Insecticidal Activity of Four Essential Oils Extracted from Chilean Patagonian Plants as Potential Organic Pesticides

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Abstract: Patagonia is a geographical area characterized by a wide plant biodiversity. Several native plant species are traditionally used in medicine by the local population and demonstrated to be sources of biologically active compounds. Due to the massive need for green and sustainable pesticides, this study was conducted to evaluate the insecticidal activity of essential oils (EOs) from understudied plants growing in this propitious area. Ciprés (Pilgerodendron uviferum), tepa (Laureliopsis philippiana), canelo (Drimys winteri), and paramela (Adesmia boronioideae) EOs were extracted through steam distillation, and their compositions were analyzed through GC–MS analysis. EO contact toxicity against Musca domestica L., Spodoptera littoralis (Boisd.), and Culex quinquefasciatus Say was then evaluated. As a general trend, EOs performed better on housefly males over females. Ciprés EO showed the highest insecticidal efficacy. The LD50 values were 68.6 (183.7) and 11.3 (75.1) µg adult−1 on housefly females and males, respectively. All EOs were effective against S. littoralis larvae; LD50 values were 33.2–66.7 µg larva−1, and tepa EO was the most effective in terms of LD50 (i.e., <100 µg larva−1). Canelo, tepa, and paramela EOs were highly effective on C. quinquefasciatus larvae, with LC50 values <100 µL L−1. Again, tepa EO achieved LD50 <100 µL L−1. This EO was characterized by safrole (43.1%), linalool (27.9%), and methyl eugenol (6.9%) as major constituents. Overall, Patagonian native plant EOs can represent a valid resource for local stakeholders, to develop effective insecticides for pest and vector management, pending a proper focus on their formulation and nontarget effects.

Keywords: bioinsecticide; green insecticide; Culex quinquefasciatus; Musca domestica; Spodoptera littoralis; contact toxicity; mosquito; moth; housefly

1. Introduction

Patagonia is a geographical region in the world’s southern hemisphere shared by Chile and Argentina. A part of this region belongs to one of the 35 world biodiversity hotspots, places of plant endemic biodiversity. In the hotspot called “Central Chile”, there are about...
four thousand native plant species, half of which are endemic and turn out to be a rich source of biologically active compounds. Between this wide biodiversity, paramela (*Adesmia boronioides* Hook.f.), canelo (*Drimys winteri* J.R.Forst. & G.Forst.), tepa (*Laureliopsis philippiana* (Looser) Schodde), and ciprés de las Guaitecas (*Pilgerodendron uviferum* (D. Don) Florin) have a well-recognized role in Patagonian traditional medicine and culture, finding large interest for their numerous biological activities.

*A. boronioides*, also known as paramela, is an aromatic and medicinal species belonging to the Fabaceae family [1,2]. It is a resinous shrub, 0.40 to 2 m high, which has been used to treat rheumatic pain and hair loss [3], as incense for the respiratory tract, as a digestive [4], as an aphrodisiac, and to alleviate menstrual discomfort [5]. This species has received an increasing interest, especially for its essential oil (EO), which has been reported for its antimicrobial, antifungal, trypanocidal, and anti-inflammatory activities [6,7].

A second noteworthy species is *L. philippiana*, known as tepa or huanhuán [8], which belongs to the Atherospermataceae family [9]. It is traditionally used as a decongestant and antibiotic agent, bronchodilator, anti-allergenic, anti-inflammatory, immunostimulant, analgesic, and to calm inflammation of varicose veins [10,11]. Recently, tepa EO fumigant insecticidal activity and the repellent and antifeedant effects have been reported as promising on adults of *Sitophilus zeamais* Motsch, *Sitophilus oryzae* L., and *Sitophilus granarius* L. (Coleoptera: Dryophthoridae) [12,13].

Another interesting species found in the Patagonian region is *D. winteri*, commonly known as canelo, which belongs to the Winteraceae family. This shrub has been used for the treatment of rheumatism, skin infections, inflammation, gastrointestinal problems, colds, and hypertension [14]. Recently, the bioactivity of canelo EO against *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) aphis has been investigated [15]; it has shown insecticidal effects against *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) and *Aegorhinus superciliosus* Guérin (Coleoptera: Curculionidae) [16].

Lastly, *P. uviferum*, also known as the ciprés de las Guaitecas, belongs to the Cupressaceae family [9] and is an endemic tree reaching diameters of up to 1.1 m and heights of more than 20 m [17]. The ciprés of the Guaitecas is used externally as a medicinal ointment for treating lumbar pain, stress, and varicose veins. *P. uviferum* EO is considered a good model in the search for raspberry weevil repellents [18], and it showed an effectiveness in reducing the adult growth of *Hylastinus obscurus* Marsham (Coleoptera: Curculionidae) [19].

The aim of this work was to investigate the insecticidal potential of the EOs from these Patagonian plants against adults of *Musca domestica* L. (Diptera: Muscidae), and third-instar larvae of *Spodoptera littoralis* Boisd. (Lepidoptera: Noctuidae) and *Culex quinquefasciatus* Say (Diptera: Culicidae). Given the need to manage invasive and dangerous arthropod vectors and pests [20], coupled with the importance to face the increasing insecticide resistance with alternative green and sustainable pesticides [21–23], these endemic and under-researched plants may represent a source of potential insecticidal products and, consequently, a chance for economic development for the region’s economy.

