Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*)

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Abstract

The essential oils isolated from nine geographical provenances of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) leaves were examined by GC–MS and their chemical constituents were compared. According to GC–MS and cluster analyses the leaf essential oils of the nine provenances and their relative contents were classified into six chemotypes—cinnamaldehyde type, cinnamaldehyde/cinnamyl acetate type, cinnamyl acetate type, linalool type, camphor type and mixed type. In addition, the antifungal activities of leaf essential oils and their constituents from six chemotypes of indigenous cinnamon were investigated in this study. Results from the antifungal tests demonstrated that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type had an excellent inhibitory effect against white-rot fungi, *Trametes versicolor* and *Lenzites betulina* and brown-rot fungus *Laetiporus sulphureus*. The antifungal indices of leaf essential oils from these two chemotypes at the level of 200 µg/ml against *T. versicolor*, *L. betulina* and *L. sulphureus* were all 100%. Among them, the IC₅₀ (50% of inhibitory concentrations) value of the essential oil of cinnamaldehyde type leaf against *L. sulphureus* was 52–59 µg/ml. Cinnamaldehyde possessed the strongest antifungal activities in comparison with other constituents of the essential oils from cinnamaldehyde type leaf, at the level of 100 µg/ml its antifungal indices against *T. versicolor*, *L. betulina* and *L. sulphureus* were 100%. The IC₅₀ values of cinnamaldehyde against *T. versicolor*, *L. betulina* and *L. sulphureus* were 73, 74 and 73 µg/ml, respectively.

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1. Introduction

Lauraceae is an economically important family consisting mostly of trees. The genus *Cinnamomum* comprises about 250 species that are distributed in Asia and Australia (Jayaprakasha et al., 2003). Indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh.) (*Cinnamomum*) is an endemic tree that grows in Taiwan's natural hardwood forests at elevations between 400 and

1500 m (Liu et al., 1998). Hu et al. (1985) analyzed composition of essential oils of *C. osmophloeum* leaves collected randomly from 21 provenances in central, southern, and eastern parts of Taiwan and found that cinnamaldehyde was the major constituent of some *C. osmophloeum* provenances and that eugenol was present in other *C. osmophloeum* provenances. Based on differences in chemical composition of leaf essential oil, *C. osmophloeum* was classified into nine types: cassia type, cinnamaldehyde type, coumarin type, linalool type, eugenol type, camphor type, 4-terpinenol type, linalool/terpinenol type, and mixed type (Hu et al., 1985).

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C. osmophloeum has been of interest to researchers because the chemical constituents of its leaf essential oil are similar to those of *Cinnamomum cassia* bark oil (Chang et al., 2001; Hu et al., 1985). Cinnamon oil is commonly used in the food industry because of its special aroma. In addition, its antimicrobial and antifungal properties have also drawn great attention from many researchers (Chang et al., 2001; Hili et al., 1997; Kim et al., 2004; Ouattara et al., 1997; Park et al., 2000; Singh et al., 1995). Our previous studies have demonstrated that leaf essential oils from cinnamaldehyde type of *C. osmophloeum* had excellent antitermite, antibacterial, antimitic, antimildew, antimosquito and antipathogenic activities (Chang et al., 2001; Chang and Cheng, 2002; Chen et al., 2002; Chen and Chang, 2002; Cheng et al., 2004a; Lee et al., 2005). However, to the best of our knowledge there is no literature concerning the antifungal activities of leaf essential oils and their constituents from different provenances of *C. osmophloeum* against wood decay fungi. In light of the possible existence of chemical polymorphism of leaf essential oils from different provenances of *C. osmophloeum*, it is interesting to study differences in the bioactivity of varieties of indigenous cinnamon leaf oils. In this study leaf essential oils from nine *C. osmophloeum* provenances (Table 1, CO1–CO9) were analyzed by GC–MS for their chemical composition and the antifungal activities of these leaf essential oils were also investigated.

2. Methods

2.1. Plant material

Leaves of nine *C. osmophloeum* provenances (CO1–CO9) were collected in September 2002 from the Lien Hua-Chin Research Center and the Da-Pin-Ting of Taiwan Sugar Farm located in Nantou County in central Taiwan. The species were identified by Mr. Yen-Ray Hsui of the Taiwan Forestry Research Institute, and voucher specimens were deposited at the laboratory of wood chemistry (School of Forestry and Resource Conservation, National Taiwan University).

