Chemical Composition and Agronomic Traits of *Allium sativum* and *Allium ampeloprasum* Leaves and Bulbs and Their Action against *Listeria monocytogenes* and Other Food Pathogens

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Abstract: In this work, we aimed to study the chemical composition of the essential oils from bulbs and leaves of two cultivars of *Allium sativum* L. and two of *A. ampeloprasum* L. var. *holmense*. Moreover, we investigated their activity against four common bacterial strains responsible for food contamination (*Listeria monocytogenes*, *Escherichia coli*, *Acinetobacter baumannii*, and *Staphylococcus aureus*) by formation of biofilms. The susceptibility of bacterial biofilms was evaluated by crystal violet assay, whereas the metabolic changes occurring in the bacterial cells were ascertained through the MTT test. The essential oils were characterized by the presence of most characteristic components, although with different composition between the species and the cultivars. The essential oils inhibited the capacity of the pathogenic bacteria to form biofilms (up to 79.85% against *L. monocytogenes* and/or acted on their cell metabolism (with inhibition of 68.57% and 68.89% against *L. monocytogenes* and *S. aureus*, respectively). The capacity of the essential oils to act against these foodborne bacteria could suggest further ideas for industrial applications and confirms the versatility of these essential oils as food preservatives.

Keywords: *Allium sativum*; *Allium ampeloprasum*; essential oil; biofilm; food pathogens

1. Introduction

The term *Allium* identifies a very large genus of monocotyledonous plants, including about 700 plant species, organized into 15 subgenera and 72 sections [1]. The subgenus *Allium* is the largest, comprising approximately 280 species [2], 114 of which make up its largest section, *Allium* [3], which includes economically important species, such as garlic (*A. sativum* L.) and leek (*A. ampeloprasum* L.). Its first use is as a condiment, but it is also employed for therapeutic purposes due to the properties attributed to it jointly by scientific investigation and traditional medicine. Due to its widespread cultivation, *A. sativum* is almost ubiquitous, with origins in central Asia but quickly spreading in the Mediterranean basin and already known in ancient Egypt [4]. *A. ampeloprasum* is native to all countries bordering the Black Sea, as well as the Adriatic and Mediterranean Seas, North Africa; it is also present in Ethiopia, Uzbekistan, Iran, and Iraq.

*Allium* plants are generally perennial and herbaceous. Their prevalent biological form is bulbous geophyte (G bulb). Roots are fasciculated and coming out from the terminal part of the bulb. The stem is characterized by a bulbaceous hypogeal part (rarely rhizomatous or simple tuberous-type roots), the bulbs of which can be singular or numerous (aggregated); small, with an elongated oval shape; or large and globose and covered by
a fibrous, reticulated, or smooth tunic surface. The epigeal part of the stem instead starts directly from the bulbs; some stems are fistulous, generally with a round section. At the base, the scape is wrapped in sheaths. Leaves are present in spirals, with an elongated, narrow, or enlarged shape but always flattened or almost cylindrical; in all cases, the length is preponderant over the width. These species have attracted human interest due to their flavor, taste, therapeutic properties, and ornamental value. For these reasons, they have been cultivated for thousands of years. Modern science confirmed that the plants of the genus *Allium* exhibit a wide variety of medicinal effects, such as defense against pathogens, prevention and treatment of cancer and cardiovascular disease, neuroprotection, hepatoprotection, and antifatigue effects [5–11].

*A. sativum* is one of the oldest cultivated species used in herbal medicine for therapeutic purposes in many cultures. Ancient medical texts documented medical applications of garlic. It has antihypertensive, anthelmintic, antioxidant, antithrombotic, antibiotic, antiseptic, and balsamic properties [12]. The species are differentiated into five cultivated vegetables, namely leek, elephant garlic, spring onion, kurran, and Persian leek.

As a plant with multiple properties, *A. ampeloprasum* is classified as an edible officinal plant and exploited for its wide therapeutic and health properties. In fact, since ancient times, it has also been used in folk medicine to promote digestion and treat malfunctioning of the intestines. It reduces blood pressure, helps in dissolving kidney stones, prevents cramps and colds, helps to lower cholesterol, and can decongest the respiratory tract [13].

The essential oils (EOs) of *A. sativum* and *A. ampeloprasum* largely reflect the general composition of the oils obtained from plants of this subgenus. Some differences distinguish the two species regarding the presence and concentration of certain compounds. *A. sativum* contains a much more varied pool of sulfur compounds than *A. ampeloprasum*. The latter is characterized by the same main compounds (especially dimethyl sulfide) but has a much smaller variety of components. Over the past 50 years, intense research evaluated the biological activity of the EOs of the genus *Allium*. The organosulfides contribute to its use as an antioxidant [14–17]. Allicin contributes to the anti-inflammatory property, and it would seem to be a good candidate for the treatment of inflammation-related neurodegenerative diseases, such as Alzheimer’s [18–22]. Allicin and sulfur compounds derived from alliin metabolism have been shown to promote apoptosis in neoplastic cells [23]. Sulfur compounds can decrease the hepatic synthesis of cholesterol and the oxidation of LDL and HDL [24,25]. Allyl propyl disulfide, allicin, cysteine sulfoxide, and S-allyl cysteine decrease blood sugar, fasting cholesterol lipids [26,27], and cellular sensitivity to insulin [28]. Some components of these EOs showed effect on obesity [29] and inhibit platelet aggregation [30–34]. *Allium* EOs have antiviral [35], antiprotozoal [36,37], antifungal [38,39], and antibacterial [40–43] activities.

In recent years, the rising occurrence of foodborne diseases has been correlated to an expansion of the presence, in foods, of some pathogenic bacteria, such as *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and the emergent pathogen *Acinobacter baumannii*, often with the capacity to exhibit the multiple-drug resistance (MDR) phenotype [44]. Several bacterial strains, including *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinobacter baumannii*, can produce biofilms, causing a serious problem for the food industry. This contamination can involve all stages of production, from harvesting and processing to storage [45–47]. The extracts of *A. sativum* exhibited clear evidence of antibacterial activity against different foodborne pathogenic bacteria [48]. Several papers reported the antibiofilm activity of *A. sativum*, but no studies reported antibiofilm activity exhibited by the EO of *A. ampeloprasum*. Caputo and colleagues reported the antibiofilm activity of extracts of two cultivars of *A. ampeloprasum* [49]. Thus, the utilization of *Allium* EOs can be of great importance in the food industry for the preservation of food from specific foodborne pathogens in all segments of the productive chain.

In the present work, we aimed to study the chemical composition of the EOs from two cultivars of *A. sativum* and two of *A. ampeloprasum* var. holmense, as well as their possible antibacterial activity against four pathogens of food interest—*Listeria monocytogenes*, *Acine-
2. Materials and Methods

2.1. Plant Material

Plants of two cultivars of *A. sativum*, cv ‘Rosso di Sulmona’ and cv ‘Rosso di Spagna’ and two cultivars of *A. ampeloprasum* var. *holmense*, cv ‘Contursi T.’ and cv ‘Irsinia’ were collected in May–June 2020. The cultivars were grown in an experimental field at Pon tecagnano (Salerno province, Southern Italy,) on a previously ploughed and fertilized fine-texture soil. Cloves of all cultivars were planted in the middle of November 2019 with a spacing of 10 cm (*A. sativum*) or 20 cm (*A. ampeloprasum*) in rows spaced 50 cm apart in order to obtain densities of 20 and 10 plants per m², for *A. sativum* and *A. ampeloprasum*, respectively. All cultivars were arranged in 5 m² plots (2.0 m × 2.5 m) according to a randomized block design with three reps. Moreover, the normal agronomic practices of local garlic growers were followed. At harvest time, samples of 10 plants randomly taken from each plot were analyzed for the morphological traits reported in Table 1.

### Table 1. Morphological traits of bulbs and cloves of garlic cultivars.

| Cultivars 1            | Species      | Bulb Skin Colour | Clove Skin Colour | Floral Stem | Bulb Mean Weight (g) | Bulb Equatorial Diameter (mm) | Cloves per Bulb | Clove Mean Weight (g) |
|------------------------|--------------|------------------|-------------------|-------------|----------------------|-------------------------------|-----------------|----------------------|
| 'Rosso di Sulmona'     | *A. sativum* | white            | red               | yes         | 41.2 (±0.8) a        | 48.1 (±0.9) b                  | 11.8 (±0.9) a    | 3.1 (±0.7) a         |
| 'Rosso di Spagna'      | *A. sativum* | cream            | red               | yes         | 50.3 (±1.4) a        | 54.6 (±1.0) a                  | 11.3 (±0.7) a    | 3.7 (±0.9) a         |
| 'Irsinia'              | *A. ampeloprasum* | cream 2 | l. brown 2 | yes         | 68.1 (±0.4) b        | 75.1 (±0.9) b                  | 5.3 (±0.6) a     | 11.4 (±0.2) b        |
| ‘Contursi T.’          | *A. ampeloprasum* | cream 2 | l. brown 2 | yes         | 75.0 (±0.9) a        | 84.2 (±1.1) a                  | 5.1 (±0.5) a     | 12.1 (±0.4) a        |

1 Means followed by the same letters in the same column and within each *Allium* species are not significantly (p ≤ 0.05) different. 2 l. brown = light brown.

