Chemical Compositions and Anti-Mildew Effects of *Cinnamomum micranthum* Leaf and Twig Essential Oils on Paper

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**Abstract**

In this study, we evaluated the anti-mildew effects of paper treated with essential oils of leaves, twigs, and their main constituents from *Cinnamomum micranthum*. The main ingredients with the greater anti-mildew effects on paper capability were also purified and identified. Fresh leaves and twigs of *C. micranthum* were hydrodistilled in a Clevenger-type apparatus, and the resulting oil characterized using GC-FID and GC-MS instruments. The leaf essential oil consisted principally of *n*-decanal (50.1%), (*E*)-β-ocimene (7.9%), (*E*)-nerolidol (6.5%), and (*E*)-β-caryophyllene (3.8%), and the twig oil’s main components were τ-cadinol (18.3%), (*E*)-β-ocimene (16.4%), α-cadinol (13.6%), *n*-decanal (10.6%), and β-selinene (5.8%). Comparing the mildew resistance of the oils on paper exhibited that twig oil was the best anti-mildew activity; at 200 μg/cm\(^2\), the twig oil completely inhibited the growth of *Aspergillus clavatus*, *Cladosporium cladosporioides*, *Chaetonium globosum*, *Myrothecium verrucaria*, and *Penicillium citrinum*. The twig oil was further divided into 8 fractions (TO1-T08). TO4 fraction had moderate anti-mildew effects; at the concentration of 200 μg/cm\(^2\), all fungi strains were totally inhibited, except *A. niger* and *Trichoderma viride*, which were 83.5%, and 93.2% inhibited, respectively. The main ingredients of TO4 fraction were τ-cadinol, and α-cadinol, so we isolated and used the for anti-mildew effect tests; τ-cadinol, and α-cadinol showed moderate anti-mildew activities. Since *C. micranthum* twig essential oil, τ-cadinol, and α-cadinol were exhibited a great anti-mildew effects on paper, they are worth further investigations and utilization.

**Keywords**

*Cinnamomum micranthum*, anti-mildew effects, essential oil, τ-cadinol, α-cadinol

Received: May 9th, 2022; Accepted: June 20th, 2022.

**Introduction**

The climate in Taiwan is warm and humid, which is ideal for mold growth. Moreover, many molds are harmful microorganisms. Mycotoxins produced by mycelium are harmful to human health, such as with food poisoning caused by aflatoxins, and produce pathogenic microorganisms that cause human diseases, such as tinea pedis, ringworm, and allergies. In addition to being detrimental to medical hygiene and food preservation, mold in the environment also causes serious harm to building materials, textiles, and paper products.\(^1\)–\(^3\) Nowadays, several methods of anti-mildew and sterilization, including sterilization with dry or steam heat, and treatment with infrared rays, ultraviolet rays, microwaves, radiation, etc. In addition, chemical agents are commonly used for sterilization and disinfection. However, the use of synthetic chemicals will cause problems such as carcinogenicity, acute toxicity, and teratogenicity to the human body, and they degrade slowly in the environment and cause problems such as environmental pollution, so the use is restricted.\(^4\) Therefore, the use of plant essential oils or compounds as natural antifungal agents has gradually attracted many researchers’ attention in recent years.

*Cinnamomum micranthum* (Hayata) is an evergreen broad-leaved tree mainly found in Taiwan, China, and Vietnam.\(^5\) The wood of *C. micranthum* can be used in furniture- and ship-making due to its straightness and good quality.\(^5\) Three reports have identified leaf, trunk, and root essential oil constituents,

