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The Composition, Anti-mildew and Anti-wood-decay Fungal Activities of the Leaf and Fruit Oils of *Juniperus formosana* from Taiwan

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In this study, anti-mildew and anti-wood-decay fungal activities of the leaf and fruits essential oil and its constituents from *Juniperus formosana* were evaluated in vitro against seven mildew fungi and four wood decay fungi, respectively. The main compounds responsible for the anti-mildew and anti-wood-decay fungal activities were also identified. The essential oil from the fresh leaves and fruits of *J. formosana* were isolated using hydrodistillation in a Cleverning-type apparatus, and characterized by GC–FID and GC–MS, respectively. The leaf oil mainly consisted of α-adams bornyl acetate (5.2%), limonene (4.3%), and myrcene (4.1%), and α-pinene (26.1%), β-myrcene (32.4%), α-thujene (5.9%) and limonene (5.9%). Comparing the anti-mildew and anti-wood-decay fungal activities of the oils suggested that the leaf oil was the most effective. For the anti-mildew and anti-wood-decay fungal activities of the leaf oil, the active source compounds were determined to be α-cadinol and elemol.

**Keywords:** *Juniperus formosana*, Essential oil, Anti-mildew activity, Anti-wood-decay fungal activity, α-Cadinol, Elemol.

*Juniperus formosana* Hayata (Cupressaceae) is a large tree mainly distributed in Taiwan, and China [1]. However, only three references were found regarding the chemical compositions of this species from China [2-4]. In Taiwan, there is no report of the essential oil composition and bioactivities for *J. formosana*. Therefore, in this study, the essential oil from the leaves and fruits was first isolated using hydrodistillation, and then analyzed. In addition, the climate of Taiwan is warm and humid, and thus conducive to the growth of mildew and wood decay fungi. Mildew growth causes problems in the preservation of cultivated crops as well as inducing allergies, asthma, bronchitis, onychomycosis, cerebral infections, pneumonia, peritonitis, and immune-deficiency syndrome [5]. The wood decay fungi can easily cause damage to wooden products. Therefore, we also applied the essential oils to seven strains of mold fungi and four of wood decay fungi to examine their interference efficacies, respectively. The second part of the study examined the anti-mildew and anti-wood-decay fungal activities of the leaf and fruit oils. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Hydrodistillation of *J. formosana* leaves and fruits produced yellow-colored oils with yields (v/w), on a moisture free basis, of 1.51 ± 0.06 and 1.86 ± 0.05, v/w, respectively. All compounds are listed in order of their elution from the DB-5 column (Table 1). A total of 49 compounds were identified from the hydrodistilled leaf oil of *J. formosana*. Monoterpene hydrocarbons were predominant (69.2%), followed by oxygenated sesquiterpenes (20.5%), sesquiterpene hydrocarbons (5.4%), oxygenated monoterpenes (3.6%), and non-terpenoids (1.2%). Of the monoterpene hydrocarbons, α-pinene (41.0%), limonene (11.5%) and β-myrcene (5.8%) were the major compounds. α-Cadinol (11.0%) and elemol (6.3%) were the chief sesquiterpene hydrocarbons. In *J. formosana* leaf oil, Yu et al. [2] found 55 compounds, mainly α-pinene (9.6%), bornyl acetate (5.2%), limonene (4.3%), and myrcene (4.1%). Adams et al. [3] found 70 compounds, mainly α-pinene (47.7%), myrcene (7.2%), limonene (4.0%), β-pinene (2.9%), γ-cadinene (2.4%), and germacrene D (2.3%). Our results differed from the above papers with α-pinene, limonene, α-cadinol, elemol, and β-myrcene as the major compounds. This is the first presentation of these compounds in *J. formosana* leaf oil.

Twenty-five components were identified from the fruit oil. Among them, monoterpane hydrocarbons were the most dominant (93.4%), followed by sesquiterpene hydrocarbons (2.5%), oxygenated sesquiterpenes (2.2%), and oxygenated monoterpenes (1.9%). α-Pinene (40.9%), β-myrcene (32.4%), α-thujene (5.9%) and limonene (5.9%) were the major monoterpane hydrocarbons. In *J. formosana* fruit oil, Yu and Xie [4] found 47 compounds mainly myrcene (27.1%), α-pinene (26.1%), γ-terpinene (10.7%), and limonene (6.0%). Our results differed from the above paper with α-pinene, β-myrcene, α-thujene and limonene as the main compounds. This is the first presentation of these compounds for *J. formosana* fruit oil.

