Chemical Compositions and In Vitro Antiphytopathogenic Fungi Activities of the Leaf and Cones Essential Oils of Cunninghamia lanceolata From Taiwan

Kuang-Ping Hsu¹, Yu-Chang Su², and Chen-Lung Ho¹

Abstract
In this study, antiphytopathogenic fungi activities of the leaf and cones essential oils and its constituents from Cunninghamia lanceolata were evaluated in vitro against 6 plant pathogenic fungi. The main compounds responsible for the antiphytopathogenic fungi activities were isolated and identified. The essential oil from the fresh leaves and cones of C. lanceolata was isolated using hydrodistillation in a Clevenger-type apparatus, and characterized by GC-FID and GC-MS, respectively. The leaf oil consisted primarily of ferruginol (10%), τ-cadinol (8.2%), and α-cadinol (6.6%); the cones oil’s main constituents were abietadiene (42.5%), abietatriene (13.1%), and α-pinene (9.6%). Comparing the antiphytopathogenic fungi activities of the oils suggested that leaf oil was the most effective one. Further fractionation of the leaf oil produced ferruginol, τ-cadinol, and α-cadinol. The 3 compounds exhibited very strong antiphytopathogenic fungi activities. For the antiphytopathogenic fungi activities of the leaf oil, the active source compounds were determined to be ferruginol, τ-cadinol, and α-cadinol.

Keywords
Cunninghamia lanceolata, essential oil, terpene, antiphytopathogenic fungi activity, ferruginol, τ-cadinol, α-cadinol

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The warm and humid island-type climate of Taiwan is rather conducive to the growth of molds or mildews; hence, they are prevalent in our environments. It causes plants in nature to be attacked by microorganisms and triggers various diseases. There are many types of microorganisms that can cause plant diseases, such as fungi, bacteria, viruses, nematodes, and among all pathogens, fungi cause the most types of diseases.¹ Plant pathogenic fungi can infect plant tissues, destroy important organs such as roots or leaves, reduce the vitality of plants, and eventually cause the death of plants.²⁻⁵ Increasing recognition of the importance of plant pathogenic fungi infections and the difficulties encountered in their treatment have stimulated the search for alternatives to synthetic chemical fungicides. However, these chemicals may cause secondary environmental pollutions.⁶⁻⁷ In recent years, scientists began looking at plant essential oils or naturally produced chemicals as fungicides. Our research team has proven on numerous occasions the antifungal efficacies of various essential oils.⁸⁻⁹ Cunninghamia lanceolata (Lamb.) Hook. (Taxodiaceae) is a traditional Chinese medicine used for the treatment of hernia, arthritis, and strangury. It is a very important tree species of silviculture in Taiwan.¹⁰ Previous studies have reported the composition and antifungal activities of wood of C. lanceolata.¹¹⁻¹⁷ However, only 1 report noted the composition of leaf essential oil, but this report did not evaluate the biological activity.¹¹ No prior study has investigated the chemical composition and biological activity of the cones essential oil. Therefore, we used hydrodistillation to collect the leaf and cones oils, and analyzed these using GC-FID and GC-MS. The second part of the study examined the antiphytopathogenic fungi activity of the essential oils and dominant constituents isolated from C. lanceolata against plant pathogenic fungi. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.
Results and Discussion