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Results and Discussions

Hydrodistillation of *C. micranthum* leaf and twigs gave yellow essential oils with yields of 0.59 ± 0.01 and 0.38 ± 0.01 mL/100 g, based on the dry weight of leaves and twigs, respectively. All identified compounds, their percentages, and their linear retention index (LRI) values listed in order of elution from the DB-5 column (Table 1). Fifty-two compounds were identified, representing 99.6% of the leaf oil. Among the groups, non-terpenoids were predominated (57.8%), followed by oxygenated sesquiterpenes (19.5%), sesquiterpene hydrocarbons (11.7%), sesquiterpenes (1.4%), and monoterpenes (0.3%). Of the non-terpenoids, (E)-nerolidol (6.5%) was the chief component, and (E)-β-caryophyllene (3.8%) was the major compound of sesquiterpene hydrocarbons. (E)-β-oicimene (7.9%) was the major component of the monoterpenes hydrocarbons. After an exhaustive search, we have found 3 reports showed that the leaf oil of *C. micranthum* and their components. These compounds have the moderate antifungal activity on paper. Therefore, the twig oil was selected for further examine its composition.

In total, 55 compounds were identified, representing 99.6% of the twig essential oil. Among the twig oil compounds, the most dominant was oxygenated sesquiterpenes (39.0%), next in order were sesquiterpene hydrocarbons (22.6%), monoterpane hydrocarbons (20.3%), non-terpenoids (14.2%), and oxygenated monoterpenes (3.5%). Of the oxygenated sesquiterpenes, α-cadinol (18.3%) and α-cadinol (18.3%) were the dominant compounds. The main component of the sesquiterpene hydrocarbons was β-selinene (5.8%), and the monoterpane hydrocarbons (E)-β-oicimene (16.4%) was the principal compound. Of the non-terpenoids, n-decanal (10.6%) was the main component. According to the literature, there is no report about the twig oil composition of *C. micranthum*. Therefore, this study is the first to report the composition of twig oil from *C. micranthum* in the literature.

In our previous research paper, essential oils and the ingredients have been shown to have inhibitory activities against bacteria, foodborne pathogens, mildew, and wood-decay fungi. Mildew damage to paper products is very serious, it will not only destroy the appearance of paper products but also reduce the mechanical strength of paper products, making paper brittle, thinner, and more easily damaged. Therefore, it is necessary to evaluate the feasibility of anti-mildew treatment of paper when applying essential oils to food wrapping paper, kitchen paper, and wallpaper. In this experiment, according to the CNS2690 and TAPPi T487 cm-93 methods, 7 strain fungi were used, viz. *Aspergillus clavatus*, *A. niger*, *Chaetonotum globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum*, and *Trichoderma viride*. These pathogens are extremely harmful to paper products, cellulosic materials, leather, and wooden product.

Figure 1 displays the antifungal activities of leaf and twig parts of *C. micranthum* oil. At the same essential oil concentration of 200 μg/cm² on paper, the twig oil demonstrated clearly that the antifungal indices on paper are better than that of the leaf oil (Figure 1). At this concentration, twig oil suppressed totally the growth of *A. clavatus*, *Ch. globosum*, *Cl. cladosporioides*, *M. verrucaria*, and *P. citrinum*.

To evaluate the half-maximal inhibitory concentration (IC₅₀) values of the oils of leaf and twig, regression analysis was performed on the antifungal indices of the 2 oils versus fungi. The results are indicated in Table 2. The table displays that the IC₅₀ of twig oil resistant 7 mold pathogens was between 56.8 and 136.5 μg/cm²; for leaf oil was >200 μg/cm². According to the above results, the anti-mildew experiments showed that twig oil has the moderate antifungal activity on paper. Therefore, the twig oil was selected for further examine its composition.

The 2 chemotypes of *Cinnamomum umbraculiflorum* essential oils, cinnamaldehyde, and cinnamaldehyde–cinnamyl acetate oils were shown to require a concentration of 175 μg/cm² to inhibit the growth of half of *T. viride* strain. Our research shows that *C. micranthum* essential oil is equally good at inhibiting molds.

