Chemical Composition and In Vitro Anti-Wood-Decay Fungal Activities of Dysphania ambrosioides Leaf Essential Oil From Taiwan

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Abstract
We evaluated the leaf essential oil in whole or fractions of Dysphania ambrosioides with respect to their resistance to wood decay fungal activities in vitro of 4 fungi. The main ingredients with the greater anti-wood decay capability were also identified. Fresh leaves of D. ambrosioides were hydrodistillated in a Clevenger-type apparatus and the resulting oil characterized using GC-FID and GC-MS instruments. The essential oil was found to consist of α-terpinene (30.5%), p-cymene (17.3%), carvacrol (16.2%), and ascaridole (15.1%). The oil showed resistance to wood decay activity of Trametes versicolor, Phanerochaete chrysosporium, Phaeolus schweinitzii, and Lenzites sulphurea. The oil had excellent resistance to wood decay fungal activities, and the active compounds were shown to be carvacrol and ascaridole.

Keywords
Dysphania ambrosioides, anti-wood-decay fungal activity, essential oil, terpene, carvacrol, ascaridole

Introduction
Wood has been loved by people and widely used for construction and furniture since ancient times. However, as an organic material, wood is easily damaged by microorganisms, especially in hot and humid climates that are conducive to the growth of wood-decaying fungi. In order to ensure the function of wood products and prolong their service life, the most wood products are treated with wood preservatives. Traditional CCA (chromated copper arsenate) is a good wood preservative, once widely used to prolong the service life of wood. But its toxicity to human and the environment causes it to be banned gradually. Hence, searching for newer, more effective, and nontoxic wood preservative is an imperative issue.

Certain essential oils have inhibitory capacities to the growth of wood-decaying fungi. Our previous studies have shown that many essential oils have such functions. For instance, bark essential oil of Cunninghamia lanceolate var. konishii and fruit oil of Liquidambar formosana have excellent resistance to wood decay fungi. These oils can fully inhibit the growth of Trametes versicolor, Phanerochaete chrysosporium, Phaeolus schweinitzii, and Lenzites sulphurea at a dose of only 100 μg/mL.

Dysphania ambrosioides (L.) Mosyakin & Clemants (epazote, Mexican tea, or wormseed) is an annual or short-lived perennial herb covered with aromatic glandular hairs and belongs to the family Amaranthaceae. The species is native to South and Central America, with a wide distribution in Africa, Europe, Australia, and Asian countries, and is also indigenous to Taiwan. According to World Health Organization (WHO), it is considered to be one of the most widely used medicinal plants in the world, widely used in folk medicine to treat skin conditions and diseases of the respiratory, digestive, vascular, urogenital, and nervous systems. Many references have showed the components and antimicrobial, insecticidal, allelopathic, antioxidant, antiparasitic, and cytotoxic activities of D. ambrosioides essential oil.

Zefzoufi et al reported that it can work against fungal phytopathogens such as Verticillium dahlia, Fusarium oxysporum f. sp. melonis and F. culmorum, and Stappen et al found that it can inhibit Colletotrichum acutatum, C. fragariae, and C. gloeosporioides.

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Based on these studies, *D. ambrosioides* is worthy of further research.

This report is comprised of 2 parts: the first part used hydrodistillation to obtain the leaf essential oil of *D. ambrosioides* indigenous to Taiwan and its chemical components were determined using GC-FID and GC-MS. The second part determined the resistance to wood-decay fungal activities of the oil. Therefore, the aim of this study was to establish a chemical basis for the effective multipurpose utilization strategies of the native species.

Results and Discussion

Hydrodistillation of *D. ambrosioides* produced a light-yellow-colored oil with yield of 0.85 ± 0.03 mL./100 g based on the dry weight of leaves. Table 1 presents the identified compounds, and their percentages, as well as their linear retention index (LRI) values listed in order of elution from the DB-5 capillary column. Twenty-five components were identified from the oil of *D. ambrosioides*. Of the essential oil components, 51.0% comprised of 6 monoterpene hydrocarbons, 47.2% were 15 oxygenated monoterpenes, and 1.5% were 3 sesquiterpene hydrocarbons, and 0.1% was one diterpene. This essential oil composition does not contain oxygenated sesquiterpenes. The major compounds of the monoterpene hydrocarbons were α-terpinene (30.5%) and p-cymene (17.3%). The main compounds of the oxygenated monoterpenes were carvacrol (16.2%) and ascaridole (15.1%). The chemical composition of *D. ambrosioides* essential oil has been extensively studied by GC and GC-MS analysis from different countries around the world (Table 2).

