Abstract: Plants and their constituents have been used to treat diverse ailments since time immemorial. Many plants are used in diverse external and internal formulations (infusions, alcoholic extracts, essential oils (EOs), etc.) in the treatment of inflammation-associated diseases, such as those affecting the respiratory tract or causing gastrointestinal or joint problems, among others. To support the traditional uses of plant extracts, EOs have been assessed for their alleged anti-inflammatory properties. However, the effect of EOs on the release of cytokines and chemokines has been much less reported. Considering their traditional use and commercial relevance in Portugal and Angola, this study evaluated the effect of EOs on the in vitro inhibition of the cytokine tumor necrosis factor-α (TNF-α) and the chemokine (C-C motif) ligand 2 (CCL2) by lipopolysaccharide (LPS)-stimulated human acute monocytic leukemia cells (THP-1 cells). Twenty EOs extracted from eighteen species from seven families, namely from Amaranthaceae (Dysphania ambrosioides), Apiaceae (Foeniculum vulgare), Asteraeaceae (Brachylaena huillensis, Solidago virgaurea), Euphorbiaceae (Spirostachys africana), Lamiaceae (Lavandula luisieri, Mentha cervina, Origanum majorana, Satureja montana, Thymbra capitata, Thymus mastichina, Thymus vulgaris, Thymus zygis subsp. zygis), Myrtaceae (Eucalyptus globulus subsp. maidenii, Eucalyptus radiata, Eucalyptus viminalis) and Pinaceae (Pinus pinaster) were assayed for the release of CCL2 and TNF-α by LPS-stimulated THP-1 cells. B. huillensis, S. africana, S. montana, T. mastichina and T. vulgaris EOs showed toxicity to THP-1 cells, at the lowest concentration tested (10 µg/mL), using the tetrazolium dye assay. The most active EOs in reducing TNF-α release by LPS-stimulated THP-1 cells were those of T. capitata (51% inhibition at 20 µg/mL) and L. luisieri (15–23% inhibition at 30 µg/mL and 78–83% inhibition at 90 µg/mL). L. luisieri EO induced a concentration-dependent inhibition of CCL2 release by LPS-stimulated THP-1 cells (23%, 54% and 82% inhibition at 10, 30 and 90 µg/mL, respectively). These EOs are potentially useful in the management of inflammatory diseases mediated by CCL2 and TNF-α, such as atherosclerosis and arthritis.

Keywords: essential oils; cytokine; chemokine; inflammation

1. Introduction

Since ancient times, man has used the plant kingdom as a source for clothing, construction, fuel, food, spices and medicines, as well as for poisons. Nowadays, around half the pharmaceutical drugs used in developed countries, such as aspirin, are of plant origin [1]. Traditional medicine is still the main source of health care for 80% of the people in developing countries, where medicinal plants are commonly used for the treatment of several ailments, notably inflammatory diseases.
Acute inflammation is a short-term reaction which is essential for survival after an infection or a physical injury. On the other hand, chronic inflammation, promoted by social, environmental and lifestyle factors (diet, smoking, alcoholism, inactivity), may trigger diverse long-term illnesses such as cardiovascular disease, cancer, diabetes mellitus, chronic kidney disease, nonalcoholic fatty liver disease and autoimmune and neurodegenerative disorders [2]. These diseases and lifestyles are associated with atherosclerosis, the early detection of which is based on peripheral artery and carotid artery thickness. In a recent study of the epidemiological burden caused by carotid atherosclerosis, Song et al. [3] estimated that, in 2020, the prevalence of increased carotid intima-media thickness in people aged 30 to 79 years was about 28%, equivalent to approximately 1070 million cases worldwide.

In Portugal, recent studies showed that as many as 740,000 adults are affected by atherosclerosis [4]. Data on other inflammatory diseases in Portugal, such as rheumatic diseases [5], showed that women (64%) are more affected by rheumatic diseases, including rheumatoid arthritis, than men (47%). These diseases are underdiagnosed in Portugal and are responsible for disability and absenteeism at work, with the consequent individual, social and economic costs [5]. Much less is known about inflammatory diseases in other Portuguese-speaking countries. There are no studies on the prevalence of rheumatic diseases in Angola [6], although the occurrence of rheumatic fever, rheumatoid arthritis or systemic lupus erythematosus is known [7].

Acute inflammation can be treated by using oral nonsteroidal anti-inflammatory drugs (NSAIDs), despite being associated with adverse gastrointestinal and cardiovascular effects [8,9]. It would be desirable to identify natural plant products with anti-inflammatory properties but with fewer adverse effects. Despite the traditional application of plants, their effects have not always been proven by scientific evidence. On the other hand, scientific research sometimes provides evidence of biological activities for which the plants in question had never been traditionally used.

Plant extracts, such as essential oils (EOs), have been used in traditional medicine as anti-inflammatories, digestives, diuretics, expectorants and sedatives, along with other applications (Table 1). Nowadays, in addition to their use in aromatherapy, essential oils find application in cosmetics, cleaning products, fragrances, foods and beverages. Essential oils have been reported to show several biological properties, including antimicrobial, antioxidant, anti-inflammatory and anticancer properties, among others [10–12]. Particularly relevant is EOs’ anti-inflammatory activity, either by inhibiting several enzymes, such as oxygenases, nitric oxide synthases and peroxidases, or by inducing the release of pro-inflammatory cytokines, like interleukins and tumor necrosis factor-α (TNF-α) [10].

Table 1. Some of the traditional applications of the species studied in the present work.

| Family/Plant Species | Common Names (pt/en) | Medicinal Use | Other Uses | Reference |
|----------------------|----------------------|---------------|------------|-----------|
| Amaranthaceae        |                      |               |            |           |
| *Dysphania ambrosioides* (L.) Mosyakin & Clemants (= *Chenopodium ambrosioides* L.) | Quenopódio/Wormseed | Against respiratory, gastrointestinal and joint inflammatory disorders | Vermifuge, emetic | [13,14] |
| Apiaceae/Umbelliferae|                      |               |            |           |
| *Foeniculum vulgare* Mill. | Funcho/Fennel | Against respiratory and gastrointestinal inflammatory disorders | Culinary (seasoning) | [15] |
| Family/Plant Species | Common Names (pt/en) | Medicinal Use | Other Uses | Reference |
|----------------------|----------------------|---------------|------------|-----------|
| **Asteraceae/Compositae** | | | | |
| Brachylaena huillensis O. Hoffm. (= Brachylaena hutchinsii Hutch., Brachylaena mullensis O.Hoffm.) | Muhuhu */Silver oak | Against schistosomiasis and roots against diabetes | Firewood, charcoal, timber, poles, posts, tool handles, carving. Perfumery (essential oil distilled from wood) | [16–18] |
| **Solidago virgaurea L.** | | | | |
| Euphorbiaceae | | | | |
| Spirostachys africana Sond. [= Excoecaria africana (Sond.) Müll.Arg., Excoecaria synandra Pax, Excoecariopsis synandra (Pax) Pa, Sapium africanum (Sond.) Kuntze, Spirostachys synandra (Pax) Pax, Stillingia africana (Sond.) Baill.] | Tambooti ** | External to treat myiasis, internal against gastrointestinal inflammatory disorders | Use of wood in furniture | [20–22] |
| **Lamiaceae/Labiatae** | | | | |
| Lavandula luisieri (Rozeira) Rivas-Martínez | Rosmaninho/butterfly lavender | External and internal against respiratory, circulatory, gastrointestinal and joint inflammatory disorders | Ornamental, aromatic, cosmetic, culinary (seasoning) | [23,24] |
| Mentha cervina L. | Poejo fino/Hart’s pennyroyal | External and internal against respiratory and gastrointestinal inflammatory disorders | Aromatic, culinary (seasoning) | [25] |
| Origanum majorana L. | Oregão/Marjoram | External and internal against nervous, respiratory and gastrointestinal inflammatory disorders | Aromatic, culinary (seasoning) | [26] |
| Satureja montana L. | Segurelha/Winter savory | External and internal against nervous, respiratory and gastrointestinal inflammatory disorders | Culinary (seasoning) | [27] |
| Thymbra capitata (L.) Cav. [= Thymus capitatus Hoffms. et Link., Thymus creticus Brot., Coridothymus capitatus Rechenb. f., Satureja capitata L.] | Tomilho-de-Creta/Conehead thyme | External and internal against spasms and nervous, respiratory and gastrointestinal disorders | Aromatic, culinary (seasoning) | [28] |
| Thymus mastichina (L.) L. | Bela-luz/Spanish marjoram | External and internal against nervous, respiratory, gastrointestinal and joint inflammatory disorders | Aromatic, culinary (seasoning) | [28] |
| Thymus pulegioides L. | Serpão/Broad-leaved thyme, lemon thyme | External and internal against nervous, respiratory and gastrointestinal inflammatory disorders | Aromatic, culinary (seasoning) | [29] |
### Table 1. Cont.

