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Antifungal property of the essential oils and their constituents from *Cinnamomum osmophloeum* leaf against tree pathogenic fungi

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Abstract: This study compares the chemical constituents of leaf essential oils from various geographical provenances of *Cinnamomum osmophloeum* and investigates their antifungal activities against six tree pathogenic fungi. According to GC-MS and cluster analyses, the leaf essential oils obtained from different geographical 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. Results from the antifungal tests show that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde–cinnamyl acetate type have excellent inhibitory effect against *Rhizoctonia solani, Collectotrichum gloeosporioides, Ganoderma australe* and *Fusarium solani*. Furthermore, among the fourteen constituents of *C osmophloeum* leaf essential oils, *Z*-cinnamaldehyde, eugenol, geraniol and citral display the best antifungal properties. Comparisons of the antifungal properties of *Z*-cinnamaldehyde congeners reveal that *Z*-cinnamaldehyde exhibits the best antifungal property of this group.

Keywords: *Cinnamomum osmophloeum*; leaf; essential oils; *Z*-cinnamaldehyde; antifungal property; tree pathogenic fungi

INTRODUCTION

Taiwan's tropical and subtropical climate has many species of microbe and they reproduce very quickly. As a result, problems with tree diseases are extremely serious and are worse for artificial forests and plantations using non-native species. In these situations, serious diseases occur more easily, and can cause heavy losses very quickly. The use of toxic fungicides, but primarily their misuse, has contributed significantly to human health and environmental pollution. As a result, simple bioactive compounds of plant origin against pathogenic fungi have been a target of interest in the search for ecologically safe products. Several naturally occurring compounds such as linalool, menthol, thymol, carvacrol, emodin, physcion and rhein have been reported to be effective against plant pathogenic fungi.[1–3]

Indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh) is an endemic tree that grows at middle elevations in Taiwan's natural hardwood forest,[4] particularly in the mountainous area of Po-Li. Since the chemical constituents of its leaf essential oil are similar to those in the well-known bark essential oil of *Cinnamomum cassia*, the potential utilization of *C osmophloeum* leaf is worth exploring.[5] Leaves may be a source of effective fungicidal compounds and may possibly be a renewable source of friendly fungicides having low human and environment toxicity. In other applications, the antibacterial property of cinnamon oils and their constituents is also valuable.[6,7]

Our previous studies have shown that leaf essential oils from *C osmophloeum* have excellent anti-bacterial, anti-termite, anti-mildew, anti-mite and anti-mosquito activities.[5,8–11] However, to the best of our knowledge, there is no literature concerning the antifungal properties of leaf essential oils and their constituents from *C osmophloeum* against tree pathogenic fungi. Due to the chemical polymorphism of leaf essential oil from different provenances of *C osmophloeum*,[12] it is of interest to study differences in the bioactivity of varieties of indigenous cinnamon leaf oils. Therefore, in this study we examine the chemical composition of leaf essential oils from six *C osmophloeum* provenances using GC-MS, followed by an investigation of their antifungal properties against tree pathogenic fungi. The antifungal activity of *Z*-cinnamaldehyde congeners was also examined to help understand the effects of chemical structure on the antifungal property.

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EXPERIMENTAL

Plant materials

Mature leaf of one *C osmophloeum* provenance (samples 1) was collected from the Da-Pin-Ting of the Taiwan Sugar Farm and mature leaves of five *C osmophloeum* provenances (samples 2–6) were collected from the Lien Hua-Chin Research Center on July 2003. Both districts are in Nantou County of central Taiwan. The Da-Pin-Ting of Taiwan Sugar Farm is at an altitude of 400–500 m. Lien Hua-Chin Research Center is at an altitude of 576–925 m. The annual average temperature is 21 °C, the annual average rainfall is 2200 mm, and the pH value of the soil is 4.2–4.7. The species were identified by Mr Yen-Ray Hsui of the Taiwan Forestry Research Institute. A voucher specimen (COLO009–COLO014) of each sample was deposited in the laboratory of wood chemistry (School of Forestry and Resource Conservation, National Taiwan University).

Isolation of essential oils

Fresh *C osmophloeum* leaves were cleaned with distilled water and air-dried to MC 40% at room temperature (27 °C). These samples (150 g each), in triplicate, were subjected to hydrodistillation for 6 h using a Clevenger-type apparatus,[5] followed by determination of oil contents. Leaf essential oils were stored in airtight containers prior to analysis by gas chromatography (GC) and gas chromatography—mass spectrometry (GC-MS).

