Review Article Open Access

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Essential Oils as Immunomodulators: Some Examples

https://doi.org/10.1515/chem-2017-0037
received August 2, 2017; accepted November 4, 2017.

Abstract: Essential oils (EOs) exhibit a wide range of pharmacological properties, which have been reported over the years in various studies. The aim of this literature review is to present the latest findings of the immunomodulatory effects of EOs. From 2008 to 2016 *in vivo*- and/or *in vitro*-studies, most of which were published in the last couple of years, have been selected based on their topic relevance, namely immunomodulatory, anti-inflammatory, antileishmanial, antiallergic, and anticancer effects of various EOs. These findings show modulation of pro- and anti-inflammatory cytokines, antiproliferative, chemotactic properties and also exert antiparasitic effects by inhibiting the pro, axenic and intramacrophagic amastigote forms of Leishmania parasites or by modulating the T_H1 and T_H2 immune responses. Furthermore, the EOs of some plants show the ability to reduce the mast cell degranulation and improve the airway inflammation and mucus obstruction in the cases of immediate hypersensitivity in murine models. Additionally, the cytotoxicity of some EOs against human melanoma, hepatoma, lung, prostate and breast cancer cell lines proposed their potential antitumor effect by an increased immunosuppressive (cytostatic) activity.

Keywords: Essential oils, immunomodulatory effects, anti-inflammatory effects, antileishmanial activity, immunosuppressive effects

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1 Introduction

In the last years there is a growing interest towards natural products in life sciences and among the medicinal society, as an alternative and complementary to common treatments. Standard drugs and treatments times show reduced beneficiary effects often with significant adverse side effects, while natural compounds exhibit a better patient compliance, less side effects and are more cost-efficient compared to many of the standard treatments. Essential oils (EOs) have shown to possess a wide range of pharmacological properties and immunomodulatory activities with promising results that could be implemented as alternative treatments to various immune system related diseases. The understanding of the mechanisms of our immune system is important to fully grasp the effect of EOs as immunomodulators and how they augment, modify and influence the immune response, either as immunosuppressors (in allergies, as anticancer agents, etc.) or as immunopotentors (in the cases of immunodeficiencies to prevent infections etc.).

The immune system offers protection from infectious agents and harmful substances by recognizing and destroying antigens on their surface, but can also attack organ transplants and in the cases of autoimmune diseases it is able to destroy substances and tissues that are normally presented in the body.

A healthy individual has two levels of defence: The innate or non-specific immunity with what an individual is born, and the acquired or adaptive immunity which is developed after exposing and specifically defending against various antigens. The immune system is organized in central (bone marrow, thymus) and peripheral (lymph nodes, spleen, and blood) lymphoid organs and tissues. It includes specialized white blood cells, the lymphocytes which differentiate in the bone marrow and thymus in B lymphocytes (B cells) and T lymphocytes (T cells) respectively. The B cells migrate through the body fluids to the lymph nodes, spleen and blood, recognizing specific foreign invaders circulating in lymph and blood, providing the body with the humoral or antibody mediated immunity. T cells mature within the thymus into several different

types, including helper, killer and suppressor cells, and are responsible for the cell mediated immunity, affected in the cases of immunodeficiency. Once formed, both the T and B cells offer a memory to the immune system for a faster response, if exposed to the same antigen next time [1].

Immunomodulation is aimed at modifying the immune response either via augmentation to prevent infections in states of immunodeficiencies or by suppressing the immune system in allergies, autoimmune diseases or organ transplantations, where the goal is to weaken the immune system.

In inflammatory process, during the innate immune response, various cells of the immune system (neutrophils, macrophages, lymphocytes) release mediators (cytokines, ROS etc.) that enhance the inflammatory process and activate the adaptive immunity. T Helper cells in turn activate various other type of cells (monocytes, B cells etc.) by releasing various cytokines, as TNF-α, IL-1β, IL-2, which affect the progression of inflammation [2,3]. Many plants exhibit an immunomodulatory effect on macrophages, natural killer (NK) cells and lymphocytes. Cytokines, like TNF-α and interleukins are used in determination of immune response in macrophage cell-based models, since macrophages as the first barrier of the immune system produce those cytokines [4]. TNF-α is a fundamental mediator of cell death, differentiation and initiation of inflammation and immune modulation [5].

2 Immunomodulatory effects of EOs

Recently there has been an interest and shift towards natural products, phytochemicals and EOs as an alternative to conventional treatment and as an additional way to boost and enhance the immune system. The positive results of the studies by Orhan et al. [6], showed strong antimicrobial activity of several EOs and their compounds against *Klebsiella pneumoniae* strains, prompted the same research group to further investigate the immunomodulatory properties of the EO and their compounds [7].

The plants used were *Foeniculum vulgare* (Lamiaceae), *Satureja cuneifolia* (Lamiaceae), *Mentha × piperita* (Lamiaceae), *Mentha spicata* (Lamiaceae), *Origanum onites* (Lamiaceae), *Origanum minutiflorum* (Lamiaceae), *Origanum majorana* (Lamiaceae) and *Origanum vulgare var. hirtum* (Lamiaceae. The compounds tested were the monoterpenoides citronellol, iso-borneol, menthol, borneol, carvacrol, menthofurane, thymol and vanillin, and were purchased from Carl Roth Chemical Company (Karlsruhe, Germany) [7]. The EOs were obtained by hydrodistillation at 1.2% for *F. vulgare*, 1.0% for *S. cuneifolia*, 3.2% for *M. × piperita*, 1.8% for *M. spicata*, 2.6% for *O. onites*, 3.1% for *O. munitiflorum*, 3.5% for *O. majorona*, and 2.8% for *O. vulgare var. hirtum* [7].

The immunomodulatory activities were evaluated based on the oxidative burst produced by reactive oxygen species (ROS) from whole blood phagocytes and isolated polymorphonuclear neutrophils (PMNs), proliferation of PHA-stimulated T-cells and production of pro-inflammatory cytokines IL-2 and TNF-α. Luminol-enhanced chemiluminescence assay was performed and the results were monitored as relative light units (RLU), while the percentage of (%) inhibition and IC_{50}, which is the concentration that inhibits 50% of ROS produced were calculated based on a formula [7].

The EOs of *F. vulgare, S. cuneifolia, and O. munitifilorum* strongly inhibited ROS produced from whole blood phagocytes, compared to the reference drug ibuprofen. Thymol potently inhibited the oxidative burst from whole blood phagocytes and isolated PMNs, among which kaempferol-3-O-β-d-galactoside, caffeic acid, and quercetin exhibited a better inhibitory effect than ibuprofen [7].

Regarding the T-cell proliferation, measured by standard thymidine incorporation assay on phytohaemagglutinin (PHA) stimulated peripheral blood cells, only *M. piperita* EO showed a moderate inhibitory effect. *M. piperita* and *M. spicata* EOs exhibited the highest inhibition on TNF-α. Menthol and methofurane, found in *M. piperita*, could be contributing to the high TNF-α suppressing effect, while carvone, the major monoterpene keton (75%) that is detected in *M. spicata* oil could also be donating its high effect. In the case of *S. cuneifolia*, its major component thymol (42%), could justify its high inhibitory effect on oxidative burst on whole blood phagocytes as well as TNF-α production, since both of them exhibit similar values in the assays performed. Lastly carvacrol, the major constituent (67-83%) [6] of *Origanum species*, demonstrating an inhibition of peripheral mediators and a decreasing effect on TNF-α levels [8] can suggest to be a donor to the immunomodulatory effect of *origanum* oils towards reduction of pro-inflammatory cytokines [7].

After a number of randomly selected EOs, terpenoid and aromatic components and polyphenolic compounds were monitored in this study. There is an evidence of their immunomodulatory effect. Nonetheless, further studies are needed to examine the mechanisms of actions that remain unclear and the connection and correlation between the EOs and their constituents and how they contribute their effect and influence their immunomodulatory properties [7].

The *Tetradenia riparia* (Hochstetter) Codd (Lamiaceae) plant, also known as *Iboza riparia* and *Moschosma*

riparium, is used in traditional medicine in Africa for the treatment of inflammations and infectious diseases. The EOs of *T. riparia* have also shown antioxidant, anticarcinogenic and antimicrobial properties. However, there were only a few studies and reports on the immunomodulatory effects of *T. riparia* and one paper [9] examined the immunomodulatory effects of *T. riparia* essential oil (TrEO) on murine peritoneal macrophages.

T. riparia EO was obtained by hydrodistillation and analysed by gas chromatography, mass spectrometry (GC-MS) and gas chromatography flame ionization detector (GC-FID). The TrEO chemical composition is shown in Table 1. Resident peritoneal macrophages were obtained from BALB/c mice (euthanized by 40% CO_2 inhalation). The cytotoxicity of the DMSO (0.005% v/v- non-toxic) diluted TrEO was determined by colorimetric analysis of tetrazolium (XTT) solution and the Trypan Blue test, with untreated cultures serving as a viability control. Cytokine mRNA expression was determined using semi quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) (Trizol reagent extracted RNA) and cytokine production and quantification using flow cytometry [9].