| Family/Plant Species | Common Names (pt/en) | Medicinal Use | Other Uses | Reference |
|----------------------|----------------------|---------------|------------|-----------|
| **Thymus vulgaris L.** | Tomilho/thyme | External and internal against nervous, respiratory and gastrointestinal inflammatory disorders | Ornamental, aromatic, culinary (seasoning) | [19] |
| **Thymus zygis Loefl. ex L. subsp. zygis** | Erva-de-Santa-Maria/Spanish red thyme | External and internal against nervous, circulatory, respiratory and gastrointestinal inflammatory disorders | Aromatic, culinary (seasoning) | [28] |
| **Myrtaceae** | | | | |
| **Eucalyptus globulus subsp. maidenii (F.Muell.) J.B.Kirkp.** | Eucalipto/Maiden’s gum | External and internal against circulatory, respiratory and gastrointestinal inflammatory disorders | Timber, fuel, paper pulp. Aromatic, culinary (seasoning) | [30] |
| **Eucalyptus radiata A.Cunn. ex DC.** | Eucalipto/Narrow-leaved peppermint eucalyptus | External and internal against mouth, respiratory and gastrointestinal inflammatory disorders | | [31] |
| **Eucalyptus viminalis Labill.** | Eucalipto/Manna gum | Internal against respiratory inflammatory disorders | Deodorant | [32] |
| **Pinaceae** | | | | |
| **Pinus pinaster Aiton** | Pinheiro-bravo/Maritime pine | External for circulatory problems, and internal against respiratory, gastrointestinal and joint inflammatory disorders | Timber and oleoresin production | [33] |

pt/en: Official two-letter codes of Portuguese and English languages, respectively. * African name adopted in Portuguese. ** African name given to the wood and adopted in Portuguese and English.

Chemokines constitute a family of chemoattractant cytokines. These are small heparin-binding proteins involved in atherosclerosis by promoting directed migration of inflammatory cells. Chemokine (C-C motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein-1 (MCP-1), has been detected in atherosclerotic lesions [34]. CCL2 is also a potent mediator of chronic inflammation, triggering, for instance, inflammation in rheumatoid arthritis [35]. In addition, inflammatory response is characterized by increased production of tumor necrosis factor-α (TNF-α) [35]. TNF-α, interleukin (IL)-1 and IL-6, secreted by macrophages, lymphocytes, natural killer cells and vascular smooth muscle cells, are considered pro-atherogenic cytokines [36]. Despite the reported anti-inflammatory potential of several EOs (Table 2), their effect on the release of CCL2 is much less reported than the release of TNF-α.
Table 2. Previously reported anti-inflammatory activity of the essential oils (EOs) from the species under study.

| Family/Species | EO/EO Components’ Anti-Inflammatory Activity | Reference |
|----------------|---------------------------------------------|------------|
| **Apiaceae**   |                                             |            |
| Foeniculum vulgare | EO inhibition of 5-lipoxygenase (IC₅₀ = 0.04 mg/mL). Fenchone inhibition of 5-lipoxygenase (IC₅₀ = 0.02 mg/mL). | [37] |
|                | EO (200 and 400 mg/kg) decreased the activity of mieloperoxidase (MPO) and the expression of TNF-α in the colon tissue previously submitted to acetic acid solution (acute colitis), and inhibited acetic acid-induced expression of p-NF-κB p65 protein. | [38] |
| **Lamiaceae**  |                                             |            |
| Lavandula luisieri | EO (50–200 mg/kg) inhibition of paw edema (31–83%) induced by carrageenan administered in male Wistar rats. EO (25 µg/mL) nitric oxide (NO) inhibition (75%) in IL-1β induced primary chondrocyte. EO reduction of iNOS in human chondrocytes and intestinal cell line C2BBe1 (54.9 and 81.0%, respectively) and phosphorylated IκB-α (87.4% and 62.3%, respectively). EO (10 µg/mL) diminished the TNF-α, IL-1β, IL-6, IL-10 and COX-2 secretion and NFkB gene expression after activation of THP-1 cells by lipopolysaccharide or human ox – LDL. The activity was attributed to cis-sabinene hydrate and terpinen-4-ol. | [39] |
|                | EO (25 µg/mL) nitric oxide (NO) inhibition (75%) in IL-1β induced primary chondrocyte. EO (25 µg/mL) inhibition of 5-lipoxygenase (IC₅₀ = 0.1 mg/mL). EO (0.5 µg/mL) inhibition (80%) of 5-lipoxygenase (IC₅₀ = 0.005 µg/mL). EO reduced the TFN-α, IL-1β, IL-8 secretion levels of THP-1 cells. EO and carvacrol suppressed lipopolysaccharide-induced COX-2 mRNA and protein expression in human macrophage-like U937 cells. EO (moderate concentration) decreased the mRNA levels of IL-1β, IL-6, granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF-α, and lowered the amount of IL-1β and IL-6 proteins in animal models of colitis. EO reduced production and gene expression of the pro-inflammatory mediators TNF-α, IL-1β and IL-6 and increased the parameters on the anti-inflammatory IL-10 cytokine. EO (5000 ppm) decreased paw edema and ear swelling, inhibited the total mRNA IL-1β expression in the mouse colon. | [40] |
|                | EO (200 mg/kg) inhibited by 28.8% the inflammatory phase of wound healing (Whittle method). EO thymol type inhibition of 5-lipoxygenase (IC₅₀ = 54 – 73 µL/L). EO linalool type inhibition of 5-lipoxygenase (IC₅₀ = 299 – 402 µL/L). EO reduced production and gene expression of the pro-inflammatory mediators TNF-α, IL-1β and IL-6 and increased the parameters on the anti-inflammatory IL-10 cytokine. | [41] |
|                | EO (200 mg/kg) inhibited by 28.8% the inflammatory phase of wound healing (Whittle method). EO thymol type inhibition of 5-lipoxygenase (IC₅₀ = 54 – 73 µL/L). EO linalool type inhibition of 5-lipoxygenase (IC₅₀ = 299 – 402 µL/L). EO reduced production and gene expression of the pro-inflammatory mediators TNF-α, IL-1β and IL-6 and increased the parameters on the anti-inflammatory IL-10 cytokine. | [42] |
|                | EO thymol type inhibition of 5-lipoxygenase (IC₅₀ = 54 – 73 µL/L). EO linalool type inhibition of 5-lipoxygenase (IC₅₀ = 299 – 402 µL/L). EO reduced production and gene expression of the pro-inflammatory mediators TNF-α, IL-1β and IL-6 and increased the parameters on the anti-inflammatory IL-10 cytokine. | [43] |
| **Myrtaceae**  |                                             |            |
| Eucalyptus globulus subsp. maidenii | EO inhibition of 5-lipoxygenase (IC₅₀ = 0.16 mg/mL). EO (0.5µg/mL) inhibition (50%) of lipoxygenase. EO (200 mg/kg) inhibited by 28.8% the inflammatory phase of wound healing (Whittle method). | [44] |
|                |                                             | [37] |
| **Pinaceae**   |                                             |            |
| Pinus pinaster | EO (100 mg/kg dose) inhibition (30.3%) of paw edema in the Whittle method using carrageenan. | [45] |

EO: Essential oil. IC₅₀: Half-maximal inhibitory concentration. LDL: Low-density lipoprotein. COX-2: Ciclo-oxigenase-2.

Essential oils are gaining commercial relevance in several countries, such as Portugal or Angola, as an additional source of income in a context of a more sustainable use of the local flora. Nevertheless, despite these essential oils being traded, national or internationally, for specific markets, it is ever more important for their added value to gather scientific support for their alleged biological properties. Given the traditional and commercial use of EOs for medicinal and cosmetic purposes and the knowledge of the ability, of
their monoterpene and sesquiterpene constituents, to act as anti-inflammatories [56,57], the present work evaluated twenty EOs obtained from eighteen plant species collected in Portugal and Angola (Table 1) for their effect on the release of CCL2 (MCP-1) and TNF-α by lipopolysaccharide (LPS)-stimulated THP-1 cells.

2. Material and Methods

2.1. Plant Material

Collective and/or individual samples, from cultivated and wild-growing medicinal and aromatic plants, were collected from mainland Portugal (Table 3). As a rule, the plant material was collected during the local producers’ harvesting season. For herbaceous species, this was usually at the flowering phase, whereas for trees, it was at landscaping time. If not immediately extracted, the plant material was stored at −20 °C until essential oil (EO) isolation. Dried aerial parts from commercially available products sold in local herbal shops were also analyzed, as well as the essential oils isolated from oleoresin, in the case of Pinus pinaster, and from the two species from Angola (Table 3). A total of twenty essential oils isolated from eighteen species from the Amaranthaceae, Apiaceae, Asteraceae, Euphorbiaceae, Lamiaceae, Myrtaceae and Pinaceae families were tested. A voucher specimen of each plant species, collected from the wild state condition, was deposited in the Herbarium of the Botanical Garden of Lisbon University, Lisbon, Portugal. For commercial plant material, a reference sample from each plant is retained at the CBV laboratory and is available upon request.