Based on the dry weight of leaves and cones, hydrodistillation of *C. lanceolata* produced yellow colored oils with yields of 1.31 ± 0.06 and 0.98 ± 0.05 mL/100 g, respectively. All compounds are listed in order of their elution from the DB-5 column (Table 1). A total of 67 compounds were identified from the hydrodistilled leaf oil of *C. lanceolata*. Among the leaf oil compounds, sesquiterpene hydrocarbons were predominant (33.6%), followed by monoterpenes hydrocarbons (23.1%), oxygenated sesquiterpenes (20.3%), oxygenated diterpene (12.4%), diterpene hydrocarbon (3.9%), oxygenated monoterpenes (3.8%), and others (2.0%). Among the sesquiterpene hydrocarbons, β-selinene (5.1%), germacrene D (5.0%), and α-selinene (4.8%) were the major compounds. Of the monoterpenes hydrocarbons, α-pinene (10.1%) and limonene (4.6%) were the main components. Monoterpene hydrocarbons τ-cadinol (8.2%) and α-cadinol (6.6%) were the major compounds; of the oxygenated diterpene, ferruginol (10%) was the chief compound. After a thorough search, we found only 1 report pertaining to the leaf oil of *C. lanceolata*. In the report, the main ingredients of the leaf oil were α-limonene (27.3%), α-pinene (18.5%), β-caryophyllene (9.6%), and β-myrcene (9.6%), which were different from our present results.

Forty-seven components were identified from the cones oil. Among the component groups, diterpene hydrocarbons were the most dominant ones (62.9%), followed by monoterpenes hydrocarbons (15.2%), sesquiterpene hydrocarbons (8.4%), oxygenated sesquiterpenes (5.8%), oxygenated diterpenes (4.3%), and oxygenated monoterpenes (2.2%). Abietadiene (42.5%) and abietatriene (13.1%) were the major compounds of the diterpene hydrocarbons. Of the monoterpenes hydrocarbons, α-pinene (9.6%) was the chief compound. The search turns up no report on the cones oil of *C. lanceolata*, however. Thus, the cones oil composition on *C. lanceolata* represents the first such report in the literature.

The leaf and cones oils of *C. lanceolata* were tested against 6 plant pathogenic fungi, including 2 seedling pathogens, *Fusarium oxysporum* and *Rhizoctonia solani*, 2 leaf pathogens, *Pestalotia funerea* and *Colletotrichum gloeosporioides*, and 2 root and stem pathogens, *Ganoderma australe* and *Fusarium solani*. Figure 1 shows the antifungal indices of leaf and cones parts of *C. lanceolata* oil (at a concentration of 500 µg/mL) against plant pathogenic fungi. The antifungal indices demonstrated clearly that the leaf oil had antifungal activities superior to those of the cones oil (Figure 1). At this concentration, the antifungal indices of the leaf oil against damping-off pathogens, *F. oxysporum* and *R. solani*, were 72.3% and 100%, respectively. As for the leaf pathogens, *P. funerea* and *C. gloeosporioides*, the antifungal indices of the leaf oil were 67.3% and 90%, respectively. The antifungal indices of the oil against root rot pathogens, *G. australis* and *F. solani*, were 100% and 100%, respectively. The results obtained showed that the leaf oil had excellent antifungal activities with values >67.3%, and suppressed totally the growth of *R. solani*, *G. australis*, and *F. solani*.

In order to evaluate the half inhibition concentrations (IC$_{50}$) of the leaf and cones oils, the antifungal indices of 2 oils vs fungi were regression analyzed. The results are shown in Table 2. As the table shows, the IC$_{50}$ of leaf oil against 6 plant pathogenic fungi was between 83.2 and 308.9 µg/mL, that of cones oil was >500 µg/mL. Summarizing the above, the antifungal tests indicate that leaf essential oil of *C. lanceolata* has the strongest antiphytopathogenic bioactivity. Thus, leaf oil is singled out for further examination of its ingredients.

To further understand the antiphytopathogenic fungi activities’ ingredients of the oil extracted from the leaf of *C. lanceolata*, we have conducted a silica gel column chromatography of the leaf essential oil with the eluents mixed by n-hexane (n-hex) and ethyl acetate (EA) in different proportions (n-hex/EA = 100/0-0/100). The eluents were separated into 7 fractions (CO1-CO7). Then, we carried out experiment using 7 fractions under antifungal tests and used Nystatin as a control at a concentration of 100 µg/mL. The results are shown in Figure 2. CO4 had the best antiphytopathogenic activity with the highest antifungal indices against 6 plant pathogenic fungi. Among the plant pathogenic fungi, *R. solani*, *P. funerea*, *C. gloeosporioides*, *G. australis*, and *F. solani* were completely inhibited by the fraction.

