Research paper

Antifungal Activities and Chemical Compositions of Essential Oils from Leaves of Four Eucalypts

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【Summary】

Essential oils from the leaves of 4 eucalypts species, i.e., *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora*, were extracted by hydrodistillation. Compositions of the essential oils were analyzed and identified. Their biological activities with respect to antifungal activity were examined. Yields of the essential oils in descending order were *E. camaldulensis* (3.48 mL 100 g^{-1}), *E. urophylla* (3.14 mL 100 g^{-1}), *E. grandis* (3.01 mL 100 g^{-1}), and *E. citriodora* (1.89 mL 100 g^{-1}). Components of individual essential oils identified that 51 compounds were present in *E. urophylla*, with γ-terpinene predominant; *E. grandis* had 65 identified compounds with 1, 8-cineole being the richest fraction; there were 62 compounds identified from the essential oil of *E. camaldulensis* with 1, 8-cineole as the main component; and for *E. citriodora*, 35 compounds were identified with citrionellal as the dominant components. Anti-mildew tests of the 4 eucalypt essential oils indicated that *E. citriodora* had the best efficacy, and was extensively effective against all tested mildew species. *E. urophylla*, on the other hand, had the poorest efficacy. On the wood decay fungus tests, the same was observed for *E. citriodora* essential oil, and this indicated that it might be an excellent choice as a wood preservative. The main reason for its effectiveness was the presence of citronellal and citronellol which had obvious benefits in fighting mildew and fungi.

Key words: *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, *E. citriodora*, essential oil, antifungal activity.

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研究報告

四種桉樹葉精油組成分及抗真菌活性之測定

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摘　要

本研究以四種不同種類之桉樹葉片，即垂尾桉(*Eucalyptus urophylla*)、玫瑰桉(*E. grandis*)、赤桉(*E. camaldulensis*)及檸檬桉(*E. citriodora*)等，以傳統水蒸氣蒸餾法萃取桉樹葉精油，並分離精油組成成分及進一步評估抗真菌活性。精油收率依序為赤桉(3.48 mL 100 g^{-1})、垂尾桉(3.14 mL 100 g^{-1})、玫瑰桉(3.01 mL 100 g^{-1})及檸檬桉(1.89 mL 100 g^{-1})。各精油的成分化合物鑑定，垂尾桉葉精油共鑑定51個化合物，最主要主成分為γ-松油烯(γ-terpinene)。玫瑰桉葉精油共鑑定65個化合物，最主要主成分為桉葉精(1, 8-cineole)。赤桉葉精油共鑑定62個化合物，主要主成分為桉葉精。檸檬桉葉精油共鑑定35個化合物，最主要成分為香茅醛(citrionellal)。抗真菌試驗結果顯示四種桉樹葉精油中，以檸檬桉抗黴菌效果為最佳，且對各試驗黴菌廣泛有效，而以垂尾桉抗黴菌效果最差。抗腐朽菌試驗結果亦顯示檸檬桉精油之抗腐朽菌活性最高，可做為極佳之抗木材腐朽劑。主要原因為檸檬桉葉精油主成分香茅醛與香茅醇(citronellol)等二化合物，此二化合物對於抗黴菌及抗腐朽菌活性有明顯的助益。

關鍵詞：垂尾桉、玫瑰桉、赤桉、檸檬桉、精油、抗真菌試驗。

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INTRODUCTION

Fragrant essential oils have found extensive uses in the industrial production of foodstuffs, scented soaps, perfumes, and cosmetics, as well as for medicinal purposes. Many essential oil-containing plants or herbs are known in folklore as possessing curative powers. There are already numerous studies devoted to the biological activities of the essential oils from medicinal plants, particularly with respect to their antibacterial, antifungal (Chaumont and Bardey 1989, Hammouchi et al. 1990, Lemos et al. 1990, Ferdous et al. 1992, Faouzia et al. 1993, Demetzos et al. 1997), and insecticidal (Kambu et al. 1982) properties. Many of the reports also isolated and identified the main ingredients mediating the antibacterial and antifungal activities (Onawunmi et al. 1984, Oloke et al. 1988, Hinou et al. 1989, Zakarya et al. 1993, Carson and Riley 1995).

Eucalyptus belongs to the family Myrtaceae, and is a globally distributed genus important as 1 of the 3 most-extensively planted pulpwood plantation species (Zobel 1988). Logs of the species are mainly used for pulping and papermaking and are grown on plantations in tropical and subtropical regions. The purposes of this study were to expand multipurpose utilization of eucalypt plantation wood, and to analyze and identify the leaf essential oils of the 4 species of *E. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* with regard to the compositions and contents of the components. Then 7 species of mildew strains and 4 species of decay-causing fungi were assayed with the essential oils to evaluate their antifungal activities. The results thus obtained can be used as references for the multipurpose utilization of locally grown eucalyptus. Results of the antibacterial and antioxidant activities of these essential oils shall be reported in another paper.

MATERIALS AND METHODS

Plant materials

Fresh leaves of felled 22-year-old eucalypts, *E. urophylla*, *E. grandis*, and *E. camal-*

dulensis were collected from the Kukeng Experimental Plantation of the Taiwan Forestry Research Institute (TFRI) in south-central Taiwan in August 2003, while fresh leaves of *E. citriodora* were collected from felled 22-year-old trees at a plantation at the Lienhwachih Research Center, TFRI in central Taiwan in August 2003. The collected leaves were immediately shipped to our Taipei headquarters, and the essential oils were extracted for subsequent analyses.

Hydrodistillation extraction

In the study, hydrodistillation was carried out using steam. One kilogram each of fresh leaves from the 4 species was placed in a round-bottomed flask, and 3 L of distilled water was added. After 8 h of steam distillation, the oil layers had separated from the water layers and were collected, and anhydrous sodium sulfate was added to remove the water. Yields of the essential oils were determined, and the oils were stored in specimen bottles.