2.2. Extraction of Essential Oils

Samples were cleaned of residues of soil and other material and dried for about one week at room temperature. The plant material was divided into aerial parts and bulbs, which, separated and classified, were extracted with methanol at room temperature. This extraction was repeated three times, renewing the solvent. The extracts were then filtered using paper filters and freed of excess methanol using a rotavapor. Subsequently, the samples, with the minimum amount of methanol, were placed in a flask half-filled with water and subjected to steam distillation, as reported in the European Pharmacopoeia [50]. The obtained essential oils were solubilized in *n*-hexane, dried in a nitrogen atmosphere, and stored in amber vials in a refrigerator at 4 °C.

2.3. Composition of the Essential Oils

The EO composition was studied by GC and GC-MS. GC analyses were performed using a Perkin-Elmer Sigma-115 gas chromatograph equipped with FID and data handling processor. A HP-5 MS fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was used, with the following operative conditions: column temperature: 40 °C, with 5 min initial hold, and then to 270 °C at 2 °C/min, 270 °C (20 min); injection mode splitless (1 µL of a 1:1000 *n*-hexane solution). Temperatures of injector and detector were 250 °C and 290 °C, respectively. Analysis was also performed with a fused silica HP Innowax polyethylene glycol capillary column (50 m × 0.20 mm i.d., 0.25 µm film thickness). In both cases, helium was used as carrier gas (1.0 mL/min). GC-MS analyses were carried out using an Agilent 6850 Ser. II apparatus, equipped with a fused silica DB-5 capillary column (30 m × 0.25 mm i.d., 0.33 µm film thickness), coupled to an Agilent Mass Selective Detector MSD 5973; ionization energy voltage 70 eV; electron multiplier voltage energy 2000 V. Mass spectra were scanned in the range 40–500 amu, scan time 5 scans/s. The GC conditions were as reported above; temperature of transfer line, 295 °C.
Most of the components were identified by comparing their Kovats indices (Ki) with those of the literature [51–53] and by analysis of the mass spectra compared to those of pure standards or to those reported in the NIST 02 and Wiley 257 mass libraries. The Kovats indices were determined related to a homologous series of n-alkanes (C10-C35), under the same operating conditions. For some compounds, the co-injection with standard samples confirmed the identification.

2.4. Antibacterial Properties of the Oils

Microorganisms and Culture Conditions

Gram-positive *Listeria monocytogenes* (ATCC 7644) and *Staphylococcus aureus* subsp. *aureus* (ATCC 25923) and Gram-negative *Acinetobacter baumannii* (ATCC 19606) and *Escherichia coli* (DSM 8579) were the tester bacterial strains. Bacteria were cultured in Luria–Bertani broth for 18 h at 37 °C (*A. baumannii* grew at 35 °C) and 80 rpm (Corning LSE, Pisa, Italy) for microbial analysis.

2.5. Minimal Inhibitory Concentration (MIC)

The MIC of each essential oil was evaluated through a resazurin microtiter-plate assay [54]. Multiwell plates were prepared in triplicate; then, they were incubated at 37 °C (35 °C for *A. baumannii*) for 24 h. The lowest concentration at which a colour change arose (from dark purple to colourless) determined the MIC value of each EO.

2.6. Biofilm Inhibitory Action of the EOs

The EOs capacity to influence the formation of bacterial biofilm was evaluated by the method of Caputo et al. [46] in flat-bottomed 96-well microtiter plates. Before the test, the overnight bacterial cultures were adjusted to 0.5 McFarland (1.5 × 10⁷ cells/mL, Densitometer cell density turbidity 0.3–15.0 McFarland, CAMLAB, Cambridge, United Kingdom) with fresh culture broth. Ten µL of the diluted cultures were placed in each well; then 10 µL/mL and 20 µL/mL of each EO and Luria-Bertani broth were added, for a final volume of 250 µL/well. Microplates were sealed with parafilm, to avoid the evaporation and incubated for 48 h at 37 °C (except for *A. baumannii*, incubated at 35 °C). Planktonic cells were removed and, subsequently, sterile PBS was used to wash the attached cells. Methanol (200 µL) was added to each well and kept for 15 min to fix the sessile cells. Methanol was discarded and the microplates were left to dry. The staining of the adhered cells was obtained addition of 200 µL of 2% *w/v* crystal violet solution, discarded after 20 min. Wells were lightly washed with sterile PBS and left to dry. Glacial acetic acid 20% *w/v* (200 µL) was added to obtain the release of the bound dye. The absorbance was measured at λ = 540 nm (Cary, Varian, Milano, Italy). The percent of adhesion was calculated respect to control; an inhibition of 0% was considered for cells without treatment. The tests were carried out in triplicate and the average results were taken for reproducibility.

2.7. Inhibition of Cell Metabolic Activity within the Biofilm

Two concentrations (10 µL/mL and 20 µL/mL) of the EOs were assessed for their capacity to inhibit the metabolic activity of the bacterial cells through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method [55]. After 48 h total of incubation, planktonic cells were removed, and 150 µL of PBS and 30 µL of 0.3% of MTT (Sigma, Milano, Italy) was added, keeping microplates at 37 °C (*A. baumannii* was incubated at 35 °C). After 2 h, the MTT solution was expelled, and two washing steps were performed with 200 µL of sterile physiological solution; then, 200 µL of dimethyl sulfoxide (DMSO, Sigma, Milano, Italy) was added to allow for the dissolution of the formazan crystals that were measured at OD = 570 nm (Cary, Varian, Milano, Italy) after 2 h.
2.8. Statistical Analysis

All assays were carried out in triplicate. Data of each experiment are expressed as the mean ± SD, and were statistically analyzed by two-way ANOVA, followed by Dunnet’s multiple comparisons test at a significance level of $p < 0.05$ using GraphPad Prism 6.0.

3. Results and Discussion

3.1. Morphological Traits of Bulbs and Cloves of Garlic Cultivars

As shown in Table 1, garlic bulbs and clove traits were significantly different among the tested cultivars. In particular, *A. ampeloprasum* showed larger bulbs and cloves than *A. sativum*. Conversely, the number of cloves per bulb was higher for *A. sativum*. Between *A. ampeloprasum* cultivars, cv ‘Contursi T.’ showed higher values for almost all traits, with the exception of the number of cloves per bulb, which remained significantly unchanged. Considering the *A. sativum* cultivars, mean weight and equatorial diameter of bulbs were significantly higher in ‘Rosso di Spagna’ compared to ‘Rosso di Sulmona’. Finally, the clove traits were not significantly different between the two cultivars.

3.2. Chemical Composition

The analysis of *A. ampeloprasum* var. *holmense* samples (Table 2) showed a quantitatively different composition between bulbs and aerial parts, even of the same cultivar. In the aerial parts of the cv. ‘Irsina’, 44 components were found, whereas in the bulbs, only 4 components were found. On the other hand, there is the opposite situation in the case of the cv ‘Contursi T.’, in which 10 components were found in the aerial parts, compared to 60 components found in the bulbs. The composition of the latter was particular, with a great variety of compounds generally present in low percentages. The composition reflects the data reported in the scarce literature available [33,56,57].

It must be emphasized that in many cases, the data on allicin do not correspond with what is reported in the literature. This is because the compound is very unstable and reactive and can rapidly decompose into other sulfur compounds. For this reason, compositional studies that have been characterized by different extraction or analysis techniques could report discordant data on the amount of allicin [58–60].