2.2. Distillation of essential oils

Fresh *C. osmophloeum* leaves were cleaned with distilled water and air-dried to MC 40% at room temperature (27 °C), the air-dried samples (150 g each), in triplicate, were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h (Chang et al., 2001), followed by determination of oil contents. Leaf essential oils were stored in airtight containers prior to analysis by gas chromatography–mass spectrometry (GC–MS).

2.3. GC–MS analysis

Leaf essential oils were analyzed by a Trace GC–PoLaris Q mass (ion source 200 °C, 70 eV) instrument, equipped with an RTx-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The oven temperature was held at 80 °C for 1 min, then programmed to increase from 80 to 200 °C at a rate of 4 °C/min and held for 5 min. Injector temperature, 250 °C; split ratio, 1:10; carrier gas, helium at a flow rate of 1 ml/min; injection volume, 1 µl. Identification of the major components of *C. osmophloeum* leaf oils was confirmed by comparison with standards, by spiking, and on the basis of their mass spectral fragmentation using the Wiley/NBS Registry of Mass Spectral library and NIST MS Search. The quantity of compounds was obtained by integrating the peak area of the spectrograms.

2.4. Antifungal assays

Two white-rot fungi, *Lenzites betulina* (CCRC 35296) and *Trametes versicolor* (CCRC 35253) and one brown-rot fungus, *Laetiporus sulphureus* (CCRC 35305), were used in this experiment. The method of Chang et al. (1999) was employed for antifungal evaluation of both the essential oil and its main constituents. Briefly, 500, 200, 100 and 50 µg/ml of essential oils were added to sterilized potato dextrose agar (PDA) in 9 cm Petri dish. After transferring the mycelium of three fungi strains, the testing dishes were incubated at 26 ± 2 °C, 70% relative humidity. When the mycelium of fungi reached the edges of control dishes (without adding essential oils or constituents) for ≈10–14 d, the antifungal indices were calculated. Each test was repeated three times, and the data averaged. The IC₅₀ values (the concentration in µg/ml that inhibited 50% of the mycelium of fungi growth) were calculated by probit analysis. Didecyl dimethyl ammonium chloride (DDAC), a commercially available fungicide, was used as a reference compound. The formula of antifungal indices was shown as follows:

$$\text{Antifungal index (\%)} = \left(1 - \frac{Da}{Db}\right) \times 100,$$

Da, the diameter of growth zone in the experimental dish (cm); Db, the diameter of growth zone in the control dish (cm).

2.5. Cluster analysis

The cluster analysis was performed with multi-variate statistical package (MVSP) software to identify relatively homogeneous groups of nine provenances (CO1–CO9) of *C. osmophloeum* based on percent composition of their essential oil samples. Euclidean distance was selected as a measure of similarity, and the unweighted

Table 1
Chemical compositions of leaf essential oils from nine provenances of *C. osmophloeum*