The cell viability of TrEO in murine macrophages according to the Trypan Blue test, was 90%, similar to that of the untreated macrophages (96%). The CC_{50} was determined at 165 ng/mL according to the XTT method, and the immunomodulatory effect of TrEO on macrophages was tested at 30ng/ml, which showed a high cell viability (97%) [9]. The mRNA expression and cytokine production was tested after 3, 6 and 24 hour incubation at 37°C. The TrEO stimulation of cytokine expression varied with the incubation period. After 3h incubation period, IL-1β, IL-10, IL-12, IL-17 and IFN-γ mRNA expression was detected, with only IL-1β showing a higher expression after 6h. TNF-α, IL-18 and IL-33 were expressed neither after 3h nor after 6h incubation and no cytokine was expressed after 24h [9].

However, TrEO modulated cytokine synthesis until 24h of incubation. IL-1β synthesis was gradually increased by TrEO, while IL-10 was decreased until 24h. IL-6 showed an increase at 3h, followed by a decrease at 6h and was normalized at 24h. IL-17, exhibited a similar modulation to IL-6. TrEO induced IL-2 production at 3h and then induced granulocyte-macrophage colony-stimulating factor (GM-CSF) production at 24h. Finally, TNF-α, IL-5 and IL-12 production did not show any modulation by TrEO [9]. These findings show that murine macrophages treated with TrEO can express and produce cytokines that is important to the immune response. TrEO induced pro-inflammatory cytokines, associated with the innate cellular immune response and suppressed the production of IL-10, which contributes to the regulation of the immune response. However, these results represent the effect of TrEO on murine macrophages and the pharmacological and immunomodulatory effects of *T. riparia* EO should be further studied *in vivo* and in humans to establish the TrEO as an alternative therapy for infectious, autoimmune and carcinogenic diseases.

Table 1: Chemical composition of TrEO.

compounds	Content (%)
Monoterpene hydrocarbons	1.05
α-pinene, limonene, *trans*-β-ocimene, n.i.	
Oxygenated monoterpenes	15.51
fenchone, *endo*-fenhol, *exo*-fenchol, camphor, borneol, terpinen-4-ol, α-terpineol, γ-terpeneol, n.i	
Sesquiterpene hydrocarbons	19.91
α-cubebene, α-copaene, β-elemene, α-gurjunene, β-caryophyllene, α-*trans*-bergamotene, α-humulene, *allo*-aromadendrene, germacrene-D, *cis*-β-guaiene, bicyclogermacrene, α-muurolene, α-(*E,E*)-farnesene, δ-amorphene, δ-cadinene	
Oxygenated sesquiterpenes	38.85
muurolol 5-en-4α-ol, spathulenol, globulol, viridiflorol, guaiol, cadinol *epi*-α, aromadendrene epoxi-*allo*, α-cadinol, 14-Hydroxy-9-*epi*-caryophyllene, (2*Z*,6*E*)-farnesol, guaiol acetate, *N*-nonadecane	
Oxygenated diterpenes	24.67
9β,13β-epoxy-7-abietene, dehydroabietane, abieta-7,13-dien-18-ol, abietol, manoyl oxide, 6,7-Dehydroroyleanone, n.i	
Identification based on retention index, NMR spectra and comparison of mass spectra. n.i.: not identified compounds. (adapted and newly draw from Demarchi et al. 2015) [9]	

Cinnamomum osmophloeum Kaneh. is an endemic tree of the family of Lauraceae and grows in Taiwan. It belongs to the Cinnamomum species found in Asia and Australia [10]. It is used as a food additive and as one of the most traditional Chinese medicine [11]. The major constituent of the leaf EO of *C. osmophloeum* is cinnamaldehyde and studies showed that the leaf EO of the cinnamaldehyde type exhibits antimicrobial [12,13], anti-inflammatory [14], antipyretic [15], antitumor cell growth effects [16,17]. It is widely used to treat influenza and other inflammatory conditions. However the *in vivo* modulatory effects of *C. osmophloeum* EO and cinnamaldehyde on cytokines remain unclear.

Lin et al. [18] examined the modulatory effects of cinnamaldehyde and the *C. osmophloeum* leaf EO on the expression of serum Th1 cytokines, IFN-γ and IL-2, and the Th2 cytokines, IL-4 and IL-10, since both type of

cytokines are related to the inflammatory process. The EO of *C. osmophloeum* was obtained by hydrodistillation and characterized using HS-GC/MS and quantitative HPLC-analysis. In this study BALB/c female mice were subjected to various concentrations of *C. ospholoeum* leaf EO or cinnamaldehyde for 4 weeks. The acute and subacute toxicity was examined after euthanizing the mice by H-E staining. The cytokine modulation was determined by ELISA immunoassay [18].

Cinnamaldehyde was identified by HS-GC/MS and quantitative HPLC-analysis as the major compound at 16 mg/ml. Benzaldehyde and 3-phenylpropionaldehyde were also identified by HS-GC/MS in the *C. osmophloeum* leaf EO. The acute and sub-acute toxicity tests showed no changes in body weight, kidney and liver function or pathology when treated with up to 1 mL/kg body weight of *C. osmophloeum* leaf EO or up to 4 mg/kg body weight of cinnamaldehyde. The mice treated with *C. osmophloeum* leaf EO showed no significant modulatory effect, but some minor variations, of IL-2, IL-4, IL-10 or IFN-γ. On the other hand, after treatment with different doses of cinnamaldehyde, there was a significant increase in the serum concentration of those cytokines. There were no time- and dose-dependent effects observed [18].

Although cinnamaldehyde is the major compound of *C. osmophloeum* leaf EO, the possibility that other constituents function as antagonists, which would explain the lack of modulatory effect of the leaf EO, cannot be excluded. It can be concluded though that cinnamaldehyde shows safe and modulatory effects on cytokines aiding at the Th1/Th2 cytokine balance which plays a significant role at the prevention of a disease.

3 Immunosuppressive effects

Part of the immune cells, the dendritic cells (DCs) are the best antigen presenting cells, responsible for the induction of the adaptive immunity [19-21]. DCs initiate the adaptive immunity by presenting a specified antigen on the surface to the T cells, which results in the differentiation of the specified antigen-specific T cells. Pathogens that have the specified antigen are attacked by the specific T cell-based immune response. Thus, dendritic cells are an ideal target to evaluate the potential immunomodulatory activity of modulators, as the essential oils [22-24].

Litsea cubeba L., also known as maqaw, belongs to the Lauraceae family and grows in China, Taiwan, Indonesia and areas of Southeast Asia. Fruits of *L. cubeba* are used in Taiwanese cuisine and its EO has been used as flavour enhancer in cosmetics, food and cigarettes. Studies have already reported the pharmacological, anti-inflammatory, microbicidal, antioxidative and anticancer effect of *L. cubeba*. Chen et al. [25] examined and reported the immunosuppressive effects of *L. cubeba* and added those to its variety of pharmacological properties. Chen et al. [25] analysed the composition of the fruit EO of *L. cubeba* (LcEO) and its immunomodulatory effect on dendritic cells and mice. The fruit EO was extracted by hydrodistillation and analysed by gas chromatography (GC) and gas chromatography/ mass microextraction (HS-SPME/GC). The chemical composition of LcEO is shown in Table 2. The immunosuppressive activity of LcEO was evaluated with bone marrow-derived dentritic cells by performing the contact hypersensitivity (CHS) responses in mice and by determining the TNF-α and IL-12 production by enzyme-linked immuno sorbent assay (ELISA) [25].

The yield of the EOs extracted was 0.4% - 3.7%, similar to that of previous studies. From the total of 48 components identified, the principal compounds were citral (neral and geranial) with 88%. The compounds were identified by comparative analysis by DI/GC and HS-SPME/GC, showing that a combination of both results leads to a more complete identification [25]. The cytotoxicity of the LcEO was determined by the viability of mouse bone marrow-derived dendritic cells after being treated with different concentrations of LcEO and it showed no toxic effect at the concentrations of $5 \times 10^{4-}$, $1 \times 10^{5-}$, $2 \times 10^{5-}$ and $4 \cdot 10^{5-}$ fold diluted LCEO and a minor toxicity at $5 \cdot 10^{5-}$. To determine the immunosuppressive effect on DCs, the effect of LcEO on TNF-α and IL-12 production by DCs stimulated lipopolysaccharides (LPS) was examined. The results showed a dose-dependent inhibition of the TNF-α and IL-12 production by LPS-induced DCs when treated with LcEO, with the IC_{50} of LcEO being $1 \times 10^{5-}$ and $2 \times 10^{5-}$ fold dilution respectively [25]. By DNFB (2,4-dinitro-1-fluorobenzene)-induced CHS, a stimulated inhibition of a cell-mediated response was examined. Mice were sensitized by DNFB paint in the presence or absence of LcEO. The results indicate that LcEO inhibits the CHS in the DNFB-sensitized mice. In addition, immunostaining analysis showed a reduction of CD3+ T cells (activated by DCs) in the presence of LcEO. These results report the effect of LcEO against delayed-type hypersensitivity/ type-4-hypersensitivity [25].

