Mosquito Larvicidal Activity, Antimicrobial Activity, and Chemical Compositions of Essential Oils from Four Species of Myrtaceae from Central Vietnam

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Abstract: Mosquitoes are important vectors of several diseases, and control of these insects is imperative for human health. Insecticides have proven useful in controlling mosquito populations, but insecticide resistance and environmental concerns are increasing. Additionally, emerging and re-emerging microbial infections are problematic. Essential oils have been shown to be promising mosquito larvicidal agents as well as antimicrobial agents. In this work, the essential oils from four species of Myrtaceae (Baeckea frutescens, Callistemon citrinus, Melaleuca leucadendra, and Syzygium nervosum) growing wild in central Vietnam have been obtained by hydrodistillation and analyzed by gas chromatographic techniques. The essential oils have been screened for mosquito larvicidal activity against Aedes aegypti, Aedes albopictus, and Culex quinquefasciatus, and for antimicrobial activity against Enterococcus faecalis, Staphylococcus aureus, and Candida albicans. Callistemon citrinus fruit essential oil, rich in α-pinene (35.1%), 1,8-cineole (32.4%), limonene (8.2%), and α-terpineol (5.8%) showed good larvicidal activity with 24-h LC50 = 17.3 µg/mL against both Ae. aegypti and Cx. quinquefasciatus, and good antibacterial activity against E. faecalis (minimum inhibitory concentration (MIC) = 16 µg/mL). The 48-h larvicidal activities of Melaleuca leucadendra leaf essential oil, rich in α-eudesmol (17.6%), guaiol (10.9%), linalool (5.1%), (E)-caryophyllene (7.0%), and bulnesol (3.6%) were particularly notable, with LC50 of 1.4 and 1.8 µg/mL on Ae. aegypti and Cx. quinquefasciatus. Similarly, M. leucadendra bark essential oil, with α-eudesmol (24.1%) and guaiol (11.3%), showed good antibacterial activity against E. faecalis. Both B. frutescens and C. citrinus leaf essential oils demonstrated anti-Candida activities with MIC values of 16 µg/mL. The results of this investigation suggest that essential oils derived from the Myrtaceae may serve as “green” alternatives for the control of mosquitoes and/or complementary antimicrobial agents.

Keywords: Baeckea frutescens; Callistemon citrinus; Melaleuca leucadendra; Syzygium nervosum
1. Introduction

Mosquitoes are important vectors of diseases and kill more humans than any other animal. *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) (Diptera: Culicidae) are vectors of the yellow fever, dengue, Zika, and chikungunya viruses [1–3]; *Culex quinquefasciatus* (Say) is the primary vector of the Saint Louis encephalitis and West Nile viruses, as well as the filarial nematode *Wuchereria bancrofti*, and may also be a vector of the Zika virus [4].

Microbial infections continue to be a problem, for humans [5], as well as for livestock and other agriculture settings [6–8]. Compounding this problem are newly emerging pathogenic microorganisms, in addition to re-emerging multidrug-resistant pathogens [9,10].

The Myrtaceae is comprised of 131 genera and around 5500 species, all of which are woody trees or shrubs and contain essential oils [11]. Several members of the family are commercially important for their medicinal essential oils, such as clove (*Syzygium aromaticum* (L.) Merr. & L.M. Perry), tea tree (*Melaleuca alternifolia* Cheel), allspice (*Pimenta dioica* (L.) Merr.), and *Eucalyptus*. In this work, we present the essential oil compositions of four species of Myrtaceae growing wild in central Vietnam, their larvicidal activities against *Ae. aegypti, Ae. albopictus*, and *Cx. quinquefasciatus*, and their antimicrobial activities against *Enterococcus faecalis, Staphylococcus aureus*, and *Candida albicans*.

*Baeckea frutescens* L. (syn. *Baeckea chinensis* Gaertn., *Baeckea cochinchinensis* Blume, *Baeckea sumatrana* Blume) is a shrub or small tree that ranges throughout southeastern China (including the provinces of Fujian, Guangdong, Guangxi, Hainan, Jiangxi, and Zhejiang), Burma, Cambodia, India, the Philippines, Thailand, and Vietnam [12].

*Callistemon citrinus* (Curtis) Skeels (syn. *Melaleuca citrina* (Curtis) Dum. Cours., *Callistemon lanceolatus* DC., *Callistemon lanceolatus* Sweet, *Metrosideros citrina* Curtis, *Metrosideros lanceolata* Sm.) is a shrub or small tree, native to Australia, but has been introduced to tropical and subtropical regions worldwide [13].

*Melaleuca leucadendra* (L.) L. (syn. *Melaleuca viridiflora* C.F. Gaertn., *Myrtus leucadendra* L.) is a tree growing as large as 40 m in height, native to tropical Australia (Queensland, Northern Territory, and Western Australia, New Guinea, and islands of eastern Indonesia [14]. The tree has been introduced to other tropical areas [12], including Vietnam, where it is grown for use as poles and construction materials [14].

*Syzygium nervosum* DC. (syn. *Cleistocalyx operculatus* (Roxb.) Merr. & L.M.Perry, *Eugenia operculata* Roxb.) is a medium-sized tree native to the Asian tropics, from southern China (Guangdong, Guangxi, Hainan, Xizang Zizhiqu, and Yunnan provinces), India, Burma, Sri Lanka, Thailand, and Vietnam [12], and south into eastern Australia [15].

Photographs of the plants presented in this work are shown in Figure 1.
Figure 1. Photographs of the plants examined in this work. A: Baeckea frutescens, B: Callistemon citrinus, C: Syzygium nervosum, D: Melaleuca leucadendra.

2. Results and Discussion

2.1. Chemical Compositions

The essential oil from the fresh leaves of Baeckea frutescens was obtained in a yield of 2.23%. The leaf essential oil composition of B. frutescens is presented in Table 1. A total of 88 compounds were identified accounting for 100% of the essential oil composition, with monoterpene hydrocarbons (55.6%) predominating. The major components were α-pinene (11.1%), β-pinene (19.0%), p-cymene (8.9%), 1,8-cineole (10.1%), γ-terpinene (11.7%), (E)-caryophyllene (7.1%), and α-humulene (9.9%). Leaf essential oil compositions have previously been reported from Vietnam [16–18], China [19], and from Malaysia [20]. The compositions of these essential oils have shown remarkable chemical variation. Nevertheless, the composition of B. frutescens in this present study is very similar to that
found in a sample collected from Đồng Hới, Quảng Bình Province [16], and sample 2 (from Sóc Sơn District, Hanoi) reported by Tam and co-workers [17].

| RI_{(cal)} | RI_{(db)} | Compounds | % | RI_{(cal)} | RI_{(db)} | Compounds | % |
|-----------|-----------|-----------|---|-----------|-----------|-----------|---|
| 992       | 997       | α-Thujene | 1.8 | 1370      | 1375      | α-Copaene | 0.2 |
| 930       | 933       | α-Piene   | 11.1 | 1398      | 1405      | (Z)-Caryophyllene | tr |
| 943       | 948       | α-Fenchne | tr  | 1401      | 1406      | α-Gurjane | tr |
| 945       | 953       | Camphene  | 0.1 | 1415      | 1417      | (E)-Caryophyllene | 7.1 |
| 968       | 972       | Sabine   | tr  | 1433      | 1438      | Aromadendrene | 0.1 |
| 975       | 978       | β-Piene   | 19.0 | 1452      | 1454      | α-Humulene | 9.9 |
| 984       | 991       | Myrene    | 0.3 | 1455      | 1457      | alio-Aromadendrene | 0.1 |
| 1000      | 1004     | p-Mentha-1(7),8-diene | tr  | 1466      | 1472      | trans-Cadin-1(6),4-diene | 0.1 |
| 1003      | 1007     | α-Phillandrene | 0.1 | 1469      | 1478      | γ-Murolene | tr |
| 1005      | 1009     | δ-3-Carene | 8.9 | 1490      | 1501      | α-Selinene | 0.1 |
| 1013      | 1018     | α-Terpinene | 0.3 | 1485      | 1490      | γ-Amorphone | tr |
| 1021      | 1025     | p-Cymeine | 8.9 | 1490      | 1501      | α-Selinene | 0.1 |
| 1025      | 1030     | Limonene  | 1.7 | 1492      | 1497      | α-Murolene | 0.1 |
| 1029      | 1030     | 1,6-Cineole | 10.1 | 1500      | 1507      | Geranyl isobutyrate | 0.1 |
| 1030      | 1034     | (Z)-β-Ocimene | tr  | 1506      | 1512      | γ-Cadinene | 0.2 |
| 1041      | 1045     | (E)-Ocimene | tr  | 1509      | 1519      | Cubebol | tr |
| 1055      | 1057     | γ-Terpinene | 11.7 | 1512      | 1518      | δ-Cadinene | 0.9 |
| 1065      | 1069     | cis-Linalool oxide (furanoïd) | tr  | 1515      | 1519      | trans-Calamenene | 0.1 |
| 1081      | 1086     | Terpinolene | 0.7 | 1516      | 1521      | Zonarene | 0.1 |
| 1085      | 1093     | p-Cymenene | tr  | 1526      | 1536      | trans-Cadin-1,4-diene | 0.1 |
| 1096      | 1101     | Linalool  | 4.4 | 1530      | 1538      | α-Cadinene | tr |
| 1098      | 1104     | Hotrienol | tr  | 1534      | 1544      | α-Calacorene | tr |
| 1114      | 1119     | endo-Fenchol | 0.1 | 1541      | 1549      | α-Elemol | tr |
| 1133      | 1139     | Nopinone  | tr  | 1545      | 1551      | (Z)-Caryphyllene oxide | 0.1 |
| 1136      | 1141     | trans-Pino-carveol | tr  | 1554      | 1562      | (E)-Nerolidol | 0.5 |
| 1150      | 1156     | Camphene hydrate | tr  | 1570      | 1576      | Spathulenol | tr |
| 1165      | 1170     | δ-Terpine | 0.1 | 1576      | 1587      | Caryophyllene oxide | 2.0 |
| 1167      | 1170     | Bornol    | 0.1 | 1579      | 1590      | Globulol | 0.1 |
| 1169      | 1171     | cis-Linalool oxide (pyranoid) | tr  | 1592      | 1592      | Humulene epoxide I | 0.3 |
| 1173      | 1179     | 2-Isopropenyl-5-methyl-4-hexenal | 0.1 | 1598      | 1605      | Ledol | 0.1 |
| 1176      | 1180     | Terpinen-4-ol | 0.7 | 1604      | 1613      | Humulene epoxide II | 2.4 |
| 1178      | 1188     | Naphthalene | tr  | 1619      | 1624      | Muurola-4,10(14)-dien-1β-ol | tr |
| 1181      | 1186     | α-Cumen-8-ol | tr  | 1621      | 1628      | 1-epi-Cubenol | 0.3 |
| 1190      | 1195     | α-Terpine | 1.7 | 1625      | 1631      | Germacra-1(10),5-dien-4α-ol | 0.3 |
| 1198      | 1203     | p-Cumenol | tr  | 1626      | 1632      | Humulenol II | 0.3 |
| 1219      | 1229     | Nerol     | tr  | 1630      | 1636      | Caryophylla-4(12),8(13)-dien-5β-ol | 0.1 |
| 1234      | 1240     | Ascaridole | tr  | 1634      | 1643      | τ-Cadinol | 0.2 |
| 1244      | 1244     | Geraniol  | 0.1 | 1636      | 1645      | τ-Murolol | 0.1 |
| 1261      | 1268     | Geraniol  | tr  | 1639      | 1651      | α-Murolol (= δ-Cadinol) | 0.1 |
| 1268      | 1275     | trans-Ascaridol glycol | tr  | 1648      | 1652      | α-Eudesmol | 0.5 |
| 1274      | 1284     | p-Cymen-7-ol | tr  | 1841      | 1837      | Homoioisobaeckol | 0.5 |
| 1284      | 1289     | Thymol    | tr  | 1861      | 1877      | Monoterpehydrocarbons | 55.6 |
| 1291      | 1399     | Carvacrol | tr  | 1862      | 1878      | Oxygenated monoterpenoids | 17.5 |
| 1298      | 1306     | Isoascaridole | tr  | 1874      | 1882      | Sesquiterpenhydrocarbons | 19.1 |
| 1314      | 1320     | Methyl geranate | 0.1 | 1883      | 1900      | Oxygeneated sesquiterpenoids | 7.3 |
| 1341      | 1349     | α-Cubebe | tr  | 1887      | 1903      | Benzenoid aromatics | 0.5 |
| 1344      | 1357     | Eugenol   | tr  | 1900      | 1916      | Others | tr |
|           |          |           |     |           |           | Total identified | 100.0 |