GC-FID analysis

The leaf essential oils were analyzed using Trace GC (Thermo, Austin, TX, USA) with FID detector and an RTx-5MS phenyl methyl siloxane column (30 m × 0.25 mm; film thickness, 0.25 μm) was used. The injector temperature was maintained at 250 °C. Injection volume was 1 μl at 1:10 split ratio and helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. The initial oven temperature was maintained at 80 °C for 1 min and programmed to increase at 4 °C min^{-1} to 200 °C (held for 5 min).

GC-MS analysis

The leaf essential oils were analyzed on a Trace GC 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 °C to 200 °C by 4 °C min^{-1} and held for 5 min. Then a PoLaris Q mass (ion source 200 °C, 70 eV) instrument (Thermo) was used to identify the major components of *C osmophloeum* under the following conditions: injector temperature, 250 °C; split ratio, 1:10; carrier gas, helium at a flow rate of 1 ml min^{-1}; 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 Spectra Library and NIST MS Search. The quantity of compounds was obtained by integrating the peak area of the chromatograms.

Fungal strains

The tree pathogenic fungi were obtained from the culture collection and research center of the Food Industry Research and Development Institute. The fungal strains used in experiments were: two seedling pathogenic fungi *Fusarium oxysporum* (CCRC32121) and *Rhizoctonia solani* (CCRC31626); two root pathogenic fungi *Ganoderma australe* (CCRC36246) and *Fusarium solani* (CCRC32458); two leaf pathogenic fungi *Pestalotiopsis funereal* (CCRC35266) and *Collectotrichum gloeosporioides* (CCRC35003). Cultures of each of the fungi were maintained on potato dextrose agar (PDA) medium and were stored at 4 °C.

Antifungal assays

The antifungal assays of *C osmophloeum* leaf essential oils were performed on the basis of methods previously described,[13] with slight modifications. Essential oils (100 μg ml^{-1}) and constituents were dissolved in ethanol and then applied to sterilized PDA in 9-cm plates (Petri dishes). After transferring the mycelium of six tree pathogen fungi, the testing plates were incubated at 26 ± 2 °C, 70% relative humidity. When the fungal mycelium of control group reached the edges of the plates (with no added essential oils or constituents), the antifungal indices were calculated. Each test was repeated three times, and the data averaged. The formula for antifungal indices was as follows: Antifungal index (%) $= (1 - Da/Db) \times 100$ [where Da is the diameter of growth zone in the experimental plate (cm), and Db is the diameter of growth zone in the control plate (cm)].

Cluster analysis

Percent composition of the essential oil samples was used to determine differences among the six provenances (1 to 6) of *C osmophloeum* by cluster analysis using the MVSP (multivariate statistical package) software. Euclidean distance was selected as a measure of similarity, and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

Statistical analysis

The Scheffe method of SAS was used to analyze the difference of antifungal properties among six *C osmophloeum* leaf essential oils and their constituents ($p < 0.05$).

RESULTS AND DISCUSSION

Yields and chemical constituents of essential oils

Distillation of six provenances of *C osmophloeum* leaves yielded from 0.1 to 4.7% (v/w) essential oils based on dry weight (Table 1). The highest oil content was found in sample 4 (4.7%), followed by sample 2

Table 1. Constituents and their relative contents (%) of leaf essential oils from six provenances of *Cinnamomum osmophloeum*