Dendritic cells influence the development of chronic inflammations which can be harmful and often leads to diseases and autoimmunity. The results provided by this study showed that the fruit essential oil of *Lutea cubeba* has promising immunosuppressive properties that could be used for the treatment of inflammation and

autoimmune diseases. Lastly it is possible that neral and geranial, the major compounds of LcEO, could contribute to the immunosuppressive effects [25].

4 Anti-inflammatory effects

Chemotaxis describes the response of cells to chemical signals, where neutrophils, being the first responders to inflammation, react to the chemotactic factors (chemoattractants) that are released at the site of inflammation. Previous studies [26] have demonstrated an interaction between granulocytes or polymorphonuclear leukocytes (PMNs) and the chemotactic factor, casein, indicating that human PMNs have a membrane receptor for casein and their interaction results in a chemotactic response. Another protein that influences the immune response is phytohaemagglutinin or PHA which is a lectin found in plants and used as a mitogen triggering T-lymphocyte cell division [27]. Florao et al. [27] examined the immunomodulatory, anti-inflammatory and anti-chemotactic properties of *Baccharis* species, based on the inhibition of the proliferation of phytohaemagglutinin-stimulated lymphocytes and casein-induced human granulocyte chemotaxis.

Baccharis L. is a genus of the *Asteraceae* family and commonly known in Brazil as 'carquejas'. The *Baccharis* genus is characterized by its plenty EOs that can be obtained from the aerial parts. It is known for its several uses in folk medicine, this study selected four species based on their medical uses for the treatment and or relief of symptoms of inflammation [27].

The plants collected were *B. articulata*, *B. genistelloides* subsp *crispa*, *B. dracunculifolia* and *B. gaudichaudiana* and their EOs were obtained by hydrodistillation and analysed by gas-chromatography mass-spectrometry (GC-MS) and GC/flame ionization detector (GC-FID) (Table 3) [27]. The major components of ba Human peripheral blood was collected in accordance with the declaration of Helsinki and approved by the Health Sciences Ethical Committee of the Federal University of Paraná. The human leukocytes were isolated and their mononuclear (MNC) and granulocyte (GNC) fractions were obtained. The cell viability and toxicity, when exposed to EOs (1 x 10^{-5}–100 µL/mL) for GNC (2 and 5 hours) and for MNC (4 and 5 days), was determined using the Trypan Blue exclusion test. The immunomodulatory activity of the EOs of the *Baccharis* species was based on flow cytometric analyses and silver staining (AgNOR), as described [27], and the anti-chemotactic properties were measured using the Boyden's chamber method migration assay, as described.

Table 2: Chemical composition of LcEO.

compounds	Content (%)	
	DI/GC	HS-SPME/GC
Monoterpenes	4.01	11.91
α-thujene, α-pinene, camphene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, *ρ*-cymene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, γ-terpinene, α-terpinolene, 1,3,8-*ρ*-menthatriene		
Sesquiterpenes	0,10	0,06
α-copaene, β-elemene, β-caryophyllene, α-humulene, δ-cadinene		
Terpene alcohols	2.75	5.22
linalool, isopulegol, verbenol, α-terpineol, *cis*-carveol, *cis*-geraniol, nerolidol		
Terpene aldehydes	89.25	75.09
citroneral, neral, geranial		
Terpene ketone	0.14	0.10
camphor, piperitone, piperitenone		
Terpene ester	0.32	0.14
methyl salicylate, bornyl acetate, terpinenyl acetate, citronellyl acetate, geranyl acetate, neryl acetate, methyl cinnamate		
Terpene oxide	0.16	0.17
1,8-cineole, *trans*-linalool oxide, *cis*-rose oxide, *trans*-rose oxide, limonene oxide, caryophyllene oxide,		
Aliphatic aldehydes	0.01	0.03
3-methyl butanal, 2-methyl butanal, pentanal, hexanal, 2,6-dimethyl hept-5-enal		
Aliphatic ketone	1.19	2.23
6-methyl-5-hepten-2-one		
Aliphatic alcohol		<0.01
2-methyl-3-buten-2-ol		
Aliphatic esters	0.01	0.01
ethyl isovalerate, isoamyl, acetate, ethyl tiglate		
Analyzed by gas chromatography with direct injection (DI/GC) and headspace-solid phase microextraction (HS-SPME/GC). (adapted and newly draw from Chen et al. 2016) [25]		

From the twelve major constituents of the EOs obtained, spathulenol predominated in *B. articulata*, *B. dracunculifolia* and *B. gaudichaudiana*, while palustrol

was the major constituent of *B. genistelloides* subsp. *crispa*. and τ-gurjunene occurs in high percentage in *B. gaudichaudiana*. The EOs of *B. genistelloides* subsp. *crispa, B. gaudichaudiana* and *B. dracunculifolia* showed no toxicity for human GNC up to 1 x 10^{-2} µL/mL, regardless the incubation period. On the other hand, GNC viability dropped when cells were exposed to *B. articulate* EO, after 5 hours of incubation at the same dose. The EOs of all four species exerted no toxicity to MNC up to 1 x 10^{-2} µL/mL, compared to the controls viability [27].

For the PHA-stimulated populations, compared to the PHA-control values (3.5± 0.4), the EOs of *B. genistelloides* subsp. *crispa* (2.2±0.2), *B.gaudichaudiana* (2.1±0.1) and *B. dracunculifolia* (2.1±0.4) exhibited a dose related drop in the cell proliferation index at 1 x 10^{-2} µL/mL, while the inhibitory effect of *B.gaudichaudiana* was effective even at 1 x10^{-3} µL/mL [27].

Antichemotactic activity inhibiting GNC migration after being exposed to the EOs and induced to migrate towards a casein gradient, was observed for the EOs of *B. articulate* and *B. dracunculifolia* with a maximum inhibitory effect at 10^{-2} µL/mL. However, the cytotoxicity of *B. articulate* EO towards GNC makes *B. dracunculifolia* EO the only one exhibiting notable anti-chemotactic effects [27].

Concluding, the findings of Florao et al., [27] showed that all the *Baccharis* EOs included except that of the *B. articulate*, inhibit the proliferation of PHA-stimulated lymphocytes and that only the EO of *B. dracunculifolia* significantly inhibited the casein induced human granulocyte chemotaxis. Florao et al., [27] also reported that the potential diversity of the immunomodulatory and anti-inflammatory properties of the EOs of the *Baccharis* species. The abundance of EOs found in the species indicates the further investigations needed to fully compromise the pharmaceutical and therapeutic use of the *Baccharis* plants.

Cympogon martini var. Motia, also known as *Palmarosa*, belongs to the family of Poaceae and is native to regions of India and Indochina. Its EO is widely used in aromatherapy and in Ayuverdic medicine, or traditional Indian medicine, for its pharmacological effects on skin conditions and pain relief.

Andrade et al. [28] evaluated the immunomodulatory effect of *C. martinii* EO and the active compound contained in the EO geraniol regarding the production of pro- and anti-inflammatory cytokines, TNF-α and IL-10 respectively by human monocytes (MNC) *in vitro*. The monocytes were incubated with the EO or geraniol obtained from *C. martini*, the cytotoxicity was determined after 18h colorimetric with the MTT- assay and the modulation of cytokine production was determined by ELISA. According to the MTT assay there was no cytotoxicity detected on monocytes. The ELISA immunoassay showed that the production of the pro-inflammatory cytokine TNF-α was not modified by *C. martini* EO or geraniol. On the contrary, all the nontoxic concentrations of *C. martinii* EO and geraniol increased the anti-inflammatory cytokine IL-10 in human monocytes [28].

Table 3: Major components of the essential oils of *B. articulata*, *B. dracunculifolia* and *B. gaudichaudiana*.

Compounds
2-*epi*-β-funebrene, α-amorphene, 10,11-epoxy-calamenene, β-bisabolene, *trans*-calamenene, palustrol, spathulenol, τ-gurjunene, humulene epoxide II, caryophylla-4(12),8(13)-dien-5-ol, α-muurolol, epi-α-bisabolol
The constituents of the essential oils were identified by GC/FID and GC/MS. (adapted and newly drawn from Florao et al. 2012) [27]

Due to the limited access to this article, not all data and mechanisms of actions, as well as the methods used were examined and studied. Based on the information presented, it is shown that *C. martini* EO and its active compound geraniol exhibit an anti-inflammatory effect by inducing the IL-10 synthesis, while geraniol seems to be donating this effect to the essential oil. These findings prove the Ayuverdic use of *Palmarosa*, or *Cympogon martini* to relief pain and against skin conditions [28].

Rosmarinus officinalis, which belongs to the Lamiaceae family and is native to the Mediterranean region, is a well-known and widely used herbal plant, having both household and medicinal uses. It is being used in anti-inflammatory drug preparations and in the treatment of headaches, colds, colic and other diseases [29]. Additionally, rosemary extracts and their compounds show a high *in vivo* anti-inflammatory activity [30], with few studies examining the anti-inflammatory effect of rosemary essential oil on the gastrointestinal apparatus. The anti-inflammatory effect of plants and EO can be examimed by carrageenan-induced mouse paw oedema [31,32,33] and possible therapies for inflammatory bowel disease are studied by means of an experimental colitis muring model, induced by chemical agents, f.e. by intrarectal trinitrobenzene sulfonic acid (TNBS) application, causing necrotic lesions, resulting to scars and fibrosis [34]. Inflammatory bowel disease therapies reveal no satisfying results and consequently alternative therapies are used, modulating the immune system and disturbing cell signalling, cytokines and proinflammatory mediators [35]. The sub optimal current therapies and the lack of studies on the anti-inflammmatory effect of

rosemary EO and its application in inflammatory bowel disease led Juhas et al. [36] to examine the dietary addition of rosemary EO in experimental TNBS colitis on a murine model.

