Comparative Evaluation of Hydrodistillation, Supercritical Fluid Extraction, and Organic Solvent Extraction on Leaf Essential Oils of *Chamaecyparis formosensis* and *C. obtusa* var. *formosana* and Their Potential as Wood-Protective Agents

Ying-Ju Chen [a], Fu-Lan Hsu [b], and Sen-Sung Cheng [c,*]

Hydrodistillation (HD), organic solvent extraction (OSE), and supercritical fluid extraction (SFE) were compared in terms of the chemical composition and antifungal activity of leaf essential oils from *Chamaecyparis formosensis* and *C. obtusa* var. *formosana*. Gas chromatography–mass spectrometry revealed notable differences among extraction methods. In *C. formosensis*, HD-derived oil was dominated by α-pinene (83.4%), SFE-derived oil by kaur-16-ene (51.1%), and OSE-derived oil by phytol (44.4%). In *C. obtusa* var. *formosana*, HD oil was rich in sabinene (36.2%) and thujopsene (22.5%), SFE oil in totarol (50.9%), and OSE oil in thujopsene (27.6%) and cedrol (24.8%). Bioassays demonstrated that OSE oil of *C. formosensis* exhibited the strongest inhibitory effects against *Trichoderma sp.*, *Trametes versicolor*, *Laetiporus sulphureus*, and *Gloeophyllum trabeum*. For *C. obtusa* var. *formosana*, HD oil was most effective against *Trichoderma sp.* and *L. sulphureus*, whereas SFE oil was most active against *G. trabeum*. These results highlight the strong influence of the extraction method on both chemical composition and antifungal efficacy of leaf essential oils.

DOI: 10.15376/biores.20.4.9332-9347

Keywords: Chamaecyparis formosensis; Chamaecyparis obtusa var. *formosana; Hydrodistillation; Organic solvent extraction; Supercritical fluid extraction; Antifungal activity*

Contact information: a: Forest Products Utilization Division, Taiwan Forestry Research Institute, Ministry of Agriculture, Taipei 100051, Taiwan; b: Taimalee Research Center, Taiwan Forestry Research Institute, Ministry of Agriculture, Taitung 963001, Taiwan; c: The Experimental Forest, College of Bio-Resources and Agriculture, National Taiwan University, Nantou 55750, Taiwan;
** Corresponding author: sscheng@ntu.edu.tw*

INTRODUCTION

Chamaecyparis formosensis Matsum. and *Chamaecyparis obtusa* var. *formosana* (Hayata) are emblematic cypresses of Taiwan's montane forests. They are prized for both their durable timber and culturally valued aroma. The chemical composition of their essential oils was first delineated in 1931 (Kafuku and Ichikawa 1931; Kafuku *et al.* 1931). Later studies showed that hydrodistilled leaf oils of *C. formosensis* are dominated by α-pinene, while reports on *C. obtusa* var. *formosana* have yielded differing results. Su *et al*. (2006) found α-pinene (76.7%) to be the predominant constituent, whereas Chen *et al*. (2011), based on samples collected across multiple regions in Taiwan, demonstrated that the leaf oils of this taxon could be classified into three distinct chemotypes—β-elemol type, (–)-thujopsene type, and *cis*-thujopsenal type—highlighting pronounced geographic

variation. Leaf essential oils of *C. obtusa* var. *formosana* have demonstrated strong antitermitic potential (Cheng *et al*. 2007). Nevertheless, compared with leaf essential oils, wood-derived oils and their constituents have been investigated more extensively, with numerous studies confirming their antimicrobial, antifungal, and antitermitic activities (Wang *et al*. 1987; Wu and Wang 1990; Wang *et al*. 2005; Chen and Chang 2017). Although previous analyses reported that leaf essential oils are rich in monoterpenes and sesquiterpenes with pesticidal properties (Su *et al*. 2006; Chen *et al*. 2011), systematic evaluations of the influence of different extraction methods—such as hydrodistillation (HD), organic solvent extraction (OSE), and supercritical fluid extraction (SFE)—on their chemical composition and bioactivity remain limited (Zhou *et al*. 2023; Karimnejad and Ghavam 2024). Moreover, valorizing leaf biomass, typically regarded as a low-value forestry byproduct, for essential oil production represents a more environmentally sustainable strategy, supporting circular bioeconomy objectives and alleviating pressure on slow-growing, economically valuable heartwood resources. As recent research has primarily focused on wood-derived oils or isolated compounds (Hsu *et al*. 2016; Lin *et al.* 2022; Chen *et al.* 2023), the biological potential and practical applications of leaf essential oils, as well as the effects of extraction techniques, have yet to be fully elucidated.

Extraction technology plays a decisive role in shaping essential oil profiles and downstream efficacy. Conventional HD is simple and inexpensive, but it may degrade heat-labile constituents and often delivers limited yields (Zhou *et al*. 2023; Wang *et al*. 2025). OSE can increase recovery of high molecular weight or less volatile compounds. Hexane is among the most commonly used solvents for OSE because its strong hydrophobicity matches the lipophilic nature of most essential oil constituents, and its relatively low boiling point (63 to 69 °C) facilitates efficient solvent removal after extraction (Ghahramanloo *et al*. 2017; Bourgou *et al*. 2021). Nevertheless, residual solvents and energy demands raise safety and environmental concerns (Ghahramanloo *et al*. 2017; Park *et al*. 2022; Wang *et al*. 2025). SFE offers tunable selectivity and solvent-free products but requires costly high-pressure equipment (Ha *et al.* 2008; Donelian *et al.* 2009; Huo *et al.* 2024). Comparative studies on other taxa (e.g., *Aquilaria spp*., *Mentha longifolia*; *Satureja bachtiarica* Bunge.) demonstrate that switching extraction methods can shift major constituents, thereby modulating antioxidant, antibacterial, and antifungal activities (Memarzadeh *et al.* 2015; Yousefi *et al.* 2019; Karimnejad *et al*. 2024; Wang *et al*. 2025). However, no systematic evaluation of HD, OSE, and SFE has been reported for Taiwanese cypress leaves, leaving a critical knowledge gap for both science and industry.

Essential oils from conifers, including Taiwanese cypresses, have been reported to exhibit antifungal activity against several wood-decaying fungi such as *Trametes versicolor*, *Laetiporus sulphureus*, and *Gloeophyllum trabeum* (Wang *et al*. 2005; Chen and Chang 2017; Chen *et al*. 2023). In particular, essential oils from *C. formosensis* and *C. obtusa* var. *formosana* have shown good inhibitory effects against a range of decay fungi in both liquid and vapor phases, underscoring their potential as natural antifungal agents. However, the extent to which extraction methods influence their antifungal performance against wood-decaying fungi has not yet been systematically investigated.