The abovementioned results have proven that CO4 exerted the best antiphytopathogenic fungi activity. We further identified the ingredients from the CO4 fraction using GC and GC-MS. The results are shown in Table 3. Thirteen compounds were identified from CO4 fraction; τ-cadinol (30.5%), α-cadinol (29.8%), and ferruginol (30.8%) were the main ones.

Furthermore, we used the isolated and purified 3 compounds (τ-cadinol, α-cadinol, and ferruginol) to conduct antifungal tests against 6 plant pathogenic fungi, respectively. Figure 3 shows the antifungal indices of the main compounds (τ-cadinol, α-cadinol, and ferruginol) to conduct antifungal tests against 6 plant pathogenic fungi, respectively. The abovementioned results have proven that CO4 exerted the best antiphytopathogenic fungi activity. We further identified the ingredients from the CO4 fraction using GC and GC-MS. The results are shown in Table 3. Thirteen compounds were identified from CO4 fraction; τ-cadinol (30.5%), α-cadinol (29.8%), and ferruginol (30.8%) were the main ones.

Furthermore, we used the isolated and purified 3 compounds (τ-cadinol, α-cadinol, and ferruginol) to conduct antifungal tests against 6 plant pathogenic fungi, respectively. Figure 3 shows the antifungal indices of the main compounds (100 µg/mL) against 6 plant pathogens. The results indicated that 3 compounds exhibited excellent activity against *F. oxysporum*, *R. solani*, *P. funerea*, *C. gloeosporioides*, *G. australis*, and *F. solani* with the highest antifungal indices ranging from 61% to 100%.

In addition, the IC$_{50}$ values of 3 compounds are shown in Table 4. IC$_{50}$ values of τ-cadinol against *F. oxysporum*, *R. solani*, *P. funerea*, *C. gloeosporioides*, *G. australis*, and *F. solani* were 93.2, 58.3, 80.3, 58.2, 48.3, and 40.1 µg/mL. For α-cadinol, the following IC$_{50}$ values were obtained against 6 plant pathogenic fungi: 38.1, 29.2, 40.8, 10.8, 40.8, and 30.8 µg/mL for ferruginol, the following IC$_{50}$ values were obtained against 6 plant pathogenic fungi: 21.5, 26.8, 69.3, 39.8, 28.6, and 59.1 µg/mL (Table 4). Previous study have showed that these compounds with the highest antifungal indices ranging from 61% to 100%.

Results suggest that τ-cadinol, α-cadinol, and ferruginol of *C. lanceolata* leaf oil could be used as potential natural fungicide for controlling fungal pathogens and worth further investigation.
Table 1. Chemical Composition of the Leaf and Cones Essential Oils of *Cunninghamia lanceolata*.