| Table 2. Chemical composition of EOs from *A. ampeloprasum* var. *holmense*, cultivars ‘Irsina’ and ‘Contursi T.’. |
|---|---|---|---|---|
| N. | ‘Irsina’ | ‘Contursi T.’ |
| | Aerial Parts | Bulbs | Aerial Parts | Bulbs | RT | KLs |
| 1 | 2,4-Dimethylhexane | - | 22.3 | - | - | 5.0 | 758 |
| 2 | 3-Methylthiophene | - | 3.4 | - | - | 7.4 | 788 |
| 3 | 2,2-bis (Methylthio)-1-propanol | - | - | 1.7 | - | 9.2 | 812 |
| 4 | 2,6-Dimethylnonane | 0.1 | - | - | - | 11.3 | 838 |
| 5 | 2,3,5,8-Tetramethyldecane | T | - | - | 1.3 | 12.2 | 851 |
| 6 | 4-Methyl-1-undecene | 0.1 | - | - | 0.3 | 12.3 | 852 |
| 7 | Diallyl disulfide | - | - | 0.1 | 0.2 | 12.9 | 860 |
| 8 | 2-Hydroxyethyl-disulfide | T | - | - | - | 15.2 | 889 |
| 9 | Borneol | 0.1 | - | - | - | 15.5 | 893 |
| 10 | Terpinen-4-ol | T | - | - | - | 15.9 | 898 |
| 11 | Tridecane | - | - | 0.3 | 0.4 | 17.9 | 925 |
| 12 | 1,1-Thiodis-1-butene | - | - | - | 0.3 | 18.2 | 928 |
| 13 | Dimethyl sulfide | 0.1 | - | - | 0.4 | 17.5 | 919 |
| 14 | (Z)-Methyl propenyl disulfide | T | - | - | 0.3 | 17.9 | 925 |
| 15 | 2,6,10-Trimethylundecane | T | - | - | 1.0 | 18.2 | 928 |
| 16 | Dodecyl sulfide | - | - | - | 0.1 | 18.5 | 932 |
| 17 | Dodecyl-7-en disulfide | T | - | - | 2.5 | 18.7 | 934 |
| 18 | Dodecyl-8-en disulfide | - | - | - | 0.4 | 18.9 | 937 |
| 19 | Methyl octane | - | - | - | 0.4 | 19.0 | 939 |
| 20 | Carvacrol | 4.1 | - | - | - | 19.2 | 941 |
| 21 | n-Heptene | - | - | - | 0.3 | 19.4 | 942 |
| N. | 'Irsina' Aerial Parts | 'Irsina' Bulbs | 'Contursi T.' Aerial Parts | 'Contursi T.' Bulbs | RT | KI |
|----|----------------------|----------------|-----------------------------|-------------------|----|----|
| 22 | 2-Methoxy-4-vinylphenol | - | - | 1.1 | 20.0 | 950 |
| 23 | Hexanal | 0.1 | - | 0.5 | 20.6 | 959 |
| 24 | (E)-Allyl propyl disulfide | 0.2 | - | 0.2 | 21.0 | 964 |
| 25 | (Z)-Allyl propyl disulfide | 0.1 | - | 0.1 | 22.1 | 979 |
| 26 | Hexanol | - | - | 0.8 | 22.2 | 979 |
| 27 | Octane | - | - | 1.2 | 22.4 | 982 |
| 28 | Decane disulfide | - | - | 0.8 | 23.1 | 992 |
| 29 | Geranyl isovalerate | - | - | 0.3 | 23.3 | 994 |
| 30 | Nonanal | 0.1 | - | 2.8 | 23.5 | 997 |
| 31 | Nonene | - | - | 3.3 | 24.0 | 1000 |
| 32 | Decene | 0.3 | - | 6.1 | 24.4 | 1005 |
| 33 | 2,4-Bis(1,1-dimethyl-ethyl)-phenol | 0.1 | - | 1.1 | 24.7 | 1009 |
| 34 | 2-Butyl-1-octanol | 0.3 | - | 1.6 | 25.0 | 1013 |
| 35 | Butyl octene | - | - | 2.2 | 25.2 | 1017 |
| 36 | n-Nonane | - | - | 1.0 | 26.8 | 1038 |
| 37 | (Z)-9-Octadecene | 0.1 | - | - | 26.5 | 1033 |
| 38 | Propyl trisulfide | - | - | 0.7 | 29.6 | 1075 |
| 39 | Ethyl 2-oxo-tetradecanoate | - | - | 2.1 | 27.8 | 1050 |
| 40 | Undecane | - | - | 0.8 | 28.4 | 1059 |
| 41 | Undecene | - | - | 0.9 | 28.6 | 1061 |
| 42 | Methyl propenyl trisulfide | - | - | 7.7 | 29.1 | 1068 |
| 43 | Methyl 12-methyltridecanoate | 0.8 | - | 0.5 | 29.5 | 1073 |
| 44 | Methyl triacontanoate | - | - | 0.7 | 29.6 | 1075 |
| 45 | Methyl 1,3,5-Trithiane | - | - | 2.1 | 27.8 | 1050 |
| 46 | Methyl pentadecanoate | 0.7 | - | - | 30.8 | 1088 |
| 47 | trans-Methyl 1,3-pentil-undadecanoate | 0.1 | - | - | 31.2 | 1097 |
| 48 | Propenyl trisulfide | 0.1 | - | 3.2 | 2.8 | 31.8 | 1098 |
| 49 | Propyl allyl disulfide | 0.9 | - | 34.4 | 14.7 | 31.9 | 1100 |
| 50 | Methyl 14-methyl-pentadecanoate | 0.4 | - | - | 32.8 | 1114 |
| 51 | Methyl 14-methyl-16-eicosanoate | 0.8 | - | - | 33.1 | 1116 |
| 52 | Methyl 11-esadecanoate | 0.9 | - | - | 33.4 | 1120 |
| 53 | 2-Hexyl-1-octanol | 0.1 | - | - | 33.5 | 1122 |
| 54 | Diallyl disulfide | 15.2 | 42.5 | - | - | 33.8 | 1126 |
| 55 | Propyl allyl trisulfide | 0.2 | - | - | 34.0 | 1129 |
| 56 | Methyl 14-methyl-esadecanoate | 0.4 | - | - | 34.9 | 1143 |
| 57 | Methyl 12-Hexyl-cyclopropan-octanoate | 0.2 | - | - | 35.0 | 1144 |
| 58 | Methyl Heptadecanoate | 0.4 | - | 0.5 | 35.5 | 1151 |
| 59 | Methyl (Z)-9-esadecanoate | 0.1 | - | 0.6 | 36.2 | 1160 |
| 60 | Allicin | 57.3 | 29.8 | 53.1 | 8.6 | 37.0 | 1171 |
| 61 | Methyl allicin | 7.0 | - | 3.2 | - | 37.3 | 1176 |
| 62 | Diallyl trisulfide | 2.6 | - | - | 1.0 | 37.6 | 1182 |
| 63 | Methyl 8,11-ottadecanoate | - | - | 1.2 | 37.9 | 1185 |
| 64 | Methyl 10-oxo-octadecanoate | - | - | 0.6 | 38.1 | 1187 |
| 65 | Methyl allyl trisulfide | - | - | 0.2 | 38.3 | 1191 |
| 66 | Methyl diallyl trisulfide | 0.7 | - | - | 0.3 | 38.4 | 1192 |
| 67 | Ethyl allyl trisulfide | - | - | - | 0.1 | 39.3 | 1199 |
| 68 | Ethyl diallyl trisulfide | 1.9 | - | 1.5 | 2.9 | 39.4 | 1199 |
| 69 | Vinyl diallyl trisulfide | 0.1 | - | - | 2.7 | 40.8 | 1221 |
| 70 | Propenyl trisulfide | 0.2 | - | - | 1.1 | 41.3 | 1229 |
| 71 | Heptadecan trisulfide | - | - | 1.8 | 41.7 | 1235 |
| 72 | Di-tert-dodecyl disulfide | T | - | - | - | 42.0 | 1241 |
| 73 | Octadecan trisulfide | - | - | 0.2 | 42.4 | 1247 |
| 74 | Pentadecan tetrasulfide | - | - | 0.3 | 0.9 | 43.4 | 1262 |
| 75 | Methyl esacosanoate | T | - | - | 0.4 | 43.9 | 1269 |
| 76 | Methyl 9,12-epithio-9,11-octadecanoate | - | - | 2.7 | 44.2 | 1274 |
| 77 | Diallyl tetrasulfide | - | - | 2.6 | 44.9 | 1284 |
| 78 | Propyl allyl tetrasulfide | - | - | 0.9 | 46.4 | 1300 |
| 79 | Methyl tetracosanoate | - | - | 1.1 | 46.8 | 1307 |
| 80 | Propyl 3-(octadeciloxi)-oleate | - | - | 0.5 | 47.1 | 1313 |
| 81 | Propyl pentyl tetrasulfide | - | - | 2.0 | 47.3 | 1315 |
| 82 | Cyclo octasulfide | - | - | 2.7 | 50.1 | 1360 |
| Total | 96.9 | 98.0 | 97.9 | 97.8 | | |
In all cases, the main compounds are sulfur compounds. Allicin appears to be the main component in the EOs from the aerial parts, with quantities that exceed 50% of the total—more precisely, 57.3% in the aerial parts of ‘Irsina’ and 53.1% in the aerial parts of ‘Contursi T.’.

The situation of bulbs is different, where allicin, despite being among the main compounds, is not the principal component. In fact, its percentages settle at 29.8% in the bulbs of ‘Irsina’ and 8.6% in the bulbs of ‘Contursi T.’.

The other main compounds differ depending on the plant. ‘Irsina’ contains high amounts of diallyl sulfide, which is the main component of the EO from the bulbs (42.5%), whereas the aerial parts contain 15.2% of this compound. The bulbs of ‘Contursi T.’ have propyl allyl sulfide as the main component (14.7%), whereas the aerial parts contain 34.4%.

These results agree with the literature, in particular with the studies by Satyal and colleagues (2017) [56] that showed that the majority of components of the EO of this species turn out to be the whole series of sulfur compounds, first of all diallyl disulfide, dipropyl disulfide, diallyl trisulfide, and dipropyl trisulfide.

The analysis of A. sativum samples showed (Table 3) a quantitatively richer composition as compared to that of the A. ampoloprasum samples. All the samples, except for the EO from the bulbs of A. sativum ‘Rosso di Sulmona’ and the aerial parts of A. sativum ‘Rosso di Spagna’, showed a very rich composition, in many cases exceeding 50 components, as in the case of the bulbs of the ‘Rosso di Spagna’ (77 components). The main components are the sulfur compounds. Allicin is once again the main component, with quantities exceeding 50%: 61.8% in ‘Rosso di Sulmona’ bulbs and 52.9% in the ‘Rosso Spagna’ bulbs. The aerial parts, on the other hand, contain lower quantities of allicin: 36.8% in ‘Rosso di Sulmona’ and 21.1% in cv ‘Rosso Spagna’. Diallyl disulfide appeared among the main components, becoming the most representative compound in the aerial parts of the ‘Rosso Spagna’ (48.5%). Other sulfur components were present: propyl allyl disulfide, contained in good amounts in the aerial parts of the ‘Rosso di Sulmona’ (30.6%).

