Chemical Composition and Antibacterial Activity of Essential Oils from \textit{Ferula} L. Species against Methicillin-Resistant \textit{Staphylococcus aureus} \\

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Abstract: Essential oils (EOs) were obtained by hydrodistillation of various parts of \textit{Ferula ovina} (Boiss.) Boiss., \textit{Ferula iliensis} Krasn. ex. Korovin, and \textit{Ferula akitschkensis} B. Fedtsch. ex Koso-Pol., collected in the flowering/budding and fruiting stages. Eight samples of EOs isolated from \textit{F. ovina} and four samples from \textit{F. akitschkensis} were analyzed by gas chromatography–mass spectrometry (GC-MS). The major constituents of \textit{F. ovina} EOs were \(\alpha\)-pinene (6.9–47.8%), \(\beta\)-pinene (1.5–7.1%), sabinene (0.1–20.5%), \(\beta\)-phellandrene (0–6.5%), \textit{trans}-verbenol (0.9–7.4%), eremophilene (3.1–12%), and 6\(Z\)-2,5,5,10-tetramethyl-undeca-2,6,9-trien-8-one (0–13.7%). The major constituents of \textit{F. akitschkensis} EOs were \(\alpha\)-pinene (0–46.2%), \(\beta\)-pinene (0–47.9%), sabinene (0–28.3%), eremophilene (0–10.6), \(\beta\)-caryophyllene (0–7.5%), himachalen-7-ol (0–28.2%), and a himachalol derivative (0–8.3%). Samples of EOs from \textit{F. ovina}, \textit{F. iliensis}, and \textit{F. akitschkensis} were evaluated for antibacterial activity against methicillin-resistant \textit{Staphylococcus aureus} (MRSA) pulse-field gel electrophoresis type USA300 (LAC). EOs from \textit{F. ovina} exhibited the highest antibacterial activity compared to samples from other \textit{Ferula} spp., with the most potent EOs being isolated from roots at the flowering and fruiting stages and stems at the fruiting stage (IC\textsubscript{50} values of 19.1, 20.9, and 22.9 \(\mu\)g/mL, respectively). Although EOs demonstrated concentration-dependent inhibition of MRSA growth, analysis of the major constituents (\(\alpha\)-pinene, \(\beta\)-pinene, and sabinene) showed that they had low activity, suggesting that other components were likely responsible for the observed bioactivity of the unfractionated EOs. Indeed, correlation of the GC-MS data with antibacterial activity suggested that the putative components responsible for antibacterial activity were, either individually or in combination, eremophilene and \textit{trans}-verbenol. Overall, these results suggest that the EOs from \textit{F. ovina} could have potential for use as alternative remedies for the treatment of infectious diseases caused by MRSA. \\

Keywords: \textit{Ferula}; essential oil; antibacterial activity; methicillin-resistant \textit{Staphylococcus aureus} (MRSA)
1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main causative agents of skin and soft tissue infections. Infections caused by MRSA have limited treatment options since these strains are resistant to the entire class of β-lactam antibiotics. Vancomycin still remains the treatment of choice for serious MRSA infections [1]; however, vancomycin must be administered intravenously, which makes administration outside of hospital or clinical settings challenging. Additionally, *S. aureus* strains that have vancomycin intermediate resistance are prevalent and although rare, vancomycin resistant *S. aureus* strains have also been isolated [2]. Thus, there is an increased interest in finding alternative methods of treatment, including natural compounds such as essential oils (EOs), that are effective against bacterial infections [3,4]. The antimicrobial properties of EOs have been reported in several studies (reviewed in [5–7]), and combination of antibiotics with EOs targeting multidrug resistant bacteria could lead to new choices to overcome the problem of bacterial resistance [8,9]. Thus, EOs offer promise as an alternative treatment option. *Ferula* spp. are a good source of biologically active compounds, such as sesquiterpenes, terpenoid coumarins, and sulfur containing compounds [10–16]. The genus *Ferula* (Apiaceae) comprises ~185 species distributed throughout Central Asia, the Mediterranean, and northern Africa, and many species of *Ferula* L. have been used in traditional medicine [13,17,18]. For example, one of the plant species in our study, *Ferula iliensis* Krasn. ex. Korovin, is a native plant of Kazakhstan that is widely used by the local population as an anti-inflammatory treatment [19]. The main constituents of most reported EOs from *Ferula* spp. exhibiting antimicrobial activity are monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpenes [20–23]. Monoterpenes and sesquiterpenes are a frequently occurring group of compounds in EOs and have a broad spectrum of pharmacological properties, including antimicrobial activity [24–28]. Previously, we reported the chemical composition and immunomodulatory activity of EOs isolated from *Ferula iliensis* and *Ferula akitschkensis* [12,19]. Likewise, several sulfur compounds, including sec-butyl disulfide derivatives, were found in EOs and/or ole-gum resins obtained from various *Ferula* spp. [15,29–32]. The fruit oil of *Ferula latissecta* contains a high amount of polysulfide compounds, of which (Z)-1-propenyl sec-butyl disulfide (65.2%) and (E)-1-propenyl sec-butyl disulfide (6.8%) are the major constituents [33]. Monoterpene hydrocarbons dominated (85.7%) over all other compound groups in EOs from *F. akitschkensis* [12]. *Ferula ovina* (Boiss.) Boiss. is a fodder plant in Kazakhstan, and there is no information on the use of this plant in traditional medicine. However, *F. ovina* is a flavoring agent used as an ingredient in Iranian spices and condiments [34]. Aqueous extracts of *F. ovina* possess anti-spasmodic, anticholinergic, and smooth muscle relaxant activities [35], and antibacterial activity of *F. ovina* EOs against *S. aureus* was demonstrated by Syed et al. [36]. Radulovic et al. reported that bornyl 4-methoxybenzoate was one of the constituents of EOs from *F. ovina*, and it was shown that this compound induces hyperalgesia in mice [34].

In the present study, the chemical composition of EOs isolated from several samples of *F. ovina* and *F. akitschkensis* was evaluated. Antibacterial activity of EOs obtained from various parts of *F. ovina*, *F. iliensis*, and *F. akitschkensis* against MRSA was also assessed. Finally, three main constituents of the EOs (α-pinene, β-pinene, and sabinene) were evaluated for antibacterial activity.

2. Results and Discussion

2.1. Chemical Composition of Ferula EOs

EOs were isolated from various parts of *Ferula* species. The yield of EOs varied depending on the plant species and plant part. Specifically, the yields (v/w) of *F. ovina* EOs were: 0.97 (FOEO_I), 0.16 (FOEO_Lg), 0.04 (FOEO_Rg), 0.95 (FOEO_Rb), 1.12 (FOEO_U/s), 0.16 (FOEO_Lb), 0.03 (FOEO_Rfr), and 0.78% (FOEO_Rfr). The yields (v/w) of *F. akitschkensis* EOs were: 0.95 (FAEO_Rb), 0.14 (FAEO_Lb), 2.52 (FAEO_Rb), and 2.24% (FAEO_Rfr) (see abbreviations for the EOs in the footnote of Table 1). The chemical composition of two additional EOs isolated from umbels with seeds and stems at
the fruiting stage of F. akitschkensis were reported previously [12]. Hydrodistillation of the umbels with seeds and stems produced 0.7 and 0.02% EOs, respectively [12]. The chemical composition of all EOs from F. iliensis were reported recently, and yields of their EOs varied from 0.4 to 1.1% [19].

The chemical composition of 8 EOs from F. ovina and 4 EOs from F. akitschkensis is summarized in Table 1, where the identified compounds are listed in order of their elution. In addition, the relative retention index (RRIexp) values obtained for the detected constituents are included for comparison with those values previously reported (RRIlit) for these compounds [37–53].

Analysis of the EOs from F. ovina revealed a total of 102 different constituents. FOEOIL was found to be the most complex, with 62 constituents, while FOEOU/s, FOEOBF, FOEOF, FOEOF, and FOEOI had 56, 53, 50, 45, 43, and 41 constituents, respectively. Sabinene, α-pinene, β-pinene, eremophilene, β-phellandrene, trans-verbenol, and 6Z-2,5,5,10-tetramethyl-undeca-2,6,9-trien-8-one (all present at >5%) were the most common volatile constituents detected. Their concentrations varied depending on plant parts. For example, the highest content of α-pinene was identified in the inflorescence (35.1%), umbels with seeds (47.4%), and roots (47.8% and 46.5%). EOs isolated from the roots had a higher content of β-pinene and eremophylene compared to other parts of the plant. The content of sabinene was 20.5% in the inflorescence, whereas it was present only in trace amounts in the roots. GC analysis of the volatiles on a Lipodex G chiral column revealed the existence of enantiomeric pairs of α-pinene and β-pinene in FOEOF, where we found (1S)-(−)-α-pinene (49%) and (1S)-(−)-β-pinene (29%).