2. Results

2.1. Chemical Characterization of Essential Oils

Table 1 shows the results obtained from the GC–MS analysis of the four EOs. Ciprés EO was composed mainly of sesquiterpene hydrocarbons (81.2%) and oxygenated sesquiterpenes (17.2%), accounting for 98.3% of the total composition. The most abundant compounds were δ-cadinene (44.9%), *trans*-cadina-1(6),4-diene (8.3%), 1-epi-cubenol (7.3%), and α-copaene (6.1%). Other constituents were cubenol (5.4%), (*E*)-caryophyllene (4.9%), α-humulene (3.9%), *trans*-calamenene (3.4%), α-muurolene (2.9%), and α-calcocorene (2.4%). Low concentrations of *trans*-cadina-1,4-diene (1.6%), gleenol (1.5%), α-muurolol (1.4%), γ-muurolene (0.8%), epizonearene (0.6%), and α-eudesmol (0.6%) were also detected.
Table 1. Chemical composition of the essential oils from *Pilgerodendron uviferum* (ciprés), *Laureliopsis philippiana* (tepa), *Drimys winteri* (canelo), and *Adesmia boronioides* (paramela).

| No | Component a | RI b | RI Lit. c | Essential Oil ID | Ciprés % d | Tepa % | Canelo % | Paramela% |
|----|-------------|------|-----------|------------------|------------|--------|---------|----------|
| 1  | 2-heptanone | 893  | 889       | Tr f             | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 2  | 2-heptanol  | 902  | 894       | Tr               | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 3  | isobutyl isobutyrate | 912 | 908 | Tr | RI, MS |
| 4  | α-thujene    | 921  | 924       | 1.0 ± 0.2        | 18.8 ± 2.8 | 7.0 ± 1.3 | Std, RI, MS |
| 5  | α-pinene     | 926  | 932       | Tr               | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 6  | ethyl tiglate| 934  | 929       | Tr               | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 7  | Camphene     | 939  | 946       | 0.5 ± 0.2        | 21.5 ± 3.5 | 1.2 ± 0.3 | Std, RI, MS |
| 8  | thuja-2,4(10)-diene | 945 | 953 | Tr | Std, RI, MS |
| 9  | α-pinene     | 945  | 953       | 0.5 ± 0.1        | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 10 | 3-p-menthene | 977  | 984       | Tr               | 0.1 ± 0.0  | 0.1 ± 0.0 | 0.1 ± 0.0 | RI, MS    |
| 11 | 2-pentyl-furan | 986 | 979 | Tr | RI, MS |
| 12 | 3-octanol    | 989  | 988       | 0.4 ± 0.2        | 0.9 ± 0.2  | 0.1 ± 0.0 | 0.1 ± 0.0 | Std, RI, MS |
| 13 | 3-octanol    | 990  | 990       | 0.1 ± 0.0        | 0.4 ± 0.1  | 0.2 ± 0.0 | Std, RI, MS |
| 14 | benzene acetaldehyde | 997 | 984 | Tr | RI, MS |
| 15 | 3-p-menthene | 997  | 988       | 0.1 ± 0.0        | 0.4 ± 0.1  | 0.2 ± 0.0 | Std, RI, MS |
| 16 | 3-octanol    | 997  | 988       | 0.1 ± 0.0        | 0.4 ± 0.1  | 0.2 ± 0.0 | Std, RI, MS |
| 17 | α-hellemandrene | 1003 | 1002 | 3.0 ± 0.6 | 0.4 ± 0.1 | 0.2 ± 0.0 | Std, RI, MS |
| 18 | δ-3-carene   | 1008 | 1008      | 0.1 ± 0.0        | 0.1 ± 0.0  | 0.1 ± 0.0 | Std, RI, MS |
| 19 | α-terpinene  | 1014 | 1014      | 0.1 ± 0.0        | 0.4 ± 0.1  | 0.2 ± 0.1 | Std, RI, MS |
| 20 | p-cymene     | 1022 | 1020      | 1.1 ± 0.2        | 0.1 ± 0.0  | 1.5 ± 0.3 | Std, RI, MS |
| 21 | limonene     | 1025 | 1024      | 0.8 ± 0.2        | 2.6 ± 0.5  | 2.1 ± 0.4 | Std, RI, MS |
| 22 | 1,8-cineole  | 1027 | 1026      | 0.5 ± 0.1        | 0.1 ± 0.0  | 0.1 ± 0.0 | Std, RI, MS |
| 23 | (Z)-β-oicmene | 1037 | 1032     | 0.1 ± 0.0        | 0.6 ± 0.2  | Tr       | Std, RI, MS |
| 24 | 3-p-menthene | 1043 | 1036      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 25 | (E)-β-oicmene | 1047 | 1044     | 0.2 ± 0.0        | Tr       | Std, RI, MS |
| 26 | γ-terpinene  | 1055 | 1054      | 0.1 ± 0.0        | 0.7 ± 0.2  | 0.4 ± 0.1 | Std, RI, MS |
| 27 | acetophenone | 1065 | 1059      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 28 | cis-linalool oxide | 1071 | 1067 | Tr | RI, MS |
| 29 | terpinolene  | 1085 | 1086      | 0.3 ± 0.1        | 0.3 ± 0.0  | 0.1 ± 0.0 | Std, RI, MS |
| 30 | p-cymene     | 1086 | 1089      | 1.1 ± 0.2        | 0.1 ± 0.0  | 1.5 ± 0.3 | Std, RI, MS |
| 31 | trans-linalool oxide | 1087 | 1084 | Tr | RI, MS |
| 32 | 6-camphenone | 1092 | 1095      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 33 | 2-nonanone   | 1094 | 1087      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 34 | linalool     | 1100 | 1095      | 27.9 ± 3.1       | 2.8 ± 0.4  | 0.3 ± 0.1 | Std, RI, MS |
| 35 | ethyl heptanoate | 1101 | 1097   | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 36 | 2-methylbutyl-2-methyl butyrate | 1106 | 1100 | Tr | RI, MS |
| 37 | 1,3,8-p-menthatriene | 1109 | 1108 | Tr | RI, MS |
| 38 | trans-thujone | 1113 | 1112      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 39 | 3-methyl-3-butenyl 3-methyl butanoate | 1115 | 1112 | Tr | RI, MS |
| 40 | α-campholenal | 1122 | 1122      | 0.7 ± 0.2        | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 41 | allo-oicmene | 1129 | 1128      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 42 | trans-pinocarveol | 1133 | 1135 | Tr | RI, MS |
| 43 | camphor      | 1138 | 1141      | 0.1 ± 0.0        | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 44 | trans-verbenol | 1141 | 1140     | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 45 | 1,4-dimethyl-4-acetyl-1-cyclohexene | 1147 | 1152 | Tr | RI, MS |
| 46 | isobutyl hexanoate | 1154 | 1149 | Tr | RI, MS |
| 47 | trans-pinocamphone | 1155 | 1158 | Tr | RI, MS |
| 48 | pinocarvone  | 1157 | 1160      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 49 | borneol      | 1161 | 1165      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 50 | δ-terpineol  | 1164 | 1162      | Tr               | 0.1 ± 0.0  | 0.4 ± 0.1 | Std, RI, MS |
| 51 | cis-pinocamphone | 1169 | 1172 | Tr | RI, MS |
Table 1. Cont.