In this work it was hypothesized that extraction technique would significantly influence (i) the yield and chemical composition of *C. formosensis* and *C. obtusa* var. *formosana* leaf essential oils and (ii) their antifungal performance against wood decay fungi. Accordingly, this study employed HD, OSE, and SFE under optimized, reproducible conditions to obtain oils, which were then profiled by GC–MS and evaluated for antifungal activity. By identifying the method that maximizes bioactive constituents while

minimizing environmental burdens, the aim was to provide a sustainable pathway for converting forestry residues into natural wood protective agents, thereby adding economic value to leaf litter and advancing sustainable forest management.

EXPERIMENTAL

Materials

Plant materials

Leaves of *C. formosensis* and *C. obtusa* var. *formosana* were collected from the Experimental Forest of National Taiwan University during the winter season. Specifically, leaves of *C. formosensis* were collected from Compartment 29 of Plantation No. 79-2 in the Duigaoyue Tract (longitude: 120°51′10.19″, latitude: 23°29′12.67″) at an elevation of 2,457 m. Similarly, leaves of *C. obtusa* var. *formosana* were collected from Compartment 21 in Plantation No. 62-1A in the Neimaopu Tract (longitude: 120°48′14.35″, latitude: 23°38′43.51″) at an elevation of 1,908 m. Once on-site collection was complete, the samples were immediately transported to the laboratory at a low temperature. The sample trees were identified by Mr. Yen-Ray Hsui (Taiwan Forestry Research Institute, TFRI). Voucher specimens were deposited at the department of Forest Products Utilization, TFRI, Taiwan. After the branches were separated from the leaves, the leaf samples were subjected to essential oil extraction procedures with HD, SFE, and OSE.

Chemicals and reagents

Ethanol (99.5%, analytical grade) and anhydrous sodium sulfate (Na_2SO_4, ≥99%) were purchased from Sigma-Aldrich (USA). Positive control reagents included didecyldimethylammonium chloride (≥98%, Sigma-Aldrich) and triadimefon (a commercially available fungicide, Bayer CropScience). Potato dextrose agar (PDA) medium was obtained from Difco Laboratories (USA). All reagents were of analytical grade and used without further purification.

Essential Oil Extraction

Hydrodistillation (HD)

Fresh leaves (approximately 1 kg per species) were transported to the TFRI and subjected to hydrodistillation using a Clevenger-type apparatus. The leaves were placed in a large round-bottom flask containing 3 L of distilled water and heated for 6 to 8 h to obtain the essential oil fraction separated from the recondensed water. Residual moisture was removed by treatment with anhydrous sodium sulfate, and the oils were subsequently stored in airtight vials until further analysis. Essential oil yields were determined gravimetrically, and all reported values represent the mean of triplicate extractions.

Supercritical fluid extraction (SFE)

Essential oils were extracted using SFE with CO_2 as the supercritical solvent in a Speed SFE system equipped with a 50 mL extraction vessel (Applied Separations, Allentown, PA, USA). Briefly, approximately 7 g of leaves was loaded into the vessel, and the vessel was packed with propylene wool. After preliminary tests were conducted, the optimal extraction conditions were determined to be a temperature of 40° C, a pressure of 150 bar, and a flow rate of approximately 4 to 6 L/min. Following a 20 min static extraction phase, a 30 min dynamic extraction phase was performed, and this cycle was repeated

twice. Finally, the supercritical fluid extract was collected, and the oil yield was calculated.

Organic solvent extraction (OSE)

Essential oils were extracted using OSE following a method outlined by Ahmadian *et al.* (2018) with some modifications. Briefly, 300 g of fresh leaves was soaked in 1 L of *n*-hexane (high-performance liquid chromatography grade) and agitated at 150 rpm for 24 h. Subsequently, the extract was filtered, and *n*-hexane was removed using a rotary evaporator at 40 °C, yielding concrete. To eliminate pigment and wax, the concrete was redissolved in 60 mL of absolute ethanol and stirred for 2 h in a water bath at 50 °C. Next, the solution was cooled to −10 °C, and the waxes were completely removed by filtration. Finally, the mixture was concentrated using a rotary evaporator, and the extraction yield was calculated on the basis of the initial dry weight. All extraction procedures were performed in triplicate.

Essential Oil Composition Analysis

The chemical compositions of the essential oils were analyzed using a PerkinElmer Clarus 600 gas chromatography mass spectrometry system (PerkinElmer Instruments, Waltham, MA, USA). Briefly, a 1-μL aliquot of diluted essential oil was injected into the system. Subsequently, separation was performed using a DB-5ms column (Crossbond 5% phenyl methylpolysiloxane, 30 m × 0.25 mm × 0.25 μm), with helium used as the carrier gas at a flow rate of 1 mL/min. Next, the injection port temperature was set to 250 °C, with an ionization voltage of 70 eV, and the mass range was scanned from *m*/*z* 40 to 450 amu. The oven temperature was programmed to be isothermal at 60 °C for 5 min, raised to 150 °C at the rate of 5 °C/min, raised to 180 °C at the rate of 2 °C/min, then to 250 °C at a rate of 25 °C/min and held for 2 min. Helium was employed as the carrier gas at a 1 mL/min flow rate. The relative content of each essential oil component was calculated based on the peak area in the chromatogram (not shown here). Component identification was performed by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) 2.0 and Wiley 8 databases. Terpenoids were further identified using the arithmetic index (AI), in conjunction with the mass spectra library and reference AI (rAI) (Adams 2007). The AI was calculated using the Eq.1,

$$\text{Arithmetic index} = 100 \times [n + (RT_{(x)} - RT_{(n)})/(RT_{(n+1)} - RT_{(n)})] \quad (1)$$

where $RT_{(n)}$ and $RT_{(n+1)}$ are the retention times of *n*-alkanes and (n + 1)-alkanes, respectively, and $RT_{(x)}$ is the retention time of the unknown compound, where $RT_{(n)} < RT_{(x)} < RT_{(n+1)}$.

Antifungal Assay

The antifungal activity of essential oils was evaluated using an agar dilution method following Cheng *et al*. (2006) with minor modifications. Samples of *Trametes versicolor* (BCRC 35253), *Laetiporus sulphureus* (BCRC 35305), *Gloeophyllum trabeum* (BCRC 31614), and *Trichoderma* sp. (BCRC 35296) were obtained from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. After essential oils were extracted, they were dissolved in 150 μL of 99.5% ethanol and added to 15 mL of sterilized potato dextrose agar in 9-cm Petri dishes. Ethanol was used as a negative control, and didecyldimethylammonium chloride and triadimefon (a commercially available fungicide) were used as positive controls. A fungal mycelial plug was placed at the center of the medium and incubated at

27 ± 2 °C at 70% relative humidity. Fungal growth was monitored on a daily basis. Once the mycelia in the control group reached the edges of the Petri dishes, the antifungal index was calculated by Eq. 2,

$$\text{Antifungal index (\%)} = (1 - D_a/D_b) \times 100 \quad (2)$$

where D_a and D_b are the diameters (in centimeters) of the fungal growth zone in the oil-treated dish and control dish, respectively. All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation.

