Article

Phytochemical Characterization and Bioactive Properties of Cinnamon Basil (Ocimum basilicum cv. ‘Cinnamon’) and Lemon Basil (Ocimum × citriodorum)

Chaimae Majdi 1,2,3, Carla Pereira 1, Maria Inês Dias 1, Ricardo C. Calhelha 1, Maria José Alves 1, Boutayna Rhourri-Frih 2, Zoubida Charrouf 3, Lillian Barros 1,*, Joana S. Amaral 1,4,* and Isabel C.F.R. Ferreira 1

1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; cmajdiy@gmail.com (C.M.); carlap@ipb.pt (C.P.); maria.ines@ipb.pt (M.I.D.); calhelha@ipb.pt (R.C.C.); maria.alves@ipb.pt (M.J.A.); iferreira@ipb.pt (I.C.F.R.F.)
2 CBMN-UMR 5248, Faculty of Pharmacy, University of Bordeaux, 33016 Bordeaux, France; boutayna.frih@u-bordeaux.fr
3 Faculté des Sciences, Université Mohammed V, Rabat 10100, Morocco; zcharrouf@yahoo.fr
4 REQUIMTE/LAQV, Faculdade de Farmácia da Universidade do Porto, 4050-313 Porto, Portugal
* Correspondence: lillian@ipb.pt (L.B.); jamaral@ipb.pt (J.S.A.)

Received: 1 April 2020; Accepted: 27 April 2020; Published: 29 April 2020

Abstract: The aim of this work was to contribute to the knowledge on the chemical composition and bioactive properties of two species of the Ocimum genus, namely O. basilicum cultivar ‘Cinnamon’ and O. × citriodorum. For this purpose, samples of these plants grown in Portugal were evaluated for their composition in phenolic and volatile compounds, and the infusion and hydroethanolic extracts were assessed for their in vitro antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities. In total, the two basil samples showed the presence of seven caffeic acid and derivatives (dimers, trimers, and tetramers) and five flavonoids, mainly glycoside derivatives of quercetin. Despite some qualitative and quantitative differences, in both samples rosmarinic acid was the major phenolic compound, and linalool the predominant volatile compound. In general, the tested extracts provided relevant bioactive properties since both basil species showed higher antioxidant activity in Thiobarbituric Acid Reactive Substances (TBARS) and Oxidative Hemolysis Inhibition (OxHLIA) assays when compared with the positive control Trolox. Despite O. × citriodorum extracts showing slightly better activity against some strains, both types of extracts evidenced similar antimicrobial activity, being more active against Gram-positive bacteria. The extracts also revealed interesting cytotoxicity, particularly the O. × citriodorum hydroethanolic extract which was also the only one exhibiting anti-inflammatory activity.

Keywords: sweet basil; lemon basil; phenolic compounds; volatile compounds; antioxidant activity; antimicrobial activity; anti-proliferative activity

1. Introduction

Many species belonging to Lamiaceae family have a long history of culinary use as aromatic herbs or spices, but also in folk medicine. Among those, several belong to the Ocimum genus, which is collectively designated as basil and includes more than 30 different species [1]. In the Mediterranean region, one of the most important and frequently consumed species is Ocimum basilicum, commonly known as sweet basil or common basil. It is a typical seasoning in many countries, with fresh leaves being consumed in large quantities as an ingredient in several dishes and food preparations. In addition, O. basilicum is also cultivated worldwide for its essential oil, with applications...
in medicine/pharmaceutical, perfume and cosmetics, and as flavoring agent. Moreover, its use as traditional medicinal herb has also been reported, mainly for treating headaches, coughs, digestive, and nervous disorders [2]. *O. basilicum* includes several varieties that have been selected and developed over many years for a variety of purposes, with the existence of at least 18 different cultivars being mentioned by the Herb Society of America [3]. Those comprise of culinary basils, such as cultivar ‘Genovese’ or ‘Italian Large Leaf’, that were selected for their leaf shape, size, aroma and flavor, while cultivars such as ‘Purple Ruffles’ were developed to enhance ornamental traits, for example, leaf color [4].

It is known that different cultivars of basil have the genetic ability of generating different chemical compounds and consequently presenting distinct chemical profiles [5]. Nevertheless, most studies so far focused mainly on the volatile compounds profile, showing the existence of a great variety of chemotypes within the same species of the *Ocimum* genus [6]. However, the chemical composition beyond volatiles and also the bioactive properties of the plants’ extracts are scarcely studied for most *O. basilicum* cultivars as well as for several other species of the *Ocimum* genus. In fact, while many studies have been conducted on *O. basilicum*, only few refer to the cultivar *O. basilicum* ‘Cinnamon’ or to the natural hybrid *Ocimum × citriodorum* Vis. under study in this work.

*O. basilicum* cv. ‘Cinnamon’, also known as Mexican spice basil or cinnamon basil due to its distinctive cinnamon taste and spicy aroma, is frequently consumed as an ingredient in infusions and baked goods but also in raw dishes, fruit salads and jellies as alternative of ground cinnamon [3,6]. Besides culinary, the plant is also grown for its essential oil, which is used in perfumeries [6]. *O. × citriodorum*, popularly known as lemon basil, is a natural hybrid between sweet basil (*O. basilicum*) and African basil (*Ocimum americanum*). This aromatic plant is frequently used both in the Mediterranean and Asian cuisines due to its lemony scent and flavor. In addition, lemon basil has also been used as raw material for the chemical, pharmaceutical and food industries, increasing its economic interest and prompting its cultivation [7].

Despite the interest and wide use of both species, as mentioned, there is still a scarcity of information regarding both plants. Thus, this work aims at filling this gap by evaluating the phytochemical composition of the two plant species, their phenolic compounds and volatiles profile, and also a screening of their biological properties, namely antioxidant, antimicrobial, cytotoxic, and anti-inflammatory activities.

2. Materials and Methods

2.1. Plant Material and Preparation of the Extracts

Commercial samples (bags of 50 g) of fragmented dried leaves of “cinnamon basil” (*O. basilicum* cv. ‘Cinnamon’) and of “lemon basil” (*Ocimum × citriodorum*), were provided by a Portuguese company (Cantinho das Aromáticas, Vila Nova de Gaia, Portugal) dedicated to the production and commercialization of dry aromatic herbs, produced in the Northern region of Portugal under organic farming. Each sample was reduced to a fine powder and stored from light until further analysis.

Two solvents, namely water and ethanol/water (80:20, v/v), were used to prepare the extracts. To prepare the infusion, 200 mL of boiled distilled water was added to each sample (600 mg) and kept for resting at room temperature for 5 min, followed by filtration, freezing, and lyophilization (FreeZone 4.5, Labconco, Kansas City, MO, USA).

For the hydroethanolic extract preparation, each sample (300 mg) was extracted by stirring with 100 mL of ethanol/water (80:20 v/v, at 25 °C at 150 rpm) for 1 h and subsequently filtered using Whatman no.4 filter paper (Sigma-Aldrich, St. Louis, MO, USA). The residue was then re-extracted using the same conditions. The combined extracts were evaporated at 35 °C under reduced pressure (rotary evaporator Büchi 3000 series, Buchi AG, Flawil, Switzerland) until the complete removal of ethanol, and afterwards the aqueous phase was frozen and lyophilized.
Additionally, the two basil samples were submitted to essential oil extraction by hydrodistillation in a Clevenger apparatus. For that purpose, approximately 40 g of freshly ground leaves were introduced in a round-bottom flask with 400 mL of distilled water and the mixture was boiled for three hours. After this period, the essential oil was separated from the water and, because a low yield was obtained, the oil was recovered with the addition of 1 mL of hexane. After being collected, the oil was dried over anhydrous sodium sulphate and stored at −20 °C until being analyzed.

2.2. Chemical Composition

2.2.1. Phenolic Compounds

The qualitative and quantitative analysis of the phenolic profile was performed by a liquid chromatography system, Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA), coupled with a diode-array in series with a Linear Ion Trap mass spectrometry detector equipped with an electrospray ionization source (LC-DAD-ESI/MSn) operating under the conditions previously described by Bessada et al. (2016) [8].