It should be noted, that the main constituents previously reported for EOs from the seeds of F. ovina collected in China were polysulfide alkanes (86.3%), sesquiterpenoids (8.3%), and monoterpenoids (0.5%) [54]. EOs from leaves of F. ovina collected in Iran were mainly monoterpenes, specifically, α-pinene (50.0%) and limonene (11.5%) [55], which is similar to the chemical composition of EOs that we isolated from F. ovina (Table 1). The volatile compounds identified in EOs isolated from buds, leaves, and roots at the budding stage and roots at the fruiting stage of F. akitschkensis are listed in Table 1. Analysis of these EOs revealed a total 105 different constituents. The most complex, FAEOIL, contained 51 constituents, while FAEOIR, FAEOF, and FAEOB had 45, 45, and 37 constituents, respectively. Predominant constituents of the EOs obtained from buds and roots at the budding and fruiting stages and umbels with seeds were monoterpen hydrocarbons (70.6–95.2%), with the main compounds being α-pinene, β-pinene, and sabinene (Table 1), whereas EOs from stems at the fruiting stages were distinguished by a high percentage of myristicin (67.9%) and 2-himachalen-7-ol (7.9%) [12]. The existence of enantiomeric pairs in EOs isolated from umbels with seeds of F. akitschkensis was reported previously, where we found (1S)-(−)-α-pinene (95%), (1S)-(−)-β-pinene (94%), and (1R,5R)-(+) sabinene (97%) [12]. A detailed chemical composition of EOs from F. iliensis was recently reported by our group, with the major constituents of the EOs from all parts of the plant being sulfur-containing compounds, including (E)-propenyl sec-butyl disulfide (15.7–39.4%) and (Z)-propenyl sec-butyl disulfide (23.4–45.0%) [19].
| RRI_{exp} | RRI_{lit} | Compound | FOEO_1 | FOEO_{Lfl} | FOEO_{Sfl} | FOEO_{Rfl} | FOEO_{U/s} | FOEO_{Lfr} | FOEO_{Sfr} | FOEO_{Rfr} | FAEO_{B} | FAEO_{Lb} | FAEO_{Rb} | FAEO_{Rfr} |
|---------|-----------|----------|--------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------|-------------|-----------|-------------|
| 1032    | 1032      | α-Pinene | 35.1   | 10.3        | 15.0        | 47.4        | 47.8        | 6.9         | 7.6         | 46.5        | 25.0      | 36.4        | 46.2      |             |
| 1035    | 1035      | α-Thujene | 1.2    | 0.3         | tr          | 0.6         | tr          | 0.9         |             |             |           |             |           |             |
| 1072    | 1070      | α-Fenchene | 0.2    | 0.6         | tr          | 0.8         | 0.4         | 0.5         | tr          | 0.5        | 0.2        | 0.5       | 1.0        |
| 1118    | 1118      | β-Pinene | 6.0    | 2.6         | 3.6         | 1.9         | 7.1         | 1.7         | 1.5         | 6.7         | 11.1      | 47.9       | 28.6      |
| 1132    | 1132      | Sabinene | 20.5   | 5.5         | 2.0         | tr          | 6.5         | 3.2         | tr          | 28.3       | tr         | 28.3      | tr         |
| 1158    | 1137      | Thuja-2,4(10)-diene | 0.3    | 0.3         | tr          | 0.1         | 0.2         | 0.2         | tr          | 0.1        |            |           |           |
| 1159    | 1159      | δ-3-Carene | 0.2    | 0.2         | 0.4         | 1.0         | 0.4         | 0.5         | 0.6         | 3.1        | 8.3       |           |           |
| 1174    | 1175      | Myrcene | 0.8    | 0.2         | 0.4         | 1.0         | 0.4         | 0.4         | 0.5         | 0.6        | 3.1       | 8.3        |           |
| 1176    | 1176      | α-Phellandrene | 0.3    | 0.2         | tr          | 0.2         | tr          | 0.2         | tr          | 0.1        |           |           |
| 1203    | 1204      | Limonene | 0.6    | 0.7         | tr          | 0.3         | 0.6         | 0.3         | 0.4         | 1.8        | 2.2       |           |           |
| 1218    | 1218      | β-Phellandrene | 3.9    | 1.7         | tr          | 6.5         | 0.6         | tr          | 0.4         | 0.8        | 2.2       |           |           |
| 1246    | 1246      | (Z)-β-Ocimene | 0.1    | tr          | 1.6         | tr          | 1.6         | tr          | 0.6         | 1.0        |           |           |
| 1255    | 1255      | γ-Terpinene | 0.7    | 0.2         | tr          | 0.3         | 0.2         | tr          | 0.6         | 0.1        |           |           |
| 1266    | 1266      | (E)-β-Ocimene | tr    | tr          | 0.3         |             |             |             |             |             |           |           |
| 1280    | 1280      | β-Cymene | 0.8    | 1.3         | 1.1         | 0.2         | 0.6         | 0.7         | 0.4         | 2.5        | 0.4       | 0.5       | 0.2       |
| 1286    | 1290      | Isoterpinolene | 0.3    | 0.1         | 0.1         | 0.1         | 0.1         | 0.2         | 0.2         | 0.1        | 0.1       |           |           |
| 1439    | 1477      | γ-Campholenaldehyde | 0.4    | 0.2         | 0.2         | 0.1         | 0.2         | 0.6         | 0.4        |           |           |
| 1474    | 1482      | Longipinene | 0.2    | 0.4         | tr          | 0.8         | 0.3         | 0.4         | 0.5         | 0.2        | 0.5       |           |           |
| 1482    | 1464      | Fenchyl acetate | 0.2    |              |             |             |             |             |             | 0.1        | 0.2       |           |           |
| 1457    | 1457      | Citronellal | 0.4    |              |             |             |             |             |             |           |           |           |
| 1492    | 1492      | Cyclosativene | 0.3    | 1.0         | 0.8         | 1.3         | 0.7         | 1.2         | 1.6         | 0.6        | 0.5       | 0.9       | 0.3       |
| 1497    | 1497      | α-Copaene | 0.3    | 0.1         | 0.8         | 0.3         | 0.1         | 0.2         | 0.3         | 0.3       | 0.3       |           |           |
| 1499    | 1500      | α-Campholenaldehyde | 0.7    | 1.6         | 0.5         | 0.4         | 0.4         |             |             |           |           |
| 1506    | 1506      | Decanal | 0.7    |              |             |              |             |             |             |           |           |           |           |
| 1512    | 1497      | Longicyclene | 0.7    |              |             |              |             |              |             |           |           |           |           |
| 1525    | 1528      | Cyperene | 0.3    |              |             |              |             |              |             |           |           |           |           |
| 1544    | 1545      | α-Gurjunene | 0.3    |              |             |              |             |              |             |           | 0.1       |           |
| 1549    | 1549      | β-Cubecene | 0.3    |              |             |              |             |              |             |           |           | 0.1       |           |
| 1553    | 1553      | Linalool | 0.3    |              |             |              |             |              |             |           |           |           |           |
| 1556    | 1571      | cis-Sabinene hydrate | 0.3    |              |             |              |             |              |             |           |           |           |           |
| 1571    | 1573      | trans-p-Menth-2-en-1-ol | 0.1    | 0.2         | 0.1         | 0.2         | 0.4         | 0.6         |           |           |
| 1586    | 1586      | Pinocarvone | 0.4    | 0.8         | 0.1         | 0.2         | tr          | 0.1         | 0.2        |           |           |
| 1587    | 1587      | 1,7-Diepi-β-cedrene | 0.2    |              |             |              |             |              |             |           |           |           |

**Table 1.** Composition of the volatile compounds identified in the essential oils from different parts of *F. ovina* and *F. akitschkensis.*
Table 1. Cont.

| RRI_{exp} | RRI_{lit} | Compound                        | Concentration in EOs (%) * |
|-----------|-----------|--------------------------------|---------------------------|
| 1589      | 1565      | Aristolene                      | FOEO_t  0.2  FOEO_{Lit}  0.4  FOEO_{Sfl}  tr  FOEO_{Rfl}  tr  FOEO_{U/s}  0.1  FOEO_{Rfr}  0.2  FOEO_{B}  0.2  FOEO_{J}  0.1 |
| 1591      | 1592      | Bornyl acetate §                |                           |
| 1595      | 1588      | Isothymol methylether           |                           |
| 1596      | 1590      | trans-β-Bergamotene             |                           |
| 1596      | 1596      | α-Guaiene §                     |                           |
| 1600      | 1600      | β-Elemene §                     |                           |
| 1604      | 1598      | Thymol methyl ether             |                           |
| 1610      | 1611      | Calarene                        |                           |
| 1611      | 1611      | Terpinen-4-ol §                 |                           |
| 1612      | 1612      | β-Caryophyllene §               | tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1 |
| 1628      | 1629      | Aromadendrene §                 |                           |
| 1638      | 1638      | cis-p-Menth-2-en-1-ol            |                           |
| 1648      | 1648      | Myrtenal §                      |                           |
| 1650      | 1650      | γ-Elemene §                     |                           |
| 1659      | 1668      | γ-Gurjunene §                   |                           |
| 1661      | 1663      | α-Himachalene §                 |                           |
| 1661      | 1661      | trans-Pinocarvyl acetate §      |                           |
| 1663      | 1663      | cis-Verbenol §                  |                           |
| 1668      | 1668      | (Z)-β-Farnesene §               |                           |
| 1672      | 1671      | trans-Pinocarvyl §              |                           |
| 1687      | 1689      | α-Humulene §                    |                           |
| 1683      | 1683      | trans-Verbenol §                | tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1  tr  0.1 |
| 1697      | 1718      | 4,6-Guaiadiene                  |                           |
| 1704      | 1704      | γ-Muurolele §                   |                           |
| 1704      | 1704      | Myrtenyl acetate                |                           |
| 1705      | 1706      | α-Terpineol §                   |                           |
| 1706      | 1706      | γ-Himachalene                   |                           |
| 1711      | 1708      | γ-Himachalene                   |                           |
| 1722      | 1722      | Dodecanal §                     |                           |
| 1725      | 1725      | Verbenone                       |                           |
| 1726      | 1726      | Germacrene D §                  |                           |
| 1730      | 1730      | Cadina-3,5-diene §              |                           |
| 1739      | 1740      | β-Himachalene                   |                           |
| 1740      | 1740      | Valencene §                     |                           |
| 1740      | 1740      | α-Muurolele §                   |                           |
| 1741      | 1741      | β-Isobolene                     |                           |
| 1742      | 1743      | β-Selinene §                    |                           |
| 1743      | 1743      | Eremophiline §                  |                           |
| 1744      | 1740      | α-Selinene §                    |                           |
| 1750      | 1750      | Dauca-8,11-diene                |                           |
| 1754      | 1754      | Himachala-2,4-diene*            |                           |
| 1768      | 1768      | cis-α-Isobolene §               |                           |
| 1771      | 1773      | γ-Isobolene §                   |                           |
Table 1. Cont.