		Specimens					
Constituents	t_R (min)	1	2	3	4	5	6
Benzaldehyde	4.14	1.8	—	2.1	—	—	—
Camphene	4.35	—	—	—	—	0.6	—
β-Pinene	4.67	—	—	—	—	2.2	—
p-Cymene	5.39	0.3	—	—	—	8.7	—
Salicylaldehyde	5.81	—	0.4	—	0.8	1.8	—
Linalool	6.91	—	—	—	90.6	—	0.5
Camphor	8.31	0.3	—	—	—	44.0	—
2-Methylbenzofuran	8.64	—	14.4	—	—	—	—
Benzenepropanal	8.70	3.4	1.2	3.7	—	—	—
Terpinene-4-ol	9.13	0.4	—	—	—	4.2	—
α-Terpineol	9.40	—	—	—	0.6	2.2	0.8
p-Allylanisole	9.61	—	0.8	—	—	—	—
Anisole	9.63	—	—	—	—	—	3.8
Anethole	9.67	1.5	—	1.3	1.0	—	—
E-Cinnamaldehyde	10.35	1.4	—	1.0	—	—	—
Geraniol	11.14	—	—	—	—	1.4	0.6
Z-Cinnamaldehyde	11.69	85.3	2.9	50.9	—	1.0	0.2
Citral	11.80	—	—	—	1.9	—	—
Bornyl acetate	12.17	0.2	1.0	1.6	—	20.8	9.8
Eugenol	14.29	0.6	—	1.6	—	2.3	—
Geranyl acetate	14.45	—	4.4	—	—	—	—
α-Cubebene	14.82	0.4	—	—	—	—	3.0
α-Fenchene	14.93	—	—	—	—	1.5	—
β-Caryophyllene	16.20	0.7	—	1.1	1.0	1.6	—
Coumarin	16.79	—	—	—	1.1	—	2.0
Cinnamyl acetate	16.81	0.4	54.4	28.5	—	0.7	—
Aromadendrene	17.40	—	—	—	—	—	3.0
T-Muurolene	17.95	0.2	—	—	—	—	1.8
α-Muurolene	18.53	—	0.4	—	—	—	1.2
δ-Cadinene	18.67	—	2.1	—	—	—	—
β-Cadinene	19.17	0.6	3.0	0.6	—	0.6	1.4
γ-Elemene	20.24	—	0.5	—	—	—	—
Isoledene	20.82	0.2	1.2	—	0.5	—	2.2
Caryophyllene oxide	20.98	0.7	3.7	0.9	0.5	0.8	8.0
T-Cadinol	22.56	—	3.4	0.7	0.6	1.6	17.5
α-Cadinol	22.96	—	—	0.6	—	1.0	11.7
Azunol	23.47	—	—	—	—	—	2.9
Rimuen	30.05	—	—	1.8	—	—	—
Verticiol	31.79	—	—	1.6	—	—	—
Sum		97.4	93.8	98.0	98.6	97.0	71.4
Oil yield (%, v/w)		1.1	1.6	1.0	4.7	0.8	0.1

t_R = Retention time (min); -: not detected.

(1.6%), sample 1 (1.1%), sample 3 (1.0%), sample 5 (0.8%) and sample 6 (0.1%).

Compositions of the six essential oils also are reported in Table 1. About 39 compounds were identified in the leaf essential oils, representing 98.6–71.4% of the essential oils, respectively. The leaf essential oil of sample 1 was found to contain mainly *Z*-cinnamaldehyde (85.3%). The leaf essential oil of sample 2 contained cinnamyl acetate as the major component (54.4%), followed by 2-methylbenzofuran (14.4%). The main constituents of leaf essential oil from sample 3 were *Z*-cinnamaldehyde (50.9%) and cinnamyl acetate (28.5%). For leaf essential oil of sample 4, its major constituent was linalool (90.6%). The major components of leaf essential oil of sample 5 were camphor (44.0%) and bornyl acetate (20.8%). The leaf essential oil of sample 6 was found to be rich in T-cadinol (17.5%), α-cadinol (11.7%), bornyl acetate (9.6%), and caryophyllene oxide (8.0%).

The major constituents identified in this study have also been reported in 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 reported to be 75% *Z*-cinnamaldehyde and 56% camphor, respectively.[14] Jayaprakasha *et al*[15–17] reported the 34 compounds from cinnamon fruits and 26 compounds from cinnamon flowers with (*E*)-cinnamyl acetate (42–54%) as their major compounds. Kaul *et al*[18] also found that the major compound isolated from different parts of cinnamon oil was (*E*)-cinnamyl acetate (22–65%).

The differences in the percentage composition of the essential oils among six provenances of *C osmophloeum* were determined using cluster analysis. Following the results obtained herein and the previous classification by Hu *et al*,[19] we classified the leaf essential oil of provenance 1 the cinnamaldehyde type, provenance 2 the cinnamyl acetate type, provenance 3 the cinnamaldehyde-cinnamyl acetate type, provenance 4 the linalool type, and provenance 5 the camphor type. However, the essential oil from provenance 6 was classified as a mixed type because of lack of a dominant compound.