| Compound            | K.I.\(^a\) | K.I.\(^b\) | Concentration (%) | Identification\(^c\)     |
|---------------------|-------------|-------------|-------------------|--------------------------|
| α-Thujene           | 930         | 929         | 3.6               | 1.2                      | MS, KI, ST               |
| α-Pinene            | 939         | 937         | 10.1              | 0.1                      | MS, KI, ST               |
| Camphene            | 954         | 952         | 0.1               | 0.1                      | MS, KI, ST               |
| Sabinene            | 975         | 974         | 0.3               | 0.1                      | MS, KI, ST               |
| β-Pinene            | 979         | 979         | 0.8               | 0.5                      | MS, KI, ST               |
| β-Myrcene           | 991         | 991         | 1.4               | 0.8                      | MS, KI, ST               |
| α-Terpinepine       | 1017        | 1017        | 0.4               | 0.2                      | MS, KI, ST               |
| p-Cymene            | 1024        | 1025        | 0.2               | 1.3                      | MS, KI, ST               |
| Limonene            | 1029        | 1030        | 4.6               | 1.0                      | MS, KI, ST               |
| γ-Terpinepine       | 1059        | 1060        | 0.9               | 0.3                      | MS, KI, ST               |
| Terpinolene         | 1088        | 1089        | 0.7               | 0.2                      | MS, KI, ST               |
| Linalool            | 1096        | 1097        | 0.1               | -                        | MS, KI, ST               |
| cis-β-Terpinecol    | 1144        | 1146        | 0.1               | -                        | MS, KI                   |
| Terpinen-4-ol       | 1177        | 1177        | 1.3               | 0.8                      | MS, KI, ST               |
| α-Terpineol         | 1188        | 1189        | 0.9               | 0.6                      | MS, KI, ST               |
| γ-Terpineol         | 1199        | 1196        | 0.1               | -                        | MS, KI                   |
| Bornyl acetate      | 1288        | 1289        | 0.5               | 0.4                      | MS, KI, ST               |
| α-Terpinyl acetate  | 1349        | 1350        | 0.7               | 0.3                      | MS, KI, ST               |
| Neryl acetate       | 1361        | 1363        | 0.1               | 0.1                      | MS, KI, ST               |
| α-Ylangene          | 1375        | 1374        | 0.1               | -                        | MS, KI                   |
| α-Copaene           | 1376        | 1375        | 0.5               | 0.1                      | MS, KI                   |
| β-Elemene           | 1390        | 1391        | 3.6               | 1.4                      | MS, KI, ST               |
| β-Caryophyllene     | 1419        | 1419        | 3.9               | 1.1                      | MS, KI, ST               |
| β-Copaene           | 1432        | 1433        | 0.3               | -                        | MS, KI                   |
| cis-β-Farnesene     | 1442        | 1442        | 0.4               | 0.1                      | MS, KI                   |
| α-Humulene          | 1454        | 1455        | 0.5               | 0.1                      | MS, KI, ST               |
| γ-Muurolene         | 1479        | 1477        | 3.7               | 0.7                      | MS, KI                   |
| Germancrene D       | 1485        | 1481        | 5.0               | 1.5                      | MS, KI, ST               |
| β-Selinene          | 1490        | 1486        | 5.1               | 1.4                      | MS, KI                   |
| α-Selinene          | 1498        | 1494        | 4.8               | 1.1                      | MS, KI                   |
| trans-β-Guaiene     | 1502        | 1501        | 0.6               | 0.4                      | MS, KI                   |
| γ-Cadinene          | 1513        | 1512        | 1.2               | 0.2                      | MS, KI                   |
| δ-Cadinene          | 1523        | 1520        | 3.3               | 0.4                      | MS, KI                   |
| trans-Cadina-1,4-diene | 1534    | 1532        | 0.3               | -                        | MS, KI                   |
| α-Cadinene          | 1538        | 1536        | 0.2               | -                        | MS, KI                   |
| α-Calacorene        | 1545        | 1543        | 0.2               | -                        | MS, KI                   |
| Elemol              | 1549        | 1549        | 0.4               | 0.1                      | MS, KI                   |
| (β)-Nerolidol       | 1563        | 1564        | 1.6               | 0.6                      | MS, KI                   |
| Dodecanoic acid     | 1566        | 1566        | 0.4               | -                        | MS, KI, ST               |
| Caryophyllene oxide | 1583        | 1581        | 0.7               | 0.5                      | MS, KI, ST               |
| Salvial-4(14)-en-1-one | 1594        | 1593        | 0.2               | -                        | MS, KI                   |
| Junecol             | 1619        | 1616        | 0.3               | -                        | MS, KI                   |
| 1-epi-Cubenol       | 1629        | 1628        | 0.3               | -                        | MS, KI                   |
| γ-Eudesmol          | 1632        | 1631        | 0.6               | 0.1                      | MS, KI                   |
| δ-Cadinol          | 1640        | 1640        | 8.2               | 0.1                      | MS, KI                   |
| α-Muurrolol         | 1646        | 1645        | 0.3               | 0.3                      | MS, KI, ST               |
| α-Eudesmol          | 1653        | 1652        | 0.2               | -                        | MS, KI                   |
| a-Cadinol           | 1654        | 1654        | 6.6               | 2.9                      | MS, KI, ST               |
| Eudesma-4(15),7-dien-1β-ol | 1688 | 1686 | 0.5 | - | MS, KI |
| Eudesm-7,11-en-4-ol | 1700        | 1699        | -                 | 1.3                      | MS, KI                   |
| Vetiselineol        | 1731        | 1733        | 0.1               | -                        | MS, KI                   |

