffin solutions (concentrations of 2%, 10% and 50% in liquid paraffin). Comparing the various 2% *Ocimum gratissimum* EO preparations investigated, the methanolic solution exhibited the highest antibacterial activity, followed by the ointment in macrogol blend base, the cream in sodium laurate monostearin base, the dispersion with 1% Tween 80 and then the white petrolatum. The simple ointment and macrogol cream did not show antibacterial activity. All the activities of these preparations were higher than those of the positive controls used in this study. *Ocimum gratissimum* EO in lipophilic semisolid bases like petrolatum and simple ointment exhibited much lower or no activity compared to its formulation in the more hydrophilic macrogol blend ointment base. However, this higher antibacterial activity could be linked to the inherent activity of the bland macrogol blend base. The preparation of *Ocimum gratissimum* EO in sodium laurate monostearin cream base exhibited considerably higher activity compared to its formulation in macrogol cream base that showed no activity at 2% oil concentration. The presence of petrolatum in the macrogol cream base possibly inhibited the release of the active principle from the EO. The intrinsic antibacterial property of sodium lauryl sulphate may have contributed to the remarkable activity of the monostearin cream preparation of the EO (Orafidiya, 2002).

4.1.2. Antioxidant activity

In a study conducted by Ben Ddjemaa and collaborators (2016) the *in vitro* potential antioxidant activity of the *Lavandula aspic L.*, known as wild lavender, was evaluated The ointment formulation consisted of white wax (5%) and white petrolatum (95%). *Lavandula aspic* EO was extracted by the steam distillation method and the compositional analysis of the volatile constituents was performed by GC-MS. In its

constitution, it was possible to identify a large percentage of oxygenated monoterpenes, followed by esters, oxygenated sesquiterpenes, monoterpenic hydrocarbons, sesquiterpenic hydrocarbons and ketones. The ointment was prepared by introducing the EO at a concentration of 4% (w/w). The assay performed was the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. This activity was compared to the activity of ascorbic acid (positive control). The IC_{50} of the EO calculated with the DPPH test was 5166.66 µg/mL, which compared to the values related to ascorbic acid, namely 20.73 µg/ml. A similar result was obtained with superoxide anion in which the IC_{50} of the EO was 16483.33 µg/mL, which is higher than the values using ascorbic acid which IC_{50} was 0.32 µg/mL. These results indicate that for the same amount of oil, the antioxidant activity of the lavender EO is much lower than the ascorbic acid. The lavender EO was further tested for its wound healing activity in rats using an excision wound model and the results proved that this formulation compared to control (commercial calming topical medicine) provide regeneration.

4.2. FORMULATIONS WITH ESSENTIAL OILS TESTED IN ANIMALS

Table 2 shows the bioactivity of the EOs in animal models and the studies ranging from the year of 2012 to the year of 2017. Four studies are presented, one of them is about the antifungal activity of the Malacalm® gel tested in dogs against *Malassezia* dermatitis, a study that was previously referred in Table 1. Two articles are focused on mosquito repellent activity of the EOs of *Cymbopogon winterianus* and *Piper aduncum* being the results remarkable. Then, a study on skin UV-B protection provided by marigold EO is presented.

Table 2 - Bioactivity of the essential oils incorporated in the formulations using animal models

Activity	*Type of formulation*	*Essential oil and concentration*	*Study design/characterization*	*Sample size*	*Evaluation*	*Results*	*Reference*
Anti-Malassezia dermatitis	Gel Malacalm®	*Citrus aurantium* 1%, *Lavandula officinalis* 1%, *Origanum vulgare* 0.5%, *Origanum majorana* 0.5%, *Mentha piperita* 0.5% and *Helichrysum italicum* var. *talicum* 0.5%	- Group A: 20 dogs treated with Malacalm® - Group B: 10 dogs treated with conventional therapy (ketoconazole) - Group C: 5 untreated dogs (control group)	35 dogs affected by dermatitis due to *Malassezia pachydermatis*	Clinical and cytological evaluation after 30 and 180 days	- The group treated with Malacalm® showed a good outcome (90%) as well as the conventional therapy (100%) - The control group did not show any significant variation in their clinical status	(Nardoni et al., 2014)
Mosquito Repellent Activity	Ointment; Cream; Gel.	*Piper aduncum Linnaeus* (10%)	- Test group: formulation with EO - Control group: formulation without EO	4 human subjects	Protection time, repellency percentage and mean landing/biting percentage on the hands after exposition to 25 nulliparous *Aedes aegypti*	- The three formulations were able to repel >65% of the *Aedes aegypti* at 4 h post-application - Protection time is longer in the ointment and in the cream, with 182.5 minutes and 162.5 minutes respectively	(Mamood et al., 2017)
	Cream	Citronella oil (1%, 5% and 10%)	- Test group: cream with EO - Control (placebo) group: cream base	60 adult female mosquitoes	Mosquito repellence percentage after 15, 30, 45, 60, 90 and 120 minutes	- Cream base and cream with 1% EO were not able to repel the mosquitoes at any time point - It was observed an increase of the activity with the time and concentration of EO (5 and 10%) in the cream - The cream (10%) has enhanced the time of the residence time but compared with the raw EO	(Yadav et al., 2014)
Skin UV-B protection	Cream	Calendula (4% and 5%)	- Without UV-B irradiation and without cream treatment - UV-B irradiated - UV-B + formulation with 4% of EO - UV-B + formulation with 5% of EO	24 albino rats	Lipid peroxidation and antioxidants as well as enzyme level of skin of albino rats against UV-B radiation	- The cutaneous application of EO prevents UV-B-induced alterations in the level of antioxidants in skin tissue	(A. K. Mishra et al., 2012)

EO, Essential oil; UV-B, Ultraviolet-B

4.2.1. Anti-Malassezia dermatitis

Malassezia pachydermatis is a lipophilic yeast, which is part of the cutaneous microflora of several vertebrates and it commonly occurs on the skin and mucosae of healthy dogs, being the factors unknown. Current therapeutic options for *Malassezia pachydermatis* canine include the topical application of anti-fungal agents and, when this is ineffective, systemic treatment is adopted. Dogs with *Malassezia* dermatitis require regular maintenance therapy to prevent relapses, which are very common. This disorder can be very worrisome for dogs and time-consuming, frustrating, and extremely costly for the pet owner. Several natural products were developed and this study intended to evaluate the *in vitro* antifungal activity of an available mixture of EOs, the gel Malacalm®. A study conducted by Nardoni *et al.*, (2014), made the clinical and mycological evaluation of a herbal antifungal formulation in canine *Malassezia* dermatitis. This study has two different parts, *in vitro* (Table 1) and *in vivo* (Table 2). As already mentioned, Malacalm® is a commercially available mixture composed of six different EOs. In the present study, this product was administered as sole therapy to treat canine *Malassezia pachydermatis*. A group of dogs that previously have been treated with conventional anti-Malassezia treatment and with constant recurrence, were randomly divided into 3 groups to be studied. One group (group A) was subjected to the treatment with Malacalm®. Twenty dogs were treated twice a day directly in affected areas for 1 month. The other group (group B) consisted of 10 animals treated with a conventional therapy based on ketoconazole 10 mg/kg/day and chlorhexidine 2% twice a week for 3 weeks. The last group with 5 dogs (group C) was the control with no treatment. The species *Malassezia pachydermatis* was identified in all dogs and at all anatomic sites. Results showed that the group with Malacalm® treatment present a good outcome in 90% of the dogs. Dogs from group C did not show any significant variation in their clinical status and there were not observed significant differences between the 2 treatment groups. On the other hand, the follow-up visit carried out on day 180 allowed to observe a recurrence of clinical signs in all dogs in group B, while no significant clinical changes were referred in the dogs of group A, which indicates that the treatment with the gel Malacalm® reduces the recurrence of infection. Antifungal activity of Malacalm® seems to be enhanced with