The chromatographic separation was carried out on a thermostatted (35 °C) Spherisorb S3 ODS-2 C18 column (3 µm, 4.6 × 150 mm, Waters, Milford, MA, EUA), using the mobile phase conditions (solvents, gradient, and flow rate) described by Bessada et al. (2016) [8].

The spectral data for all peaks were recorded at 280 and 370 nm as preferred wavelengths. The phenolic compounds were identified by comparing their retention times, UV-vis and mass spectra with those obtained with commercial standard compounds, when available. Otherwise, compounds were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a 7-level calibration curve for each available phenolic standard (caffeic acid, rosmarinic acid, quercetin-3-O-rutinoside, and quercetin-3-O-glucoside, Extrasynthese, Genay, France) was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of another compound from the same phenolic group. Each extract was analyzed in triplicate and the results were expressed as mg per g of extract.

2.2.2. Volatile Compounds

The essential oil was analyzed by gas chromatography coupled to mass spectrometry detection (GC-MS) using a GC-2010 Plus (Shimadzu, Kyoto, Japan) system equipped with a AOC-20iPlus (Shimadzu, Kyoto, Japan) automatic injector and a SH-RXi-5ms (30 m × 0.25 mm × 0.25 µm; Shimadzu, Kyoto, Japan, USA) column. The conditions regarding the temperature set for the injector, oven, transfer line, and ion source temperatures were the same as previously described by Spréa et al. (2020) [9]. The volume of the injected sample was 1 µL with a split ratio of 1:10. Helium was used as the carrier gas, adjusted to a linear velocity of 30 cm/s. The ionization energy was 70 eV, scan range was 35–500 u, with a scan time of 0.3 s. The compounds’ identification was based on a comparison of the obtained mass spectra with those of the Nacional Institute of Standards and Technology (NIST2017) mass spectra library and linear retention index (LRI), calculated using the retention times of an n-alkane series (C8-C40, ref. 40147-U, Supelco), and analyzed under identical conditions. The calculated LRI was compared with previously published data [10]. Compounds were quantified as a relative percentage of total volatiles using the relative area values directly obtained from peak total ion current (TIC). Analyses were performed in triplicate.
2.3. Bioactive Properties

2.3.1. Evaluation of In Vitro Antioxidant Properties

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS assay was performed following a procedure previously described by Pinela et al. (2012) [11]. Briefly, porcine brain homogenates were prepared and added to a mixture with 0.2 mL of samples extracts, 0.1 mL of FeSO$_4$ (0.01 mM), and 0.1 mL of ascorbic acid (0.1 mM). The test tubes were incubated at 37 °C for one hour and, after that, 0.5-mL trichloroacetic acid (28% w/v) was added to stop the reaction. To visualize the extent of oxidation, 0.38 mL of thiobarbituric acid (2%, w/v) was added and the mixture was heated at 80 °C for 20 min. After the formation of the pink thiobarbituric acid-malondialdehyde complex (TBA-MDA), the tubes were centrifuged at 14,000 rpm for five minutes to remove the precipitated protein and the color intensity of the complex was measured at 532 nm. The inhibition ratio (%) was calculated using the formula,

$$\text{Inhibition ratio (\%)} = \left( \frac{A - B}{A} \right) \times 100 \quad (1)$$

where A and B were the absorbance of the control and the extract solution, respectively. Trolox was used as a positive control (in concentrations ranging from 25 to 500 µg/mL). The results were expressed as IC$_{50}$ values (extract concentration able to provide 50% of antioxidant activity, µg/mL).

Oxidative Hemolysis Inhibition Assay (OxHLIA)

The OxHLIA assay was performed as described by Lockowandt et al. (2019) [12]. Briefly, 200 µL of a freshly prepared sheep’s erythrocyte suspension (2.8% in PBS, v/v) was mixed with either 400 µL of extracts dissolved in PBS, water (for complete hemolysis), or PBS solution (as control), using flat-bottom, 48-well microplates. After incubating the plates at 37 °C for 10 min while being shaken, 200 µL (160 mM) of the oxidizer 2, 2’-Azobis(2-amidinopropane) dihydrochloride (AAPH) was added to each well. The plate was again incubated at 37 °C with agitation and the optical density was measured at 690 nm every 10 min. The following equation was used to evaluate the erythrocyte population that remained intact (P):

$$P (\%) = \left( \frac{S_t - CH0}{S_0 - CH0} \right) \times 100 \quad (2)$$

where S0 and St are the optical density of the sample at 0 and t min, respectively, and CH0 is the optical density of the complete hemolysis at zero minutes. The delayed time of hemolysis (Δt) is calculated as follows:

$$\Delta t (\text{min}) = H_{t50} \text{(sample)} - H_{t50} \text{(control)} \quad (3)$$

where Ht50 corresponds to the 50% hemolytic time (min) graphically obtained from the hemolysis curve of each antioxidant sample concentration. The Δt values were then linearly correlated to the tested concentrations and from the correlation obtained, the inhibition concentration able to promote a Δt hemolysis delay of 60 min (IC$_{50}$ (60 min), µg/mL) was calculated for each sample. Trolox was used as positive control (in concentrations from 12.5 to 400 µg/mL).

2.3.2. Antimicrobial Activity

The antibacterial activity was assessed by using the microdilution broth method coupled to the rapid p-iodonitrotetrazolium chloride (INT) colorimetric assay. The antibacterial activity was assessed against clinical isolates from patients hospitalized in various departments of the Local Health Unit of Bragança, Portugal, comprising five Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Pseudomonas aeruginosa, and Proteus mirabilis, isolated from urine and expectoration) and three Gram-positive bacteria (methicillin-resistant Staphylococcus aureus (MRSA), Listeria monocytogenes, and Enterococcus faecalis). Prior to the assay, the extracts were re-dissolved...
in 250-µL dimethylsulfoxide (DMSO) and 750 µL of medium and water to obtain a stock solution of 20 mg/mL. Afterwards, they were submitted to further dilution (10 mg/mL to 0.625 mg/mL) using culture medium. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as previously described by Pires et al. (2018). Ampicillin (20 mg/mL), Imipenem (1 mg/mL), and Vancomycin (1 mg/mL) were used as positive controls. Muller Hinton Broth added with 5% DMSO inoculated with each bacterium was used as negative control.

2.3.3. Cytotoxic Activity

The antiproliferative activity of the samples was evaluated by using the Sulforhodamine B (SRB) colorimetric assay as previously described by Abreu et al. (2011) [13]. To obtain stock solutions of 8 mg/mL, each of the lyophilized extracts were re-dissolved in water, and further dilutions from 0.4 until 0.06 mg/mL were prepared. The test was performed using the following human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma), and HepG2 (hepatocellular carcinoma), obtained from DSMZ (Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). To maintain the tumor cells they were kept in a nutritive medium, namely RPMI-1640 (HyClone, Logan, USA) medium containing 10% heat-inactivated FBS (Fetal bovine serum, HyClone, Logan, UT, USA) and 2 mM glutamine for MCF-7, NCI-H460 and HCT-15, while HeLa and HepG2 cells were kept in Dulbecco’s Modified Eagle Medium (DMEM, HyClone, Logan, UT, USA) supplemented with 10% FBS, 2-mM glutamine and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin, HyClone, Logan, UT, USA). Briefly, the cells were incubated at 37 °C in a humidified air incubator containing 5% CO2 until reaching a sufficient density, after which they were plated in 96-well plates to a density of 1.0 × 10^4 cells/well. The cells were left to attach for 24 h and, then, the different sample concentrations were added, and the mixture incubated at the same conditions for 48 h. After that, 100 µL of cold 10% trichloroacetic acid were added followed by another incubation at 4 °C for 60 min to fix the cells. After washing with deionized water (4×) and drying, 100 µL of SRB solution (0.1% in 1% acetic acid) was added to each plate well, which was then incubated at room temperature for 30 min. After removing the unbound SRB with the addition of 1% acetic acid, the bounded SRB was re-solubilized in 200 µL of 10-mM Tris base solution with agitation for five minutes, and the absorbance was measured at 515 nm using a microplate reader Biotek ELX800 (BioTek Instruments, Inc., Winooski, VT, USA). The results were calculated as GI50 values (concentration that inhibits 50% of cell growth). Ellipticine was used as a positive control (in concentrations from 0.3 to 10 µg/mL).