To further understand the anti-mildew fungi activities of ingredients of the oil extracted from the twig of *C. micranthum*, we performed antifungal experiments using the 8 separate fractions (TO1-TO8) of the twig essential oil obtained in the above experiments. The results are shown in Figure 2. TO4 fraction had the best anti-mildew fungi activity with the highest antifungal indices against 8 mildew fungi. Among the mildew fungi, at a concentration of 200 μg/cm², *A. clavatus*, *Ch. globosum*, *Cl. cladosporioides*, *M. verrucaria*, and *P. citrinum* were completely inhibited by this fraction.

The abovementioned results prove that TO4 exerted the best antibacterial activity. We further identified the ingredients from the TO4 fraction using GC and GC-MS. The results are shown in Table 3. Eight compounds were identified from the TO4 fraction; α-cadinol (36.6%) and α-cadinol (36.6%) were the main ones. Finally, we used the 2 isolated and purified compounds (α-cadinol and α-cadinol) to conduct antifungal tests against 7 mildew fungi. The MIC and IC₅₀ values are shown in Table 4. The results indicated that the active source compounds were α-cadinol and τ-cadinol. Previous studies support the contention that these compounds have significant activity for suppressing microbial growth.
| Compounds identified | LRILit<sup>a</sup> | LRIExp<sup>b</sup> | Leaf | Twig | Identification<sup>c</sup> |
|----------------------|------------------|------------------|------|------|--------------------------|
| n-Hexanal            | 801              | 801              | -d   | 0.2  | MS, LRI, CO-ST          |
| n-Nonane             | 900              | 900              | -    | 0.2  | MS, LRI, CO-ST          |
| α-Thujene            | 930              | 928              | 0.4  | 0.2  | MS, LRI, CO-ST          |
| α-Pinene             | 939              | 938              | -    | 0.5  | MS, LRI, CO-ST          |
| Camphene             | 954              | 954              | 0.4  | 0.7  | MS, LRI, CO-ST          |
| Sabinenene           | 975              | 975              | 0.1  | -    | MS, LRI, CO-ST          |
| n-Octanal            | 988              | 1002             | 0.5  | -    | MS, LRI, CO-ST          |
| α-Terpineene         | 1017             | 1018             | -    | 0.3  | MS, LRI, CO-ST          |
| β-Cymene             | 1024             | 1026             | -    | 0.3  | MS, LRI, CO-ST          |
| 1,8-Cineole          | 1031             | 1034             | 0.2  | 0.4  | MS, LRI, CO-ST          |
| α-Thujene            | 1055             | 1055             | 7.9  | 16.4 | MS, LRI, CO-ST          |
| Linalool             | 1096             | 1098             | 0.6  | 1.6  | MS, LRI, CO-ST          |
| n-Nonanal            | 1100             | 1101             | 0.7  | 0.9  | MS, LRI, CO-ST          |
| allo-Ocimene         | 1132             | 1132             | -    | 0.8  | MS, LRI, CO-ST          |
| trans-Pino carveol   | 1139             | 1139             | -    | 0.4  | MS, LRI                 |
| n-Nonanol            | 1169             | 1172             | 1.0  | MS, LRI |
| Terpinen-4-ol        | 1177             | 1180             | -    | 0.4  | MS, LRI, CO-ST          |
| n-Decanal            | 1201             | 1203             | 50.1 | 10.6 | MS, LRI, CO-ST          |
| Bornyl acetate       | 1288             | 1285             | 0.5  | 0.7  | MS, LRI, CO-ST          |
| 2-Undecanone         | 1294             | 1293             | 0.2  | -    | MS, LRI                 |