respect to its individual EOs probably because the combination can show synergistic effects.

4.2.2. Mosquito repellence

Mamood *et al.*, (2017) developed a study that aimed to evaluate the effectiveness of 10% *Piper aduncum* EO as a repellent in ethanol and three different semisolid formulations: ointment, cream and gel. The EO was extracted by HD of the leaves of *P. aducum* cultivated in Malaysia and the chemical constituents were analysed by GC-MS. The major constituent of the EO was apiole, which represented about 38.01% of the constituents detected in the EO. The other main constituents were methyl isobutyl ketone, piperitone and caryophyllene. The test consisted of the application of the three formulations to the subject's hands, which were then inserted into a cage containing 25 nulliparous *Aedes aegypti*. The number of mosquitoes landing on or biting each subject's hand was recorded, and repellency percentage, landing/biting percentage and protection time for each of the formulations were compared. Between the semisolid EO formulations, there were no statistically significant differences. All three semisolid EO formulations were able to repel >65% of the *Aedes aegypti* at 4 h post-application. Directly after application, EO in ethanol was able to repel >95% of the mosquitoes. However, as expected, 30 min after application the effectiveness of the EO in ethanol started to decrease due to the volatility of the active ingredients. When the EO was prepared using three semisolid formulations, its effectiveness as a repellent increased because the volatile compounds evaporated more slowly. The EO incorporated in the ointment and cream formulations repelled 100% of the *Aedes aegypti* mosquitoes for 1 h, compared with only 30 min when it was in the gel formulation. All three semisolid formulations provided good efficacy against *Aedes aegypti*, repelling >80% of the mosquitoes, for up to 210 min post-application in the case of the cream formulation and 180 min post-application, and in the case of the gel and ointment formulations. As the EO cream and ointment formulations displayed better repellent properties than the EO gel formulation, they appear to be the most promising *Piper aduncum* EO formulations to be developed and commercialized as alternatives to synthetic repellents. The ointment

formulation of the EO offered 3 h of protection time, approximately the same as the cream formulation, while the gel yielded a lower protection time.

EOs can have repellent activity and Yadav *et al.,* (2014) conducted a study that consisted in the development of an effective mosquito repellent cream formulation containing citronella (*Cymbopogon winterianus*). Several formulations were developed and it were evaluated their appearance, application and stability (in terms of emulsification), pH and consistency. The repellent activity was tested in mosquitoes. The base cream was compared with the formulations with 1%, 5% and 10% of EO. As shown in Table 2, after 15 minutes the EO showed 60% repellence activity. The formulations with the EO incorporated have shown activity after 30 minutes. The formulations with 5% and 10% of the EO showed significant mosquito repellence after 30 minutes. At the end of one hour, all the formulations and the EO have shown repellence. The results showed that the EO alone has a shorter action time than the EO incorporated in the formulations. The EO reached 100% mosquito repellence from 45 minutes to 60 minutes, that is, it maintains its maximum repellence for 15 consecutive minutes. Formulations with concentrations of 5% and 10% of EO acquired 100% repellence at 60 minutes, maintaining the maximum repellence level for another 30 and 60 minutes, respectively. The formulation in the concentration of 5% of EO, lasted for 30 minutes only with a reduction of 5% of repellence (95%). Then, it can be concluded that the cosmetic formulations that incorporate EOs, despite the time of action being longer, have the advantage of not being released quickly. More than 50% mortality was observed after 1 h in the case of pure citronella EO, while in the case of the formulations with the concentration of 5% and 10% of the EO it was seen after 2 h and 1.5 h, respectively. In this study also was performed a safety test and there was a significant difference in the primary irritation index of vehicle control and standard irritant group, which indicates the irritation potential of lactic acid in the animals. Pure citronella EO was significantly different from the vehicle control group on a scale of 0.0 to 8.0. Cream comprising of Citronella EO was found to be 0.45, which is non-significantly different from the vehicle or placebo control group. The results categorized this formulation into the category of irritation barely perceptible and make it appropriate for topical application. The primary irritation index of the formulation was significantly less in comparison to the primary irritation index of citronella EO and the standard irritant group. Skin irritation

characteristic of citronella EO in terms of erythema and edema hampers its utility and acceptability for topical application.

4.2.3. Protection against UV-B radiation

In the study conducted by Mishra *et al.*, (A. K. Mishra et al., 2012) was evaluated the effect of a cream (O/W) containing the EO of *Calendula officinalis* on biochemical parameters of the skin of albino rats against ultraviolet-B (UV-B) radiation. The effects were measured in terms of lipid peroxidation marker enzyme level and nonenzymatic antioxidant parameters which include reduced glutathione, ascorbic acid level, total protein level and antioxidant enzymes as superoxide dismutase and catalases. The treatment with creams containing 4% and 5% of Calendula EO are responsible for a significant decrease in the malondialdehyde level, whereas the levels of catalase, glutathione, superoxide dismutase, ascorbic acid, and the total protein level were significantly increased after 1 month of daily irradiation and treatment when compared to untreated control groups. Therefore, these results suggest that the cutaneous application of the EO of Calendula prevents UV-B-induced alterations in the level of antioxidants in skin tissue.

4.3. FORMULATIONS WITH ESSENTIAL OILS IN CLINICAL STUDIES

Table 3 shows the clinical studies using the different formulations with EOs (test groups) comparing with control groups. Studies are from the year of 1999 to the year of 2018. There is represented a study relative to the antifungal activity of tea tree EO in the treatment of onychomycosis. The anti-psoriatic activity of the *kunzea* EO was evaluated with Psoriasis Area and Severity Index (PASI). The promising results of the use of *Matricaria chamomilla* OE in migraine pain relief and the significant decrease of the side effects of this condition are also reported. Another study evaluated the potential capacity of the *Curcuma aeruginosa* EO to inhibit the axillary hair-growth, and equally to lighten the skin. The preservative activity was also one of the bioactivities tested in the clinical studies and comprised the comparison of different EOs.