Statistical Analysis

All statistical analyses were conducted using SPSS version 17.0 (SPSS, Chicago, IL, USA). One-way analysis of variance was conducted to compare the antifungal activity of various essential oils obtained using different extraction methods. Scheffé's post hoc test was used to identify significant differences, with the confidence interval set at 95%.

RESULTS AND DISCUSSION

Essential Oil Yield Obtained from Different Extraction Methods

Table 1 presents the average essential oil yields of *C. formosensis* and *C. obtusa* var. *formosana* obtained using different extraction methods. For *C. formosensis*, OSE (7.2 ± 0.1%) was associated with the highest essential oil yield, followed by SFE (1.0 ± 0.2%) and HD (0.3 ± 0.0%). For *C. obtusa* var. *formosana*, OSE (7.5 ± 0.1%) was associated with the highest essential oil yield, followed by SFE (2.2 ± 1.2%) and HD (1.4 ± 0.4%). Among all methods, OSE was associated with the highest essential oil yield, consistent with the findings of previous studies. In a study on *Ocimum basilicum* L. essential oils, HD, OSE, and SFE were reported to yield 0.26, 2.39, and 0.43% essential oils, respectively (de Barros *et al.* 2014). Similarly, in a study on *Artemisia annua* essential oils, HD was reported to yield 0.49% essential oils, OSE was reported to yield 7.28% essential oils, and SFE was reported to yield 5.27 and 5.73% essential oils at 30 °C/150 bar and 50 °C/300 bar, respectively (Zhou *et al.* 2023).

Each extraction method has a unique effect on the yield of essential oils. This variability is primarily attributable to differences in the solubility, diffusion rates, and extraction efficiency of volatile compounds, which affect their final yield. Additionally, different extraction methods exhibit varying degrees of effectiveness depending on plant material and essential oil composition. Therefore, selecting an appropriate extraction method is crucial and should be guided by the target essential oil composition and quality requirements.

Among the traditional extraction methods, HD is typically associated with the lowest yield of essential oils, particularly because essential oils contain both nonpolar and high-molecular-weight compounds, and the high polarity of water facilitates the extraction of polar compounds, making it less effective for nonpolar compounds. HD is regarded as the most suitable method for extracting essential oils with boiling points exceeding 100 °C, which are insoluble or only slightly soluble in water (de Barros *et al.* 2014; Zhou *et al.* 2023). Prolonged heating during distillation may cause the loss of volatile aromatic compounds and may change the composition of essential oils instead of improving their yield. High temperatures may also cause the degradation of heat-sensitive compounds (Huo *et al.* 2024) or the loss of volatile compounds (Manzoor *et al.* 2019), further reducing their

yield. Despite these limitations, HD remains one of the most commonly used extraction methods because of its relatively low equipment cost, operational simplicity, and high reproducibility (Zhou *et al.* 2023). Compared with HD, OSE is associated with a higher essential oil yield and enables the extraction of a wider range of compounds (Fekri *et al.* 2021). Previous studies have shown that hexane can be efficiently recovered and reused through conventional evaporation or distillation (Burger *et al*. 2019; Bourgou *et al*. 2021). Nevertheless, the final extracts may still contain residual solvents or suffer from purity issues, which pose potential risks to human health (Fekri *et al.* 2021; Zhou *et al.* 2023).

Compared with HD and OSE, SFE has been reported to have a higher extraction rate and extraction efficiency and is more capable of mitigating the loss of active ingredients (Zhang *et al.* 2016; Zhou *et al.* 2023). Because of its relatively low critical temperature and pressure requirements, SFE can effectively extract heat-resistant compounds under mild conditions, which in turn reduces the risk of thermal degradation. In addition, SFE involves carbon dioxide as the extraction medium. Carbon dioxide is a colorless, odorless, inert, nonflammable, and inexpensive gas that can be rapidly removed after extraction, resulting in a pure and pollutant-free essential oil. Therefore, SFE is particularly suitable for the extraction of heat-sensitive compounds and can facilitate high-purity, high-efficiency extraction while addressing the problem of solvent residues. The main limitations of SFE are its high equipment cost and its precise pressure and temperature control requirements. In summary, each extraction method is suitable for different types of plant materials and target components. Therefore, when selecting an extraction method, factors such as plant material characteristics, target component stability, extraction efficiency, and operational cost should be carefully considered.

Table 1. Average Essential Oil Yields of *C. formosensis* and *C. obtusa* var. *formosana* Obtained Using Different Extraction Methods

Species	Yields (%)		
	HD	SFE	OSE
C. formosensis	0.3 ± 0.0	1.0 ± 0.2	7.2 ± 0.1
C. obtusa var. *formosana*	1.4 ± 0.4	2.2 ± 1.2	7.5 ± 0.1

Note: The results are presented as means of three replicates with standard deviations.

Chemical Composition of Leaf Essential Oils

C. formosensis

As shown in Table 2, the chemical compositions of the *C. formosensis* leaf essential oils significantly varied depending on the extraction method used. For instance, the essential oils extracted by HD were dominated by α-pinene (83.4%) followed by β-myrcene (4.4%) and β-pinene (3.0%). The essential oils extracted by SFE were dominated by kaur-16-ene (51.1%) followed by germacrene D (23.0%). The essential oils extracted by OSE were dominated by phytol (44.4%), terpinen-4-ol (11.2%), and an unidentified component (19.7%).

The leaf essential oils of *C. formosensis* were categorized into four major classes: monoterpene hydrocarbons, sesquiterpene hydrocarbons, diterpene hydrocarbons, and oxygenated diterpenes (Table 2). The predominant compound types varied depending on the extraction method used. HD primarily yielded monoterpene hydrocarbons (92.4%), SFE produced a mixture dominated by diterpene hydrocarbons (51.1%) and sesquiterpene hydrocarbons (34.2%), while OSE resulted mainly in oxygenated diterpenes (44.4%).

Table 2. Chemical Compositions of *C. formosensis* Leaf Essential Oils Obtained Using Different Extraction Methods