These studies on *D. ambrosioides* oil have suggested that there are many chemotypes, for example, India oils, with the following major components: α-terpinene (65.4%), and p-cymene (29.4%)\(^{38}\); α-terpinene (63.6%), p-cymene (19.5%), and ascaridole (6.2%)\(^{39}\); α-terpinene (44.7%), p-cymene (21.3%), and ascaridole (17.9%)\(^{40}\); ascaridole (38.0%), α-terpinene (37.7%), and p-cymene (16.7%)\(^{41}\); ascaridole (40.7%), isoascaridole (22.7%), α-terpinene (17.9%), and p-cymene (8.5%)\(^{42}\); p-cymene (36.3%) and ascaridole (31.2%)\(^{21}\); α-terpinene (37.2%), isoascaridole (20.5%), and ascaridole (14.8%)\(^{43}\); and α-terpinene (72.5%), p-cymene (20.6%), and terpinolene (2.5%)\(^{44}\). In China, the *D. ambrosioides* oil was found to contain the following major components: menthol (31.3%), and α-terpinene (13.2%)\(^{45}\); bornylcne (42.6%), p-cymene (21.8%), ascaridole (18.4%), and α-terpinene (11.7%)\(^{46}\); p-cymene (49.6%), α-terpinene (26.8%), and isoscalarene (8.2%)\(^{47}\); (Z)-ascaridole (29.7%), isoscalarene (13.0%), and α-terpinene (12.7%)\(^{48}\); and α-terpinene (32.9%) and p-cymene (24.2%)\(^{49}\). In Brazil, the main components were (Z)-ascaridole (61.4%) and isoascaridole (18.6%)\(^{11}\); α-terpinolone (69.9%) and ascaridole (17.1%)\(^{50}\); α-terpinene (40.7%), p-cymene (21.8%), and isoascaridole (12.5%)\(^{51}\); α-terpinene (30.5%), thymol (18.1%), and α-cymene (13.7%)\(^{52}\); and cis-piperitone oxide (30.3%), isoascaridole (18.2%), p-cymene (13.2%), and α-terpinene (12.5%)\(^{33}\); whereas in the Iran, the oil contained mainly α-terpinyl acetate (73.9%), p-cymene (4.3%)\(^{53}\); and ascaridole (43.4%), α-terpinene (15.9%), and camphor (12.4%)\(^{54}\). In Cameroon, the main components of the species was α-isascaridole (35.4%), p-cymene (29.2%), and isoascaridole (26.0%)\(^{55}\); while in the Nigeria, the main components of the species were α-terpinene (56.0%), α-terpinyl acetate (15.7%), and p-cymene (15.5%)\(^{56}\); α-terpinene (63.1%), p-cymene (26.4%), ascaridole (3.9%)\(^{57}\); and α-terpinene (48.7%), p-cymene (21.7%), α-terpinyl butyrate (17.1%), and ascaridole (5.7%)\(^{58}\); and in the Cuban oil had carvacrol (62.4%), and ascaridole (22.5%) as the main components\(^{59}\). In Mexico, the main components were limonene (32.5%), and trans-pinocarveol (26.7%)\(^{60}\); and ascaridole epoxide (45.5%) and α-isascaridole (34.2%)\(^{60}\), while the Morocco, the main components of the oil was α-terpinene (61.0%), 4-carene (13.6%), and p-cymene (12.9%)\(^{14}\), and in the Tunisian oil had α-isascaridole (60.3%) and m-cymene (22.2%)\(^{12}\). It can be seen from the above literature that the chemical composition of *D. ambrosioides* essential oil are many chemotypes, which may be due to different geographical locations, harvest time, extraction methods, etc.\(^{61}\) Moreover, as reported by Cavalli et al\(^{62}\) by GC analysis, ascaridole undergoes a partial thermal isomerisation to isoascaridole and hence the amount of ascaridole is underestimated by GC analysis. In this study, *D. ambrosioides* oil from Taiwan were found to have main constituents α-terpinene, p-cymene, carvacrol, and ascaridole. These results are different from those of the abovementioned studies and represent the first report of this *D. ambrosioides* oil.