RI_{(cal)}: Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. RI_{(db)}: Retention indices obtained from the databases [21–23]. tr: trace (< 0.05%).

The leaf and fruit essential oils of Callistemon citrinus were obtained in yields of 0.62% and 0.34%, respectively. A total of 53 compounds were identified in the leaf essential oil of C. citrinus, and 63 compounds were identified in the fruit essential oil, accounting for 99.6% and 99.4% of the compositions, respectively. Monoterpehydrocarbons (27.6% and 53.8%) and oxygenated monoterpenoids (69.9% and 41.3%) dominated the leaf and fruit oils, respectively. The major components in C. citrinus leaf
and fruit essential oils were α-pinene (18.1% and 35.1%, respectively), limonene (5.4% and 8.2%), 1,8-cineole (56.3% and 32.4%), and α-terpineol (11.2% and 5.8%) (Table 2). There have been several previous examinations of the composition of *C. citrinus* leaf essential oil from various geographical locations [24–34]. An agglomerative hierarchical cluster analysis based on the compositions of the leaf essential oils (Figure 2) reveals three well-defined clusters: (#1) 1,8-cineole >> α-pinene > α-terpineol, (#2) 1,8-cineole > α-terpineol >> eugenol, and (#3) α-pinene > 1,8-cineole > α-terpineol. The *C. citrinus* leaf essential oil from Vietnam (this study) falls into cluster #1.

**Table 2.** Chemical compositions of the leaf and fruit essential oils of *Callistemon citrinus* from central Vietnam.

| RI (calc) | RI (db) | Compound                              | % Composition |
|----------|---------|---------------------------------------|---------------|
|          |         |                                       | Leaf | Fruit |
| 793      | 791     | 2,4-Dimethyl-3-pentanone              | 0.3   | tr    |
| 912      | 913     | Isobuty1 isobutyrate                  | 0.2   | 0.3   |
| 924      | 927     | α-Thujene                             | 0.3   | 0.8   |
| 932      | 933     | α-Pinene                              | 18.1  | 35.1  |
| 946      | 948     | α-Fenchene                            | tr    | tr    |
| 948      | 953     | Camphene                              | 0.1   | 0.1   |
| 971      | 972     | Sabine ne                             | tr    | tr    |
| 976      | 978     | β-Pinene                              | 0.6   | 0.7   |
| 987      | 989     | Myrcene                               | 0.1   | 0.5   |
| 999      | 1000    | δ-2-Carene                            | tr    | 0.1   |
| 1004     | 1004    | p-Mentha-1(7),8-diene                 | 0.1   | 0.1   |
| 1006     | 1007    | α-Phellandrene                        | 0.4   | 1.6   |
| 1008     | 1009    | δ-3-Carene                            | 0.1   | 0.1   |
| 1011     | 1014    | Isoamyl isobutyrate                   | 0.2   | 0.3   |
| 1014     | 1018    | α-Terpine                            |       | 0.2   |
| 1014     | 1015    | 2-Methylbutyl isobutyrate             | tr    | 0.1   |
| 1021     | 1022    | Ethyl 3-methylbut-3-enyl carbonate    | 0.1   | 0.1   |
| 1024     | 1025    | p-Cymene                              | 2.2   | 4.6   |
| 1029     | 1030    | Limonene                              | 5.4   | 8.2   |
| 1030     | 1032    | 1,8-cineole                           | 56.3  | 32.4  |
| 1032     | 1034    | (Z)-β-Ocimene                         |       | 0.1   |
| 1044     | 1046    | (E)-β-Ocimene                         | tr    | 0.2   |
| 1051     | 1050    | Prenyl isobutyrate                    | tr    | 0.1   |
| 1057     | 1057    | γ-Terpine                            | 0.3   | 1.0   |
| 1084     | 1087    | Terpinolene                           | 0.1   | 0.6   |
| 1088     | 1093    | p-Cymene                              |       | 0.1   |
| 1099     | 1101    | Linalool                              | 0.5   | 1.4   |
| 1119     | 1119    | *endo*-Fenchol                        | 0.1   | 0.1   |
| 1140     | 1141    | *trans*-Pinocarveol                   | 0.3   | tr    |
| 1155     | 1156    | Camphene hydrate                      | tr    | tr    |
| 1163     | 1164    | Pinocarvone                           | tr    | —     |
| 1170     | 1170    | δ-Terpine                            | 0.2   | 0.1   |
| 1170     | 1165    | *iso*-Borneol                         |       | 0.1   |
| 1173     | 1173    | Borneol                               | 0.1   | 0.1   |
| 1179     | 1179    | 2-Isopropenyl-5-methyl-4-hexenal      | 0.1   | tr    |
| 1180     | 1180    | Terpinen-4-ol                        | 0.5   | 0.6   |
| 1185     | 1188    | Naphthalene                          | 0.1   | —     |
| 1186     | 1189    | *p*-Cymen-8-ol                       | —     | tr    |
| 1188     | 1187    | *trans*-p-Mentha-1(7),8-dien-2-ol     | 0.1   | —     |
| 1194     | 1195    | α-Terpineol                          | 11.2  | 5.8   |
| 1202     | 1202    | *cis*-Sabinol                        | —     | 0.1   |
| 1219     | 1223    | *trans*-Carv eol                      | 0.1   | tr    |
| 1230     | 1230    | *cis*-p-Mentha-1(7),8-dien-2-ol       | tr    | —     |
| 1249     | 1249    | Geraniol                             | 0.5   | 0.6   |
| 1298     | 1300    | Carvacrol                            | tr    | 0.1   |
| 1351     | 1356    | Eugenol                              | 0.1   | 0.1   |
| 1385     | 1390    | β-Elemene                            | —     | 0.1   |
| 1392     | 1395    | Phenylethyl isobutyrate              | tr    | tr    |
Table 2. Cont.

| RI_{(calc)} | RI_{(db)} | Compound                  | % Composition |
|-------------|-----------|---------------------------|---------------|
|             |           |                           | Leaf | Fruit |
| 1417        | 1417      | (E)-Caryophyllene         | 0.1  | 0.2   |
| 1436        | 1438      | Aromadendrene             | 0.1  | 0.2   |
| 1452        | 1454      | α-Humulene                | —    | 0.1   |
| 1458        | 1458      | allo-Aromadendrene        | 0.1  | 0.1   |
| 1477        | 1480      | Germacrene D              | —    | tr    |
| 1487        | 1491      | Viridiflorene             | —    | 0.1   |
| 1500        | 1503      | (E,E)-α-Farnesene         | —    | 0.1   |
| 1505        | 1507      | Geranyl isobutyrate       | 0.1  | —     |
| 1505        | 1508      | β-Bisabolene              | —    | 0.1   |
| 1514        | 1518      | s-Cadinene                | —    | tr    |
| 1535        | 1539      | Flavesone                 | 0.3  | 0.3   |
| 1557        | 1561      | (E)-Nerolidol             | —    | 0.1   |
| 1575        | 1578      | Spathulenol               | 0.4  | 1.3   |
| 1580        | 1577      | Caryophyllene oxide       | tr   | 0.1   |
| 1584        | 1590      | Globulol                  | 0.1  | 0.2   |
| 1593        | 1594      | Viridiflorol              | 0.1  | 0.1   |
| 1595        | 1599      | Cubeban-11-ol             | tr   | 0.1   |
| 1609        | 1614      | iso-Leptospernone         | tr   | 0.1   |
| 1619        | 1626      | Leptospernone             | tr   | 0.2   |
| 1629        | 1629      | iso-Spathulenol           | —    | 0.2   |

Monoterpene hydrocarbons: 27.6 53.8
Oxygenated monoterpenoids: 69.9 41.3
Sesquiterpene hydrocarbons: 0.2 0.8
Oxygenated sesquiterpenoids: 0.5 2.0
Others: 1.4 1.4
Total identified: 99.6 99.4

RI_{(calc)}: Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. RI_{(db)}: Retention indices obtained from the databases [21–23]. tr: trace (<0.05%).