Table 3. Chemical composition of the EOs of A. sativum, cultivars ‘Rosso di Sulmona’ and ‘Rosso di Spagna’.

| N. | Rosso di Sulmona | Rosso di Spagna |
|---|---|---|
|  | Aerial Parts | Bulbs | % Aerial Parts | Bulbs | RT | KI<sub>a</sub> |
| 1 | 2,4-Dimethylhexane | - | 1.7 | 17.1 | 0.1 | 5.0 | 757 |
| 2 | 3,31-Thiobis-1-propane | - | - | - | - | T | 6.4 | 776 |
| 3 | 3-Methyl-thiophene | - | - | - | - | T | 7.4 | 788 |
| 4 | 2,3-Dimethyl- thiophene | - | - | - | - | T | 7.7 | 792 |
| 5 | Methyl-2-propenyl-disulfide | - | - | - | - | T | 8.1 | 796 |
| 6 | α-Pinene | - | - | - | - | T | 8.5 | 803 |
| 7 | 2,2-Bis (Methylthio)-1-propanol | - | - | - | 0.6 | 9.2 | 812 |
| 8 | (-)-β-Pinene | - | - | - | T | 9.9 | 819 |
| 9 | 2,6-Dimethylnonane | - | - | - | T | 11.2 | 837 |
| 10 | D-Limonene | - | - | - | T | 11.4 | 840 |
| 11 | 1,1-Dimetoxy-cyclohexane | - | - | - | - | T | 12.2 | 850 |
| 12 | 2,3,5,8-Tetramethyl-decane | 0.7 | - | - | T | 12.3 | 851 |
| 13 | 4-Methyl-1-undecene | - | - | - | - | T | 12.4 | 853 |
| 14 | Butyl propenyl sulfide | - | - | - | T | 12.5 | 855 |
| 15 | Diallyl disulfide | - | - | - | 0.2 | 12.9 | 859 |
| 16 | 4-Ethenyl-1,2-dimethyl-benzene | - | - | - | T | 14.3 | 877 |
| 17 | Allyl-1-propenyl sulfide | - | - | - | T | 14.7 | 882 |
| 18 | 9-Hydroxystereryl-ethyl-disulfide | - | - | - | 0.1 | 15.1 | 888 |
| 19 | 2- Hydroxyethyl- disulfide | 0.2 | - | - | - | 15.2 | 889 |
| 20 | Benzyl methyl sulphide | - | - | - | T | 15.3 | 889 |
| 21 | 3,4-Dimethyl-thiophene | - | - | - | 0.1 | 15.4 | 891 |
| 22 | 2-Ethyl-5-[(2-ethylbuthyl) thio]-thiophene | 0.1 | - | - | 0.2 | 16.1 | 901 |
| 23 | Bis(1,1-dimethylpropyl) -dilsulfide | - | - | - | 0.1 | 16.4 | 905 |
| 24 | Tridecane | 0.4 | - | - | - | 16.6 | 907 |
| 25 | 1,1-Thiobis-1-butine | - | - | - | T | 16.9 | 910 |
| N. | Rosso di Sulmona | Rosso di Spagna | RT | KI<sub>a</sub> |
|----|-----------------|----------------|----|-------------|
| 27 | Dimethyl disulfide | 0.2 - - 0.1 | 17.6 | 921 |
| 28 | (Z)-Methyl propenyl disulfide | 2.9 - - T | 17.9 | 925 |
| 29 | (E)-Methyl propenyl disulfide | - - - | 18.0 | 926 |
| 30 | 2,6,10-Trimethyl-dodecane | - - - | 18.2 | 928 |
| 31 | Dodecyl sulfide | - - - | 18.5 | 931 |
| 32 | Dodecyl-7-en disulfide | - - - | 18.6 | 934 |
| 33 | Dodecyl-8-en disulfide | - - - | 18.9 | 937 |
| 34 | 2-Methoxy-4-vinylphenol | - - 0.1 | 19.8 | 948 |
| 35 | (E)-Allyl propyl disulfide | 0.4 - 0.6 | 21.0 | 965 |
| 36 | (Z)-Allyl propyl disulfide | 3.9 - 0.3 | 22.1 | 979 |
| 37 | Methyl 9-oxo-nonanoate | - - T | 22.8 | 983 |
| 38 | Decane disulfide | - - T | 23.1 | 992 |
| 39 | Geranyl isovalerate | - - T | 23.3 | 994 |
| 40 | Nonene | 1.1 - 2.7 | 24 | 1000 |
| 41 | Decene | 1.3 - 0.4 | 24.2 | 1002 |
| 42 | 2,4-Bis(1,1-dimethyl-ethyl)-phenol | 1.1 - 0.7 0.3 | 24.7 | 1009 |
| 43 | 2-Butyl-1-octanol | 1.5 - 0.6 | 25 | 1013 |
| 44 | (E)-9-Octadecene | 0.2 - T | 26.5 | 1033 |
| 45 | 4-Methyl-1-undecene | 0.5 - 0.1 | 26.5 | 1034 |
| 46 | 1,3,5-Trithiane | 2.1 - 0.2 | 27.8 | 1051 |
| 47 | Methyl propenyl disulfide | - - 0.6 | 28.6 | 1064 |
| 48 | Methyl 12-methyl-tridecanoate | 2.6 - 0.2 | 29.5 | 1073 |
| 49 | Methyl tricatanoate | - - 0.1 | 29.6 | 1074 |
| 50 | Ethyl 2-oxo-tetradecanoate | - - T | 30 | 1079 |
| 51 | Methyl pentadecanoate | - - 0.1 | 30.8 | 1091 |
| 52 | Methyl 12-methyl-tetradecanoate | - - 0.2 | 31 | 1093 |
| 53 | Methyl trans-3-pentil-oxiran-undecanoate | - - 0.3 | 31.6 | 1094 |
| 54 | Propenyl trisulfide | 0.8 - 0.2 | 31.8 | 1096 |
| 55 | Propyl allyl disulfide | - - 0.1 | 31.9 | 1099 |
| 56 | Propyl allyl trisulfide | 30.6 - 4.9 - | 32.0 | 1100 |
| 57 | Vinyl trisulfide | - - 0.1 | 32.5 | 1108 |
| 58 | Methyl 14-methyl-pentadecanoate | - - 0.2 | 3.8 | 1113 |
| 59 | Methyl (Z)-9-esadecanoate | - - 0.7 | 33.1 | 1116 |
| 60 | Diallyl disulfide | - - 34.5 48.5 | 12.6 | 1125 |
| 61 | Methyl 2-hexyl-cyclopentanopropanoate | - - 0.2 | 35.1 | 1144 |
| 62 | Tridecan trisulfide | 0.3 - 0.2 | 35.5 | 1151 |
| 63 | Sulfide cyclooctatomic | - - T | 35.9 | 1156 |
| 64 | Methyl (Z)-11-octadecanoate | - - T | 36.2 | 1160 |
| 65 | Allyl<sub>c</sub>c | 36.8 61.8 21.1 | 52.9 | 1170 |
| 66 | Methyl allin<sub>c</sub>c<sub>c</sub> | 9.8 - 1.8 | 37.4 | 1177 |
| 67 | Methyl 8,11-octadecadienoate | - - - | 43.7 | 1182 |
| 68 | Diallyl trisulfide | - - - | 53.8 | 1183 |
| 69 | Methyl diallyl trisulfide | 0.4 - 0.8 | 39.0 | 1194 |
| 70 | Ethyl diallyl trisulfide | 0.1 - 5.1 | 39.6 | 1203 |
| 71 | Vinyl diallyl trisulfide | - - 0.2 | 40.8 | 1222 |
| 72 | Heptadecan trisulfide | - - 0.5 | 41.6 | 1234 |
| 73 | Di-tert-dodecyl disulfide | 0.2 - 0.8 | 42.0 | 1241 |
| 74 | Octadecan trisulfide | - - 1.7 | 42.5 | 1248 |
| 75 | Tridecan tetrasulfide | - - 0.1 | 43.1 | 1258 |
| 76 | Pentadecan tetrasulfide | - - 0.6 | 43.2 | 1262 |
| 77 | Methyl exacosanoate | - - 0.1 | 43.9 | 1269 |
| 78 | Methyl 9,12-epithio-9,11-octadecanoate | - - 0.7 | 44.0 | 1270 |
| 79 | Diallyl tetrasulfide | - - 0.3 | 44.9 | 1282 |
| 80 | Propyl allyl tetrasulfide | - - 0.1 | 45.4 | 1292 |
| 81 | Methyl triacontanoate | - - 0.4 0.3 | 45.5 | 1294 |
| 82 | Methyl tetraicosanoate | - - 0.1 | 46.8 | 1308 |
| 83 | Propyl 3-octadeclioxi-oleate | - - 0.4 | 47.1 | 1312 |

RT = retention time; KI = Kovats Index on an HP5 MS capillary column; T = traces, less than 0.05%; - = absent.
In this case, the results are in agreement with the literature [61,62], which reported a massive presence of sulfur compounds among which stand out dimethyl disulfide, diallyl disulfide, allyl methyl disulfide, propyl allyl disulfide, methyl propenyl disulfide, and diallyl trisulfide.

3.3. Biofilm Inhibitory Capacity of the EOs

The capacity of the EOs to inhibit bacterial biofilm formation and the metabolism of the bacterial cells within biofilm was assessed through crystal violet and MTT tests, respectively, using two concentrations—10 \( \mu \)L/mL and 20 \( \mu \)L/mL, amply lower than the minimal inhibitory concentration—calculated by the resazurin test and shown in Tables 4 and 5.