Antifungal properties of six chemotypes of *C osmophloeum* leaf essential oils

Antifungal properties of seedling pathogens

Figure 1 shows the antifungal indices of leaf essential oils at a dosage of 100 $\mu g\,ml^{-1}$ from six chemotypes of *C osmophloeum* against the two seedling pathogens (*Fusarium oxysporum* and *Rhizoctonia solani*). The antifungal properties of leaf essential oils of six chemotypes against *F oxysporum* did not exceed 18.0% at a dosage of 100 $\mu g\,ml^{-1}$, indicating that the leaf essential oils of six chemotypes had no significant antifungal effects against seedling pathogen *F oxysporum*. On the other hand, the leaf essential oil from cinnamaldehyde type and cinnamaldehyde–cinnamyl acetate type at a dosage of 100 $\mu g\,ml^{-1}$ showed a strong inhibitory effect against seedling pathogen *R solani*, with antifungal indices of 85.7 and 67.1%, respectively. This confirms that *C osmophloeum* leaf essential oils of cinnamaldehyde type and cinnamaldehyde–cinnamyl acetate type have a strong antifungal action against seedling pathogen *R solani*. Since cinnamaldehyde-type leaf oil contained a high amount of *Z*-cinnamaldehyde and also showed a strong antifungal activity, it is clear that the antifungal property of *C osmophloeum* leaf oil is directly affected by the cinnamaldehyde content. It has been reported that the antifungal property of essential oils from *Helichrysum italicum* ssp *microphyllum* against *R solani* and *F oxysporum*, and that the MICs were higher than

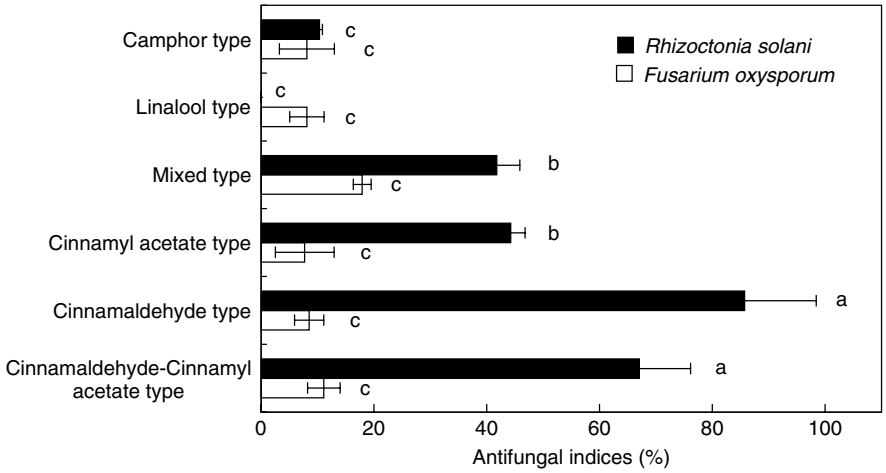

Figure 1. Antifungal indices of leaf essential oils (100 μg ml^{-1}) from six chemotypes of *Cinnamomum osmophloeum* against two seedling pathogens. Numbers followed by different letters (a–c) are significantly different at the level of $p < 0.05$ according to the Scheffe test.

1000 μg ml^{-1}.[20] Accordingly, the antifungal effects of leaf essential oils from cinnamaldehyde type of *C osmophloeum* are worth further detailed investigation.

Antifungal properties of root pathogens

In this study *Ganoderma australe* and *Fusarium solani* were selected to assay the antifungal properties of *C osmophloeum* leaf essential oils. Figure 2 shows the antifungal indices of leaf essential oils at a dosage of 100 μg ml^{-1} from six chemotypes of *C osmophloeum* against two root pathogens. In comparison with the data, it can be seen that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type had better antifungal properties against the root pathogens *F solani* and *G australe*. When treated with essential oils of cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type, the antifungal indices against root pathogen *G australe* were 100.0 and 72.0%, respectively, while the antifungal indices against root pathogen *F solani* were both 100.0%. It is clear that cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type had significant antifungal properties against the root pathogens *F solani* and *G australe* at a dosage of 100 μg ml^{-1}. Pitarokili *et al*[21] demonstrated that complete growth inhibition of *F solani* and *G australe* was achieved only by the application of essential oil from 1,8-cineole-type *Salvia fruticosa* at a concentration of 2000 μl l^{-1}.

In the case of root pathogen *G australe*, a higher antifungal index for the cinnamaldehyde-type essential oil suggests that the antifungal effect against *G australe* might be affected by the cinnamaldehyde content in the essential oil. However, antifungal indices of both cinnamaldehyde type and cinnamaldehyde-cinnamyl acetate type against root pathogen *F solani* were 100%, and the major constituents of these two *C osmophloeum* leaf oils were cinnamaldehyde and cinnamyl acetate. It is therefore concluded that *Z*-cinnamaldehyde and cinnamyl acetate might be the main compounds inhibiting the growth of root pathogen *F solani*.