Figure 2. Dendrogram obtained from the agglomerative hierarchical cluster analysis of Callistemon citrinus leaf essential oil compositions.
Essential oils were obtained from six different tissues of *Melaleuca leucadendra*, young leaves, old leaves, stem bark, fruits, and branch tips, in yields of around 1%. A total of 104 compounds were identified in the *M. leucadendra* essential oils. Sesquiterpene hydrocarbons (18.8%–31.0%) and oxygenated sesquiterpenoids (35.6%–69.5%) were the dominant chemical classes. The essential oil compositions are compiled in Table 3.

| RI_{calc} | RI_{db} | Compound | Young Leaf | Old Leaf | % Composition | Stem Bark | Fruit | Branch Tips |
|----------|---------|----------|-----------|---------|---------------|-----------|-------|-------------|
| 923      | 927     | α-Thujene | 0.8       | 0.4     | 0.1 tr        | 1.2       |       |             |
| 931      | 933     | α-Pinene  | 0.7       | 0.6     | 0.8 0.2       | 1.4       |       |             |
| 947      | 953     | Camphene  | —         | 0.1     | tr            | tr        |       |             |
| 960      | 960     | Benzaldehyde | 0.1     | 0.1     | —             | tr        |       |             |
| 975      | 978     | β-Pinene  | 0.1       | 0.2     | 0.3 0.1       | 0.1       | 0.1   |             |
| 987      | 991     | Myrcene   | 0.2       | 0.3     | 0.2 0.1       | 0.2       | 0.2   |             |
| 1003     | 1004    | p-Mentha-1(7),8-diene | — | —     | tr — —       |            | 1.0   |             |
| 1005     | 1007    | α-Phellandrene | 0.3     | 0.2     | —             | 0.3       |       |             |
| 1007     | 1009    | 3,5-carene | 0.1       | tr      | tr —         | 0.1       |       |             |
| 1015     | 1018    | α-Terpine | 0.4       | 0.3     | —             | 0.4       |       |             |
| 1023     | 1025    | α-Cymene  | 3.9       | 1.7     | 1.3 0.5       | 8.7       |       |             |
| 1027     | 1030    | Limonene  | 0.3       | 0.8     | 1.4 0.4       | 0.7       |       |             |
| 1029     | 1031    | β-Phellandrene | 0.1     | 0.1     | tr            | 0.1       |       |             |
| 1030     | 1030    | 1,8-cineole | —         | 5.2     | 1.8 0.2       | tr        |       |             |
| 1033     | 1034    | (Z)-β-Ocimene | —        | tr      | —             | tr        |       |             |
| 1043     | 1045    | (E)-β-Ocimene | —        | tr      | —             | tr        |       |             |
| 1056     | 1057    | γ-Terpine | 2.2       | 1.3     | tr            | 3.3       |       |             |
| 1068     | 1069    | cis-Linalool oxide (furanoid) | — | —       | —             | tr        |       |             |
| 1084     | 1086    | Terpinolene | 3.0       | 1.6     | 0.1 tr       | 4.4       |       |             |
| 1089     | 1093    | p-Cymene  | 0.1       | tr      | tr —         | 0.2       |       |             |
| 1099     | 1101    | Linalool  | 4.9       | 5.1     | 1.4 0.4       | 4.2       |       |             |
| 1103     | 1107    | Nonanal   | —         | —       | 0.1           | —         |       |             |
| 1110     | 1110    | 1,3,8-p-Menthatriene | tr | tr      | —             | 0.2       |       |             |
| 1112     | 1124    | cis-p-Menth-2-en-1-ol | tr | tr      | —             | —         |       |             |
| 1141     | 1142    | Epoxyterpinolene | 0.3     | tr      | —             | 0.6       |       |             |
| 1147     | 1149    | iso-Pulegol | —        | tr      | —             | tr        |       |             |
| 1168     | 1170    | δ-Terpinol | —        | —       | —             | —         |       |             |
| 1170     | 1170    | Bornol    | —         | —       | tr            | —         |       |             |
| 1177     | 1177    | 2-Isopropenyl-5-methyl-4-hexenal | 0.2     | 0.1     | —             | 0.3       |       |             |
| 1179     | 1180    | Terpinen-4-ol | 0.9     | 0.4     | tr 0.1       | 1.1       |       |             |
| 1183     | 1188    | Naphthalene | —         | —       | 0.1 0.1       | 0.2       |       |             |
| 1184     | 1188    | 4'-Methylacetophenone | 0.1     | tr      | —             | 0.1       |       |             |
| 1186     | 1188    | p-Cymen-8-ol | 1.0     | 0.2     | 0.1 0.1       | 1.2       |       |             |
| 1194     | 1195    | α-Terpine | 0.7       | 1.8     | 0.5 0.1       | 0.6       |       |             |
| 1198     | 1195    | p-Menth-3-en-7-al | — | —       | —             | 0.1       |       |             |
| 1202     | 1203    | p-Cumenol | 0.1       | 0.1     | —             | 0.1       |       |             |
| 1222     | 1222    | iso-Ascaridol | —        | tr      | —             | 0.1       |       |             |
| 1223     | 1226    | Nerol     | —         | tr      | tr            | —         |       |             |
| 1225     | 1227    | Citronellol | —         | tr      | tr 0.1       | 0.1       |       |             |
| 1248     | 1249    | Geraniol  | 0.2       | 0.6     | 0.4 0.1       | 0.2       |       |             |
| 1266     | 1266    | Geranial  | —         | tr      | tr            | —         |       |             |
| 1273     | 1275    | trans-Ascaridol glycol | 0.2     | tr      | —             | 0.1       |       |             |
| 1290     | 1291    | cis-Ascaridol glycol | 0.1     | —       | —             | 0.1       |       |             |
| 1293     | 1305    | Benzophenone | —        | —       | tr            | —         |       |             |
| 1318     | 1318    | 3-Hydroxyxineole | 0.2     | —       | —             | 0.1       |       |             |
| 1348     | 1356    | Eugenol    | —         | 0.1     | —             | —         |       |             |
| 1367     | 1371    | α-Ylangene | 0.4       | 0.6     | 0.9 0.6       | 0.7       |       |             |
| 1373     | 1375    | α-Copaene | 0.2       | 0.3     | 0.8 0.3       | 0.3       |       |             |
| 1375     | 1380    | Geranyl acetate | —       | 0.1     | 0.2 tr       | 0.1       |       |             |
| 1381     | 1382    | β-Bourbonene | —        | —       | tr            | —         |       |             |
| 1387     | 1390    | β-Elemene  | 0.1       | 0.1     | 0.1 tr       | 0.1       |       |             |
| 1389     | 1394    | Sativone  | 0.1       | 0.1     | 0.1 tr       | 0.1       |       |             |
| 1401     | 1405    | (Z)-Caryophyllene | —        | tr      | tr            | —         |       |             |
| 1417     | 1417    | (E)-Caryophyllene | 3.8     | 7.0     | 5.5 4.3       | 5.7       |       |             |
Table 3. Cont.

| RI_{(calc)} | RI_{(db)} | Compound | % Composition | Young Leaf | Old Leaf | Stem Bark | Fruit | Branch Tips |
|-------------|-----------|----------|---------------|------------|----------|-----------|-------|-------------|
| 1421        | 1426      | 8-Hydroxycarvotanacetone | 0.1 | — | — | 0.1 |
| 1426        | 1427      | γ-Elemene | 0.2 | 0.3 | 0.1 | 0.1 |
| 1432        | 1436      | α-Guaiene | 0.1 | 0.2 | 0.2 | 0.2 |
| 1438        | 1444      | Guai-6,9-diene | 0.2 | 0.2 | 0.1 | 0.2 |
| 1444        | 1448      | cis-Murola-3,5-diene | 0.2 | 0.2 | 0.1 | 0.2 |
| 1446        | 1447      | iso-Germacrene D | 0.1 | 0.2 | 0.1 | 0.2 |
| 1453        | 1454      | α-Humulene | 2.8 | 4.4 | 3.5 | 2.8 |
| 1467        | 1473      | Drima-7,9(11)-diene | 0.1 | 0.2 | 0.2 | 0.2 |
| 1474        | 1476      | γ-Gurjunene | 0.6 | 1.1 | 1.1 | 0.9 |
| 1476        | 1479      | α-Amorphene | 0.7 | 1.2 | 1.5 | 0.9 |
| 1484        | 1488      | δ-Selinene | 1.0 | 1.6 | 0.7 | 1.3 |
| 1487        | 1492      | β-Selinene | 2.4 | 3.7 | 4.8 | 3.1 |
| 1490        | 1490      | γ-Amorphone | 0.2 | 0.3 | 0.4 | 0.3 |
| 1494        | 1501      | α-Selinene | 2.1 | 3.7 | 3.6 | 2.5 |
| 1495        | 1496      | trans-Murola-4(14),5-diene | — | 0.2 | — | — |
| 1496        | 1497      | α-Murolene | — | 0.2 | 0.1 | — |
| 1499        | 1505      | α-Bulnesene | — | 0.1 | 0.1 | 0.2 |
| 1499        | 1506      | β-Amorphone | — | 0.2 | — | — |
| 1500        | 1502      | trans-β-Guaiene | — | 0.3 | — | — |
| 1501        | 1501      | β-Dihydroagarofuran | — | 0.2 | 0.2 | — |
| 1515        | 1518      | δ-Cadinene | — | 0.2 | 0.1 | — |
| 1517        | 1519      | trans-Calamenene | — | 0.7 | 0.4 | — |
| 1534        | 1540      | Selina-4(15),7(11)-diene | 0.5 | 0.6 | 0.6 | 0.6 |
| 1539        | 1541      | α-Calacorene | 0.3 | 0.6 | 0.8 | 0.5 |
| 1539        | 1546      | Selina-3,7(11)-diene | 0.3 | 0.2 | — | 0.3 |
| 1545        | 1546      | α-Elemol | 0.3 | 0.1 | 0.3 | 0.4 |
| 1556        | 1557      | Germacrene B | 0.4 | 0.4 | 0.1 | — |
| 1580        | 1587      | Caryophyllene oxide | 1.8 | 2.3 | 3.3 | 3.2 |
| 1590        | 1600      | Khusimone | 0.2 | 0.3 | 0.4 | 0.3 |
| 1595        | 1603      | Guaiol | 12.5 | 10.9 | 11.3 | 10.4 |
| 1607        | 1613      | Humulene epoxide II | 0.8 | 0.9 | 1.5 | 1.3 |
| 1610        | 1619      | Rosilol | 0.5 | 0.4 | 0.5 | 0.5 |
| 1620        | 1621      | Germaca-1(10),5-dien-4-ol | 0.3 | 0.2 | 0.2 | — |
| 1623        | 1624      | Selina-6-en-48-ol | 2.0 | 1.6 | 1.7 | 2.2 |
| 1624        | 1629      | iso-Spathulenol | 0.2 | — | — | — |
| 1628        | 1631      | Eremoligenol | 3.4 | 3.4 | 4.9 | 6.5 |
| 1630        | 1633      | γ-Eudesmol | 3.9 | 2.8 | 3.5 | 5.3 |
| 1632        | 1634      | cis-Cadin-4-en-7-ol | 3.5 | 3.0 | 3.3 | 3.5 |
| 1635        | 1636      | Caryophylla-4(12),8(13)-dien-5-ol | — | 0.2 | 0.2 | 0.1 |
| 1638        | 1645      | Hinesol | 1.0 | 0.9 | 1.2 | 1.6 |
| 1645        | 1644      | Selina-3,11-dien-6-ol | — | 0.2 | 0.3 | — |
| 1653        | 1652      | α-Eudesmol | 21.2 | 17.6 | 24.1 | 30.7 |
| 1657        | 1660      | Selin-11-en-4-ol | 1.9 | 1.5 | 1.3 | 1.6 |
| 1663        | 1673      | Bulnesol | 5.3 | 3.6 | 3.3 | 4.4 |
| 1668        | 1671      | 14-Hydroxy-9-epi-(E)-caryophyllene | — | 0.5 | — | — |
| 1670        | 1677      | Cadalene | — | 0.3 | 0.2 | — |
| 1695        | 1696      | Juniper camphor | — | 0.2 | 0.1 | 0.2 |
| 1918        | 1929      | Carisone | — | 0.1 | 0.4 | — |