(Continued)
Experimental

Plant Materials

Fresh leaves and cones of *C. lanceolata* were collected in January 2018 from Guanyin Mt in central Taiwan (Taichung County, elevation 600 m, N 24°28′69″, 120°90′51″). The samples were compared with specimen no. ou-9886 from the herbarium of National Chung-Hsing University (NCHU) and were positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-068) was deposited in the NCHU herbarium.

Isolation of the Leaf and Cones Essential Oil

Leaves and cones of *C. lanceolata* (1 kg) were distilled for 3 hours using a Clevenger-type apparatus and hydrodistillation technique. The essential oil obtained was dried with anhydrous sodium sulfate. The oil yields and all test data are the average of triplicate analyses.

Essential Oil Analysis

A Hewlett-Packard (HP) 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness, J&W Scientific) and a FID detector

### Table 1. Continued

| Compound                        | K.I.\(^a\) | K.I.\(^b\) | Leaf | Cones | Identification\(^c\) |
|---------------------------------|------------|------------|------|-------|----------------------|
| Tetradecanoic acid              | 1768       | 1766       | 0.4  | -     | MS, KI, ST           |
| Hinesol acetate                 | 1784       | 1783       | 0.2  | -     | MS, KI               |
| 1-Octadecene                    | 1790       | 1793       | 0.1  | -     | MS, KI               |
| Pentadecanoic acid              | 1867       | 1863       | 0.2  | -     | MS, KI, ST           |
| Isopimara-(9,11),15-diene        | 1905       | 1903       | 0.1  | -     | MS, KI               |
| Oxacycloheptadecan-2-one         | 1934       | 1932       | 0.3  | -     | MS, KI               |
| Cembrene                        | 1938       | 1940       | -    | 0.5   | MS, KI               |
| Phytol                          | 1943       | 1942       | 0.7  | -     | MS, KI, ST           |
| Hexadecanoic acid               | 1968       | 1968       | 0.4  | -     | MS, KI, ST           |
| Sandaracompimara-(8,14),15-diene | 1969       | 1969       | -    | 0.9   | MS, KI               |
| Manool oxide                    | 1987       | 1984       | 0.6  | 0.4   | MS, KI               |
| 1-Eicosene                      | 1988       | 1986       | 0.3  | -     | MS, KI               |
| 13-epi-Dolabradiene             | 2000       | 2001       | -    | 1.0   | MS, KI               |
| Abieta-(8,12)-diene             | 2022       | 2020       | -    | 2.3   | MS, KI               |
| Kaurene                         | 2043       | 2041       | 1.3  | -     | MS, KI, ST           |
| Abietatriene                    | 2056       | 2054       | 0.3  | 13.1  | MS, KI               |
| Abietadiene                     | 2087       | 2085       | 2.1  | 42.5  | MS, KI               |
| Neuzkol                         | 2133       | 2129       | 0.8  | -     | MS, KI               |
| Abieta-(8,14),13(15)-diene      | 2154       | 2150       | 0.2  | 2.6   | MS, KI               |
| Sandaracompimarinal             | 2184       | 2185       | 0.3  | 1.8   | MS, KI               |
| Abietal                         | 2313       | 2314       | -    | 0.8   | MS, KI               |
| Ferruginol                      | 2371       | 2366       | 10.0 | -     | MS, KI, ST           |
| Abietol                         | 2401       | 2399       | -    | 1.2   | MS, KI               |
| Compound identified             |            |            |      |       |                      |
| Monoterpene hydrocarbon         |            |            | 23.1 | 15.2  |                      |
| Oxygenated monoterpene          |            |            | 3.8  | 2.2   |                      |
| Sesquiterpene hydrocarbon       |            |            | 33.6 | 8.4   |                      |
| Oxygenated sesquiterpene        |            |            | 20.3 | 5.8   |                      |
| Diterpene hydrocarbon           |            |            | 3.9  | 62.9  |                      |
| Oxygenated diterpene            |            |            | 12.4 | 4.3   |                      |
| Others                          |            |            | 2.0  | -     |                      |
| Yield (mL/100 g)                |            |            | 1.31 ± 0.06 | 0.98 ± 0.05 |