No.	Compounds	AI (exp.)	AI (lit.)	Relative contents (%)		
				HD	SFE	OSE
1	α-Pinene	931	932	83.4 ± 3.5	-	-
2	β-Pinene	978	974	3.0 ± 0.1	-	-
3	β-Myrcene	986	988	4.4 ± 0.2	-	-
4	Unknown	1007	-	0.1 ± 0.1	-	-
5	Limonene	1026	1024	0.4 ± 0.1	-	-
6	β-Phellandrene	1025	1025	1.0 ± 0.1	-	-
7	Terpinolene	1082	1086	0.2 ± 0.0	-	-
8	Camphor	1141	1141	-	-	8.8 ± 3.4
9	Terpinen-4-ol	1177	1174	-	-	11.2 ± 0.5
10	Unknown	1193	-	-	-	3.2 ± 0.2
11	Bornyl acetate	1283	1284	0.2 ± 0.1	-	-
12	Safrole	1286	1285	-	-	4.2 ± 0.3
13	Unknown	1337	-	-	-	5.2 ± 0.8
14	Unknown	1415	-	0.3 ± 0.1	1.4 ± 0.1	-
15	α-Humulene	1453	1452	2.4 ± 0.1	6.3 ± 3.1	-
16	γ-Muurolene	1475	1478	0.4 ± 0.2	1.8 ± 0.2	-
17	Germacrene D	1483	1485	1.8 ± 0.8	23.0 ± 2.6	-
18	γ-Cadinene	1514	1513	0.6 ± 0.4	-	-
19	δ-Cadinene	1522	1522	0.9 ± 0.2	3.1 ± 0.8	-
20	Unknown	1531	-	-	5.6 ± 2.0	-
21	Unknown	1666	-	0.3 ± 0.1	-	3.3 ± 1.1
22	Unknown	1680	-	-	7.7 ± 2.5	19.7 ± 2.7
23	Kaur-16-ene	2040	2042	0.6 ± 0.2	51.1 ± 16.8	-
24	(*E*)-Phytol	2095	2103	-	-	44.4 ± 11.5
	Monoterpene hydrocarbons (%)			92.4	0.0	0.0
	Oxygenated monoterpenes (%)			0.2	0.0	20.0
	Sesquiterpene hydrocarbons (%)			6.1	34.2	0.0
	Diterpenes hydrocarbons (%)			0.6	51.1	0.0
	Oxygenated diterpenes (%)			0.0	0.0	44.4
	Phenolics (%)			0.0	0.0	4.2
	Others (%)			0.7	14.7	31.4

RT (min): Retention time (minute); AI (exp.): Arithmetic indices on DB-5ms column; AI (lit.): Arithmetic indices from the literature; HD: Hydrodistillation; SFE: Supercritical fluid extraction; OSE: Organic solvent extraction
Note: The results are presented as means of three replicates with standard deviations.

Research into the leaf essential oils of *C. formosensis* dates back to 1931, when Kafuku and Ichikawa (1931) first analyzed the chemical composition of these essential oils and confirmed that α-pinene was the main compound. Similarly, Su *et al.* (2006) identified α-pinene (71.6%) as the primary compound in *C. formosensis* leaf essential oils, followed by δ-2-carene (4.6%), β-myrcene (4.1%), γ-muurolene (3.1%), β-pinene (2.7%), and α-caryophyllene (2.0%). Chen *et al.* (2011) reported that the leaf essential oils extracted from *C. formosensis* in different regions of Taiwan primarily consist of monoterpene hydrocarbons (87.1 to 93.7%), with α-pinene (71.6 to 86.0%) as the primary compound, followed by β-pinene (2.7 to 3.3%), β-myrcene (2.5 to 3.7%), and Δ^3-carene (0.0 to 6.7%).

Table 3. Chemical Compositions of *C. obtusa* var. *formosana* Leaf Essential Oils Obtained Using Different Extraction Methods

No.	Compound	AI (exp.)	AI (lit.)	Relative contents (%)		
				HD	SFE	OSE
1	α-Thujene	927	924	1.8 ± 0.4	-	-
2	α-Pinene	931	932	2.3 ± 0.5	-	0.3 ± 0.1
3	Sabinene	972	969	36.2 ± 3.74	0.3 ± 0.1	0.4 ± 0.2
4	β-Myrcene	986	988	5.2 ± 1.23	-	-
5	α-Terpinene	1014	1014	4.2 ± 4.5	-	-
6	Limonene	1026	1024	0.8 ±0.1	-	-
7	β-Phellandrene	1025	1025	0.4 ± 0.1	-	-
8	γ-Terpinene	1055	1054	6.6 ± 2.2	-	-
9	Terpinolene	1082	1086	2.0 ± 0.75	-	-
10	Unknown	1151	-	-	-	0.7 ± 0.2
11	Camphor	1141	1141	-	-	3.2 ± 0.2
12	Terpinen-4-ol	1177	1174	8.1 ± 3.2	0.2 ± 0.0	6.1 ± 0.6
13	α-Terpineol	1186	1186	0.4 ± 0.2	0.7 ± 0.2	0.6 ± 0.0
14	L-Bornyl acetate	1283	1284	-	-	0.2 ± 0.0
15	Anethole	1279	1282	-	-	0.8 ± 0.0
16	Safrole	1286	1285	-	-	0.4 ± 0.1
17	α-Terpinyl acetate	1316	1316	-	-	0.4 ± 0.1
18	Unknown	1417		-	-	0.3 ± 0.1
19	α-Cedrene	1411	1410	-	-	1.1 ± 0.1
20	β-Cedrene	1419	1419	-	-	0.9 ± 0.1
21	Thujopsene	1429	1429	22.5 ± 3.45	3.2 ± 1.1	27.6 ± 7.6
22	β-Chamigrene	1476	1476	0.4 ± 0.1	0.2 ± 0.0	2.1 ± 0.7
23	Unknown	1534	-	0.5 ± 0.2	-	0.8 ± 0.1
24	Elemol	1543	1548	4.6 ± 0.4	15.7 ± 6.2	17.7 ± 5.2
25	Unknown	1589	-	-	17.7 ± 5.6	3.9 ± 1.3
26	Unknown	1598	-	0.6 ± 0.2	-	-
27	Cedrol	1603	1600	1.6 ± 0.5	2.1 ± 1.7	24.8 ± 0.1
28	γ-Eudesmol	1628	1630	0.4 ± 0.1	1.2 ± 0.6	0.4 ± 0.2
29	α-Eudesmol	1650	1652	1.1 ± 0.4	-	2.9 ± 0.7
30	Beyerene	1931	1931	0.3 ± 0.0	4.6 ± 1.9	3.4 ± 0.6
31	Phytol	1942	1942	-	-	1.0 ± 0.1
32	Unknown	2182	-	-	3.2 ± 1.2	-
33	Totarol	2315	2314	-	50.9 ± 8.7	-
	Monoterpene hydrocarbons (%)			59.5	0.3	0.7
	Oxygenated monoterpenes (%)			8.5	0.9	10.5
	Sesquiterpene hydrocarbons (%)			22.9	3.4	31.7
	Oxygenated sesquiterpenes (%)			7.7	19	45.8
	Diterpenes hydrocarbons (%)			0.3	4.6	3.4
	Oxygenated diterpenes (%)			0.0	50.9	1.0
	Phenolics (%)			0.0	0.0	1.2
	Others (%)			1.1	20.9	5.7

RT (min): Retention time (minute); AI (exp.): Arithmetic indices on DB-5ms column; AI (lit.): Arithmetic indices from the literature; HD: Hydrodistillation; SFE: Supercritical fluid extraction. OSE: Organic solvent extraction
Note: The results are presented as means of three replicates with standard deviations.

The compositions of HD-extracted essential oils observed in this study were consistent with those reported previously. Few studies have examined the biological activity of *C. formosensis* leaf essential oils, and their chemical compositions obtained by SFE or OSE remain unexplored. While comparative studies on extraction methods and the

antimicrobial activity of essential oils have been reported for other taxa, to our knowledge this is the first study to compare the leaf essential oils of *C. formosensis* and *C. obtusa* var. *formosana* across different extraction methods.