| n-Undecanal          | 1306             | 1306             | 0.2  | -    | MS, LRI, CO-ST          |
| Decanoic acid        | 1366             | 1366             | 0.7  | -    | MS, LRI, CO-ST          |
| α-Copaene            | 1376             | 1377             | -    | 0.7  | MS, LRI                 |
| (E)-β-Damascenone    | 1384             | 1383             | 0.1  | -    | MS, LRI                 |
| β-Elemene            | 1390             | 1389             | 0.1  | 0.5  | MS, LRI                 |
| n-Dodecanal          | 1408             | 1408             | 1.4  | -    | MS, LRI, CO-ST          |
| α-cis-Bergamotene    | 1412             | 1413             | 0.4  | -    | MS, LRI                 |
| (E)-β-Caryophyllene  | 1419             | 1421             | 3.8  | 0.5  | MS, LRI                 |
| Aromadendrene        | 1441             | 1441             | 0.3  | 0.6  | MS, LRI, CO-ST          |
| α-Humulene           | 1454             | 1453             | 0.6  | 1.5  | MS, LRI, CO-ST          |
| (E)-β-Famesene       | 1456             | 1454             | 0.2  | -    | MS, LRI                 |
| β-Acoradiene         | 1470             | 1469             | -    | 0.4  | MS, LRI                 |
| trans-Cadina-1(6),4-diene | 1477   | 1476             | -    | 1.6  | MS, LRI                 |
| γ-Muurolene          | 1479             | 1478             | 0.6  | -    | MS, LRI                 |
| α-Caracemone         | 1480             | 1480             | -    | 1.1  | MS, LRI                 |
| α-Amorphene          | 1484             | 1483             | 0.3  | -    | MS, LRI                 |
| β-Selinene           | 1490             | 1490             | 1.8  | 5.8  | MS, LRI                 |
| α-Selinene           | 1498             | 1496             | 0.8  | 2.7  | MS, LRI                 |
| β-Bisabolene         | 1505             | 1506             | 0.9  | 1.9  | MS, LRI                 |
| γ-Cadinene           | 1513             | 1513             | 0.2  | 0.6  | MS, LRI                 |
| δ-Cadinene           | 1523             | 1521             | 0.6  | 2.1  | MS, LRI                 |
| Calamenene<sup>e</sup> | 1529           | 1528             | -    | 0.9  | MS, LRI                 |
| Zonarene             | 1529             | 1529             | -    | 0.4  | MS, LRI                 |
| trans-γ-Bisabolene   | 1531             | 1531             | 0.3  | 0.3  | MS, LRI                 |
| α-Cadinene           | 1538             | 1535             | 0.8  | 0.5  | MS, LRI, CO-ST          |
| α-Calacorene         | 1545             | 1545             | -    | 0.5  | MS, LRI                 |
| (E)-Nerolidol        | 1563             | 1563             | 6.5  | 0.6  | MS, LRI, CO-ST          |
| Dodecanoic acid      | 1566             | 1568             | -    | 0.5  | MS, LRI, CO-ST          |
| Dendrolasin          | 1571             | 1571             | 0.5  | -    | MS, LRI                 |
| Caryophyllenyl alcohol | 1572           | 1572             | -    | 1.9  | MS, LRI, CO-ST          |
| Spathulenol          | 1578             | 1580             | -    | 0.2  | MS, LRI, CO-ST          |
| Caryophyllene oxide  | 1583             | 1582             | 0.7  | 1.4  | MS, LRI, CO-ST          |
| Octadecanoic acid, diethyl ester | 1585   | 1584             | 3.7  | 0.5  | MS, LRI                 |
| Globulol             | 1590             | 1588             | 0.5  | -    | MS, LRI, CO-ST          |
| Carotol              | 1594             | 1594             | 0.5  | -    | MS, LRI                 |
Conclusion