R. officinalis EO (RoEO) was added to the laboratory mice diet at three concentrations (1250, 2500 and 5000 ppm) and the ICR mice were fed ad libitum 14 days before TNBS administration. Dexamethasone, which exhibits anti-inflammatory effects and which is used in many murine inflammatory models, was used here (carrageenan paw oedema and TNBS colitis) as well [36].

A 50 μL portion of carregeenan 1% (w/v) in saline was administrated subplantar to the right paw, while 50 μL of saline was administrated to the left paw of the male ICR mice and subsequently the carrageenan paw oedema was examined by the increase in the paw volume between the right (carrageenan) and the left (saline) [36]. TNBS colitis was induced intrarectal at 120 mg/kg, with a total volume of 40 μL, and the colitis development was examined daily based on the body weight. After 3 days of TNBS administration the mice were killed, the was colon removed and opened (longitudinal). The mucosal damage was assessed using colon macroscopic scoring [37,38], with the colon to body weight was used as a colonic inflammation marker. The most affected segment was removed and the cytokines expression of IL-1β and IL-6 were determined using ELISA assay, with Bradford protein assay to determine the total protein in the tissue supernatants and the myeloperoxidase activity, connected to the neutrophil infiltration in the tissue was spectrophotometrically determined, both in the paws (carrageenan) and colonic samples (TNBS colitis) [36].

A concentration of 5000 ppm of RoEO dietary supplementation showed an increase of carrageenan paw oedema after 2h, compared to the control group, while inhibiting the extend of the oedema after 24h. At a 2500 ppm concentration of RoEO, there was a significant decrease in paw oedemas observed similar to that of the dexamethasone-group. Paw weight and swelling and MPO activity were decreased compared to the control groups with all three concentrations of RoEO [36].

The dexamethasone treated group showed a decrease at the colon weight: body weight ratio, but did not exhibit a colon mucosa protective effect with a minor decrease in the macroscopic damage scores. RoEO dietary supplementation on the other hand exhibited a significant decrease in macroscopic scores at 5000 ppm RoEO diet, compared to the control mice (TNBS group). Furthermore, in the DEX-group the colon MPO activity and IL-1β concentration decreased in a non-significant manner compared to the control group, while the groups on 2500 ppm and 5000 ppm RoEO diet showed comparable non-significant results. However, in the 1500 ppm RoEO enriched diet group, there was a significant reduction in colon MPO activity and IL-6 cytokine [36].

The results of this study reveal the anti-inflammatory effect of RoEO dietary supplementation in a dose-and time dependent manner in an inflammatory murine model which could support its use as an alternative therapy for inflammatory bowel diseases. Further research is needed to fully understand its mechanisms of actions.

Thymus vulgaris of the Lamiaceae family has a long traditional culinary and medicinal use and besides its antiseptic, anti-oxidative and antimicrobial effects, it also exhibits immunomodulatory effects of certain interest. To examine its anti-inflammatory properties a study was performed [39], driven by the few *in vivo* and *in vitro* existing studies evaluating these properties and in specifically the effects of thyme extracts on the gastrointestinal system, performed contact hypersensitivity (CHS) experimental models, carrageenan-induced mouse paw odema and TNBS-induced experimental colitis on a murine model. The anti-inflammatory intestinal effect of thyme essential oil (TEO) was examined in a form of a dietary supplementation of three different concentrations (1250, 2500 and 5000 ppm) on a TNBS induced colitis in mice and was compared to the two other anti-inflammatory murine models, the DTH (delayed type hypersensitivity or CHS) and the carrageenan paw oedema [39].

Balb/c mice were fed ad libitum 7 days before TNBS administration and 5 days before hypersensitisation with 1250, 2500 and 5000 ppm thyme EO enriched standard laboratory diet. The DTH reaction was induced in mice by topical application of a 2% oxazolone solution in acetone/olive oil (4:1 V/V), 50 μL on the abdomen and 5 μL on each paw [40], and five days following the right ears were challenged by topical application of 10 μL of a 1% oxazolone solution, where the increase in ear thickness was measured using a Mitutoyo thickness gauge at 24 and 48h after challenge. The carrageenan paw oedema and the TNBS colitis were induced as described in sub chapter "1.7 Anti-Inflammatory Effects of *Rosmarinus officinalis* Essential Oil in Mice." as well as the colon mucosal damaged examined using colon macroscopic scoring system in a similar manner, determining colon to body weight ratio and quantifying IL-1β and IL-6 mRNA expression by PCR which was statistically presented [39].

There were differences observed in the DTH/CHS reaction in mice in a dose dependent manner between the TEO dietary supplementation groups. At 1250 ppm TEO diet, there was an increase in ear swelling both after 24h and 48h, while the 5000 ppm diet showed decreased ear

inflammations, but not in a significant manner compared to the control group after 48h. After the mouse paw oedema induction there were dose-dependent differences at the oedema development indicated between the 5000 ppm TEO diet and the control mice with an significant decrease in paw swelling after 2, 4 and 24h while an increase in paw inflammation was observed again at the 1250 ppm TEO diet after 2 and 4h and an insignificant decrease after 24h. The 2500 ppm TEO diet exerted insignificant changes, both increases and decreases in ear and paw swelling at different times [39]. The macroscopic and microscopic damage scores, as well as the colon weight to body weight ratio, showed comparable scores to that of the TNBS group at 2500 ppm while at 1250 ppm TEO diet, there was an insignificant decrease in colon weight to body weight ratio. On the contrary at 5000 ppm TEO diet, both the macro- and microscopic damage scores and the colon weight to body weight ratio were significant decreased compared to the TNBS group. At the same diet group, the expression of IL-1β and IL-6 cytokines were decreased while only IL-1β decreases could be considered significant, indicating a decrease in colon inflammation when at 5000 ppm TEO dietary supplementation [39].

These results indicate in the same way as with the EO of *Rosmarinus officinalis* that thyme essential oil shows dose and time dependent changes in murine experimental inflammatory models and could be applied in inflammatory bowel diseases as well, always with caution, regarding its contraindications and further research.

5 Antileishmanial activity

Leishmaniasis is a parasitic disease caused by protozoan parasites of the genus Leishmania and transmitted by haematophagous anthropods species of sand fly of the genus Phlebotomus (Old World Leishmaniasis) and genus Lutzomyia (New World Leishmaniasis). The life cycle of these parasites includes two distinct forms: an amotile extracellular promastigote form found in the sand fly vector and a non-flagellated intracellular amastigote form which can be found within the mononuclear phagocytes in the mammalian host [41,42]. The clinical manifestation differs in compromising the skin (cutaneous Leishmaniasis), mucosa (muco-cutaneous Leishmaniasis) and internal organs (visceral Leishmaniasis). Currently there is no vaccine to prevent any of the manifestations and the chemotherapy used is not satisfactory. Having also considering the toxic side effects of the first-line drugs for the treatment of leishmaniasis, there is a need for alternative strategies in countering this disease.

Syzygium cumini (L) Skeels, known as "jambolão" in Brazil, is an evergreen tropical tree of the family Myrtaceae, originated in the Indian subcontinent and regions of Asia. The most common use of *S.cumini* is for the treatment of diabetes. In addition, *S. cumini* has demonstrated, among other (antiviral, antibacterial, antioxidant, antiallergic etc.), antileishmanial properties. A study [43] was conducted, which evaluated the effects, cytotoxicity and possible mechanisms of action of *S. cumini* EO (ScEO) and its major component α-pinene on *Leishmania amazonensis*. The life cycle of Leishmania differentiates in the promastigote found in the parasite, and the amastigote form within the phagocytes in the host which is also responsible for the clinical manifestation of leishmaniasis. The findings of this study [43] report the anti-leismanial activity of ScEO and α-pinene on the amastigote form of *L. amazonensis*. ScEO was extracted by steam hydrodistillation and characterized using gas chromatography-mass spectrometry (GC-MS) as described in a previous study [45]. Extracellular amastigote-like forms were obtained by *in vitro* differentiation of promastigotes of *L. amazonensis* in the stationary growth phase by increasing the temperature to 32°C and decreasing the pH to 4.6 [44] and murine peritoneal macrophages were collected from mice. The promastigotes or axenic amastigotes were cultured with increasing concentrations of α-pinene and ScEO and incubated and macrophages were harvested and plated on culture plates to be incubated for cell adhesion [43]. Adhered macrophages were infected with promastigotes at a ratio 10 promastigotes to 1 macrophage and incubated with a range of concentrations of ScEO, α-pinene and Glucantime® (as a positive control) that were reported not to be toxic to macrophages. The cytotoxic effect on macrophages, haemolysis and immunomodulatory activity, both lysosomal activity and phagocytosis, and nitric oxide production were determined by means of increasing concentrations [43].