**Table 4.** Minimal inhibitory concentration (\( \mu \)L/mL) of the EOs from cultivars of *A. ampeclusum* var. *holmense* necessary to inhibit the growth of the pathogenic bacterial strains *Listeria monocytogenes*, *Escherichia coli*, *Acinetobacter baumannii*, and *Staphylococcus aureus*.

|          | *A. baumannii* | *E. coli* | *L. monocytogenes* | *S. aureus* |
|----------|----------------|-----------|--------------------|-------------|
| ‘Irsina’ | Aerial parts   | 30 \( \pm \) 2 | 40 \( \pm \) 3     | 30 \( \pm \) 3 | 30 \( \pm \) 3 |
|          | Bulbs          | 30 \( \pm \) 3 | 30 \( \pm \) 3     | 30 \( \pm \) 2 | 30 \( \pm \) 3 |
| “Contursi T.” | Aerial parts | 30 \( \pm \) 3 | 28 \( \pm \) 2     | 40 \( \pm \) 3 | 28 \( \pm \) 2 |
|          | Bulbs          | 40 \( b \) \( \pm \) 2 | 35 \( a \) \( \pm \) 3 | 30 \( \pm \) 2 | 30 \( \pm \) 2 |
| Tetracycline |                | 31 \( \pm \) 1 | 24 \( \pm \) 3     | 39 \( \pm \) 2 | 38 \( \pm \) 2 |

The experiments were performed in triplicate and reported as the mean (\( \pm \)SD). \( a: p < 0.1; b: p < 0.0001; c: p < 0.0001 \) compared with the tetracycline used as control (ANOVA followed by Dunnett’s multiple comparison test).

**Table 5.** Minimal inhibitory concentration (\( \mu \)L/mL) of the EOs from two cultivars of *A. sativum* necessary to inhibit the growth of the pathogenic bacterial strains *Listeria monocytogenes*, *Escherichia coli*, *Acinetobacter baumannii*, and *Staphylococcus aureus*.

|          | *A. baumannii* | *E. coli* | *L. monocytogenes* | *S. aureus* |
|----------|----------------|-----------|--------------------|-------------|
| ‘Rosso di Sulmona’ | Aerial parts | 30 \( \pm \) 2 | 40 \( a \) \( \pm \) 3 | 30 \( \pm \) 3 | 40 \( \pm \) 2 |
|          | Bulbs          | 30 \( \pm \) 2 | 30 \( b \) \( \pm \) 3 | 30 \( \pm \) 3 | 40 \( \pm \) 2 |
| ‘Rosso di Spagna’ | Aerial parts | 30 \( \pm \) 4 | 30 \( b \) \( \pm \) 2 | 30 \( \pm \) 4 | 30 \( \pm \) 2 |
|          | Bulbs          | 30 \( \pm \) 2 | 35 \( a \) \( \pm \) 2 | 30 \( \pm \) 3 | 28 \( \pm \) 3 |
| Tetracycline |                | 31 \( \pm \) 1 | 24 \( \pm \) 3     | 39 \( \pm \) 2 | 38 \( \pm \) 2 |

The experiments were performed in triplicate and reported as the mean (\( \pm \)SD). \( a: p < 0.1; b: p < 0.0001 \) compared with the tetracycline used as control (ANOVA followed by Dunnett’s multiple comparison test).

A biofilm is an amassing of microorganisms on animate and inanimate surfaces with the support of extracellular polymeric substance (formed by proteins, polysaccharides, and nucleic acids), which has an important function in infection and bacterial resistance [61]. Biofilm formation facilitates such survival in the body [62]. Biofilms are considered important with respect to microbial survival and growth in the food industry. In fact, microbial growth in biofilms protects microorganisms against clean-up and sterilization and makes them more difficult to remove [63]. The antibacterial activity of the essential oil of *A. sativum* against many pathogenic bacteria, including antibiotic-resistant bacteria, such as the Shiga toxin-producing *E. coli* (STEC) [64] and the methicillin-resistant *Staphylococcus aureus* (MRSA), is well documented [65–67]. The EOs of the *Allium* variety bulbs were generally able to inhibit the formation of biofilm by the Gram-positive *L. monocytogenes*, which is an ubiquitous pathogen representing a major alarm for the food industry because it is an agent of the serious foodborne illness listeriosis. This bacterium can contaminate food products during different phases of processing, introduced to food industry environments by many means. *L. monocytogenes* may grow in biofilms, so it can be more protected against the environmental factors that tend to eradicate it. Some studies reported that the adherence to surfaces by *L. monocytogenes* is very important for its survival and persistence.
in food. When included in biofilm, this bacterium becomes more difficult to be removed. In recent decades, different approaches have been proposed to impede the adhesion of \textit{L. monocytogenes}; however, they are difficult to be applied due to high costs and problems of resistance by the bacterium \cite{68}. Thus, the world of natural biomolecules has been studied to find new solutions to limit the proliferation and virulence of \textit{L. monocytogenes} during the steps concurring with food production \cite{69}. From this point of view, therefore, our results seem very interesting. Because the crucial point of the growth and virulence of \textit{L. monocytogenes} is its ability to adhere to surfaces (organic or inorganic), our data demonstrate that some of the extracts tested are capable of limiting such bacterial capacity. In fact, Table 6 indicates that the EO from the leaves of “Irsina” was capable of inhibiting up to 79.95\% of the adhesive capacity of \textit{L. monocytogenes}; such capacity was observed, although weaker, by testing 20 \mu L/mL of the EO from the bulbs of ‘Contursi T.’. The cultivars ‘Rosso di Sulmona’ and ‘Rosso di Spagna’ were capable of inhibiting the adhesion capacity of \textit{L. monocytogenes}, with percentages of inhibition up to 64.11\% and 61.22\%, respectively. The action exhibited by these EOs vs. \textit{L. monocytogenes} is in agreement with the literature. JadHAV and colleagues \cite{70} and Sandasi and colleagues \cite{71} showed that different EOs obtained from culinary and/or medicinal plants are capable of acting in reducing the attack of \textit{L. monocytogenes} cells ab origine and therefore of influencing the formation of a subsequent biofilm by this microorganism. Recently, Somrani and colleagues \cite{72} reported an excellent inhibitory biofilm activity by commercial EOs of \textit{A. sativum} and \textit{A. cepa}. However, biochemical variations of plants, which also affect their biological properties, can be related to the effects of genetic diversity, geographical origin, time of harvest, and the procedural methods used for the extraction \cite{73}.

In our experiments, the EOs from \textit{A. sativum} were generally able to inhibit the formation of biofilms of all the bacterial strains tested. Furthermore, except in a few cases, all EOs were able to inhibit the formation of the biofilm of \textit{A. baumannii}, a Gram-negative coccus known to cause nosocomial infections \cite{74}, where it provokes up to 30\% mortality \cite{75}. The EOs of \textit{A. sativum} were also capable of inhibiting biofilm formation by \textit{S. aureus}. In this case, the behavior exhibited by the EOs was different. In fact, the inhibitory action exhibited by the EO from the bulbs of ‘Rosso di Spagna’ was stronger than that of the EO from aerial parts (70.29\% and 44.39\%, respectively). Conversely, the EO of the aerial parts of ‘Rosso di Sulmona’ were more capable of inhibiting the \textit{S. aureus} biofilm, with an inhibition value of 33.48\% (with respect to 1.12\% shown by the EO from bulbs, which was almost completely ineffective against \textit{S. aureus}).

It is also important to emphasize the inhibitory efficacy exerted by the EOs vs. \textit{E. coli}. In fact, at the highest concentration of EO used in the experiments, all the EOs of \textit{A. sativum} proved capable of inhibiting, albeit with greater or lesser vigor, the biofilm of this bacterium, reaching inhibition percentages up to 54.09\% (EO of the bulb of ‘Rosso di Spagna’). Our data disagree with those reported by Yang and colleagues. \cite{76}. On the other hand, the cv ‘Rosso di Sulmona’, the bulbs of which contained more than double the allicin (61.8\%), was slightly less effective in inhibiting the biofilm formed by this microorganism (41.20\% inhibition). The two cultivars of \textit{A. ampeloprasum} proved capable of inhibiting the formation of bacterial biofilms with varying effectiveness. The EO from the cv. “Irsina” proved to be more effective than the EO from the cv ‘Contursi T.’ in the sense that it was able to inhibit—more or less with the same effectiveness—the formation of the biofilms of the four bacteria. The EO obtained from the aerial parts of ‘Contursi T.’, although ineffective vs. \textit{L. monocytogenes}, was able to achieve up to 81.88\% inhibition of the \textit{E. coli} biofilm and up to 73.47\% of that formed by \textit{S. aureus}. The EO obtained from the bulbs of ‘Contursi T.’ was ineffective vs. \textit{A. baumannii} but managed to inhibit the biofilm of the other three pathogens, with inhibition percentages ranging between 25.39\% (vs. \textit{E. coli}) and 61.41\% (vs. \textit{S. aureus}). Few reports reported the antibacterial effects of the EOs from \textit{A. ampeloprasum}. Methanolic extracts from bulbs and aerial parts of this species demonstrated biofilm-inhibitory activity against \textit{L. monocytogenes}, \textit{E. coli}, \textit{P. aeruginosa}, and \textit{S. aureus} \cite{49}. 
Table 6. Inhibitory activity of the EOs from the cultivars of *A. ampeloprasum* var. *holmense* and *A. sativum* on the biofilm formation capacity of four pathogenic strains.