| No | Component          | RI       | RI Lit. | Ciprèss % | Tepa % | Canelo % | Paramela % |
|----|--------------------|----------|---------|-----------|--------|----------|------------|
| 52 | ethyl benzoate     | 1170     | 1169    | Tr        | RI, MS |
| 53 | terpinen-4-ol      | 1172     | 1174    | 0.2 ± 0.0 | 0.6 ± 0.2 | 1.0 ± 0.2 | Std, RI, MS |
| 54 | p-cymen-8-ol       | 1183     | 1179    | Tr        | RI, MS |
| 55 | α-terpineol        | 1187     | 1186    | 2.1 ± 0.4 | 0.4 ± 0.1 | 0.3 ± 0.1 | Std, RI, MS |
| 56 | myrtenal           | 1190     | 1195    | 0.2 ± 0.0 | Tr      | Std, RI, MS |
| 57 | methyl chavicol    | 1196     | 1195    | Tr        | RI, MS |
| 58 | verbenone          | 1204     | 1204    | 0.1 ± 0.0 | Tr      | RI, MS |
| 59 | trans-carveol      | 1218     | 1215    | 0.1 ± 0.0 | Tr      | RI, MS |
| 60 | carvone            | 1240     | 1239    | 0.1 ± 0.0 | Tr      | Std, RI, MS |
| 61 | bornyl acetate     | 1281     | 1287    | Tr        | Std, RI, MS |
| 62 | safrole            | 1284     | 1285    | 43.1 ± 3.9 | Tr | RI, MS |
| 63 | theaspirane        | 1290     | 1298    | Tr        | RI, MS |
| 64 | indane derivative  | 1336     | 1336    | 0.2 ± 0.0 | Tr      | MS |
| 65 | α-cubebene         | 1343     | 1345    | Tr        | RI, MS |
| 66 | eugenol            | 1355     | 1356    | 1.1 ± 0.9 | Tr | Std, RI, MS |
| 67 | α-copaene          | 1367     | 1374    | 6.1 ± 0.9 | Tr | RI, MS |
| 68 | β-bourbonene       | 1376     | 1387    | Tr        | RI, MS |
| 69 | β-elemente         | 1385     | 1389    | Tr        | 1.2 ± 0.3 | Std, RI, MS |
| 70 | α-gurjunene        | 1400     | 1409    | Tr        | RI, MS |
| 71 | methyl eugenol     | 1406     | 1403    | 6.9 ± 1.1 | Tr | RI, MS |
| 72 | (E)-caryophyllene | 1409     | 1417    | 4.9 ± 0.8 | 0.2 ± 0.0 | 1.4 ± 0.3 | Std, RI, MS |
| 73 | caryophyllane      | 1412     | 1415    | Tr        | RI, MS |
| 74 | α-guaiane          | 1431     | 1437    | 0.2 ± 0.0 | Tr | RI, MS |
| 75 | 6,9-guaia diene    | 1436     | 1442    | 4.3 ± 0.6 | Tr | RI, MS |
| 76 | aromadendrene      | 1440     | 1439    | 0.7 ± 0.2 | Tr | RI, MS |
| 77 | α-humulene         | 1443     | 1452    | 3.9 ± 0.7 | Tr | 0.2 ± 0.0 | Std, RI, MS |
| 78 | allo-aromadendrene | 1450     | 1458    | Tr        | RI, MS |
| 79 | (E)-β-farnesene    | 1452     | 1454    | 0.1 ± 0.0 | Tr | Std, RI, MS |
| 80 | sesquisabinene     | 1456     | 1457    | Tr        | RI, MS |
| 81 | trans-cadina-1(6),4-diene | 1466 | 1475 | 8.3 ± 1.2 | Tr | RI, MS |
| 82 | γ-muurolene        | 1469     | 1478    | 0.8 ± 0.2 | Tr | RI, MS |
| 83 | germacrene D       | 1471     | 1484    | 0.1 ± 0.0 | Tr | RI, MS |
| 84 | β-selinene         | 1475     | 1489    | 0.1 ± 0.0 | 0.3 ± 0.1 | Std, RI, MS |
| 85 | β-dihydroagarofuran | 1487 | 1496 | 1.8 ± 0.4 | Tr | RI, MS |
| 86 | bicyclgermacrene   | 1487     | 1500    | 0.1 ± 0.0 | 2.6 ± 0.4 | RI, MS |
| 87 | epizonarene        | 1491     | 1501    | 0.6 ± 0.2 | Tr | RI, MS |
| 88 | α-muurolene        | 1493     | 1500    | 2.9 ± 0.6 | Tr | RI, MS |
| 89 | esquel-6-en-9-one  | 1494     | 1494    | 30.7 ± 3.1 | Tr | MS |
| 90 | epishyobunone      | 1502     | 1498    | 0.2 ± 0.0 | Tr | RI, MS |
| 91 | γ-cadinene         | 1505     | 1513    | 0.1 ± 0.0 | Tr | RI, MS |
| 92 | trans-calamenene   | 1519     | 1521    | 3.4 ± 0.6 | Tr | RI, MS |
| 93 | δ-cadinene         | 1521     | 1522    | 44.9 ± 2.9 | 0.1 ± 0.0 | Tr | RI, MS |
| 94 | trans-cadina-1,4-diene | 1525 | 1533 | 1.6 ± 0.3 | Tr | RI, MS |
| 95 | γ-dehydro-ar-himachalene | 1533 | 1530 | 0.6 ± 0.2 | Tr | RI, MS |
| 96 | α-agarofuran       | 1533     | 1540    | 0.6 ± 0.2 | Tr | RI, MS |
| 97 | α-calacorene       | 1535     | 1542    | 2.4 ± 0.2 | Tr | RI, MS |
| 98 | furopelargone A    | 1536     | 1538    | 1.1 ± 0.3 | Tr | RI, MS |
| 99 | hedycaryol         | 1543     | 1546    | 0.3 ± 0.1 | 18.2 ± 2.9 | Std, RI, MS |
| 100| β-calacorene       | 1555     | 1564    | 0.1 ± 0.0 | Tr | RI, MS |
| 101| cryolan-8-ol       | 1558     | 1573    | Tr        | RI, MS |
| 102|(E)-nerolidol      | 1562     | 1561    | 0.1 ± 0.0 | Tr | Std, RI, MS |
| 103| spathulenol        | 1567     | 1577    | 0.1 ± 0.0 | Tr | RI, MS |
| 104| caryophyllene oxide| 1571     | 1583    | 0.7 ± 0.2 | Tr | Std, RI, MS |
### Table 1. Cont.