| Monoterpeine hydrocarbons | 11.9 | 7.2 | 4.2 | 1.3 | 21.2 |
| Oxygenated monoterpenoids | 8.8 | 13.5 | 4.4 | 0.8 | 9.0 |
| Sesquiterpene hydrocarbons | 18.8 | 30.8 | 30.5 | 23.4 | 31.0 |
| Oxygenated sesquiterpenoids | 56.9 | 47.6 | 59.1 | 69.5 | 35.6 |
| Benzenoid aromatics | 0.2 | 0.1 | 0.0 | 0.0 | 0.1 |
| Others | 0.0 | 0.0 | 0.2 | 0.1 | 0.2 |
| Total identified | 96.6 | 99.3 | 98.4 | 95.2 | 97.1 |

RI_{(calc)}: Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. RI_{(db)}: Retention indices obtained from the databases [21–23]. tr: trace (<0.05%).

Brophy has described two different chemotypes of M. leucadendra from Australia, based on leaf essential oil composition [14]. Chemotype I, from Western Australia, is rich in monoterpenoids, e.g.,
1,8-cineole (10–45%), p-cymene (5–22%), α-pinene (4–19%), limonene (3–6%), and α-terpineol (6–9%). Chemotype II, from eastern Australia, is dominated by phenylpropanoids, which was divided into two subtypes: IIa, eugenol methyl ether (95%–97%), and IIb, (E)-iso-eugenol methyl ether (74%–88%) subtype). Chemotype IIa has also been represented by samples from Minas Gerais, Brazil [35], and from Lahore, Pakistan [36]. There is a third chemotype, dominated by (E)-nerolidol (>90%), which has been described from Uttarakhand, India [37] and from Pernambuco, Brazil [38]. Chemotype I has also been found in Cuba [39] and Rio de Janeiro, Brazil [40]. They were both dominated by 1,8-cineole (43.0% and 48.7%, respectively), but these two samples were also rich in viridiflorol (24.2% and 27.8%, respectively), and therefore, may represent a subtype of chemotype I.

An agglomerative hierarchical cluster analysis was carried out using the *M. leucadendra* leaf essential oil compositions reported in the literature [14,36–50] (Figure 3). The cluster analysis reveals two sub-types of chemotype I, the two sub-types of chemotype II, as described by Brophy [14], and chemotype III, the nerolidol chemotype. The leaf essential oils of *M. leucadendra* from Vietnam, fall into sub-type Ib; the leaf oils were rich in α-eudesmol (17.6%–21.2%), guaiol (10.9%–12.5%), with lesser concentrations of linalool (4.9%–5.1%), (E)-caryophyllene (3.8%–7.0%), and bulnesol (3.6%–5.3%). Concentrations of 1,8-cineole were low (0.0%–5.2%), and (E)-nerolidol and viridiflorol were not observed at all.

![Figure 3. Dendrogram obtained from the agglomerative hierarchical cluster analysis of Melaleuca leucadendra leaf essential oil compositions.](image-url)
The leaf essential oil of *Syzygium nervosum* was obtained in 0.2% yield. A total of 61 compounds were identified in the leaf oil of *S. nervosum*, accounting for 90.9% of the composition, with 31.7% monoterpenoid hydrocarbons, 24.3% sesquiterpenoid hydrocarbons, and 27.9% oxygenated sesquiterpenoids predominating. The leaf essential oil of *S. nervosum* was rich in (Z)-β-ocimene (20.3%), caryophyllene oxide (13.2%), (E)-caryophyllene (12.1%), and α-pinene (5.2%) (Table 4). The leaf essential oil composition is qualitatively similar, but quantitatively different, to a previous report on the leaf essential oil from Lê Mao District, Vinh City, Vietnam [51]. Both samples had relatively high concentrations of α-pinene, (Z)-β-ocimene, (E)-β-ocimene, and (E)-caryophyllene (3.7%, 32.1%, 9.4%, and 14.5%, respectively, in the Vinh City sample), but the concentration of myrcene was much higher (24.6%) in the sample from Vinh City. The leaf essential oil *S. nervosum* from Nepal showed a very different composition with myrcene (69.7%), (E)-β-ocimene (12.2%), (Z)-β-ocimene (4.8%), and linalool (4.1%) [52].

### Table 4. Chemical compositions of essential oils from *Syzygium nervosum* from central Vietnam.

| RI<sub>(calc)</sub> | RI<sub>(db)</sub> | Compound                  | %   | RI<sub>(calc)</sub> | RI<sub>(db)</sub> | Compound                  | %   |
|-------------------|------------------|----------------------------|-----|-------------------|------------------|----------------------------|-----|
| 930               | 933              | α-Pinene                   | 5.2 | 1486              | 1492             | β-Selinene                 | 0.9 |
| 968               | 971              | Tetrahydrofururfuryl acetate | 0.2 | 1492              | 1501             | α-Selinene                 | 0.9 |
| 975               | 978              | β-Pinene                   | 1.0 | 1494              | 1500             | α-Murolene                 | 0.4 |
| 986               | 991              | Myrcene                    | 0.4 | 1509              | 1512             | γ-Cadinene                 | 0.9 |
| 1022              | 1025             | p-Cymene                   | 0.1 | 1514              | 1518             | β-Cadinene                 | 1.0 |
| 1027              | 1030             | Limonene                   | 0.2 | 1533              | 1538             | α-Cadinene                 | 0.4 |
| 1033              | 1034             | (Z)-β-Ocimene              | 20.3| 1538              | 1541             | α-Calcoene                 | 0.4 |
| 1043              | 1045             | (E)-β-Ocimene              | 3.5 | 1557              | 1560             | (E)-Nerolidol              | 0.1 |
| 1089              | 1091             | Rosefuran                  | 0.7 | 1559              | 1560             | β-Calcoene                 | 0.5 |
| 1092              | 1101             | α-Pinene oxide             | 1.3 | 1573              | 1576             | Spathulenol                | 0.6 |
| 1097              | 1101             | Linalool                   | 0.3 | 1579              | 1587             | Caryophyllene oxide        | 13.2|
| 1101              | 1102             | 6-Methyl-3,5-heptadien-2-one| 0.5 | 1582              | 1590             | Globulol                   | 1.2 |
| 1125              | 1127             | ally-Ocimene               | 0.8 | 1591              | 1592             | Viridiflorol               | 0.4 |
| 1127              | 1128             | (Z)-Epoxy ocimene (= (Z)-Myroxide) | 0.5 | 1593              | 1593             | Guaiol                     | 0.5 |
| 1137              | 1137             | (E)-Epoxy ocimene (= (E)-Myroxide) | 0.4 | 1595              | 1592             | Humulene epoxide I         | 0.2 |
| 1167              | 1169             | Rosefuran epoxide          | 0.3 | 1603              | 1607             | β-Oplopenone               | 0.8 |
| 1170              | 1171             | p-Mentha-1,5-dien-8-ol     | 0.2 | 1606              | 1613             | Humulene epoxide II        | 1.8 |
| 1182              | 1188             | Naphthalene                | 0.4 | 1623              | 1624             | Selina-6-en-β-ol           | 3.4 |
| 1193              | 1195             | α-Terpineol                | 0.1 | 1624              | 1628             | 1-epi-Cubanol              | 0.6 |
| 1199              | 1205             | (3Z)-Octenyl acetate       | 0.4 | 1631              | 1634             | cis-Cadin-4-en-7-ol        | 0.4 |
| 1206              | 1207             | (3E)-Octenyl acetate       | 0.7 | 1638              | 1643             | Caryophylla-4(12),8(13)-dien-5β-ol | 0.5 |
| 1333              | 1349             | α-Terpinyl acetone         | 0.7 | 1640              | 1644             | τ-Cadinol                  | 0.2 |
| 1366              | 1367             | Cyclostavine               | 0.2 | 1643              | 1651             | α-Murolol (= δ-Cadinol)    | 0.2 |
| 1372              | 1375             | α-Copaene                  | 0.4 | 1645              | 1645             | Selina-3,11-dien-6-ol      | 0.4 |
| 1374              | 1380             | Geranyl acetate            | 0.4 | 1652              | 1655             | α-Cadinol                 | 1.7 |
| 1417              | 1417             | (E)-Caryophyllene          | 12.1| 1655              | 1660             | Selin-11-en-κ-ol           | 0.6 |
| 1426              | 1433             | β-Copaene                  | 0.3 | 1698              | 1697             | (E)-trans-α-Bergamota-2,10-dien-12-ol | 0.4 |
| 1435              | 1438             | Aromadendrene              | 0.6 |                   |                  | Monoterpene hydrocarbons  | 31.7|
| 1452              | 1454             | α-Humulene                 | 2.7 |                   |                  | Oxygenated monoterpenoids  | 4.9 |
| 1471              | 1478             | γ-Murolene                 | 0.9 |                   |                  | Sesquiterpene hydrocarbons| 24.3|
| 1473              | 1476             | γ-Gurjuneine               | 1.4 |                   |                  | Oxygenated sesquiterpenoids| 27.9|
| 1475              | 1482             | α-Amorphene                | 0.3 |                   |                  | Others                     | 2.1 |