To evaluate the cytotoxicity against non-tumoral cells, namely hepatocytes, an identical procedure was performed using freshly harvested porcine liver cells (PLP2), established as described by Abreu et al. (2011) [13].

2.3.4. Anti-inflammatory Activity

The evaluation of the anti-inflammatory activity was determined as described by Taofiq et al. (2015) [14] using a mouse macrophage-like cell line (RAW264.7) cultured in DMEM medium/HIGH GLUCOSE, at 37 °C under 5% CO2, in humidified air. Briefly, after cells were seeded in the plates, the media was removed, the extracts were added, and plates incubated for 60 min. Subsequently, the cells were stimulated by adding 30 µL of a bacterial lipopolysaccharide solution (10 µg/mL) and culture media to reach a volume of 300 µL. After incubation (24 h, 37 °C, 5% CO2, humidified air), the supernatant of each well was transferred to another plate and Griess reagent was added. After 5 min resting at ambient temperature and protected from light, the produced nitric oxide was determined at 515 nm using a microplate reader Biotek ELX800 (BioTek Instruments, Inc., Winooski, VT, USA). Dexamethasone (Sigma-Aldrich, St Louis, MO, USA) was used as positive control (in concentrations from 0.3 to 10 µg/mL). The effect of all the tested samples in the absence of LPS was also evaluated, to observe if they induced changes in nitric oxide basal levels. In negative controls, no LPS was added.
The results were calculated by comparison with the standard calibration curve obtained with nitrite solution and expressed as EC$_{50}$.

2.4. Statistical Analysis

The results were expressed as mean ± standard deviation (SD) of triplicate analysis. The differences between the different extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference post hoc test with $\alpha = 0.05$, coupled with Welch’s statistic. Differences among extractions and samples were assessed by applying the Student’s t-test at a 5% significance level using the SPSS Statistics software (IBM SPSS Statistics for Windows, v. 23.0, IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Chemical Composition

3.1.1. Phenolic Compounds Profile

The characteristic data (retention times, $\lambda_{\text{max}}$, pseudomolecular ion, main fragment ions in MS2) of the phenolic compounds identified in the prepared extracts are listed in Table 1. In total, the two basil samples showed the presence of seven caffeic acid and derivatives (dimers, trimers and tetramers of rosmarinic acid) and five flavonoids, mainly glycoside derivatives of quercetin. Caffeic acid (compound 2), quercetin-3-O-rutinoside (compound 5), quercetin-3-O-glucoside (compound 7), and rosmarinic acid (compound 10) were positively identified according to their retention, mass spectra and UV-vis characteristics in comparison with commercial standards. Compounds 1 ([M − H]$^{-}$ at m/z 341) and 4 ([M − H]$^{-}$ at m/z 473) presented a fragmentation pattern that allowed assigning them as caffeic acid hexoside and chicoric acid (dicafeoyltartaric acid), respectively. This last compound, as well as compounds 2 and 10 (caffeic acid and rosmarinic acid, respectively), have been described by several authors as being the main phenolic acids in basil [15–20]. Compounds 6 and 11 ([M − H]$^{-}$ at m/z 717) presented a fragmentation pattern with successive losses of 198 u (danshensu) or 180 u (caffeic acid) units, coherent with salvianolic acid B (also known as lithospermic acid B) [21,22]. Compound 12 ([M − H]$^{-}$ at m/z 537) presented a UV spectrum and fragmentation pattern consistent with the caffeic acid trimer lithospermic acid A. Salvianolic acids H/I, with the same molecular weight as lithospermic acid A, were discarded as possible identities because they present quite a different fragmentation pattern [22,23]. Furthermore, the presence of lithospermic acid A in an unnamed sweet basil cultivar was already reported by Lee & Scagel [16]. Based on the obtained data, the remaining compounds (peaks 4, 8, and 9) were assigned to quercetin glycosides derivatives. Compound 4 ([M − H]$^{-}$ at m/z 595) released two MS$^2$ fragments at m/z 463 ([M − H-132]$^{-}$ and 301 ([M − H-162]$^{-}$, which revealed the alternative loss of a pentosyl and hexosyl moieties, being tentatively identified as quercetin-O-pentoside-O-hexoside. Meanwhile, compounds 8 and 9 ([M − H]$^{-}$ at m/z 549) presented three MS$^2$ fragments at m/z 505, 463, and 301 (quercetin; [M − H-44-42-162]$^{-}$, loss of an malonyl-hexoside moiety), being assigned as quercetin-O-malonyl-hexoside.
Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λmax), mass spectrometric data, tentative identification, and quantification (mg/g of extract) of the phenolic compounds in Ocimum basilicum 'Cinnamon' and Ocimum × citriodorum hydroethanolic extracts and infusion preparations.

| Peak | Rt (min) | λmax (nm) | (M − H)⁺ (m/z) | MS² (m/z) | Tentative Identification | Ocimum basilicum cv. 'Cinnamon' | Ocimum × citriodorum |
|------|----------|-----------|----------------|-----------|-------------------------|-------------------------------|----------------------|
|      |          |           |                |           |                         | EtOH:H₂O Infusion | EtOH:H₂O Infusion |
| 1    | 4.81     | 326       | 341            | 179(100),161(62),135(34) | Caffeic acid hexoside A | tr  | tr  | tr  | tr  |
| 2    | 9.89     | 323       | 179            | 135(100)  | Caffeic acid A          | 1.194 ± 0.002 c  | 0.63 ± 0.02 d  | 1.41 ± 0.03 b  | 3.1 ± 0.1 a  |
| 3    | 13.54    | 327       | 473            | 311(100),293(98),179(8),149(5),135(5) | Chioric acid A | 0.51 ± 0.03 d  | 0.61 ± 0.01 c  | 0.64 ± 0.01 b  | 1.09 ± 0.05 a  |
| 4    | 15.88    | 350       | 595            | 463(25),301(100) | Quercetin-O-glucoside-O-hexoside B | 2.07 ± 0.06 a  | 1.54 ± 0.02 b  | nd  | nd  |
| 5    | 17.63    | 355       | 609            | 301(100)  | Quercetin-3-O-rutinoside B | 4.53 ± 0.01 a  | 3.24 ± 0.04 b  | 1.525 ± 0.003 d | 2.85 ± 0.02 c  |
| 6    | 18.21    | 340       | 717            | 537(40),519(100),493(15),359(10),339(8),321(5) | Salvinolic acid B isomer 1 C | nd  | nd  | 2.23 ± 0.04 b  | 2.47 ± 0.03 a  |
| 7    | 18.53    | 341       | 463            | 301(100)  | Quercetin-3-O-glucoside B | 3.405 ± 0.003 a  | 2.22 ± 0.04 d  | 2.91 ± 0.08 b  | 2.43 ± 0.02 c  |
| 8    | 20.05    | 337       | 549            | 505(5),463(28),301(100) | Quercetin-O-malonyl-hexoside B | 3.4 ± 0.1 a  | 2.82 ± 0.03 b  | 1.67 ± 0.02 d  | 2.11 ± 0.03 c  |
| 9    | 20.45    | 337       | 549            | 505(5),463(48),301(100) | Quercetin-O-malonyl-hexoside B | nd  | nd  | 1.58 ± 0.02 b  | 1.84 ± 0.05 b  |
| 10   | 21.75    | 339       | 359            | 197(25),179(41),161(100),135(5) | Rosmarinic acid C | 77 ± 1 a  | 41.0 ± 0.2 d  | 50 ± 1 c  | 59.1 ± 0.3 b  |
| 11   | 25.79    | 329       | 717            | 537(5),519(100),493(5),339(5),321(7),295(5) | Salvinolic acid B isomer 2 C | 5.2 ± 0.1 c  | 7.3 ± 0.4 a  | nd  | 6.9 ± 0.1 b  |
| 12   | 30.44    | 284/329   | 537            | 493(100),439(5),359(62),197(5),179(10),161(15) | Lithospermic acid A C | 7.11 ± 0.02 a  | 2.82 ± 0.06 b  | nd  | nd  |