| No | Component a | RI b | RI Lit. c | Ciprés % d | Tepa % | Canelo % | Paramela% |
|----|--------------|------|----------|------------|--------|----------|-----------|
| 105 | gleenol | 1578 | 1586 | 1.5 ± 0.2 | RI, MS |
| 106 | allo-hedycariol | 1579 | 1580 | 0.2 ± 0.0 | RI, MS |
| 107 | furopelargone B | 1583 | 1588 | 7.0 ± 1.1 | RI, MS |
| 108 | esquel-7-en-9-one | 1589 | 1590 | 10.2 ± 1.4 | MS |
| 109 | humulol | 1591 | 1609 | Tr | RI, MS |
| 110 | 5-epi-7-epi-α-eudesmol | 1596 | 1607 | 0.1 ± 0.0 | 0.4 ± 0.1 | RI, MS |
| 111 | α-corocalene | 1615 | 1622 | 0.2 ± 0.0 | RI, MS |
| 112 | 1-epi-cubenol | 1620 | 1627 | 7.3 ± 0.9 | RI, MS |
| 113 | 10-epi-γ-eudesmol | 1608 | 1622 | 0.4 ± 0.1 | 0.5 ± 0.1 | 2.2 ± 0.4 | RI, MS |
| 114 | eremoligenol | 1619 | 1629 | 0.6 ± 0.2 | RI, MS |
| 115 | γ-eudesmol | 1622 | 1630 | 6.6 ± 1.1 | RI, MS |
| 116 | hinesol | 1629 | 1640 | 0.3 ± 0.0 | RI, MS |
| 117 | cubenol | 1634 | 1645 | 5.4 ± 1.0 | RI, MS |
| 118 | 4-α-hydroxy-dihydroagarofuran | 1634 | 1651 | 2.2 ± 0.5 | RI, MS |
| 119 | α-muurolol | 1639 | 1644 | 1.4 ± 0.3 | RI, MS |
| 120 | β-eudesmol | 1639 | 1649 | 5.2 ± 1.0 | RI, MS |
| 121 | α-eudesmol | 1643 | 1652 | 0.6 ± 0.2 | 6.3 ± 1.1 | RI, MS |
| 122 | α-cadinol | 1645 | 1652 | 0.3 ± 0.1 | RI, MS |
| 123 | 7-epi-α-eudesmol | 1646 | 1662 | 0.2 ± 0.0 | RI, MS |
| 124 | bulnesol | 1656 | 1670 | 0.5 ± 0.1 | RI, MS |
| 125 | cadalene | 1665 | 1675 | 0.3 ± 0.1 | RI, MS |
| 126 | kaurene | 2039 | 2042 | 1.3 ± 0.3 | RI, MS |
| 127 | n-heneicosane | 2100 | 2100 | 0.1 ± 0.0 | Std, RI, MS |
| 128 | n-tricosane | 2300 | 2300 | Tr | Std, RI, MS |

Total identified (%) Grouped compounds (%)

- **Monoterpene hydrocarbons**: Tr 18.0 48.7 13.6
- **Oxygenated monoterpenes**: 30.3 4.4 3.5
- **Sesquiterpene hydrocarbons**: 81.2 0.4 5.9 5.3
- **Oxygenated sesquiterpenes**: 17.2 Tr 39.0 57.2
- **Phenylpropanoids**: 51.1 Tr Tr
- **Others**: 0.2 1.4 0.6

- The order of components is according to their elution from a HP-5MS column (30 m l. × 0.25 mm i.d., 0.1 mm f.t.).
- Retention index value taken from Adams and/or NIST17 libraries.
- Peak area percentage as the mean of three injections ± standard deviation.
- Peak assignment method: Std, comparison of RT, RI, and MS with those of analytical standard (Sigma, Milan, Italy); RI, coherence of the experimentally determined RI with respect to those stored in ADAMS, NIST17, and FFNSC3 libraries; MS, mass fragmentation overlapping because of matching with ADAMS, WILEY275, FFNSC3, and NIST17 spectral libraries.