Antifungal properties of leaf pathogens

Figure 3 shows the antifungal indices of leaf essential oils (100 μg ml^{-1}) from six chemotypes of *C osmophloeum* against two leaf pathogens *Pestalotiopsis*

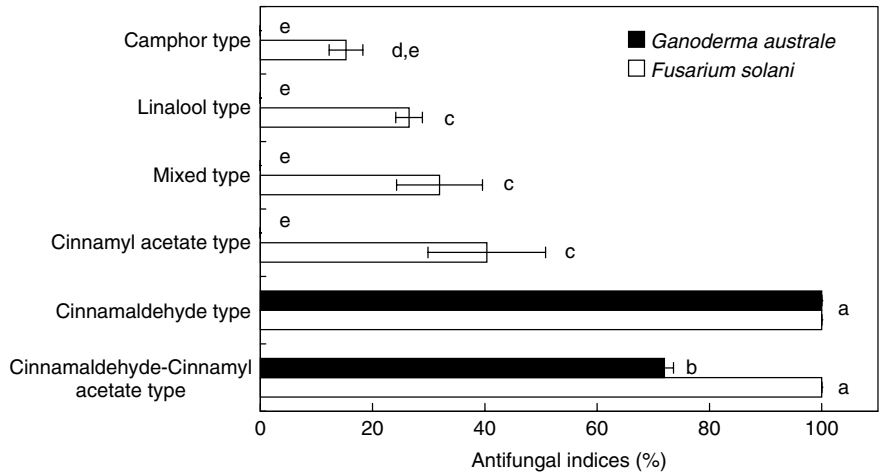

Figure 2. Antifungal indices of leaf essential oils (100 μg ml^{-1}) from six chemotypes of *Cinnamomum osmophloeum* against two root pathogens. Numbers followed by different letters (a–e) are significantly different at the level of $p < 0.05$ according to the Scheffe test.

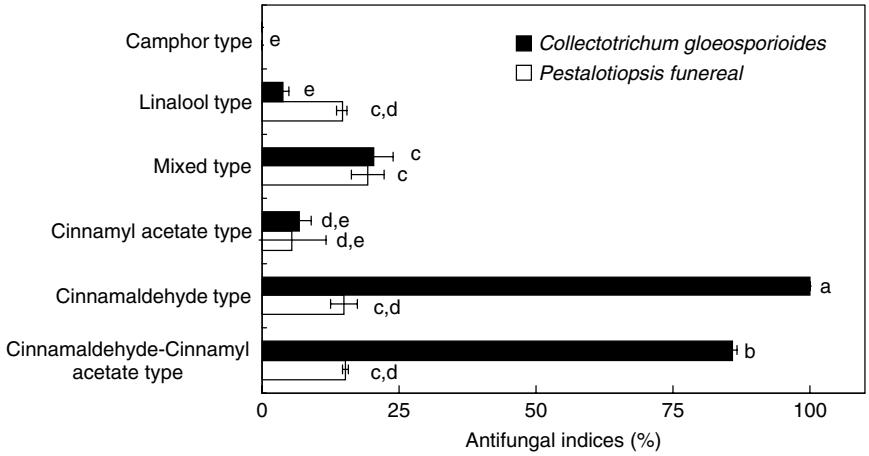

Figure 3. Antifungal indices of leaf essential oils (100 μg ml^{-1}) from six chemotypes of *Cinnamomum osmophloeum* against two leaf pathogens. Numbers followed by different letters (a–e) are significantly different at the level of $p < 0.05$ according to the Scheffe test.

funereal and *Collectotrichum gloeosporioides*. The antifungal indices against *P funereal* did not exceed 20% for all six chemotypes, indicating that none of the leaf essential oils of six chemotypes could inhibit the fungal growth of leaf pathogen *P funereal*. In contrast, the antifungal indices of essential oils of both cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type at a dosage of 100 μg ml^{-1} against leaf pathogen *C gloeosporioides* were 85.8 and 100.0%, respectively, indicating that both cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type had significant antifungal effects against leaf pathogen *C gloeosporioides* at a dosage of 100 μg ml^{-1}. Since the major constituents of these two *C osmophloeum* were *Z*-cinnamaldehyde and cinnamyl acetate, we infer that growth inhibition of leaf pathogen *C gloeosporioides* might be caused by the presence of *Z*-cinnamaldehyde and cinnamyl acetate.