RI<sub>(calc)</sub>: Retention indices determined with respect to a homologous series of n-alkanes on a ZB-5ms column. RI<sub>(db)</sub>: Retention indices obtained from the databases [21–23].

### 2.2. Mosquito Larvicidal Activity

The 24-h and 48-h larvicidal activities are presented in Tables 5 and 6, respectively. The Myrtaceae essential oils presenting the best 24-h larvicidal activities were *C. citrinus* fruit essential oil (LC<sub>50</sub> = 17.3 µg/mL against both *Ae. aegypti* and *Cx. quinquefasciatus*), *M. leucadendra* stem bark essential oil (LC<sub>50</sub> = 17.1, 19.3, and 21.4 µg/mL against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*,...
respectively), *M. leucadendra* fruit essential oil (LC$_{50}$ = 13.9, 19.2, and 26.2 µg/mL against *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*, respectively), and, especially, *M. leucadendra* old leaf essential oil (LC$_{50}$ = 7.4 and 6.6 µg/mL against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively). The 48-h larvicidal activities of *M. leucadendra* old leaf essential oil are particularly notable with LC$_{50}$ of 1.4 and 1.8 µg/mL on *Ae. aegypti* and *Cx. quinquefasciatus*.

The larvicidal activities of *M. leucadendra* essential oils are likely due to the high concentrations of α-eudesmol and guaiol, or possibly synergistic effects involving these compounds. Unfortunately, there appear to be no reports on the larvicidal activities of these compounds.

It is tempting to suggest that the sensitivity of mosquito larvae to *C. citrinus* fruit essential oil is due to the combination of α-pinene and 1,8-cineole. 1,8-Cineole, (+)-α-pinene, and (–)-α-pinene have been screened against *Ae. aegypti* larvae, and showed modest larvicidal activities (LC$_{50}$) of 74.9, 50.9, and 64.8 µg/mL, respectively [53]. Furthermore, *Hedychium bousigonianum* cv. “Tai Emperor” rhizome essential oil, with 16.7% α-pinene and 25.5% 1,8-cineole, showed only marginal larvicidal activity against *Ae. aegypti* (80% lethality at 125 µg/mL) [54]. In addition, Pavela has shown that α-pinene has marginal larvicidal activity against *Cx. quinquefasciatus* (LC$_{50}$ = 95 µg/mL), 1,8-cineole is inactive (LC$_{50}$ > 250 µg/mL), and a binary mixture of the two compounds does not demonstrate synergistic activity [55]. The observed larvicidal activities of *C. citrinus* fruit essential oil is apparently due to synergistic activities involving minor components. It has been shown that *Musca domestica* preferentially metabolizes the major components in an essential oil, which leaves the components of lower concentrations to act as the toxic agents [56].

| Table 5. Twenty-four-hour mosquito larvicidal activities of Myrtaceae essential oils. |
|---------------------------------------------------------------|
| Essential Oil                  | LC$_{50}$ (95% Fiducial Limits) | LC$_{90}$ (95% Fiducial Limits) | χ$^2$ | p     |
|--------------------------------|---------------------------------|---------------------------------|-------|-------|
|                                | Aedes aegypti                   |                                 |       |       |
| *Baeckea frutescens* leaf EO  | 23.00 (20.38–25.75)             | 40.05 (35.75–46.71)             | 6.512 | 0.039 |
| *Callistemon citrinus* leaf EO| 22.37 (18.62–25.88)             | 37.44 (30.00–49.06)             | 0.6655| 0.717 |
| *Melaleuca leucadendra* young leaf EO | nt                        | nt                              |       |       |
| *Melaleuca leucadendra* old leaf EO | 7.40 (6.30–8.612)             | 18.29 (16.05–21.47)             | 30.77 | 0.000 |
| *Melaleuca leucadendra* stem bark EO | 17.14 (14.73–19.21)         | 36.25 (32.42–42.31)             | 2.244 | 0.326 |
| *Melaleuca leucadendra* fruit EO | 13.90 (11.03–16.02)            | 31.76 (28.40–37.25)             | 0.5750| 0.750 |
| *Melaleuca leucadendra* branch tip EO | 21.99 (19.80–24.57)         | 37.63 (33.67–43.39)             | 2.277 | 0.517 |
| *Syzygium nervosum* leaf EO   | 28.63 (24.83–32.87)             | 61.41 (53.99–72.38)             | 3.792 | 0.285 |
|                                | Aedes albopictus                |                                 |       |       |
| *Baeckea frutescens* leaf EO  | 25.73 (23.68–28.39)             | 37.01 (33.33–43.13)             | 0.4209| 0.810 |
| *Callistemon citrinus* leaf EO| nt                             | nt                              |       |       |
| *Callistemon citrinus* fruit EO| nt                             | nt                              |       |       |
| *Melaleuca leucadendra* young leaf EO | nt                        | nt                              |       |       |
| *Melaleuca leucadendra* old leaf EO | nt                        | nt                              |       |       |
| *Melaleuca leucadendra* stem bark EO | 19.31 (16.83–21.60)         | 40.91 (36.56–47.59)             | 0.9866| 0.741 |
| *Melaleuca leucadendra* fruit EO | 21.97 (19.69–21.32)            | 39.08 (34.96–45.47)             | 4.7420| 0.093 |
| *Melaleuca leucadendra* branch tip EO | nt                        | nt                              |       |       |
| *Syzygium nervosum* leaf EO   | nt                             | nt                              |       |       |
|                                | Culex quinquefasciatus         |                                 |       |       |
| *Baeckea frutescens* leaf EO  | 81.72 (76.16–87.75)             | 112.7 (104.7–123.6)             | 3.097 | 0.078 |
| *Callistemon citrinus* leaf EO| 73.60 (64.87–85.83)             | 172.2 (135.9–249.1)             | 0.5710| 0.000 |
| *Callistemon citrinus* fruit EO| 17.30 (11.04–22.56)            | 77.42 (66.07–95.50)             | 63.93 | 0.000 |
| *Melaleuca leucadendra* young leaf EO | 46.62 (42.65–51.45)         | 70.10 (62.93–82.10)             | 0.2083| 0.648 |
| *Melaleuca leucadendra* old leaf EO | 6.618 (3.635–9.183)         | 32.80 (27.99–40.13)             | 5.4714| 0.361 |
| *Melaleuca leucadendra* stem bark EO | 21.35 (13.62–28.02)         | 100.2 (84.4–126.2)              | 86.78 | 0.000 |
| *Melaleuca leucadendra* fruit EO | 26.20 (19.47–32.30)            | 91.81 (78.04–114.46)            | 46.32 | 0.000 |
| *Melaleuca leucadendra* branch tip EO | 43.69 (40.13–47.81)         | 64.43 (58.27–74.71)             | 0.0218| 0.883 |
| *Syzygium nervosum* leaf EO   | 46.09 (40.59–52.38)             | 95.07 (84.44–109.96)            | 1.061 | 0.786 |

LC$_{50}$ and LC$_{90}$ in µg/mL. nt = not tested.
1,8-cineole (10.1% and 56.3%, respectively). The leaf oil of *Callistemon citrinus* fruit essential oil are qualitatively similar. It is not obvious why the larvicidal activities of *Plants* 2020 only marginal larvicidal activities against *Ae. aegypti* larvae (LC$_{90}$= 34.69 (30.31–42.30) µg/mL). Unfortunately, we have found no larvicidal screening of (E)-$\alpha$-pinene, (–)$\beta$-pinene, and (–)ocimene in the literature. Note, however, that *Culex quinquefasciatus* is diicult to explain. Both $\beta$-pinene and $\gamma$-terpinene have shown good larvicidal activity against *Cx. pipiens pallens* with 24-h LC$_{50}$ of 21.1, 12.9, and 12.6 µg/mL for (+)$\beta$-pinene, (–)$\beta$-pinene, and $\gamma$-terpinene, respectively [53]. (E)-Caryophyllene showed only weak larvicidal activity (LC$_{50}$ = 93.7 µg/mL), however [53], and $\alpha$-humulene was found to be inactive against this mosquito [57]. The major components of *C. citrinus* leaf essential oil and *C. citrinus* fruit essential oil are qualitatively similar. It is not obvious why the larvicidal activities of these two oils against *C. quinquefasciatus* are so different, but it may be due to synergistic effects of minor components present in the fruit essential oil but absent in the leaf essential oil. Apparently, there is more involved in the larvicidal activities of these essential oils than the major components.