Tepa EO presented a different composition profile, being mainly constituted by phenylpropanoids (51.1%), with a dominance of safrole (43.1%) and methyl-eugenol (6.9%). Oxygenated monoterpenes and monoterpene hydrocarbons were also important fractions of this EO, with percentages of 30.3 and 18.0%, respectively. Among them, linalool (27.9%) and 1,8-cineole (8.5%), and α-phellandrene (3.0%), β-pinene (1.3%), and p-cymene (1.1%) were the most representative compounds, respectively.

Canelo EO obtained from *D. winteri* appeared to be mainly constituted by monoterpene hydrocarbons (48.7%) and oxygenated sesquiterpenes (39.0%). Lower concentrations of
oxygenated monoterpenes and sesquiterpenes hydrocarbons were also detected, with percentages of 4.4 and 5.9%, respectively. Among monoterpe hydrocarbons, \( \alpha \)-pinene and \( \beta \)-pinene were the most abundant ones (18.8% and 21.5%, respectively), whereas hedycaryol (18.2%), \( \alpha \)-eudesmol (6.3%), \( \beta \)-eudesmol (5.2%), and \( \gamma \)-eudesmol (6.6%) have been found as the most representative of the sesquiterpene class.

Paramela EO from \( A. \) boronioides has been found to be mainly composed of oxygenated sesquiterpenes (57.2%) and monoterpene hydrocarbons (13.6%), accounting for 79.9% of the total composition. Oxygenated monoterpenes and sesquiterpene hydrocarbons were also detected in lower concentrations (3.5 and 5.3%, respectively). In detail, the principal compounds identified were the oxygenated sesquiterpenes esquel-6-en-9-one (30.7%) and esquel-7-en-9-one (10.2%).

Figure 1 shows the chemical structure of the main bioactive compounds of ciprés, tepa, canelo and paramela EOs.

2.2. Insecticidal Activity

2.2.1. Insecticidal Activity against Houseflies

The tested EOs provided promising efficacy against \( M. \) domestica adults (Table 2). Generally, it can be noted that males showed a significantly higher sensitivity; significantly lower lethal doses were estimated for them (except for tepa EO), if compared with females. On the other hand, despite the significant difference in efficacy between the housefly sexes, no difference was observed in terms of efficacy between individual EOs, as the confidence intervals overlapped at least in one LD\(_{50}\) parameter in each case.
Table 2. Insecticidal activity of the four essential oils from Patagonian plants against adults (females and males) of Musca domestica; df = degrees of freedom, ns = not significant (p > 0.05).

| Essential Oil | M. domestica Female | M. domestica Male |
|---------------|----------------------|-------------------|
|               | LC$_{50}$ (µg adult$^{-1}$) | CI$_{95}$ | LC$_{90}$ (µg adult$^{-1}$) | CI$_{95}$ | $\chi^2$ (df = 3) | p-Value  |
| Canelo        | 76.7 | 60.1–96.5 | 296.5 | 259.7–321.5 | 1.766 | 0.622 ns | 19.3 | 13.3–25.1 | 140.8 | 121.5–165.9 | 0.697 | 0.705 ns |
| Tepa          | 88.7 | 81.3–94.2 | 128.6 | 118.5–139.7 | 1.564 | 0.457 ns | 24.6 | 18.5–29.7 | 119.3 | 89.4–139.7 | 1.096 | 0.777 ns |
| Paramela      | 65.2 | 51.7–78.1 | 195.1 | 156.5–220.1 | 2.583 | 0.273 ns | 11.1 | 8.5–15.5 | 113.1 | 98.7–120.5 | 5.958 | 0.113 ns |
| Ciprés        | 68.6 | 58.2–75.8 | 183.7 | 152.5–211.1 | 1.782 | 0.257 ns | 11.3 | 8.4–15.5 | 75.1 | 48.9–95.5 | 3.893 | 0.273 ns |

Nevertheless, considering the lowest LD$_{50(90)}$ values, the following two EOs provided the best results: ciprés EO, with LD$_{50(90)}$ estimated as 68.6 (183.7) and 11.3 (75.1) µg adult$^{-1}$ for females and males, respectively, and paramela EO, with LD$_{50(90)}$ estimated as 65.2 (195.1) and 11.1 (113.1) µg adult$^{-1}$ for females and males, respectively.

2.2.2. Insecticidal Activity against Moths

The efficacy of EOs in terms of acute toxicity for S. littoralis larvae is presented in Table 3. All EOs provided promising efficacy; their LD$_{50}$ values ranged from 33.8 to 66.7 µg larva$^{-1}$, while LD$_{90}$ values ranged from 72.3 to 124.5 µg larva$^{-1}$. Nevertheless, tepa EO provided the highest efficacy where the confidence interval for LD$_{90}$ was estimated as less than 100 µg larva$^{-1}$.

Table 3. Insecticidal activity of the four essential oils from Patagonian plants against 3rd-instar larvae of Spodoptera littoralis; df = degrees of freedom, ns = not significant (p > 0.05).

| Essential Oil | LC$_{50}$ (µg larva$^{-1}$) | CI$_{95}$ | LC$_{90}$ (µg larva$^{-1}$) | CI$_{95}$ | $\chi^2$ (df = 3) | p-Value |
|---------------|-----------------------------|----------|-----------------------------|----------|------------------|---------|
| Canelo        | 39.7 | 28.5–51.7 | 110.1 | 82.5–128.7 | 0.505 | 0.917 ns |
| Tepa          | 35.2 | 29.1–40.6 | 72.3 | 59.5–93.2 | 0.569 | 0.966 ns |
| Paramela      | 66.7 | 55.1–77.2 | 124.5 | 104.6–142.8 | 2.676 | 0.444 ns |
| Ciprés        | 33.8 | 26.5–41.7 | 106.3 | 89.7–127.6 | 1.861 | 0.761 ns |

2.2.3. Insecticidal Activity against Mosquitoes

Significant differences in the EO efficacy were observed on C. quinquefasciatus larvae (Table 4). In terms of mosquito insecticidal efficacy, highly promising EOs were canelo, tepa, and paramela, with LC$_{50}$ values estimated as less than 100 µL L$^{-1}$.