Total Phenolic Acids: 91 ± 1 a 52.4 ± 0.4 d 55 ± 1 c 72.7 ± 0.3 b
Total Flavonoids: 13.4 ± 0.2 a 9.8 ± 0.1 b 7.9 ± 0.1 d 9.2 ± 0.1 c
Total Phenolic Compounds: 105 ± 1 a 62.2 ± 0.1 c 63 ± 2 e 81.9 ± 0.2 b

nd—not detected. tr—traces. Standard calibration curves used for compounds' quantification: λ—caféic acid (y = 388345x + 406369, R² = 0.9939, limit of detection (LOD) and limit of quantitation (LOQ) = 0.78 and 1.97 μg/mL, respectively); C—quercetin-3-O-glucoside (y = 13343x + 7675, R² = 0.9998, LOD and LOQ = 0.18 and 0.65, respectively); A—rosmarinic acid (y = 191291x − 652903, R² = 0.999, LOD and LOQ = 0.15 and 0.68, respectively); B—quercetin-3-O-glucoside (y = 34843x − 160173, R² = 0.9998, LOD and LOQ = 0.21 and 0.71, respectively). Different letters (lowercase letters) correspond to significant differences (p < 0.05).
Comparing the two basil samples under study, a higher content of total phenolic compounds (TPC) was obtained for the hydroethanolic extract of cinnamon basil, while for lemon basil higher amounts were obtained for the infusion. Lithospermic acid A was only identified in \textit{O. basilicum} cv. ‘Cinnamon’ while, interestingly, different Salvianolic acid B isomers were found in each basil species. In both samples, irrespective of the extraction method, rosmarinic acid was the main phenolic compound, which is in good agreement with previous works that reported this phenolic acid as being the main compound present in \textit{O. basilicum} \cite{16,24–26}. Nevertheless, most of the previous works on the phenolic composition of \textit{O. basilicum} samples do not mention the cultivar used or refer to commercial samples, also without specifying the cultivar. In the study performed by Simeoni et al. (2018) \cite{27} using a commercial \textit{O. basilicum} sample (non-identified cultivar), 13 phenolic acids and three flavonoids were identified and quantified, among which chicoric acid was the major compound, followed by rosmarinic acid. The major flavonoid was isoquercetin, while in the present work it was found to be quercetin-3-O-rutinoside. Different flavonoids, such as kaempferol, apigenin, and luteolin derivatives, and phenolic acids, such as chlorogenic acid, were reported by Jayasinghe et al. (2003) \cite{15} in the fraction of a methanolic extract prepared with \textit{O. basilicum} grown in Sri Lanka, but again the cultivar was not discriminated, which can possibly explain, at least partially, the different profiles. Similarly, Hossain et al. (2010) \cite{17} identified a total of 33 compounds in a basil hydroethanolic (80%) extract, of which 24 were described for the first time, despite no quantitative data being given. The identified compounds included several flavonoids and phenolic acids distinct of the ones found in the present study. However, the authors only mentioned that it was a commercial basil spice sample bought from Turkey, without even referring to the species used. As far as the literature consulted on the phenolic composition of sweet basil, only one previous study was found that included the cultivar ‘Cinnamon’ \cite{18}. The study included 15 different cultivars of \textit{O. basilicum}; nevertheless, only the four major phenolic acids were evaluated. The ‘Cinnamon’ cultivar presented rosmarinic acid as the main phenolic acid, followed by chicoric acid, which is in good agreement with the present results. Nevertheless, the third major compound was caftaric acid, which was not detected in the present study. Despite the phenolic acids content reported by Kwee and Niemeyer (2011) \cite{18} in \textit{O. basilicum} cultivar ‘Cinnamon’ being much lower than the ones presented on Table 1, these values are not comparable because the results are differently expressed.

In what concerns \textit{O. × citriodorum} phenolic composition, data from the literature was also found to be very scarce. Hakkim et al. (2008) \cite{28} evaluated the phenolic composition, using HPLC-DAD, of the methanolic extracts of eight different \textit{Ocimum} species, including \textit{O. × citriodorum}. The authors reported the presence of seven phenolic acids, namely rosmarinic, lithospermic, vanillic, p-coumaric, hydroxybenzoic, ferulic, and cinnamic acids, in \textit{O. × citriodorum}. Contrary to the results obtained in the present work (Table 1), hydroxybenzoic (0.20 mg/g dry extract) and p-coumaric (0.19 mg/g dry extract) were found to be the main compounds. In addition, in the present study, the contents of rosmarinic and lithospermic acids were much higher than those reported by Hakkim et al. (2008) \cite{28}.

### 3.1.2. Volatile Compounds

Table 2 lists the compounds identified by GC-MS in the essential oil of cinnamon basil and lemon basil. A total of 59 and 75 compounds were detected for \textit{O. basilicum} cultivar ‘Cinnamon’ and \textit{O. × citriodorum}, respectively, representing 99.2% and 88.3% of the total compounds in each essential oil (Table 2). Despite the qualitative and quantitative differences between the composition of the two oils, linalool was found to be the major compound in both samples. In cinnamon basil, it was followed by (E)-methyl-cinnamate, which presented also a very high amount (24.7%), and τ-cadinol (7.4%), while in lemon basil by caryophyllene oxide (6.2%) and trans-α-bergamotene (5.7%). Considering relative percentages, lemon basil presented a higher content of oxygenated monoterpenes and of sesquiterpenes whereas cinnamon basil was rich in the cinnamic acid derivative methyl-cinnamate, which was absent in the former essential oil, and presented slightly higher amounts of oxygenated sesquiterpenes and monoterpenes. When compared to previously reported data, the profile of \textit{O. basilicum} cultivar
'Cinnamon' was very similar to that described by Wesolowska and Jadczak (2016) [6] regarding the same cultivar cultivated in North-western Poland, but completely distinct to that reported by Tsasi et al. (2017) [29]. The chemical profile of *O. basilicum* cultivar 'Cinnamon' samples collected in the Island of Kefalonia, Greece, showed methyl chavicol as major compound (ranging from 60.2–75.1%) followed by linalool (0.6–5.7%) and germacrene D (3.2–5.0%), while in the present study the content of methyl chavicol was much lower (2.5%). According to Vieira and Simon (2006) [4] who studied the volatile profile of different basil species, *O. basilicum* cv. 'Cinnamon' can be differentiated from three other *Ocimum* species (*O. americanum, O. × citriodorum* and *O. minimum*) and 14 *O. basilicum* cultivars by the high amount of (E)-methyl-cinnamate, which is in good agreement with the data reported by Wesolowska and Jadczak (2016) [6] and the present study.