The leaf and fruit oils of *J. formosana* were tested against seven mildew fungi (*Aspergillus clavatus* (A. c.), *A. niger* (A. n.), *Chaetomium globosum* (Ch. g.), *Cladosporium cladosporioides* (Cl. c.), *Myrothecium verrucaria* (M. v.), *Penicillium citrinum* (P. c.), and *Trichoderma viride* (T. v.). The antifungal indexes demonstrated clearly that the leaf oil had antifungal activities superior to those of the fruit oil (Table 1). Among the fungi tested, the leaf oil was totally inhibitory of mycelial growth of *A. clavatus*, *Cl. cladosporioides*, *Ch. globosum*, and *M. verrucaria* at a 1 mg/mL concentration. The leaf oil was superior to the anti-mildew fungal activities of the essential oils from *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, *E. citriodora* [5], *Lithsea cubeba* [6], *L. coreana* [7], and *Neolitsea parvigemma* [8]. The results verified that *J. formosana* leaf oil has notable antifungal activities.

However, to ascertain the source compounds responsible for *J. formosana* antifungal activities, the main components were...
individually tested for their antifungal activities (Fig. 2). As for α-pinene, β-myrcene and limonene, very low levels of activity were found against the seven mold fungi; none of the antifungal indices exceeded 30%. However, the sesquiterpenoids, elemol and α-cadinol exhibited better activities. Elemol and α-cadinol exhibited significant activity for suppressing microbial growth [8,10].

Previous studies support the contention that these compounds have significant activity for suppressing microbial growth [8,10].

This study also tested the anti-wood-decay fungal activities of the major components of J. formosana leaf oil to ascertain its source compounds. Results indicated that the anti-wood-decay fungal activities were due to α-cadinol and elemol. At a concentration of 50 μg/mL, α-cadinol and elemol showed total growth inhibition.
Experimental

The leaf essential oil of *Juniperus formosana* was isolated from fresh leaves and fruits of the species. The essential oils of the leaves and fruits (1 kg) were hydrodistilled for 3 h using a Clevenger-type apparatus. After distillation, the volume of oils obtained was measured, and the essential oils were stored in glass containers, hermetically sealed with rubber lids, covered with aluminum foil to protect the contents from light, and kept refrigerated at < 4°C until used. 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 x 0.25 mm x 0.25 μm film thickness, J&W Scientific) and a FID detector was used for the quantitative determination of oil components. Oven temperature was programmed as follows: 50°C for 2 min, rising to 250°C at 5°C/min. Injector temperature: 270°C. Carrier gas: He 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 ethyl acetate) were injected manually in the split mode. Identification of the oil components was based on their retention indices and mass spectra, obtained from GC/MS analysis on a Hewlett-Packard HP 6890/HP973 equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness, J&W Scientific). The GC analysis parameters listed above and the MS were obtained (full scan mode: scan time: 0.3 s, mass range was m/z 23-300) in the EI mode at 70 eV. All data were the average of triplicate analyses.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [6], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [9,18].

Antifungal assays: The method of Su et al. [5] was adopted. Mold and wood decay fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. References of ASTM G21, JIS Z 2911 and AATCC test method 30 were consulted for the mold fungal strains; 7 strains {A. clavatus (ATCC 1007), A. niger (ATCC 6275), *Ch. globosum* (ATCC 6205), *Cl. cladosporioides* (ATCC 13276), *M. verrucaria* (ATCC 9095), *P. citrinum* (ATCC 9849) and *T. viride* (ATCC8678)} were tested. The wood decay fungi used were *T. versicolor* (BCRC 35253), *Phae. chrysosporium* (BCRC 36200), *Phae. schweinitzii* (BCRC 35365) and *L. sulphureus* (BCRC 35305). Antifungal assays were carried out in triplicate and data were averaged. Different concentrations of the essential oils (12.5-1000 μg/mL) were added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27°C. When the mycelium of fungi reached the edge of the control plate, the antifungal index was calculated as follows:

\[
\text{Anti-fungal index (\%) = (1- Da/Db) X 100}
\]

where Da is the diameter of the growth zone in the control dish (cm) and Db is the diameter of the growth zone in the control dish (cm).

### Table 2: Anti-wood-decay fungal indices of leaf and fruit essential oils of *J. formosana*.

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Dosage (μg/mL)</th>
<th>Anti-fungal index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Trametes versicolor</strong></td>
<td><strong>Phaeo. chrysosporium</strong></td>
</tr>
<tr>
<td>Leaf</td>
<td>12.5</td>
<td>89 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>100 ± 0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100 ± 0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>100 ± 0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>Fruit</td>
<td>12.5</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>25 ± 3.3</td>
</tr>
</tbody>
</table>

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