Table 4. Insecticidal activity of the four essential oils from Patagonian plants against 3rd-instar larvae of Culex quinquefasciatus; df = degrees of freedom, ns = not significant (p > 0.05).

| Essential Oil | LC$_{50}$ (µL L$^{-1}$) | CI$_{95}$ | LC$_{90}$ (µL L$^{-1}$) | CI$_{95}$ | $\chi^2$ (df = 3) | p-Level | df |
|---------------|--------------------------|----------|--------------------------|----------|------------------|---------|----|
| Canelo        | 48.6 | 33.5–62.8 | 111.2 | 98.5–126.9 | 3.129 | 0.536 ns | 4 |
| Tepa          | 52.2 | 39.8–61.1 | 81.5 | 71.8–92.7 | 1.452 | 0.325 ns | 4 |
| Paramela      | 77.3 | 72.5–82.1 | 110.6 | 101.5–124.3 | 1.762 | 0.623 ns | 3 |
| Ciprés        | 261.7 | 232.8–287.6 | 685.1 | 601.2–723.5 | 5.497 | 0.241 ns | 4 |

Tepa EO was the most efficient one with the confidence interval for LD$_{90}$ estimated as less than 100 µL L$^{-1}$.

3. Discussion

In general, the composition of the EOs from ciprés, tepa, canelo, and paramela are strongly influenced by the geographic area of distribution within Patagonia. Indeed, concerning ciprés EO, in a study conducted by Malizia et al. [24], the analyzed EO was obtained by plants collected in Argentinian Patagonia spontaneous forests and was mainly...
constituted by monoterpenes (54.1%), which instead are present only in traces in the EO analyzed in our study, although with a large presence of sesquiterpenes (40.4%). The sesquiterpene nature of the EO presented in this work is consistent with the ones reported by Oyarzun and Garbarino [25] and Espinoza et al. [18]; in both studies, the analyzed EOs were obtained from Chilean varieties of \textit{P. uviferum}. However, differences in the main constituents of the EO have been detected: \(\delta\)-cadinene and \(\alpha\)-copaene were present in a lower amount (10.8 and 0.7%, respectively), while cubenol, which was present in a low concentration, was the most abundant compound (22.6%) [18,19], suggesting that the difference in composition can be due to other factors such as environmental stress and season of collection.

On the other hand, the usual composition of tepa EO is in accordance with previous studies from Norambuena et al. [13] and Madrid et al. [26], in which phenylpropanoid compounds are the most abundant class, with 78.4 and 67.6%, respectively, with safrole and methyl eugenol as the major compounds, though with different concentrations (17.0% and 24.4% for safrole and 61.4% and 7.12% for methyl eugenol, respectively). At the same time, in two independent studies, no sesquiterpenes were detected from the GC–MS analysis of the EO, and monoterpenes were the dominant class of compounds, with 1,8-cineole (13.89–37.4%) and linalool (32.3%) resulting as the principal compounds [10,11]. These differences in the composition may be related to the field collection season and the trees’ geographical area [13].

\(\alpha\)-Pinene and \(\beta\)-pinene are the most abundant constituents of canelo EO in accordance with data reported in the literature [16,27–29], although in different proportions. In the study from Barrero et al. [27], both \(\alpha\)-pinene and \(\beta\)-pinene were present in lower percentages than those found in the presented work (14.9 and 5.9%, respectively). From the analysis of Monsalvez et al. [28], \(\alpha\)-pinene was the most abundant compound with a percentage of 71.2%, while \(\beta\)-pinene had a lower concentration (14.2%). The composition of \(\alpha\)-pinene and \(\beta\)-pinene is likely dependent on the collection area. In fact, in a study conducted by Muñoz et al. [29], both insular and continental \textit{D. winteri} EOs were analyzed, resulting in a different monoterpene profile; the EO obtained from plants collected in Chiloé island (southern Chile) was constituted by high levels of monoterpene hydrocarbons (92%), particularly \(\alpha\)-pinene (23.1%) and \(\beta\)-pinene (43.6%), while the EO from plants collected in the metropolitan region of Santiago (central Chile) presented much lower \(\alpha\)-pinene and \(\beta\)-pinene percentages (2.9 and 1.3%, respectively) and higher percentages of sesquiterpenes (32%) and phenylpropanoids (27%). This tendency was confirmed by other studies in which the material was collected in different regions. For example, Verdeguer et al. [30] analyzed a \textit{D. winteri} EO obtained from plants collected in the V region of central Chile and found out that the percentage of monoterpenes was very low (\(\beta\)-pinene had a percentage of 2.7%, while \(\alpha\)-pinene was completely absent). On the other side, Monsalvez et al. [28] obtained their EO rich in monoterpenes by collecting the plant material in the Nuble Province of Chile, in the southern regions.

Paramela EO extracted from \textit{A. boronioides} was mainly characterized by the presence of esquel-6-en-9-one and esquel-7-en-9-one. In our analysis, these compounds were identified only through the matching of the mass fragmentation with MS-spectral libraries. Their actual presence was then confirmed by data reported in the literature [31], indicating the presence of esquel-6-en-9-one and esquel-7-en-9-one in percentages of 19.1 and 12.5%, respectively. In addition, \(\alpha\)-pinene was detected in the study by Gonzalez et al. [32] even though this monoterpene was less abundant than in the present study (3%). The composition of \textit{A. boronioides} is susceptible to various conditions, as reported by Gonzalez et al. [7]. The sesquiterpene class remains the principal one characterizing this EO.