To test the wood-decay fungal resistant activities of *D. ambrosioides* essential oil, 4 wood-decay fungi were tested, including 2 white-rot fungi (*T. versicolor* and *Phae. chrysorropium*) and 2 brown-rot fungi (*Phae. schweinitzii* and *L. sulphureus*). Table 3 lists the wood-decay fungal resistant activity of *D. ambrosioides* essential oil at various concentrations. For complete inhibition, an oil concentration is only 50 μg/mL for *Phae. schweinitzii* and *L. sulphureus*, 100 μg/mL for *Phae. chrysorropium*, and 200 μg/mL for *T. versicolor*.

The wood essential oil of Cunninghamia konishii, which major compound was cedrol, required a concentration of 400 μg/mL to fully inhibit *T. versicolor*, and *L. sulphureus*\(^{63}\), and so our study shows that *D. ambrosioides* oil is better at inhibiting wood-rot fungus.

From the above experiments, it is known that *D. ambrosioides* essential oil has excellent inhibitory activity against wood-rot fungi. Therefore, the major compounds of *D. ambrosioides* essential oil were tested for their anti-wood-decay fungal activities, respectively. The ascaridole compound was isolated from *D. ambrosioides* essential oil by silica gel column chromatography. Ascaridole compound was identified based on infrared radiation, \(^{13}\)C NMR, \(^{1}H\) NMR spectroscopic, and mass spectrometric data.\(^{16,64}\) The Carvacrol, α-terpinene, and p-cymene were from isolates found by Hsu and Ho (2019) in a study of *Plectranthus amboinicus*\(^{65}\) essential oil. Table 4 are shown the MIC and IC\(_{50}\) values of the 4 compounds. The experiment
results showed that the carvacrol and ascaridole were the main active source compounds. Previous references support the argument that carvacrol compound presents significant activity in inhibiting the growth of microorganisms. No studies have been published on the inhibition of wood decay fungus by the ascaridole compound, so this is the first such publication.

Conclusion

This study indicates that the *D. ambrosioides* essential oil and its major compounds of α-terpinene, p-cymene, carvacrol, and ascaridole possess pronounced antifungal activity against wood-decay fungi. The essential oil is mainly composed of monoterpene hydrocarbons and oxygenated monoterpenes. Among these ingredients, carvacrol and ascaridole have been shown to have excellent activities against wood-decay fungi. Therefore, the focus of future studies will be on the development of various natural wood-decay fungi-resistant products.

### Experimental

#### Plant Materials

The *D. ambrosioides* botanical material was obtained from Nei-Heng Mt in north Taiwan in October 2021 (New Taipei City, altitude 350 m, latitude (N) 25°24′036″, longitude (E) 121°55′65″). The plant was identified by Professor Yu Hang-Ming from the Taiwan Forestry Research Institute (TFRI). A voucher specimen (WCCLH-086) has been deposited at the laboratory of the Wood Cellulose Department, TFRI, Taiwan.

#### Isolation of *D. ambrosioides* Essential Oil

The fresh leaves of *D. ambrosioides* (10 kg) were hydrodistilled using a Clevenger-type apparatus for 3 h. The essential oil was dried using anhydrous sodium sulfate and stored in a sealed vial at 4 °C until needed. The yield of essential oil and

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### Table 1. Chemical Composition of Leaf Essential Oil From *Dysphania ambrosioides*