2.2. Extraction and Chemical Analysis of the Essential Oils

Essential oils were extracted by hydrodistillation for 3 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia [59], and stored at −20 °C until analysis. The EOs were analyzed by gas chromatography (GC) for component quantification and gas chromatography coupled to mass spectrometry (GC-MS) for component identification.

2.2.1. Gas Chromatography (GC)

Gas chromatographic analyses were performed using a Perkin Elmer Clarus 400 gas chromatograph equipped with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column ((50% phenyl)-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.15 μm; J & W Scientific Inc.). The oven temperature was programmed from 45 to 175 °C, at 3 °C/min, and subsequently at 15 °C/min up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures were 280 °C and 300 °C, respectively; the carrier gas, hydrogen, was adjusted to a linear velocity of 30 cm/s. The samples were injected using a split sampling technique, ratio 1:50. The volume of injection was 0.1 μL of n-pentane-essential oil solution (1:1). The percentage composition of the volatiles was computed, by the normalization method from the GC peak areas, and calculated as the mean values of two injections, from each sample, without using the response factors.
Table 3. List of the evaluated species, sampling year, plant part used for hydrodistillation, plant source, essential oil yield and main components (≥10%).

| Family/Species     | Code | Sampling Date | Plant Part | Collection Place/Source | EO yield (%,
v/w) | Main Components     |
|--------------------|------|---------------|------------|-------------------------|-----------------|---------------------|
| **Amaranthaceae**  |      |               |            |                         |                 | iso-Ascaridole 51,  |
| *Dysphania ambrosioides* | Da   | 2013          | FF         | Monsaraz                | 0.56             | ascaridole 16       |
| **Apiaceae**       |      |               |            |                         |                 | α-Pinene 27, trans-anethole 18, Limonene 11 |
| *Foeniculum vulgare* | Fv   | 2013          | DV         | Herbal shop             |                 | Methyl chavicol 79, limonene 12 |
|                    |      |               |            |                         |                 |                     |
| *Foeniculum vulgare* | Fv s | 2013          | Seeds      | Herbal shop             | 1.16             |                     |
| **Asteraceae**     |      |               |            |                         |                 |                     |
| *Brachylaena huillensis* | Bh   | 2013          | EO         | Angola                  | n.a.             | Copae-15-ol * 14    |
|                    |      |               |            |                         |                 | Copae-15-ol * 12    |
| *Solidago virgaurea* | Sv   | 2013          | FF         | Pinheiro da Cruz        | 0.72             | β-Pinene 22, α-pinene 21, germacrene D 15, limonene 12 |
| **Euphorbiaceae**  |      |               |            |                         |                 |                     |
| *Spirostachys africana* |      |               |            |                         |                 |                     |
| **Lamiaceae**      |      |               |            |                         |                 |                     |
| *Lavandula luisieri* | Li   | 2013          | DF         | Herbal shop             | 0.44             | 5-Methylene-2,3,4,4-tetramethylcyclopent-2-enone 18, 1,8-cineole 16 |
| *Mentha cervina*   |      |               |            |                         |                 |                     |
| *Origanum majorana* | Om   | 2013          | DV         | Herbal shop             | 0.98             | Terpinen-4-ol 18, carvacrol 17, γ-terpinene 13, carvacrol methyl ether 13 |
| *Satureja montana* |      |               |            |                         |                 |                     |
| *Thymbra capitata* |      |               |            |                         |                 |                     |
| *Thymus mastichina* | Thm  | 2013          | FF         | Bragança                | 1.35             | Carvacrol 77        |
| *Thymus pulegioides* | Thp  | 2013          | DL         | Herbal shop             | 0.49             | Carvacrol 71        |
| *Thymus vulgaris*  |      |               |            |                         | 1.20             | Thymol 32, ρ-cymene 22 |
| *Thymus zygis subsp. zygis* | Thzz | 2013          | FF         | Bragança                | 0.71             | Thymol 45, ρ-cymene 21, γ-terpinene 16 |
| **Myrtaceae**      |      |               |            |                         |                 |                     |
| *Eucalyptus globulus* subsp. maidenii | Eg   | 2013          | FL         | MEE                      | 3.20             | α-Pinene 15, 1,8-Cineole 46, Limonene 23 |
| *Eucalyptus radiata* | Er   | 2012          | FL         | MEE                      | 7.20             | 1,8-Cineole 49      |
| *Eucalyptus viminalis* | Ev   | 2012          | FL         | MEE                      | 2.50             | α-Pinene 10, 1,8-Cineole 69 |
| **Pinaceae**       |      |               |            |                         |                 |                     |
| *Pinus pinaster*   |      |               |            |                         | 29.76            | α-Pinene 62, β-pinene 23 |

Unless otherwise specified, the collection place was in Portugal. * Detailed composition of EOs reported in Faria et al. [58]. Commercialized as Lavandula stoechas L. n.a.: Information not available. * Identification based on mass spectra only. DF: Dry, flowering phase aerial parts. DL: Dry leaves. DV: Dry, vegetative phase aerial parts. EO: Essential oil supplied by the producer, obtained from the wood. EO*: In-lab re-distilled essential oil supplied by the producer, due to some turbidity of the original sample. FF: Fresh, flowering phase aerial parts. FL: Fresh leaves from fruiting phase. MEE: Mata Experimental do Escaroupim.

2.2.2. Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS unit consisted of a Perkin Elmer Clarus 600 gas chromatograph, equipped with a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; J & W Scientific, Inc.), and interfaced with a Perkin Elmer 600T mass spectrometer (software version 5.4.2.1617, Perkin Elmer, Shelton, CT, USA). Injector and oven temperatures were as above; transfer line temperature, 280 °C; ion source temperature, 220 °C; the carrier gas, helium, was adjusted to a linear velocity of 30 cm/s; split ratio, 1:40; ionization energy,
70 eV; scan range, 40–300 u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to n-alkane indices and GC-MS spectra from a lab-made library, created with reference essential oils, laboratory-synthesized components, laboratory-isolated compounds and commercially available standards.

2.3. In Vitro Inhibition of TNF-α and CCL2

This assay was performed according to Campana et al. [60]. Briefly, THP-1 cells (ATCC TIB-202) were cultivated in RPMI 1640 medium supplemented with 0.05 mM 2-mercaptoethanol, 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 µg/mL gentamicin at 37°C in an atmosphere containing 5% CO₂. The medium was renewed twice a week when the cell concentration reached 1.0 × 10⁶ cells/mL. The cells were transferred to a 96-well microplate at a concentration of 100,000 cells per well and incubated for 18 h with RPMI supplemented with 1% FBS to initiate serum starvation, which was kept throughout the experiment.

The cells were pre-treated with EOs at three concentrations for 3 h. To determine each EO working concentration, the toxicity of the EOs on THP-1 cells was accessed by measuring cell viability using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method and untreated cells as the reference for viability [61]. EOs were considered nontoxic for the THP-1 cell line, and adequate for further analysis, when cell viability was higher than 90%. The EO concentrations ranged from 3 µg/mL to 90 µg/mL (Dysphania ambrosioides, Eucalyptus globulus, E. radiata, E. viminalis, Foeniculum vulgare, Lavandula stoechas, Mentha cervina, Origanum majorana, Pinus pinaster, Solidago virgaurea, Thymus mastichina, Th. pulegioides (Thymus abbreviated to Th., to avoid confusion with T. from Thymbra), Th. vulgaris), from 3 µg/mL to 30 µg/mL (Brachylaena huillensis, Satureja montana, Spirostachys africanus, Th. zygis) and from 5 µg/mL to 90 µg/mL (Thymbra capitata) (Table 4).