Table 3 - Clinical studies using the different formulations with essential oils comparing with control groups

Activity/clinical outcome	***Type of formulation***	***Essential oil***	***Study design/characterization***	***Sample size (age range)***	***Outcome***	***Results***	***Reference***
Pain relief in migraine	Oleogel	*Matricaria chamomilla* (2%)	- Test group: oleogel containing the EO - Control (placebo) group: oleogel not containing the EO	72 patients (18-65 years)	Visual analogue scale (VAS) questionnaires for each migraine attack (including the status of pain and other complications like nausea, vomiting, photophobia, and phonophobia)	- A statistically significant decrease of pain before and after 2 h were observed in the test group compared to the placebo - A statistically significant decrease the of side effects (pain, nausea, vomiting, photophobia, and phonophobia) in the test group on the patients after 30 min	(Zargaran et al., 2018)
Axillary hair-growth inhibition and lightening activity	Lotion	*Curcuma aeruginosa* Roxb. (1 or 5%)	- Test group: lotion containing the EO - Control (placebo) group: base lotion	60 females (18-23 years)	Reduction in axillary hair-growth rate after 14 weeks	- One armpit contained the lotion with curcuma EO and the other placebo - Both concentrations of the EO reduced axillary hair-growth - Axillary skin brightness increased robustly and this continued for at least 2 weeks after treatment ceased	(Srivilai et al., 2017)
Anti-psoriatic activity	Ointment and/or scalp lotion	*Kunzea oil* (20%)	- Test group: ointment and/or scalp lotion containing the EO - Control group: control medications not containing EO	30 patients (25-74 years)	PASI after 8 weeks	- There was a reduction of Psoriasis Area and Severity Index in both groups after 8 weeks but the efficacy was not presented statistically significant difference	(Thomas et al., 2015)
Preservative activity	Cream	*Lavandula officinalis, Melaleuca alternifolia, Cinnamomum zeylanicum* (2.5%)	- Test group: cosmetic emulsion containing the EOs - Control group: cosmetic emulsion with methylparaben	40 female volunteers (25-50 years)	Number of viable microorganisms bacteria, yeast and mould in cosmetic formulations after application of the volunteers on the forearm skin using fingers two times a day for 8 weeks	- In the test group it was observed a complete inhibition of the growth of bacteria, yeast and mould, in the cosmetic emulsion compared to the control group - Cinnamon EO added to cosmetic emulsion at concentration 2.5 % completely inhibited *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* growth after 4 weeks of incubation during a challenge test	(Herman, 2014)

Anti-onychomycotic effect	Cream	*Melaleuca alternifolia* (5%)	- Test group: cream containing 5% of the EO and butenafine hydrochloride (2%) - Control group: cream containing 5% of the EO	60 outpatients (39 males, 21 females; 18-80 years)	Treatment of toenail onychomycosis after 8 weeks	- The eight-week treatment may have been reduced to prove the action of Melaleuca EO in placebo. The formulation that included the synergy of EO and butenafine hydrochloride had an effect on onychomycosis	(Syed et al., 1999)

EO, Essential oil; Psoriasis Area and Severity Index, PASI.

4.3.1. Pain relief in migraine

A clinical trial conducted by Zargaran *et al.,* (2018) evaluated the efficacy of an oleogel on migraine attacks. This study was developed with 72 patients between 18 and 65 years of age, where the test group applied the oleogel with *Matricaria chamomilla* (chamomile) EO and the control group applied a mixture of 10% traditional chamomile oil in liquid paraffin (placebo), to evaluate migraines relief during 24 h. Visual Analog Scale (VAS) questionnaires were filled in by the patients and scores were given, ranging from 0 to 10, based on the severity of pain. Other complications like nausea, vomiting, photophobia and phonophobia were also monitored. Results showed a significant decrease in pain after using EO as compared to using placebo. After 30 minutes, it was observed a significant effect of EO in decreasing nausea, vomiting, photophobia, and phonophobia, as compared to placebo. The activity of the chamomile oleogel was significantly compared to placebo group. Results supported the efficacy of chamomile oleogel as pain relief in migraines without aura. These effects can be due to chamomile constituents such as chamazulene, and apigenin and its derivatives as these compounds can reduce nitric oxide release and synthesis by inhibiting nitric oxide synthase expression in activated macrophages. Nitric oxide stimulates central sensitization and induces migraine headaches and can trigger inflammation at the site of action blocking its synthesis can reduce migraine pain.

4.3.2. Axillary hair-growth inhibition and lightening activity

A single center, randomized, double-blind, placebo-controlled study during 10 weeks was carried out in 60 women between 18 and 23 years. Participants were divided randomly into groups, to use either 1 or 5% (w/w) turmeric (*Curcuma aeruginosa*) from rhizome EO lotion or only placebo lotion, to evaluate the growth and the density of the hair as well as the brightness. On one axilla it was applied the lotion with the EO, and in the other base-lotion (placebo) for 10 weeks followed by placebo in both axillae for 2 weeks. From weeks 5-11 of trial, 1 and 5% (w/w) of EO retarded growth by 13% and 16% respectively, while placebo was ineffective. *Curcuma aeruginosa* EO had no

influence on hair density. Both concentrations of *Curcuma aeruginosa* EO rapidly and equally effectively brightened skin within 3 weeks which persisted 2 weeks after treatment ceased, while placebo darkened the skin. These actions are compatible with anti-androgenic activity in the *Curcuma aeruginosa* EO. In addition, no adverse reactions ascribed to EO were detected or reported. In general, participants were satisfied with the treatment and reported reduced hairiness, freedom from any discomforts, but the product odour attracted some negative comments (Srivilai et al., 2017).

4.3.3. Anti-psoriatic activity

A randomized and controlled clinical trial was carried out in patients diagnosed with psoriasis. Patients randomly received an ointment and/or scalp lotion containing 20% kunzea oil (test group) or control medications not containing kunzea oil (control group). Formulations also contained 3% salicylic acid (keratolytic agent) and cod liver oil was incorporated into the formulation as an emollient. Each patient was examined at the beginning of treatment, and again after 2, 4 and 8 weeks of therapy, by a single blinded clinical investigator. The severity of the disease was measured using the PASI. After 8 weeks of treatment, both topical treatment regimens showed significant improvement in psoriasis signs (redness, thickness and scaliness). From baseline to the end of treatment, PASI score decreased by 51% in the test group and by 60% in the control group. The VAS score of pruritus decreased by 77% for the test group and 72% for the control group. However, the results did not support the use of kunzea oil for the management of psoriasis may be because of the presence of excipients, such as the emollient cod liver oil and the keratolytic agent salicylic acid, that might have contributed towards the slightly increased efficacy observed. This study demonstrated that both treatment formulations were safe and effective in managing mild to moderate cases of psoriasis (Thomas et al., 2015).