| Compound             | LRI<sup>LRI</sup><br>    | LRI<sup>LRI</sup><br>Exp | Concentration (%) | Identification<sup>c</sup> |
|----------------------|--------------------------|--------------------------|---------------------|----------------------------|
| α-Terpinene          | 1014                     | 1017                     | 30.5                | MS, LRI, CO-ST             |
| p-Cymene             | 1020                     | 1024                     | 17.3                | MS, LRI, CO-ST             |
| Limonene             | 1025                     | 1029                     | 1.0                 | MS, LRI, CO-ST             |
| γ-Terpinene          | 1066                     | 1059                     | 1.5                 | MS, LRI, CO-ST             |
| Terpinolene          | 1090                     | 1088                     | 0.3                 | MS, LRI, CO-ST             |
| p-Cymene             | 1093                     | 1091                     | 0.3                 | MS, LRI, CO-ST             |
| Linalool             | 1102                     | 1096                     | 0.4                 | MS, LRI, CO-ST             |
| α-Thujone            | 1110                     | 1102                     | 0.3                 | MS, LRI                   |
| trans-p-Mentha-2,8-dien-1-ol | 1126 | 1122                     | 0.1                 | MS, LRI                   |
| trans-Pinocarveol    | 1140                     | 1139                     | 0.5                 | MS, LRI                   |
| Camphor              | 1152                     | 1146                     | 0.3                 | MS, LRI, CO-ST             |
| Terpinen-4-ol        | 1185                     | 1177                     | 0.2                 | MS, LRI, CO-ST             |
| Ascaridole           | 1234                     | 1237                     | 15.1                | MS, LRI, CO-ST             |
| α-Piperitone epoxide | 1259                     | 1254                     | 1.7                 | MS, LRI                   |
| α-Carvone oxide      | 1266                     | 1263                     | 3.9                 | MS, LRI                   |
| trans-Carvone oxide  | 1283                     | 1276                     | 2.2                 | MS, LRI                   |
| Thymol               | 1304                     | 1290                     | 0.6                 | MS, LRI, CO-ST             |
| Carvacrol            | 1314                     | 1299                     | 16.2                | MS, LRI, CO-ST             |
| Isoascaridole        | 1320                     | 1304                     | 4.9                 | MS, LRI                   |
| Piperitenone         | 1343                     | 1343                     | 0.5                 | MS, LRI                   |
| Piperitenone oxide   | 1367                     | 1368                     | 0.3                 | MS, LRI                   |
| (E)-β-Caryophyllene  | 1426                     | 1419                     | 1.0                 | MS, LRI                   |
| Germacrene D         | 1486                     | 1485                     | 0.3                 | MS, LRI, CO-ST             |
| Drim-8(12)-ene       | 1494                     | 1491                     | 0.2                 | MS, LRI                   |
| Phyto1               | 1942                     | 1943                     | 0.1                 | MS, LRI, CO-ST             |
| Monoterpene hydrocarbons (%) | 51.0                  |                         |                     |
| Oxygenated monoterpenes (%) | 47.2            |                         |                     |
| Sesquiterpene hydrocarbons (%) | 1.5               |                         |                     |
| Oxygenated sesquiterpenes (%) | s                 |                         |                     |
| Diterpenes (%)       |                         | 0.1                     |                     |
| Oil Yield (ml/100 g)  |                         | 0.85 ± 0.03             |                     |

<sup>a</sup>LRI<sub>LRI</sub><br>LRI<sub>LRI</sub><br>Lin = Linear retention index on DB-5 capillary column from the literature.<<sup>38</sup>

<sup>b</sup>LRI<sub>LRI</sub><br>LRI<sub>LRI</sub><br>Exp = Determined linear retention index relative to n-alkanes (C<sub>8</sub>-C<sub>30</sub>) on DB-5 capillary column.

<sup>c</sup>Identification by; MS = NIST 17 and Wiley 11 libraries spectra, and the literature.

<sup>d</sup>LRI = linear retention index (LRI) same as literature.<<sup>35</sup>-37

<sup>e</sup>CO-ST = co-injection and comparison with the linear retention index and mass spectra of standards.

<sup>f</sup>d- Not detected.
Table 2. Comparison of the Chemical Composition of D. ambrosioides Essential Oil From Different Countries.