EOs are complex mixtures, even of several dozens of compounds; several factors can have a significant impact on their insecticidal efficacy, including their chemical composition [33], mutual synergistic relationships among the EO constituents [34,35], the mechanisms of action of active substances [33], and the mode of application and post-application conditions [36,37]. Regarding the tested EOs, it can be noted that they were
very complex mixtures where the content of none of the major compounds was higher than 50%. Thus, it is difficult to determine which of the compounds was responsible for the highest biological activity as not only the above-mentioned synergistic, but also antagonistic relationships between the present substances may have played a role and may have reduced the final insecticidal efficacy [34,35].

This is the first report on the insecticidal efficacy of EOs obtained from these plant species against M. domestica, S. littoralis, and C. quinquefasciatus. However, some of these EOs have already been tested for insecticidal efficacy against other target organisms. For example, the EO from P. uviferum with δ-cadinol (24.16%), cubenol (22.64%), 15-copaenol (15.46%), and δ-cadinene (10.81%) as the major compounds has been tested against Haematobia irritans (L.) (Diptera: Muscidae) [38]. The authors reported very good insecticidal efficacy in their fumigation tests with LC$_{50}$ values for P. uviferum EO of 9.41 and 1.02 µL L$^{-1}$ air at 1 and 4 h, respectively. The authors also found a promising repellent efficacy of this EO. Our tests extended the insecticidal efficacy of this EO on other insect species, which are highly important in agricultural and public health settings. Additionally, the EO from L. philippiana has been previously assessed for its insecticidal efficacy against key stored product beetles S. oryzae (L.), S. zeamais, and S. granarius [13]. L. philippiana EO, mainly composed by methyl eugenol (61.38%) and safrole (14.76%), was tested on adults of Sitophilus spp., showing that the highest contact toxicity was achieved on S. oryzae at 4.0% (v/w). The same EO concentration also achieved a >80% antifeedant effect. The exposure to the EO led to a marked reduction in F$_1$ emergence, which was at a maximum of 60% for S. granarius and S. oryzae, and 36% for S. zeamais. Sitophilus spp. have been found highly sensible to the fumigant toxicity and repellent effect of the above-mentioned EO. Similarly, the EO from D. winteri was successfully tested for its efficacy against stored product pests [39,40] as well as on the aphid A. pisum in deterrent bioassays [15]. Our work adds knowledge to the pool of information on the biological efficacy of this EO. Of note, we provided new information on the promising insecticidal efficacy of the EO from A. boronioides, which has not yet been studied for insecticidal activity.

As shown by our tests, the tested EOs showed promising insecticidal efficacy but, in many cases, it was not possible to identify the most effective one. Despite different chemical compositions, it could be hypothesized that very complex mixtures of several dozens of substances may be detrimental to their individual biological efficacies, most likely due to possible antagonistic relationships between the contained substances [34,35]. This phenomenon can, thus, result in the suppression of better biological efficacy of the major compounds. Although this hypothesis will have to be clarified in the tested EOs, this claim is supported by the work of other authors. δ-Cadinene can be mentioned as an example: this compound showed a major level of 45% in the tested EO from P. uviferum. As found by Govindarajan et al. [41], its level in the Kadsura heteroclita (Roxb.) EO was 18.3%, together with other major chemical components such as calarene (14.8%) and δ-4-carene (12.5%). This EO was tested for insecticidal activity on Anopheles stephensi Liston, Aedes aegypti (L.), and C. quinquefasciatus larvae, with LC$_{50}$ values ranging from 103 to 122 µg mL$^{-1}$. However, for the isolated substances δ-cadinene, calarene, and δ-4-carene, a higher efficacy was determined on A. stephensi (LC$_{50}$ = 8, 12, and 16 µg mL$^{-1}$, respectively), A. aegypti (LC$_{50}$ = 9, 13, and 18 µg mL$^{-1}$), and C. quinquefasciatus (LC$_{50}$ = 10, 14, and 19 µg mL$^{-1}$).

Despite the very promising insecticidal effects found for the tested EOs, we are very aware that further studies will be required to explore the effect of these EOs on nontarget organisms [42] to estimate the environmental impact of the areal application of botanical insecticides based on these EOs. Similarly, it will be necessary to study the impact of lethal and sublethal doses or concentrations on target species, considering that, as we know, even such applications can result in a significant reduction in subsequent population densities of target organisms; in practice, this can be utilized particularly to reduce the population density of flies and mosquitoes [43,44]. Additionally, possible ways of increasing the efficacy using nanoemulsions or encapsulation will be the subject of further studies [45].
4. Materials and Methods

4.1. Plant Collection

Plant materials used for isolation of the four EOs were collected in different areas of the Aysén region, Patagonia, Chile. Samples of *L. philippiana*, *P. uviferum*, and *D. winteri* were collected in December 2019, March 2019, and January 2019, respectively, in the locality of Valle Mirta (property Walwalun, La Junta, Aisén Region, Chile) at about 220 m a.s.l. (−43.88483222302501, −72.30618713473653). *A. boronioides* samples were collected in March 2019 in Puerto Ibañez along the carretera austral (Aisén Region, Chile) (−43.88483222302501, −72.30618713473653). Plants were identified by one of us (Daniela Santibañez Nieto) and deposited at the Herbarium of University of Antioquia. Selected parts of the plants used for EOs extraction were as follows: fresh green leaves, flowers, and resinous stems for *A. boronioides*; dry wood shavings for *P. uviferum*; dry leaves and flowering tops for *D. winteri*; fresh leaves and flowering tops for *L. philippiana*. Of note, the Patagonian plants subjected to hydrodistillation are quite rare and difficult to collect; due to the wet climate of the area, in situ drying is often difficult. For this reason, whenever possible, we processed the material as fresh and when not as dry material.