Gas chromatography (GC) and GC–MS analysis

A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific) and a FID detector were used for the percentage determination of oil components. The oven temperatures were programmed as follows: 50℃ for 2 min, rising to 250℃ at 5℃ min^{-1}. The injector temperature was 270℃. The carrier gas was He with a flow rate of 1 mL min^{-1}. The detector temperature was 250℃. The split ratio was 1: 10, and 1 μl of sample was injected. Identification of the oil components was based on their retention indices and mass spectra, obtained from the GC–MS analysis on a Hewlett-Packard HP6890/HP5973 instrument equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness, J&W Scientific). The GC analytical parameters were the same as those listed above, and the MS was operated (full-scan mode) in the EI mode at 70 eV.

Component identification

Identification of the chemical constituents was based on comparisons of their Kovats indices (KI) (Van den Dool and Kratz 1963, Adams 2001), retention times (RT), and mass spectra with those obtained from authentic standards, the NIST and Wiley libraries of spectra, and the literature (Adams 1995).

Fungal strains

The mildew and wood decay fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu, Taiwan. For the mildew strains, references of ASTM G21, JIS Z 2911, and ATCC test method 30 were consulted, and 7 strains including *Aspergillus clavatus* (ATCC 1007), *A. niger* (ATCC 6275), *Chaetomium globosum* (ATCC 6205), *Cladosporium cladosporioides* (ATCC 13276), *Myrothecium verrucaria* (ATCC 9095), *Penicillium citrinum* (ATCC 9849), and *Trichoderma viride* (ATCC 8678), were tested. For the wood decay fungi, related standard methods of CNS (Chinese National Standard) and JIS were consulted, and 2 strains of white rot, *Trametes versicolor* (CCRC 35253) and *Phanerochaete chrysosporium* (ATCC 24725), as well as 2 strains of brown rot, *Phaeolus schweinitizii* (ATCC 38047) and *Lenzites sulphureus* (CCRC 35305), were tested. Cultures of each of the fungi were maintained on potato dextrose agar (PDA) medium and were stored at 4℃.

Antifungal assay

The methods of Kofujita et al. (2001) and Ohtani et al. (2001) were adopted. Essential oils diluted with isobutyl ether to specific concentrations were added to the PDA medium and homogeneously dispersed, allowing natural penetration of the solution into the gel medium. After inoculation of fungal strains, the Petri dishes were kept in a growth chamber maintained at 27℃ and 70% RH. The radii of the fungal hypha growth of the test groups were compared with those of the control groups and used to calculate the antifungal indices and IC_{50} concentrations. Each treatment was replicated 5 times. The formula for the antifungal index is:

antifungal index (%) = (1 - *D*a/*D*b) × 100;

where *D*a is the diameter of the growth zone in the experimental dish (cm), and *D*b is the diameter of the growth zone in the control dish (cm).

RESULTS AND DISCUSSION

Yields, compositions, contents, and identification of the leaf essential oils

Yields of leaf essential oils from the hydrodistillation of *E. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* were 3.14, 3.01, 3.48, and 1.89 mL 100 g^{-1}, respectively. The 1st 3 species had roughly comparable essential oil yields, while *E. citriodora* had a markedly lower yield.

Contents of the components of the leaf oils of *E. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* are shown in Table 1. Discussion of the results for each species is presented below.

Fifty-four compounds were identified from the leaf essential oil of *E. urophylla*. The dominant component was γ-terpinene at 26.16% of the total; followed by *p*-cymene (22.32%) and 1,8-cineole (13.90%). The results differ from information, in the literature as Cimanga et al. (2002), Dagne et al. (2000), and Shieh (1998) all suggested that the main components of *E. urophylla* were α-pinene and 1,8-cineole; while the γ-terpinene and *p*-cymene contents were very low. In our results, the quantities of α-pinene and 1,8-cineole made up 1.84% and 13.90%, respectively, far less than the proportions of γ-terpinene and *p*-cymene. In Singh et al. (1988) who reported the leaf oil of *E. urophylla* from India, the dominant components were *p*-cymene (75%), α-pinene (7%) and γ-terpinene (4%). The in results also showed differences with those of the previous literature. We assume that the differences might have been caused by differences in the chemotype of the species.

Sixty-seven compounds were identified from the leaf essential oil of *E. grandis*. The dominant compound was 1,8-cineole, making up 19.77% of the essential oil. When we compared the results with those in the literature, *E. grandis* from Uruguay (Dellacassa et al. 1990), Australia (Brophy et al. 1991), and Turkey (Azcan et al. 1995) all had similar leaf essential oils with 1,8-cineole predominating.

There were 65 compounds identified from the leaf essential oil of *E. camaldulensis*. The dominant compound was 1,8-cineole, accounting for 29.58% of the total. No *p*-cymene or cryptone was found. The results were similar to those from Algeria (Benayache et al. 2001), Mozambique (Pagula et al. 2000), Morocco (Farah et al. 2002), Burundi (Dethier et al. 1994), the Congo (Cimanga et al. 2002), Greece (Tsiri et al. 2003), and Taiwan (Shieh 1996) for *E. camaldulensis*, all of which reported 1,8-cineole as the main component with small or no traces of *p*-cymene or cryptone.