Compounds	RT ^a	CO1	CO2	CO3	CO4	CO5	CO6	CO7	CO8	CO9
<i>Monoterpene hydrocarbons (%)</i>										
Camphene	4.33	0.0 ^b	0.0	9.0	0.0	12.3	0.0	0.0	0.0	0.0
β-Pinene	4.65	—	—	9.0	—	0.6	—	—	—	—
<i>p</i> -Cymene	5.38	—	—	—	—	2.2	—	—	—	—
Limonene	5.45	—	—	—	—	8.7	—	—	—	—
α-Terpinene	6.74	—	—	—	—	0.8	—	—	—	—
<i>Oxygenated monoterpenes (%)</i>										
Linalool oxide	6.77	4.7	1.6	1.4	12.0	73.4	87.5	0.7	1.0	5.4
Linalool	6.93	—	—	—	0.5	—	0.5	—	—	—
Camphor	8.29	—	—	0.8	—	43.8	—	—	—	—
Borneol	8.80	—	—	—	0.3	0.9	—	—	—	—
Terpinene-4-ol	9.11	—	—	—	—	4.3	—	—	—	—
α-Terpineol	9.42	—	—	0.2	0.8	2.0	0.4	—	—	—
Geraniol	11.13	—	—	—	0.6	1.7	—	—	—	—
Citral	11.78	—	—	—	—	—	—	—	—	—
Bornyl acetate	12.15	4.7	1.6	0.4	9.8	20.6	—	0.7	1.0	1.0
Geranyl acetate	14.44	—	—	—	—	—	—	—	—	4.4
<i>Sesquiterpene hydrocarbons (%)</i>										
Cyclosativene	14.57	7.9	2.6	1.9	19.7	4.6	2.5	1.1	1.7	7.2
α-Cubebene	14.84	—	—	—	0.4	—	—	—	—	—
α-Fenchene	14.94	0.6	—	—	3.0	—	—	—	—	—
Humulene	15.30	—	—	—	—	1.5	—	—	—	—
β-Caryophyllene	16.19	—	—	—	0.2	—	—	—	—	—
α-Caryophyllene	17.23	4.7	1.1	1.0	—	1.6	1.3	0.8	1.3	—
Aromadendrene	17.42	0.8	—	—	—	—	—	—	—	—
T-Murolene	17.82	—	—	—	3.0	—	—	—	—	—
Valencene	17.82	—	—	—	1.8	—	—	—	—	—
α-Murolene	17.95	—	—	—	0.5	—	—	—	—	—
δ-Cadinene	18.51	—	—	—	1.2	—	—	—	—	0.4
γ-Murolene	18.65	—	—	—	—	—	0.7	0.3	0.4	2.1
β-Cadinene	18.94	—	—	—	1.8	—	—	—	—	—
γ-Elemene	19.19	0.6	0.6	0.7	1.4	0.6	—	—	—	3.0
Isoledene	20.23	0.7	0.5	0.2	0.5	0.9	—	—	—	0.5
α-Guaiene	20.80	0.5	—	—	2.2	—	0.5	—	—	1.2
Copaene	21.54	—	—	—	0.8	—	—	—	—	—
Azunol	22.25	—	0.4	—	—	—	—	—	—	—
Oxygenated sesquiterpenes (%)	23.48	—	—	—	2.9	—	—	—	—	—
Caryophyllene oxide	—	5.2	2.2	1.1	48.9	3.9	0.7	0.4	0.8	7.1
Guaiol	20.98	3.6	0.9	0.2	8.0	0.8	—	0.4	0.8	3.7
Unknown	21.32	0.7	—	—	0.8	0.5	—	—	—	—
T-Cadinol	22.20	—	—	—	10.2	—	—	—	—	—
α-Cadinol	22.55	0.9	0.7	0.4	17.8	1.6	0.7	—	—	3.4
β-Cadinol	22.94	—	0.6	0.5	11.5	1.0	—	—	—	—
Diterpene hydrocarbons (%)	26.67	—	—	—	0.6	—	—	—	—	—
Rimuen	—	1.4	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Labda-8(20),12,14,-triene	30.03	1.4	1.8	—	—	—	—	—	—	—
Kaur-16-ene	30.39	—	0.4	—	—	—	—	—	—	—
Oxygenated diterpenes (%)	32.82	—	0.4	—	—	—	—	—	—	—
Verticiol	—	1.1	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other (%)	31.79	1.1	1.6	—	—	—	—	—	—	—
Benzaldehyde	—	79.2	89.3	86.4	6.0	5.8	8.6	97.8	96.6	72.9
Salicylaldehyde	4.12	1.7	2.2	0.8	—	—	—	3.8	3.4	—
Benzyl alcohol	5.83	—	—	—	—	1.8	0.6	—	—	0.4
2-Methylbenzofuran	8.32	—	0.4	—	—	—	—	—	—	—
Benzenepropanal	8.65	—	—	—	—	—	—	—	—	14.4
<i>p</i> -Allylanisole	8.71	4.5	3.6	1.1	—	—	—	6.3	5.4	—
Anethole	9.60	—	—	—	3.8	—	—	—	—	0.8
<i>cis</i> -Cinnamaldehyde	9.64	1.8	1.1	0.6	—	—	0.9	1.6	1.6	—
4-Allylphenol	10.33	1.1	1.0	0.5	—	—	—	1.0	1.0	—
<i>trans</i> -Cinnamaldehyde	11.29	—	—	0.3	—	—	—	0.4	0.6	—
	11.66	60.3	50.7	34.1	0.2	1.0	3.1	81.0	76.7	2.9

Table 1 (continued)

Compounds	RT ^a	CO1	CO2	CO3	CO4	CO5	CO6	CO7	CO8	CO9
Cinnamyl alcohol	12.77	–	–	0.2	–	–	–	–	–	–
Eugenol	14.29	2.4	1.6	4.5	–	2.3	–	2.8	2.5	–
Coumarin	16.79	–	–	–	2.0	–	4.1	–	–	–
Cinnamyl acetate	16.83	7.4	28.7	44.5	–	0.7	–	0.9	5.4	54.4
Identified components (%)		99.5	99.0	100.0	86.6	99.9	99.8	100.0	100.0	92.6
Oil yield (% v/dry wt)		1.0	1.2	2.4	0.1	0.8	2.9	1.1	1.0	1.6

^a Retention time (min).^b Not detected.

pair-group method with arithmetic average (UPGMA) was used for cluster definition (Pereira et al., 2003).