Antifungal properties of constituents from *C osmophloeum* leaf essential oils

The above results show that the *C osmophloeum* leaf essential oils have significant antifungal properties against four tree pathogens: *R solani, F solani, G australe* and *C gloeosporioides*. To understand further the relative antifungal strength of the constituents in the leaf essential oils of *C osmophloeum*, we selected fourteen constituents in *C osmophloeum* to analyze their antifungal performance.

Table 2 shows the antifungal indices of the constituents of *C osmophloeum* leaf essential oils against seedling pathogen *R solani*. Among the fourteen constituents examined, *Z*-cinnamaldehyde, eugenol, citral and geraniol all displayed a strong inhibitory effect against seedling pathogen *R solani*. Their antifungal indices were 71.7, 83.9, 78.3 and 74.5%, respectively. The antifungal indices of all other constituents against *R solani* did not exceed 47.6%, indicating they could not effectively inhibit the growth of seedling pathogen *R solani*. Comparing the antifungal indices of the four compounds with cinnamaldehyde-type *C osmophloeum* leaf essential oil, the inhibitory effects of the four compounds were less than that of cinnamaldehyde-type *C osmophloeum* leaf essential oil against *R solani* (85.7%), suggesting that the antifungal property of

Table 2. Antifungal indices of the constituents (100 μg ml^{-1}) of *Cinnamomum osmophloeum* leaf essential oils against the tree pathogenic fungi: *Rhizoctonia solani* (*Rs*); *Ganoderma australe* (*Ga*); *Fusarium solani* (*Fs*); *Collectotrichum gloeosporioides* (*Cg*)

	Pathogens			
Constituents	*Rs*	*Ga*	*Fs*	*Cg*
Borneol	$2.5 \pm 3.4^{j,k}$	0.0 ± 0.0^{k}	$2.6 \pm 1.1^{j,k}$	$6.8 \pm 4.2^{h,i,j,k}$
Cinnamyl acetate	$31.5 \pm 4.8^{e,f,g,h}$	$22.2 \pm 7.9^{f,g,h,i,j,k}$	$3.0 \pm 2.4^{j,k}$	$4.4 \pm 3.1^{i,j,k}$
Benzenepropanal	$47.6 \pm 2.1^{d,e}$	0.0 ± 0.0^{k}	$39.7 \pm 5.6^{e,f}$	$19.8 \pm 2.0^{f,g,h,i,j,k}$
Camphor	$3.2 \pm 0.8^{j,k}$	0.0 ± 0.0^{k}	$13.2 \pm 1.1^{g,h,i,j,k}$	$7.0 \pm 0.0^{h,i,j,k}$
Camphene	0.0 ± 0.0^{k}	0.0 ± 0.0^{k}	$12.3 \pm 4.3^{g,h,i,j,k}$	0.0 ± 0.0^{k}
Anethole	0.0 ± 0.0^{k}	0.0 ± 0.0^{k}	$34.0 \pm 3.5^{e,f,g}$	$11.2 \pm 4.1^{g,h,i,j,k}$
Z-Cinnamaldehyde	$71.7 \pm 3.7^{b,c,d}$	$49.1 \pm 3.7^{d,e}$	$28.9 \pm 1.8^{e,f,g,h,i}$	100.0 ± 0.0^{a}
Geraniol	$74.5 \pm 2.9^{b,c}$	$6.2 \pm 0.0^{i,j,k}$	$72.3 \pm 0.7^{b,c,d}$	$2.4 \pm 0.9^{j,k}$
Citral	$78.3 \pm 3.4^{a,b}$	100.0 ± 0.0^{a}	$65.7 \pm 7.6^{b,c,d}$	$10.2 \pm 5.2^{g,h,i,j,k}$
α-Terpineol	$15.2 \pm 2.7^{f,g,h,i,j,k}$	$14.2 \pm 7.7^{g,h,i,j,k}$	$3.0 \pm 0.0^{j,k}$	$2.9 \pm 0.0^{j,k}$
Linalool	$16.9 \pm 1.1^{f,g,h,i,j,k}$	$8.0 \pm 5.0^{h,i,j,k}$	0.0 ± 0.0^{k}	$2.9 \pm 0.0^{j,k}$
Benzaldehyde	$29.1 \pm 6.2^{e,f,g,h,i}$	0.5 ± 0.4^{k}	$4.0 \pm 3.5^{i,j,k}$	0.8 ± 0.7^{k}
Eugenol	$83.9 \pm 0.6^{a,b}$	$49.9 \pm 3.3^{c,d,e}$	$11.5 \pm 2.4^{g,h,i,j,k}$	100.0 ± 0.0^{a}
Coumarin	$26.4 \pm 1.8^{e,f,g,h,i,j}$	$5.3 \pm 4.1^{i,j,k}$	0.0 ± 0.0^{k}	$3.0 \pm 0.2^{j,k}$