In total, 44 compounds were identified from the leaf essential oil of *E. citriodora*, and the main components were citronellal and

Table 1. Composition of the leaf oils of *Eucalyptus urophylla* (*E. uro*), *E. grandis* (*E. gra*), *E. camaldulensis* (*E. cam*), and *E. citriodora* (*E. cit*)

Peak no.	Consituent	K.I.[a]	Concentration (%)				Identification[b]	Peak no.	Consituent	K.I.[a]	Concentration (%)				Identification[b]
			E. uro	*E. gra*	*E. cam*	*E. cit*					*E. uro*	*E. gra*	*E. cam*	*E. cit*	
1	α-thujene	930	-[c]	-	0.2	0.1	MS, KI	50	neryl acetate	1362	-	0.1	-	-	MS, KI
2	α-pinene	939	1.8	11.4	9.7	1.3	MS, KI, ST	51	*iso*-ledene	1376	-	-	0.1	-	MS, KI
3	camphene	954	0.1	0.2	0.3	-	MS, KI, ST	52	α-copaene	1377	0.1	0.8	0.1	-	MS, KI
4	β-pinene	979	0.1	0.2	9.9	2.2	MS, KI, ST	53	geranyl acetate	1381	0.1	1.0	-	0.2	MS, KI
5	β-myrcene	991	0.1	0.2	1.1	0.3	MS, KI, ST	54	β-elemene	1391	-	0.2	0.1	0.1	MS, KI, ST
6	α-phellandrene	1003	0.9	0.4	0.2	-	MS, KI, ST	55	α-gurjunene	1410	0.1	0.7	1.1	0.4	MS, KI, ST
7	α-terpinene	1017	0.3	0.1	0.1	0.1	MS, KI, ST	56	α-cedrene	1412	-	0.1	0.1	0.2	MS, KI, ST
8	*p*-cymene	1025	22.3	2.1	-	0.2	MS, KI, ST	57	β-caryophyllene	1419	1.4	6.9	0.3	5.6	MS, KI, ST
9	limonene	1029	2.2	4.7	15.2	0.7	MS, KI	58	β-copaene	1432	-	-	0.1	-	MS, KI
10	β-phellandrene	1030	-	-	-	1.2	MS, KI	59	β-gurjunene	1434	-	-	0.4	-	MS, KI
11	1,8-cineole	1031	13.9	19.8	29.6	1.9	MS, KI	60	aromadendrene	1441	0.3	1.2	3.9	0.1	MS, KI, ST
12	*cis*-β-ocimene	1037	0.3	0.5	-	0.2	MS, KI, ST	61	α-neo-clovene	1454	-	-	0.1	-	MS, KI
13	γ-terpinene	1060	26.2	1.5	0.6	1.0	MS, KI	62	α-caryophyllene	1455	0.3	1.2	0.1	0.4	MS, KI
14	*trans*-linalool oxide (furanoid)	1073	0.2	-	-	-	MS, KI, ST	63	*allo*-aromadendrene	1460	0.2	0.8	1.0	0.2	MS, KI
15	*cis*-linalool oxide (furanoid)	1087	0.1	-	-	-	MS, KI, ST	64	γ-gurjunene	1477	0.1	0.3	0.1	-	MS, KI
16	terpinolene	1089	1.3	2.2	3.0	0.9	MS, KI	65	γ-muurolene	1480	-	0.1	0.1	-	MS, KI
17	*p*-cymenene	1091	0.1	0.4	0.3	-	MS, KI, ST	66	*epi*-cubebol	1494	-	0.3	0.4	-	MS, KI
18	linalool	1097	0.3	0.3	0.1	0.1	MS, KI, ST	67	viridiflorene	1497	0.3	1.6	0.9	0.4	MS, KI
19	isopentyl 2-methyl butanoate	1100	0.3	0.2	trace[d]	-	MS, KI	68	bicyclogermacrene	1500	0.6	0.6	0.1	0.1	MS, KI
20	*cis*-rose oxide	1108	-	-	-	trace	MS, KI	69	α-muurolene	1500	-	0.4	0.1	-	MS, KI, ST
21	exo-fenchol	1122	-	0.2	0.2	-	MS, KI	70	(E,E)-α-farnesene	1506	-	-	0.1	0.1	MS, KI
22	*allo*-ocimene	1132	0.2	0.3	-	0.1	MS, KI	71	γ-cadinene	1514	-	0.1	0.1	-	MS, KI
23	*trans*-pinocarveol	1139	-	0.2	0.2	-	MS, KI	72	δ-cadinene	1523	0.5	2.2	0.2	0.2	MS, KI
24	*cis*-verbenol	1141	-	-	0.1	-	MS, KI	73	*trans*-calamenene	1529	0.4	1.2	-	-	MS, KI
25	camphor	1146	0.3	1.6	0.8	-	MS, KI	74	*trans*-cadina-1(2), 4-diene	1535	0.1	0.5	-	-	MS, KI
26	camphene hydrate	1150	-	-	trace	-	MS, KI, ST	75	α-calacorene	1546	-	0.2	-	-	MS, KI
27	citronellal	1153	-	-	-	49.5	MS, KI, ST	76	(E)-nerolidol	1563	-	0.2	trace	-	MS, KI, ST
28	*iso*-isopulegol	1160	-	-	-	10.4	MS, KI, ST	77	*epi*-globulol	1564	0.1	0.2	1.1	-	MS, KI, ST
29	borneol	1169	0.1	0.6	0.4	-	MS, KI, ST	78	ledol	1569	0.3	0.5	0.4	0.1	MS, KI, ST
30	neoiso-isopulegol	1171	-	-	-	2.2	MS, KI	79	spathulenol	1578	1.8	0.7	0.1	0.1	MS, KI, ST
31	1,8-menthadien-4-ol	1173	-	-	1.6	0.3	MS, KI	80	caryophyllene oxide	1583	0.2	0.5	0.1	0.1	MS, KI, ST
32	terpinen-4-ol	1177	6.3	1.3	-	0.3	MS, KI, ST	81	globulol	1585	1.5	2.4	4.7	0.3	MS, KI
33	diethyl succinate	1179	-	0.2	-	-	MS, KI	82	viridiflorol	1593	1.0	1.4	1.1	0.1	MS, KI
34	*p*-cymen-8-ol	1183	0.2	0.4	0.5	-	MS, KI	83	β-cedrene epoxide	1623	-	-	0.2	-	MS, KI
35	α-terpineol	1189	1.3	5.0	4.0	0.8	MS, KI, ST	84	10-*epi*-γ-eudesmol	1624	0.5	0.7	0.5	0.1	MS, KI
36	n-decanal	1202	-	0.2	-	-	MS, KI	85	*trans*-isolongifolanone	1627	-	-	0.2	-	MS, KI
37	*p*-cymen-9-ol	1205	-	-	0.1	-	MS, KI	86	leptospermone	1631	0.4	-	-	-	MS, KI
38	citronellol	1226	-	-	-	11.9	MS, KI, ST	87	γ-eudesmol	1632	1.4	2.0	0.8	0.1	MS, KI
39	*cis*-*p*-mentha-1(7), 8-dien-2-ol	1231	-	0.1	0.2	-	MS, KI	88	*cis*-cadin-4-en-7-ol	1637	0.5	0.4	-	-	MS, KI
40	piperitone	1253	0.1	0.3	0.1	-	MS, KI	89	epoxy-allo-alloaromadendrene	1641	0.5	0.6	0.2	-	MS, KI
41	butyrophenone	1253	-	-	0.1	-	MS, KI	90	δ-cadinol	1646	0.6	0.6	0.1	-	MS, KI
42	*iso*-bornyl acetate	1286	-	0.3	-	-	MS, KI, ST	91	α-cadinol	1654	0.3	-	0.2	0.1	MS, KI
43	safrole	1287	-	0.2	trace	-	MS, KI	92	neo-intermedeol	1660	0.1	0.3	0.1	-	MS, KI
44	thymol	1290	2.0	-	-	-	MS, KI, ST		*Monoterpene hydrocarbons*	55.9	24.1	40.5	8.3		
45	carvacrol	1299		0.1	-	-	MS, KI, ST		*Oxygenated monoterpenes*	27.5	45.3	38.1	83.0		
46	*iso*-verbanol acetate	1310	-	0.3	-	-	MS, KI		*Sesquiterpene hydrocarbons*	4.4	19.1	9.0	7.8		
47	exo-2-hydroxy-cineole acetate	1345	0.2	0.7	-	-	MS, KI		*Oxygenated sesquiterpenes*	9.2	10.6	10.1	0.9		
48	α-terpinyl acetate	1349	4.2	12.8	0.1	0.4	MS, KI, ST		*Others*	2.0	0.4	0.1	0.0		
49	citronellyl acetate	1353	-	-	-	5.2	MS, KI, ST		Yield (ml/100 g)	3.14	3.01	3.48	1.8		