Table 4. Inhibition of TNF-α and CCL2 production by lipopolysaccharide (LPS)-activated THP-1 monocytic cells elicited by the evaluated essential oils (EOs).

| Family/Species and Control | Concentrations (µg/mL) | TNF-α Inhibition (% ± S.D., n = 3) | CCL2 Inhibition (% ± S.D., n = 3) |
|---------------------------|------------------------|------------------------------------|-----------------------------------|
| Control                   | LPS (200 ng) DMSO (0.1%) | 2428.1 ± 587.8 *                   | 2382.3 ± 1480.8 *                  |
| Amaranthaceae             |                        |                                    |                                   |
| Dysphania ambrosioides    | 90                     | 48.6 ± 2.1 ***                   | 15.6 ± 0.7 ***                   |
|                           | 30                     | 30.9 ± 1.5 *                     | 9.4 ± 0.3 **                      |
|                           | 10                     | 13.6 ± 1.0                       | 7.5 ± 0.3 *                       |
| Apiaceae                  |                        |                                    |                                   |
| Foeniculum vulgare        | 90                     | 22.3 ± 1.9 ***                   | NI                                |
|                           | 30                     | 0.5 ± 0.0                        | NI                                |
|                           | 10                     | NI                                | NI                                |
| Asteraceae                |                        |                                    |                                   |
| Brachylaena huillensis    | 30                     | NI                                | 18.8 ± 2.3 **                     |
| (re-distilled EO)         | 10                     | 4.4 ± 0.8                        | 9.0 ± 0.5                         |
|                           | 3                      | NI                                | 5.4 ± 0.2                         |
Table 4. Cont.

| Family/Species and Control | Concentrations (µg/mL) | TNF-α Inhibition (% ± S.D., n = 3) | CCL2 Inhibition (% ± S.D., n = 3) |
|---------------------------|------------------------|-----------------------------------|---------------------------------|
| Brachylaena huillensis    | 30                     | ND                                | ND                              |
|                           | 10                     | ND                                | ND                              |
|                           | 3                      | ND                                | ND                              |
| Solidago virgaurea       | 90                     | ND                                | ND                              |
|                           | 30                     | 5.0 ± 0.2                         | ND                              |
|                           | 10                     | NI                                | 4.9 ± 0.1 *                     |
|                           | 3                      | ND                                | 8.0 ± 0.1 **                    |
| Euphorbiaceae             |                        |                                   |                                 |
| Spirostachys africanus   | 30                     | ND                                | ND                              |
|                           | 10                     | ND                                | ND                              |
|                           | 3                      | ND                                | ND                              |
| Lamiaceae                |                        |                                   |                                 |
| Lavandula luisieri        | 90                     | 82.9 ± 8.2 ***                    | 82.0 ± 12.4 ***                 |
|                           | 30                     | 23.2 ± 1.1 ***                    | 54.3 ± 3.0 ***                  |
|                           | 10                     | 2.5 ± 0.1                         | 22.7 ± 1.0 ***                  |
|                           | 3                      | 8.0 ± 0.1                         |                                 |
| Mentha cervina           | 90                     | NI                                | NI                              |
|                           | 30                     | NI                                | NI                              |
|                           | 10                     | 2.5 ± 0.0                         |                                 |
| Origanum majonara        | 90                     | NI                                | NI                              |
|                           | 30                     | NI                                | NI                              |
|                           | 10                     | 4.8 ± 0.1                         |                                 |
| Satureja montana         | 90                     | ND                                | ND                              |
|                           | 30                     | ND                                | ND                              |
|                           | 10                     | ND                                | ND                              |
|                           | 3                      | 0.2 ± 0.0                         |                                 |
| Thymbra capitata         | 90                     | ND                                | NI                              |
|                           | 30                     | 51.1 ± 6.5 ***                    | ND                              |
|                           | 10                     | 29.5 ± 1.7 ***                    | 0.4 ± 0.0                       |
|                           | 5                      | 9.1 ± 0.1 *                       |                                 |
| Thymus mastichina        | 90                     | ND                                | ND                              |
|                           | 30                     | ND                                | ND                              |
|                           | 10                     | ND                                | ND                              |
| Thymus pulegioides       | 90                     | ND                                | ND                              |
|                           | 30                     | NI                                | 0.9 ± 0.0                       |
|                           | 10                     | NI                                | 8.4 ± 0.5                       |
| Thymus vulgaris          | 90                     | ND                                | ND                              |
|                           | 30                     | ND                                | ND                              |
|                           | 10                     | ND                                | ND                              |
| Thymus zygis ssp. sygis  | 90                     | ND                                | 8.9 ± 1.0                       |
|                           | 30                     | NI                                | 2.7 ± 0.2                       |
|                           | 10                     | NI                                |                                 |
|                           | 3                      | NI                                | 0.2 ± 0.0                       |
| Myrtaceae                |                        |                                   |                                 |
| Eucalyptus globulus subsp. maidenii | 90                     | 6.5 ± 0.4                         | 1.7 ± 0.0                       |
|                           | 30                     | 4.4 ± 0.3                         | NI                              |
|                           | 10                     | 2.4 ± 0.2                         | NI                              |
| Eucalyptus radiata       | 90                     | 12.0 ± 0.1 *                      | NI                              |
|                           | 30                     | NI                                | NI                              |
|                           | 10                     | NI                                | NI                              |
| Eucalyptus viminalis     | 90                     | 3.3 ± 0.2                         | NI                              |
|                           | 30                     | 0.2 ± 0.0                         | NI                              |
|                           | 10                     | NI                                | 1.4 ± 0.0                       |
| Pinaceae                 |                        |                                   |                                 |
| Pinus pinaster (oleoresin) | 90                     | ND                                | ND                              |
|                           | 30                     | 6.3 ± 0.1                         | NI                              |
|                           | 10                     | 6.8 ± 0.3                         | NI                              |

*a Inflammatory mediator production (absolute values in pg/mL). NI: No inhibition. ND: Not determined due to toxicity (cell viability ≤ 90%). * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001: Indicates significant inhibition of TNF-α or CCL2 release in comparison to LPS-stimulated cells (ordinary one-way ANOVA/Newman–Keuls multiple comparison test: GraphPad Prism).
Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 (Sigma), added at 200 ng/mL, was employed as the inflammatory stimulus. The plate was incubated at 37°C overnight. After this period, the plate was centrifuged (1800 g, 5 min, 16°C), the supernatant collected and TNF-α release measured using the cytokine-specific sandwich quantitative ELISA according to the manufacturer’s instructions (TNF-α duo set, DY210, R&D Systems, Minneapolis, MN, USA). CCL2 release was measured using the cytokine-specific sandwich quantitative ELISA according to the manufacturer’s instructions (Human CCL2/MCP-1 duo set, DY279, R&D Systems, Minneapolis, MN, USA). Dexamethasone was employed as positive control (0.3 µM). The statistical significance of differences was calculated employing the software GraphPad Prism, version 5.0 (GraphPad Software Inc., San Diego, CA, USA), using ordinary one-way ANOVA/Newman–Keuls multiple comparison test. All the experiments were performed in triplicate.

3. Results and Discussion

3.1. Chemical Composition of the Essential Oils

All essential oils were fully chemically characterized. Table 3 reports only their main constituents (≥10%), since, in some cases, duly marked in Table 3, the detailed composition was previously reported, or their composition was overall very similar to data from prior studies. In the case of EOs from Angola, the complexity of the EOs still requires additional characterization for the full identification of some minor components.

Although commercialized as *Lavandula stoechas* L., the presence of necrodane derivatives, such as 5-methylene-2,3,4,4-tetramethylcyclopent-2-enone (18%) in the analyzed essential oil, undoubtedly indicated that the butterfly lavender tested was *L. luisieri* and not *L. stoechas* (Table 3). The chemical composition of *L. stoechas* essential oil is characterized by large variations in fenchone, camphor and 1,8-cineole amounts, whereas necrodane derivatives are characteristic of *L. luisieri* [23,24,62]. Even though some variations in their contents were observed, the remaining essential oil compositions were in accordance with previous studies carried out with *Foeniculum vulgare* [15,63], *Mentha cervina* ([25] and references therein), *Origanum majorana* [64], *Satureja montana* [58,65], *Thymbra capitata*, *Thymus mastichina*, *Th. pulegioides*, *Th. vulgaris* and *Th. zygis* subsp. *zygis* [28,64–66], *Eucalyptus* species [67,68] and *Pinus pinaster* [33,69].

Although a few studies evaluated the EO composition from *Brachylaena huillensis* aerial parts, only three studies reported the essential oil composition from the wood or saw powder of this species [16,17,70]. Although no detailed composition has been reported, α-amorphene was the dominant constituent in the studies of Klein and Schmidt [70] and of Maitai et al. [16] (17% and 15%, respectively), whereas β-caryophyllene (19%) was the major constituent described by Oliva et al. [17]. In the present study, α-amorphene was the second main component, together with gleenol (both 6%), whereas β-caryophyllene was found only in trace amounts. Baarschers et al. [20] reported the isolation of diterpenes from *Spirostachys africana* wood, but no previous studies addressed the EO composition from the wood.

3.2. In Vitro Inhibition of TNF-α Release by LPS-Stimulated THP-1 Cells

The potential anti-inflammatory activity of essential oils (EOs) was investigated by measuring TNF-α release by lipopolysaccharide (LPS)-stimulated THP-1 cells by employing an immunoassay. The toxicity of the EOs on THP-1 cells was accessed to determine the adequate EO working concentrations. When the cell viability of THP-1 cells was higher than 90%, samples were considered non-cytotoxic and adequate for further analysis. According to the availability of EO, at least three concentrations were checked for each essential oil (Table 4). Data in Table 4 also include information on EOs which were not assessed further due to being toxic (ND) to differentiate them from those that showed no inhibition (NI).