| RRI_{exp} | RRI_{lit} | Compound                        | FOEO_L | FOEO_Lfl | FOEO_Sfl | FOEO_B | FOEO_Bfl | FOEO_Bfr | FOEO_Rfr | FAEO_B | FAEO_L | FAEO_R | FAEO_Rfr |
|-----------|-----------|---------------------------------|--------|----------|----------|--------|----------|----------|----------|--------|--------|--------|----------|
| 1771      | 1771      | cis-Piperitol §                  |        |          |          |        |          |          |          |        |        |        |          |
| 1772      | 1774      | Citronellol §                    | 1.5    |          |          |        |          |          |          |        |        |        |          |
| 1773      | 1774      | δ-Cadinene §                     |        |          |          |        |          |          |          |        |        |        |          |
| 1778      | 1783      | β-Sesquiphellandrene §           |        |          |          |        |          |          |          |        |        |        |          |
| 1784      | 1786      | (E)-α-Bisabolene                 |        |          |          |        |          |          |          |        |        |        |          |
| 1786      | 1786      | ar-Curcumene §                   |        |          |          |        |          |          |          |        |        |        |          |
| 1788      | 1782      | 1-Decanol §                      | 0.2    |          |          |        |          |          |          |        |        |        |          |
| 1796      | 1790      | Selina-3,7(11)-diene             |        |          |          |        |          |          |          |        |        |        |          |
| 1804      | 1804      | Myrtenol §                       | 0.5    | 0.1      | 1.3      | 1.8    |          |          |          |        |        |        |          |
| 1849      | 1849      | Cuparene §                       |        |          |          |        |          |          |          |        |        |        |          |
| 1853      | 1853      | cis-Calamenene                   |        |          |          |        |          |          |          |        |        |        |          |
| 1854      | 1853      | Germacrene B §                   | 1.0    | 1.0      | 1.0      | 1.7    |          |          |          |        |        |        |          |
| 1864      | 1864      | β-Cymen-8-ol §                   |        |          |          |        |          |          |          |        |        |        |          |
| 1868      | 1868      | (E)-Geranyl acetone §            |        |          |          |        |          |          |          |        |        |        |          |
| 1869      | 1869      | Neophytadiene                    | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 1871      | 1871      | Neryl isovalerate                |        |          |          |        |          |          |          |        |        |        |          |
| 1878      | 1878      | 2,5-Dimethoxy-p-cymene           |        |          |          |        |          |          |          |        |        |        |          |
| 1882      | 1882      | α-Dehydro-ar-himachalene         | 0.3    | 0.1      | 0.1      | 0.1    |          |          |          |        |        |        |          |
| 1888      | 1888      | ar-Himachalene §                 | 1.0    | 1.3      | 1.3      | 1.3    |          |          |          |        |        |        |          |
| 1925      | 1930      | γ-Dehydro-ar-himachalene         |        |          |          |        |          |          |          |        |        |        |          |
| 1933      | 1933      | Neryl valerate                   | 0.1    | 1.9      | 1.9      | 1.9    |          |          |          |        |        |        |          |
| 1941      | 1941      | α-Calacorene-I                   | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 1956      | 1954      | (E)-β-Ionone                     | 0.3    |          |          |        |          |          |          |        |        |        |          |
| 1973      | 1973      | 1-Dodecanol §                    | 0.2    |          |          |        |          |          |          |        |        |        |          |
| 1982      | 1984      | α-Calacorene-II                  | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 2001      | 2001      | Isoxaryophyllene oxide           | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 2004      | 2004      | Oxidohimachalene                 |        |          |          |        |          |          |          |        |        |        |          |
| 2008      | 2008      | Caryophyllene oxide §            | 0.1    | 0.1      | 0.1      | 0.1    |          |          |          |        |        |        |          |
| 2030      | 2030      | Methyl eugenol §                 | 0.4    |          |          |        |          |          |          |        |        |        |          |
| 2044      | 2044      | 6,7-Epoxy-himachalene            |        |          |          |        |          |          |          |        |        |        |          |
| 2056      | 2056      | α-Copaene-8-ol §                 | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 2071      | 2071      | Humulene epoxide II              | 0.3    |          |          |        |          |          |          |        |        |        |          |
| 2080      | 2080      | Junenol                          | 0.1    |          |          |        |          |          |          |        |        |        |          |
| 2131      | 2131      | 1-α-(H)-himachal-4-en-1-β-ol     | 0.3    |          |          |        |          |          |          |        |        |        |          |
| 2165      | 2165      | Hexahydro-farnesylacetone §      |        |          |          |        |          |          |          |        |        |        |          |
| 2169      | 2169      | DMF                              | 0.4    |          |          |        |          |          |          |        |        |        |          |
| 2179      | 2179      | 6-epi-Cubenol                    | 0.7    | 0.5      | 0.5      | 0.5    |          |          |          |        |        |        |          |
| 2219      | 2219      | Torreyl                          | 0.5    |          |          |        |          |          |          |        |        |        |          |
| 2221      | 2221      | Dimyrcene II-a                   |        |          |          |        |          |          |          |        |        |        |          |
| 2232      | 2232      | α-Bisabolol                      | 0.6    | 0.3      | 0.3      | 0.3    |          |          |          |        |        |        |          |
| 2240      | 2240      | epi-α-Bisabolol                  | 1.5    | 1.5      | 1.5      | 1.5    |          |          |          |        |        |        |          |
| 2245      | 2245      | Elemicine §                      |        |          |          |        |          |          |          |        |        |        |          |
Table 1. Cont.

| RRI\exp | RRI\lit | Compound                              | Concentration in EOs (%) | FOEO\_I | FOEO\_Lfl | FOEO\_Sfl | FOEO\_Rfl | FOEO\_U/s | FOEO\_Lfr | FOEO\_Sfr | FOEO\_Rfr | FOEO\_Rb | FAEO\_Lb | FAEO\_Rb | FAEO\_Rfr |
|---------|---------|---------------------------------------|--------------------------|---------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------|---------|----------|
| 2248    | 2246    | Himachalol §                          | 2.4                      | 2.4     | 2.9       | 5.4       | 2.1       | 2.1       | 5.8       | 2.3       | 6.9       | 28.2     |         |         |          |
| 2249    |         | β-Himachalol *                         |                          |         |           |           |           |           |           | 0.3       | 0.3       | 0.3       | 1.0     |         |          |
| 2252    |         | Himachalol derivative *                |                          |         |           |           |           |           |           |           |           | 0.9      | 4.1     |         |          |
| 2254    |         | 2-Himachalen-7-ol                     |                          |         |           |           |           |           |           |           |           |          |         |         |          |
| 2256    | 2256    | Cadalene                              |                          | 0.2     | 2.2       |           |           |           |           |           |           |          |         |         |          |
| 2296    | 2296    | Myristicine §                         |                          |         |           |           |           |           |           |           |           | 0.3      | 1.5     |         |          |
| 2297    |         | Allohimachalol §                       |                          | 0.1     |           |           |           |           |           | 0.1       | 3.2       | 0.3       | 0.3     | 1.0     |          |
| 2298    |         | Torilenol                              |                          | 0.7     | 2.5       | 3.0       | 1.0       |           |           |           | 1.0       |         |         |         |          |
| 2300    | 2300    | (E)-Longipinane §                      |                          |         |           |           |           |           |           |           |           |          |         |         |          |
| 2303    |         | 8,9-Dehydroxysolongifolene *          |                          |         |           |           |           |           |           |           |           |          |         |         |          |
| 2304    |         | TMCMP §                               |                          | 1.7     |           |           |           |           |           |           |           |          |         |         |          |
| 2308    | 2332    | Khusinol                               |                          | 1.0     |           |           |           |           |           |           |           |          |         |         |          |
| 2376    |         | 10-Hydroxy-calamenene                  |                          | 0.2     |           |           |           |           |           |           |           | 1.9      |         |         |          |
| 2456    |         | Oxygenated sesquiterpene *            |                          | 0.2     |           |           |           |           |           |           |           | 8.4      |         |         |          |
| 2467    |         | GTO §                                 |                          |         |           |           |           |           |           |           |           | 3.7      |         |         |          |
| 2468    |         | Marsupellol                            |                          |         |           |           |           |           |           |           |           |          |         |         |          |
| 2482    |         | Dauca-8(14),11-dien-9 α-ol            |                          | 2.0     |           |           |           |           |           |           |           |          |         |         |          |
| 2500    | 2500    | Pentacosane §                          |                          | 1.2     |           |           |           |           |           |           |           | 0.4      |         |         |          |
| 2533    |         | γ-Costol                              |                          |         |           |           |           |           |           |           |           | 0.6      |         |         |          |
| 2542    |         | Eudesma-4(15),7-dien-1-ol             |                          | 0.5     |           |           |           |           |           |           |           | 0.6      |         |         |          |
| 2565    |         | 1-Hexadecanol                         |                          |         |           |           |           |           |           |           |           | 0.4      |         |         |          |
| 2575    |         | 10-Hydroxy-calamenene isomer *        |                          |         |           |           |           |           |           |           |           | 0.5      |         |         |          |
| 2606    | 2607    | β-Costol                              |                          |         |           |           |           |           |           |           |           | 0.4      |         |         |          |
| 2620    | 2619    | Phytol §                              |                          | 0.6     |           |           |           |           |           |           |           | 0.6      |         |         |          |
| 2700    |         | Heptacosane §                          |                          |         |           |           |           |           |           |           |           | 1.0      |         |         |          |
| 2900    | 2900    | Nonacosane §                          |                          |         |           |           |           |           |           |           |           | 4.4      |         |         |          |
| 2931    | 2931    | Hexadecanoic acid §                   |                          | 1.3     |           |           |           |           | 0.6       | 1.7       | 0.9       | 0.5      | 0.5     |         |          |
| 2932    |         | TMUTO *                               |                          | 4.7     | 4.7       |           |           |           |           |           |           |          |         |         |          |