\(^{a}\)Kováts retention indices on a DB-5 column with reference\(^{18–21}\) to \(\text{n}-\)alkanes.

\(^{b}\)Kováts retention indices, experimental: \(\text{n}-\)alkanes (C\(_9\)-C\(_{24}\)) were used as reference points in the calculation of relative retention indices.

\(^{c}\)MS, NIST and Wiley library spectra, and the literature; KI, Kováts index; ST, authentic standard compounds.

\(^{d}\)Not detected.
was used for quantitative determination of the oil components. The percentage composition of the oil was computed from the GC peak areas without any corrections. Oven temperature was programmed as follows: 50 °C for 2 minutes, rising to 250 °C at 5 °C/min; injector temperature: 270 °C; carrier gas: helium with a flow rate of 1 mL/min; detector temperature: 250 °C, split ratio: 1:10. Diluted samples (1.0 µL, 1/100, v/v, in EA) were injected manually in the split mode. Identification of the oil components was based on their Kováts indices and mass spectra obtained from GC-MS analysis on a HP 6890/HP 5973 equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness, J&W Scientific). The GC analysis parameters are listed above and the MS were obtained (full scan mode: scan time was 0.3 seconds and mass range was \( m/z \) 30-500) in the EI mode at 70 eV. All data were the average of triplicate analyses.

Component Identification

Identification of the essential oil constituents from the leaf and cones was based on comparisons of the Kováts retention indices, and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and the literature.

Isolation and Purification of Leaf Oil Components

The leaf essential oil (20 g) mixed with silica gel (60 g) (Merck 7734, Merck Co., Germany) was chromatographed on a silica gel open column (600 g) and eluted with a stepped gradient consisting of \( n \)-hex and EA (ranging from \( n \)-hex/EA = 100:0-0:100). The samples collected were screened by thin-layer chromatography (TLC) (Silica gel 60F254, Merck Co., Germany).

### Table 2. IC\(_{50}\) and minimum inhibitory concentration (MIC) Values (µg/mL) of Leaf and Cones Oils From Cunninghamia lanceolata Against the Plant Pathogenic Fungi.