The EOs of *T. capitata*, *L. luisieri*, *F. vulgare* and *D. ambrosioides* significantly reduced TNF-α release by LPS-stimulated THP-1 cells, in comparison to the control cells. From
these four EOs, those of T. capitata and L. luisieri were the most effective. T. capitata EO showed an inhibition percentage of TNF-α release of 51 ± 7% at 20 µg/mL, whereas that of L. luisieri EO was 23 ± 1% at 30 µg/mL and 83 ± 8% at 90 µg/mL (Table 4). These inhibition percentages were higher than those of D. ambrosioides EO (49 ± 2% at 90 µg/mL), or F. vulgare EO (22 ± 2% at 90 µg/mL) (Table 4).

The potential anti-inflammatory activity of L. luisieri, F. vulgare and T. capitata EOs has been previously reported using different in vitro and in vivo models (Table 2), but as far as we know, the anti-inflammatory potential of D. ambrosioides EO has not been addressed to date. Recently, the anti-inflammatory activity of alcoholic or hydroalcoholic extracts of D. ambrosioides was reported as showing the ability to reduce interleukin 6 (IL-6), myeloperoxidase (MPO), nitric oxide (NO) and adenosine-deaminase (ADA) activity and TNF-α and, therefore, they are potentially useful in wound healing and in the treatment of arthritic processes [13,71]. The oxygen-containing monoterpenic ascaridole was identified as a constituent of D. ambrosioides ethanolic extract by Grassi et al. [13], a compound also identified in the essential oils evaluated in the present work (Table 3).

Despite carvacrol being the main compound of T. capitata EO (Table 3), this phenol-like oxygen-containing monoterpenic may not be the only compound accountable for T. capitata EO activity. Indeed, other carvacrol-rich EOs, such as those of S. montana and Th. zygis (Table 3), were not able to reduce TNF-α release. The presence of antagonists in these EOs can also not be ignored. Moreover, it is relevant to highlight the important role of the minor compounds and/or some of the compounds’ enantiomeric ratio in the overall activity of EOs. Often overlooked, these factors can contribute to synergistic or antagonistic actions determining differences in the EOs’ activities [56,72]. These results make it difficult to predict the effect of different species’ essential oils that share the same major component for TNF-α release.

Th. pulegioides and, particularly, Th. vulgaris EOs, with thymol, an isomer of carvacrol as the main component (Table 3), were toxic for THP-1 cells, even at lower concentrations (Table 4). Th. vulgaris EO has been reported to show anti-inflammatory activity, including the capacity of reducing TNF-α release, this activity being related solely to the higher carvacrol content [37,49,50,52]. On the other hand, Th. zygis and Th. vulgaris EOs, which have thymol as the main constituent, have been reported to decrease TNF-α secretion by human macrophages derived from THP-1 monocytes and activated by oxidized (ox)-LDLs [51]. Dexamethasone at 0.3 µM had > 90% inhibition.

3.3. In Vitro Inhibition of CCL2 Release by LPS-Stimulated THP-1 Cells

Inflammatory changes in arterial lesions are characterized by the recruitment and activation of monocytes/macrophages, which are regulated by CCL2. This chemoattractant cytokine has been shown to play a vital role in the initiation and progression of atherosclerotic lesions in experimental animals [73]. The effect of the essential oils on CCL2 release by LPS-stimulated THP-1 cells was also evaluated.

Of the four essential oils with the ability to inhibit CCL2 release, only L. luisieri EO had remarkable activity, with inhibition percentages of 23 ± 1%, 54 ± 3% and 82 ± 12% at 10, 30 and 90 µg/mL, respectively (Table 4). The major compound of L. luisieri EO, 5-methylene-2,3,4,4-tetramethylcyclopent-2-ene, a necrodane derivative, may have contributed to this activity, along with 1,8-cineole. Nevertheless, the absence of the activity of other EOs in which 1,8-cineole was also present, even in a much higher percentage, such as Th. mastichina or Eucalyptus EOs (Table 3), may suggest that 5-methylene-2,3,4,4-tetramethylcyclopent-2-ene plays an important role in the inhibition of both TNF-α and CCL2 release (Table 4). The inhibitory activities elicited by D. ambrosioides, S. virgaurea or B. huillensis EOs were much lower (Table 4). Dexamethasone at 0.3 µM had > 90% inhibition.

Reports regarding the action of essential oils and/or their main components on the production of CCL2 are scarce. Limonene isolated from Citrus junos EO was able to inhibit CCL2 production on diesel exhaust particle (DEP)-stimulated human eosinophilic leukemia HL-60 clone 15 cells [74]. Artemisia argyi EO, mainly constituted by 1,8-cineole
(33%), camphor (17%), (-)-borneol (13%) and α-thujone (13%), reduced TNF-α, IL-6, IFN-β and CCL2 in LPS-induced RAW264.7 macrophages [75]. Xiao Qing Long Tang essential oil was able to suppress CCL2, IL-1β, IL-6, IL-10 and TNF-α expression and production by LPS-stimulated RAW264.7 cells [76]. In addition, Park et al. [77] also reported that (-)-linalool was able to inhibit microglial migration induced by CCL2, a chemokine released by oxygen-glucose deprivation/reoxygenation (OGD/R) in cortical cells from 17-day-old embryos of Sprague-Dawley rats.

Along with L. luisieri EO, the ascaridole- and iso-ascaridole-rich D. ambrosioides EO was also able to reduce CCL2 release by LPS-stimulated THP-1 cells, as observed for TNF-α, although in a weaker manner (Table 4). The absence of these compounds in the remaining non-active EOs may suggest that these volatile compounds have an important role in the suppression of some inflammatory processes in which TNF-α and CCL2 are involved. Despite the traditional application of D. ambrosioides as a vermifuge and against vomiting [14], this is the first report on the effect of its essential oil on the release of the pro-inflammatory cytokine TNF-α and the chemokine CCL2. For this reason, this EO and its main component ascaridole, and/or its isomers, should be further investigated to explore their anti-inflammatory activity.

4. Conclusions

Inflammatory disorders are usually treated with steroidal anti-inflammatory drugs (SAIDs) or non-SAIDs (NSAIDs). Nevertheless, because these drugs present multiple negative side effects, it is important to assess and validate the use of other potential anti-inflammatory agents, namely, essential oils. Moreover, some of these essential oils are by-products from landscaping activities or other industries, thus constituting an added value to countries’ local flora.

This study suggests that T. capitata and L. luisieri EOs, mainly constituted by carvacrol and 5-methylene-2,3,4,4-tetramethylcyclopent-2-enone and 1,8-cineole, respectively, were the most effective to inhibit TNF-α release by LPS-stimulated THP-1 cells, whereas only L. luisieri EO had the ability to inhibit CCL2 release by LPS-stimulated THP-1 cells.

EOs’ chemical complexity and variability (existence of chemotypes and/or the enantiomeric ratio of some components), their hydrophobicity and, sometimes, their scarcity, have been considered some of the limitations to their use in diverse formulations. Nevertheless, EOs are Generally Regarded as Safe (GRAS), and the knowledge on their biological properties should be further explored, in solo formulations and in combination therapies, as potential anti-inflammatory agents. This approach would contribute to the goal of decreasing the use of SAIDs and, therefore, preventing or diminishing these drugs’ adverse side effects.

Author Contributions: M.G.M., L.F., C.I.d.S., F.C.B. and A.C.F.: Conceptualization, methodology, formal analysis and investigation, M.G.M., F.C.B., L.F., C.I.d.S. and A.C.F.: writing—original draft preparation, review and editing; M.G.M., F.C.B. and A.C.F.: funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: The European Commission under the Seventh Framework Programme (FP7) of the European Union, Marie Curie International Research Staff Exchange Scheme (MC-IRSES). Project PEOPLE MC-IRSES, FP7-PEOPLE-2011-IRSES, PIRSES-GA-2011-295251 Fundação para a Ciência e Tecnologia (FCT/MCTES), FEDER, PT2020 PA, Compete 2020. Projects MED UIDB/05183/2020 and CESAM UIDP/50017/2020 + UIDB/50017/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: All authors are indebted to Wolfgang Kreis, from the Friedrich Alexander Universität Erlangen, Nürnberg, Germany, as the main supervisor of the international project under which part of this work was performed (DIGITALIS: The genus Digitalis: Molecular taxonomy, preservation, active constituents and therapeutic applications. Project PEOPLE MC-IRSES, FP7-
PEOPLE-2011-IRSES, PIRSES-GA-2011-295251, funded by the European Commission under the Seventh Framework Programme (FP7) of the European Union, Marie Curie International Research Staff Exchange Scheme (MC-IRSES)). Maria Graça Miguel and Carina Isabel da Silva are grateful for the grants under research contract PEOPLE MC-IRSES, FP7-PEOPLE-2011-IRSES, PIRSES-GA-2011-295251. The authors acknowledge the Instituto da Conservação da Natureza e das Florestas (ICNF) and particularly Eng João Sanches (Mata Experimental do Escaroupim (MEE)) from the Centro Nacional de Sementes Florestais (CENASEF) for kindly allowing the sampling of all studied species from MEE. This study was partially funded by Fundação para a Ciência e Tecnologia (FCT / MCTES), under MED UIDB/05183/2020, and CESAM UIDP/50017/2020 + UIDB/50017/2020, FEDER, PT2020 PA and Compete 2020.