C. obtusa var. formosana

As shown in Table 3, the chemical compositions of *C. obtusa* var. *formosana* leaf essential oils varied depending on the extraction method used. For instance, the essential oils extracted by HD were dominated by sabinene (36.2%) and thujopsene (22.5%); the essential oils extracted by SFE were dominated by totarol (50.9%), elemol (15.7%), and an unidentified compound (17.7%). The essential oils extracted by OSE were dominated by thujopsene (27.6%), cedrol (24.8%), and elemol (17.7%).

The leaf essential oils of *C. obtusa* var. *formosana* were divided into four categories based on their structural characteristics (Table 3). HD primarily yielded monoterpene hydrocarbons (59.6%), SFE produced mainly oxygenated diterpenes (50.9%), and OSE resulted in a high proportion of oxygenated sesquiterpenes (45.8%) and sesquiterpene hydrocarbons (31.8%).

Multiple studies have examined the chemical compositions of *C. obtusa* var. *formosana* essential oils. Su *et al.* (2006) compared the leaf essential oil compositions of five coniferous species in Taiwan and discovered that α-pinene was the main compound in *C. obtusa* var. *formosana*, *C. formosensis*, and *Calocedrus formosana*. They also reported that the leaf essential oils of *C. obtusa* var. *formosana* contained α-pinene (76.7%), β-myrcene (5.7%), β-pinene (3.2%), γ-muurolene (2.8%), δ-2-carene (2.1%), and β-phellandrene (2.1%). Cheng *et al.* (2007) extracted various essential oils from the heartwood, bark, and leaves of *C. obtusa* var. *formosana* and examined their insecticidal activity against termites (*Coptotermes formosanus*). However, they did not provide details on the chemical composition of each essential oil.

Chen *et al.* (2011) compared the chemical compositions of *C. obtusa* var. *formosana* leaf essential oils from different regions and reported that the concentration of each component varied depending on the region. Using cluster analysis, they classified these essential oils into three chemotypes depending on their chemical composition: β-elemol, thujopsene, and *cis*-thujopsene. According to this classification, the essential oil composition obtained in the present study belongs to the category of thujopsene.

According to the aforementioned results, for both *C. formosensis* and *C. obtusa* var. *formosana*, HD is primarily associated with monoterpene compounds, whereas SFE and OSE are primarily associated with high-molecular-weight oxygenated sesquiterpenes and diterpenes. Research into the extraction of essential oils from *Satureja bachtiarica* has indicated a significant effect of the extraction method used on the chemical composition of the essential oils extracted from aerial plant parts ($p < 0.01$) (Memarzadeh *et al.* 2015). This effect may be attributable to the loss or enhancement of certain compounds, potentially as a result of oxidation, glycoside hydrolysis, esterification, or other chemical processes (Ghasemi Pirbalouti *et al.* 2013; Memarzadeh *et al.* 2015).

The bioactivity of essential oils primarily depends on their chemical composition, specifically on whether they are alcohols, aldehydes, acids, phenols, esters, or terpenes (Zhou *et al.* 2023). In addition, the extraction conditions significantly influence the chemical composition of essential oils, which in turn affects their bioactivity. Many studies have indicated that the essential oils extracted by SFE have excellent antimicrobial activity. For example, Glišić *et al.* (2007) reported that the fruit essential oils of *Daucus carota* L. extracted by SFE were significantly more effective in killing *Bacillus cereus* compared

with those extracted by HD, indicating the ability of SFE to retain compounds with antimicrobial activity.

Although SFE can enhance the antimicrobial and antioxidant activity of essential oils in many cases, few studies have confirmed the superiority of SFE to HD (Glišić *et al.* 2007). Yousefi *et al.* (2019) reported that the *Aquilaria crassna* essential oils extracted by HD had lower minimum inhibitory concentrations against *Staphylococcus aureus* and *Candida albicans* compared with those extracted by SFE. This discrepancy in findings may be attributable to the different concentrations of volatile compounds in essential oils, because many compounds with antioxidant and antibacterial activity are often volatile, and HD-extracted essential oils tend to contain a high concentration of volatile compounds (Huo *et al.* 2024). In summary, the extraction method used directly affects the composition and antibacterial and antioxidant capacity of the essential oils extracted.

Antifungal Activity of *C. formosensis* and *C. obtusa* var. *formosana* Leaf Essential Oils

Poisoned food assay

Figure 1 depicts the antifungal effects of essential oils (800 µg/mL) extracted from *C. formosensis* and *C. obtusa var. formosana* leaves against *Trichoderma sp.*, *T. versicolor*, and two brown-rot fungi, namely *L. sulphureus* and *G. trabeum*. Among all essential oils, the essential oils extracted from *C. formosensis* leaves by OSE exhibited the strongest antifungal activity against *Trichoderma sp.*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*, with inhibition indices of 76.1, 81.8, 79.7, and 67.4%, respectively (Fig. 1). The essential oils extracted by HD achieved an inhibition index of 71.5% against *L. sulphureus,* but they did not achieve an inhibition index greater than 70% against the other fungal strains. As shown in Table 2, the *C. formosensis* leaf essential oils extracted by OSE were dominated by (*E*)-phytol (44.4%), which is a common antifungal compound. Multiple studies have indicated that essential oils rich in (*E*)-phytol have high antifungal activity (Rajab *et al.* 1998; Kobaisy *et al.* 2001). These findings suggest that the strong antifungal effect of OSE-extracted essential oils is attributable to their high concentration of (*E*)-phytol.

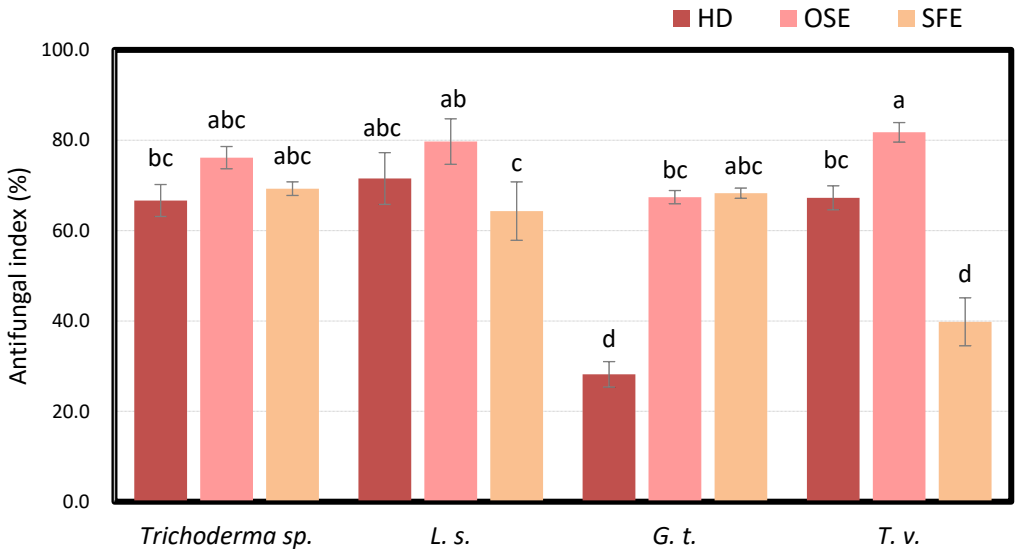

Fig. 1. Antifungal activity of *C. formosensis* leaf essential oils obtained using different extraction methods. Data are presented as mean ± SD of five replicates. Abbreviations: *L.s., Laetiporus sulphureus; G.t., Gloeophyllum trabeum; T.v., Trametes versicolor.*