The results of this study suggested that α-pinene showed a higher cytotoxic effect on promastigotes of *Leishmania amazonensis* than the reference drug Glucantime®, while the positive effect of ScEO has been previously determined [45]. A low activity of Glucantime® on axenic amastigotes was observed. ScEO and α-pinene showed a significant inhibition with α-pinene having the most effective activity against axenic amastigote forms. ScEO and α-pinene were also effective against intracellular amastigotes in macrophages infected with *Leishmania amazonensis* with α-pinene being more effective than ScEO, but Glucantime® showing higher activity [43]. Furthermore, ScEO demonstrated minor cytotoxicity against human

erythrocytes and murine macrophages, while *α*-pinene reduced the viability of both cells. Compared to the selectivity of the substance for the parasite rather than the mammalian cells, *α*-pinene proved safer for macrophages and ScEO for human erythrocyte (type O^+) due to its low observed toxicity against these cells, while Glucantime® showed the highest toxicity to erythrocytes than parasites [43]. Regarding the increase in lysosomal volume and phagocytosis, ScEO promoted a volume increase in the endocytic compartment. *α*-Pinene increased the retention of the neutral red in the secretory vesicles of macrophages. The phagocytic activity of macrophages was augmented by both treatmens with ScEO and *α*-pinene at 200 and 400 μg/mL and 100, 200 and 400 μg/mL respectively [43].

It is safe to conclude that although Glucantime® exhibits a higher activity against the intracellular amastigote form, due to its high toxicity against both the human erythrocytes and macrophages and selectivity for erythrocytes over parasites, the need for a better alternative could be substituted with the treatments of ScEO and *α*-pinene.

Xylopia discreta, of the family of Annonacea, is an evergreen tree, growing in S. America with its active compounds showing microbicidal and antitumoral properties. Until recently there have not been any reports on the antiparasitic activity of *X. discreta*. Kemp et al. [46] exhibited one of the first reports about the antiparasitic effect of *X. discreta* on *Leishmania panamensis*, responsible for the majority of cutaneous leishmaniasis cases in Colombia. This study [46] determined the *in vitro* antileishmanial activity of the EO and extracts of *Xylopia discreta* by determining the *median lethal concentration* (LC_{50}) on exposed macrophages (J774-murine and U927-human) and the *median effective concentration* (EC_{50}) by the reduction of *Leishmania panamensis* infected cells. As a reference for an antileishmanial activity a *selectivity index* (SI) (LC_{50}/EC_{50}) above 20 was chosen. Infected cultures were incubated for 72h in presence of *X. discreta* EO, extracts and controls. The antileishmanial activity of *X. discreta* extracts and EO was different between the murine (J774) and human (U937) macrophages. Both, in the LC_{50} and the EC_{50}, the activity of the extracts and EO showed different effects on the murine and human macrophages. On the human cell lines (U937) the ethanolic and methanolic extracts of leaves and the leaf EO exerted the highest activity with an SI of 40, 23 and 26 respectively [46]. On treating the murine macrophages (J774) only the leaf methanolic extract and the EO maintained their antiparasitic effect (Si 65 and 110 respectively). The ethyl acetate extract of leaves also showed an evident effect when treating the murine cell lines (SI=32.6). Comparing the SI values the *X. discreta* treatment proved to be more efficient on the J774 cellular line. In both lines pentaminide, a known therapeutic antileishmanial medicament, was used as a positive control. Based on the information already presented, the acetate extract, the methanol extract and the EO were analysed to quantify the pro-inflammatory mediators [46].

The immunomodulatory activity of the substances was assessed by quantifying the mediators (IL12 p70 fraction, IL10, IL5, MCP-1 and TNF-α) produced after treating the infected macrophages by flow cytometry [46].

In the case of *cutaneous leishmaniasis*, in the experimental murine model, there has been established a correlation between the secretion of pro-inflammatory cytokines and the subsequent activation of Th1-phenotype T cells (associated with resistance to infection), while the release of anti-inflammatory cytokines is associated with the Th2 type (associated with susceptibility to infection) [47, 48, 49, 50]. This indicates the positive effects of the dual, antimicrobial and immunomodulatory, activity of *X. discreta*. In cases of visceral leishmaniasis, there is a mixture of both Th1 and Th2 types related to the infection [47]. The Th1 immune response is a natural resistance mechanism to the parasitic infection, associated with the secretion of pro-inflammatory mediators, inducing macrophage microbicidal activity, while the Th2 phenotype is associated with the production of anti-inflammatory cytokines, deactivating the microbicidal properties of macrophages [50].

There was no statistical activity observed in the IL12, TNF-α and IL10 production when treated with the *X. discreta* extracts and EO. On the contrary, there was an increase in the MCP-1 production in murine-infected macrophages when treated with the methanolic extract of leaves, with the EO and even with the ethanolic extract of seed, which although showed a low anti-leishmanial activity, exhibits an immunomodulatory effect that could be positive in the optimum control of leishmaniasis [46]. The difference in MCP-1 secretion in infected and in uninfected cells, both receiving treatment with the methanolic extract of leaf and the EO, indicates a synergism between the parasite and those substances in *X. discreta* which increases this pro-inflammatory chemokine production and activates the microbicidal effect in the infected macrophages. Lastly, according to this study, a tendency between the extract concentration and cytokine production was observed. Higher concentrations induced lower cytokine or chemokine levels while lowers showed a higher induction [46].

This study [46] revealed that substances contained in the methanolic extracts and in the EO reduced the parasite

load and were also able to activate the macrophage microbicidal activity, leading to parasite clearance as well as to a lasting immune response. In this study, the methanolic extracts and the essential oil of *X. discreta* induced an immunostimulatory effect over the infected macrophages increasing the production of *monocyte chemoattractant protein-1* (MCP-1), a pro-inflammatory chemokine, relevant to the Th-1 phenotype and an important healing and resistance mediator in the cases of cutaneous and visceral leishmaniasis, reducing parasite load in a dose-dependent manner. It is safe to assume that MCP-1 is an important healing and resistance mediator in the cases of cutaneous and visceral leishmaniasis.

The inadequate antileishmanial effect and side effects of the antimonial and second-line drug treatments for cutaneous leishmaniasis have led Demarchi et al. [51] to research and evaluate the plant *Tetradenia riparia*, which has shown promising results for the treatment of infectious diseases in folk medicine, as an alternative treatment for CL [9]. This study [51] evaluated the *in vitro* antileishmanial activity and immunomodulatory effects of TrEO on cytokine modulation by *L. amazonensis* infected peritoneal fluid murine macrophages [51]. Peritoneal fluid cells were infected with Leishmania and incubated with *TrEO* for 3, 6 and 24h. Cytokines were screened using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) and flow cytometry. The antileishmanial activity was evaluated at 24h by microscopic counting and quantitative PCR (qPCR) [51].

Leishmania parasites are able to modify both innate and adaptive immune responses by inhibition of the antimicrobial mechanisms of host cells and by causing an imbalance between T-Helper cells. During treatment with TrEO, the EO and the infection lead to the induction and inhibition of the immune response and macrophage functions. Different cytokines promote the activation or inhibition of primary cells, as macrophages, neutrophils, T-Helper cells (T_H1, T_H17, T_H2), which are involved in cutaneous leishmaniasis.

L. amazonensis infection induced IL-1β, IL-4, IL-6, IL-10 and TNF, among other factors (IL-5, IL-17, GM-CSF), leading to macrophage inhibition and T_H2 and T_H17 cell activation, which favours the persistence of the infection and the progression of the disease [51]. TNF secretion promotes neutrophil recruitment and enhances inflammation. *TrEO* treatment inhibited TNF, IL-4 and IL-10 productions, following by T_H2 and T_H17 cell inhibition leading to the control of the inflammation and disease progression. Furthermore, *L. amazonensis* infection inhibits the protective cytokines interferon-γ (IFN-γ), IL-12 and IL-18 which promote macrophage and T_H1 cell activation. *T. riparia* EO reversed the inhibitory effects of the parasitic infection and enhanced mainly the IFN-γ secretion and led via microbicidal mediator activation, as nitric oxide (NO) and other reactive oxygen species, to parasite death [51].

According to this study [51], the infection index for untreated and infected macrophages was 112. After TrEO treatment, the infection index was reduced to 54 at 30 ng/mL, 68 at 0.3 μg/mL and 79 at 3 μg/mL. TrEO was reported to be most effective against *L. amazonensis* at 30 ng/mL inducing 50% death of *Leishmania* amastigotes after 24-h incubation, and also did not present cytotoxicity in murine macrophages (>95% viable cells). On the contrary, higher doses were less effective and presented a higher toxicity. These results suggest that TrEO could be used as an alternative antileishmanial therapy.

6 Modulation on immediate hypersensitivity

The prevalence of allergic diseases, such as allergic asthma, allergic rhinitis, food allergies and anaphylaxis among others of the immediate or type I hypersensitivity has been on the increase worldwide [52]. Immediate hypersensitivity is an allergic reaction after exposure to foreign antigens, referred to as allergens. In the presence of allergens B cells, stimulated by CD4+ and Th2 cells, produce IgE antibodies specific to an antigen and the immediate hypersensitivity is triggered, followed of the activation of basophils and mast cells [53]. Degranulation of mast cells and basophils results to the release of a range of mediators and generation of Th2 cytokines as IL-4 and IL-13, both in mice and men which makes them key modulators of the Th2-immune response [54].