|                     | A. baumannii | E. coli | L. monocytogenes | S. aureus |
|---------------------|--------------|---------|------------------|-----------|
|                     | 47.18 ± 1.59 | 20.65 ± 3.2 | 11.11 ± 1.8 | 73.47 ± 1.91 |
| 'Irsina' Aerial parts 10 µL/mL | 72.68 ± 1.42 | 22.58 ± 0.93 | 79.85 ± 1.05 | 57.97 ± 1.11 |
| 'Irsina' Bulbs 10 µL/mL | 7.35 ± 1.73 | 18.82 ± 3.08 | 52.58 ± 1.25 | 41.96 ± 1.15 |
| 'Irsina' Bulbs 20 µL/mL | 52.83 ± 1.14 | 45.95 ± 0.81 | 63.24 ± 1.72 | 50.17 ± 0.82 |
| 'Contursi T.' Aerial parts 10 µL/mL | 45.86 ± 1.31 | 81.88 ± 1.21 | 0 | 61.41 ± 1.61 |
| 'Contursi T.' Bulbs 10 µL/mL | 0 | 25.39 ± 1.28 | 40.69 ± 1.57 | 25.52 ± 1.59 |
| 'Contursi T.' Bulbs 20 µL/mL | 12.25 ± 2.35 | 24.73 ± 1.76 | 37.60 ± 1.40 | 1.12 ± 0.13 |
| 'Rosso di Sulmona' Aerial parts 10 µL/mL | 48.55 ± 1.52 | 41.20 ± 3.37 | 42.03 ± 0.54 | 9.47 ± 0.67 |
| 'Rosso di Sulmona' Aerial parts 20 µL/mL | 46.08 ± 2.56 | 41.56 ± 3.34 | 40.98 ± 4.32 | 43.49 ± 2.16 |
| 'Rosso di Sulmona' Bulbs 10 µL/mL | 3.17 ± 3.17 | 36.31 ± 1.47 | 64.11 ± 0.74 | 33.48 ± 2.16 |
| 'Rosso di Spagna' Aerial parts 10 µL/mL | 49.91 ± 2.75 | 54.09 ± 1.77 | 46.88 ± 0.71 | 44.39 ± 1.44 |
| 'Rosso di Spagna' Aerial parts 20 µL/mL | 26.62 ± 3.02 | 0 | 44.84 ± 4.64 | 15.25 ± 0.38 |
| 'Rosso di Spagna' Bulbs 20 µL/mL | 56.97 ± 1.73 | 27.16 ± 1.66 | 61.22 ± 2.09 | 70.29 ± 0.14 |

Results are expressed as percentages (mean ± SD) and calculated assuming the control (untreated bacteria, for which we assumed an inhibitory value = zero). a: p < 0.1 compared with the control (ANOVA followed by Dunnett's multiple comparison test).

Action of EOs against Bacterial Metabolism

Through the MTT test, the potential of EOs to inhibit the metabolism of bacterial cells present within the biofilm was also evaluated. The results are shown in Table 7. The EOs from both the aerial parts and bulbs of *A. ampeloprasum* were overall able to act on the metabolism of the microbial cells present within the biofilm. In the case of the tests carried out against *L. monocytogenes*, the results obtained with the EOs from ‘Irsina’ and ‘Contursi T.’ corroborated the already interesting data obtained by the crystal violet test. In fact, in this case, the EOs demonstrated an ability not only to limit the adhesive capacity of *L. monocytogenes* but also to affect, albeit more weakly, its metabolism. The EO from ‘Contursi T.’ showed an inhibitory effect of up to 25.28%; the EO from ‘Irsina’ was slightly more labile when tested against *L. monocytogenes* and *S. aureus*. However, bearing in mind that the action of the oils was particularly effective on the formation of the biofilm of these microorganisms, we can affirm that the two EOs of *A. ampeloprasum* tested turned out to be able to fight the pathogenicity of these four microbial strains, either by acting on the formation of the biofilm or by inhibiting those biochemical changes that affect the cells enveloped and protected by the biofilm and which determine the triggering of a series of biochemical events that lead
the bacterium to prove itself more resistant, even to antibiotics [69]. This was also observed with the EOs of *A. sativum*, which, in some cases, in the face of an incisive biofilm-inhibitory activity, did not exhibit an equal activity on cellular metabolism. This was the case, for example, of the action exerted by the EOs vs. *E. coli*, as in the case of the EOs from ‘Rosso di Sulmona’. On the other hand, the EOs from aerial parts of ‘Rosso di Spagna’ did not show the same inhibitory capacity, being able to exert an inhibition of, at most, 18.27% and only at the highest dose tested.

**Table 7.** Inhibitory activity of the EOs from the cultivars of *A. ampeloprasum* var. *holmense* and *A. sativum* on the cell metabolism of the pathogenic strains within the biofilm.

|                | A. baumannii | E. coli | L. monocytogenes | S. aureus |
|----------------|--------------|---------|------------------|-----------|
| 'Irsina' Aerial parts 10 µL/mL | 0           | 18.26±1.52 | 0                | 0         |
| 'Irsina' Aerial parts 20 µL/mL | 30.13±0.28  | 71.08±2.47 | 31.43±0.31       | 55.99±1.1 |
| 'Irsina' Bulbs 10 µL/mL          | 0           | 0       | 0                | 39.17±1.15|
| 'Irsina' Bulbs 20 µL/mL          | 14.48±0.13  | 0      | 30.25±0.68       | 47.81±0.82|
| 'Contursi T.' Aerial parts 10 µL/mL | 76.15±0.91 | 63.86±2.13 | 8.65±0.68       | 6.55±0.23 |
| 'Contursi T.' Aerial parts 20 µL/mL | 89.47±0.86 | 76.71±0.97 | 24.58±1.37      | 36.07±2.32|
| 'Contursi T.' Bulbs 10 µL/mL     | 69.28±1.37  | 65.08±0.99 | 8.46±0.55       | 7.97±0.94 |
| 'Contursi T.' Bulbs 20 µL/mL     | 81.14±0.27  | 79.15±0.43 | 25.28±0.88      | 36.19±2.18|
| 'Rosso di Sulmona' Aerial parts 10 µL/mL | 16.94±1.13 | 68.57±0.89 | 30.34±2.16      | 0         |
| 'Rosso di Sulmona' Aerial parts 20 µL/mL | 36.46±0.68 | 0    | 8.19±1.43       | 0         |
| 'Rosso di Sulmona' Bulbs 10 µL/mL | 65.16±0.85  | 0        | 11.13±1.65      | 0         |
| 'Rosso di Sulmona' Bulbs 20 µL/mL | 45.88±1.50  | 4.27±0.69 | 13.61±1.65      | 68.89±1.45|

Results are expressed as percentages (average ± SD) and calculated assuming the control (untreated bacteria, for which we assumed an inhibitory value= zero). a: p < 0.1 compared with the control (ANOVA followed by Dunnett’s multiple comparison test).

The different effects of the four EOs confirmed once again that the EOs can act as antibiofilm agents, as amply demonstrated with other essential oils [77,78]. Our data show that the antibiofilm activity of these EOs is probably due to the ample presence of allicin and diallyl disulfide. These data are in agreement with the recent literature [79–82].

**4. Conclusions**

In this work, we showed that there is diversity in the chemical composition between the two species of *A. ampeloprasum* var. *holmense* and *A. sativum* and within the same species between the cultivars. The chemical compositions confirmed the presence of the main and most characteristic compounds as allicin and sulfur compounds, as reported in literature. These compounds were responsible for biological activities. The essential oils obtained, although differing in efficacy, demonstrated their capability to act against the formation of new biofilms, which is a key step in the increase in virulence of pathogenic bacteria, mainly for *L. monocytogenes*. Our results comfort us about the possibility of using these essential oils as potential preserving agents in food manufacturing, for instance, in the manufacturing of fermented meats, where the taste and smell of *Allium* EOs (both *A. sativum* and *A. ampeloprasum*) used as ingredients at the right concentrations do not have a negative effect from a sensorial point of view and can safeguard the products without affecting their quality. Moreover, from our data, it is possible hypothesize the use of these EOs both
during the manufacturing processes and on the finished product; on this latter, they can be used as a food additive to maintain the biological properties described above. However, the EOs must be used mainly during the manufacturing process to avoid the formation of biofilms on the total product. In fact, if EOs were used only on the finished product, there would be an antibacterial action only on the external parts. The most promising EOs appear to be those extracted from aerial parts and bulbs of A. ampioprasum ‘Irsina’ and from aerial parts of A. sativum ‘Rosso di Spagna’.