Studies on the bioactivity of *C. formosensis* essential oils have primarily focused on wood-derived instead of leaf-derived essential oils. Wang *et al.* (2005) analyzed the chemical composition of *C. formosensis* heartwood essential oils and identified α-eudesmol as the main compound. They reported that α-eudesmol had a strong antifungal effect against *L. sulphureus* and *T. versicolor*, achieving complete inhibition at concentrations of 50 and 100 μg/mL, respectively. Similarly, Kuo *et al.* (2007) analyzed the chemical composition of *C. formosensis* heartwood essential oils and identified myrtenal as the main compound. They reported that myrtenal had an insecticidal effect against *Lepisma saccharina* (silverfish). Although the antifungal activity of *C. formosensis* leaf essential oils are lower than that of wood-derived oils, leaf essential oils remain a promising alternative from a sustainability perspective. Utilizing these essential oils ensures high resource efficiency while maintaining bioactivity. OSE not only produces large quantities of essential oils but also enhances their antifungal activity by enriching bioactive compounds.

In this study, *C. obtusa* var. *formosana* essential oils exhibited stronger antifungal effects than those of *C. formosensis* essential oils, regardless of the extraction method. At an essential oil concentration of 800 μg/mL**,** the OSE-extracted essential oils had inhibition indices of 65.7, 84.3, 86.5, and 68.6% against *Trichoderma sp.*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*, respectively (Fig. 2). At the same concentration, the SFE-extracted essential oils had inhibition indices of 81.4, 78.9, 88.4, and 82.1% against *Trichoderma sp.*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*, respectively, and the HD-extracted essential oils had inhibition indices of 100.0, 77.9, 100.0, and 75.2% against *Trichoderma sp.*, *T. versicolor*, *L. sulphureus*, and *G. trabeum*, respectively (Fig. 2). At an essential oil concentration of 400 μg/mL, the inhibition indices remained at 77.8, 74.8, 97.4, and 34.8%, respectively (data not shown).

These results indicated that the essential oils extracted in this study exhibited varying inhibitory effects on each fungus. At a concentration of 800 μg/mL, the HD-extracted essential oils exhibited the strongest inhibitory effect against *Trichoderma sp.* and *L. sulphureus*, achieving 100% inhibition. At the same concentration, the SFE-extracted essential oils exhibited the strongest inhibitory effect against *G. trabeum*, achieving 82.1% inhibition, and the OSE-extracted essential oils exhibited the strongest inhibitory effect against *T. versicolor*, achieving 84.3% inhibition.

The fungi tested in this study (*T. versicolor*, *L. sulphureus*, *G. trabeum*, and *Trichoderma* sp.) are all important wood-decay species; therefore, the inhibitory effects of the leaf essential oils indicate their potential as natural wood-protective agents. Similar studies have also demonstrated that leaf essential oils from other tree species, such as *Litsea coreana* (Ho *et al.* 2010), *Metasequoia glyptostroboides*, and *Melaleuca leucadendron* (Bajpai and Kang 2010; Rini *et al.* 2012), exhibit pronounced antifungal activities against both white-rot and brown-rot fungi. These findings are consistent with the present results and further highlight the potential of leaf essential oils as natural wood protectants, particularly in substituting for chemical preservatives, promoting the sustainable use of wood products, and reducing reliance on heartwood resources, thereby aligning with the principles of environmental sustainability.

In summary, the results of this work demonstrate that the method selected for extraction plays a decisive role in shaping both the chemical composition and antifungal efficacy of cypress leaf essential oils. While wood-derived oils of cypresses have long been studied for their bioactivities, the present findings provide the first systematic comparison of *C. formosensis* and *C. obtusa* var. *formosana* leaf essential oils obtained by HD, OSE,

and SFE. Beyond antifungal activity, it is worth noting that leaf powders and methanolic extracts of *C. obtusa* have also been reported to exhibit weed-suppression potential through allelochemicals such as (−)-hinokiic acid and (+)-dihydrosesamin, which were not detected in the present essential oil fractions (Kato-Noguchi *et al*. 2024). This underscores that extraction approaches targeting different chemical classes can lead to distinct biological applications, highlighting the broader value of leaf biomass as a renewable resource.

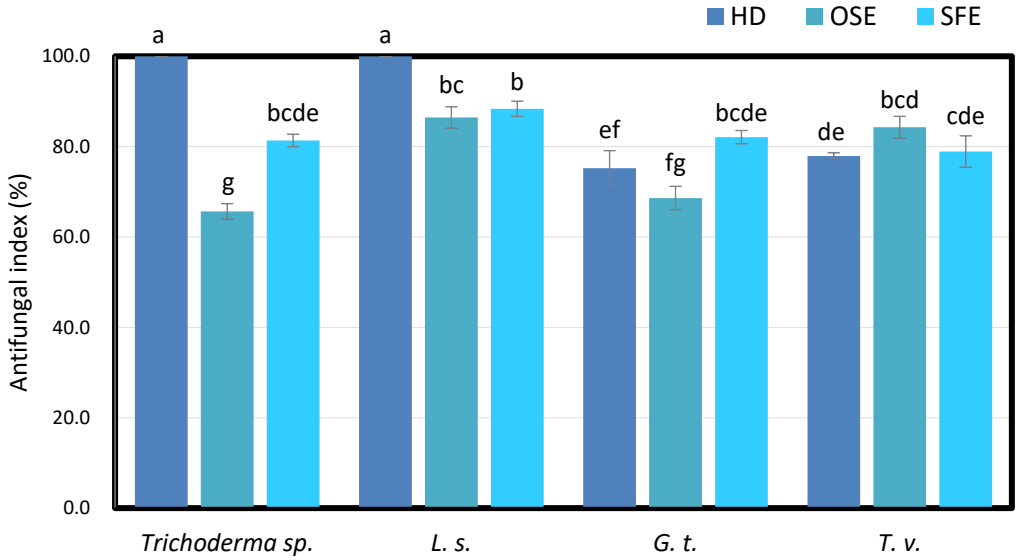

Fig. 2. Antifungal activity of *C. obtusa* var. *formosana* leaf essential oils obtained using different extraction methods. Data are presented as mean ± SD of five replicates. Abbreviations: *L.s., Laetiporus sulphureus; G.t., Gloeophyllum trabeum; T.v., Trametes versicolor.*