2.6. Statistical analysis

The Scheffe multiple comparison procedure of SAS statistical program was used to analyze the difference of antifungal activities among nine *C. osmophloeum* leaf essential oils and their constituents ($p < 0.05$). All results were obtained from three independent experiments and expressed as mean $\pm$ SD.

3. Results and discussion

3.1. Yields and chemical polymorphism of essential oils from different provenances

The essential oil contents of nine provenances of *C. osmophloeum* leaves were obtained by hydrodistillation from a minimum of 0.1% dry weight to a maximum of 2.9% dry weight (Table 1). The highest oil yield was found in CO6 (2.9%), followed by CO3 (2.4%), CO9 (1.6%), CO2 (1.2%), CO7 (1.1%), CO8 (1.0%), CO1 (0.9%), CO5 (0.8%) and CO4 (0.1%).

Compositions of the nine essential oils are also reported in Table 1. A total of 56 compounds were identified in the nine leaf essential oils, representing 86.6–100.0% of the essential oils, respectively. The leaf essential oils of CO1, CO7 and CO8 were found to contain mainly cinnamaldehyde (60.3%, 81.0% and 76.7%, respectively). The main constituents of leaf essential oils from CO2 and CO3 were cinnamaldehyde (50.7% and 34.1%) and cinnamyl acetate (28.7% and 44.5%). The leaf essential oil of CO9 contains cinnamyl acetate as the major components (54.4%) followed by 2-methylbenzofuran (14.4%). The leaf essential oil of CO4 was found to be rich in T-cadinol (17.8%) and α -cadinol (11.5%). The major components of leaf essential oil of CO5 were camphor (43.8%) and bornyl acetate (20.6%). For leaf essential oil of CO6, its major constituent was linalool (86.6%).

The major constituents identified in this study have also been reported in some previous studies related to

the chemical analyses of *Cinnamomum* species. For instance, major compounds present in stem-bark oil and root-bark oil from cinnamon were 75% cinnamaldehyde and 56% camphor, respectively (Senanayake et al., 1978). Kaul et al. (2003) found that the major compound isolated from different parts of cinnamon oil was (*E*)-cinnamyl acetate (22–65%). In another investigation, Jayaprakasha et al. (1997, 2000, 2003) also reported the 34 compounds from cinnamon fruits and 26 compounds from cinnamon flowers with (*E*)-cinnamyl acetate (42–54%) as their major compounds.

The differences in the percent composition of the essential oils among nine provenances of *C. osmophloeum* were determined by using cluster analysis (Fig. 1). Following the results obtained herein and the previous classification by Hu et al. (1985), we have classified the leaf essential oils into six main groups, cinnamaldehyde type (CO1, CO7 and CO8), cinnamaldehyde/cinnamyl acetate type (CO2 and CO3), cinnamyl acetate type (CO9), camphor type (CO5) and linalool type (CO6), while the essential oil from CO4 was classified as a mixed type because of lack of a dominant compound.

3.2. Antifungal activity of essential oils from different provenances

Fig. 2 shows the antifungal activities of the leaf essential oils from six chemotypes of *C. osmophloeum* against

Fig. 1. Dendrogram obtained by cluster analysis of the percent composition of essential oils from nine provenances of *C. osmophloeum*.

Fig. 2. Antifungal activities of leaf essential oils (200 µg/ml) from six chemotypes of *C. osmophloeum* against white-rot fungi *T. versicolor* and *L. betulina* and brown-rot fungus *L. sulphureus*. CO1, CO7, CO8 = cinnamaldehyde type; CO2, CO3 = cinnamaldehyde/cinnamyl acetate type; CO9 = cinnamyl acetate type; CO4 = mixed type; CO6 = linalool type; CO5 = camphor type. Each experiment was performed $\times 3$ and the data averaged ($N = 3$). Numbers followed by different letters (a–i) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