**Total % Based on Chemical Class**

| Monoterpene hydrocarbons | 70.5 | 23.6 | 21.7 | 51.3 | 71.1 | 16.9 | 9.1 | 55.1 | 70.6 | 0.4 | 95.2 | 89.1 |
|--------------------------|------|------|------|------|------|------|-----|------|------|-----|------|------|
| Oxygenated monoterpenes  | 3.9  | 12.6 | 17.3 | 3.5  | 3.0  | 6.7  | 5.4 | 4.6  | 3.6  | 3.2 | 1.4  | 1.8  |
| Sesquiterpene hydrocarbons | 7.9 | 17.2 | 19.8 | 25.9 | 10.9 | 18.9 | 19.2 | 19.4 | 6.8 | 37.4 | 1.5 | 2.2 |
| Oxygenated sesquiterpenes | 5.2 | 13.1 | 12.4 | 7.4 | 4.6 | 13.7 | 30.9 | 5.5 | 7.5 | 42.3 | 0.0 | 0.0 |
| Miscellaneous compounds | 1.7 | 9.9 | 4.7 | 4.3 | 0.6 | 3.0 | 14.9 | 0.9 | 0.5 | 10.5 | 1.0 | 4.5 |

* The data are presented as a relative percentage by weight for each component in EOs isolated from *F. ovina* inflorescences (FOEO\_I), leaves at the flowering stage (FOEO\_Lfl), stems at the flowering stage (FOEO\_Sfl), roots at the flowering stage (FOEO\_Rfl), umbels with seeds (FOEO\_U/s), leaves at the budding stage (FAEO\_Lb), stems at the budding stage (FAEO\_Sb), and roots at the budding stage (FAEO\_Rb). RRI\_exp, relative retention indices calculated against n-alkanes, % calculated from FID data. RRI\_lit, published RRI values for the volatile compounds [37–53]. § Compounds identified by co-injection. Trace amount (tr) were present at <0.1%. * Tentatively identified from the Wiley mass spectrum library. DMPF, 3,4-dimethyl-5-pentylidene-2(5H)-furanone; TMCMP (1E)-1-[2,6,6-trimethylcyclohex-1-enyl]-3-methyl-1,4-pentadien-3-ol; GTO, germacr-4(15),5,10(14)-trien-1a-ol; TMUTO, 6\(^Z\)-2,5,5,10-tetramethyl-undeca-2,6,9-trien-9-one.

§ Compounds identified by co-injection. Trace amount (tr) were present at <0.1%. * Tentatively identified from the Wiley mass spectrum library. DMPF, 3,4-dimethyl-5-pentylidene-2(5H)-furanone; TMCMP (1E)-1-[2,6,6-trimethylcyclohex-1-enyl]-3-methyl-1,4-pentadien-3-ol; GTO, germacr-4(15),5,10(14)-trien-1a-ol; TMUTO, 6\(^Z\)-2,5,5,10-tetramethyl-undeca-2,6,9-trien-9-one.
2.2. Antibacterial Activity of the EOs and Their Main Components

Eight samples of EOs isolated from *F. ovina*, seven samples from *F. iliensis*, and six samples from *F. akitsckensis* were evaluated for growth inhibitory activity in MRSA cultures, and the IC$_{50}$ values are shown in Table 2. The results show that *F. ovina* EOs, especially FOEO$_{Rfl}$, FOEO$_{Rfr}$, and FOEO$_{Sfr}$, had the highest growth inhibitory activity against MRSA, as compared to EOs from other *Ferula* spp. Low inhibitory activity was observed for all seven EOs isolated from *F. iliensis*. Likewise, EOs isolated from buds and leaves of *F. akitschkensis* had weak activity, while EOs isolated from other plant parts had no activity against the bacteria.

| Plant Species                  | Part of Plant | EO Name   | IC$_{50}$ (µg/mL) |
|--------------------------------|---------------|-----------|-------------------|
| *F. ovina*, flowering stage    | inflorescence | FOEO$_I$  | 28.2 ± 2.8        |
|                                | leaf          | FOEO$_Ll$ | 29.8 ± 2.9        |
|                                | stem          | FOEO$_Sf$ | 35.9 ± 2.0        |
|                                | root          | FOEO$_Rf$ | 19.1 ± 2.9        |
| umbels with seeds              | FOEO$_{U/s}$ | 43.7 ± 4.1|
| leaf                          | FOEO$_{Lf}$  | 35.3 ± 1.9|
| stem                          | FOEO$_{Sf}$  | 22.9 ± 0.8|
| root                          | FOEO$_{Rf}$  | 20.9 ± 1.2|
| *F. iliensis*, flowering stage | inflorescence | FOEO$_I$  | 55.0 ± 10.2       |
|                                | leaf          | FOEO$_Ll$ | 94.3 ± 11.1       |
|                                | stem          | FOEO$_Sf$ | 79.1 ± 8.9        |
|                                | root          | FOEO$_Rf$ | 58.1 ± 6.1        |
| umbels with seeds              | FOEO$_{U/s}$ | 49.8 ± 3.8|
| stem                          | FOEO$_{Sf}$  | 48.0 ± 2.0|
| root                          | FOEO$_{Rf}$  | 48.7 ± 5.5|
| *F. akitsckensis*, budding stage | bud          | FAEO$_b$  | 46.5 ± 6.7        |
|                                | leaf          | FAEO$_Lb$ | 47.8 ± 4.7        |
|                                | root          | FAEO$_Rb$ | N.A.              |
| umbels with seeds              | FAEO$_{u/s}$ | N.A.      |
| stem                          | FAEO$_{Sm}$  | N.A.      |
| root                          | FAEO$_{Rf}$  | N.A.      |

N.A., no activity was observed, even at the highest tested concentration (100 µg/mL). IC$_{50}$ values are presented as the mean ± S.D. of three independent experiments.

Based on chemical composition and biological activity of the EO samples tested (Tables 1 and 2), three major constituents were selected for further analysis (α-pinene, β-pinene, and sabinene). The specific enantiomers were available from commercial sources: α-pinene and sabinene as racemic mixtures and the (−)-enantiomer of β-pinene. The effects of α/β-pinenes and sabinene on MRSA growth are presented in Table 2.

Antibacterial activity of the most active samples (FOEO$_{Rfl}$, FOEO$_{Rfr}$, and α/β-pinenes) against MRSA was also evaluated by enumerating the number of colony-forming units (CFU). Following a 1-h incubation of bacteria with the selected EOs, the bacteria were plated on solid media and incubated overnight. FOEO$_{Rfl}$ and FOEO$_{Rfr}$ significantly inhibited growth of MRSA, even at the lowest concentrations tested (6.25 µg/mL), and only a few bacterial colonies were observed at the highest tested concentrations (100 µg/mL) (Figure 1A). However, the individual constituents (±)-α-pinene and (−)-β-pinene demonstrated much weaker activity, even at the highest concentrations tested (Figure 1B).
To date, more than 70 species of *Ferula* have been chemically investigated [56–58]; however, there are only a few reports on the biological activity of EOs isolated from *Ferula* spp. In some studies, the bacteriostatic properties of EOs from *Ferula* spp. were associated with a high content of α-pinene and β-pinene or polysulfides [56]. EOs from *F. assa-foetida* contained sulfur compounds and had antimicrobial activity against *S. aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [57], while EOs from *F. latisecta* were active against *S. aureus* and *Candida albicans* [33]. However, disulfides exhibited much lower antimicrobial activity than other sulfur containing compounds [58]. In the present studies, EOs from *F. iliensis*, which also mainly contain sulfur compounds, did not demonstrate a high level of antibacterial activity against MRSA. Likewise, Iranshahi et al. reported that EOs from the fruits of *F. latisepta*, which have a high content of polysulfides (mainly sec-butyl-(Z)-propenyl disulfide), exhibited only moderate antibacterial activity against *S. aureus* (ATCC 6538p) [33].

**Figure 1.** *Ferula ovina* essential oils (EOs) and their constituents inhibit MRSA growth in a dose-dependent manner. MRSA strain LAC USA300 was grown to mid exponential phase then resuspended in TSB (2 × 10^5 CFU) and incubated with varied concentrations of EOs or constituents. CFUs were recovered following a 1 h incubation with the indicated concentrations of *F. ovina* EOs from roots at flowering (FOEO_R) and fruiting (FOEO_F) stages Panel (A) or EO constituents (+)-α-pinene and (−)-β-pinene Panel (B). *p < 0.001*, as determined by one-way ANOVA with Dunnett’s test compared to LAC grown in DMSO. Data are from three separate experiments.