[a] Kovats index on a DB-5 column in reference to n-alkanes (Adams 1995, 2001). [b] MS, NIST and Wiley libraries spectra and the literature; KI, Kovats index; ST, authentic standard compounds. [c] Not detected. [d] trace < 0.1%.

citronellol, respectively making up 49.45% and 11.86% of the total. Studies carried out in Australia (Bignell et al. 1997), Ethiopia (Dagne et al. 2000), Burundi (Dethier et al. 1994), India (Rao et al. 2003), the Congo (Menut et al. 1992), and Uruguay (Dellacassa et al. 1990) all found citronellal to be the main ingredient in the leaf oil of *E. citriodora*; with citronellol the next most-abundant component, having contents which ranged from 6.3% to 20.4%. Thus, these results are largely comparable.

The compositional analysis of the 4 eucalypts leaf oils found that monoterpenes predominated. *E. citriodora* and *E. grandis* leaf oils had greater amounts of more oxygenated monoterpenes while *E. urophylla* and *E. camaldulensis* had more of the monoterpene hydrocarbons. All 4 species had relatively low sesquiterpene contents, as also noted by several studies (Dellacassa et al. 1990, Brophy et al. 1991, Dethier et al. 1994, Azcan et al. 1995, Bignell et al. 1997, Benayache et al. 2001).

Antifungal activities of leaf oils of eucalypts against mildew

Seven mildew fungi were selected for the anti-mildew fungal tests. They were *A. clavatus*, *A. niger*, *C. globosum*, *C. cladosporioides*, *M. verrucaria*, *P. citrinum*, and *T. viride*. Most of these fungi can reap havoc with organic cultural relics, and certain species can be harmful to human health, such as inducing allergic reactions, asthma, bronchitis (Grant et al. 1976, Blyth et al. 1977, Blyth 1978), onychomycosis (Naidu et al. 1991, Naidu 1993, Hattori et al. 2000), cerebral infections (Anandi et al. 1989, Abbott et al. 1995, Kleinschmidt 2002), pneumonia (Prentice et al. 1996), peritonitis, and immunodeficient syndrome (Chouaki et al. 2002).

In the preparatory experiments, we screened the minimum inhibitory concentration (MIC) values of the essential oils against the mildew fungi and established that a concentration of 10 mg $disc^{-1}$ or above was needed; hence, a 10 mg $disc^{-1}$ concentration was employed to test for the antifungal indices and durability of the effectiveness. Growth diameters of the fungal colonies were measured daily, and on days 3, 7, 14, and 21, the antifungal index was recorded as shown in Fig. 1. Results show that for *A. clavatus* and *A. niger*, after 7 d of inoculation, *E. grandis* at 92.16% and 60.76% and *E. citriodora* at 90.72% and 54.65% had the best fungal growth suppression, followed by *E. camaldulensis* at 79.66% and 24.05% and *E. urophylla* at 72.32% and 5.70%, which were less effective. After 21 d of culture, however, the results indicated that *E. grandis* had the best durability (56.47% and 16.08%), followed by *E. citriodora* (28.24% and 0%). As for *C. globosum*, except for *E. camaldulensis*, the leaf essential oils of the other 3 species could fully suppress its fungal growth even at 21 d. For *C. cladosporioide*, after 7 d of culture, *E. citriodora* produced the best suppression (100%), followed by *E. grandis* (85.39%), *E. urophylla* (68.54%), and *E. camaldulensis* (64.04%), which was the least effective. After 21 d of culture, however, all 4 leaf essential oils had antifungal indices of < 50%, indicating poor durability against *Cladosporium* sp., with *E. urophylla*, in particular showing the weakest residual effect. As for *M. verrucaria*, after 7 d of culture, the leaf essential oil of *E. citriodora* showed the best result with total suppression; *E. grandis* leaf oil was next with 87.5% suppression; and *E. urophylla* and *E. camaldulensis* achieved about 60% suppression. Even after 21 d, *E. citriodora* could still fully suppress fungal growth; while *E. grandis* could manage about 54%, and the other 2 species showed < 50% effectiveness.