Common therapies against immediate hypersensitivity are based on the use of corticoids and histamines which in some cases show no effectiveness and several adverse effects [55]. These facts indicate the need of new immunomodulators effective against immediate hypersensitivity and with minimal adverse effects.

Minthostachys belongs to the *Lamiaceae* family and is a complex genus found in South America. *Mintostachy verticillata* (Griseb.) Epling, also known as “piperina” is found in Venezuela, Colombia, Ecuador, Peru and regions of Argentina and has been used in the traditional medicine as a digestive, for spasms, sedative and interestingly for bronchitis and asthma [56]. Studies have shown that the *M. verticillata* EO (MvEO) exhibits antimicrobial, antiviral activity [57] and antiallergic properties being able to

stimulate B cells, T cells, CD4+ and CD8+, as well as to promote IFN-γ production and IL-13 inhibition on human cells [58,59].

Carridi et al. [60], the same group that showed the antiallergic properties of *M. verticillata* EO [58,59], examined the modulatory activity of the main components of MvEO, both *in vivo* and *in vitro*, on immediate hypersensitivity. *M. verticillata* EO was obtained by hydrodistillation and it was identified and analyzed by gas chromatography (GC) flame ionisation detector (FID) by comparing it against the standard pure compounds, pulegone, menthone and limonene, which were purchased from Sigma Aldrich. The EO and monoterpenes were diluted in DMSO for the immunological *in vitro* assay. The sample tested included PBMCs and basophils from 10 male and 36 female patients allergic to dust mites, whereas the PBMCs from healthy individuals were used to set the dose response of the compounds. IgE and the IL-13 were analyzed using a commercial ELISA IgE quantification and ELISA human interleukin 13 kit respectively. The cellular proliferation was assessed by MTT cell proliferation assay kit and the antiallergic effect was assessed by passive cutaneous anaphylaxis reaction (PCA) on BALB/c mice [60].

The yield of *M. verticillata* EO was 4.8%. The main components identified were pulegone (63.4%), menthone (15.9%), limonene (2.1%) and other minor terpenoid components. The compounds stimulated the proliferation of human lymphocytes *in vitro*. The EO showed the highest stimulation at 6 μg/mL, pulegone at 62 μg/mL, menthone at 60 μg/mL and limonene at 55 μg/mL, while there was no synergistic effect observed when the compounds were combined [60]. The IL-13 production after stimulation with the EO or monoterpenes, at 6 μg/mL essential oil, 62 μg/mL pulegone, 60 μg/mL menthone, 55 μg/mL limonene, and the allergen was reduced compared to the production after stimulation with only the allergen. A combination of pulegone, menthone and limonene exerted a significant inhibition of the *in vitro* spontaneous IL-13 production from cells of allergic patients [60]. The EO (10 μg/mL) showed higher inhibition on the β-hexosaminidase enzyme liberation compared to the monoterpenes (40 μg/mL pulegone, 40 μg/mL menthone, 20 μg/mL limonene), alone or together and to standard drugs. The monoterpenes alone or in combination also exhibited higher inhibition than standard drugs [60].

According to the PCA model on BALB/c mice the EO showed a dose-depenent inhibition of the PCA reaction with the best inhibitory effect at 200mg/kg, but this was still lower than that of dexamethasone. On the other hand limonene was the only monoterpene to inhibit the PCA reaction in a dose-dependent way at 250 mg/kg, exhibiting a higher inhibition than the EO similar to that of dexamethasone [60]. The PCA model mimics an acute mast cell mediated reaction and is a perfect way to examine *in vivo* the effects on immediate hypersensitivity. *In vivo* limonene showed a mast cell mediated reaction suppression as potent as that of dexamethasone which supports the findings of studies suggesting its anti-inflammatory effect on bronchial asthma by inhibiting NOS, NO and PG-E_2 production [61]. Concluding, the reduction of IL-13 and β-hexosaminidase in T cells and basophils, as well as the mast-cell degranulation inhibition in a murine model, and the lack of toxic properties and adverse effects [62,63], suggests in agreement with previous results, that both the essential oil and the monoterpenes, mainly limonene, could be used in the treatment of allergic diseases.

Mast cells and basophils play an important role by IgE in progression of allergic diseases. The interaction of allergens with surface-bound IgE promotes the liberation of mediators and results in the production of cytokines that activate the migration of macrophages and neutrophils which in turn induce inflammation [64,65]. The direct connection of allergies to inflammation, as it is an inevitable outcome, led Mitoshi et al. [66] to examine the anti-allergic and anti-inflammatory properties of 20 EOs from herbal plants and citrus fruits.

Around 17 herbal plant EOs were obtained by hydrodistillation and they include essential oils of *Ocimum basilicum* L. (basil) Lamiaceae, *Carum carvi* L. (caraway) Apiaceae, *Daucus carota* L. (carrot seed) Apiaceae, *Apium graveolens* L. (celery seed) Apiaceae, *Matricaria chamomilla* L. (chamomile) Asteraceae, *Cympogon winterianus* Jowitt (citronella) Poaceae, *Salvia sclarea* L. (clary sage) Lamiaceae, *Syzigium aromaticum* L. (clove) Myrtaceae, *Cuminum cyminum* L. (cumin) Apiaceae, *Eucalyptus globulus* L. (eucalyptus) Myrtaceae, *Cympogon citratus* (DC.) Stapf (lemongrass) Poaceae, *Majorana hortensis* Moench (marjoram) Lamiaceae, *Myristica fragans* Houtt (nutmeg) Myristicaceae, *Salvia officinalis* L. (sage) Lamiaceae, *Santalum album* L. (sandalwood) Santalaceae, *Mentha spicata* L. (spearmint) Lamiacaeae and *Thymus vulgaris* L. (thyme) Lamiaceae. The 3 EOs derived from citrus fruits were cold-pressed and included *Citrus limon* L. (lemon), *Citrus aurantifolia* Swingle (lime) and *Citrus sinensis* L. (orange) [66].

The mast cell degranulation was determined by a β-hexosaminidase release assay, performed on RBL-2H3 rat basophilic leukemia cell lines and the inhibitory effect of the EOs on TNF-α was examined on RAW264.7 murine macrophage cell lines. The anti-anaphylactic effects were measured by PCA (passive cutaneous anaphylaxis) reaction on mice and the anti-inflammatory activity by TPA

(a chemical oedema inducer, 12-O-tetradecanoylphorbol-13-acetate) application, a method described in a previous study [67]. Nuclear proteins and whole cell lysates were subjected to western blot analysis to evaluate the nuclear translocation of NF-κB and the protein expression of IκB-α [66]. The EOs (100 μg/mL) did not influence the growth of RBL-2H3 cells nor inhibited β-hexosaminidase enzyme activity. The degranulation was calculated by determining the β-hexosaminidase activity in the supernatant and cell lysate. From the 20 species of Eos, a significant inhibition of mast cell degranulation, assessed by β-hexosaminidase release was observed from chamomile, lemongrass and sandalwood at over 40% inhibition, with lemongrass showing the strongest one, whereas the EOs of lime and eucalyptus EOs did not show any effect. The EOs from chamomile, lemongrass and sandalwood exhibited also the most significant inhibition of TNF-α which initiates the inflammatory response by the NF-κB signalling pathway. The lemongrass EO exerted the highest effect on TNF-α production [66]. By comparing the effects of the 20 EOs on TNF-α inhibition in macrophages and β-hexosaminidase inhibition in mast cells there was a correlation observed between them. The anti-allergic effects and anti-inflammatory activity may be related within a shared pathway [66].

Since the EO derived from lemongrass showed the strongest inhibitory effects, both in β-hexosaminidase release and TNF-α production, this study focused on further examining it. The lemongrass EO was identified by gas chromatography mass spectrometry (GC-MS). The chemical composition of the CcEO is shown in Table 4. The 2 main components were geranial and neral. From the compounds identified citral, geraniol, geranyl acetate, linalool and camphene were obtained by isolation from the oils, whereas geranial was chemically synthesized, and all were tested on β-hexosaminidase release by mast cells and TNF-α production by macrophages [66]. Citral and the chemically synthesized geranial showed a similar inhibition of β-hexosaminidase and TNF-α (ca. 55% and >60% respectively) to that of the EO, while the rest of the compounds showed moderate or weak activity (<35%) in both the β-hexosaminidase release and TNF-α production. Citral and geranial proved to be the active components and were further investigated on the inhibitory activity against LPS-induced inflammatory responses in cultured macrophages. By means of Western Blot Analysis it was shown that 10μg/ml of citral and geranial inhibited the LPS-induced NF-κB nuclear translocation (at around 60% and 50% respectively), similar to the lemongrass EO inhibition. Citral and geranial did not affect the IκB-α expression which is induced by LPS stimulation and associated with the NF-κB translocation, showing that lemongrass EO, citral and geranial suppress the NF-κB nuclear translocation by a IκB-α independent mechanism [66].