Convention on Biodiversity: The authors obtained, and acknowledge, the appropriate authority to access plant samples, other than commercially available plant material, essential oils or oleoresin, used for research as required under the framework of the United Nations Convention on Biodiversity.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| CCL2         | Chemokine (C-C motif) ligand 2 |
| COX-2        | Cyclooxygenase-2 |
| DF           | Dry, flowering phase aerial parts |
| DL           | Dry leaves |
| DV           | Dry, vegetative phase aerial parts |
| EOs          | Essential oils |
| FBS          | Fetal bovine serum |
| FF           | Fresh, flowering phase aerial parts |
| FL           | Fresh leaves from fruiting phase |
| IC50         | Half-maximal inhibitory concentration |
| LDL          | Low-density lipoprotein |
| LPS          | Lipopolysaccharide |
| MCP-1        | Monocyte chemoattractant protein-1 |
| MEE          | Mata Experimental do Escaroupim |
| MTT          | 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide |
| THP-1        | Human acute monocytic leukemia cell line |
| TNF-α        | Tumor necrosis factor-α |

References

1. Vane, J.R.; Botting, R.M. The mechanism of action of aspirin. *Thromb. Res.* 2003, 110, 255–258. [CrossRef]
2. Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D.W.; Fasano, A.; Miller, G.W.; et al. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* 2019, 25, 1822–1832. [CrossRef] [PubMed]
3. Song, P.; Fang, Z.; Wang, H.; Cai, Y.; Rahimi, K.; Zhu, Y.; Fowkes, F.G.R.; Fowkes, F.J.L.; Rudan, I. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: A systematic review, meta-analysis, and modelling study. *Lancet Glob. Health* 2020, 8, e721–e729. [CrossRef]
4. CCAP. Custo e Carga da Aterosclerose em Portugal.Centro de Estudo de Medicina Baseada na Evidência da FML, Centro de Estudos Aplicados da Universidade Católica e Sociedade Portuguesa de Aterosclerose. 2019. Available online: https://spatersclerose.org/highlights-de-2018/item/274-custo-e-carga-da-aterosclerose-em-portugal.html (accessed on 13 October 2020). (In Portuguese)
5. Branco, J.C.; Faustino, A.; Carvalho, B.; Araújo, F.; Canhão, H.; Brito, I.; da Silva, J.A.P.; Costa, J.A.; Costa, L.; Mauricio, L.; et al. Rede Nacional de especialidade hospitalar e de referenciação de reumatologia. 2015. Available online: https://www.sns.gov.pt/wp-content/uploads/2016/05/rede-referencia%C3%A7%C3%A3o-hospitalar-reumatologia.pdf (accessed on 13 October 2020). (In Portuguese)
6. Usenbo, A.; Kramer, V.; Young, T.; Musekiwa, A. Prevalence of arthritis in Africa: A systematic review and meta-analysis. *PLoS ONE* 2015, 10, e0133858. [CrossRef]
7. SESPA. Secretário de Estado para a Saúde Pública de Angola. 2018. Available online: http://jornaldeangola.sapo.ao/sociedade/angola_so_tem_oito_medicos_formados_em_reumatologia (accessed on 13 October 2020). (In Portuguese)
8. Boudreault, J.; Desmeules, F.; Roy, J.-S.; Dionne, C.; Frémond, P.; MacDermid, J.C. The efficacy of oral non-steroidal anti-inflammatory drugs for rotator cuff tendinopathy: A systematic review and meta-analysis. *J. Rehabil. Med.* 2014, 46, 294–306. [CrossRef]
9. Wallace, J.L. Eicosanoids in the gastrointestinal tract. *Br. J. Pharmacol.* 2019, 176, 1000–1008. [CrossRef]
10. Miguel, M.G. Antioxidant and anti-inflammatory activities of essential oils: A short review. *Molecules* 2010, 15, 9252–9287. [CrossRef]
11. Dandlen, S.; Lima, A.; Mendes, M.; Miguel, M.; Faleiro, M.L.; Sousa, M.; LG, P.; JG, B.; Figueiredo, A. Antimicrobial activity, cytotoxicity and intracellular growth inhibition of Portuguese *Thymus* essential oils. *Rev. Bras. Farmacog.* 2011, 21, 1012–1024. [CrossRef]

12. De Lima, VT; Vieira, M.C.; Kassuya, C.A.L.; Cardoso, C.A.L.; Alves, J.M.; Foglio, M.A.; de Carvalho, J.E.; Formagio, A.S.N. Chemical composition and free radical-scavenging, antiproliferation and anti-inflammatory activities of the essential oil from *Ocimum kilimandscharicum*. *Phytomedicine* 2014, 21, 1298–1302. [CrossRef]

13. Grassi, L.T.; Malheiro, A.; Meyre-Silva, C.; Buss, Z.S.; Monguilhot, E.D.; Fröde, T.S.; da Silva, K.A.B.S.; de Souza, M.M. From popular use to pharmacological validation: A study of the anti-inflammatory, anti-nociceptive and healing effects of *Chenopodium ambrosioides* extract. *J. Ethnopharmacol.* 2013, 145, 127–138. [CrossRef]

14. Alonso-Castro, A.J.; Domínguez, F.; Ruiz-Padilla, A.J.; Campos-Xolalpa, N.; Zapata-Mora, J.R.; Carranza-Alvarez, C.; Maldonado-Miranda, J.J. Medicinal plants from North and Central America and the Caribbean considered toxic for humans: The other side of the coin. *Evid. Based Complement. Alternat. Med.* 2017, 2017, 9439868. [CrossRef] [PubMed]

15. Miguel, M.G.; Cruz, C.; Faleiro, L.; Simões, M.T.F.; Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G. *Foeniculum vulgare* essential oils: Chemical composition, antioxidant and antimicrobial activities. *Nat. Prod. Commun.* 2010, 5, 319–328. [CrossRef] [PubMed]

16. Maitai, C.K.; Talalaj, S.; Talalaj, D. *Chenopodium ambrosioides* extract. *Phytother. Res.* 2014, 28, 135–140. [CrossRef] [PubMed]

17. Oliva, M.M.; Demo, M.S.; Malele, R.S.; Mutayabarwa, C.; Chinhota, I.; Chipanganya, P.; Chibaya, C.; Schmidt, R.J. Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products—Part 4: *Solidago virgaurea*—*Vitis vinifera*. *Contact Dermat.* 2017, 77, 67–87. [CrossRef]

18. Mrema, J.P. Conservation of *Brachylaena hugillensis* O.Hoffm (Asteraceae) in Dindili Forest Reserve, Morogoro, Tanzania. Ph.D. Thesis, Addis Ababa University, Addis Ababa, Ethiopia, 2006.

19. Minciullo, P.L.; Calapai, G.; Miroddi, M.; Mannucci, C.; Chinou, I.; Gangemi, S.; Schmidt, R.J. Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products—Part 4: *Solidago virgaurea*—*Vitis vinifera*. *Contact Dermat.* 2017, 77, 67–87. [CrossRef]

20. Baarschers, W.H.; Horn, D.H.; Johnson, L.R.F. The structure of some diterpenes from tambooti wood, *Spirostachys africana* Sandon. *J. Chem. Soc. 1967, 10, 4046–4055.* [CrossRef]

21. Mathabe, M.C.; Hussein, A.A.; Nikolova, R.V.; Basson, A.E.; Meyer, J.J.M.; Lall, N. Antibacterial activities of terpenoids isolated from *Spirostachys africana*. *J. Ethnopharmacol.* 2008, 116, 194–197. [CrossRef]

22. Mukandwiwa, L.; McGaw, L.J.; Eloff, J.N.; Naidoo, V. Extracts of four plant species used traditionally to treat myiasis influence pupation rate, pupal mass and adult blow fly emergence of *Lucilia cuprina* and *Chrysomya marginalis* (Diptera: Calliphoridae). *J. Ethnopharmacol.* 2012, 143, 812–818. [CrossRef]

23. Zuzarte, M.; Gonçalves, M.J.; Cruz, M.T.; Cavaleiro, C.; Canhoto, J.; Vaz, S.; Pinto, E.; Salgueiro, L. *Lavandula luisiensis* essential oil as a source of antifungal drugs. *Food Chem.* 2012, 135, 1505–1510. [CrossRef]