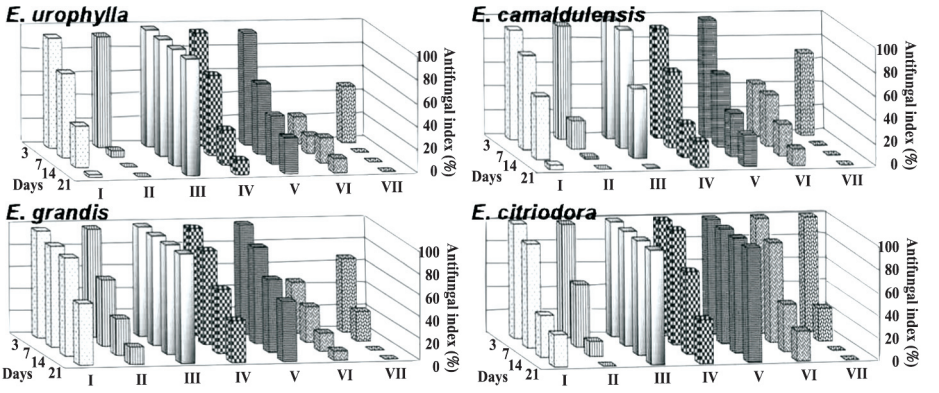

Fig. 1. Antifungal index (%) of the essential oils (10 mg disc^{-1}) from *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* leaves after 3, 7, 14, and 21 d of incubation.
***I, *Aspergillus clavatu*; II. *A. niger*; III, *Chaetomium globosum*; IV, *Cladosporium cladosporioides*; V, *Myrothecium verrucaria*; VI, *Penicillium citrinum*; VII, *Trichoderma viride*.**

With regard to the growth suppression of *P. citrinum*, except for *E. citriodora* leaf oil which maintained an 86% effectiveness on the 7 d of culture, the other 3 species had leaves of effectiveness of < 50%. After 21 d of culture, even *E. citriodora* had an antifungal index of only 26.4%, showing a lack of durability. This particular mildew fungus was quite resistant to the leaf essential oils of the 4 eucalypts as well. As for *T. viride*, after 3 d of culture, the leaf essential oil of *E. citriodora* achieved full suppression, followed by *E. grandis*, *E. camaldulensis*, and *E. urophylla*, which was the least effective. After 7 d of culture, however, only the first 2 oils maintained an antifungal index of about 27%, while the others exhibited an antifungal index of 0. On day 10, even the indices of *E. citriodora* and *E. grandis* dropped to 0.

Furthermore, in order to determine the IC_{50} values of the 4 eucalypt leaf essential oils against the 7 mildew fungi, different concentrations of the oils were applied, and antifungal indices at different concentrations were used to calculate the IC_{50} values. The results are shown in Table 2. IC_{50} values of the 4 leaf essential oils against the 7 mildew fungi suggest that *E. citriodora* was superior to the other oils, followed in order by *E. grandis*, *E. camaldulensis*, and *E. urophylla*. Ohtani et al. (2001) experimented using the leaf essential oil of the conifer *Chamaecyparis obtusa* against several mildew fungi. For both 7 and 14 d of culture, at equivalent essential oil concentrations of 10 mg disc^{-1}, our *E. citriodora* leaf essential oil performed better than that of *C. obtusa* for suppression of the activities of *A. niger*, *C. globosum*, *C. cladosporioides*, and *P. citrinum*.

Antifungal activities of leaf oils of eucalypts against wood decay fungi

In order to carry out the anti-decay fungal tests using the 4 eucalypt leaf essential oils, 4 fungi were tested. They were 2 strains of white rot of *T. versicolor* and *P. chrysosporium*, and 2 strains of brown rot of *P. schweintzii* and *L. sulphureus*. All of these fungi can cause decay in wood. In the preparatory experiments, we screened the MIC values of the essential oils against the decay fungi and established that a concentration of at least 10 mg disc^{-1} was required; hence, a 10 mg disc^{-1} concentration was employed to test

Table 2. IC_{50} values (mg $disc^{-1}$) of leaf essential oils from 4 *Eucalyptus* species against 7 mildew species

Fungal species	Essential oils			
	E. urophylla	*E. grandis*	*E. camaldulensis*	*E. citriodora*
Aspergillus clavatus	8.14	4.14	6.29	3.89
Aspergillus niger	> 20	9.30	14.49	6.98
Cladosporium cladosporioides	8.91	8.22	8.97	3.93
Chaetomium globosum	8.04	5.56	4.74	3.93
Myrothecium verrcaria	8.92	6.71	7.72	2.87
Penicillium citrinum	> 20	11.83	11.38	6.46
Trichoderma virdide	11.38	4.86	6.34	3.15

for fungal suppression indices and durability of the effectiveness. The results are shown in Fig. 2. For suppression of *T. versicolor*, at 7 d after inoculation, the leaf essential oil of *E. citriodora* exhibited the best antifungal index of 89.8%, followed by *E. grandis* at 62.4%, *E. urophylla* at 28.4%, and *E. camaldulensis* at 12.8%. After culture for 21 d, *E. citriodora* maintained a fair antifungal index, while the other leaf oils maintained the same order of performance. *E. camaldulensis* was the least effective, losing bioactivity against this fungus after 14 d. As for the growth suppression of *P. chrysosporium*, after 3 d of culture, except for *E. camaldulensis* oil which was capable of reaching an antifungal index of 87.1%, the 3 other essential oils had 100% efficacy. After 7 d, *E. citriodora* was still capable of 100% suppression, whereas *E. grandis* had dropped to 91.4% and *E. urophylla* to 24.7%. On the 14th day, *E. citriodora* still maintained its effectiveness, while the other 3 oils had all lost their suppression efficacy. Even after 21 d, *E. citriodora* was still 100% effective. On the suppression of *P. schweintizii*, the leaf essential oils of *E. citriodora* and *E. grandis*