*Syzgium nervosum* essential oil larvicidal activity is also difficult to explain. There were high concentrations of (Z)$\beta$-ocimene (20.3%), (E)-caryophyllene (12.1%), and caryophyllene oxide (13.2%). Unfortunately, we have found no larvicidal screening of (Z)$\beta$-ocimene in the literature. Note, however, that *Syzgium jambolana* essential oil, rich in (Z)$\beta$-ocimene (27.2%), was inactive against *Ae. aegypti* larvae (LC$_{50}$ = 433 µg/mL) [58]. Furthermore, (E)-caryophyllene and caryophyllene oxide have shown only marginal larvicidal activities against *Ae. aegypti* or *Cx. pipiens pallens* [53,57].
2.3. Antimicrobial Activity

The Myrtaceae essential oils were screened for antibacterial activity against *Enterococcus faecalis* (ATCC 29912) and *Staphylococcus aureus* (ATCC 25923), and for antifungal activity against *Candida albicans* (ATCC 10231). The antimicrobial activities are summarized in Table 7.

| Sample                        | Enterococcus faecalis | Staphylococcus aureus | Candida albicans |
|-------------------------------|-----------------------|-----------------------|------------------|
|                                | MIC (µg/mL)           |                       |                  |
| Baeckea frutescens leaf EO    | 64                    | nt                    | 16               |
| Callistemon citrinus leaf EO  | 32                    | 256                   | 16               |
| Callistemon citrinus fruit EO | 16                    | nt                    | 128              |
| Melaleuca leucadendra old leaf EO | 32              | 64                    | 128              |
| Melaleuca leucadendra stem bark EO | 16              | 64                    | 64               |
| Melaleuca leucadendra fruit EO | 32                  | 64                    | 256              |
| Syzygium nervosum leaf EO     | 32                    | nt                    | 128              |
| Streptomycin                  | 256                   | 256                   | nt               |
| Nistatin                      | nt                    | nt                    | 8                |
|                                | IC₅₀ (µg/mL)          |                       |                  |
| Baeckea frutescens leaf EO    | 33.56                 | nt                    | 8.67             |
| Callistemon citrinus leaf EO  | 16.67                 | 128.00                | 8.67             |
| Callistemon citrinus fruit EO | 8.89                  | nt                    | 32.67            |
| Melaleuca leucadendra old leaf EO | 16.72             | 33.23                 | 65.56            |
| Melaleuca leucadendra stem bark EO | 8.32             | 32.23                 | 34.22            |
| Melaleuca leucadendra fruit EO | 15.98               | 32.89                 | 128.35           |
| Syzygium nervosum leaf EO     | 17.00                 | nt                    | 65.33            |

MIC = minimum inhibitory concentration, EO = essential oil, nt = not tested, IC₅₀ = median inhibitory concentration.

The leaf essential oils of *B. frutescens* and *C. citrinus* both showed excellent anti-*Candida* activity, with minimum inhibitory concentration (MIC) values of 16 µg/mL. van Zyl and co-workers have screened several monoterpenoids against *C. albicans*, and many of the major components that were found in *B. frutescens* and *C. citrinus* leaf essential oils did show notable activities, including α-pinene (MIC 12.0 µg/mL), β-pinene (MIC 1.0 µg/mL), limonene (MIC 10.0 µg/mL), and γ-terpinene (MIC 6.0 µg/mL) [59]. 1,8-Cineole and α-terpineol are relatively inactive against *C. albicans*, however [60,61].

A perusal of the literature reveals a broad spectrum of reported antimicrobial activities for terpenoid constituents against *E. faecalis*, *S. aureus*, and *C. albicans* (Table 8). There are several potential reasons for the apparent discrepancies, including variation in antimicrobial assay protocols, different susceptibilities of different strains of a particular microorganism, mathematical errors in calculating dilutions and MIC values.

Callistemon citrinus fruit essential oil, dominated by α-pinene (35.1%) and 1,8-cineole (32.4%), was particularly active against *E. faecalis*. Neither of these compounds have shown notable activity against *E. faecalis*, however (Table 8); the activity observed for *C. citrinus* fruit essential oil must be attributed to synergistic activity of less abundant components. Melaleuca leucadendra bark essential oil, which was rich in α-eudesmol (24.1%) and guaiol (11.3%), also exhibited notable activity against *E. faecalis*, possibly due to the high concentrations of sesquiterpene alcohols present.
3. Materials and Methods

3.1. Plant Collection

Plant materials were collected from wild-growing plants in the Hoa Vang and Hoa Khanh districts of Da Nang city. The plants were identified by Do Ngoc Dai. In each case, the fresh plant material was chopped, and 2.0 kg was subjected to hydrodistillation using a Clevenger-type apparatus (Table 9).
Table 9. Collection details and essential oil yields of four species of Myrtaceae from central Vietnam.

| Species                  | Vietnamese Name                              | Collection Site                                                                 | Voucher Number | Part            | % Yield |
|--------------------------|---------------------------------------------|---------------------------------------------------------------------------------|----------------|----------------|---------|
| Baeckea frutescens L.    | Chổi xıl, Chổi         | Hoa Vang district, Da Nang city (16°1’10.1" N, 108°06’01.3" E, elev. 27 m), in January 2019. | NHH7           | Leaf           | 2.23    |
| Melaleuca leucadendra (L.) L. | Trầm lă dài, trầm lă hęp | Hoa Vang district, Da Nang city (16°1’10.1" N, 108°06’01.3" E, elev. 27 m), in February 2019. | NHH4           | Young leaf     | 1.22    |
| Callistemon citrinus (Curtis) Skeels | Trầm bông đỗ, Trầm liểu, Kiều nhuy, Kiều  | Garden for Medicinal Plant Conservation, Duy Tan University, Hoa Khanh district, Da Nang city (16°02’57.6” N, 108°09’34.5” E, elev 8 m), in November 2018. | NHH6           | Fruit          | 0.34    |
| Syzygium nervosum DC.    | Vối, Trầm với, Trầm nắp   | Garden for Medicinal Plant Conservation, Duy Tan University, Hoa Khanh district, Da Nang city (16°02’57.6” N, 108°09’34.5” E, elev. 8 m), in January 2019. | NHH10          | Leaf           | 0.20    |

3.2. Gas Chromatographic – Mass Spectral Analysis

Each of the essential oils was analyzed by gas chromatography-mass spectrometry (GC-MS), as previously reported [81], using a Shimadzu GCMS-QP2010 Ultra, fitted with a ZB-5 column. Identification of the oil components was based on their retention indices determined by reference to a homologous series of \( n \)-alkanes, and by comparison of their mass spectral fragmentation patterns with those in the NIST [21] and FFSNC [22] databases and our own Sat-Set library [23].

3.3. Mosquito Larvicidal Assays

Mosquito colonies of *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* were obtained and maintained as previously described [82].

Larvicidal activities of the essential oils were evaluated according to the protocol of Liu and co-workers [83] with slight modifications. For each assay, 150 mL of water containing 20 fourth-instar mosquito larvae was placed into 250-mL beakers and aliquots of the essential oils dissolved in EtOH (1% stock solution) were then added. A set of controls using EtOH only (negative control) and permethrin (positive control) were included for comparison. Mortality was recorded after 24 h and after 48 h of exposure, during which no nutritional supplement was added. The experiments were carried out at 25 ± 2 °C. Each test was conducted in quadruplicate with five concentrations (100, 50, 25, 12.5 and 6 µg/mL). The data obtained were subjected to log-probit analysis [84] to obtain \( LC_{50} \) values, \( LC_{90} \) values and 95% confidence limits using Minitab® 19 (Minitab, LLC, State College, PA, USA).

3.4. Antimicrobial Screening

The antimicrobial activity of the essential oils was evaluated using two bacteria (*Enterococcus faecalis*, ATCC 299212, and *Staphylococcus aureus*, ATCC 25923) and one yeast (*Candida albicans*, ATCC 10231) using the microdilution broth susceptibility assay, as previously reported [82]. Stock solutions of the each of the essential oils were prepared in dimethylsulfoxide. Dilution series were prepared from 16,384 to 2 µg/mL (\( 2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^8, 2^7, 2^5, 2^3 \) and \( 2^1 \) µg/mL) in sterile distilled water in micro-test tubes from where they were transferred to the 96-well microtiter plates for the assays.
3.5. Agglomerative Hierarchical Cluster Analysis

The essential oil compositions from this work and from the published literature were treated as operational taxonomic units (OTUs). The percentage composition of the major components of the essential oils was used to determine the chemical relationship between the various essential oil samples by agglomerative hierarchical cluster (AHC) analysis, using the XLSTAT software, version 2018.1.1.6097 (Addinsoft™, Paris, France). Euclidean distance was used to measure dissimilarity, and Ward’s method was used for cluster definition.

4. Conclusions

Essential oils derived from *Baeckea frutescens*, *Callistemon citrinus*, *Melaleuca leucadendra*, and *Syzygium nervosum* have shown larvicidal activities against the mosquito species tested. In most cases, the larvicidal activities cannot be attributed to the major components, and synergistic interactions with minor components are likely responsible. Likewise, all of the Myrtaceae essential oils examined for antimicrobial activity showed promise. Thus, these essential oils may serve as “green” vector control agents and/or complementary antimicrobial agents, as well as providing value-added commodities for harvested timbers (e.g., *Melaleuca leucadendra*).