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References

1. Friesen, N.; Fritsch, R.M.; Blattner, F.R. Phylogeny and new intrageneric classification of Allium (Alliaceae) based on nuclear ribosomal DNA ITS sequences. J. Syst. Evol. Bot. 2006, 22, 372–395. [CrossRef]
2. Hanelt, P.; Schultze-Motel, J.; Fritsch; Kruse, J.R.; Maass, H.I.; Ohle, H.; Pistrick, K. Infrageneric grouping of Allium—The Gatersleben approach. In Proceedings of the International Symposium, Gatersleben, Germany, 11–13 June 1991; pp. 107–123.
3. Mathew, B. A Review of Allium Section Allium; Royal Botanic Gardens: Kew, UK, 1996.
4. Londhe, V.P. Role of garlic (Allium sativum) in various diseases: An overview. Angiogenesis 2011, 12, 13.
5. Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B.; Smith, B. Onions—A global benefit to health. Phytother. Res. 2002, 16, 603–615. [CrossRef]
6. Chauhan, N.B. Multiplicity of garlic health effects and Alzheimer’s disease. J. Nutr. Health Aging 2005, 9, 421–432.
7. Morihara, N.; Nishihama, T.; Ushijima, M.; Ide, N.; Takeda, H.; Hayama, M. Garlic as an antifatigue agent. Mol. Nutr. Food Res. 2007, 51, 1329–1334. [CrossRef] [PubMed]
8. Iciek, M.; Kwiecien, I.; Włodek, L. Biological properties of garlic and garlic-derived organosulfur compounds. Environ. Mol. Mutagen. 2009, 50, 247–265. [CrossRef]
9. Nicastro, H.L.; Ross, S.A.; Milner, J.A. Garlic and onions: Their cancer prevention properties. Cancer Prev. Res. 2015, 8, 181–189. [CrossRef]
10. Guan, M.J.; Zhao, N.; Xie, K.Q.; Zeng, T. Hepatoprotective effects of garlic against ethanol-induced liver injury: A mini-review. Food Chem. Toxicol. 2018, 111, 467–473. [CrossRef] [PubMed]
11. Zhu, Y.; Anand, R.; Geng, X.; Ding, Y. A mini review: Garlic extract and vascular diseases. Neurol. Res. 2018, 40, 421–425. [CrossRef] [PubMed]
12. Bhandari, P.R. Garlic (Allium sativum L.): A review of potential therapeutic applications. Int. J. Green Pharm. 2012, 6, 118–129. [CrossRef]
13. Pignatti, S. Flora d’Italia; Edagricole: Bologna, Italy, 1982.
14. Agusti, K.T. Therapeutic and medicinal values of onions and garlic. In Onions and Allied Crops; Rabinovitch, H.D., Ed.; Routledge: Milton Park, UK, 2017; pp. 99–104.
15. Kim, J.H. Anti-bacterial action of onion (Allium cepa L.) extracts against oral pathogenic bacteria. J. Nihon Univ. Sch. Dent. 1997, 39, 136–141. [CrossRef] [PubMed]
16. Yin, M.C.; Cheng, W.S. Antioxidant activity of several Allium members. J. Agric. Food Chem. 1998, 46, 4097–4101. [CrossRef]
17. Siegers, C.P.; Röbbe, A.; Pentz, R. Effects of garlic preparations on superoxide production by phorbol ester activated granulocytes. Phytotherapy 1999, 6, 13–16. [CrossRef]
18. Hobauer, R.; Frass, M.; Gmeiner, B.; Kaye, A.D.; Frost, E.A. Garlic extract (Allium sativum) reduces migration of neutrophils through endothelial cell monolayers. Middle East J. Anesthesiol. 2000, 15, 649–658. [PubMed]
19. Sela, U.R.I.; Ganor, S.; Hecht, I.; Brill, A.; Miron, T.; Rabinkov, A.; Wilchek, M.; Mirelman, D.; Lider, O.; Hershkoviz, R. Allicin inhibits SDF-1α-induced T cell interactions with fibronectin and endothelial cells by down-regulating cytoskeleton rearrangement, Pyk2 phosphorylation and VLA-4 expression. *Immunology* 2004, 111, 391–399. [CrossRef]

20. Jin, P.; Kim, J.A.; Choi, D.Y.; Lee, Y.J.; Jung, H.S.; Hong, J.T. Anti-inflammatory and anti-amyloidogenic effects of a small molecule, 2, 4-bis (p-hydroxyphenyl)-2-butenal in Tg2576 Alzheimer’s disease mice model. *J. Neuroinflamm.* 2013, 10, 767. [CrossRef]

21. Jeong, Y.Y.; Ryu, J.H.; Shin, J.H.; Kang, M.J.; Kang, J.R.; Han, J.; Kang, D. Comparison of anti-oxidant and anti-inflammatory effects between fresh and aged black garlic extracts. *Molecules* 2016, 21, 430. [CrossRef] [PubMed]

22. Abdel-Daim, M.M.; Abdel-Rahman, H.G.; Dessouki, A.A.; Ali, H.; Khodeer, D.M.; Bin-Jumah, M.; Alhader, M.S.; Alkahtani, S.; Aleya, L. Impact of garlic (*Allium sativum*) oil on cisplatin-induced hepatorenal biochemical and histopathological alterations in rats. *Sci. Total Environ.* 2020, 710, 136338. [CrossRef] [PubMed]

23. Bayan, L.; Kouliand, F.H.; Gorji, A. Garlic: A review of potential therapeutic effects. *Avicenna J. Phytomed.* 2014, 4, 1.

24. Iweala, E.E.J.; Akubugwo, I.E.; Okeke, C.U. Effects of ethanolic extracts of *Allium sativum* Linn. Liliaceae (Garlic) on serum cholesterol and blood sugar levels of albino rabbits. *J. Plant Res.* 2005, 9, 14–18.

25. Qidwai, W.; Ashfaq, T. Role of garlic usage in cardiovascular disease prevention: An evidence-based approach. *Evid. Based Complement. Altern. Med.* 2013, 2013, 125649. [CrossRef] [PubMed]

26. Patel, D.K.; Prasad, S.K.; Kumar, R.; Hemalatha, S. An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pac. J. Trop. Biomed.* 2012, 2, 320–330. [CrossRef]

27. Faroughi, F.; Mohammad-Alizadeh Charandabi, S.; Javadzadeh, Y.; Mirghafourvand, M. Effects of garlic pill on blood glucose level in borderline gestational diabetes mellitus: A triple blind, randomized clinical trial. *Iran. Red Crescent Med. J.* 2018, 20, 1–7. [CrossRef]

28. Zhai, B.; Zhang, C.; Sheng, Y.; Zhao, C.; He, X.; Xu, W.; Huang, K.; Luo, Y. Hypoglycemic and hypolipidemic effect of S-allylcysteine sulfoxide (alliin) in DIO mice. *Sci. Rep.* 2018, 8, 3527. [CrossRef] [PubMed]

29. Keophiphath, M.; Priem, F.; Jacquemond-Collet, I.; Clément, K.; Lacasa, D. 1, 2-vinylidithin from garlic inhibits differentiation and inflammation of human preadipocytes. *J. Nutr.* 2009, 139, 2055–2060. [CrossRef]

30. Apitz-Castro, R.; Escalante, J.; Vargas, R.; Jain, M.K. Ajoene, the antiplatelet principle of garlic, synergistically potentiates the antiaggregatory action of prostacyclin, forskolin, indomethacin and dypiridamole on human platelets. *Thromb. Res.* 1986, 43, 302–311. [CrossRef]

31. Bordía, A.; Verma, S.K.; Srivastava, K.C. Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. *Prostaglandins Leukot. Essent. Fat. Acids* 1998, 58, 257–263. [CrossRef]

32. Ariga, T.; Tsuji, K.; Seki, T.; Morimoto, T.; Yamamoto, J.I. Antithrombotic and antineoplastic effects of phyto-organosulfur compounds. *Biofactors* 2000, 13, 251–255. [CrossRef] [PubMed]

33. Ascrizzi, R.; Flamini, G. Leek or garlic? A chemical evaluation of elephant garlic volatiles. *Molecules* 2020, 25, 2082. [CrossRef] [PubMed]

34. Rendu, F.; Brohard-Bohn, B.; Pain, S.; Bachelot-Loza, C.; Auger, J. Thiosulfimates inhibit platelet aggregation and microparticle shedding at a calpain-dependent step. *J. Thromb. Haemost.* 2001, 86, 1284–1291.

35. Gruhlke, M.C.; Nicco, C.; Batteux, F.; Slusarenko, A.J. The effects of allicin, a reactive sulfur species from garlic, on a selection of mammalian cell lines. *Antioxidants* 2017, 6, 1. [CrossRef] [PubMed]

36. Gu, X.; Wu, H.; Fu, P. Allicin attenuates inflammation and suppresses HLA-B27 protein expression in ankylosing spondylitis mice. *Biomed Res. Int.* 2013, 2013, 171573. [CrossRef] [PubMed]

37. Gallwitz, H.; Bonse, S.; Martinez-Cruz, A.; Schlichting, I.; Schumacher, K.; Krauth-Siegel, R.L. Ajoene is an inhibitor and subversive substrate of human glutathione reductase and trypanothione reductase: Crystallographic, kinetic, and spectroscopic studies. *J. Med. Chem.* 1999, 42, 364–372. [CrossRef] [PubMed]

38. Zhen, H.; Fang, F.; Ye, D.Y.; Shu, S.N.; Zhou, Y.F.; Dong, Y.S.; Nie, X.C.; Li, G. Experimental study on the action of allitrudin against human cytomegalovirus in vitro: Inhibitory effects on immediate-early genes. *Antivir. Res.* 2006, 72, 68–74. [CrossRef] [PubMed]

39. Pai, S.T.; Platt, M.W. Antifungal effects of *Allium sativum* (garlic) extract against the *Aspergillus* species involved in otomycosis. *Lett. Appl. Microbiol.* 1995, 20, 14–18. [CrossRef]

40. Naganawa, R.; Iwata, N.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Suzuki, A. Inhibition of microbial growth by ajoene, a sulfur-containing compound derived from garlic. *Appl. Environ. Microbiol.* 1996, 62, 4238–4242. [CrossRef]

41. Yoshida, H.; Iwata, N.; Katsuzaki, H.; Naganawa, R.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Suzuki, A. Antimicrobial activity of a compound isolated from an oil-macerated garlic extract. *Biosci. Biotechnol. Biochem.* 1998, 62, 1014–1017. [CrossRef]

42. Anki, S.; Mirelman, D. Antibacterial properties of allicin from garlic. *Microbes Infect.* 1999, 1, 125–129. [CrossRef]

43. Yoshida, H.; Katsuzaki, H.; Ohta, R.; Ishikawa, K.; Fukuda, H.; Fujino, T.; Suzuki, A. Antimicrobial activity of the thiosulfimates isolated from oil-macerated garlic extract. *Biosci. Biotechnol. Biochem.* 1999, 63, 591–594. [CrossRef]

44. De Amorim, A.M.B.; dos Santos Nascimento, J. *Acinetobacter*: An underrated foodborne pathogen? *J. Infect. Dev. Ctries* 2017, 11, 111–114. [CrossRef]