| Essential oils | Plant pathogenic fungi | \( IC_{50} \) | MIC | \( IC_{50} \) | MIC | \( IC_{50} \) | MIC | \( IC_{50} \) | MIC | \( IC_{50} \) | MIC |
|---------------|------------------------|--------------|-----|--------------|-----|--------------|-----|--------------|-----|--------------|-----|
| Leaf          | **Fusarium oxysporum** | 198.5        | >500.0 | 95.3         | 250 | 308.9        | >500.0 | 132.1        | >500.0 | 90.3         | 125  |
|               | **Rhizoctonia solani** | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 |
|               | **Pestalotiopsis funerea** |            |       |              |     |              |      |              |      |              |      |
|               | **Colletotrichum gloeosporioides** |    |     |              |     |              |      |              |      |              |      |
|               | **Ganoderma australe**   |             |     |              |     |              |      |              |      |              |      |
|               | **Fusarium solani**      |             |     |              |     |              |      |              |      |              |      |
| Cones         | **Fusarium oxysporum**   | >500.0      | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 |
|               | **Rhizoctonia solani**   | >500.0      | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 | >500.0       | >500.0 |
|               | **Pestalotiopsis funerea** |            |       |              |     |              |      |              |      |              |      |
|               | **Colletotrichum gloeosporioides** |    |     |              |     |              |      |              |      |              |      |
|               | **Ganoderma australe**   |             |     |              |     |              |      |              |      |              |      |
|               | **Fusarium solani**      |             |     |              |     |              |      |              |      |              |      |
According to the antiphytopathogenic fungi activity assay, the CO4 exerted the best antiphytopathogenic fungi activity. Ferruginol, τ-cadinol, and α-cadinol were isolated and purified from CO4 fraction by semipreparative HPLC (column: Si-60 column, mobile phase: EtOAc/n-C₆H₁₄ = 30/70, flow rate: 1 mL/min). Ferruginol (retention time (RT): 14.3 minutes), τ-cadinol (RT: 22.1 minutes), and α-cadinol (RT: 31.2 minutes) were separately obtained. The structures of 3 compounds were confirmed by comparing physical and spectral data (including ¹H-NMR, ¹³C-NMR, and EI-MS) with the previously reported

![Antiphytopathogenic fungi activities of CO1-CO7 fractions (100 µg/mL) against 6 plant pathogenic fungi.](image)

**Table 3. Constituents and Contents of CO4 Fraction From Leaf Essential Oil of Cunninghamia lanceolata.**

| Constituents       | K.I.a | K.I.b | CO4 (%) | Identification^c |
|--------------------|------|-------|---------|------------------|
| γ-Muurolene        | 1479 | 1477  | 1.3     | MS, KI           |
| Germacrene D       | 1485 | 1481  | 1.6     | MS, KI, ST       |
| β-Selinene         | 1490 | 1486  | 0.8     | MS, KI           |
| α-Selinene         | 1498 | 1494  | 0.3     | MS, KI           |
| γ-Cadinene         | 1513 | 1513  | 0.6     | MS, KI           |
| δ-Cadinene         | 1523 | 1520  | 0.9     | MS, KI           |
| τ-Cadinol          | 1640 | 1640  | 30.5    | MS, KI           |
| α-Muurolol         | 1646 | 1645  | 0.2     | MS, KI, ST       |
| α-Eudesmol         | 1653 | 1652  | 0.3     | MS, KI           |
| α-Cadinol          | 1654 | 1654  | 29.8    | MS, KI, ST       |
| Kaurene            | 2043 | 2041  | 2.1     | MS, KI, ST       |
| Sandaracompimarinal| 2184 | 2185  | 0.1     | MS, KI           |
| Ferruginol         | 2371 | 2366  | 30.8    | MS, KI, ST       |
| Identified components (%) | 99.3 |       |         |                  |