24. Figueiredo, A.C.; Pedro, L.G.; Barroso, J.G.; Trindade, H.; Sanches, J.; Oliveira, C.; Correia, M. *Lavandula luisiensis* (Rozeira) Rivas-Martínez and *Lavandula pedunculata* (Mill.) Cav. *Agrotec.*

25. Rodrigues, L.; Duarte, A.; Figueiredo, A.C.; Brito, L.; Teixeira, G.; Moldão, M.; Monteiro, A. Chemical composition and antibacterial activity of the essential oils from the medicinal plant *Menitua cervina* L. grown in Portugal. *Med. Chem. Res.* 2012, 21, 3485–3490. [CrossRef]

26. Costa, D.C.; Costa, H.S.; Albuquerque, T.G.; Ramos, F.; Castilho, M.C.; Sanches-Silva, A. Advances in phenolic compounds analysis of aromatic plants and their potential applications. *Trends Food Sci. Technol.* 2015, 45, 336–354. [CrossRef]

27. Mastelić, J.; Jerković, I. Gas chromatography-mass spectrometry analysis of free and glyconjugated aroma compounds of seasonally collected *Satureja montana* L. *Food Chem.* 2003, 80, 135–140. [CrossRef]

28. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G.; Salgueiro, L.; Miguel, M.G.; Faleiro, M.L. Portuguese *Thymbra capitata, Thymbra capitata* volatiles: Chemical composition and biological activities. *Curr. Pharm. Des.* 2008, 14, 3120–3140. [CrossRef] [PubMed]

29. Fernandes, A.S.F.; Barros, L.; Carvalho, A.M.; Ferreira, I.C.F.R. Lipophilic and hydrophilic antioxidants, lipid peroxidation, inhibition and radical scavenging activity of two Lamiaceae food plants. *Eur. J. Lipid Sci. Technol.* 2010, 112, 1115–1121. [CrossRef]

30. Figueiredo, A.C.; Pedro, G.; Barroso, J.G.; Sanches, J.; Correia, M. *Oleos essenciais de espécies de Eucalyptus*. *Agrotec.* 2013, 8, 96–100.

31. Mahumane, G.D.; van Vuuren, S.F.; Kamatou, G.; Sandasi, M.; Viljoen, A.M. Chemical composition and antimicrobial activity of *Eucalyptus radiata* leaf essential oil, sampled over a year. *J. Essent. Oil Res.* 2016, 28, 475–488. [CrossRef]

32. Toltacheva, A.A.; Rogozhin, E.A.; Deryabin, D.G. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. *Acta Pharm.* 2014, 64, 173–186. [CrossRef]

33. Figueiredo, A.C.; Pedro, L.G.; Barroso, J.G.; Trindade, H.; Sanches, J.; Oliveira, C.; Correia, M. *Pinus pinaster* Aiton and *Pinus pinea* L. *Agrotec* 2014, 12, 23–27.

34. Takeya, M.; Yoshimura, T.; Leonard, E.J.; Takahashi, K. Detection of monocyte chemoattractant protein-1 in human atherosclerotic lesions by an anti-monoocyte chemoattractant protein-1 monoclonal antibody. *Hum. Pathol.* 1993, 24, 534–539. [CrossRef]

35. Bakheet, S.A.; Alrwashed, B.S.; Ansari, M.A.; Nadeem, A.; Attia, S.M.; Alansari, M.M.; Aldossari, A.A.; Assiri, M.A.; Mahmood, H.M.; Al-Mazroua, H.A.; et al. CXC chemokine receptor 3 antagonist AMG487 shows potent anti-arthritis effects on collagen-induced arthritis by modifying B cell inflammatory profile. *Immunol. Lett.* 2020, 225, 74–81. [CrossRef]
36. Tousoulis, D.; Oikonomou, E.; Economou, E.K.; Crea, F.; Kaski, J.C. Inflammatory cytokines in atherosclerosis: Current therapeutic approaches. Eur. Heart J. 2016, 37, 1723–1735. [CrossRef]

37. Azzaa, S.; Lyoussi, B.; Megias, C.; Cortés-Giraldo, I.; Vioque, J.; Figueiredo, A.C.; Miguel, M.G. Anti-oxidant, anti-inflammatory and anti-proliferative activities of Moroccan commercial essential oils. Nat. Prod. Commun. 2014, 9, 587–594. [CrossRef] [PubMed]

38. Rezayat, S.M.; Dehpour, A.-R.; Motamed, S.M.; Yazdanparast, M.; Chamanara, M.; Rashidian, A.; Sahebgharani, M. Foeniculum vulgare essential oil ameliorates acetic-induced colitis in rats through the inhibition of NF-kB pathway. Inflammopharmacology 2018, 26, 851–859. [CrossRef] [PubMed]

39. Arantes, S.; Candeias, F.; Lopes, O.; Lima, M.; Pereira, M.; Tórico, T.; Cruz-Morais, J.; Martins, M.R. Pharmacological and toxicological studies of essential oil of Lavandula stoechas subsp. Iuuiieri. Planta Med. 2016, 82, 1266–1273. [CrossRef] [PubMed]

40. Rufino, A.T.; Ribeiro, M.; Sousa, C.; Judas, F.; Salgueiro, L.; Cavaleiro, C.; Mendes, A.F. Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis. Eur. J. Pharmcol. 2015, 750, 141–150. [CrossRef]

41. Rufino, A.T.; Ferreira, I.; Judas, F.; Salgueiro, L.; Lopes, M.C.; Cavaleiro, C.; Mendes, A.F. Differential effects of the essential oils of Lavandula latifolia and Eryngium duriaeif subsp. juresianum in cell models of two chronic inflammatory diseases. Pharm. Biol. 2015, 53, 1220–1230. [CrossRef]

42. Arranz, E.; Jaime, L.; López de las Hazas, M.C.; Reglero, G.; Santoyo, S. Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil. Ind. Crops Prod. 2015, 67, 121–129. [CrossRef]

43. Azzaa, S.; El-Guendouz, S.; Miguel, M.G.; Antunes, M.D.; Faleiro, M.L.; Correia, A.I.; Figueiredo, A.C. Antioxidant, anti-inflammatory and anti-hyperglycaemic activities of essential oils from Thymbra capitata, Thymus albicans, Thymus caespiptitus, Thymus cinnomoos, Thymus lotocepalus and Thymus mastichina from Portugal. Nat. Prod. Commun. 2016, 11, 1029–1038. [CrossRef]

44. Carrasco, A.; Perez, E.; Cutillas, A.-B.; Martinez-Gutierrez, R.; Tomas, V.; Tudela, J. Originsum vulgare and Thymbra capitata essential oils from Spain: Determination of aromatic profile and bioactivities. Nat. Prod. Commun. 2016, 11, 113–120. [CrossRef]

45. Wei, A.; Shibamoto, T. Antioxidant/lipoxygenase inhibitory activities and chemical compositions of selected essential oils. J. Agric. Food Chem. 2010, 58, 7218–7225. [CrossRef]

46. Tsai, M.-L.; Lin, C.-C.; Lin, W.-C.; Yang, C.-H. Antimicrobial, antioxidant, and anti-inflammatory activities of essential oils from five selected herbs. Biosci. Biotech. Bioch. 2017, 81, 1977–1983. [CrossRef]

47. Abdelli, W.; Bahri, F.; Romane, A.; Höferl, M.; Wanner, J.; Schmidt, E.; Jirovetz, L. Chemical composition and anti-inflammatory activity of Algerian Thymus vulgaris essential oil. Nat. Prod. Commun. 2017, 12, 611–614. [CrossRef] [PubMed]

48. Fachini-Queiroz, F.C.; Kummer, R.; Estevinho, S.; Carvalho, N.; Cunha, J.M.; Grespan, R.; Bersani-Amado, C.A.; Cuman, R.K.N. Effects of thymol and carvacrol, constituents of Thymus vulgaris L. essential oil, on the inflammatory response. Evid. Based Complement. Alternat. Med. 2012, 2012, 657026. [CrossRef] [PubMed]

49. Hotta, M.; Nakata, R.; Katsukawa, M.; Hori, K.; Takahashi, S.; Inoue, H. Carvacrol, a component of thyme oil, activates PPARα and Gamma and suppresses COX-2 expression. J. Lip. Res. 2010, 51, 132–139. [CrossRef] [PubMed]

50. Bukovská, A.; Čikoš, Š.; Juňás, Š.; Il’kóvá, G.; Rehák, P.; Koppel, J. Effects of a combination of thyme and oregano essential oils on TNBS-induced colitis in mice. Mediat. Inflamm. 2007, 23296. [CrossRef] [PubMed]

51. Ocaña, A.; Reglero, G. Effects of thyme extract from Gymnosporangium sabinae (from Thymus vulgaris, Thymus zygis and Thymus hyemalis) on cytokine production and gene expression of oxLDL-stimulated THP-1 macrophages. J. Obes. 2012, 2012, 104706. [CrossRef] [PubMed]

52. Juňás, Š.; Bujáková, D.; Rehák, P.; Čikoš, Š.; Veselá, J.; Il’kóvá, G.; Koppel, J. Anti-inflammatory effects of thyme essential oil in mice. Acta Vet. Brno 2008, 77, 327–334. [CrossRef]

53. Cutillas, A.-B.; Carrasco, A.; Martinez-Guiterrez, R.; Tomas, V.; Tudela, J. Thyme essential oils from Spain: Aromatic profile ascertained by GC-MS, their anti-lipoxygenase and antimicrobial activities. J. Food Drug Anal. 2018, 26, 529–544. [CrossRef]

54. Tümen, I.; Guragac, F.T.; Keles, H.; Reunanan, M.; Kupeli-Akkol, E. Characterization and wound repair potential of essential oil of Eucalyptus globulus Labill. Fresenius Environ. Bull. 2017, 26, 6390–6399.