4.3.4. Preservative activity

Other different clinical trials consisted in comparing the preservative effectiveness of plant extracts (*Matricaria chamomilla*, *Aloe vera*, *Calendula officinalis)* and EOs

(*Lavandula officinalis*, *Melaleuca alternifolia*, *Cinnamomum zeylanicum*) with methylparaben in cream formulation against skin microflora during 2 months of application by volunteers. Forty female volunteers, in the age range 25–50, were assigned to one of the eight treatment groups: EOs, plant extracts and methylparaben free cream formulation, cream formulation with methylparaben, cosmetic emulsion with *Cinnamomum zeylanicum*/*Lavandula officinalis*/*Melaleuca alternifolia* oils, cosmetic emulsion with *Matricaria chamomilla/Aloe vera/Calendula officinalis* extracts. The cosmetics were applied by volunteers on the forearm skin using fingers two times a day. Results showed that cinnamon EO at the concentration of 2.5% completely inhibited bacteria, yeast and mould growth in a cream formulation more firmly than methylparaben and plant extracts during 2 months of application by volunteers. During the challenge test, the study showed that cinnamon EO added to the cream formulation at a concentration of 2.5% completely inhibited *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* growth after 4 weeks of incubation. The findings of the present study suggest that cinnamon EO inhibits the growth of microorganisms in cream formulations more effectively than methylparaben and plant extracts (Herman, 2014).

4.3.5. Antifungal activity

A randomized, double-blind, controlled study examined the clinical efficacy and tolerability of 2% butenafine hydrochloride and 5% *Melaleuca alternifolia* EO incorporated in a cream to manage toenail onychomycosis in a cohort. Sixty outpatients aged 18–80 years with 6–36 months duration of disease were randomized to two groups (40 and 20), test group (2% butenafine hydrochloride and 5% *Melaleuca alternifolia* EO incorporated in cream and control (with only 5% *Melaleuca alternifolia* EO incorporated in a cream) groups, respectively. After 16 weeks, 80% of patients using medicated cream were cured, as opposed to none in the placebo group. Four patients in the test group experienced subjective mild inflammation without discontinuing treatment. The tea tree EO alone in the placebo did not show the expected response, possibly the treatment duration (8 weeks) was insufficient to render its full potency (Syed et al., 1999).

5. DISCUSSION

Despite being used since ancient times, in recent years, the use of EOs has increased remarkably. In the cosmetic industry, EOs are widely used in cosmetics as a fragrance, with the purpose of improving the aromatic component of the formulation and also increasing the acceptability of the product. In addition to a wide range of unique and pleasant aromas, EO can act as bioactive agents exhibiting several different activities, including antimicrobial (antibacterial and antifungal) and antioxidant ones. Cosmetics require preservation against microbial contamination to guarantee consumer's safety and to increase their shelf-life (Halla et al., 2018). It happens that the preservatives commonly used can have toxic effects.

Nowadays, customers have more access to scientific information, are more aware and read the product labels, which are filled with preservatives potentially harmful for human health, existing reports that the accumulation of parabens in the human organism could lead to estrogenic effects (Matwiejczuk et al., 2020).

The greater its concentration, the greater the degree of preservation. However, when used in large concentrations, they can have toxic effects for the consumer (Halla et al., 2018) and in low concentrations, it has been reported that microbes developed microbial resistance (Dao et al., 2018; Halla et al., 2018). Preservatives, including parabens, are a leading cause of allergic and irritant contact dermatitis, and there is increasing evidence that some may even cause toxic effects including endocrinological effects (Mayekiso et al., 2006). Other recent alternatives are the acid preservatives such as phenoxyethanol, benzoic acid and sorbic acid. These have the disadvantage of requiring a low pH to be active and causing allergies in sensitive skin. They are commonly used in combination with other preservatives, increasing the skin irritation factor (Lundov et al., 2009).

To meet customers' expectations, the cosmetics industry are looking for new and innovative methods of cosmetic production. In these terms, are gaining some relevance the use of natural ingredients, currently more commonly incorporated in cosmetic formulas, as well as biologically active substances such as the use of EOs in cosmetic formulations, which can be for example alternatives for the synthetic preservatives (Matwiejczuk et al., 2020).

Indeed, one approach to minimizing these effects is the use of self-preserving or preservative-free cosmetic formulations using in alternative EOs in their composition. In respect to this, in some studies, the EOs were compared with commercial preservatives and the efficacy was higher, being results very promising.

EOs are added to cosmetic formulations due to their recognized properties, such as: anti-inflammatory and antimicrobial antioxidants, emollients, dyes, humectants, healing, anti-mutagenic, anti-ageing, protective against damage caused by UV-B radiation. The limitations imposed on the use of EOs as antimicrobials in cosmetic preparations are mainly due to their volatility, lipophilic aspects and strong odour (Halla et al., 2018).

The fact is that from the several studies *in vitro* studies reported in the literature it was observed relevant effects of antibacterial and antifungal activities against the main potentially pathogenic representatives of Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), mould (*Aspergillus niger)* and yeast (*Candida albicans*). In most of the studies, the antimicrobial effect was observed due to the EOs efficacy on the microorganisms even when present at low levels of concentration (below 5% of EOs). Although EOs were used in low concentrations, they have more benefits compared to many synthetic preservatives.

It should be highlighted some of them namely for its preservative efficacy, *Calamintha officinalis* and *Lavandula angustifolia* and its antimicrobial effect the *Cinnamomum zeylanicum*, *Artemisia afra*, *Pteronia incana*, *Lavandula officinalis*, *Rosmarinus officinalis* and its antifungal activity of a gel Malacalm® containing *Citrus aurantium*, *Lavandula officinalis*, *Origanum vulgare*, *Origanum majorana*, *Mentha piperita* and *Helichrysum italicum*. Regarding antimicrobial activity, some of the individual components present in EOs such as 1,8-cineole, carvacrol and thymol are known to be promising compounds in combating the proliferation of microorganisms that can compromise the formulation, the person's health and the treatment (Nazzaro et al., 2013).

In relation to the formulations, it also should be highlighted that several different formulations were tested being evident that the antimicrobial activity was higher in the

more hydrophilic ones compared to the more lipophilic ones. There is an example of the study conducted by Tomczykowa *et al.*, (2018) that analysed their different formulations, one oil-base, other water-based and a mixture of the two. The EO was uniformly dispersed in the hydrogel and oleogel formulations. At the bigel formulation (hydrogel + oilgel), the EO was added to the oil phase (oleogel). Analysing the results, the antifungal activity of EO was better in the hydrogel, followed by the bigel and the oleogel. The affinity of EOs for lipophilic compounds may decrease its activity. It is clear that the EO in a formulation with water as the majority base exhibits greater biological activity on the skin. Another study, conducted by Gemeda *et al*., (Gemeda et al., 2018) compared the antimicrobial activity of EO at different formulations bases and the results showed higher activity against all tested bacteria and fungi with the EO incorporated in the hydrophilic ointment. The EO was easily diffused or the bioactive constituents in the formulations were easily released, comparing with the other formulations. The lipophilic affinity of EO with paraffin could be a factor that impairs the release of the EO. Analysing the ointments formulations with better results, constituents of hydrophilic and macrogol blends are very different. Probably, the results are due to the presence of sodium lauryl sulfate and polyethylene, respectively, that improve the solubility in water and can enhance the diffusion of bioactive constituents. In addition, a relevant aspect related to the formulations was the possibility that some of them could present their preservative efficacy regarding its composition even that the EOs were not added. This is an interesting aspect that was observed in one study and that naturally be important as the EDTA present in the formulation could help to demonstrate this activity. Additionally, one *in vitro* study was also evaluated the antioxidant effects. However, the results demonstrated lower activity than the ascorbic acid (known antioxidant used as control).