4.2. Isolation of Essential Oils

The EOs extraction from the selected plant materials were obtained through steam distillation. In detail, a 200 L alembic was filled with 50 L of water, and the plant material was placed over a metal mesh. When the alambique marked 60 °C, cold water was added to cool down the serpentine and allow the hydrolate to come out. For *L. philippiana*, 50 kg of leaves and stems were distilled to obtain 89 mL of EO, while, for *D. winteri*, 50 kg of leaves and stems were distilled to obtain 20 mL of EO. For *A. boronioides*, 100 kg of buds and some flowers were distilled to obtain 46 mL of EO, while for *P. uviferum*, 60 kg of wood shavings were distilled for the achievement of 45 mL of EO. Table 5 reports the yields (v/w) obtained for the four EOs calculated on a dry basis.

| Plant Species | *L. philippiana* | *D. winteri* | *A. boronioides* | *P. uviferum* |
|---------------|-----------------|--------------|-----------------|--------------|
| Yield         | 0.06            | 0.04         | 0.04            | 0.07         |

4.3. Essential Oils Chemical Characterization

The chemical characterization of the four EOs was performed using an Agilent 6890 N gas chromatograph equipped with a single-quadrupole 5973 N mass spectrometer and an auto-sampler 7863 (Agilent, Wilmington, DE, USA). The separation of EO components was achieved using an HP-5 MS capillary column (30 m, 0.25 mm i.d., 0.1 µm film thickness; 5% phenylmethylpolysiloxane), supplied by Agilent (Folsom, CA, USA). The analytical conditions and chromatogram analysis were the same as reported by Benelli et al. [45].

4.4. Insecticidal Activity

4.4.1. Insecticidal Activity against Houseflies

Adults of *M. domestica* (males and females, 3–5 days old, from established laboratory colonies, >20 generations) were selected for the experiments. Contact toxicity of the four Patagonian EOs was evaluated through their topical application on the pronotum of *M. domestica* males and females. The four Patagonian EOs were prepared in acetone (Sigma-Aldrich, Schnelldorf, Germany) to obtain a concentration series (corresponding to the applied doses for females of 30, 50, 70, 100, and 150 µg adult⁻¹ and for males of 10, 30, 50, 80, and 110 µg adult⁻¹). Subsequently, 1 µL of EO was applied on each CO₂-anesthetized fly through a micro-electric applicator. Acetone alone was used as the negative control. Then, the flies were moved to rearing containers sized 15 × 12 × 8 cm with a perforated lid (at 25 ± 1 °C, 70 ± 3% R.H., and 16:8 h (L:D)), containing their usual food.
The experiment was replicated 4 times in total (20 insects per replication). Mortality was assessed 24 h after treatment. Insects failing to respond were considered dead.

4.4.2. Insecticidal Activity against Moths

Larvae of *S. littoralis* (3rd instar, mean larval weight 12 ± 3 mg, from established laboratory colonies, >20 generations) were selected for the experiments. Contact toxicity of the four Patagonian EOs was evaluated through topical application on the dorsum of *S. littoralis* larvae. The EOs were prepared in acetone (Sigma-Aldrich, Germany) to obtain a concentration series (1 µL was applied using a micro-electric applicator to the dorsum, corresponding to the applied doses of 20, 40, 70, 90, and 110 µg larva⁻¹). Acetone was used the negative control. Then, the larvae were moved to rearing containers sized 15 × 12 × 8 cm with a perforated lid (at 25 ± 1 °C, 70 ± 3% R.H., and 16:8 h (L:D)) and containing their usual food. The experiment was replicated 4 times in total (20 insects per replication). Mortality was assessed 24 h after treatment. Insects failing to respond were considered dead.

4.4.3. Insecticidal Activity against Mosquitoes

*C. quinquefasciatus* larvae (3rd instar) were exposed to four Patagonian EOs diluted in dimethyl sulfoxide (DMSO) relying on the WHO protocol [46] with minor changes by Pavela and Sedlak [47]; the tested concentrations were 20, 30, 50, 80, and 100 µL mL⁻¹. Distilled water with the same amount of DMSO as that used for dissolving the EOs was the negative control. The experiments were carried out at 25 ± 1 °C, 70 ± 3% R.H., and 16:8 h (L:D). The experiments were replicated 4 times in total (25 insects per replication). Mortality was assessed 24 h after treatment. Insects failing to respond were considered dead.

4.5. Data Analysis

To calculate the EO lethal doses/concentrations on each target insect, we used a minimal series of at least 5 different doses/concentrations, which resulted in mortality rates in the range of 10–90%. Mortality was corrected using Abbott’s formula [48], and the lethal concentration values (LC₅₀ and LC₉₀) and associated 95% confidence limits for each treatment were estimated using probit analysis [49].

5. Conclusions

This study supports evidence of the insecticidal potential of EO obtained from ciprés (*P. uviferum*), tepa (*L. philippiana*), and canelo (*D. winteri*), and demonstrates for the first time the insecticidal efficacy of the EO from paramela (*A. boronioides*). The four Patagonian EOs are active against *M. domestica*, *S. littoralis*, and *C. quinquefasciatus* with promising LC₅₀,(90).

However, it is not possible to establish which are the main responsible compounds for the biological activity, as the chemical composition is varied, and the different components could act synergistically or antagonistically. Notably, this study shows that these Patagonian plants can represent a local source of potential insecticidal products for the control of insects of medical and agricultural importance. Further studies are needed to explore the effects of these EOs on other target species, with special reference to the invasive moth pest *Spodoptera frugiperda* (J. E. Smith) [50], to determine their single components’ contribution to the insecticidal activity, and to evaluate their possible synergistic or antagonistic effect.

Semi-field and field evaluation of the insecticidal activity of the most active botanical products will also be conducted.

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