| Compound | RT  | LRI a | LRI b | Quantification c (Relative %) | O. basilicum cv. | O. × citriodorum |
|----------|-----|-------|-------|-----------------------------|-------------------|------------------|
| 1-Hexanal | 7.90 | 800   | 801   | −                           | 0.014 ± 0.001     |
| 2-Hexenal | 10.23 | 850  | 846   | −                           | 0.012 ± 0.001     |
| α-Thujene | 13.90 | 926   | 924   | 0.07 ± 0.003               | 0.044 ± 0.002     |
| α-Pinene | 14.22 | 932   | 932   | 0.036 ± 0.001              | 0.245 ± 0.005     |
| Camphene | 14.96 | 947   | 946   | 0.0157 ± 0.0004           | 0.022 ± 0.003     |
| Benzaldehyde | 15.55 | 958   | 952   | 0.049 ± 0.003             | 0.012 ± 0.001     |
| Sabinene | 16.30 | 972   | 969   | 0.019 ± 0.001             | 0.014 ± 0.003     |
| β-Pinene | 16.43 | 975   | 974   | 0.064 ± 0.002              | 0.092 ± 0.002     |
| 1-Octen-3-ol | 16.69 | 980   | 974   | −                           | 0.138 ± 0.002     |
| 2-Hexenal | 10.23 | 850  | 846   | −                           | 0.014 ± 0.001     |
| α-Thujene | 13.90 | 926   | 924   | 0.07 ± 0.003               | 0.044 ± 0.002     |
| α-Pinene | 14.22 | 932   | 932   | 0.036 ± 0.001              | 0.245 ± 0.005     |
| Camphene | 14.96 | 947   | 946   | 0.0157 ± 0.0004           | 0.022 ± 0.003     |
| Benzaldehyde | 15.55 | 958   | 952   | 0.049 ± 0.003             | 0.012 ± 0.001     |
| Sabinene | 16.30 | 972   | 969   | 0.019 ± 0.001             | 0.014 ± 0.003     |
| β-Pinene | 16.43 | 975   | 974   | 0.064 ± 0.002              | 0.092 ± 0.002     |
| 1-Octen-3-ol | 16.69 | 980   | 974   | −                           | 0.138 ± 0.002     |
| Compound | RT  | LRI a | LRI b | Quantification c (Relative %) |
|----------|-----|-------|-------|-------------------------------|
|          |     |       |       | O. basilicum cv. 'Cinnamon' | O. × citroidorum |
| 41       | 27.70 | 1196  | 1192  | – | 0.09 ± 0.01 |
| 42       | 27.79 | 1198  | 1195  | 2.46 ± 0.01 | 0.285 ± 0.009 |
| 43       | 28.40 | 1211  | 1211  | 0.019 ± 0.004 | 0.132 ± 0.002 |
| 44       | 28.84 | 1221  | 1218  | 0.028 ± 0.003 | – |
| 45       | 29.19 | 1234  | 1227  | 0.036 ± 0.002 | 3.70 ± 0.05 |
| 46       | 29.93 | 1244  | 1239  | 0.0355 ± 0.0001 | 0.34 ± 0.01 |
| 47       | 29.98 | 1245  | 1235  | – | 2.21 ± 0.04 |
| 48       | 30.60 | 1258  | 1249  | – | 1.60 ± 0.01 |
| 49       | 31.16 | 1270  | 1264  | 0.012 ± 0.001 | 2.68 ± 0.02 |
| 50       | 31.71 | 1282  | 1280  | – | 0.054 ± 0.001 |
| 51       | 31.93 | 1287  | 1284  | 0.38 ± 0.01 | – |
| 52       | 32.69 | 1303  | 1298  | – | 0.202 ± 0.008 |
| 53       | 32.83 | 1306  | 1299  | 3.14 ± 0.02 | 0.054 ± 0.005 |
| 54       | 33.64 | 1324  | 1322  | – | 0.078 ± 0.001 |
| 55       | 33.72 | 1326  | 1324  | 0.0214 ± 0.0004 | – |
| 56       | 34.45 | 1343  | 0.037 ± 0.003 | – |
| 57       | 34.84 | 1352  | 1345  | 0.157 ± 0.003 | 0.456 ± 0.003 |
| 58       | 35.16 | 1359  | 1356  | 2.18 ± 0.04 | 0.323 ± 0.001 |
| 59       | 35.46 | 1366  | 1359  | – | 1.04 ± 0.01 |
| 60       | 36.02 | 1379  | 1374  | 0.40 ± 0.02 | 1.011 ± 0.005 |
| 61       | 36.46 | 1384  | 1376  | 24.70 ± 0.06 | – |
| 62       | 36.47 | 1389  | 1387  | – | 0.313 ± 0.002 |
| 63       | 36.78 | 1395  | 1389  | 3.00 ± 0.05 | 0.27 ± 0.03 |
| 64       | 36.98 | 1400  | 1400  | – | 0.012 ± 0.001 |
| 65       | 37.78 | 1419  | 1411  | – | 0.012 ± 0.001 |
| 66       | 37.93 | 1423  | 1419;149 | 0.335 ± 0.009 | – |
| 67       | 37.98 | 1424  | 1417  | – | 4.32 ± 0.03 |
| 68       | 38.37 | 1434  | 1430  | – | 0.120 ± 0.002 |
| 69       | 38.57 | 1438  | 1432  | 0.66 ± 0.03 | 5.76 ± 0.04 |
| 70       | 38.72 | 1442  | 1437  | 1.26 ± 0.02 | – |
| 71       | 39.05 | 1450  | 1448  | 0.011 ± 0.001 | – |
| 72       | 39.21 | 1454  | 1453  | – | 0.109 ± 0.002 |
| 73       | 39.44 | 1459  | 1454;1542 | – | 1.14 ± 0.03 |
| 74       | 39.53 | 1461  | 1457  | – | 0.056 ± 0.002 |
| 75       | 40.39 | 1482  | 1478  | – | 0.10 ± 0.01 |
| 76       | 40.53 | 1486  | 1480  | 3.17 ± 0.03 | 3.70 ± 0.02 |
| 77       | 40.75 | 1491  | 1489  | 0.28 ± 0.02 | 0.585 ± 0.003 |
| 78       | 41.14 | 1500  | 1493;1492 | – | 0.54 ± 0.01 |
| 79       | 41.53 | 1510  | 1509  | 2.26 ± 0.03 | – |
| 80       | 41.58 | 1511  | 1505  | – | 0.49 ± 0.04 |
| 81       | 41.87 | 1519  | 1513  | 3.76 ± 0.03 | 0.41 ± 0.04 |
| 82       | 42.18 | 1527  | 1522  | 0.63 ± 0.05 | – |
| 83       | 42.22 | 1528  | 1522  | – | 0.71 ± 0.03 |
| 84       | 42.49 | 1534  | 1533  | 0.15 ± 0.01 | – |
| 85       | 42.77 | 1541  | 1537  | 0.09 ± 0.01 | – |
| 86       | 43.68 | 1565  | 1561  | 0.87 ± 0.03 | – |
| 87       | 43.79 | 1567  | 1561  | – | 0.46 ± 0.02 |
| 88       | 44.38 | 1583  | 1577  | 1.375 ± 0.003 | – |
| 89       | 44.61 | 1589  | 1582  | 0.35 ± 0.04 | 6.225 ± 0.008 |
| 90       | 45.07 | 1600  | 1594  | – | 0.209 ± 0.006 |
| 91       | 45.69 | 1616  | 1608  | – | 0.151 ± 0.001 |
| 92       | 45.82 | 1620  | 1618  | 1.33 ± 0.07 | – |
| 93       | 46.80 | 1646  | 1638  | 7.44 ± 0.03 | 0.521 ± 0.002 |
| 94       | 47.17 | 1656  | 1649  | 0.33 ± 0.01 | – |
| 95       | 47.28 | 1659  | 1652  | 0.6 ± 0.2 | – |
Table 2. Cont.

| Compound                        | RT     | LRI a | LRI b | Quantification c (Relative %) |
|---------------------------------|--------|-------|-------|-----------------------------|
|                                 |        |       |       | O. basilicum cv.            |
|                                 |        |       |       | ‘Cinnamon’                  |
| a-Bisabolol                     | 48.33  | 1687  | 1685  | 0.499 ± 0.004               |
| Hexahydrofarnesyl acetone      | 52.07  | 1849  | 1847  | 0.105 ± 0.006               |
| Total identified                | 92.2 ± 0.2 | 88.3 ± 0.2 |            |                            |
| Monoterpenes                   | 4.6 ± 0.1 | 3.12 ± 0.06 |            |                            |
| Oxygenated monoterpenes        | 31.8 ± 0.2 | 55.1 ± 0.3 |            |                            |
| Sesquiterpenes                 | 13.1 ± 0.2 | 20.2 ± 0.1 |            |                            |
| Oxygenated sesquiterpenes      | 12.4 ± 0.2 | 8.1 ± 0.1 |            |                            |
| Other                           | 30.3 ± 0.5 | 1.79 ± 0.07 |            |                            |

a LRI, linear retention index determined on a SH-RX-5ms fused silica column (Shimadzu) relative to a series of n-alkanes (C8–C40). b linear retention index reported in literature (Adams, 2017) [10]. c relative % is given as mean ± SD, n = 3.