CONCLUSIONS

1. The essential oil yields of both *C. formosensis* and *C. obtusa* var. *formosana* varied markedly depending on the extraction method, with organic solvent extraction (OSE) consistently producing the highest yields, followed by supercritical fluid extraction (SFE) and hydrodistillation (HD). In addition to these yield differences, the chemical composition also depended strongly on extraction method: HD predominantly yielded volatile monoterpenes, whereas SFE and OSE produced higher-molecular-weight sesquiterpenes and diterpenes.
2. Essential oils obtained by OSE exhibited the strongest antifungal activity against *Trichoderma sp*., *T. versicolor*, *L. sulphureus*, and *G. trabeum*, which may be attributable to their high (*E*)-phytol content.
3. Similarly, the essential oils extracted by HD from *C. obtusa* var. *formosana* had the strongest inhibitory effect against *Trichoderma* sp. and *L. sulphureus*, which may be attributable to their high sabinene and thujopsene content.
4. The findings of this study provide valuable insights into the potential applications of *C. formosensis* and *C. obtusa* var. *formosana* leaf essential oils, which may serve as eco-friendly wood preservatives.

ACKNOWLEDGMENTS

The authors express special thanks to the Experimental Forest of National Taiwan University for providing the materials, to Mr. Yen-Ray Hsui of Taiwan Forestry Research Institute for his technical assistance.

Conflicts of Interest

The authors have no conflicts of interest to declare.

REFERENCES CITED

Adams, R. P. (2007). "Identification of essential oil components by gas chromatography - mass spectrometry," 4th Ed., Allured Publ. Corporation, Carol Stream, IL, USA.

Ahmadian, M., Ahmadi, N., Babaei, A., Naghavi, M. R., and Ayyari, M. (2018). "Comparison of volatile compounds at various developmental stages of tuberose (*Polianthes tuberosa* L. cv. Mahallati) flower with different extraction methods," *Journal of Essential Oil Research* 30(3), 197-206. DOI: 10.1080/10412905.2018.1424651.

Bajpai, V. K., and Kang, S. C. (2010). "Antifungal activity of leaf essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu," *Journal of the American Oil Chemists' Society* 87(3), 327-336. DOI: 10.1007/s11746-009-1500-6.

Bourgou, S., Bettaieb Rebey, I., Ben Kaab, S., Hammami, M., Dakhlaoui, S., Sawsen, S., Msaada, K., Isoda, H., Ksouri, R., and Fauconnier, M.-L. (2021). "Green solvent to substitute hexane for bioactive lipids extraction from black cumin and basil seeds," *Foods* 10(7), article 1493. DOI: 10.3390/foods10071493.

Burger, P., Plainfossé, H., Brochet, X., Chemat, F., and Fernandez, X. (2019). "Extraction of natural fragrance ingredients: History overview and future trends," *Chemistry & Biodiversity* 16(10), article e1900424. DOI: 10.1002/cbdv.201900424.

Chen, Y. J., and Chang, S. T. (2017). "Distribution and characteristic comparisons of the endemic cypresses in Taiwan," *Taiwan Journal of Forest Science* 32(1), 71-86.

Chen, Y. J., Lin, C. Y., Cheng, S. S., and Chang, S. T. (2011). "Phylogenetic relationships of the genus *Chamaecyparis* inferred from leaf essential oil," *Chemistry & Biodiversity* 8(6), 1083-1097. DOI: 10.1002/cbdv.201000348.

Chen, Y. J., Yeh, R. Y., and Cheng, S. S. (2023). "Antifungal potential and chemical composition of *Chamaecyparis formosensis* and *Chamaecyparis obtusa* var. *formosana* essential oils in liquid and vapor phases against 4 fungal species," *Natural Product Communications* 18(10), 1-7. DOI: 10.1177/1934578x231208816.

Cheng, S. S., Chang, H. T., Wu, C. L., and Chang, S. T. (2007). "Anti-termitic activities of essential oils from coniferous trees against *Coptotermes formosanus*," *Bioresource Technology* 98(2), 456-459. DOI: 10.1016/j.biortech.2006.01.006.

Cheng, S. S., Liu, J. Y., Hsui, Y. R., and Chang, S. T. (2006). "Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*)," *Bioresource Technology* 97(2), 306-312. DOI: 10.1016/j.biortech.2005.02.030.

de Barros, N. A., de Assis, A. V., and Mendes, M. F. (2014). "Basil oil extraction using supercritical fluid: experimental and mathematical analysis," *Ciencia Rural* 44(8), 1499-1505. DOI: 10.1590/0103-8478cr20130917.

Donelian, A., Carlson, L. H. C., Lopes, T. J., and Machado, R. A. F. (2009). "Comparison of extraction of patchouli (*Pogostemon cablin*) essential oil with supercritical CO_2 and by steam distillation," *Journal of Supercritical Fluids* 48(1), 15-20. DOI: 10.1016/j.supflu.2008.09.020.

Fekri, N., El Amir, D., Owis, A., and AbouZid, S. (2021). "Studies on essential oil from rose-scented geranium, *Pelargonium graveolens* L'Hérit. (Geraniaceae)," *Natural Product Research* 35(15), 2593-2597. DOI: 10.1080/14786419.2019.1682581.

Ghahramanloo, K. H., Kamalidehghan, B., Javar, H. A., Widodo, R. T., Majidzadeh, K., and Noordin, M. I. (2017). "Comparative analysis of essential oil composition of Iranian and Indian *Nigella sativa* L. extracted using supercritical fluid extraction and solvent extraction," *Drug Design Development and Therapy* 11, 2221-2226. DOI: 10.2147/dddt.S87251.

Ghasemi Pirbalouti, A., Hossayni, I., and Shirmardi, H.-A. (2013). "Essential oil variation, antioxidant and antibacterial activity of mountain fennel (*Zaravschanica membranacea* (Boiss.) M. Pimen.)," *Industrial Crops and Products* 50, 443-448. DOI: 10.1016/j.indcrop.2013.07.053.

Glišić, S. B., Mišić, D. R., Stamenić, M. D., Zizovic, I. T., Ašanin, R. M., and Skala, D. U. (2007). "Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity," *Food Chemistry* 105(1), 346-352. DOI: 10.1016/j.foodchem.2006.11.062.

Ha, H. K. P., Maridable, J., Gaspillo, P., Hasika, M., Malaluan, R., and Kawasaki, J. (2008). "Essential oil from lemongrass extracted by supercritical carbon dioxide and steam distillation," *Philippine Agricultural Scientist* 91(1), 36-41.

Ho, C. L., Hsu, K. P., Tseng, Y. H., Liao, P. C., Wang, E. I. C., Jeyashoke, N., Chien, T. C., Dong, W. C., and Su, Y. C. (2010). "Composition and antifungal activities of the leaf essential oil of *Litsea coreana* from Taiwan," *Natural Product Communications* 5(10), 1677-1680.