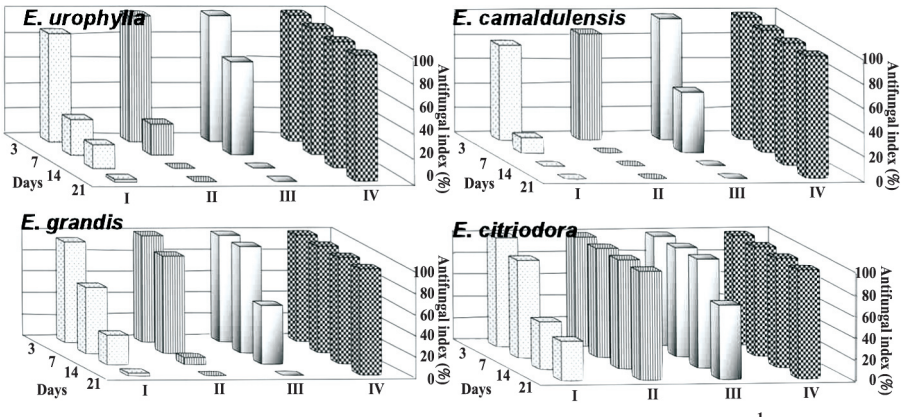

Fig. 2. Anti-wood-rot-fungal index (%) of the essential oils (10 mg $disc^{-1}$) from *Eucalyptus. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* leaves after 3, 7, 14, and 21 d of incubation.

*I, ***Trametes versicolor*; II, *Phanerochaete chrysosporium*; III, *Phaeolus schweinitizii*; IV *Lenzites sulphureus*.**

could still effect total suppression after 7 d of culture. *E. urophylla* was less effective, and *E. camaldulensis* was the poorest. After 21 d of continuous culture, *E. citriodora* leaf oil showed better durability, whereas the 3 other oils had all lost their activities. Regarding *L. sulphureus*, after 21 d of culturing, all leaf essential oils exhibited 100% suppression of fungal growth, indicating excellent bioactivity against this fungus.

The results of IC_{50} values of the 4 leaf oils against the 4 decay fungi indicate that once again (Table 3), *E. citriodora* leaf oil was the best, followed in order by *E. grandis*, *E. urophylla*, and *E. camaldulensis*, which was the least effective. All of these results suggest that the leaf essential oil of *E. citriodora* is an excellent anti-decay fungus substance.

In summary, the results of the anti-mildew and anti-decay fungus tests indicated that based on the most-dominant component of the 4 eucalypt leaf oils, 1,8-cineole, apparently was not a main player, as the proportions of this component showed no direct correlation with the antifungal performances. Thus, other components in the essential oils must have influenced the antifungal activities. These results are similar to the conclusions drawn by several other studies (Knobloch et al. 1989, Maria et al. 1989, Inouye et al. 2001, Cimanga et al. 2002). Those researchers proposed that the antifungal activities of the monoterpene hydrocarbons in the essential oils, such as α-pinene, β-pinene, *p*-cymene, limonene, myrcenes, and γ-terpinene, are generally not very effective. Based on our results, the monoterpene hydrocarbons content of the 4 leaf oils were in the order of *E. urophylla* > *E. camaldulensis* > *E. grandis* > *E. citriodora*; while the antifungal activities were in the order of *E. citriodora* > *E. grandis* > *E. camaldulensis* > *E. urophylla*. Thus the higher the content of monoterpene hydrocarbons was, the less effective the antifungal activities of the essential oils were, in congruency with the study results of the above-listed researchers.

It is also mentioned in the literature that certain oxygenated monoterpenes, such as citronellal, citronellol, nerol, geraniol, borneol, and genranial, and several phenolic compounds, such as thymol, eugenol, carvacrol, etc., all significantly contribute to the antifungal activities (Chaumont et al. 1989, Mahmoud et al. 1994, Viollon and Chaumont 1994, Griffin et al. 1999, Delespaul et al. 2000, de Billerbeck et al. 2001, Inouye et al. 2001). From the previous composition analyses, we know that the combined content of citronellal and citronellol in the leaf essential oil of *E. citriodora* was as high as 61%, whereas the 3 other eucalypts had no trace of these compounds. Thus, the champion of antifungal activities among the essential oils belongs rightly to *E. citriodora*, and citronellal and citronellol were the main contributors to such activities. Total phenolic compounds in the 4 essential oils were also analyzed but

Table 3. IC_{50} values (mg $disc^{-1}$) of leaf essential oils from 4 *Eucalyptus* species against 4 wood decay fungi

Fungal species	Essential oils			
	E. urophylla	*E. grandis*	*E. camaldulensis*	*E. citriodora*
Trametes versicolor	> 20	9.16	17.76	3.97
Phaneochaete chrysosporium	5.46	4.96	6.06	3.19
Phaeolus schweintizii	4.09	6.01	10.04	2.59
Lenzites sulphureu	4.21	3.37	4.29	1.00

showed no direct correlation with the antifungal activities. Thus, we deemed that for these 4 eucalypts, phenolic compounds in their essential oils were not important players in the antifungal activities.

As for the contribution of sesquiterpenes to the antifungal activities, no correlation was found between the performance and proportions of either sesquiterpene hydrocarbons or oxygenated sesquiterpenes. Furthermore, their proportional contents were low; hence we deemed that they had no relation to the antifungal activities of the essential oils.

CONCLUSIONS

In this study, we extracted the leaves of the 4 species of *Eucalyptus* trees of *E. urophylla*, *E. grandis*, *E. camaldulensis*, and *E. citriodora* using a hydrodistillation method. Yields of the oils were 3.48 mL 100 g^{-1} for *E. camaldulensis*, 3.14 mL 100 g^{-1} for *E. urophylla*, 3.01 mL 100 g^{-1} for *E. grandis*, and 1.89 mL 100 g^{-1} for *E. citriodora*. In the *E. urophylla* leaf essential oil, γ-terpiene and *p*-cymene were the main components; in *E. grandis* and *E. camaldulensis*, 1,8-cineole predominated; and in *E. citriodora*, citronellal and citronellol were the main compounds. All 4 oils had high proportions of monoterpenes; but the sesquiterpene contents were low.