The effect on the immediate hypersensitivity reaction was examined by means of the IgE-mediated PCA reaction *in vivo*. Compared to Tranilast® (100 mg/kg) (close to 20%), an anti-allegic drug, lemongrass EO, citral and geranial showed stronger inhibition of the PCA reaction (60.7. 57.7, 77.4% respectively; more than twice the effect) at the same concentration [66]. The anti-inflammatory effects of lemongrass EO and its two main compounds were examined on a TPA-induced inflammatory oedema of the mouse ear *in vivo*. With a pre-treatment of 500μg/ear there was an inhibition of the inflammation similar to the inhibition of the *in vivo* PCA-reaction. The anti-inflammatory effects of lemongrass EO, geranial and citral were again shown to be more effective in a similar way to the PCA reaction inhibition than that of glycyrrhetinic acid, a common anti-inflammatory agent, at the same concentration [66].

The findings of this study [66] suggest that citral donates its anti-allergic and anti-inflammatory properties to *C. citratus* (DC.) Stapf (lemongrass) EO. This study indicated a correlation of the β-hexosaminidase release and TNF-α production by comparing the inhibition by the 20 EOs which suggests that the way that the EOs inhibit the mast cell degranulation should be further investigated. Furthermore, the fact that the lemongrass EO and its 2 major components inhibited the NF-κB nuclear translocation by an IκB-α independent mechanism, although an LPS stimulation results in IκB-α inhibiting NF-κB and the NF-κB transcription factor [68], suggests further investigation of the NF-κB pathways affected from the EOs. Lastly, both the PCA and the TPA reactions suggest that lemongrass EO is a promising anti-allergic and anti-inflammatory alternative, when compared to the existing common drugs and with no observation of toxicity or adverse effects.

Atopic asthma is the result of an increased immune response to allergens and induces airway inflammation and obstruction, as well as airway hyperresponsiveness (AHR) [70] which are featured in murine asthma as well. AHR and airway inflammation are associated with cytokines produced by the Th2 response, as IL-4, IL-5 and IL-13 [71]. The airway obstruction results partly from mucus hypersecretion, with Muc5ac and Muc5b being secreted in murine models [72,73].

Studies have shown that lavender EO inhibits the mast cell induced ear inflammation and the anti-DNP IgE passive cutaneous anaphylaxis in mice [74]. Mast cell degranulation can promote AHR, accumulation of bronchial inflammation and mucus cell hyperplasia [73,75].

Table 4: Chemical composition of *Cympogon citratus* EO.

Compounds	Content (%)
geranial	40.16
neral	34.24
geraniol	5.11
geranyl acetate	2.89
linalool	1.45
methylheptanone	1.44
others	14.71

Identified by gas chromatography mass spectrometry (GC-MS). (Adapted and newly drawn from Mitoshi et al. 2014) [65]

A study [76] based on these previous reports, examined the effect of lavender EO on allergic inflammation. The chemical composition of lavender EO is shown in Table 5. Ovalbumin (OVA) diet free BALB/c mice were divided in lavender (lvn) -asthma group (5µl or 20µl) and control-asthma group and were sensitized intraperitoneally by injection with 20µg ovalbumin. A non-asthma group was injected with 0.1 ml saline intraperitoneal [76]. The lvn-asthma group was subjected in inhalation cages with lavender EO (5 µl or 20 µl), while the non-asthma and control-asthma groups were treated with water [76].

To determine the pulmonary function the lung resistance (RL) was measured to increasing doses of inhaled methacholine by a small animal ventilator. The bronchoalveolar lavage (BAL) fluid was obtained and the lungs were fixed in 10% formalin and used for histological studies. By random staining of periodic acid-Schiff (PAS) and hematoxylin-eosin (HE), peribronchial and perivascular tissue were examined for mucus containing and inflammatory cells as eosinphyles, lymphocytes and neutrophils, respectively, using the NIH image analysis system (National Institutes of Health, Bethesda, MD, USA). The cytokine concentration in the BAL fluid supernatants were measured by FlowCytomix and total cellular RNA was obtained from lung tissues and RT-PCR analysis was performed [76]. An enzyme-linked immunosorbent assay (ELISA) was used to measure the anti-OVA IgE antibodies in the serum. A higher increase in lung resistance in the control-asthma group, compared to the non-asthma group was observed, while the mice treated with lavender EO showed improved pulmonary function with reduced AHR increase [76].

After OVA challenges the inflammatory cells in the airway were determined from the BAL fluid. In the control-asthma group the total cell numbers, lymphocytes and eosinophils were higher than in the non-asthma group. Total cells in the lvn-asthma group treated with 20 µL lavender EO were significantly reduced or similar to that of the non-asthma group and eosinophils were at even lower numbers. There was no difference observed between 20 µL and 5 µL treatment. HE staining in the peribronchial and perivascular tissues showed similar results in total cell numbers and eosinophils, but no significant differences in neutrophils and lymphocytes between the three groups were recorded [76]. By means of the periodic acid-Schiff staining the non-asthma group showed no PAS-positive cells and areas, while they were detected in the control-asthma and showing significant lower numbers in the lvn-asthma group. Treatment with lavender EO resulted in inflammatory cell accumulation and mucus cell hyperplasia inhibition [76]. On the other hand, treatment with lavender EO did not decrease IgE levels in serum, exhibiting higher levels in both the lvn-asthma and control-asthma groups than in the non-asthma group [76].

The IL-5 and IL-13 cytokine levels in BAL fluids in the control-asthma group were higher than those in the non-asthma group and the lvn-asthma group. IL-4, IL-5 and IL-13 mRNA expression levels in lung tissue showed similar results, with IL-13 mRNA expression at almost the same levels in control and lvn-asthma group. Lastly, there was a significant increase in the Muc5ac and Muc5b in the control-asthma group compared to the non-asthma group. The lvn-asthma group showed only a significantly reduced Muc5b expression compared to the control-asthma group [76]. The results of this study [76] showed that treatment with *L. angustifolia* EO improved the pulmonary function and inhibited AHR, inflammatory cell accumulation in BAL fluids and peribronchial and perivascular tissues and mucus cell hyperplasia. Additionally, lavender EO reduced the cytokine levels and their mRNA expression in BAL fluids and lung tissue respectively, but did not decrease serum IgE levels. These findings revealed that *L. angustifolia* EO in a murine model of asthma suppressed the allergic airway inflammation and mucous cell hyperplasia, providing new insights of the effect of lavender which should be further investigated in the future [76].

7 Immunosuppressive activity on cancer cells

Cancer affects many people and their life quality worldwide with different forms of abnormal cell growth. People are being subjected to many carcinogens daily, as tobacco, UV and ionizing radiation and viral, bacterial and parasitic infections, making cancer the second leading death cause

Table 5: Chemical composition of *Lavandula angustifolia* EO.

Compounds	Content (%)
Linalyl acetat	31.78
Linalool	25.56
cis-β-ocimene	4.89
β-caryophyllene	4.78
lavandulyl acetate	4.7
terpinene-4-ol	4.03
cis-β-farnesene	3.7
trans-β-ocimene	2.77
Other	<2

The lavender essential oil was analysed by gas chromatography mass spectrometry. (adapted and newly drawn from Ueno-lio et al. 2014) [76]

after heart disease [77]. Standard cancer chemotherapies with immunosuppressive acting cytostatic/cytotoxic agents are the most common treatment targeting the signal transduction pathways with many life-threatening side effects, while new plant-derived compounds and natural products are used for the development of antitumor agents in cancer prevention and therapy [78]. The existing cancer therapies show no satisfying effectiveness which urges the need for new drugs with a strong immunosuppressive activity but with low cytotoxicity and adverse effects. EOs have shown promising effects against cancer cells, which could justify a shift towards natural products in the fight against cancer [79].

Various studies have reported the anticancer effects of EOs against tumor cell lines, as *Lavandula stoechas ssp. stoechas* EO against human colon carcinoma (COL-2) [80] and *Citrus paradise* EO against human leukemic (HL-60) cells [79].

Zu et al. [81] examined the antitumor activity of ten EOs on human lung carcinoma (A459), human prostate carcinoma (PC-3) and human breast cancer (MCF-7) cancer cell lines. The EOs that were used were of *Mentha spicata* (mint) Lamiaceae, *Zingiber officinale* (ginger) Zingiberaceae, *C. limonum* (lemon) Rutaceae, *C. paradisi* (grapefruit) Rutaceae, *Jasminum grandiflorum* (jasmine) Oleaceae, *L. stoechas* (lavender) Lamiaceae, *Anthemis nobilis* (chamomile) Asteraceae, *Thymus vulgaris* (thyme) Lamiaceae, *Rosa centifolia* (rose) Rosaceae and *Cinnamomum zeylanicum* (cinnamon) Lauraceae [81].