wood decay fungi at a concentration of 200 µg/ml. Results showed that the leaf essential oils of cinnamaldehyde type (CO1, CO7 and CO8) and cinnamaldehyde/cinnamyl acetate type (CO2 and CO3) completely inhibited the growth of brown-rot fungus (*L. sulphureus*) and white-rot fungi (*L. betulina* and *T. versicolor*) at the concentration of 200 µg/ml. The IC_{50} values of both cinnamaldehyde type (CO1, CO7 and CO8) and cinnamaldehyde/cinnamyl acetate type (CO2 and CO3) leaf essential oils against *L. sulphureus*, *L. betulina* and *T. versicolor* were 52–112 µg/ml, 141–144 µg/ml and 131–136 µg/ml, respectively (Table 2). In addition, the antifungal indices of mixed type (CO4) against *L. sulphureus* and cinnamyl acetate type (CO9) against *L. betulina* were 100% (Fig. 2), and their IC_{50} values were 79 and 150 µg/ml, respectively (Table 2). However, the antifungal indices of both camphor type (CO5) and linalool type (CO6) did not exceed 50% (Fig. 2), and their IC_{50}

Fig. 3. Antifungal activities of the main constituents (100 µg/ml) of leaf essential oils from cinnamaldehyde type of *C. osmophloeum* against white-rot fungi *T. versicolor* and *L. betulina* and brown-rot fungus *L. sulphureus*. Each experiment was performed $\times 3$ and the data averaged ($N = 3$). Numbers followed by different letters (a–g) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

values were >204 µg/ml (Table 2), indicating none of the leaf essential oils of these two chemotypes could inhibit the growth of brown-rot fungus (*L. sulphureus*) and white-rot fungi (*L. betulina* and *T. versicolor*).

On the basis of the above results, it is found that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type have the strongest antifungal activities more than the other chemotypes. Since cinnamaldehyde type (CO1, CO7 and CO8) and cinnamaldehyde/cinnamyl acetate type (CO2) leaf essential oils contained a higher amount of cinnamaldehyde (Table 1) and also displayed strong antifungal activities, it is clear that the antifungal activities of these two leaf essential oils are directly affected by the cinnamaldehyde content. Singh and co-workers have proven that cinnamaldehyde is a strong antifungal agent against human pathogens (Singh et al., 1995). Furthermore, results from Table 2 also revealed that both chemotypes against brown-rot fungus displayed much stronger antifungal activity than against white-rot fungi. In addition, the

Table 2

IC_{50} values of leaf essential oils from six chemotypes of *C. osmophloeum* against white-rot fungi *T. versicolor* and *L. betulina* and brown-rot fungus *L. sulphureus*

Chemotypes	Brown-rot fungus		White-rot fungi	
	<i>L. sulphureus</i> ^a		<i>L. betulina</i> ^a	<i>T. versicolor</i> ^a
Cinnamaldehyde type (CO7)	52 ± 3		142 ± 1	135 ± 2
Cinnamaldehyde type (CO8)	58 ± 2		141 ± 1	131 ± 2
Cinnamaldehyde type (CO1)	59 ± 2		143 ± 1	133 ± 2
Cinnamaldehyde/cinnamyl acetate type (CO2)	68 ± 1		141 ± 1	132 ± 2
Cinnamaldehyde/cinnamyl acetate type (CO3)	112 ± 3		144 ± 0	136 ± 2
Cinnamyl acetate type (CO9)	273 ± 5		150 ± 0	221 ± 10
Mixed type (CO4)	79 ± 5		284 ± 9	148 ± 10
Linalool type (CO6)	204 ± 4		301 ± 10	256 ± 8
Camphor type (CO5)	412 ± 4		448 ± 8	>500

^a Unit: µg/ml.

Table 3

IC₅₀ values of the main constituents of leaf essential oils from cinnamaldehyde type of *C. osmophloeum* against white-rot fungi *T. versicolor* and *L. betulina* and brown-rot fungus *L. sulphureus*

Compounds	Brown-rot fungus	White-rot fungi	
	<i>L. sulphureus</i> ^a	<i>L. betulina</i> ^a	<i>T. versicolor</i> ^a
Benzaldehyde	>500	300 ± 9	495 ± 10
Benzenepropanal	190 ± 3	203 ± 4	263 ± 5
Anethole	>500	>500	>500
Cinnamaldehyde	73 ± 2	74 ± 1	73 ± 2
Bornyl acetate	>500	>500	>500
Eugenol	71 ± 2	95 ± 2	92 ± 1
β-Caryophyllene	>500	>500	>500
Cinnamyl acetate	458 ± 10	300 ± 9	328 ± 8
Caryophyllene oxide	264 ± 6	147 ± 5	279 ± 7
DDAC ^b	1 ± 0	11 ± 1	11 ± 0

^a Unit: µg/ml.