Although there are several reports on the antibacterial activity of EOs against *S. aureus* (e.g., see [59–61]), many of these studies involved high EO concentrations and only a few studies evaluated the effects of EOs at concentrations below 50 μg/mL. For example, Yamani et al. reported...
that EOs from Ocimum tenuiflorum at 2.25–2.5 µg/mL had bacteriostatic activity against two S. aureus strains, including MRSA [62]. The main volatile constituents of O. tenuiflorum EOs are monoterpenes and sesquiterpenes [62]. Likewise, EOs of Aloysia polystachya at 3.64, 7.28, and 29.13 µg/mL inhibited S. aureus ATCC 25923, S. aureus ATCC 29213, and MRSA, respectively [63]. The main compounds in A. polystachya EOs are carvone (78.9%) and limonene (14.2%) [63]. Here we found that EOs from F. ovina exhibited antibacterial activity against MRSA, with FOEO<sub>Rfl</sub>, FOEO<sub>Rfr</sub>, and FOEO<sub>Sfr</sub> at concentrations of 19–22 µg/mL (Table 2). Thus, this is the first study showing effective antibacterial activity of EOs from F. ovina against a clinically-relevant MRSA strain (USA300).

Studies on the antimicrobial activity of monoterpenes showed that only the (+)-enantiomers of α-pinene and β-pinene had antibacterial activity against C. albicans, Cryptococcus neoformans, Rhizopus oryzae, and MRSA [27]. In our experiments, (±)-α-pinene and (−)-β-pinene demonstrated lower activity compared to unfractionated F. ovina EOs, and (±)-sabinene also had low activity. The highest percentage of the (+)-enantiomer of β-pinene was in FOEO<sub>Sfr</sub>. Although it could be suggested that this enantiomer was responsible for the antibacterial activity of unfractionated F. ovina EOs, some active EO samples (FOEO<sub>Rfl</sub>, FOEO<sub>Rfr</sub>, and FOEO<sub>Sfr</sub>) had lower levels of β-pinene (1.9 and 1.5%, respectively) (Table 1), which is not consistent with this conclusion. Additionally, α-pinene is present at high levels in F. akitschkensis EOs, yet these EOs had no antibacterial activity [12]. Thus, it is unlikely that (±)-α-pinene and (−)-β-pinene contribute significantly to the overall antibacterial activity observed.

The most active EOs from F. ovina were characterized by a high content of monoterpane hydrocarbons (9.1–71.1%), oxygenated monoterpenes (3.0–17.3%), and sesquiterpene hydrocarbons (7.9–25.9%). However, the various F. akitschkensis EOs, which had weak or no antibacterial activity, also had a similar range of monoterpane hydrocarbons (0.4% to 89.1%), oxygenated monoterpenes (1.4% to 3.6%), and sesquiterpene hydrocarbons (1.5% to 37.4%). In an effort to identify putative component compounds responsible for the observed antibacterial activity, we conducted a linear regression analysis based on antibacterial activity of the EOs evaluated and GC-MS data for their major (>5%) constituents (Tables 1 and 2 and our previous publications [12,19]), as described previously [64]. Correlation was not analyzed for the amounts of 6Z,2,5,5,10-tetramethyl-undeca-2,6,9-trien-8-one, himachalol derivative, 10-epi-γ-eudesmol, (E/Z)-propenyl sec butyl disulfides, and myristicin because these compounds were found only in 1-7 samples of the EOs (see Table 1 and [12,19]). As a result of this analysis, relatively good correlations were obtained for trans-verbenol, eremophilene, α-pinene, the sum of α- and β-pinenes, and the total amount of monoterpane hydrocarbons and sesquiterpenes by plotting the logarithms of antibacterial activity (IC<sub>50</sub>) of the EOs versus their GC-MS data (Table 3).

To account for inactive EOs from F. akitschkensis, we also plotted the reciprocal values of antibacterial activity (1/IC<sub>50</sub>), where inactive samples were assigned a value of zero, and obtained a good linear correlation for trans-verbenol and eremophilene (Table 3 and Figure 2A,B). Antibacterial activity also correlated with the total quantity of sesquiterpenes present in the EO samples (Figure 2C), supporting the finding for eremophilene, an eremophilane-type sesquiterpene [65]. Moreover, various EOs isolated from Verbenaceae spp., which have a high amount of sesquiterpenes, were highly active against S. aureus (reviewed in [6]). Although we did not find a correlation with total amount of oxygenated monoterpenes (Table 3), our finding of trans-verbenol supports previous studies showing that oxygenated terpenoids may have more antimicrobial activity than some other EO constituents [66]. For the remaining major constituents, including α/β-pinenes and other chemical classes, no significant correlation between antibacterial activity and their concentrations in the EOs was found (Table 3). This is also consistent with previous studies showing that the presence of α/β-pinenes does not correlate with antimicrobial/antifungal activities [67,68]. Overall, trans-verbenol and eremophilene seem to represent reasonable targets for further analysis to define the anti-MRSA activity of the active EOs.
pinenes does not correlate with antimicrobial/antifungal activities [67,68]. Overall, trans-verbix and eremophilene seem to represent reasonable targets for further analysis to define the anti-MRSA activity of the active EOs.

Figure 2. Plots of antibacterial activity versus the concentrations of eremophilene Panel (A), trans-verbix Panel (B), and total sesquiterpene hydrocarbons Panel (C) in the EOs based on GC-MS data. Activities are represented as inverse (1/IC\textsubscript{50}) values to account for the four inactive EO samples from \textit{F. akitschkensis}. These samples, indicated as closed circles, were omitted from the regression calculation and are shown as outliers. Dashed lines indicate area of the 95% confidence band. FAEO\textsubscript{Lb}, EO isolated from \textit{F. akitschkensis} leaves at the budding stage; FOEO\textsubscript{Sf}, EO isolated from \textit{F. ovina} stems at the flowering stage.
Table 3. Correlation coefficients of a linear regression analysis between antibacterial activity of the EOs and their compound composition based on GC-MS data.

| Major Constituents/Chemical Class | Antimicrobial Activity of EOs Expressed As | Spearman Rank Correlation Coefficient (r) and Significance Level (p) |
|----------------------------------|---------------------------------------------|---------------------------------------------------------------------|
|                                  | Log[IC$_{50}$]                               | 1/[IC$_{50}$]                                                       |
| Compound                         |                                             |                                                                     |
| α-pinene                         | −0.64 (p < 0.01)                            | 0.26 (n.s. $^a$)                                                   |
| β-pinene                         | −0.37 (n.s.)                               | −0.27 (n.s.)                                                       |
| α/β-pinenes                      | −0.62 (p < 0.01)                            | 0.21 (n.s.)                                                        |
| sabinene                         | −0.29 (n.s.)                               | 0.13 (n.s.)                                                        |
| β-phellandrene                   | −0.13 (n.s.)                               | 0.03 (n.s.)                                                        |
| β-caryophyllene                  | −0.15 (n.s.)                               | 0.51 (p < 0.03)                                                   |
| trans-verbenol                   | −0.76 (p < 0.001)                           | 0.74 (p < 0.002)                                                  |
| eremophilene                     | −0.81 (p < 0.0001)                          | 0.76 (p < 0.0001)                                                 |
| 2-himachalen-7-ol                | −0.07 (n.s.)                               | 0.07 (n.s.)                                                        |
|                                 |                                             |                                                                     |
| Chemical Class                   |                                             |                                                                     |
| monoterpene hydrocarbons         | −0.50 (p < 0.05)                            | 0.13 (n.s.)                                                        |
| oxygenated monoterpenes          | −0.08 (n.s.)                               | 0.27 (n.s.)                                                        |
| sesquiterpene hydrocarbons       | −0.85 (p < 0.0001)                          | 0.76 (p < 0.0001)                                                 |
| oxygenated sesquiterpenes        | −0.47 (n.s.)                               | 0.32 (n.s.)                                                        |

Concentration of compound(s) in EO samples are expressed as relative %. $^a$ n.s., no correlation (p > 0.05).

Unfortunately, these compounds are not commercially available and will require isolation, which is difficult due to their low concentrations, or possibly synthesis. Therefore, further studies are clearly warranted and are the focus of our ongoing research.

In general, our analysis performed using two activity representations (LogIC$_{50}$ and 1/IC$_{50}$) suggests that anti-MRSA activity of the EOs could be attributed to the presence of eremophilene and/or trans-verbenol and/or their additive or synergistic effect with α/β-pinenes, sabinene, and other constituents. Thus, compounds present in the greatest proportions are not necessarily responsible for the largest share of the antibacterial activity, and involvement of less abundant constituents should be considered. For example, evaluation of the major compounds of *Piper hispidinervum* EOs showed that a low quantity of terpinolene increased the nematicidal effect of safrole when binary combinations of these compounds were tested [69]. However, the interactive effects of major active constituents of EOs from *Glossogyne tenuifolia* (linalool, 4-terpineol, α-terpineol, ρ-cymene) were additive instead of synergistic, as determined by checkerboard analysis with pathogenic bacteria, including *S. aureus* [70].