Numbers followed by different letters (a–k) are significantly different at the level of $p < 0.05$ according to the Scheffe test.

C osmophloeum leaf essential oils against seedling pathogen *R solani* probably results from additive effects of the other constituents.

The antifungal indices of the constituents of *C osmophloeum* leaf essential oils against the root pathogens *G australe* and *F solani* are presented in Table 2. The antifungal index of the citral against root pathogen *G australe* was 100%, revealing that citral had an excellent inhibitory effect against *G australe*. Geraniol and citral showed quite significant inhibitory effects against the root pathogen *F solani*, with antifungal indices of 72.3 and 65.7%, respectively. It is clear that citral had an excellent inhibitory effect against both root pathogens *G australe* and *F solani*, and that geraniol only had an inhibitory effect against the root pathogen *F solani*. Cinnamaldehyde, the major compound of cinnamaldehyde type *C osmophloeum*, surprisingly did not show better inhibitory effects than *C osmophloeum* leaf essential oils. We infer that the antifungal property of *C osmophloeum* leaf essential oils against root pathogens *G australe* and *F solani* is probably due to other effects of the constituents, a topic which is worth further investigation.

With regard to the antifungal property of the fourteen constituents of *C osmophloeum* leaf essential oils against the leaf pathogen *C gloeosporioides*, the results shown in Table 2 show that *Z*-cinnamaldehyde and eugenol displayed a remarkable inhibitory effect against the leaf pathogen *C gloeosporioides*, yielding an antifungal index of 100% at a dosage of $100\,\mu g\,ml^{-1}$. Compared with the previous results obtained from the antifungal tests of *C osmophloeum* essential oils, the leaf essential oils of cinnamaldehyde-type *C osmophloeum* had an excellent antifungal effect against *C gloeosporioides*. Its major compound, *Z*-cinnamaldehyde, also exhibited a remarkable antifungal effect against *C gloeosporioides*. It was therefore concluded that the antifungal property of *C osmophloeum* leaf oil against *C gloeosporioides* is mostly attributable to *Z*-cinnamaldehyde.

Cakir *et al*[22] found that β-caryophyllene oxide and α-terpineol from *Hypericum* species essential oils were inhibitory to the growth of ten agricultural pathogenic fungi (five *Fusarium* species and five anastomosis groups of *R solani*) at a concentration of $1\,mg\,ml^{-1}$. Muller-Riebau *et al*[2] also found that only concentrations greater than $100\,\mu g\,ml^{-1}$ of thymol and carvacrol led to a complete inhibition of fungi (*Fusarium moniliforme, R solani, Sclerotinia sclerotorum* and *Phytophthora capsici*) growth. Results of this present study suggest that *Z*-cinnamaldehyde, eugenol, citral and geraniol are all potential agents for the development of natural fungicides and fumigants.

Antifungal properties of *Z*-cinnamaldehyde's congeners

To examine the structure—antifungal activity relationships, three compounds, cinnamic acid, cinnamyl acetate and cinnamyl alcohol (Fig 4), whose chemical

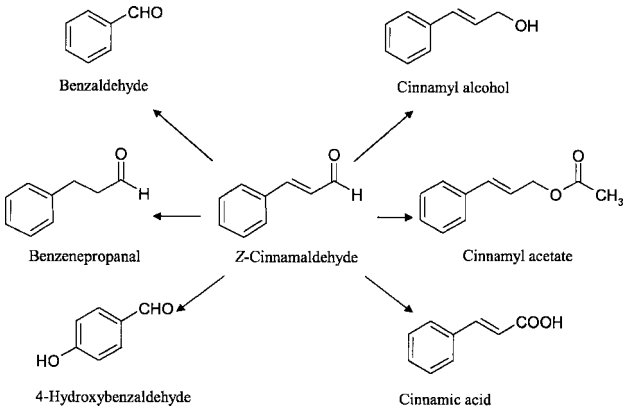

Figure 4. Structures of cinnamaldehyde's congeners.