This study investigated the leaf and twigs essential oils extracted from *C. micranthum*, with the leaf oil main components of *n*-decanal, (**E**)-**β**-ocimene, (**E**)-nerolidol, and (**E**)-**β**-caryophyllene, and the twig oil’s major components were **τ**-cadinol, (**E**)-**β**-ocimene, **α**-cadinol, *n*-decanal, and **β**-selinene, by which the twig oil possesses pronounced anti-mildew effects on paper. The twig essential oil is mainly composed of oxygenated sesquiterpenes. Among these compounds, **τ**-cadinol and **α**-cadinol have been proved to have moderate anti-mildew activity on paper. In the future, studies should focus on determining the half-life of the essential oil on paper and developing formulations to extend the longevity of effective essential oil concentrations on paper to prevent mildew.

Experimental

**Plant Materials**

The plant material of leaves and twigs of *C. micranthum* were harvested from Lienhuachih Experimental Forest of the Taiwan Forestry Research Institute (TFRI) (elevation 350 m, N 23° 93’ 30”, E 120° 92’ 25”) in July 2021. The plant was identified by Dr Wu Chia-Chen from the TFRI. A voucher specimen (WCCLH-092) has been deposited at the department of wood cellulose, TFRI, Taiwan.

**Isolation of the Leaf and Twigs Essential Oil**

The fresh leaves and twigs of *C. micranthum* (10 kg) were used a Clevenger-type apparatus for 3 h. The leaf and twigs essential

| Compounds identified       | LRILit a | LRILExp b | Leaf  | Twig  | Concentration(%) | Identification c |
|----------------------------|----------|-----------|-------|-------|-----------------|------------------|
| Cubeban-11-ol              | 1595     | 1596      | 0.3   | 0.2   | MS, LRI         |
| Khusimone                  | 1604     | 1603      | 0.2   | -     | MS, LRI         |
| Humulene epoxide II        | 1608     | 1608      | 0.3   | 0.1   | MS, LRI         |
| Junenol                    | 1619     | 1619      | 0.2   | -     | MS, LRI         |
| 10-epi-**E**-Eudesmol      | 1623     | 1624      | 1.3   | 0.8   | MS, LRI         |
| 1-epi-Cubenol              | 1628     | 1629      | 1.0   | 0.3   | MS, LRI         |
| **τ**-Cadinol              | 1640     | 1638      | 0.9   | 18.3  | MS, LRI, CO-ST |
| **α**-Cadinol              | 1654     | 1653      | 1.2   | 13.6  | MS, LRI, CO-ST |
| Sein-11-en-4-**α**-ol      | 1660     | 1659      | -     | 0.5   | MS, LRI         |
| **α**-**β**-Bisabolol       | 1671     | 1670      | 0.6   | -     | MS, LRI         |
| **α**-Bisabolol            | 1685     | 1685      | 1.9   | -     | MS, LRI         |
| 2,3-dihydro-Farnesol       | 1689     | 1689      | 0.3   | 0.1   | MS, LRI         |
| Eudesm-7(11)-en-4-ol       | 1700     | 1699      | -     | 0.4   | MS, LRI         |
| (**E**)-Nerolidyl acetate  | 1717     | 1712      | 0.2   | 0.1   | MS, LRI         |
| **γ**-Costol               | 1746     | 1745      | 1.0   | 0.2   | MS, LRI         |
| dihydro-Columellarin       | 1900     | 1903      | 1.4   | 0.3   | MS, LRI         |
| *n*-Hexadecanoic acid      | 1960     | 1960      | 0.3   | 0.3   | MS, LRI, CO-ST |

**Figure 1.** Inhibitory effects of leaf and twig parts essential oils (200 µg/cm²) from *Cinnamomum micranthum* against 7 mildew fungi. *A. c.*, *Aspergillus clavatus*; *A. n.*, *A. niger*; *Ch. g.*, *Chaetonium globosum*; *Cl. c.*, *Cladosporium cladosporoides*; *M. v.*, *Myrothecium verrucaria*; *P. c.*, *Penicillium citrinum*; *T. v.*, *Trichoderma viride*.

Table 1. Continued.