^b Didecyl dimethyl ammonium chloride.

mixed type leaf essential oil also exhibited significant effectiveness against *L. sulphureus*. It may be associated with the main constituents (17.8% of T-cadinol and 11.5% of α-cadinol) of essential oil. In our previous studies on the antifungal performance of *Taiwania cryptomerioides* extractives and *Calocedrus formosana* leaf essential oil, it has been proven that α-cadinol and T-cadinol exhibited the highest antifungal activity against *L. sulphureus* (Chang et al., 1998, 2000; Cheng et al., 2004b).

3.3. Antifungal activity of the constituents in essential oils

The above-mentioned results showed that the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type leaf essential oils had significant antifungal activities against wood decay fungi. To further understand the relative antifungal activity of the constituents in the leaf essential oils of cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type, we selected nine constituents in *C. osmophloeum* to analyze their antifungal performance. As shown in Fig. 3, cinnamaldehyde and eugenol exhibited the strongest activity against *L. sulphureus*, *L. betulina* and *T. versicolor* amongst the nine compounds tested. Both cinnamaldehyde and eugenol totally inhibited the growth of *L. sulphureus* and *L. betulina* at the concentration of 100 µg/ml. Concerning *T. versicolor*, however, eugenol (antifungal index = 74.5%) showed a lower activity than cinnamaldehyde (antifungal index = 100%). The IC₅₀ values of all these compounds in terms of antifungal activity against *L. betulina* and *T. versicolor* is ranked as cinnamaldehyde (74 and 73 µg/ml) > eugenol (95 and 92 µg/ml) > caryophyllene oxide (147 and 279 µg/ml) > benzenepropanol (203 and 263 µg/ml) > cinnamyl acetate (300 and 328 µg/ml) > benzaldehyde (300 and 495 µg/ml) > anethole (>500 µg/ml) = bornyl acetate (>500 µg/ml) = β-caryophyllene (>500 µg/ml) (Table 3). On the other hand, the order of IC₅₀ values against *L. sulphureus* is eugenol (71 µg/

ml) > cinnamaldehyde (73 µg/ml) > benzenepropanol (190 µg/ml) > caryophyllene oxide (264 µg/ml) > cinnamyl acetate (458 µg/ml) > benzaldehyde (>500 µg/ml) = anethole (>500 µg/ml) = bornyl acetate (>500 µg/ml) = β-caryophyllene (>500 µg/ml) (Table 3). Although cinnamaldehyde and eugenol all possess a stronger antifungal activity, eugenol exists in only minor quantities in the leaf essential oils of all *C. osmophloeum* provenances (CO1–CO9). Compared to DDAC, the standard fungicide which is known to be very effective against *L. sulphureus*, *L. betulina* and *T. versicolor* with IC₅₀ values are 1, 11 and 11 µg/ml. It is interesting to note that all fungi were sensitive to cinnamaldehyde and the IC₅₀ values are 73, 74 and 73 µg/ml. Therefore, cinnamaldehyde clearly is responsible for the excellent antifungal activity of cinnamaldehyde type (CO1, CO7 and CO8) leaf essential oils. A similar observation also was noted in our previous studies on the antitermite, antibacterial, antimite, antimildew, antimosquito and antipathogenic activities of *C. osmophloeum* (Chang et al., 2001; Chang and Cheng, 2002; Chen et al., 2002; Chen and Chang, 2002; Cheng et al., 2004a; Lee et al., 2005).

4. Conclusions

According to GC–MS and cluster analyses the leaf essential oils of the nine provenances and their relative contents were classified into six chemotypes—cinnamaldehyde type, cinnamaldehyde/cinnamyl acetate type, cinnamyl acetate type, linalool type, camphor type and mixed type. In addition, this study demonstrates that the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type of *C. osmophloeum* leaf essential oils have excellent antifungal activities and that cinnamaldehyde is responsible for such activity. Therefore, cinnamaldehyde as well as the cinnamaldehyde type and cinnamaldehyde/cinnamyl acetate type leaf essential oils are renewable natural products and may be further explored

as a potential source for the development of wood preservatives against decay fungi or fumigants in the near future.

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