The *in vitro* cytotoxicity of increasing concentrations of EOs on A549, PC-3 and MCF-7 human cancer cell lines was determined by measuring the cell viability by MTT (3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Different cytotoxic activities in a dose dependent way were observed among the three human cancer cell lines [81]. At 0.002% (v/v), the cell viability of all three human cancer cell lines compared to untreated control cells was at over 80%, showing no significant cytotoxic effects by the essential oils [81]. At 0.200% (v/v), the cell viability of the PC-3 cells was lower than 4%, exhibiting strong cytotoxic effects of all EOs. Most EOs reduced the viability of A549 cells, while mint EO exerted no effect, and cinnamon, thyme, chamomile and jasmine EOs showed the highest cytotoxicities (5.31%, 3.47%, 6.93% and 4.34% respectively) towards the MCF-7 cell lines. Grapefruit and ginger EOs did not exhibit a significant effect with cell viability at 75.03% and 81.85% respectively [81]. The strongest cytotoxic effect against PC-3, A549 and MCF-7 cells was observed by thyme EO, with IC_{50} values of 0.010%, 0.011% and 0.030% (v/v) respectively. Similar IC_{50} values against PC-3 and A549 were observed by cinnamon and jasmine EOs (0.012% and 0.022% (v/v) against PC-3 and 0.017% and 0.012% (v/v) against A549) [81].

In conclusion, thyme EO exhibited the strongest cytotoxicity against A549, PC-3 and MCF-7 human cancer cell lines, while cinnamon and jasmine EO showed high cytotoxicity on A549 and PC-3. The cytotoxic profile of the EOs could be attributed to their composition and components, like thymol in thyme EO which has been reported to exhibit antimicrobial activities [82] or eugenol in cinnamon EO which also attributed antimicrobial activities [83] lending them antitumor activity on these three human cancer cell lines. Thyme, cinnamon and jasmine EOs can be used as potent antitumor agents in the future. Further investigation to support their anticancer effect and the potential contribution of their main components to that effect should be carried out [81].

Pituranthos tortuosus belongs to the Apiaceae family and is a plant found in Tunisia and native to Egypt and regions of North Africa. *P. tortuosus* has been used in traditional medicine to treat fever, digestion problems and rheuma [84]. Studies have examined the properties of its EO which demonstrates antimicrobial [85], as well as promising antimutagenic, cytotoxic and apoptotic activities [86]. The lack of information on the antitumor effects of the *P. tortuosus* EO against melanomas led to a study [87] to examine the cytotoxic and apoptotic effects of the EO from the aerial parts on B16F10 melanoma cells. The *P. tortuosus* EO was obtained by hydrodistillation of the air-dried, powdered aerial parts of the plants and the EO was then identified by gas chromatography mass spectrometry (GC-MS) using flame ionization detectors (FID) [87].

The B16F10 tumor cell viability was measured by MTT assay to determine the cytotoxicity of *P. tortuosus*

EO, expressed in IC_{50} values in comparison to untreated control cells [88]. The apoptotic effect was determined by assessing nuclei cell morphology after acridine orange (AO)/ ethidium bromide double-staining and evaluating the presence of apoptotic cells by fluorescence microscopy [87]. *P. tortuosus* EO exhibited a dose and time dependent cytotoxicity, inhibiting B16F10 cell proliferation with a maximum inhibition of 91.3±3.6 % after 48 h of incubation at 400 µg/mL [87].

By means of AO/EB staining and fluorescence microscopy, a significant increase of apoptotic cells compared to the untreated control group was observed. Apoptosis was induced in a dose-dependent way with 160 µg/mL and 320 µg/mL EO increasing the apoptotic cells to approximately 70% and 95% respectively [87].

In conclusion, *P. tortuosus* EO exhibited *in vitro* a dose/time dependent inhibition of cell proliferation and an increase of apoptotic cells. These findings agree with previous studies demonstrating the antitumor and apoptotic activity of *P. tortuosus* EO. The immunomodulatory effect of this EO, with the potential increase of splenocyte proliferation, could suggest a synergistic effect against the development and progression of melanoma, since it is a form of cancer associated with immune suppression [88].

Plants of the genus *Ballota* belong to the *Lamiaceae* family, can be found in regions of Europe, especially in the mediterranean region. *Ballota* species have been used in traditional medicine and possess antimicrobial [89], antifungal [90] and antioxidant activities [91] among others. A study [92] examined the inhibitory effect of *Ballota undulata* (Sieber ex Fresen.) Benth, *B. saxatilis* (Sieber ex C.Presl) and *B. nigra* ssp. *foetida* (Vis.) Hayek on human hepatoma HepG2 and human breast cancer MCF-7 cell lines based on the information on their traditional use and potent antioxidant activities. The chemical composition of the essential oils of these plants is shown in Table 6. The antiproliferative activity of these three Ballota species against the HepG2 and MCF-7 cell lines was measured by MTT assay in comparison to a non-treated control group. The MTT assay showed a dose dependent inhibition of the essential oils on HepG2 and MCF-7 cell proliferation. *B. undulata* EO exhibited the highest inhibition against HepG2 cells of approx. 81% at 100 µg/mL. Against MCF-7, there was no significant inhibition observed, with *B. saxatilis* EO showing the highest inhibition at approx. 24% [92]. The antioxidant potential of the EOs derived of the three *Ballota* species was confirmed by DPPH test showing radical inhibition. The results of this study indicated the effectiveness *in vitro* of the *B. undulata*, *B. saxatilis*, *B. nigra* *ssp. foetida* EOs by inhibiting the human hepatoma HepG2 cell proliferation in a dose dependent way. The MCF-7 inhibition was not significant though. Since this was the first report on the antiproliferative effect of *Ballota* species on cancer cells further investigation is required to prove this effect [92].

Table 6: Chemical composition of the essential oils of *B. nigra* ssp. foetida (Bn), *B. saxatilis* (Bs) and *B. undulata* (Bu).

Compounds	Content (%)		
	Bn	Bs	Bu
Monoterpene hydrocarbons	3.9	8.4	2.6
α-thujene, α-pinene, sabinene, myrcene, α-terpinene, *p*-cymene, limonene, (*Z*)-β-Ocimene, γ-terpinene, terpinolene			
Oxygenated monoterpenes	3.8	17.4	8.6
linalool, 1,8-cineole, *cis*-linalool oxide, fenchone, *trans*-linalool oxide, α-campholenal, *trans*-pinocarveol, *cis*-verbenol, camphor, *trans*-verbenol, borneol, terpinen-4-ol, *trans*-verbenone, others			
Sesquiterpene hydrocarbons	55.9	15.1	39.3
(*E*)-β-caryophyllene, germacrene D, α-cubebene, cyclosativene, α-ylangene, α-copaene, daucene, β-bourbonene, β-elemene, α-gurjunene, δ-cadinene, others			
Oxygenated sesquiterpenes	14.0	8.9	7.3
caryophyllene oxide, caryophylladienol I, caryophylla-3,8(13)-dien-5α-ol, spathulenol, γ-eudesmol, (*E*)-nerolidol, others			
Phenols	Trace	6.3	4.8
4-vinylguaiacol, thymol, carvacrol, eugenol, *p*-vinyl anisole			
Carbonyllic compounds	8.5	12.6	5.8
(*E*)-2-Hexenal, nonanal, hexahydrofarnesyl acetone, (*E*,*E*)-farnesyl acetone, others			
Fatty acids and esters	3.1	14.5	15.8
hexadecanoic acid, (*Z*,*Z*)-9,12-octadecadienoic acid, (*Z*,*Z*,*Z*)-9,12,15-octadecatrienoic acid, tetradecanoic acid, (*Z*)-9-octadecenoic acid, others			
Hydrocarbons	1.0	7.4	2.5
heptacosane, nonacosane, pentacosane, pentacosane, hentriacontane, others			
Others	4.9	4.4	5.0
Identified by comparison of retention index, by comparison of mass spectra with MS libraries or by comparison with authentic compounds. (Adapted and newly drawn from Rigano et al. 2016) [91]			

8 Conclusion

The results of the studies examined and cited in this literature review strengthen the potential of EOs as immunomodulators and as alternative treatments for infectious, immune and carcinogenic diseases. The EOs examined show satisfying immunomodulatory, anti-inflammatory, antileishmanial, antiallergic, and anticancer effects. These pharmacological properties could be explained by means of the main components of the EOs. In most cases the major constituents of an EO exhibit a greater immunomodulatory effect than the EO itself, like α-pinene which shows greater cytotoxic effects than *S. cumini* EO against pro-, axenic and intramacrophagic amastigotes [45]. In other cases, like that of *C. ospholeum* EO, the major constituent exhibited a cytokine induction (IL-2, IL-4, IL-10 and IFN-γ) that the EO did not have [23]. This fact could indicate that the compounds of EOs can work both synergistically and as antagonists, donating or inhibiting pharmacological properties.

Furthermore, EOs exert similar but not identical effects in the *in vitro* murine and human investigations. F.e. *X. discreta* inhibits the monocyte chemoattractant protein -1 (MCP-1), a pro-inflammatory chemokine only tested in the murine and not in the human macrophages [46]. Thus, more investigation of the EO effect in human cells is needed to fully understand their mechanism of action. The properties and application forms of the EOs are also need to be considered. EOs are known to irritate the skin and thus need to be diluted when applied as emollients, especially in open wounds. Nonetheless, EOs and natural products in general, show promising results with pharmacological properties and mechanisms yet to be conquered and understand. With common standard treatments, growing ineffectively and inefficiently and with more adverse side effects, EOs could soon complement and perhaps even replace those conventional treatments.

Conflict of interest: Authors state no conflict of interest.

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