The volatile profile obtained for the O. × citriodorum sample showed striking differences when compared to other previous studies. Vieira and Simon (2006) [4] analyzed the essential oil from four accessions of O. × citriodorum grown in Purdue, USA, together with several O. basilicum cultivars, and reported the existence of two types of O. × citriodorum, one rich in citral isomers (neral + geranial) and another rich in methyl-chavicol, with three accessions belonging to the first type and one to the second. Despite presenting qualitative and quantitative differences, other studies supported the citral-rich type as the most commonly observed chemotype of O. × citriodorum [5,7,30,31], although other studies also confirmed the existence of methyl-chavicol-rich type [32]. Nevertheless, the volatile profile of the present studied sample does not fit either type as it presented linalool as major compound (32.8%) and citral being only the fifth major compound, representing 4.9% of total volatiles. Curiously, the obtained profile is much closer to the one reported by Vieira and Simon (2006) [4] for O. basilicum cultivar ‘Mrs. Burns’ Lemon’, which is also a basil plant with a strong lemon aroma [3].

3.2. Bioactive Properties

3.2.1. Antioxidant Activity

Table 3 shows the results obtained for the TBARS and OxHLIA assays. In what concerns the former assay, in general, the extracts of the two studied species were more efficient in inhibiting the formation of TBARS (resulting from the oxidation of the brain cell membranes, with consequent formation of malondialdehyde) when compared to the synthetic antioxidant Trolox, a water-soluble analogue of vitamin E. The IC_{50} of the extracts were significantly lower than that of Trolox, in a magnitude of approximately 10x for almost all extracts. This suggest a high efficacy in inhibiting the formation of TBARS, particularly for the infusions that showed the lowest IC_{50} values (8.9 ± 0.4 µg/mL for cinnamon basil and 14.1 ± 0.7 µg/mL for lemon basil).

Table 3. Results of the antioxidant activity assays (TBARS and OxHLIA) obtained for the infusion and hydroethanolic extracts of basil samples (mean ± SD, n = 3).

| Sample                      | TBARS (IC_{50} µg/mL) | OxHLIA (IC_{50} µg/mL) Δt = 60 min |
|-----------------------------|-----------------------|-----------------------------------|
| O. basilicum cv. ‘Cinnamon’ |                       |                                   |
| Infusion                    | 8.9 ± 0.4             | 27.6 ± 0.9                        |
| EtOH:H_{2}O                 | 23.8 ± 0.8            | 48 ± 2                            |
| O. × citriodorum            |                       |                                   |
| Infusion                    | 14.1 ± 0.7            | 26.9 ± 0.4                        |
| EtOH:H_{2}O                 | 15.6 ± 0.6            | 54 ± 1                            |
| Trolox                      | 139 ± 5               | 85 ± 2                            |
In the OxHLIA assay, in general, the $\Delta t$ values were well correlated to the tested extract concentrations, since higher concentrations promoted a higher hemolysis delay. Trolox, used as positive control, presented an IC$_{50}$ value of 85 ± 2 µg/mL, while the IC50 values for all the tested basil extracts were much lower. Additionally, in this assay, the infusions performed better than the hydroethanolic extracts, with the former requiring approximately half the concentration of the last to protect 50% of the erythrocyte population from the haemolytic action of the oxidative agent after 60 min. These results suggest that both the studied species have high antioxidant properties, therefore presenting a noteworthy potential to confer beneficial health effects when consumed in the infusion form.

To the best of our knowledge, there are no previous reports on the antioxidant activity of the infusions of these plants, assessed through cell-based assays and using Trolox as positive control. Nevertheless, previous studies have been conducted on the ability of the ethanolic extracts of $O$. basilicum cv. ‘Cinnamon’ and methanolic extracts of $O. \times$ citriodorum to act as antioxidants. Contrary to the results obtained in the present study, the extract of $O$. basilicum presented a lower antioxidant activity than t-butylhydroxytoluene (BHA) in the screening assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenge (Abramović et al., 2018), but the results are not directly comparable to the ones obtained in the present study because they are expressed as percentage of antioxidant activity and no IC$_{50}$ values are provided. Additionally, Hakkim et al. (2008) [28] found that the extract of $O. \times$ citriodorum tend to possess lower antioxidant activity than the positive control butylated hydroxy anisole (BHA) in DPPH, reducing power, superoxide anion scavenging activity and $\beta$-carotene-linoleic acid bleaching assays. On the other hand, in the study of Kaurinovic et al. (2011) [33], the aqueous extract of a non-identified cultivar of $O$. basilicum showed better antioxidant activity compared to BHT and BHA in the DPPH assay, and better activity than BHT in the neutralization of NO radical and H$_2$O$_2$. Additionally, the extracts were able to inhibit the lipid peroxidation in liposomes, with the largest inhibitory activity being exhibited by the ethyl acetate extract. Likewise, Touiss et al. (2019) [34] reported that a rosmarinic acid-rich extract prepared from a commercial sample of $O$. basilicum (non-identified cultivar) was able to significantly decrease the plasma total cholesterol, triglycerides, and LDL-cholesterol in high fat diet-induced hyperlipidemic mice and also prevent lipoprotein oxidation by 93% at a dose of 25µg/ml. These results are in accordance with the ones obtained in the present study, which corroborate a strong antioxidant activity for the studied Ocimum plant species.

3.2.2. Antimicrobial Activity

The results obtained for the antimicrobial activity of the two types of extracts prepared from $O$. basilicum and $O. \times$ citriodorum can be observed on Table 4. The results evidenced that all extracts present antibacterial activity since they were able to inhibit the growth of all tested strains, with the exception of P. mirabilis. Nevertheless, none of the extracts showed bactericidal activity at the tested concentrations. In general, better results were obtained for Gram-positive bacteria, with the lowest MIC (5 mg/mL) being observed for MRSA, a pathogenic nosocomial bacterium. These results are in line with previous studies on the antimicrobial activity of hydroethanolic extracts obtained from other plant species [35]. Observing the obtained MIC values (Table 4), one can conclude that the antimicrobial activity between the two types of extracts is very similar against most of the assayed strains, with the exception of MRSA for which lower MICs were obtained for the hydroethanolic extract. Better results were also obtained for the hydroethanolic extract of cinnamon basil against L. monocytogenes and for the hydroethanolic extract of lemon basil for against P. aeruginosa. Notwithstanding, the two basil samples showing similar results for most of the tested bacteria, slightly better activity was evidenced by the extracts of $O. \times$ citriodorum, in particular against the Gram-negative E. coli and K. pneumoniae. The antimicrobial activity evidenced by the extracts may be related to the presence of rosmarinic acid as major compound in these extracts since previous studies reported several biological properties associated to this phenolic acid, including antibacterial activity [36].
Table 4. Antimicrobial activity of the extracts obtained from the basil samples (mg/mL, mean ± SD, n = 3).

| Antimicrobial Activity | O. basilicum cv. 'Cinnamon' | O. × citriodorum | Ampicillin (20 mg/mL) | Imipenem (1 mg/mL) | Vancomycin (1 mg/mL) |
|------------------------|-----------------------------|-----------------|----------------------|-------------------|--------------------|
|                        | EtOH/H₂O Infusion           | EtOH/H₂O Infusion |                     |                   |                    |
| Gram-negative bacteria  |                             |                 |                      |                   |                    |
| E. coli                | 20                          | >20             | 10                   | >20               | <0.15              | <0.15              | <0.0078             | <0.0078             | n.t.               | n.t.               |
| K. pneumoniae          | 20                          | >20             | 10                   | >20               | 10                 | 20                 | <0.0078             | <0.0078             | n.t.               | n.t.               |
| M. morganii            | 10                          | >20             | 10                   | >20               | 10                 | 20                 | <0.0078             | <0.0078             | n.t.               | n.t.               |
| P. mirabilis           | >20                         | >20             | >20                  | >20               | >20                | >0.15              | <0.0078             | <0.0078             | n.t.               | n.t.               |
| P. aeruginosa          | 20                          | >20             | 20                   | >20               | >20                | >0.15              | <0.0078             | <0.0078             | n.t.               | n.t.               |
| Gram-positive bacteria |                             |                 |                      |                   |                    |
| E. faecalis            | 10                          | >20             | 10                   | >20               | <0.15              | <0.15              | n.t.               | n.t.               | <0.0078             | <0.0078             |
| L. monocytogenes       | 10                          | >20             | 10                   | >20               | <0.15              | <0.15              | <0.0078             | <0.0078             | n.t.               | n.t.               |
| MRSA                   | 5                           | >20             | 5                    | >20               | <0.15              | <0.15              | n.t.               | n.t.               | 0.25               | 0.5                |