* Monoterpane hydrocarbons (%) 9.2 20.3
* Oxygenated monoterpenes (%) 1.4 3.5
* Sesquiterpene hydrocarbons (%) 11.7 22.6
* Oxygenated sesquiterpenes (%) 19.5 39.0
* Non-terpenoids (%) 57.8 14.2
* Oil yield (mL/100 g) 0.59 ± 0.01 0.38 ± 0.01

Notes:

aLRILit, linear retention indices from a previous study.10
bLRILExp, computed linear retention indices against a mixture of a continuous series of *n*-alkane hydrocarbons *n*-alkanes (C8-C30) run on DB-5 capillary column.
Identification by: MS, comparison of NIST, and Wiley libraries mass spectra databases; LRI, linear retention index (LRI) is the same as the previous findings10–12; CO-ST, co-injection/comparison to LRI and MS of standards.
dNot detected.
eCorrect isomer not determined.
oils were then dried using anhydrous sodium sulfate and stored in a sealed vial at 4 °C until needed, respectively. The yields of essential oils and all experiment data were calculated and shown as the mean ± standard deviation of triplicate analyses.

**Essential Oils Analysis**

The *C. micranthum* leaves and twigs oils were analyzed by GC-FID and GC-MS. For GC-FID analysis was carried out on a Hewlett-Packard 6890 gas chromatograph with a flame ionization detector (FID), fitted with a DB-5 capillary columns (5% phenyl 95% methylpolysiloxane, length = 30 m, ID = 0.25 mm, film thickness = 0.25 μm). The oven temperature was programmed from 50 °C constant for 2 min, and then increased at 5 °C/min until reached 250 °C. The injector and detector temperatures were programmed at 270 °C and 250 °C, respectively. The carrier gas was hydrogen (H2) with a flow rate of 1 mL/min. The split ratio was 1:10, and 1 μL of samples (1/100, vol/vol, in ethyl acetate) were injected. Linear retention indices (LRI) values for all compounds were calculated with reference to the C8-C30 n-alkanes homologous series. The relative proportions of essential oil compositions were computed by internal normalization from the GC-FID peak areas without using correction for response factors. Results are summarized in Table 1 and are reported according to their eluted in order of the compounds on the DB-5 capillary column.

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

**Component Identification**

Compounds identification were based on calculated LRI comparison with either those in the literature or with the standard pure compounds and by matching their MS with those obtained in the “NIST 17” and “WILEY 11” libraries and in some components by co-injection with standard pure compounds.

**Anti-Mildew Assays**

According to the regulations of CNS2690 and TAPPI T487 cm-93 methods, *A. clavatus* (ATCC 1007), *A. niger* (ATCC 6275), *Ch. globose* (ATCC 6205), *Cl. cladosporioides* (ATCC 13276), *M. verrucaria* (ATCC 9095), *P. citrinum* (ATCC 9849),

| Parts | IC50 | MIC | IC50 | MIC | IC50 | MIC | IC50 | MIC | IC50 | MIC | IC50 | MIC |
|-------|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|
| Leaf  | >200 | >200| >200 | >200| >200 | >200| >200 | >200| >200 | >200| >200 | >200|
| Twig  | 56.8 | 100 | 136.5| >200| 63.9 | 125 | 105.8| 125 | 82.3 | 125 | 103.2| 200 |

Abbreviations: *A. c.*, *Aspergillus clavatus*; *A. n.*, *A. niger*; *Ch. g.*, *Chaetonium globose*; *Cl. c.*, *Cladosporium cladosporioides*; *M. v.*, *Myrothecium verrucaria*; *P. c.*, *Penicillium citrinum*; *T. v.*, *Trichoderma viride*.
Table 3. Constituents and Contents of TO4 Fraction From Twig Essential Oil of Cinnamomum micranthum.

| Compounds identified      | LRIExp<sup>a</sup> | LRIExp<sup>b</sup> | TO4 Concentration(%) | Identification<sup>c</sup> |
|---------------------------|--------------------|--------------------|----------------------|---------------------------|
| β-Selinene                | 1490               | 1490               | 2.1                  | MS, LRI                   |
| α-Selinene                | 1498               | 1496               | 1.2                  | MS, LRI                   |
| β-βisabolene              | 1505               | 1506               | 0.9                  | MS, LRI                   |
| δ-Cadinene               | 1523               | 1521               | 0.7                  | MS, LRI                   |
| Caryophyllenyl alcohol    | 1572               | 1572               | 0.8                  | MS, LRI, CO-ST            |
| Caryophyllene oxide       | 1583               | 1582               | 0.9                  | MS, LRI, CO-ST            |
| τ-Cadinol                | 1640               | 1638               | 56.8                 | MS, LRI, CO-ST            |
| α-Cadinol                | 1654               | 1653               | 36.6                 | MS, LRI, CO-ST            |