In conclusion, we report that EOs isolated from selected *Ferula* species have antibacterial activity against MRSA USA300, which is a relevant clinical strain. The most active EOs were isolated from *F. ovina* and were characterized by an abundance of monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes. On the other hand, *F. iliensis* EOs had low antibacterial activity, suggesting that (E)-propenyl sec-butyl disulfide and (Z)-propenyl sec-butyl disulfide do not have significant activity against MRSA. Finally, *F. akitsckensis* EOs possessed weak or no antibacterial activity. Although EOs from *F. ovina* demonstrated concentration-dependent inhibition of MRSA growth, their major constituents (α-pinene, β-pinene, and sabinene) had low activity, suggesting that they were not responsible for the observed bioactivity of the unfraccionated EOs. On the other hand, correlation of the GC-MS data with antibacterial activity suggested that the sesquiterpene hydrocarbon eremophilene and the oxygenated monoterpane trans-verbenol could be the constituents responsible for antibacterial activity. Further studies are clearly necessary to evaluate efficacy and elucidate the exact mechanisms by which EOs from *F. ovina* exhibit their antibacterial effects.
3. Materials and Methods

3.1. Chemicals and Materials

Three compounds found in EOs were obtained from commercial sources. (±)-α-Pinene was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). (1S)-(−)-β-Pinene was from Alfa Aesar (Ward Hill, MA, USA). (±)-Sabinene was from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The compounds were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich Chemical Co.; 10 mM stock solutions) and stored at −20 °C. S. aureus was grown using tryptic soy broth (TSB) and tryptic soy agar (TSA) (EMD Millipore, Burlington, MA, USA) containing 0.5% glucose (Sigma-Aldrich).

3.2. Plant Material

F. ovina (Boiss.) Boiss., F. iliensis, and F. akitschkensis B. Fedtsch. ex Koso-Pol. were collected from the Almaty region of Kazakhstan in May–July 2015 at two stages: F. ovina and F. iliensis were collected during the flowering and fruiting stages, and F. akitschkensis was collected at the budding and fruiting stages. GPS coordinates: F. iliensis was collected at an altitude of 695 m above sea level (latitude, N 43°35′29″; longitude, W 78°36′95″). F. ovina was collected at an altitude of 1014 m above sea level (latitude, N 43°31′52″; longitude, W 78°35′17″). F. akitschkensis was collected at an altitude of 1525 m above sea level (latitude, N 43°16′70″; longitude, W 77°42′86″). Voucher specimens were deposited at the Institute of Plant Biology and Biotechnology (Almaty, Kazakhstan). Separately collected plant parts (buds, inflorescences, leaves, stems, roots, and umbels with seeds) were air-dried for 7–14 days at room temperature in shaded, well-aired rooms. Weighed samples were cut under laboratory conditions before hydrodistillation.

3.3. Isolation of EOs

EOs were obtained from air-dried plant material (30–60 g depending on plant parts) by hydrodistillation for 3 h using a Clevenger-type apparatus. For the hydrodistillation, the conditions accepted by the European Pharmacopoeia (European Directorate for the quality of Medicines, Council of Europe, Strasbourg, France, 2014) were applied. The yield of EOs was calculated on a dry weight basis. Solutions of the EOs were prepared in DMSO (10 mg/mL) for antibacterial evaluation and n-hexane (10% w/v) for gas chromatographic analysis.

3.4. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

Chemical composition of the EOs was determined as reported previously [11] using GC-FID and GC-MS. GC-MS analysis was performed with an Agilent 5975 GC-MSD system (Agilent Technologies, Santa Clara, CA, USA). An Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness) was used with He as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min, increased to 220 °C at a rate of 4°C/min, kept constant at 220 °C for 10 min, and then increased to 240 °C at a rate of 1 °C/min. The split ratio was adjusted to 40:1, and the injector temperature was 250 °C. MS were collected at 70 eV with a mass range from m/z 35 to 450. GC analysis was performed using an Agilent 6890N GC system. To obtain the same elution order as with GC-MS, simultaneous injection was performed using the same column and appropriate operational conditions. Flame ionization detector (FID) temperature was 300 °C. The EO components were identified by co-injection with standards (whenever possible), which were purchased from commercial sources or isolated from natural sources. In addition, compound identities were confirmed by comparison of their mass spectra with those in the Wiley GC-MS Library (Wiley, New York, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany), Adams Library, and NIST Library. Confirmation was also achieved using the in-house “Başer Library of Essential Oil Constituents” database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C8–C40 n-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples for
the determination of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

3.5. Chiral GC-MS Analysis

Chromatographic separation on a chiral column was performed for α-pinene, β-pinene, and sabinene. GC-MS analysis of the enantiomers in the oil was performed with an Agilent 7890 GC equipped with a FID and 5975 MSD with a triple-axis detector and an Agilen G 4513 autoinjector, integrated with a Gerstel CIS (Gerstel, Mühlheim an der Ruhr, Germany; SEM Ltd., Istanbul, Turkey). Chiral separation was performed on a Lipodex G column (25 m × 0.25 mm × 0.125 µm film thickness; Macherey-Nagel, Düren, Germany) with He as the carrier gas (65 min at 5 mL/min, average velocity 77.985 cm/s). Injection quantity was 1 µL (10% in hexane). The temperature program for separation of α-pinene, β-pinene, and sabinene enantiomers was 50 min at 35 °C and then increased 40 °C/min to 200 °C for 10.875 min. Run time was 65 min. The split ratio was adjusted to 40:1, and the injector temperature was at 250 °C. FID temperature was 250 °C.

3.6. Bacterial Strain and Culture

MRSA pulse-field gel electrophoresis type USA300 cultures were grown in TSB containing 0.5% glucose. Overnight cultures of bacteria were diluted 1:200 in 20 mL TSB in a 125 mL flask and grown at 37 °C with shaking at 250 rpm. For all experiments, cultures were grown to mid-exponential growth phase (optical density at 600 nm [OD$_{600}$] = 1.5).

3.7. Bacterial Growth Inhibition Assays

For analysis of antibacterial activity in culture, bacteria (2.5 × 10$^7$ CFU/mL) were resuspended in TSB and incubated for 4 h at 37 °C with 5 different concentrations of EOs (6.25, 12.5, 25, 50, and 100 µg/mL) or with each of the constituents (α-pinene, β-pinene, and sabinene at 31.25, 62.5, 125, 250, and 500 µg/mL) in 96-well tissue culture plates. EOs or pure compounds diluted in DMSO were added to the wells (final concentration of DMSO was 1%). DMSO was used as a negative control. The growth suppression of bacteria was monitored as absorbance ($\lambda$ = 600 nm) every 5 min for 4 h using a SpectraMax 190 microplate reader. Spectinomycin was used as positive control, and 50 µg/mL of this antibiotic completely inhibited bacteria growth.

For analysis of EO or constituent effects on bacterial survival, bacteria (2 × 10$^5$) were resuspended in TSB and added to 96-well tissue culture plates with different concentrations of compounds diluted in TSB. The plates were incubated for 1 h at 37 °C, and the samples were plated onto TSA in Petri dishes. At the indicated time points, samples were serially diluted (1:10) in water, and CFU were enumerated the next day, as reported previously [71].

3.8. Statistical Analyses

The inhibitory effect of EOs against MRSA USA300 (LAC) was determined by calculation of the inhibitory concentration values (IC$_{50}$) as the mean ± S.D. of three independent experiments. To calculate median IC$_{50}$, curve fitting was performed by nonlinear regression analysis of the dose–response curves generated using Prism 7 (GraphPad Software, Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was performed on the datasets, followed by Dunnett’s test. For correlation analyses, the Spearman rank correlation coefficient ($r$) was calculated.

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**References**

1. Holmes, N.E.; Howden, B.P. What’s new in the treatment of serious MRSA infection? *Curr. Opin. Infect. Dis.* **2014**, *27*, 471–478. [CrossRef] [PubMed]

2. McGuinness, W.A.; Malachowa, N.; DeLeo, F.R. Vancomycin resistance in *Staphylococcus aureus*. *Yale J. Biol. Med.* **2017**, *90*, 269–281. [PubMed]

3. DeLeo, F.R.; Otto, M.; Kreiswirth, B.N.; Chambers, H.F. Community-associated meticillin-resistant *Staphylococcus aureus*. *Lancet* **2010**, *375*, 1557–1568. [CrossRef]

4. Dryden, M.S.; Dailly, S.; Crouch, M. A randomized, controlled trial of tea tree topical preparations versus a standard topical regimen for the clearance of MRSA colonization. *J. Hosp. Infect.* **2004**, *56*, 283–286. [CrossRef] [PubMed]

5. Bakkali, F.; Averbeck, S.; Averbeck, D.; Waomar, M. Biological effects of essential oils—A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef] [PubMed]

6. Pérez Zamora, C.M.; Torres, C.A.; Nuñez, M.B. Antimicrobial Activity and Chemical Composition of Essential Oils from Verbenaceae Species Growing in South America. *Molecules* **2018**, *23*, 544. [CrossRef] [PubMed]

7. Pandey, A.K.; Singh, P. The genus *Artemisia*: A 2012–2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. *Medicines* **2017**, *4*, 68. [CrossRef] [PubMed]

8. Aghraz, A.; Benameur, Q.; Gervasi, T.; Ait Dra, L.; Ben-Mahdi, M.H.; Larhsini, M.; Markouk, M.; Cicero, N. Antibacterial activity of *Cladanthus arabisicus* and *Bubonium imbricatum* essential oils alone and in combination with conventional antibiotics against Enterobacteriaceae isolates. *Lett. Appl. Microbiol.* **2018**, *55*, 1–8. [CrossRef] [PubMed]