Furthermore, it was also compiled the *in vivo* studies in animal models in which were evaluated the anti-malassezia effects, the mosquito repellent activity, the skin UV-B protection and also, the clinical studies which were evaluated a huge diversity of effects, namely the pain relief in migraine, axillary hair-growth inhibition and lightning activity, anti-psoriatic activity, preservative activity into the formulation and anti-onychomycotic effect. The diverse activities evaluated are related to the different EOs incorporated in the different formulations that were tested and interestingly in almost all cases, the biological activities were compared with a control (placebo) group and

highlighting some effects it could present repellent against mosquitoes of *Cymbopogon winterianus*, of axillary hair-growth inhibition and lightening activity of *Curcuma aeruginosa* and pain relief in migraine of *Matricaria chamomilla*.

The finished products of cosmetic formulations can be presented in several forms such as liquid (products that flow freely); gels that may be water or oil based and are thicker than a liquid, having limited flow properties, there may also be foaming gels like shampoos and liquid body washes, with a limited flow capacity; serums with varied viscosity, water or oil based; lotions with low viscosity emulsions (the viscosity can be altered through the use of different emulsifying agents, consistency factors or gums and thickening agents), they are a mixture of immiscible substances and are easy to apply to large areas; emulsions of medium to high viscosity like creams that can be O/W or W/O emulsions,; powders utilised in the colour cosmetics industry; ointments, that are semi-solid preparations of hydrocarbons with a strong emollient and occlusive effect that enhances penetration of substances on the skin and improves the product efficacy by providing a protective film on the skin, these form contains no water and not require preservative agents; paste is a mixture of powder and ointment; and other like balms, muds and scrubs (Carli, 2020). Despite the diversity of cosmetic formulations, it should be highlighted that the semi-solid creams (O/W emulsion) were the most predominant ones in the studies found. However, other formulations were also used such as lotion, shampoo, hydrogel, oleogel and bigel and ointment.

A very important property of cosmetic products is their viscosity because it is important to make them easy to apply, to dispense and use the right amount, and to suit consumer perceptions (Carli, 2020). Viscosity is also important in the action of EO. The use of topical dermocosmetics provides skin hydration and restoration of the damaged lipid barrier (Olszanecka-Glinianowicz, 2015).

Another practical aspect in the formulation conception is that the EOs usually are added at the end of the preparation of formulation because these compounds are very volatile and can react in the presence of environmental factors such as light, heat, moisture and oxygen, changing their properties.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

In cosmetic products, EOs can optimize such as their properties as well as their preservation acting as bioactive cosmetic ingredients. However, there are some limitations in the use of EOs in cosmetics, as they are volatile and can react in the presence of environmental factors. On other hand, the use of EOs is considered safer than synthetic preservatives. It is necessary for the cosmetic industry to innovate and continuously improve its products, making available on the market products that meet the needs and use trends of consumers.

Considering the various studies already mentioned and discussed, there seems to be an enormous potential for the future and broader use of EOs in formulations, continuing to explore their different biological activities, namely antimicrobial, antioxidant, anti-repellent, skin UV-B protection activity ones, that were showed through *in vitro* studies and *in vivo* in animals. It is also worth highlighting the effects that demonstrated considerable evidence of the evaluation of EOs as a repellent against mosquitoes, as an inhibitor of hair-growth and lightning activity and pain relief in migraine. Regarding its bioactivity, terpenes and phenolic compounds are the chemical constituents of the most promising EOs.

More research must be carried out in order to increase the understanding of the possible mechanisms of action of the compounds present in the EOs. The methodologies used for the classification and quantification of chemical compounds, as well as for the biological evaluation of EOs, must be standardized and then compared. The active compounds present in a EO and its amount can be different depending on the location, climate, soil and several other characteristics that determine it. So, there is a growing need to standardize all the processes of the preparation and extraction of the EOs to understand whether there will be significant consequences at the level of applicability. EOs are not as dangerous to health as used synthetic preservatives (such as parabens and isothiazolinones), which are associated with endocrinal disturbs and allergic contact dermatitis. There is a great need to change the market for several reasons and it is also necessary to review the legislation and standardize.

As a final remark, considering the several studies already developed, there seems to be enormous potential for further research and explore the use of EOs in formulations in the cosmetic and also pharmaceutical industries, exploiting their different biological activities as an appealing alternative to the synthetic compounds.

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SUPPLEMENTARY MATERIALS

SUPPLEMENTARY MATERIAL I – Description of the main characteristics of the plants from which were obtained the essential oils

Artemisia afra essential oil

- **Scientific and common name:** *Artemisia afra* Jacq. *ex* Willd. (African absinthe) (van Wyk, 2008);
- **Family:** Asteraceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Leaves (Muyima et al., 2002)
- **Origin Local:** Africa (Muyima et al., 2002);
- **Composition:** Berbenome, borneol, camphene, camphor, 1,8-cineole and isopropyl-3-methylbenzene as major components. Linalool acetate, pentylbenzene, β-phellandrene, β-pinene, 2-pinene, α-terpinene, β-thujene, 3-thujanone, others components like monoterpens and sesquiterpenes (Muyima et al., 2002);
- **Biological activities:** Antimicrobial activity, against *Mycobacterium* species, diabetes, antiplasmodial activity and malaria (du Toit & van der Kooy, 2019). It is used to treat coughs and colds, chills, dyspepsia, loss of appetite, stomach ache and other gastric derangements, colic, croup, whooping-cough, gout and as purgative, flu, headaches, inflammation, gout, sore throat, malaria, diabetes, bladder and kidney disorders, asthma, constipation as well as numerous other health problems. The most common use is the insertion of fresh leaves into the nostrils to clear blocked nasal passages (van Wyk, 2008). Focusing on malaria, the plant is taken for "fevers and in 'blood poisoning" and "the plant is used by the European, the Southern Sotho and the Zulu for measles and other fevers, including malaria" (du Toit & van der Kooy, 2019).