In the anti-mildew tests, the leaf oil of *E. citriodora* showed the best efficacy and was widely effective against all tested fungi. *E. urophylla*, on the other hand, fared the worst in the tests. Thus, the leaf oil of *E. citriodora* can potentially be used in the preservation of cellulosic products, leather goods, and wood artifacts. It might also be used as an additive to drugs to counter the occurrence of asthma, pneumonia, peritonitis, etc. In the anti-decay fungus tests, *E. citriodora* again had the highest activities, and thus may be an excellent wood preservative. The oxygenated monoterpenes which it contained, such as citronellal and citronellol, were particularly effective against fungal activities. As eucalypts are often felled to supply pulpwood needs, the study suggests that leaves of the trees might also be collected to supply essential oils and fulfill the nonwood multi-purpose utilization of forest resources.

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LITERATURE CITED

Abbott SP, Sigler L, McAleer R, McGough DA, Rinaldi MG, Mizell G. 1995. Fatal cerebral mycoses caused by the ascomycete *Chaetomium strumarium*. J Clin Microbiol 33:2692-8.

Adams RP. 1995. Identification of essential oil components by gas chromatography-massspectroscopy. Carol Stream, IL: Allured. 302 pp.

Adams RP. 2001. Identification of essential oil components by gas chromatography / quadrupole mass spectroscopy. Carol Stream, IL: Allured. 456 pp.

Anandi V, John TJ, Walter A, Shastry JC, Lalitha MK, Padhye AA, Ajello L, Chandler FW. 1989. Cerebral phaeohyphomycosis caused by *Chaetomium globosum* in a renal transplant recipient. J Clin Microbiol 27:2226-9.

Azcan M, Kara M, Baser KHC, Kinmer N, Kurkcuoglu M, Ozek T, Gulbaba G, Aslan S, Gunses K. 1995. A systematic study of essential oils of Eucalyptus species grown in Turkey. In Proceedings of the 13th International Congress of Flavours and Fragrances and Essential Oil. Vol. 2., Istanbul, Turkey. p 181.

Benayache S, Benayache F, Benyahia S, Chalchat JC, Garry RP. 2001. Leaf oils of some Eucalyptus species growing in Algeria. J Essent Oil Res 13:210-3.

Bignell CM, Dunlop PJ, Brophy JJ. 1997. Volatile leaf oils of some Queensland and northern Australian species of the genus Eucalyptus (Series II) Part II. Subgenera (a) Blakella, (b) Corymbia, (c) Unnamed, (d) Idiogenes, (e) Monocalyptus and (f) Symphyomyrtus. Flav Fragr J 12:277-84.

Blyth W, Grant IW, Blackadder ES, Greenberg M. 1977. Fungal antigens as a source of sensitization and respiratory disease in Scottish maltworkers. Clin Allergy 7:549-62.

Blyth W. 1978. The occurrence and nature of alveolitis-inducing substances in *Aspergillus clavatus*. Clin Exp Immunol 32:272-82.

Brophy JJ, House APN, Boland DJ, Lassak EV. 1991. Digests of the essential oils of 111 species from northern and eastern Australia. In: Boland DJ, Brophy JJ, House APN, editors. Eucalyptus leaf oils -- use, chemistry, distillation and marketing. Melbourne: lnkata Press. p 29-155.

Carson CF, Riley TV. 1995. Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol 78:264-9.

Chaumont JP, Bardey I. 1989. Activites antifongiques in vitro de sept huiles essentielles. Fitoterapia 60:263-6.

Chouaki T, Lavarde V, Lachaud L, Raccurt CP, Hennequin C. 2002. Invasive infections due to Trichoderma species: report of 2 cases findings of in vitro susceptibility testing and review of the literature. Clin Infect Dis 35:1360-7.

Cimanga K, Apers S, Bruyne T, Van Miert S, Hermans N, Totte J, Pieters L, Vlietinck AJ. 2002. Chemical composition and antifungal activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Essent Oil Res 14:382-7.

Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, TottéL, Pieters J, Vlietinck AJ. 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 79:213-20.

Dagne E, Bisrat D, Alemayehu M, Worku T. 2000. Essential oils of twelve Eucalyptus species from Ethiopia. J Essent Oil Res 12:467-70.

de Billerbeck VG, Roques CG, Bessiere JM. 2001. Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Can J Microbiol 47:9-17.

Delespaul Q, de Billerbeck VC, Boques CG, Michel G, Marquier-Vluales C, Bessiere JM. 2000. The antifungal activity of essential oils as determined by different screening methods. J Essent Oil Res 12:256-66.

Dellacassa E, Menendez P, Moyna P. 1990. Chemical composition of Eucalyptus essential oils grown in Uruguay. Flav Fragr J 5:91-5.

Demetzos CN, Katerinopoulos H, Kouvarakis A, Stratigukis N, Loukis A, Ekomomakis C, Spiliotis V, Tsaknis J. 1997. Composition and antimicrobial activity of essential oil of *Cistus creticus* subsp. *eriocyhalus*. Planta Med 63:477-8.

Dethier M, Nduwimana A, Coerdier Y, Menut C, Lamaty G. 1994. Aromatic plants of tropical central Africa. XVI. Studies on essential oils of five Eucalyptus species grown in Burundi. J Essent Oil Res 6:469-73.

Faouzia H, Souad FT, Tantaoui-Elaraki A. 1993. Antimicrobial activity of twenty-one Eucalyptus essential oils. Fitoterapia 64:71-7.

Farah A, Fechtal M, Chaouch A, Zrira S. 2002. The essential oils of *Eucalyptus camaldulensis* and its natural hybrid (clone 583) from Morocco. Flav Fragr J 17:395-7.