9. Benameur, Q.; Gervasi, T.; Pellizzeri, V.; Pfuchtová, M.; Tali-Maama, H.; Assaous, F.; Guettou, B.; Rahal, K.; Grufiová, D.; Dugo, G.; et al. Antibacterial activity of *Thymus vulgaris* essential oil alone and in combination with cefotaxime against blaESBL producing multidrug resistant Enterobacteriaceae isolates. *Nat. Prod. Res.* **2018**, *1–8*. [CrossRef] [PubMed]

10. Iranshahi, M.; Arfa, P.; Ramezani, M.; Jafarifar, M.R.; Sadeghian, H.; Bassarello, C.; Piacente, S.; Pizza, C. Sesquiterpene coumarins from *Ferula szovitsiana* and in vitro antileishmanial activity of 7-prenyloxycoumarins against promastigotes. *Phytochemistry* **2007**, *68*, 554–561. [CrossRef] [PubMed]

11. Schepetkin, I.A.; Kushnarenko, S.V.; Ozek, G.; Kirpotina, L.N.; Utegenova, G.A.; Kotukhov, Y.A.; Danilova, A.N.; Ozek, T.; Basar, K.H.; Quinn, M.T. Inhibition of human neutrophil responses by the essential oil of *Artemisia kotuchovii* and its constituents. *J. Agric. Food Chem.* **2015**, *63*, 4999–5007. [CrossRef] [PubMed]

12. Schepetkin, I.A.; Kushnarenko, S.V.; Ozek, G.; Kirpotina, L.N.; Sinharoy, P.; Utegenova, G.A.; Abidkulova, K.T.; Ozek, T.; Basar, K.H.; Kovrizhina, A.R.; et al. Modulation of human neutrophil responses by the essential oils from *Ferula akitschkensis* and their constituents. *J. Agric. Food Chem.* **2016**, *64*, 7156–7170. [CrossRef] [PubMed]

13. Iranshahy, M.; Iranshahi, M. Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)—A review. *J. Ethnopharmacol.* **2011**, *134*, 1–10. [CrossRef] [PubMed]

14. Iranshahi, M.; Amanolahi, F.; Schneider, B. New sesquiterpene coumarin from the roots of *Ferula latisecta*. *Avicenna J. Phytomed.* **2012**, *2*, 133–138. [PubMed]

15. Kasaian, J.; Asili, J.; Iranshahi, M. Sulphur-containing compounds in the essential oil of *Ferula allicacea* roots and their mass spectral fragmentation patterns. *Pharm. Biol.* **2016**, *54*, 2264–2268. [CrossRef] [PubMed]

16. Zhou, Y.; Xin, F.; Zhang, G.; Qu, H.; Yang, D.; Han, X. Recent advances on bioactive constituents in *Ferula*. *Drug Dev. Res.* **2017**, *78*, 321–331. [CrossRef] [PubMed]

17. Mahboubi, M. *Ferula gummosa*, a traditional medicine with novel applications. *J. Diet. Suppl.* **2016**, *13*, 700–718. [CrossRef] [PubMed]
18. Akaberi, M.; Iranshahy, M.; Iranshahi, M. Review of the traditional uses, phytochemistry, pharmacology and toxicology of giant fennel (Ferula communis L. subsp. communis). Iran. J. Basic Med. Sci. 2015, 18, 1050–1062. [PubMed]

19. Ozek, G.; Schepetkin, I.A.; Utegenova, G.A.; Kirpotina, L.N.; Andrei, S.R.; Ozek, T.; Basar, K.H.C.; Abidkulova, K.T.; Kushnarenko, S.V.; Khlebnikov, A.I.; et al. Chemical composition and phagocyte immunomodulatory activity of Ferula iliensis essential oils. J. Leukoc. Biol. 2017, 101, 1361–1371. [CrossRef] [PubMed]

20. Zellagui, A.; Gherraf, N.; Rouhauti, S. Chemical composition and antibacterial activity of the essential oils of Ferula vesicariensis Coss et Dur. leaves endemic in Algeria. Org. Med. Chem. Lett. 2012, 2, 31. [CrossRef] [PubMed]

21. Dehghan, G.; Solaimanian, R.; Shahverdi, A.R.; Amin, G.; Abdollahi, M.; Shafiee, A. Chemical composition and antimicrobial activity of essential oil of Ferula szovitsiana DC. Flavour Fragr. J. 2007, 22, 224–227. [CrossRef]

22. Maggi, F.; Cecchini, C.; Cresci, A.; Coman, M.M.; Tirillini, B.; Sagratini, G.; Papa, F. Chemical composition and antimicrobial activity of the essential oil from Ferula glauca L. (F. communis L. subsp. glauca) growing in Marche (Central Italy). Fitoterapia 2009, 80, 68–72. [CrossRef] [PubMed]

23. Elghwaji, W.; El-Sayed, A.M.; El-Deeb, K.S.; ElSayed, A.M. Chemical composition, antimicrobial and antitumor potentiality of essential oil of Ferula tingitana L. Apiaceae grow in Libya. Pharmacogn. Mag. 2017, 13, S446–S451. [PubMed]

24. Geroushi, A.; Auzzi, A.A.; Elhwuegi, A.S.; Elzawam, F.; Elsherif, A.; Nahar, L.; Sarker, S.D. Antiinflammatory sesquiterpenes from the root oil of Ferula hermonis. Phytother. Res. 2011, 25, 774–777. [CrossRef] [PubMed]

25. De Cassia da Silveira e Sa, R.; Andrade, L.N.; de Sousa, D.P. A review on anti-inflammatory activity of monoterpenes. Molecules 2013, 18, 1227–1254. [CrossRef] [PubMed]

26. Bayala, B.; Bassole, I.H.; Scifo, R.; Goula, C.; Morel, L.; Lobaccaro, J.M.; Simpore, J. Anticancer activity of essential oils and their chemical components—A review. Am. J. Cancer Res. 2014, 4, 591–607. [PubMed]

27. Da Silva, A.C.R.; Lopes, P.M.; de Azevedo, M.M.B.; Costa, D.C.M.; Alviano, C.S.; Alviano, D.S. Biological activities of alpha-pinene and beta-pinene enantiomers. Molecules 2012, 17, 6305–6316. [CrossRef] [PubMed]

28. Marchese, A.; Arciola, C.R.; Barbieri, R.; Silva, A.S.; Nabavi, S.F.; Tsetegho Sokeng, A.J.; Izadi, M.; Jafari, N.J.; Suntar, I.; Daglia, M.; et al. Update on monoterpenes as antimicrobial agents: A particular focus on p-cymene. Materials 2017, 10, 947. [CrossRef] [PubMed]

29. Kavoosi, G.; Rowshan, V. Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from Ferula assa-foetida oleo-gum-resin: Effect of collection time. Food Chem. 2013, 138, 2180–2187. [CrossRef] [PubMed]

30. Zhi-da, M.; Qi-fi, M.; Mizuno, M.; Tanaka, T.; Iinuma, M. Polysulfanes in the volatile oils of Ferula species. Planta Med. 1987, 53, 300–302. [CrossRef] [PubMed]

31. Iranshahi, M.; Amin, G.R.; Amini, M.; Shafiee, A. Sulfur containing derivatives from Ferula persica var. latisecta. Phytochemistry 2003, 63, 965–966. [CrossRef]

32. Iranshahi, M.; Mojarab, M.; Sadeghian, H.; Hanafi-Bojd, M.Y.; Schneider, B. Polar secondary metabolites of Ferula persica roots. Phytochemistry 2008, 69, 473–478. [CrossRef] [PubMed]

33. Iranshahi, M.; Hassanzadeh-Khayat, M.; Bazzaz, B.S.F.; Sabeti, Z.; Enayati, F. High content of polysulfides in the volatile oil of Ferula latisecta Rech. f. et Ael. fruits and antimicrobial activity of the oil. J. Essent. Oil Res. 2008, 20, 183–185. [CrossRef]

34. Radulovic, N.S.; Zlatkovic, D.B.; Randjelovic, P.J.; Stojanovic, N.M.; Novakovic, S.B.; Akhlaghi, H. Chemistry of spices: Bornyl 4-methoxybenzoate from Ferula ovina (Boiss.) Boiss. (Apiaceae) induces hyperalgesia in mice. Food Funct. 2013, 4, 1751–1758. [CrossRef] [PubMed]

35. Alkhali, S.; Aqel, M.; Afifi, F.; Alesawi, D. Effects of an aqueous extract of Ferula ovina on rabbit and guinea-pig smooth-muscle. J. Ethnopharmacol. 1990, 30, 35–42. [CrossRef]

36. Syed, M.; Hanif, M.; Chaudhary, F.M.; Bhatty, M.K. Antimicrobial activity of the essential oils of Umbelliferae family. Part IV. Ferula narthex, Ferula ovina and Ferula otopoda. Pak. J. Sci. Ind. Res. 1987, 30, 19–23.