Bidens tripartita essential oil

- **Scientific and common name:** *Bidens tripartita* L. (bur marigold) (Tomczykowa et al., 2018);
- **Family:** Asteraceae (Cytobiologica et al., 2008; Tomczykowa et al., 2018);
- **Part of the plant used:** Aerial parts (Tomczykowa et al., 2018);
- **Origin Local:** Bielsk Podlaski (Poland) from July 28 to August 06, 2016. (Tomczykowa et al., 2018);
- **Composition:** The oil was mainly composed of ρ-cymene, β-linalool, (Z)-cymene and α-fellandrene (Tomczykowa et al., 2018). A study conducted in Poland analysed the major compounds of the oil collected during 3 years characterized and observing both quantitative and qualitative compounds differences. In this period, the main compounds were α-pinene, ρ-cymene, β-ocimene, β-elemene, iso and α-caryophyllenes and α-bergamotene (Kaškonienė et al., 2013);
- **Biological activities:** It has significant strong antifungal activity. It is used in folk medicine as a diuretic, sudorific and anti-inflammatory agent. It is very useful in the treatment of skin diseases, in treating fevers, gravel, stone, and bladder and kidney troubles and the methylene chloride extract of this plant has been demonstrated to have high activity in the inhibition of cancer (Cytobiologica et al., 2008). It has been known as a remedy for chronic dysentery (Tomczykowa et al., 2018). The herb is valued for its diuretic and astringent properties, it could be used for bladder and kidney troubles and it is also claimed to be an excellent remedy for ruptured blood vessels (Kaškonienė et al., 2013).

Calamintha officinalis essential oil

- **Scientific and common name** Clinopodium nepeta subsp. glandulosum (Req.) Govaerts (synonym *Calamintha officinalis* Moench)(calamint) (Monforte et al., 2011);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Aerial parts (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012; Nostro et al., 2002);
- **Origin Local:** Western Europe to Central Asia and North America (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Monoterpenic ketones (carvone and pulegone) predominate (Monforte et al., 2011). Chemotypes of this species on the market generally have a high content of citral and citronellol isomers (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). In Madeira, it were found the constituents: cis-isopulegone, pulegone, *neoiso*-isopulegol and trans-isopulegone (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).;
- **Biological activities:** This plant is used like an antispasmodic, digestive, expectorant and exciting. The British Herbal Pharmacopeia indicates this plant for the treatment of flatulence in children and flu. The EO is widely used in aromatherapy in case of flatulence, colic and nervous dyspepsia. Diluted can be used for muscle pain and rheumatism (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). A study *in vivo* reports that the aerial part extract of calamint has antidiabetic action, confirming the use that this plant has in Morocco (Lemhadri et al., 2010). The local population of Madeira Island uses the leaves of calamint as a mouth freshener and to alleviate headaches and toothache. This study found that the EO of this plant is very active against *Escherichia coli* and active against *Agrobacterium tumefasciens* and *S. aureus* (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). A study showed antibacterial activity against oral and dental pathogenic microorganisms such as *Streptococcus mutans* and *Streptococcus sanguis*, with low toxicity *in vitro* (Monforte et al., 2011).

Calendula officinalis essential oil

- **Scientific and common name:** *Calendula officinalis* L. (calendula) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012; A. K. Mishra et al., 2012);
- **Family:** Asteraceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Flowers (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012; A. K. Mishra et al., 2012);
- **Origin Local:** Southern Europe and Egypt. Grown all over the world, sub-spontaneous in the Azores and casually in mainland Portugal (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** EO of *Calendula* consists mainly of α-thujene, α-pinene, 1,8-cineole, dihydrotagetone and τ-muurolol. Other constituents of the flowers are sesquiterpenes and triterpenes. Most of the flavonoids present in the plant are the glycoside derivatives of quercetin and isorhamnetion (A. K. Mishra et al., 2012) The EO has in its composition majority sesquiterpenic hydrocarbons like sesquiterpenols, being the main compounds α-cadinene, α-cadinol and epi-α-muurolol (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** Studies refer to the antifungal activity of this EO. This plant has anti-inflammatory, anticancer and immunomodulating action. In vivo studies report the action of calendula ethanol extracts in wound healing. In rats, healing was 25% greater than in the control and in rabbits, they report that there is a fast action in combating infection by Staphylococcus epidermitis. *Calendula* EO is used for skin conditions such as eczema and inflammation (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Cinnamomum zeylanicum essential oil

- **Scientific and common name:** Cinnamomum verum J.Presl (synonym *Cinnamomum zeylanicum* Blume) (cinnamon) (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lauraceae (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Dry shells (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Sri Lanka and India (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Cinnamic aldehyde, eugenol, cinnamyl acetate, cineole, linalool, caryophyllene, monoterpenes and sesquiterpenes (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).
- **Biological activities:** The EO is antiseptic and antifungal (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011). Several studies show that the antifungal activity is due to the presence of cinnamic aldehyde, being that this oil is very often used to fight cutaneous mycoses. This EO has antibacterial properties described (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Citrus aurantium essential oil

- **Scientific and common name:** *Citrus sinensis* (L.) Osbeck (bergamot) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Rutaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** The recent pericarp of the plant without heating (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). Ripe fruit rind, flower (neroli essential oil) and leaves (bitter orange essential oil) (Sarrou et al., 2013);
- **Origin Local:** South Vietnam and China (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** EO of three parts of the plant were evaluated and found that the EO from the rind of the fruit had as main constituents limonene, followed by myrcene, linalool, β-pinene and α-pinene. Regarding the EO of neroli (flower), it had as major components linalool, β-pinene, limonene, trans-β-ocimene and E-farnesol. As for the composition of the EO of bitter orange leaves, higher concentrations were detected in linalool, α-terpineol, geranyl acetate, neryl acetate, trans-β-ocimene (Sarrou et al., 2013);
- **Biological activities:** In general, the EOs of this plant have antioxidant activity. According to the study by Sarrou *et al.*, (2013), the oldest leaves are the part of the plant with the greatest antioxidant activity. This study also reports that the essential oil of bitter orange also exerts anti-inflammatory and antimicrobial action. The leaf of this plant has antispasmodic, digestive, antiseptic and bronchial secretion-stimulating properties. The leaves and flowers have a sedative action (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Citrus limon essential oil

- **Scientific and common name:** *Citrus limon* (L.) Osbeck (lemon) (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Family:** Rutaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Fruit (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Origin Local:** Temperate zones (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Dipentene, α-pinene, β-pinene, citral, citronellal, terpineol, camphene, phellandrene, flavonoids and carbohydrates (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Biological activities:** On the skin tissue the EO is antiseptic. In the cosmetic formulations this can be used as nutritive exfoliant and dandruff shampoo (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011).

Curcuma aeruginosa essential oil

- **Scientific and common name:** *Curcuma aeruginosa* Roxb. (turmeric) (Srivilai et al., 2017);
- **Family:** Zingiberaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Rhizome (Srivilai et al., 2017);
- **Origin Local:** Thailand (Srivilai et al., 2017);
- **Composition**: Isocurcumenol, β-eudesmol, curdione, curcumenol, curcumanolides, dehydrocurdione and curcumenone (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** In general, all oils, even in different species, exhibit antiseptic action and can be used as an insect repellent (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). It is used for treating gastrointestinal, uterine, and postpartum while having being antimicrobial and anti-inflammatory. It has an anti-inflammatory and anti-androgenic activity *in vitro* and *in vivo* (Srivilai et al., 2017).