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References

1. Tilak, R.; Ray, S.; Tilak, V.W.; Mukherji, S. Dengue, chikungunya … and the missing entity—Zika fever: A new emerging threat. *Med. J. Armed Forces India* 2016, 72, 157–163. [CrossRef] [PubMed]
2. Mayer, S.V.; Tesh, R.B.; Vasilakis, N. The emergence of arthropod-borne viral diseases: A global prospective on dengue, chikungunya and zika fevers. *Acta Trop.* 2017, 166, 155–163. [CrossRef] [PubMed]
3. Wilder-Smith, A.; Gubler, D.J.; Weaver, S.C.; Monath, T.P.; Heymann, D.L.; Scott, T.W. Epidemic arboviral diseases: Priorities for research and public health. *Lancet Infect. Dis.* 2017, 17, e101–e106. [CrossRef]
4. Samy, A.M.; Elaagip, A.H.; Kenawy, M.A.; Ayres, C.F.J.; Peterson, A.T.; Soliman, D.E. Climate change influences on the global potential distribution of the mosquito *Culex quinquefasciatus*, vector of West Nile virus and lymphatic filariasis. *PLoS ONE* 2016, 11, e0163863. [CrossRef] [PubMed]
5. Miró-Canturri, A.; Ayrerbe-Algaba, R.; Smani, Y. Drug repurposing for the treatment of bacterial and fungal infections. *Front. Microbiol.* 2019, 10, 41. [CrossRef]
6. Yang, Y.; Ashworth, A.J.; Willett, C.; Cook, K.; Upadhyay, A.; Owens, P.R.; Ricke, S.C.; DeBruyn, J.M.; Moore, P.A. Review of antibiotic resistance, ecology, dissemination, and mitigation in U.S. broiler poultry systems. *Front. Microbiol.* 2019, 10, 2639. [CrossRef]
7. Bennani, H.; Mateus, A.; Mays, N.; Eastmure, E.; Stäkr, K.D.C.; Häslar, B. Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. *Antibiotics* 2020, 9, 49. [CrossRef]
8. Vidovic, N.; Vidovic, S. Antimicrobial resistance and food animals: Influence of livestock environments on the emergence and dissemination of antimicrobial resistance. *Antibiotics* 2020, 9, 52. [CrossRef]
9. Geddes-McAlister, J.; Shapiro, R.S. New pathogens, new tricks: Emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. *Ann. N. Y. Acad. Sci.* 2019, 1435, 57–78. [CrossRef]
10. Mulani, M.S.; Kamble, E.E.; Kumkar, S.N.; Tawre, M.S.; Pardesi, K.R. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. *Front. Microbiol.* 2019, 10, 539. [CrossRef]
11. Mabberley, D.J. *Mabberley’s Plant-Book*, 3rd ed.; Cambridge University Press: Cambridge, UK, 2008.
Plants 2020, 9, 544

12. Missouri Botanical Garden Tropicos.org. Available online: www.tropicos.org (accessed on 11 February 2020).
13. Brophy, J.J.; Craven, L.A.; Doran, J.C. Melaleuca: Their Botany, Essential Oils and Uses; Australian Centre for International Agricultural Research: Canberra, Australia, 2013; Volume 156, ISBN 978192137517.
14. Brophy, J.J. Potentially commercial Melaleucas. In The Genus Melaleuca; Southwell, I., Lowe, R., Eds.; Harwood: Amsterdam, The Netherlands, 1999; pp. 247–274.
15. Hyland, B.P.M.; Whiffen, T. Australian Tropical Rainforest Trees, Volume 2; CSIRO Publications: East Melbourne, Australia, 1993.
16. Son, P.T.; Giang, P.M.; Van, N.B.; Chien, N.Q.; Dung, N.Q. Study on chemical composition of essential oil of Baeckea frutescens L. from Vietnam. Pharm. J. 1998, 12, 7–8.
17. Tam, N.T.; Thuam, D.T.; Bighelli, A.; Castola, V.; Muselli, A.; Richomme, P.; Casanova, J. Baeckea frutescens leaf oil from Vietnam: Composition and chemical variability. Flavour Fragr. J. 2004, 19, 217–220. [CrossRef]
18. Dai, D.; Thang, T.; Olayiwola, T.; Ogunwande, I. Chemical composition of essential oil of Baeckea frutescens L. Int. Res. J. Pure Appl. Chem. 2015, 8, 26–32. [CrossRef]
19. Ji, X.; Zhao, G.; Pu, Q.; Cai, Q.; Jiang, D. GC/MS Analysis of the essential oil of Baeckea frutescens Linn. Acta Pharm. Sin. 1980, 15, 766–768.
20. Jantan, I.; Ahmad, A.S.; Bakar, S.A.A.; Ahmad, A.R.; Trockenbrodt, M.; Chak, C.V. Constituents of the essential oil of Baeckea frutescens L. From Malaysia. Flavour Fragr. J. 1998, 13, 245–247. [CrossRef]
21. NIST17; National Institute of Standards and Technology: Gaithersburg, MD, USA, 2017.
22. Mondello, L. FFNSC 3; Shimadzu Scientific Instruments: Columbia, MD, USA, 2016.
23. Satyal, P. Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active Novel Chemotypes in Essential Oils. Ph.D. Thesis, University of Alabama in Huntsville, Huntsville, AL, USA, 2015.
24. Riaz, M.; Chaudhary, F.M. The chemical composition of Pakistani Callistemon citrinus oils. J. Essent. Oil Res. 1999, 2, 327–328. [CrossRef]
25. Brophy, J.J.; Goldsack, R.J.; Forster, P.I.; Craven, L.A.; Lepschi, B.J. The leaf essential oils of the Australian members of the genus Callistemon (Myrtaceae). J. Essent. Oil Res. 1998, 10, 595–606. [CrossRef]
26. Andola, H.C.; Haider, S.Z.; Negi, P.S.; Arunachalam, K. Composition of the essential oil of Callistemon citrinus (Curtis) Skeels from Uttarakhand (India). Natl. Acad. Sci. Lett. 2017, 40, 389–392. [CrossRef]
27. Chane-Ming, J.; Vera, R.R.; Fraisse, D.J. Chemical composition of essential oil of Callistemon citrinus (Curtis) Skeel from Reunion. J. Essent. Oil Res. 1998, 10, 429–431. [CrossRef]
28. Srivastava, S.K.; Ahmad, A.; Jain, N.; Aggarwal, K.K.; Syamasundar, K.V. Essential oil composition of Callistemon citrinus leaves from the lower region of Himalayas. J. Essent. Oil Res. 2001, 13, 359–361. [CrossRef]
29. Sharma, R.K.; Kotoky, R.; Bhattacharyya, P.R. Volatile oil from the leaves of Callistemon lanceolatus D.C. grown in north-eastern India. Flavour Fragr. J. 2006, 21, 239–240. [CrossRef]
30. Oyedeji, O.O.; Lawal, O.A.; Shode, F.O.; Oyedeji, A.O. Chemical composition and antibacterial activity of the essential oils of Callistemon citrinus and Callistemon viminalis from South Africa. Molecules 2009, 14, 1990–1998. [CrossRef]
31. Silva, C.J.; Barbosa, L.C.A.; Demuner, A.J.; Montanari, R.M.; Pinheiro, A.L.; Dias, I.; Andrade, N.J. Chemical composition and antibacterial activities from the essential oils of Myrtaceae species planted in Brazil. Quim. Nova 2010, 33, 104–108. [CrossRef]
32. Zandi-Sohani, N.; Hojjati, M.; Carbonell-Barrachina, Á.A. Insecticidal and repellent activities of the essential oil of Callistemon citrinus (Myrtaceae) against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae). Neotrop. Entomol. 2013, 42, 89–94. [CrossRef] [PubMed]
33. Shrestha, S.; Poudel, A.; Satyal, P.; Dosoky, N.S.; Chhetri, B.K.; Setzer, W.N. Chemical composition and biological activity of the leaf essential oil from Callistemon citrinus from Nepal. Am. J. Essent. Oils Nat. Prod. 2015, 2, 29–33.
34. Kumar, D.; Sukapaka, M.; Babu, G.D.K.; Padwad, Y. Chemical composition and in vitro cytotoxicity of essential oils from leaves and flowers of Callistemon citrinus from western Himalayas. PLoS ONE 2015, 10, e0133823. [CrossRef] [PubMed]
35. Silva, C.J.; Barbosa, L.C.A.; Maltha, C.R.A.; Pinheiro, A.L.; Ismail, F.M.D. Comparative study of the essential oils of seven Melaleuca (Myrtaceae) species grown in Brazil. Flavour Fragr. J. 2007, 22, 474–478. [CrossRef]
36. Siddique, S.; Parveen, Z.; Firdaus-e-Bareen; Mazhar, S. Chemical composition, antibacterial and antioxidant activities of essential oils from leaves of three *Melaleuca* species of Pakistani flora. *Arab. J. Chem.* 2020, 13, 67–74. [CrossRef]

37. Padalia, R.C.; Verma, R.S.; Chauhan, A.; Chanotiya, C.S. The essential oil composition of *Melaleuca leucadendra* L. grown in India: A novel source of (E)-nerolidol. *Ind. Crops Prod.* 2015, 69, 224–227. [CrossRef]

38. Da Silva, M.M.C.; da Camara, C.A.G.; de Moraes, M.M.; de Melo, J.P.R.; dos Santos, R.B.; Neves, R.C.S. Insecticidal and acaricidal activity of essential oils rich in (E)-nerolidol from *Melaleuca leucadendron* occurring in the state of Pernambuco (Brazil) and effects on two important agricultural pests. *J. Braz. Chem. Soc.* 2020, 31, 813–820. [CrossRef]

39. Pino, J.; Bello, A.; Urquiola, A.; Aguero, J.; Marbot, R. Chemical composition of cajuput oil (*Melaleuca leucadendra* L.) from Cuba. *J. Essent. Oil Res.* 2002, 14, 10–11. [CrossRef]

40. Siani, A.C.; Nakamura, M.J.; das Neves, G.P.; Monteiro, S.D.S.; Ramos, M.F.S. Leaf essential oil from three exotic Mytaceae species growing in the botanical garden of Rio de Janeiro, Brazil. *Am. J. Plant Sci.* 2016, 7, 834–840. [CrossRef]

41. Kumar, A.; Tandon, S.; Yadav, A. Chemical composition of the essential oil from fresh leaves of *Melaleuca leucadendron* L. from north India. *J. Essent. Oil Bear. Plants* 2005, 8, 19–22. [CrossRef]

42. Pino, J.A.; Regalado, E.L.; Rodríguez, J.L.; Fernández, M.D. Phytochemical analysis and in vitro free-radical-scavenging activities of essential oils from leaf and fruit of *Melaleuca leucadendra* L. *Chem. Biodivers.* 2010, 7, 2281–2288. [CrossRef]

43. Rini, P.; Ohtani, Y.; Ichиura, H. Antioxidant, anti-hyaluronidase and antifungal activities of *Melaleuca leucadendron* Linn. leaf oils. *J. Wood Sci.* 2012, 58, 429–436. [CrossRef]

44. Adjalian, E.; Sessou, P.; Yehouenou, B.; Bothon, F.T.D.; Noudogbessi, J.-P.; Kossou, D.; Menut, C.; Sohounhloue, D. Anti-oviposition and repellent activity of essential oil from *Melaleuca leucadendron* leaf acclimated in Bénin against the Angoumois grain moth. *Int. J. Biol. Pharm. Allied Sci.* 2015, 4, 797–806.