37. Babushok, V.I.; Linstrom, P.J.; Zenkevich, I.G. Retention indices for frequently reported compounds of plant essential oils. J. Phys. Chem. Ref. Data 2011, 40, 043101-1–043101-47. [CrossRef]
39. Brophy, J.J.; Davies, N.W.; Southwell, I.A.; Stiff, I.A.; Williams, L.R. Gas chromatographic quality control for oil of Melaleuca terpinen-4-ol type (Australian tea tree). J. Agric. Food Chem. 1989, 37, 1330–1335. [CrossRef]

40. Ferrari, B.; Tomi, F.; Casanova, J. Composition and chemical variability of Ferula communis essential oil from Corsica. Flavour Fragr. J. 2005, 20, 180–185. [CrossRef]

41. Arze, J.B.L.; Garneau, F.X.; Collin, G.; Jean, F.I.; Gagnon, H. Essential oils from Bolivia. I. Asteraceae: Baccharis tricuneta (L.f.) Pers. var. ruiziana Cuatrecassas. J. Essent. Oil Res. 2004, 16, 429–431. [CrossRef]

42. Formisano, C.; Senatore, F.; Bancheva, S.; Bruno, M.; Rosselli, S. Volatile components from aerial parts of Centaurea spinosociliata Seenus ssp. Cristata (Bartl.) Dostal and Centaurea spinosociliata Seenus ssp. Spinosociliata growing wild in Croatia. Croat. Chem. Acta 2010, 83, 403–408.

43. Baser, K.H.C.; Demirci, B.; Özek, T.; Akalin, E.; Özhataý, N. Micro-distilled volatile compounds from Ferulago species growing in western Turkey. Pharm. Biol. 2002, 40, 466–471. [CrossRef]

44. Lopes-Lutz, D.; Alviano, D.S.; Alviano, C.S.; Kolodziejczyk, P.P. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry 2008, 69, 1732–1738. [CrossRef] [PubMed]

45. Sütçü, S.; Özbek, G.; Meriçli, A.H.; Baser, K.H.C.; Haliloglu, Y.; Özbek, T. Chemical and biological diversity of the leaf and rhizome volatiles of Acorus calamus L. from Turkey. J. Essent. Oil Bear. Plants 2017, 20, 646–661. [CrossRef]

46. Ortet, R.; Thomas, O.P.; Regalado, E.L.; Pino, J.A.; Filippi, J.J.; Fernández, M.D. Composition and biological properties of the volatile oil of Artemisia gorgonum Webb. Chem. Biodivers. 2010, 7, 1325–1332. [CrossRef] [PubMed]

47. Belanger, A.; Collin, G.; Garneau, F.X.; Gagnon, H.; Pichette, A. Aromas from Quebec. II. Composition of the essential oil and the rhizomes of Asarum canadense L. J. Essent. Oil Res. 2010, 22, 164–169. [CrossRef]

48. Garneau, F.X.; Collin, G.J.; Jean, F.I.; Gagnon, H.; Arze, J.B.L. Essential oils from Bolivia. XIII. Myrtaceae: Blepharocalyx salicifolius (Kunth.) O. Berg. J. Essent. Oil Res. 2013, 25, 166–170. [CrossRef]

49. Weyerstahl, P.; Marschall, H.; Thefeld, K.; Subba, G.C. Constituents of the essential oil from the rhizomes of Hedychium gardnerianum Roscoe. Flavour Fragr. J. 1998, 13, 377–388. [CrossRef]

50. Orte, R.; Thomas, O.P.; Regalado, E.L.; Pino, J.A.; Filippi, J.J.; Fernández, M.D. Composition and biological properties of the volatile oil of Artemisia gorgonum Webb. Chem. Biodivers. 2010, 7, 1325–1332. [CrossRef] [PubMed]

51. Noorizadeh, H.; Farmany, A. Exploration of linear and nonlinear modeling techniques to predict of retention index of essential oils. J. Chin. Chem. Soc. 2010, 57, 1268–1277. [CrossRef]

52. Maggio, A.; Riccobono, I.; Spadaro, V.; Scialabba, A.; Bruno, M.; Senatore, F. Chemical composition of the essential oils of three endemic species of Anthemis Sect. Hiorthia (DC.) R. Fern. growing wild in Sicily and chemotaxonomic volatile markers of the genus Anthemis L.: An update. Chem. Biodivers. 2014, 11, 652–672. [CrossRef] [PubMed]

53. Özbek, G.; Suleimen, Y.; Tabanca, N.; Doudkin, R.; Gorovoy, P.G.; Göger, F.; Wedge, D.E.; Ali, A.; Khan, I.A.; Baser, K.H.C. Chemical diversity and biological activity of the volatiles of five Artemisia species from Far East Russia. Rec. Nat. Prod. 2014, 8, 242–261. [CrossRef]

54. Li, X.; Wang, Y.; Zhu, J.; Xiao, Q. Essential oil composition analysis of three cultivars seeds of Resina ferulae from Xinjiang, China. Pharmacogn. Mag. 2011, 7, 116–120. [CrossRef] [PubMed]

55. Rahmani, B.; Shiraz, N.Z.; Masnabadi, N.; Masoudi, S.; Monfared, A.; Lajiani, K.; Rustaiyan, A. Volatile constituents of Alocarpum erianthum (DC) H. Riedl & Kuber, Ferula ovina (Boiss.) Boiss. and Pimpinella affinis Ledeb. Three Umbelliferae herbs growing in Iran. J. Essent. Oil Res. 2008, 20, 232–235.

56. Sahebkar, A.; Iranshahi, M. Biological activities of essential oils from the genus Ferula (Apiaceae). Asian Biomed. 2010, 4, 835–847. [CrossRef]

57. Samadi, N.; Shahani, S.; Akbarzadeh, H.; Mohammad-Motamed, S.; Safaripour, E.; Farjadmand, F.; Eftekhari, M.; Monser-Esfahani, H.R.; Khanavi, M. Essential oil analysis and antibacterial activity of Ferula assa-foetida L. aerial parts from Neishabour mountains. Res. J. Pharmacogn. 2016, 3, 35–42.

58. Kim, S.; Kubec, R.; Musah, R.A. Antibacterial and antifungal activity of sulfur-containing compounds from Petiveria alliacea L. J. Ethnopharmacol. 2006, 104, 188–192. [CrossRef] [PubMed]

59. Chao, S.; Young, G.; Oberg, C.; Nakaoka, K. Inhibition of methicillin-resistant Staphylococcus aureus (MRSA) by essential oils. Flavour Fragr. J. 2008, 23, 444–449. [CrossRef]
60. Doran, A.L.; Morden, W.E.; Dunn, K.; Edwards-Jones, V. Vapour-phase activities of essential oils against antibiotic sensitive and resistant bacteria including MRSA. *Lett. Appl. Microbiol.* 2009, 48, 387–392. [CrossRef] [PubMed]
61. Zouhir, A.; Jridi, T.; Nefzi, A.; Ben Hamida, J.; Sebei, K. Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by antimicrobial peptides (AMPs) and plant essential oils. *Pharm. Biol.* 2016, 54, 3136–3150. [CrossRef] [PubMed]
62. Yamani, H.A.; Pang, E.C.; Mantri, N.; Deighton, M.A. Antimicrobial activity of Tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. *Front. Microbiol.* 2016, 7, 681. [CrossRef] [PubMed]
63. Pérez-Zamora, C.M.; Torres, C.A.; Aguado, M.I.; Bela, A.J.; Nuñez, M.B.; Bregni, C. Antibacterial activity of essential oils of *Aloysia polystachya* and *Lippia turbinata* (Verbenaceae). *Bol. Latinoam. Caribe Plantas Med. Aromat.* 2016, 15, 199–205.
64. Orchard, A.; Sandasi, M.; Kamatou, G.; Viljoen, A.; van Vuuren, S. The in vitro antimicrobial activity and chemometric modelling of 59 commercial essential oils against pathogens of dermatological relevance. *Chem. Biodivers.* 2017, 14. [CrossRef] [PubMed]
65. Yuyama, K.T.; Fortkamp, D.; Abraham, W.R. Eremophilane-type sesquiterpenes from fungi and their medicinal potential. *Biol. Chem.* 2017, 399, 13–28. [CrossRef] [PubMed]
66. Zengin, H.; Baysal, A.H. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* 2014, 19, 17773–17798. [CrossRef] [PubMed]
67. Cimanga, K.; Kambu, K.; Tona, L.; Apers, S.; De Bruyne, T.; Hermans, N.; Totte, J.; Pieters, L.; Vlietinck, A.J. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J. Ethnopharmacol.* 2002, 79, 213–220. [CrossRef]
68. Feyaerts, A.F.; Mathe, L.; Luyten, W.; De Graeve, S.; Van Dyck, K.; Broeckx, L.; Van Dijck, P. Essential oils and their components are a class of antifungals with potent vapour-phase-mediated anti-candida activity. *Sci. Rep.* 2018, 8, 3958. [CrossRef] [PubMed]
69. Andres, M.F.; Rossa, G.E.; Cassel, E.; Vargas, R.M.F.; Santana, O.; Diaz, C.E.; Gonzalez-Coloma, A. Biocidal effects of *Piper hispidinervum* (Piperaceae) essential oil and synergism among its main components. *Food Chem. Toxicol.* 2017, 109, 1086–1092. [CrossRef] [PubMed]
70. Yang, T.S.; Chao, L.K.; Liu, T.T. Antimicrobial activity of the essential oil of *Glossogyne tenuifolia* against selected pathogens. *J. Sci. Food Agric.* 2014, 94, 2965–2971. [CrossRef] [PubMed]
71. Long, D.R.; Mead, J.; Hendricks, J.M.; Hardy, M.E.; Voyich, J.M. 18β-Glycyrrhetinic acid inhibits methicillin-resistant *Staphylococcus aureus* survival and attenuates virulence gene expression. *Antimicrob. Agents Chemother.* 2013, 57, 241–247. [CrossRef] [PubMed]

**Sample Availability:** Samples of the essential oils are available from the authors.

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