<sup>a</sup>LRIExp, linear retention indices from a previous study.10

<sup>b</sup>LRIExp, computed linear retention indices against mixture of a continuous series of n-alkane hydrocarbons n-alkanes (C8-C30) run on DB-5 capillary column.

<sup>c</sup>Identification by: MS, comparison of NIST, and Wiley libraries mass spectra databases; LRI, linear retention index (LRI) is the same as the previous findings<sup>10-12</sup>; CO-ST, co-injection/comparison to LRI and MS of standards.

Table 4. The MIC and IC<sub>50</sub> Values (μg/cm<sup>2</sup>) of 2 Major Compounds From Cinnamomum micranthum Against 7 Mildew Fungi.

| Compounds | A. c. | A. n. | Cl. c. | Ch. g. | M. v. | P. c. | T. v. |
|-----------|-------|-------|--------|--------|-------|-------|-------|
| τ-Cadinol | 23.8  | 37.5  | 53.1   | 75     | 31.8  | 50    | 45.8  | 75   |
| α-Cadinol | 20.2  | 37.5  | 51.8   | 75     | 13.6  | 25    | 21.9  | 50   |
| Nystatine | <12.5 | <12.5 | <12.5  | <12.5  | <12.5 | <12.5 | <12.5 | <12.5|

Abbreviations: A. c., Aspergillus clavatus; A. n., A. niger; Ch. g., Chaetonium globosum; Cl. c., Cladosporium cladosporioides; M. v., Myrothecium verrucaria; P. c., Penicillium citrinum; T. v., Trichoderma viride.

and T. viride (ATCC 8678) molds were tested. The mildew fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu, Taiwan. These fungal strains were inoculated on potato-dextrose agar (PDA) medium, respectively, and placed in an incubator at 28 °C for 10 days. Then, they were placed in sterilized boxes and the spores are scraped onto the cultural medium using a platinum wire. Next, spores were shaken in 10 mL sterile water until they were distributed evenly, and then filter to complete the preparation of a single spore suspension by: MS, comparison of NIST, and Wiley libraries mass spectra databases; LRI, linear retention index (LRI) is the same as the previous findings<sup>10-12</sup>; CO-ST, co-injection/comparison to LRI and MS of standards.

Isolation and Purification of Twig Oil Components

The crude twig essential oil of C. micranthum (20 g) mixed with silica gel 60 (60 g) (200-300 mesh, Fluka) was chromatographed on a silica gel column (600 g) by gradient elution with n-hexane and ethyl acetate (the proportion of 2 solvents changed from 100:0 to 0:100). Then, collected fractions were monitored by TLC and those with similar profile were combined to produce 8 subfractions (TO1-TO8). The yields of each fraction were 5.3% (TO1), 8.2% (TO2), 18.3% (TO3), 42.8% (TO4), 10.3% (TO5), 8.2% (TO6), 4.1% (TO7), and 2.7% (TO8). According to the anti-mildew assay, the TO4 fraction shown the best antifungal activity. The pure τ-cadinol (RT: 22.1 min) and α-cadinol (RT: 31.2 min) compounds were separately obtained from fraction TO4 by semipreparative HPLC (column: Si-60 column, mobile phase: EtOAc/n-C<sub>6</sub>H<sub>14</sub> = 30/70, flow rate: 1 mL/min). The structures of 2 compounds were confirmed by comparing <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and EI-MS spectral data with the our previously literature values.<sup>25</sup>

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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