55. Tümen, I.; Akkol, E.K.; Taştan, H.; Sünart, I.; Kurtca, M. Research on the antioxidant, wound healing, and anti-inflammatory activities and the phytochemical composition of maritime pine (Pinus pinaster Ait.). J. Ethnopharmacol. 2018, 211, 235–246. [CrossRef]

56. Sá, R.C.S.; Andrade, L.N.; de Sousa, D.P. A review on anti-inflammatory activity of monoterpenes. Molecules 2013, 18, 1227–1254.

57. Sá, R.C.S.; Andrade, L.N.; de Sousa, D.P. Sesquiterpenes from essential oils and anti-inflammatory activity. Nat. Prod. Commun. 2015, 10, 1767–1774.

58. Faria, J.M.S.; Sena, I.; Ribeiro, B.; Rodrigues, A.M.; Maleita, C.M.N.; Abrantes, I.; Bennett, R.N.; Mota, M.; Figueiredo, A.C. First report on Meloidogyne chitwoodi hatching inhibition activity of essential oils and essential oils fractions. J. Pest. Sci. 2016, 89, 207–217. [CrossRef]

59. Council of Europe (COE). European Directorate for the Quality of Medicines. European Pharmacopoeia, 7th ed.; COE: Strasbourg, France, 2007.

60. Campana, P.R.V.; Mansur, D.S.; Gusman, G.S.; Ferreira, D.; Teixeira, M.M.; Braga, F.C. Anti-TNF-α activity of Brazilian medicinal plants and compounds from Ouratea semiserrata. Phytother. Res. 2015, 29, 1509–1515. [CrossRef] [PubMed]
61. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 1983, 65, 55–63. [CrossRef]

62. Matos, F.; Miguel, M.G.; Duarte, J.; Venâncio, F.; Meiteiro, C.; Correia, A.I.D.; Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G. Antioxidant capacity of the essential oils from *Lavandula luisieri*, *L. stoechas* ssp. *luisitana*, *L. stoechas* ssp. *luisitana* x *L. luisieri* and *L. viridis* grown in Algarve (Portugal). *J. Essent. Oil Res.* 2009, 21, 327–336. [CrossRef]

63. Lopes, V.R.; Barata, A.M.; Farias, R.; Mendes, M.D.; Lima, A.S.; Pedro, L.G.; Barroso, J.G.; Figueiredo, A.C. Morphological and essential oil variability from nine Portuguese fennel (*Foeniculum vulgare* Mill.) accessions. *Acta Hort.* 2010, 860, 33–50. [CrossRef]

64. Faria, J.M.S.; Barbosa, P.; Bennett, R.N.; Mota, M.; Figueiredo, A.C. Bioactivity against *Bursaphelenchus xylophilus*: Nematotoxics from essential oils, essential oil fractions and decoction waters. *Phytochemistry* 2013, 94, 220–228. [CrossRef]

65. Barros, A.; Faria, J.M.S.; Barbosa, P.M.; Bennett, R.N.; Mota, M.; Figueiredo, A.C. Antioxidant capacity of the essential oils from *Lavandula stoechas* ssp. *luisitana* grown in Algarve (Portugal). *J. Agric. Food Chem.* 2010, 58, 12312–12329. [CrossRef] [PubMed]

66. Figueiredo, A.C.; Barroso, J.G.; Pedro, L.G. Volatiles from *Thymbra* and *Thymus* Species of the Western Mediterranean Basin, Portugal and Macaronesia. *Nat. Prod. Commun.* 2010, 5, 1465–1476. [CrossRef]

67. Faria, J.M.S.; Lima, A.S.; Mendes, M.D.; Leiria, R.; Geraldes, D.A.; Figueiredo, A.C.; Trindade, H.; Pedro, L.G.; Barroso, J.G.; Sanches, J. Essential oil of *Eucalyptus* from Mata Experimental do Escaroupim (Portugal): Evaluation of the essential oil composition from sixteen species. *Acta Hort.* 2011, 925, 61–66. [CrossRef]

68. Miguel, M.G.; Gago, C.; Antunes, M.D.; Lagoas, S.; Faleiro, M.L.; Mégias, C.; Cortés-Giraldo, I.; Vioque, J.; Figueiredo, A.C. Antibacterial, antioxidant and antiproliferative activities of *Corymbia citriodora* and eight *Eucalyptus* species essential oils. *Medicines* 2018, 5, 61. [CrossRef]

69. Rodrigues, A.M.; Mendes, M.D.; Lima, A.S.; Barbosa, P.M.; Ascensão, L.; Barroso, J.G.; Pedro, L.G.; Mota, M.M.; Figueiredo, A.C. Pinus halepensis, *Pinus pinaster*, *Pinus pinea* and *Pinus sylvestris* essential oils chemotypes and monoterpene hydrocarbon enantiomers, before and after inoculation with the pinewood nematode *Bursaphelenchus xylophilus*. *Chem. Biodivers.* 2017, 14, e1600153. [CrossRef] [PubMed]

70. Klein, E.; Schmidt, W. Structure of brachyl oxide. *J. Agric. Food Chem.* 1971, 19, 1115–1117. [CrossRef]

71. Pereira, W.S.; da Silva, G.P.; Vigliano, M.V.; Leal, N.R.F.; Pinto, F.A.; Fernandes, D.C.; Santos, S.V.M.; Martinho, T.; Nascimento, J.R.; de Azevedo, A.P.S.; et al. Anti-arthritic properties of crude extract from *Bursaphelenchus xylophilus* enantiomers, before and after inoculation with the pinewood nematode *Bursaphelenchus xylophilus*. *J. Ethnopharmacol.* 2011, 178, 198–208. [CrossRef] [PubMed]

72. Figueiredo, A.C. Biological properties of essentials oils and volatiles. Sources of variability. *Nat. Volatiles Essent. Oils* 2017, 10, 4, 1–13.

73. Ni, W.; Kitamoto, S.; Ishibashi, M.; Usui, M.; Inoue, S.; Huasa, K.-I.; Zhao, Q.; Nishida, K.-I.; Takeshita, A.; Eghashira, K. Monocyte chemoattractant protein-1 is an essential inflammatory mediator in angiotensin II-induced progression of established atherosclerosis in hypercholesterolemic mice. *Arter. Thromb. Vasc. Biol.* 2004, 24, 534–539. [CrossRef] [PubMed]

74. Hirota, R.; Roger, N.N.; Nakamura, H.; Song, H.-S.; Sawamura, M.; Suganuma, N. Anti-inflammatory effects of limonene from *Yuzu* (*Citrus junos* Tanaka) essential oil on eosinophils. *J. Food Sci.* 2010, 75, H87–H92. [CrossRef]

75. Chen, L.-L.; Zhang, H.J.; Chao, J.; Liu, J.F. Essential oil of *Artemisia argyi* suppresses inflammatory responses by inhibiting JAK/STATs activation. *J. Ethnopharmacol.* 2017, 204, 107–117. [CrossRef]

76. Luo, G.; Kong, J.; Cheng, B.-Y.; Zhao, H.; Fu, X.-Q.; Yan, L.-S.; Ding, Y.; Liu, Y.-L.; Pan, S.-Y.; Zhang, S.-F.; et al. Xiao Qing Long Tang essential oil exhibits inhibitory effects on the release of pro-inflammatory mediators by suppressing NF-Î±B, AP-1, and IRF3 signalling in the lipopolysaccharide-stimulated RAW264.7 cells. *Rsc Adv.* 2019, 9, 12977–12989. [CrossRef]

77. Park, H.; Seol, G.H.; Ryu, S.; Choi, I.-Y. Neuroprotective effects of (−)-linalool against oxygen-glucose deprivation-induced neuronal injury. *Arch. Pharmacal Res.* 2016, 39, 555–564. [CrossRef]