45. Abdallah, M.; Benoliel, C.; Drider, D.; Dhubster, P.; Chihib, N.E. Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. *Arch. Microbiol.* 2014, 196, 453–472. [CrossRef]

46. Camargo, A.C.; Woodward, J.J.; Call, D.R.; Nero, L.A. *Listeria monocytogenes* in food-processing facilities, food contamination, and human listeriosis: The Brazilian scenario. *Foodborne Pathog. Dis.* 2017, 14, 623–636. [CrossRef] [PubMed]
47. Carter, M.Q.; Louie, J.W.; Feng, D.; Zhong, W.; Brandl, M.T. Curli fimbriae are conditionally required in *Escherichia coli* O157: H7 for initial attachment and biofilm formation. *Food Microbiol.* 2016, 57, 81–89. [CrossRef] [PubMed]

48. Haindongo, N.; Anyogu, A.; Ekwebelem, O.; Anumudu, C.; Onyeaka, H. Antibacterial and antibiofilm effects of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and mint (*Mentha piperita*) on *Escherichia coli* biofilms. *Appl. Food Biotechnol.* 2021, 4, 166–176. [CrossRef]

49. Caputo, L.; Amato, G.; Fratianni, F.; Coppola, R.; Candido, V.; De Feo, V.; Nazzaro, F. Chemical characterization and antibiofilm activities of bulbs and leaves of two aglione (*Allium ampeloprasum var. holmense* Asch. et Graebn.) landraces grown in Southern Italy. *Molecules* 2020, 25, 5486. [CrossRef] [PubMed]

50. Council of Europe. *European Pharmacopeia*, 5th ed.; Council of Europe: Strasbourg, France, 2004.

51. Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, NY, USA, 1980.

52. Davies, N.W. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. *J. Chromatogr. A* 1990, 503, 1–24. [CrossRef]

53. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing Co.: Carol Stream, IL, USA, 2007.

54. Sarker, S.D.; Nahar, L.; Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods* 2007, 42, 321–324. [CrossRef]

55. Kairo, S.K.; Bedwell, J.; Tyler, P.C.; Carter, A.; Corbel, M.J. Development of a tetrazolium salt assay for rapid determination of viability of BCG vaccines. *Vaccine* 1999, 17, 2423–2428. [CrossRef]

56. Borlinghaus, J.; Albrecht, F.; Gruhlke, M.C.; Nwachukwu, I.D.; Slusarenko, A.J. Allicin: Chemistry and biological properties. *Molecules* 2020, 25, 63–66. [CrossRef]

57. Satyal, P.; Craft, J.D.; Dosoky, N.S.; Setzer, W.N. The chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*). *Foods* 2017, 6, 63. [CrossRef] [PubMed]

58. Amagase, H. Clarifying the real bioactive constituents of garlic. *J. Nutr.* 2006, 136, 716S–725S. [CrossRef] [PubMed]

59. Stoll, A.; Seebeck, E. *Allium* compounds. I. Alliin the true mother compound of garlic oil. *Helv. Chim. Acta* 1948, 31, 189–210. [CrossRef] [PubMed]

60. Amagase, H.; Petesch, B.L.; Matsuura, H.; Kasuga, S.; Itakura, Y. Intake of garlic and its bioactive components. *J. Nutr.* 2001, 131, 955S–962S. [CrossRef] [PubMed]

61. Saxena, P.; Joshi, Y.; Rawat, K.; Bisht, R. Biofilms: Architecture, resistance, quorum sensing and control mechanisms. *Indian J. Microbiol.* 2019, 59, 3–12. [CrossRef] [PubMed]

62. Schwalm, N.D.; Groisman, E.A. Navigating the gut buffet: Control of polysaccharide utilization in *Bacteroides* spp. *Trends Microbiol.* 2017, 25, 1005–1015. [CrossRef] [PubMed]

63. Kumar, C.G.; Anand, S.K. Significance of microbial biofilms in food industry: A review. *Int. J. Food Microbiol.* 1998, 42, 9–27. [CrossRef]

64. Bhatwalkar, S.B.; Gound, S.S.; Mondal, R.; Srivastava, R.K.; Anupam, R. Anti-biofilm and antibacterial activity of *Allium sativum* against drug resistant shiga-toxin producing *Escherichia coli* (STEC) isolates from patient samples and food Sources. *Indian J. Microbiol.* 2019, 59, 171–179. [CrossRef]

65. Borlinghaus, J.; Albrecht, F.; Gruhlke, M.C.; Nwachukwu, I.D.; Slusarenko, A.J. Allicin: Chemistry and biological properties. *Molecules* 2014, 19, 12591–12618. [CrossRef] [PubMed]

66. Rattanachaikunsopon, P.; Phumkhachorn, P. Diallyl sulfide content and antimicrobial activity against food-borne pathogenic bacteria of chives (*Allium schoenoprasum*). *Biosci. Biotechnol. Biochem.* 2008, 72, 2987–2991. [CrossRef]

67. Li, G.; Ma, X.; Deng, L.; Zhao, X.; Wei, Y.; Gao, Z.; Jia, J.; Xu, J.; Sun, C. Fresh garlic extract enhances the antimicrobial activities of antibiotics on resistant strains in vitro. *Jundishapur J. Microbiol.* 2015, 8, 14814. [CrossRef]

68. Moretto, T.; Langsrud, S. *Listeria monocytogenes*: Biofilm formation and persistence in food-processing environments. *Biofilms* 2004, 1, 107–121. [CrossRef]

69. Nazzaro, F.; Fratianni, F.; De Martino, L.; Coppola, R.; De Feo, V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* 2013, 6, 1451–1474. [CrossRef] [PubMed]

70. Jadhav, S.; Shah, R.; Bhave, M.; Palombo, E.A. Inhibitory activity of yarrow essential oil on *Listeria* planktonic cells and biofilms. *Food Control* 2014, 29, 125–130. [CrossRef]

71. Sandasi, M.; Leonard, C.M.; Viljoen, A.M. The effect of five common essential oil components on *Listeria monocytogenes* biofilms. *Food Control* 2008, 19, 1070–1075. [CrossRef]

72. Somrani, M.; Inglés, M.C.; Debbabi, H.; Abidi, F.; Palop, A. Garlic, onion, and cinnamon essential oil anti-biofilms’ effect against *Listeria monocytogenes*. *Foods* 2020, 9, 567. [CrossRef] [PubMed]

73. Martins, N.; Petropoulos, S.; Ferreira, I.C. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre-and post-harvest conditions: A review. *Food Chem.* 2016, 211, 41–50. [CrossRef] [PubMed]

74. Zarrilli, R.; Giannouli, M.; Tomasoni, F.; Triassi, M.; Tsakris, A. Carbapenem resistance in *Acinetobacter baumannii*: The molecular epidemic features of an emerging problem in health care facilities. *J. Infect. Dev. Ctries* 2009, 3, 335–341. [CrossRef]

75. Perez, F.; Hujer, A.M.; Hujer, K.M.; Decker, B.K.; Rather, P.N.; Bonomo, R.A. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* 2007, 51, 3471–3484. [CrossRef]
76. Yang, X.; Sha, K.; Xu, G.; Tian, H.; Wang, X.; Chen, S.; Wang, Y.; Li, J.; Chen, J.; Huang, N. Subinhibitory concentrations of allicin decrease uropathogenic *Escherichia coli* (UPEC) biofilm formation, adhesion ability, and swimming motility. *Int. J. Mol. Sci.* 2016, 17, 979. [CrossRef]

77. De Martino, L.; Amato, G.; Caputo, L.; Nazzaro, F.; Scognamiglio, M.R.; De Feo, V. Variations in composition and bioactivity of *Ocimum basilicum* cv ‘Aroma 2’ essential oils. *Ind. Crops Prod.* 2021, 172, 114068. [CrossRef]

78. Caputo, L.; Smeriglio, A.; Trombetta, D.; Cornara, L.; Trevena, G.; Valussi, M.; Fratianni, F.; De Feo, V.; Nazzaro, F. Chemical composition and biological activities of the essential oils of *Leptospermum petersonii* and *Eucalyptus gunnii*. *Front. Microbiol.* 2020, 11, 409. [CrossRef] [PubMed]

79. Zhang, H.; Li, S.; Cheng, Y. Antibiofilm Activity of Allicin and Quercetin in Treating Biofilm-Associated Orthopaedics Infection. *Appl. Biochem. Biotechnol.* 2022. [CrossRef] [PubMed]

80. Libhua, L.; Jianhui, W.; Jialin, Y.; Yayin, L.; Guanxin, L. Effects of allicin on the formation of *Pseudomonas aeruginosa* biofilm and the production of quorum-sensing controlled virulence factors. *Pol. J. Microbiol.* 2013, 62, 243–251. [CrossRef] [PubMed]

81. Jin, Z.; Li, L.; Zheng, Y.; An, P. Diallyl disulfide, the antibacterial component of garlic essential oil, inhibits the toxicity of *Bacillus cereus* ATCC 14579 at sub-inhibitory concentrations. *Food Control* 2021, 126, 108090. [CrossRef]

82. Li, W.R.; Ma, Y.K.; Shi, Q.S.; Xie, X.B.; Sun, T.L.; Peng, H.; Huang, X.M. Diallyl disulfide from garlic oil inhibits *Pseudomonas aeruginosa* virulence factors by inactivating key quorum sensing genes. *Appl. Microbiol. Biotechnol.* 2018, 102, 7555–7564. [CrossRef]