MRSA- Methicillin resistant Staphylococcus aureus; MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration; n.t.: not tested.
3.2.3. Cytotoxic Activity and Anti-inflammatory Activity

The results for the anti-proliferative activity, hepatotoxicity, and anti-inflammatory activity of the aqueous and hydroethanolic extracts of the studied basil samples are shown in Table 5. The results are expressed in terms of GI50 values corresponding to sample concentration providing 50% of cell growth inhibition. As can be observed, the hydroethanolic extract of O. × citriodorum was the only that showed cytotoxicity against the four human tumor cell lines used with GI50 values ranging from 89 to 161 µg/mL. This was also the only extract that presented anti-inflammatory activity in the mouse macrophage-like cell line (RAW264.7) assay (Table 5). Nevertheless, it also exhibited cytotoxicity towards non-tumoral hepatocytes, despite presenting a higher GI50 value (234 ± 21 µg/mL) compared to the one obtained for cancer cell lines. Regarding the three other extracts, all of them were able to inhibit the growth of all tumoral cells with exception of non-small cell lung cancer (NCI-H460 cell line), without exhibiting cytotoxicity for non-tumor cells at the tested concentrations.

Table 5. Cytotoxic and anti-inflammatory activities of extracts obtained from the basil samples (mean ± SD, n = 3).

| Samples              | Extracts | Cytotoxic Activity GI50 Values (µg/mL) | Anti-Inflammatory Activity EC50 (µg/mL) |
|----------------------|----------|----------------------------------------|----------------------------------------|
| O. basilicum cv.     |          |                                        |                                        |
| 'Cinnamon'           | Infusion | >400                                   | >400                                   |
|                     | EtOH:H2O | 255 ± 6                                | 271 ± 8                                |
| O. × citriodorum     |          |                                        |                                        |
|                     | Infusion | >400                                   | >400                                   |
|                     | EtOH:H2O | 273 ± 14                               | 310 ± 5                                |

GI50 values correspond to the sample concentration achieving 50% of growth inhibition in human tumor cell lines or in liver primary culture PLP2. Ellipticine GI50 values: 1.21 µg/mL (MCF-7); 1.03 µg/mL (NCI-H460); 0.91 µg/mL (HeLa); 1.10 µg/mL (HepG2), and 2.29 µg/mL (PLP2). Dexamethasone EC50 value = 1.6 ± 0.2 µg/mL (RAW294.7).

Recently, Qamar et al. (2020) [37] screened the activity of O. basilicum (aerial parts non-identified cultivar) methanolic extract and fractions against several human cancer cell lines (HT-144, MCF-7, NCI-H460 and SF-268) using the same methodology as the one in this work. The authors reported that both the methanolic extract and the petroleum ether insoluble fraction showed growth inhibitory effects against all the four cell lines tested, with both exhibiting a selectively greater inhibition against the MCF-7 cell line. This is in good agreement with the results of the present work, in which MCF-7 cell line also presented the lowest GI50 value.

4. Conclusions

Until now, different studies are reported in the literature regarding Ocimum species, but few focused specifically on the two studied in this work, O. basilicum cv. Cinnamon and O. × citriodorum. The obtained results demonstrated that both plants are of great interest, both for their aromatic characteristics and composition in bioactive compounds, particularly of phenolic acids such as rosmarinic acid, but mainly for their bioactive properties. All the extracts presented relevant antioxidant activity with the infusions of both plants evidencing remarkable results in TBARS and OxLIA assays. The extracts of both basil species were able to inhibit different tumor cell lines, with the majority not affecting the normal liver cells. In addition, the hydroethanolic extract of lemon basil (O. × citriodorum) showed anti-inflammatory activity. In general, the overall results obtained for O. basilicum and O. × citriodorum support the use of both species in traditional medicine and confirm the relevance of these plants as a natural source of bioactive compounds both when consumed as foods or infusions.

Author Contributions: Conceptualization, L.B., J.S.A., I.C.F.R.F., Z.C. and B.R.-F.; Methodology, C.P., M.I.D., R.C.C., M.J.A.; Investigation, Č.P., M.I.D., R.C.C., M.J.A., C.M.; Resources, I.C.F.R.F.; Writing—first draft, L.B., J.S.A., Č.P., C.M.; Writing—review & editing—J.S.A., L.B., Č.P.; Supervision, I.C.F.R.F., J.S.A., Z.C.; All authors have read and agreed to the published version of the manuscript.
Funding: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020) and to national funding by FCT, P.I., through the institutional scientific employment program-contract for M. I. Dias, L. Barros and R. Calhelha contracts and C. Pereira contract though the celebration of program-contract foreseen in NO. 4, 5 and 6 of article 23º of Decree-Law NO. 57/2016, of 29th August, amended by Law NO. 57/2017, of 19th July. The authors are also grateful to the Interreg España-Portugal for financial support through the project 0377_Iberphenol_6_E and TRANSCoLAB 0612_TRANS_CO_LAB_2_P.

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

References
1. Rewers, M.; Jedrzejczyk, I. Genetic characterization of Ocimum genus using flow cytometry and inter-simple sequence repeat markers. Ind. Crops Prod. 2016, 91, 142–151. [CrossRef]
2. Ch, M.A.; Naz, S.B.; Sharif, A.; Akram, M.; Saeed, M.A. Biological and Pharmacological Properties of the Sweet Basil (Ocimum basilicum). Br. J. Pharm. Res. 2015, 7, 330–339. [CrossRef]
3. Meyers, M. Basil: An Herb Society of America Guide; The Herb Society of America: Kirtland, OH, USA, 2003; pp. 6–7.
4. Vieira, R.F.; Simon, J.E. Chemical characterization of basil (Ocimum spp.) based on volatile oils. Flavour Fragr. J. 2006, 21, 214–221. [CrossRef]
5. Avetisyan, A.; Markosian, A.; Petrosyan, M.; Sahakyan, N.; Babayan, A.; Aloyan, S.; Trchounian, A. Chemical composition and some biological activities of the essential oils from basil Ocimum different cultivars. BMC Complement. Altern. Med. 2017, 17, 60. [CrossRef] [PubMed]
6. Wesolowska, A.; Jadczak, D. Composition of the Essential Oils from Inflorescences, Leaves and Stems of Ocimum basilicum ‘Cinnamon’ Cultivated in North-western Poland. J. Essent. Oil Bear. Plants 2016, 19, 1037–1042. [CrossRef]
7. Paulus, D.; Valmorbida, R.; Ramos, C.E. Productivity and chemical composition of the essential oil of Ocimum x citriodorum Vis. according to ontogenetic and diurnal variation. J. Appl. Res. Med. Aroma. 2019, 12, 59–65. [CrossRef]
8. Bessada, S.M.; Barreira, J.C.; Barros, L.; Ferreira, I.C.; Oliveira, M.B.P. Phenolic profile and antioxidant activity of Coleostephus myconis (L.) Rchb. f.: An underexploited and highly disseminated species. Ind. Crops Prod. 2016, 89, 45–51. [CrossRef]
9. Spréa, R.M.; Fernandes, Â.; Calhelha, R.C.; Pereira, C.; Pires, T.C.S.P.; Alves, M.J.; Canan, C.; Barros, L.; Amaral, J.S.; Ferreira, I.C. Chemical and bioactive characterization of the aromatic plant Levisticum officinale WDJ Koch: A comprehensive study. Food Funct. 2020, 11, 1292–1303. [CrossRef]
10. Adams, R.P. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 5th ed.; Allured Pub Corp: TX, USA, 2017.
11. Pinela, J.; Barros, L.; Dueñas, M.; Carvalho, A.M.; Santos-Buelga, C.; Ferreira, I.C. Antioxidant activity, ascorbic acid, phenolic compounds and sugars of wild and commercial Tuberaria lignosa samples: Effects of drying and oral preparation methods. Food Chem. 2012, 135, S1028–S1035. [CrossRef]
12. Lockowandt, L.; Pinela, J.; Roriz, C.L.; Pereira, C.; Abreu, R.M.V.; Calhelha, R.C.; Alves, M.J.; Barros, L.; Bredol, M.; Ferreira, I.C.F.R. Chemical features and bioactivities of cornflower (Centaurea cyanus) L. capitula: The blue flowers and the unexplored non-edible part. Ind. Crops Prod. 2019, 128, 496–503. [CrossRef]
13. Abreu, R.M.; Ferreira, I.C.; Calhelha, R.C.; Lima, R.T.; Vasconcelos, M.H.; Adega, F.; Queiroz, M.J.R. Anti-hepatocellular carcinoma activity using human HepG2 cells and hepatotoxicity of 6-substituted methyl 3-aminothieno[3, 2-b] pyridine-2-carboxylate derivatives: In vitro evaluation, cell cycle analysis and QSAR studies. Eur. J. Med. Chem. 2011, 46, 5800–5806. [CrossRef] [PubMed]
14. Taofiq, O.; Calhelha, R.C.; Heleno, S.; Barros, L.; Martins, A.; Santos-Buelga, C.; Ferreira, I.C.F.R. The contribution of phenolic acids to the anti-inflammatory activity of mushrooms: Screening in phenolic extracts; individual parent molecules and synthesized glucuronated and methylated derivatives. Food Resear. Int. 2015, 76, 821–827. [CrossRef]
15. Jayasinghe, C.; Gotoh, N.; Aoki, T.; Wada, S. Phenolics composition and antioxidant activity of sweet basil (Ocimum basilicum L.). J. Agric. Food Chem. 2003, 51, 4442–4449. [CrossRef] [PubMed]
16. Lee, J.; Scagel, C.F. Chicoric acid found in basil (Ocimum basilicum L.) leaves. Food Chem. 2009, 115, 650–656. [CrossRef]
17. Hossain, M.B.; Rai, D.K.; Brunton, N.P.; Martin-Diana, A.B.; Barry-Ryan, C. Characterization of phenolic composition in Lamiaceae species by LC-ESI-MS/MS. J. Agric. Food Chem. 2010, 58, 10576–10581. [CrossRef] [PubMed]