45. Liu, B.; Peng, W. Component analysis of essential oil from *Melaleuca leucadendron* L. *J. Essent. Oil Res.* 1999, 11, 31–38. [CrossRef]

46. Muchtaridi, M.; Tjiraresmi, A.; Febriyanti, R. Analysis of active compounds in blood plasma of mice after inhalation of cajuput essential oil (*Melaleuca leucadendron* L.). *Indones. J. Pharm.* 2016, 26, 219–227. [CrossRef]

47. Garcia, J.J.; Lassak, E.V. *Melaleuca leucadendra* L. leaf oil: Two phenylpropanoid chemotypes. *Flavour Fragr. J.* 1988, 3, 43–46. [CrossRef]

48. Tia, E.V.; Lopez, P.; Menut, C.; Lozano, Y.F.; Martin, T.; Niamké, S.; Adima, A.A. Potentialité des huiles essentielles dans la lutte biologique contre la mouche blanche *Bemisia tabaci* genn. *Phytotherapie* 2013, 11, 31–38. [CrossRef]

49. Muchtaridi, M.; Tjiraresmi, A.; Febriyanti, R. Analysis of active compounds in blood plasma of mice after inhalation of cajuput essential oil (*Melaleuca leucadendron* L.). *Indones. J. Pharm.* 2016, 26, 219–227. [CrossRef]

50. Brophy, J.J.; Lassak, E.V. *Melaleuca leucadendra* L. leaf oil: Two phenylpropanoid chemotypes. *Flavour Fragr. J.* 1988, 3, 43–46. [CrossRef]

51. Dung, N.X.; Van Luu, H.; Khoi, T.T.; Leclercq, P.A. GC and GC/MS analysis of the leaf oil of *Cleistocalyx operculatus* Roxb. *Merr. et Perry* (syn. *Eugenia operculata* Roxb.; *Syzygicum mervosum* DC.). *J. Essent. Oil Res.* 1994, 6, 661–662. [CrossRef]

52. Perumalsamy, H.; Kim, N.-J.; Ahn, Y.-J. Larvicidal activity of compounds isolated from *Asarum heterotropoides* against *Culex pippins pullens*, *Aedes aegypti*, and *Ochlerotatus togoi* (Diptera: Culicidae). *J. Med. Entomol.* 2009, 46, 1420–1423. [CrossRef] [PubMed]

53. Sakhanokho, H.F.; Sampson, B.J.; Tabanca, N.; Wedge, D.E.; Demirci, B.; Baser, K.H.C.; Bernier, U.R.; Tsikolia, M.; Agramonte, N.M.; Becnel, J.J.; et al. Chemical composition, antifungal and insecticidal activities of *Hedyotis* essential oils. *Molecules* 2013, 18, 4308–4327. [CrossRef]

54. Pavela, R. Acute toxicity and synergistic and antagonistic effects of the aromatic compounds of some essential oils against *Culex quinquefasciatus* Say larvae. *Parasitol. Res.* 2015, 114, 3835–3853. [CrossRef]
56. Scalrandi, E.; Flores, G.A.; Palacio, M.; Defagó, M.T.; Carpinella, M.C.; Valladares, G.; Bertoni, A.; Palacios, S.M. Understanding synergistic toxicity of terpenes as insecticides: Contribution of metabolic detoxification in *Musca domestica*. Front. Plant Sci. 2018, 9, 1579. [CrossRef]

57. Lee, D.C.; Ahn, Y.J. Laboratory and simulated field bioassays to evaluate larvicidal activity of *Pinus densiflora* hydrodistillates, its constituents and structurally related compounds against *Aedes albopictus*, *Aedes aegypti* and *Culex pipiens pallens* in relation to their inhibitory effects on acetylcholinesterase activity. *Insects* 2013, 4, 217–229.

58. Cavalcanti, E.S.B.; de Morais, S.M.; Lima, M.A.A.; Santana, E.W.P. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti*. *L. Mem. Inst. Oswaldo Cruz* 2004, 99, 541–544. [CrossRef]

59. Van Zyl, R.L.; Seatlholo, S.T.; van Vuuren, S.F.; Viljoen, A.M. The biological activities of 20 nature identical essential oil constituents. *J. Essent. Oil Res.* 2006, 18, 129–133. [CrossRef]

60. Schmidt, J.M.; Noletto, J.A.; Vogler, B.; Setzer, W.N. Abaco bush medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. *J. Herbs Spices Med. Plants* 2006, 12, 43–65. [CrossRef]

61. Hammer, K.A.; Carson, C.F.; Riley, T.V. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. *J. Appl. Microbiol.* 2003, 95, 853–860. [CrossRef]

62. Ojeda-Sana, A.M.; van Baren, C.M.; Elechosa, M.A.; Juárez, M.A.; Moreno, S. New insights into antibacterial and antioxidant activities of rosemary essential oils and their main components. *Food Control* 2013, 31, 189–195. [CrossRef]

63. Crevelin, E.J.; Caixeta, S.C.; Dias, H.J.; Groppo, M.; Cunha, W.R.; Martins, C.H.G.; Crotti, A.E.M. Antimicrobial activity of juniper berry oil and its selected components. *Phyther. Res.* 2006, 20, 371–373. [CrossRef]

64. Rather, M.A.; Dar, B.A.; Dar, M.Y.; Wani, B.A.; Shah, W.A.; Bhat, B.A.; Ganai, B.A.; Bhat, K.A.; Anand, R.; Qurishi, M.A. Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juniperus regia* L. and its constituents. *Phytomedicine* 2012, 19, 1185–1190. [CrossRef]

65. Filipowicz, N.; Kamiński, M.; Kurlenda, J.; Asztemborska, M.; Ochocka, J.R. Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phyther. Res.* 2003, 17, 227–231. [CrossRef]

66. Reichling, J.; Suschke, U.; Schnee, J.; Geiss, H.K. Antibacterial activity and irritation potential of selected essential oil components—Structure-activity relationship. *Nat. Prod. Commun.* 2006, 1, 1003–1012. [CrossRef]

67. Tampieri, M.P.; Galuppi, R.; MacChioni, F.; Carelle, M.S.; Falcioni, L.; Cioni, P.L.; Morelli, I. The inhibition of *Candida albicans* by selected essential oils and their major components. *MycoPathologia* 2005, 159, 339–345. [CrossRef] [PubMed]

68. Jirovetz, L.; Bail, S.; Buchbauer, G.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Schmidt, E.; Geissler, M. Antimicrobial testings, gas chromatographic analysis and olfactory evaluation of an essential oil of hop cones (*Humulus lupulus* L.) from Bavaria and some of its main compounds. *Sci. Pharm.* 2006, 74, 189–201. [CrossRef] [PubMed]

69. 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]

70. Höferl, M.; Buchbauer, G.; Jirovetz, L.; Schmidt, E.; Stoyanova, A.; Denkova, Z.; Slavchev, A.; Geissler, M. Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. *J. Essent. Oil Res.* 2009, 21, 459–463. [CrossRef]

71. Vardar-Ünlü, G.; Ünlü, M.; Dönmez, E.; Vural, N. Chemical composition and in vitro antimicrobial activity of the essential oil of *Origanum minutiflorum* O Schwarz & H Davis. *J. Sci. Food Agric.* 2007, 87, 255–259.

72. Carson, C.F.; Riley, T.V. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. *J. Appl. Bacteriol.* 1995, 78, 264–269. [CrossRef]
76. Mulyaningsih, S.; Sporer, F.; Zimmermann, S.; Reichling, J.; Wink, M. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytotherapy* 2010, 17, 1061–1066. [CrossRef]

77. Chang, S.-T.; Chen, P.-F.; Chang, S.-C. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. *J. Ethnopharmacol.* 2001, 77, 123–127. [CrossRef]

78. Schmidt, E.; Bail, S.; Friedl, S.M.; Jirovetz, L.; Buchbauer, G.; Wanner, J.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Geissler, M. Antimicrobial activities of single aroma compounds. *Nat. Prod. Commun.* 2010, 5, 1365–1368. [CrossRef]

79. Juliani, H.R.; Biurrun, F.; Koroch, A.R.; Oliva, M.M.; Demo, M.S.; Trippi, V.S.; Zygalio, J.A. Chemical constituents and antimicrobial activity of the essential oil of *Lantana xenica*. *Planta Med.* 2002, 68, 762–764. [CrossRef] [PubMed]

80. Duarte Moreira, R.R.; Zimmermann Martins, G.; Teixeira Botelho, V.; dos Santos, L.E.; Cavaleiro, C.; Salgueiro, L.; Andrade, G.; Gomes Martins, C.H. Composition and activity against oral pathogens of the essential oil of *Melampodium divaricatum* (Rich.) DC. *Chem. Biodivers.* 2014, 11, 438–444. [CrossRef] [PubMed]

81. Hung, N.H.; Satyal, P.; Hie, H.V.; Chuong, N.T.H.; Dai, D.N.; Huong, L.T.; Tai, T.A.; Setzer, W.N. Mosquito larvicidal activity of the essential oils of *Erechtites* species growing wild in Vietnam. *Insects* 2019, 10, 47. [CrossRef] [PubMed]

82. Dai, D.N.; Chung, N.T.; Huong, L.T.; Hung, N.H.; Chau, D.T.M.; Yen, N.T.; Setzer, W.N. Chemical compositions, mosquito larvicidal and antimicrobial activities of essential oils from five species of *Cinnamomum* growing wild in north central Vietnam. *Molecules* 2020, 25, 1303. [CrossRef] [PubMed]

83. Liu, Z.L.; He, Q.; Chu, S.S.; Wang, C.F.; Du, S.S.; Deng, Z.W. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol. Res.* 2012, 110, 2125–2130. [CrossRef] [PubMed]

84. Finney, D. *Probit Analysis*; Reissue Edition; Cambridge University Press: Cambridge, UK, 2009; ISBN 978-0521135900.

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