Ferdous AJ, Islam SM, Ahsan M, Hasan CM, Ahmed ZV. 1992. In vitro antibacterial activity of the volatile oil of *Nigella satia* seeds against multiple drug resistant isolates of Shigella, *V. cholerae* and *E. coli*. Phytother Res 6:137-40.

Grant IW, Blackadder ES, Greenberg M, Blyth W. 1976. Extrinsic allergic alveolitis in Scottish maltworkers. Br Med J 1:490-93.

Griffin SG, Wyllie SG, Markham JL, Leach DN. 1999. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. Flav Fragr J 14:322-32.

Hammouchi M, Tantaoui-Elaraki A, Es-Safi N, Agoumi A. 1990. Mise en évidence des propriétés antibactériennes et antifongiques des huiles essentielles d'Eucalyptus. Plantes M éd Phytothér 24:278-9.

Hattori N, Adachi M, Kaneko T, Shimozuma M, Ichinohe M, Iozumi K. 2000. Case report. Onychomycosis due to *Chaetomium globosum* successfully treated with itraconazole. Mycoses 43:89-92.

Hinou JB, Harvala CE, Hinou EB. 1989. Antimicrobial activity of 32 common constituents of essential oils. Pharmazie 44:302-3.

Inouye S, Uchida K, Yamaguchi H, Miyara T, Gomi S, Amano M. 2001. Volatile aroma constituents of three labiatae herbs growing wild in the Karakoram-Himalaya district and their antifungal activity by vapor contact. J Essent Oil Res 13:68-72.

Kambu K, Di Phanzu N, Coune C, Wauters JN, Angenot L. 1982. Contribution a` l'étude des propriétés insecticides et chimiques *d' Eucalyptus saligna* du Zaý¨re. Plantes Méd Phytothér 16:34-8.

Kleinschmidt DBK. 2002. Central nervous system aspergillosis: a 20-year retrospective series. Hum Pathol 33:116-24.

Knobloch K, Pauli A, Lberl B, Weigand H, Weis N. 1989. Antibacterial and antifungal properties of essential oil components. J Essent Oil Res 1:119-28.

Kofujita H, Fujino Y, Sasaki T, Hasebe M, Ota M, Suzuki K. 2001. Antifungal activity of the bark of *Cryptomeia japonica* and its relevant components. Mokuzai Gakkaishi 47:479-86.

Lemos TLG, Matos FJA, Alencar JW, Craveiro AA, Clark AM, McChesney JD. 1990. Antimicrobial activity of essential oils of Brazilian plants. Phytother Res 4:82-4.

Mahmoud ALE. 1994. Antifungal action and antiaflatoxigenic properties of some essential oil constituents. Lett Appl Microbiol 19:110-3.

Maria LB, Deans SG, Eaglesham E. 1998. Relationship between bioactivity and chemical composition of commercial essential oils. Flav Fragr J 13:98-104.

Menut C, Lamaty G, Malanda Kiyabou G, Bessiere JM. 1992. Aromatic plants of tropical Central Africa. VIII. Individual selection of Eucalyptus for essential oil production in the Congo. J Essent Oil Res 4:427-9.

Naidu J, Singh SM, Pouranik M. 1991. Onychomycosis caused by *Chaetomium globosum* Kunze. Mycopathologia 113:31-4.

Naidu J. 1993. Growing incidence of cutaneous and fungal infections by non-dermatophyte fungi at Jabalpur (M.P.). Indian J Pathol Microbiol 36:113-8.

Ohtani Y, Kirishima M, Sameshima K. 2001. Effects of hydration on termiticidal and antifungal activities of wood essential oils. Mokuzai Gakkaishi 47:267-75.

Oloke JK, Kolawole BO, Erhun WO. 1988. The antibacterial and antifungal activities of certain components of *Aframomum melegueta*. Fitoterapia 59:384-8.

Onawunmi GO, Yisak WAB, Ogunlana EO. 1984. Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf. J Ethnopharmacol 12:279-86.

Pagula FP, Baser KHC, Kurkcuoglu M. 2000. Essential oil composition of *Eucalyptus*

camaldulensis Dehn. from Mozambique. J Essent Oil Res 12:333-5.

Prentice AV, Kibbler HG, Yeghen CC, Fenelon T, Campbell L, Warnock CK, et al. 1996. *Chaetomium pneumonia* in a patient with acute myeloid leukaemia. J Clin Pathol 49:184-6.

Rao BRR, Kaul PN, Syamasundar KV, Ramesh S. 2003. Comparative composition of decanted and recovered essential oils of *Eucalyptus citriodora* Hook. Flav Fragr J 18:133-5.

Shieh JC. 1996. Yields and chemical components of essential oils in *Eucalyptus camaldulensis* leaves. Taiwan J For Sci 11:149-57. [in Chinese with English summary].

Shieh JC. 1998. Yields and chemical components of essential oils in *Eucalyptus urophylla* leaves. Taiwan J For Sci 13:291-300. [in Chinese with English summary].

Singh AK, Brophy JJ, Gupta KC. 1988. The essential oil of *Eucalyptus urophylla*: a rich source of *p*-cymene. India Perfumer 32:201-4.

Tsiri D, Kretsi O, Chinou IB, Spyropoulos CG. 2003. Composition of fruit volatiles and annual changes in the volatiles of leaves of *Eucalyptus camaldulensis* Dehn. growing in Greece. Flav Fragr J 18:244-7.

Van den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11:463-71.

Viollon C, Chaumont JP. 1994. Antifungal properties of essential oils and their main components upon *Cryptococcus neoformans*. Mycopathologia 128:151-3.

Zakarya D, Fkih-Tetouani S, Hajji F. 1993. Chemical composition antimicrobial activity relationship of Eucalyptus essential oils. Plantes Méd Phytothér 26:331-9.

Zobel B. 1988. Eucalyptus in the forest industry. TAPPI J 71(12):42-6.