^aKováts retention indices on a DB-5 column with reference to n-alkanes.18–21
^bKováts retention indices, experimental: n-alkanes (C₉-C₂₄) were used as reference points in the calculation of relative retention indices.
^cMS, NIST and Wiley library spectra, and the literature; KI, Kováts index; ST, authentic standard compounds.
values. Ferruginol: Yellow oil, EI-MS for C_{20}H_{30}O (EI-MS: 286), \(^1\)H NMR (in CDCl\(_3\)): \(\delta\) (ppm) 0.88 (3H, s, H-18), 0.91 (3H, s, H-19), 1.16 (3H, s, H-20), 1.22 (3H, d, \(J = 7.0\) Hz, H-16), 1.30 (3H, d, \(J = 7.0\) Hz, H-17), 2.76 (1H, ddd, \(J = 17.0, 10.5, 7.0\) Hz, H-7a), 2.83 (1H, ddd, \(J = 2.0, 6.5, 17.0\) Hz, H-7b), 3.13 (sept, \(J = 7.0\) Hz, H-15), 6.62 (1H, s, H-11), 6.83 (1H, s, H-14); \(^{13}\)C NMR: \(\delta\) (ppm) 19.10 (C-6), 19.19 (C-2), 21.45 (C-19), 22.52 (C-16), 22.68 (C-17), 24.63 (C-20), 26.48 (C-15), 29.61 (C-7), 33.16 (C-18), 33.21 (C-4), 37.26 (C-10), 38.65 (C-1), 41.58 (C-3), 50.21 (C-5), 110.88 (C-11), 126.46 (C-14), 126.25 (C-8), 131.68 (C-13), 148.18 (C-9), 150.96 (C-12). \(\tau\)-Cadinol: Yellow oil, EI-MS for C_{15}H_{26}O (EI-MS: 222), \(^1\)H-NMR (in CDCl\(_3\)): \(\delta\) (ppm) 0.75 (d, \(J = 7.0\), H-12), 0.87 (d, \(J = 7.0\), H-13), 1.16 (s,H-14), 1.68 (s, H-15), 2.15 (m, H-11) 5.50 (s, H-4). \(^{13}\)C-NMR: \(\delta\) (ppm) 15.10 (C-12), 20.76 (C-14), 21.50 (C-13), 22.00 (C-1), 22.66 (C-7), 23.80 (C-15), 26.02 (C-11), 30.96 (C-2), 39.86 (C-5), 42.21 (C-6), 46.75 (C-6), 50.06 (C-10), 72.46 (C-9), 122.33 (C-4), 134.95 (C-3).

**Antiphytopathogenic Fungi Activity Assays**

The plant pathogenic fungi used were *F. oxysporum* f. sp. melonis Snyder & Hansen (BCRC32121), *R. solani* Kuhn (BCRC31626), *P. funerea* (Desmazieres) Steyaert (BCRC35266), *C. gloeosporioides* Penzig (BCRC35003), *Ganoderma australe* (Fries) Paterson

![Antifungal index](image)

**Figure 3.** Antiphytopathogenic fungi activities of 3 compounds (100 µg/mL) from *Cunninghania lanceolata* leaf oil against 6 plant pathogenic fungi.

| Compound   | *F. oxysporum* IC_{50} | *F. oxysporum* MIC | *R. solani* IC_{50} | *R. solani* MIC | *P. funerea* IC_{50} | *P. funerea* MIC | *C. gloeosporioides* IC_{50} | *C. gloeosporioides* MIC | *G. australe* IC_{50} | *G. australe* MIC | *F. solani* IC_{50} | *F. solani* MIC |
|------------|------------------------|-------------------|----------------------|----------------|----------------------|-------------------|---------------------------|------------------------|----------------------|----------------------|---------------------|-------------------|
| \(\tau\)-Cadinol | 93.2                | 500               | 58.3                  | 250            | 80.3                  | 500              | 58.2                      | 250                    | 48.3                 | 125                  | 40.1                | 125               |
| \(\alpha\)-Cadinol | 38.1                | 125               | 29.2                  | 62.5           | 40.1                  | 125             | 10.8                      | 62.5                   | 40.8                 | 125                  | 30.8                | 62.5              |
| Ferruginol  | 21.5                | 62.5              | 26.8                  | 62.5           | 69.3                  | 250             | 39.8                      | 125                    | 28.6                 | 62.5                 | 39.1                | 250               |
control plate, the antifungal index was calculated as follows:

\[
\text{Antifungal index (\%) = (1 - D_s/D_b) \times 100}
\]

where \( D_s \) is the diameter of the growth zone in the experimental dish (cm) and \( D_b \) is the diameter of the growth zone in the control dish (cm).

Each test was repeated 5 times and the data were averaged. The IC\(_{50}\) values (the concentration in mg/mL that inhibited 50% of mycelium growth) were calculated by a probit analysis.

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**ORCID ID**

Chen-Lung Ho https://orcid.org/0000-0002-0354-034X

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