Cymbopogon martini essential oil

- **Scientific and common name:** *Cymbopogon martini* (Roxb.) W. Watson (palm rose) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Poaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Aerial part (Gemeda et al., 2018);
- **Origin Local:** Native to the Indian Himalayas and Pakistan. Grown in India, Africa, Indonesia, Brazil and the Comoros Islands (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** It has in the composition monoterpenes, sesquiterpenes, monoterpenols, sesquiterpenols, aldehydes, ketones, oxides, esters and ethers. According to ISO/TC 45, ISO 4727:1988 the major constituents of palm-rose must be: β-myrcene, limonene, cis-β-ocimene, trans-β-ocimene, terpinolene, metil heptanone, linalool, linalyl acetate, terpineol, geranyl acetate, nerol, geraniol, nerolidol, farnesol and hexadecanol (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** Several works have reported the antifungal, particularly antidermatophytic activity of geraniol (Gemeda et al., 2018).

Cymbopogon winterianus essential oil

- **Scientific and common name:** *Cymbopogon winterianus* Jowitt *ex* Bor (citronella) (Ganjewala, 2009);
- **Family:** Poaceae (Ganjewala, 2009; Labinas & Crocomo, 2002);
- **Part of the plant used:** Leaves (Ganjewala, 2009);
- **Origin Local:** Sri Lanka (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Major constituents identified are citronellal, trans-geraniol and citronellol (Ganjewala, 2009);
- **Biological activities:** It is used to protect cartons containing muesli and wheat germ from beetles. Relative to this gender many biological activities have been reported like anticancer activity, antimicrobial (Ganjewala, 2009), insecticidal and repellent Studies report the antifungal activity of this OE, highlighting its activity against *Candida Albicans*(Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);

Helichrysum italicum essential oil

- **Scientific and common name:** *Helichrysum italicum* (Roth) G. Don (immortelle) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Asteraceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Aerial flowered parts (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Mediterranean region(Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** α-pinene, limonene and neryl acetate predominate in oil, and lower concentrations other esters (isovalerate, capryolate and neryl butyrate), nerol, geraniol, ketones (italidiones), aldehydes, β-caryophyllene and other sesquiterpenes. Neryl acetate can reach high percentages in the various common species (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** In Portugal is used in dermatomycosis. Antibacterial, antifungal, anti-inflammatory and healing properties are attributed to the EO of this plant. It is also used in dermatological preparations in cases of psoriasis and urticaria (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Kunzea ambigua essential oil

- **Scientific and common name:** *Kunzea ambigua* (Sm.) Druce (kunzea) (Thomas et al., 2010, 2015; Wyatt et al., 2005);
- **Family:** *Myrtaceae* (Thomas et al., 2010; Wyatt et al., 2005);
- **Part of the plant used:** Twigs (Wyatt et al., 2005), leaves and aerial parts (Thomas et al., 2010)**;**
- **Origin Local:** Australia and New Zealand (Thomas et al., 2010);
- **Composition:** Monoterpenes, α-pinene and 1,8-cineole as major components (Thomas et al., 2010). Depending on the species, could be found other components like β-pinene, ρ-cymene, γ-terpinene, bicyclogermacrene, spathulenol, globulol, viridiflorol, ledol (Thomas et al., 2010; Wyatt et al., 2005) *and* cis-jasmone and menth-1-en-4,8-diol (Wyatt et al., 2005);
- **Biological activities:** This plant exhibit biological activity in a variety of *in vitro* assays like antibacterial, antitumour and anti-inflammatory bioactivities (Wyatt et al., 2005)

Lavandula angustifolia essential oil

- **Scientific and common name:** *Lavandula angustifolia* Mill. (lavender) (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Flowering aerial parts and leaves (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Origin Local:** Cultivated all over the world, especially in France (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** The main constituents are linalool, linalyl acetate and caryophyllene. It contains low amounts of monoterpenes and other oxygenated monoterpenes (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Biological activities:** This plant has the greatest activity in its EO and many cosmetic formulations use it in dermatological formulations used in pediculosis, skin itching, atopic eczema, acne and others. In the cutaneous tissue, it has an antiseptic, stimulating action on the circulation (activating the peripheral circulation) and anti-inflammatory activity. (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011).

Lavandula aspic essential oil

- **Scientific and common name:** *Lavandula aspic* L., herein noted L. *aspic*, also known as *Lavandula latifolia* Medikus, sin. L. *spica* (Cav.) (wild lavender) (Ben Djemaa et al., 2016; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lamiaceae (Ben Djemaa et al., 2016)**;**
- **Part of the plant used:** Inflorescence (Ben Djemaa et al., 2016)**;**
- **Origin Local:** Spontaneous but cultivated in low altitude regions (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Monoterpenes, sesquiterpenes, monoterpenols, ketones, esters and oxides. Consists mainly of terpenes and terpenoids, with linalool, 1,8-cineole, and camphor being the major components (Ben Djemaa et al., 2016). Species with higher content of 1,8-cineole and camphor (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). This species differs from *Lavandula officinalis* due to its higher content in cineole and camphor (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Biological activities:** The EO and the plant have several beneficial effects, including antioxidant, antiviral and antibacterial activities (Ben Djemaa et al., 2016). This type of spontaneous lavender, is more used as an expectorant and mucolytic (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Matricaria chamomilla essential oil

- **Scientific and common name:** *Matricaria chamomilla* L. (chamomile) (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Family:** Asteraceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Flowers (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Mediterranean Europe and Middle East (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Sesquiterpenes predominate with farnesenes, azulenes (camazulene and guaiazulen), α-bisalol, bisalol and bisalone oxides, epyroethers, espatulol, monoterpenes and sesquiterpenic lactones (matricin and matricarin) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** The EO has antibacterial, antifungal, sedative and anti-neuralgic properties. It is used with digestive activity in liquors (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). In cosmetics, the EO is used as a sunscreen, eyelid swelling and dark circles. Plant as anti-inflammatory and spasmolytic activity. In herbal medicine, it is used internally as a bitter tonic, in gastrointestinal spasms, inflammatory bowel diseases and as a sedative. Externally it is used in skin inflammation, irritation of the buccopharyngeal mucosa and in stomatitis (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011; Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Melaleuca alternifolia essential oil

- **Scientific and common name:** *Melaleuca alternifolia* (Maiden & Betche) Cheel (tea tree) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Myrtaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Leaves (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Australia, Papua New Guinea (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Its major components are terpinen-4-ol, γ-terpinene and α-terpinene that predominates in the plants of this species. The components of this species contain: α-pinene, sabinene, α-terpinene, limonene, ρ-cymene, 1,8-cineole, y-terpinene, terpinen-4-ol and α-terpineol (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** It is used for its antiseptic action (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012). It has an antibacterial action on both Gram-negative and Gram-positive bacteria, including *Propionibacterium acnes*. The activity against this microorganism has allowed its introduction in cosmetics for the treatment of acneic skin with inflammatory and non-inflammatory lesions (Warnke et al., 2013). This EO is effective against strains that are multiresistant to conventional antibiotics, being a potential alternative in severe infectious situations (Warnke et al., 2013). This plant has shown good results in studies of its action against the *Herpes simplex* virus (Farag et al., 2004). It is also very common to use this EO in the treatment of mycoses and other types of fungal infections, having shown excellent action against *Candida albicans* (Ninomiya et al., 2012). In addition to the antimicrobial activities, its antioxidant, anti-inflammatory (Nogueira et al., 2014) and anxiolytic activities (da Cunha & Roque, 2013) can also be highlighted.