| Country      | Major compound                  | Reference          |
|--------------|---------------------------------|--------------------|
| Taiwan       | α-Terpinene (30.5%), p-cymene (17.3%), carvacrol (16.2%), ascaridole (15.1%) | This study         |
| India        | α-Terpinene (65.4%), p-cymene (29.4%) | 39                 |
|              | α-Terpinene (63.6%), p-cymene (19.5%), ascaridole (6.2%) | 40                 |
|              | α-Terpinene (44.7%), p-cymene (21.3%), ascaridole (17.9%) | 41                 |
|              | Ascaridole (38.0%), α-terpinene (37.7%), p-cymene (16.7%) | 42                 |
|              | Ascaridole (40.7%), isoascaridole (22.7%), α-terpinene (17.9%), p-cymene (8.5%), ascaridole (31.2%) | 43                 |
|              | α-Terpinene (37.2%), isoascaridole (20.5%), ascaridole (14.8%) | 44                 |
|              | α-Terpinene (72.5%), p-cymene (20.6%), terpinolene (2.5%) | 45                 |
| China        | Menthol (31.3%), α-terpinene (13.2%) | 46                 |
|              | Bornylene (42.6%), p-cymene (21.8%), ascaridole (18.4%), α-terpinene (11.7%), p-Cymene (49.6%), α-terpinene (26.8%), isoascaridole (8.2%) | 47                 |
|              | (Z)-Ascaridole (29.7%), isoascaridole (13.0%), p-terpinene (12.7%) | 48                 |
|              | α-Terpinene (32.9%), p-cymene (24.2%) | 49                 |
| Brazil       | (Z)-Ascaridole (61.4%), isoascaridole (18.6%) | 50                 |
|              | α-Terpinene (69.9%), ascaridole (17.1%) | 51                 |
|              | α-Terpinene (40.7%), p-cymene (21.8%), isoascaridole (12.5%) | 52                 |
|              | α-Terpinene (30.5%), thymol (18.1%), α-cymene (13.7%) | 53                 |
|              | α-Ascaridole oxide (30.3%), isoascaridole (18.2%), p-cymene (13.2%), α-terpinene (12.5%) | 54                 |
| Iran         | α-Terpinyl acetate (73.9%), p-cymene (4.3%), Ascaridole (43.4%), α-terpinene (15.9%), camphor (12.4%) | 55                 |
| Cameroon     | α-Ascaridole (35.4%), p-cymene (29.2%), isoascaridole (26.0%) | 56                 |
| Nigeria      | α-Terpinene (56.0%), α-terpinyl acetate (15.7%), and p-cymene (15.5%) | 57                 |
|              | α-Terpinene (63.1%), p-cymene (26.4%), ascaridole (3.9%) | 58                 |
|              | α-terpinyl butyrate (17.1%), ascaridole (5.7%) | 59                 |
| Cuba         | Carvacrol (62.4%), ascaridole (22.5%) | 60                 |
| Mexico       | Limonene (32.5%), trans-pinocarveol (26.7%) | 61                 |
|              | Ascaridole epoxide (45.5%), α-ascaridole (34.2%) | 62                 |
| Morocco      | α-Terpinene (61.0%), 4-carene (13.6%), p-cymene (12.9%) | 63                 |
| Tunisian     | α-Ascaridole (60.3%), m-cymene (22.2%) | 64                 |

all experiment data were calculated and shown as the mean ± standard deviation of triplicate analyses.

Table 3. The Wood-Decay Fungal Resistant Activity of D. ambrosioides Essential Oil at Various Dosages.

| Concentration (μg/mL) | Trametes versicolor | Phaeolus chrysosporium | Phanerococcus spinosus | Lenzites sulphureus |
|-----------------------|--------------------|------------------------|------------------------|--------------------|
| 12.5                  | 33 ± 1.5           | 37 ± 2.0               | 46 ± 1.5               | 48 ± 0.6           |
| 25                    | 62 ± 1.5           | 73 ± 2.5               | 82 ± 1.2               | 74 ± 1.2           |
| 50                    | 85 ± 1.5           | 86 ± 1.5               | 100 ± 0.2              | 100 ± 0.2          |
| 100                   | 92 ± 1.7           | 100 ± 0.0              | 100 ± 0.2              | 100 ± 0.0          |
| 200                   | 100 ± 0            | 100 ± 0.0              | 100 ± 0.0              | 100 ± 0.0          |

Essential Oil Analysis

Analysis of the essential oil of D. ambrosioides was performed by analytical GC-FID and GC-MS. For GC-FID analysis, a Hewlett-Packard 6890 gas chromatograph was used, equipped with a DB-5 capillary columns (5% phenyl 95% methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm film thickness), and the oil components were quantitatively determined with an FID. The oven temperature program was 50 °C constant for 2 min, and then increased at 5 °C/min until reached 250 °C. The injector temperature was heated 270 °C, the detector temperature at 250 °C, and the split ratio was 1:10, hydrogen (H2) being used as carrier gas with a flow rate of 1 mL/min. Samples (1.0 μL, 1/100, vol/vol, in ethyl acetate) were injected in split mode using ALS. The LRI for all compounds was calculated with reference to a homologous series of C6-C30 n-alkanes. Relative proportions of the oil components were calculated based on GC-FID peak areas measured on the DB-5 capillary column without the use of correction factor. Results are summarized in Table 1 and are reported according to their elution order on the DB-5 capillary column.