Hsu, C. Y., Lin, C. Y., and Chang, S. T. (2016). "Antitermitic activities of wood essential oil and its constituents from *Chamaecyparis formosensis*," *Wood Science and Technology* 50(4), 663-676. DOI: 10.1007/s00226-016-0811-7.

Huo, Y. J., Deng, W. J., Sun, X. Y., Zhou, L. L., Zhang, Q. H., and Hu, J. (2024). "Extract toolkit for essential oils: State of the art, trends, and challenges," *Food Chemistry* 461, article 140854. DOI: 10.1016/j.foodchem.2024.140854.

Kafuku, K., and Ichikawa, N. (1931). "Studies on the volatile constituents of the leaf of *Chamaecyparis formosensis*, Matsum," *Bulletin of the Chemical Society of Japan* 6(4), 94-102. DOI: 10.1246/bcsj.6.94.

Kafuku, K., Nozoe, T., and Hata, C. (1931). "Studies on the constituents of the volatile oil from the leaf of *Chamaecyparis obtusa*, Sieb. et Zucc., F. formosana, Hayata, or Arisan-Hinoki. I," *Bulletin of the Chemical Society of Japan* 6(2), 40-53. DOI: 10.1246/bcsj.6.40.

Karimnejad, M., and Ghavam, M. (2024). "Comparison of quantity, quality and antibacterial activity of essential oil *Mentha longifolia* (L.) L. under different traditional and modern extraction methods," *Plos One* 19(7), article e0301558. DOI: 10.1371/journal.pone.0301558.

Kato-Noguchi, H., Mori, K., Iwasaki, A., and Suenaga, K. (2024). "Allelopathy and allelochemicals in *Chamaecyparis obtusa* leaves for the development of sustainable agriculture," in: *Agronomy*, Vol. 14.

Kobaisy, M., Tellez, M. R., Webber, C. L., Dayan, F. E., Schrader, K. K., and Wedge, D. E. (2001). "Phytotoxic and fungitoxic activities of the essential oil of kenaf (*Hibiscus cannabinus* L.) leaves and its composition," *Journal of Agricultural and Food Chemistry* 49(8), 3768-3771. DOI: 10.1021/jf0101455.

Kuo, P. M., Chu, F. H., Chang, S. T., Hsiao, W. F., and Wang, S. Y. (2007). "Insecticidal activity of essential oil from *Chamaecyparis formosensis* Matsum," *Holzforschung* 61(5), 595-599. DOI: 10.1515/Hf2007.087.

Lin, C. Y., Cheng, S. S., and Chang, S. T. (2022). "Chemotaxonomic identification of *Chamaecyparis formosensis* Matsumura and *Chamaecyparis obtusa* var. *formosana* (Hayata) Rehder using characteristic compounds of wood essential oils," *Biochemical Systematics and Ecology* 105, article 104525. DOI: 10.1016/j.bse.2022.104525.

Manzoor, M. F., Ahmad, N., Ahmed, Z., Siddique, R., Zeng, X. A., Rahaman, A., Aadil, R. M., and Wahab, A. (2019). "Novel extraction techniques and pharmaceutical activities of luteolin and its derivatives," *Journal of Food Biochemistry* 43(9). DOI: 10.1111/jfbc.12974.

Memarzadeh, S. M., Ghasemi Pirbalouti, A., and AdibNejad, M. (2015). "Chemical composition and yield of essential oils from Bakhtiari savory (*Satureja bachtiarica* Bunge.) under different extraction methods," *Industrial Crops and Products* 76, 809-816. DOI: 10.1016/j.indcrop.2015.07.068.

Park, C., Garland, S. M., and Close, D. C. (2022). "Yield and profile of essential oil of *Kunzea ambigua*: Prediction using solvent extraction and effects of distillation time," *Journal of Essential Oil Bearing Plants* 25(2), 262-272. DOI: 10.1080/0972060x.2022.2058329.

Rajab, M. S., Cantrell, C. L., Franzblau, S. G., and Fischer, N. H. (1998). "Antimycobacterial activity of (*E*)-phytol and derivatives: A preliminary structure-activity study," *Planta Medica* 64(1), 2-4. DOI: 10.1055/s-2006-957354.

Rini, P., Ohtani, Y., and Ichiura, H. (2012). "Antioxidant, anti-hyaluronidase and antifungal activities of Melal*euca leucadendron Linn*. leaf oils," *Journal of Wood Science* 58(5), 429-436. DOI: 10.1007/s10086-012-1270-x.

Su, Y. C., Ho, C. L., and Wang, E. I. C. (2006). "Analysis of leaf essential oils from the indigenous five conifers of Taiwan," *Flavour and Fragrance Journal* 21(3), 447-452. DOI: 10.1002/ffj.1685.

Wang, S.-F., Wang, C.-L., Hsieh, T.-C., and Chu, C.-C. (1987). "The antitermite characters of major native woods in Taiwan and the termite-controlling properties of chemicals," *Bulletin of the Taiwan Forest Research Institute* 2(2), 117-127.

Wang, S. Y., Wu, C. L., Chu, F. H., Chien, S. C., Kuo, Y. H., Shyur, L. F., and Chang, S. T. (2005). "Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. wood," *Holzforschung* 59(3), 295-299. DOI: 10.1515/hf.2005.049.

Wang, X., Chan, S. W., Singaram, N., Teoh, M.-L., Mah, S. H., and Looi, C. Y. (2025). "Essential oil from *Aquilaria* spp. (agarwood): a comprehensive review on the impact of extraction methods on yield, chemical composition, and biological activities," *Journal of Essential Oil Research* 37(1), 110-144. DOI: 10.1080/10412905.2024.2447706.

Wu, K. T., and Wang, C. C. (1990). "The antimicrobial activity of the essential oils from heartwood of Taiwan yellow cypress and Taiwan red cypress," *Bulletin of Experimental Forest and Department of Forestry of National Chung Hsing University* 12(1), 187-192.

Yousefi, M., Rahimi-Nasrabadi, M., Pourmortazavi, S. M., Wysokowski, M., Jesionowski, T., Ehrlich, H., and Mirsadeghi, S. (2019). "Supercritical fluid extraction of essential oils," *Trac-Trends in Analytical Chemistry* 118, 182-193. DOI: 10.1016/j.trac.2019.05.038.

Zhang, Z. L., Yang, T., Mi, N., Wang, Y., Li, G. Y., Wang, L. H., and Xie, Y. J. (2016). "Antifungal activity of monoterpenes against wood white-rot fungi," *International Biodeterioration & Biodegradation* 106, 157-160. DOI: 10.1016/j.ibiod.2015.10.018.

Zhou, W. W., Li, J., Wang, X. F., Liu, L., Li, Y., Song, R., Zhang, M. X., and Li, X. M. (2023). "Research progress on extraction, separation, and purification methods of plant essential oils," *Separations* 10(12), article 596. DOI: 10.3390/separations10120596.

Article submitted: July 24, 2025; Peer review completed: August 16, 2025; Revised version received and accepted: August 28, 2025; Published: September 4, 2025.
DOI: 10.15376/biores.20.4.9332-9347