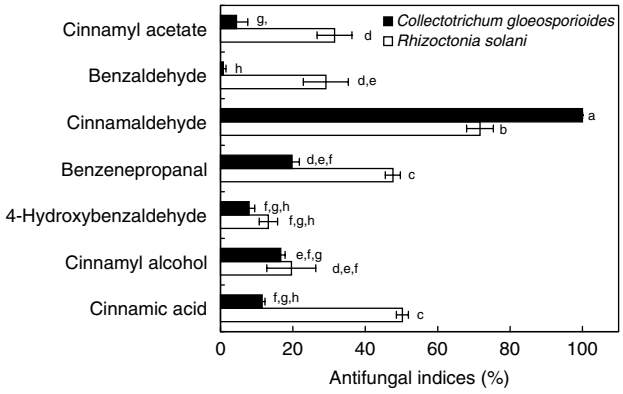

Figure 5. Antifungal activities of the congeners of cinnamaldehyde ($100\,\mu g\,ml^{-1}$). Numbers followed by different letters (a–h) are significantly different at the level of $p < 0.05$ according to the Scheffe test.

structures are similar to *Z*-cinnamaldehyde, were studied for antifungal properties against tree pathogens *R solani* and *C gloeosporioides*. Figure 5 presents the results of antifungal activity of these compounds against both tree pathogens *R solani* and *C gloeosporioides* at a dose of $100\,\mu g\,ml^{-1}$. It is clear that *Z*-cinnamaldehyde still has stronger antifungal activity than the other compounds. The effectiveness of all these compounds in terms of antifungal activity against *R solani* at a dose of $100\,\mu g\,ml^{-1}$ is ranked as *Z*-cinnamaldehyde (71.7%) > cinnamyl acid (50.3%) > cinnamyl acetate (31.5%) > cinnamyl alcohol (19.6 %). In contrast, the order of antifungal indices against *C gloeosporioides* is *Z*-cinnamaldehyde (100%) > cinnamyl alcohol (16.7%) > cinnamyl acid (11.5%) > cinnamyl acetate (4.4%). It is clear that compounds with an aldehyde group have the best antifungal activity. Thus, the antifungal activity of 4-hydroxybenzaldehyde, benzenepropanal and benzaldehyde (Fig 4) that had the same aldehyde group but different carbon–hydrogen bond lengths also were studied. The results shown in Fig 5 demonstrate that *Z*-cinnamaldehyde still has the best antifungal property. These results suggest that an aldehyde compound having a conjugated double bond and a long CH chain outside the ring, such as *Z*-cinnamaldehyde, has much better antifungal activity. A similar observation also

was noted in our previous studies on the antibacterial, antitermitic and antimosquito activities of *C osmophloeum*.[5,8,11]

CONCLUSION

In this study the effectiveness of six chemotypes of *C osmophloeum* leaf essential oils and their constituents against six tree pathogens were compared. The *C osmophloeum* leaf essential oils' major compound, *Z*-cinnamaldehyde, and the congeners of *Z*-cinnamaldehyde, were also studied to compare their antifungal properties.

Results obtained from antifungal tests demonstrated that the leaf essential oils of cinnamaldehyde type and cinnamaldehyde—cinnamyl acetate type had an excellent inhibitory effect against seedling pathogenic fungus *R solani*, leaf pathogenic fungus *C gloeosporioides* and two root pathogenic fungi *G australe* and *F solani*. Furthermore, comparison of the antifungal properties of the fourteen constituents in *C osmophloeum* leaf essential oil showed that citral, geraniol, eugenol, and *Z*-cinnamaldehyde had better antifungal properties than the others. The antifungal index of citral to *G australe* was 100%, those of geraniol and eugenol to *R solani* were 74.5 and 83.9%, respectively, and *Z*-cinnamaldehyde and eugenol to *C gloeosporioides* were also 100%. In addition, results from the antifungal tests of *Z*-cinnamaldehyde congeners revealed that an aldehyde compound having a conjugated double bond and a long carbon-hydrogen chain outside the benzene ring has a stronger antifungal property than other structures.

C osmophloeum leaf essential oils of both cinnamaldehyde type and cinnamaldehyde–cinnamyl acetate type and also *Z*-cinnamaldehyde may be further explored as potential agents for the development of natural fungicides and fumigants.

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