18. Kwee, E.M.; Niemeyer, E.D. Variations in phenolic composition and antioxidant properties among 15 basil (Ocimum basilicum L.) cultivars. Food Chem. 2011, 128, 1044–1050. [CrossRef]

19. Harnafi, H.; Ramchoun, M.; Tits, M.; Wauters, J.; Frederich, M.; Angenot, L.; Aziz, M.; Alem, C.; Amrani, S. Phenolic acid-rich extract of sweet basil restores cholesterol and triglycerides metabolism in high fat diet-fed mice: A comparison with fenofibrate. Biomed. Prev. Nut. 2013, 3, 399–397. [CrossRef]

20. Koca, N.; Karaman, Ş. The effects of plant growth regulators and L-phenylalanine on phenolic compounds of sweet basil. Food Chem. 2015, 166, 515–521. [CrossRef]

21. Ruan, M.; Li, Y.; Li, X.; Luo, J.; Kong, L. Qualitative and quantitative analysis of the major constituents on L. Extracts. Origanum vulgare L. and prevents plasma lipid oxidation. Physiol, Pharmacol. 2019, 104(14), 2015–2022. [CrossRef] [PubMed]

22. Barros, L.; Dueñas, M.; Dias, M.I.; Sousa, M.J.; Santos-Buelga, C.; Ferreira, I.C.F.R. Phenolic profile of cultivated, in vitro cultured and commercial samples of Melissa officinalis L. Infusions. Food Chem. 2013, 136, 1–8. [CrossRef]

23. Chen, H.; Zhang, Q.; Wang, X.; Yang, J.; Qang, Q. Qualitative analysis and simultaneous quantification of phenolic compounds in the aerial parts of Salvia miltiorrhiza by HPLC-DAD and ESI/MSn. Phytochem. Anal. 2011, 22, 247–257. [CrossRef] [PubMed]

24. Javanmardi, K.A.; Kashi, A.; Bais, H.P.; Vivanco, J.M. Chemical characterization of Basil (Ocimum basilicum L.) found in local accessions and used in traditional medicines in Iran. J. Agric. Food Chem. 2002, 50, 5878–5883. [CrossRef] [PubMed]

25. Nguyen, P.M.; Kwee, E.M.; Niemeyer, E.D. Potassium rate alters the antioxidant capacity and phenolic concentration of basil (Ocimum basilicum L.) leaves. Food Chem. 2010, 123, 1235–1241. [CrossRef]

26. Zgórka, G.; Glowniak, K. Variation of free phenolic acids in medicinal plants belonging to the Lamiaceae family. J. Pharm. Biomed. Anal. 2008, 59, 184–189. [CrossRef]

27. Simeoni, M.C.; Pellegrini, M.; Sergi, M.; Pittia, P.; Ricci, A.; Compagnone, D. Analysis of Polyphenols in the Composition of Essential Oils from Five Varieties of Ocimum basilicum L. Cultivated in the Island of Kefalonia, Greece. Plants 2017, 6, 41. [CrossRef]

28. Carovic-Stanko, K.; Liber, Z.; Besendorfer, V.; Javornik, B.; Bohanec, B.; Kolak, I.; Satovic, Z. Genetic relations among basil taxa (Ocimum L.) based on molecular markers nuclear DNA content, and chromosome number. Plant Syst. Evol. 2010, 283, 13–22. [CrossRef]

29. Al-Kateb, H.; Mottram, D.S. The relationship between growth stages and aroma composition of lemon basil Ocimum citriodorum vis. Food Chem. 2014, 152, 440–446. [CrossRef]

30. Tangpao, T.; Chung, H.; Somnano, S.R. Aromatic Profiles of Essential Oils from Five Commonly Used Thai Basils. Foods 2018, 7, 175. [CrossRef]

31. Kaurinovic, B.; Popovic, M.; Vlaisavljevic, S.; Trivic, S. Antioxidant Capacity of Ocimum basilicum L. and Origanum vulgare L. Extracts. Molecules 2011, 16, 7401–7414. [CrossRef] [PubMed]

32. Touiss, I.; Harnafi, M.; Khatib, S.; Bekkouch, O.; Ouguirrem, K.; Amrani, S.; Harnafi, H. Rosmarinic acid-rich extract from Ocimum basilicum L. decreases hyperlipidemia in high fat diet-induced hyperlipidemic mice and prevents plasma lipid oxidation. Physiol. Pharmacol. 2019, 23, 197–207.

33. Silva, V.; Falco, V.; Dias, M.I.; Barros, L.; Silva, A.; Capita, R.; Alonso-Calleja, C.; Amaral, J.S.; Igrejas, G.; Ferreira, I.C.F.R.; et al. Evaluation of the phenolic profile of Castanea sativa Mill. by-products and their antioxidant and antimicrobial activity against multiresistant bacteria. Antioxidants 2020, 9, 87. [CrossRef] [PubMed]
36. Matejczyk, M.; Świsłocka, R.; Golonko, A.; Lewandowski, W.; Hawrylik, E. Cytotoxic, genotoxic and antimicrobial activity of caffeic and rosmarinic acids and their lithium, sodium and potassium salts as potential anticancer compounds. *Adv. Med. Sci.* 2018, 63, 14–21. [CrossRef]

37. Qamar, K.A.; Farooq, A.D.; Siddiqui, B.S.; Kabir, N.; Begum, S. Antiproliferative Effects of *Ocimum basilicum* Methanolic Extract and Fractions, Oleanolic Acid and 3-epi-Ursolic Acid. *Curr. Tradit. Med.* 2020, 6, 134–146. [CrossRef]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).