GC-MS analyses a Hewlett-Packard 6890/5973 GG-MS system was used, equipped with a DB-5 capillary columns (the same parameters as that used in the GC-FID analysis). The carrier gas was helium (He) (99.995% purity) with a flow rate of 1 mL/min. The mass spectra conditions were ionization voltage 70 eV, full scan mode: scan time: 0.3 s, and mass range was m/z 30-500.

Component Identification

Comounds identification was based on calculated LRI comparison with those obtained in the NIST library “NIST 17” and “WILEY 11,” and in some components by co-injection with standard pure compounds.

Isolation and Purification of Ascaridole

The essential oil of D. ambrosioides (20 g) mixed with silica gel 60 (60 g) (200-300 mesh, Fluka) was chromatographed on a silica
gel open column (600 g) and eluted with a stepwise gradient of n-hexane-ethyl acetate was used to give 9 fractions. The pure ascaridole compound was obtained from Fraction III (n-hexane-ethyl acetate (98:2), 2.20 g). The other fractions were mixtures.

The structure of the ascaridole compound was confirmed by comparing $^1$H-NMR, $^{13}$C-NMR, and EI-MS spectral datas with the previous literature values.\textsuperscript{16,64} Ascaridole: Yellow oil, EI-MS for $C_9H_{16}O_2$ (EI-MS: 168), $^1$H NMR (300 MHz, CDCl$_3$) (BCRC 36200), and 2 brown-rot fungi Phane. chrysosporium strain was 2 white-rot fungi (BCRC 35305)). These fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan.

\textbf{Anti-Wood-Decay Fungal Assays}

The method of Su et al\textsuperscript{68} was employed. The wood-decay fungal strain was 2 white-rot fungi (\textit{T. versicolor} (BCRC 35253), and \textit{Phan. chrysosporium} (BCRC 36200)), and 2 brown-rot fungi (\textit{Phano. schweinitzii} (BCRC 35365) and \textit{L. sulphureus} (BCRC 35305)). These fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. Antifungal assays were performed 3 times. Briefly, 500, 250, 200, 100, 50, 25, and 12.5 μg/mL of essential oil or compounds were added to sterilized potato dextrose agar (PDA) in 9 cm Petri dishes. After the fungal mycelium reached the edges of control plates by incubating at 27 °C, the anti-wood-decay fungal index was calculated as follows:

\[
\text{Anti-wood-decay fungal index ('%) = } \left(1 - \frac{D_a}{D_b}\right) \times 100,
\]

where \(D_a\) = the diameter of the mycelium growth zone in the experimental plate (cm) and \(D_b\) = the mycelium diameter of the growth zone in the control plate (cm). The positive control was Nystatin. Fifty% inhibitory of mycelium growth concentrations (μg/mL) (IC$_{50}$ values) were measured for all the tested compounds by probit analysis.

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\textbf{Table 4.} The MIC and IC$_{50}$ Values (μg/mL) of 4 Major Components From \textit{Dysphania ambrosioides} Oil Against 4 Wood-Decay Fungi.

| Compounds | \textit{Trametes versicolor} | \textit{Phanerochaete chrysosporium} | \textit{Phaeo. chrysosporium} | \textit{L. sulphureus} |
|-----------|----------------------------|----------------------------------|-----------------------------|-----------------------|
|           | IC$_{50}$ | MIC   | IC$_{50}$ | MIC   | IC$_{50}$ | MIC   | IC$_{50}$ | MIC   |
| α-Terpine | >500     | >500  | >500     | >500  | >500     | >500  | >500     | >500  |
| p-Cymene  | >500     | >500  | >500     | >500  | >500     | >500  | >500     | >500  |
| Ascaridole| 135.8    | 150   | 128.3    | 150   | 69.8     | 100   | 36.3     | 50    |
| Carvacrol | 82.3     | 100   | 70.6     | 100   | 53.9     | 100   | 36.3     | 50    |
| Nystatin  | <12.5    | <12.5 | <12.5    | <12.5 | <12.5    | <12.5 | <12.5    | <12.5 |

\textsuperscript{a}Nystatine was used as a positive control.
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