Mentha piperita essential oil

- **Scientific and common name:** *Mentha* x *piperita* L. (peppermint) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012), (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012), (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Part of the plant used:** Aerial part (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Europe, Asia and North America (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011). The cultivated hybrid that is considered to come from *Mentha longifolia* (L.) L. or *M. suaveolens* Ehrh. and *M. aquatica* L. and therefore, propagated only vegetatively (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** The main constituents are menthol and its acetic and isovaleric acid esters and menthone. In smaller quantities, isomenthone, menthofuran, 1,8-cineole, limonene, carvone and pulegone (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Biological activities:** Several studies prove that EO of peppermint has an antispasmodic action, proving in some studies to be more effective than conventional therapy and with fewer adverse effects. In irritable colon syndrome, it has proven to have significant benefits. Other studies prove that the EO has benefits at the gastrointestinal and respiratory levels. It is widely used as a decongestant for upper airways and in oral hygiene products. More than half of the production of this oil is in dentifrices (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Ocimum gratissimum essential oil

- **Scientific and common name:** *Ocimum gratissimum* L. (clove basil) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lamiaceae (Orafidiya, 2002);
- **Part of the plant used:** Leaves (Orafidiya, 2002);
- **Origin Local:** Nigeria (Orafidiya, 2002);
- **Composition:** Predominates thymol and eugenol (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** Inhibitory activity against strains of *Escherichia coli* that are implicated in the aetiology of persistent, infantile and travellers' diarrhoea (Orafidiya, 2002).

Origanum majorana essential oil

- **Scientific and common name:** *Origanum majorana* L. (marjoram) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);.
- **Part of the plant used:** Flowery sums and leaves (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Mediterranean region and the Near East (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** α-terpineol predominates, terpenic ketones, phenols such as thymol and carvacrol and monoterpenes such as terpinene and pinenes (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** Antimicrobials, antifungals, antioxidants, urinary stimulating, antispasmodic and digestive activity is reported. Externally it is also antiseptic and healing (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Origanum vulgare essential oil

- **Scientific and common name:** *Origanum vulgare* L. (oregano) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Leaves and flowers (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Origin Local:** Native to Europe and Middle East (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** Predominates thymol and carvacrol, plus ρ-cymene, borneol, linalool, linalyl acetate, α-pinene, α-terpinene, β-bisabolol, β-caryophyllene (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** Due to its large amount of thymol and carvacrol it presents antibacterial, antimycotic and antiviral activity. It is used topically as an anti-inflammatory of the oropharynx and toothaches and in rheumatic pain. It is a mild diuretic (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).

Piper aduncum essential oil

- **Scientific and common name:** *Piper aduncum* L. (spiked pepper);
- **Family:** Piperaceae (FAZOLIN, M.; ESTRELA, J. L. V.; CATANI, V.; COSTA, 2006);
- **Part of the plant used:** Leaves (FAZOLIN, M.; ESTRELA, J. L. V.; CATANI, V.; COSTA, 2006);
- **Origin Local:** Central America (Hartemink, 2010);
- **Composition:** Prenylated benzoic acid derivatives, chromenes or benzopyrans, flavonoids, alkaloids, amides, monoterpenes, sesquiterpenes and phenylpropanoids (FAZOLIN, M.; ESTRELA, J. L. V.; CATANI, V.; COSTA, 2006);
- **Biological activities:** It was proved the antioxidant activity of the plant. Alcoholic extracts of this plant as an antibiotic activity against a lot of common species. The essential oil is used in the preparation of semi-synthetic derivatives with activity against adult *Aedes aegypti* larvae known as a vector of dengue hemorrhagic fever. The occurrence of mortality was observed in all species of insects subjected to experimentation proving its insecticidal action. The plant as an astringent, stimulating tonic, digestive, diuretic, sedative, laxative, in the treatment of haemorrhoids, normalizer of menstrual bleeding, dysentery suppressor, an anaesthetic for toothache, stomach pain relief, antiseptic for cuts in the skin and hemostatic (FAZOLIN, M.; ESTRELA, J. L. V.; CATANI, V.; COSTA, 2006).

Pteronia incana essential oil

- **Scientific and common name:** *Pteronia incana* (Burm.) DC. (blue bush) (Hulley et al., 2010; Mayekiso et al., 2006);
- **Family:** Asteraceae (Mayekiso et al., 2006);
- **Part of the plant used:** Leaves (Hulley et al., 2010);
- **Origin Local:** Southern Africa (Hulley et al., 2010);
- **Composition:** The major compounds are several monoterpenes as well as sesquiterpenes like β-pinene, sabinene, limonene, 1,8-cineole, myrcene, spathulenol and ρ-cymene (Hulley et al., 2010);
- **Biological activities:** This plant has been used for the treatment of influenza, fever, kidney ailments and backache (Hulley et al., 2010).

Rosmarinus officinalis essential oil

- **Scientific and common name:** *Rosmarinus officinalis* L. (rosemary) (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Family:** Lamiaceae (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Part of the plant used:** Aerial parts and leaves (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011);
- **Origin Local:** Mediterranean coastal region and in the centre and south of the European continent (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Composition:** α-Pinene, camphor and 1,8-cineole (eucalyptol) as major compounds (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011). In its constitution it also has camphene, myrcene, p-myrcene, bornyl acetate, borneol, verbenone(Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012);
- **Biological activities:** In the cutaneous tissue, the EO has an antiseptic action, stimulating the circulatory because it activates the peripheral circulation (Cunha, A. Proença; Roque, Odete Rodrigues; da Silva, Alda Pereira; Cunha, 2011). Cosmetics, creams with glycolic extracts of the florid plants are used in the treatment of stretch marks and seborrheic dermatitis. The hydroalcoholic lotions of the leaves are used to combat dandruff, darken hair and baldness. It also has action on the nervous system and anti-inflammatory action (Cunha, A. Proença; Roque, Odete Rodrigues; Nogueira, 2012).