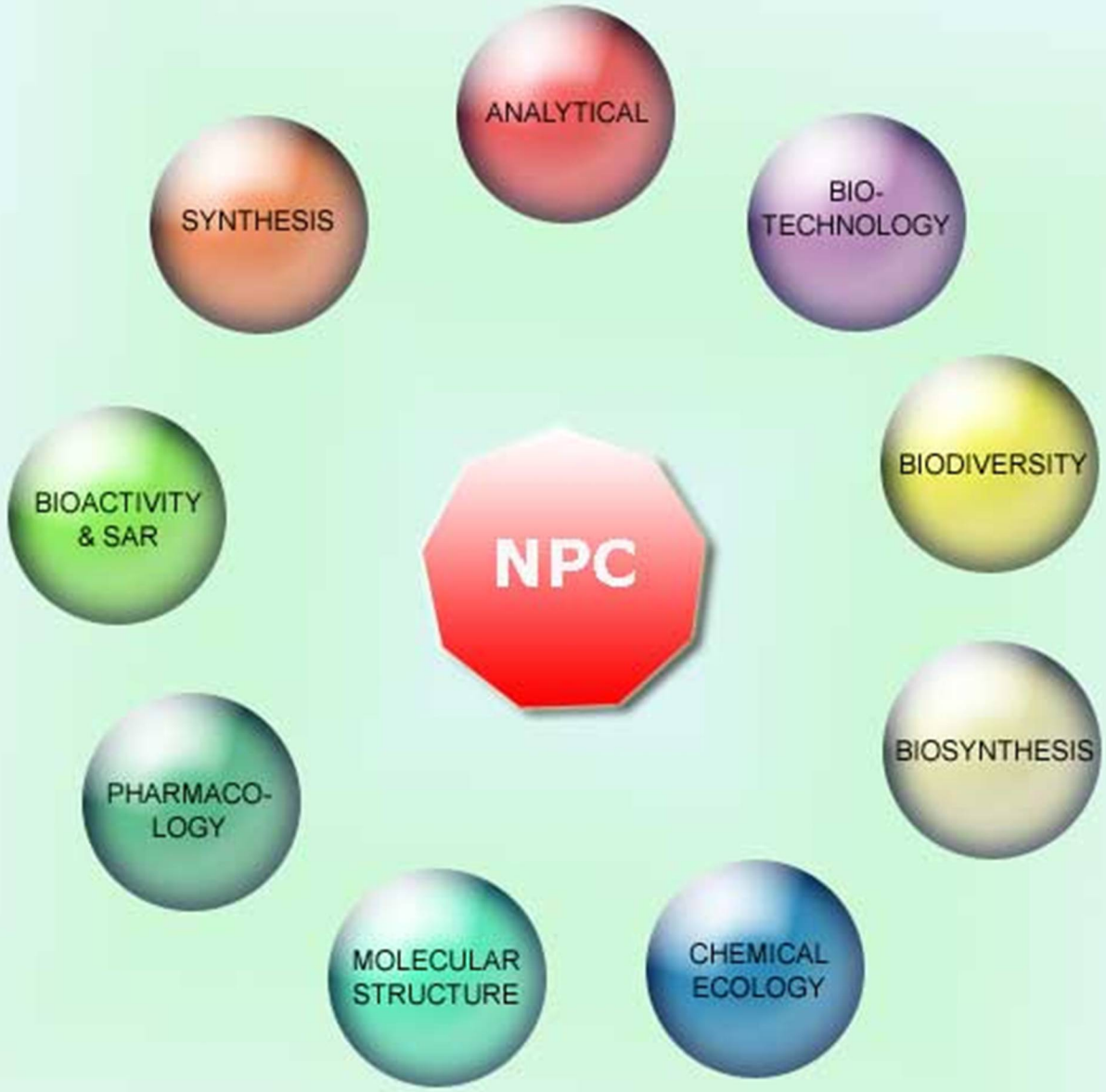
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 6. Issue 1. Pages 1-158. 2011
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

To Subscribe: Natural Product Communications is a journal published monthly. 2011 subscription price: US$1,995 (Print, ISSN# 1934-578X); US$1,995 (Web edition, ISSN# 1555-9475); US$2,495 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2011
Vol. 6
No. 1
133 - 136

Composition, Antioxidant and Antimicrobial Activities of the Seed Essential Oil of *Calocedrus formosana* from Taiwan

Chen-Lung Ho[a,b], Yen-Hsueh Tseng[a], Eugene I-Chen Wang[a], Pei-Chun Liao[c], Ju-Ching Chou[c], Chien-Nan Lin[c] and Yu-Chang Su[a*]

[a]*Division of Wood Cellulose, Taiwan Forestry Research Institute. 53, Nanhai Rd., Taipei, Taiwan 100*

[b]*Department of Forestry, National Chung Hsing University, 250 Kuo Kuang Rd., Taichung, Taiwan 402*

[c]*Institute of Biotechnology, National Ilan University, 1 Shen-Lung Rd., Ilan, Taiwan 260*

ycsu@nchu.edu.tw

Received: October 9th, 2010; Accepted: October 27th, 2010

The hydrodistillated seed essential oil of *Calocedrus formosana* was analyzed to determine its composition and yield. Twenty-seven compounds were identified, the main ones being α-pinene (63.8%), totarol (9.9%) and ferruginol (8.9%). Monoterpene hydrocarbons (73.5%) and oxygenated diterpenes (18.8%) were the predominant groups of compounds. The seed essential oil exhibited excellent antioxidant, antimicrobial and anti-wood-decay fungal activities.

Keywords: *Calocedrus formosana*, Cupressaceae, essential oil, antioxidant activity, antimicrobial activity, anti-wood-decay fungal activity, totarol, ferruginol.

Calocedrus formosana Florin (Cupressaceae) is one of the five most valuable conifers in Taiwan [1]. Many previous studies have demonstrated that the leaf, bark and heartwood essential oils and extractives of *C. formosana* have inhibitory effects against termites, mildew, and fungi, as well as functioning as an antioxidant, anti-inflammatory, and anti-mosquito larvicide [2a-2f]. However, no prior study has investigated the chemical composition and biological activity of the essential oil of *C. formosana* seed. Thus, this was obtained by hydrodistillation, analyzed for its chemical composition, and evaluated for its antioxidant, antimicrobial and anti-wood-decay fungal activities.

A dark-yellow oil was obtained from the seeds in a yield of 3.4 ± 0.03%. Twenty-seven compounds were identified (Table 1), of which monoterpene hydrocarbons were predominant (73.5%), followed by oxygenated diterpenes (18.8%), oxygenated monoterpenes (4.9%), non-terpenoids (2.2%), and monoterpene hydrocarbons (0.7%). Among the monoterpene hydrocarbons, α-pinene (63.8%) was the major compound, and of the oxygenated diterpenes, totarol (9.9%) and ferruginol (8.9%) were the chief components.

The seed essential oil of *C. formosana* was tested for its DPPH free radical scavenging capability. Ascorbic acid was used as a positive control. The IC_{50} of the DPPH free radical scavenging capability of the seed essential oil was 81.3 μg/mL. The individual main components of the seed essential oil, α-pinene, totarol and ferruginol, were also compared for their DPPH free radical scavenging capability. The results showed that the DPPH free radical scavenging capabilities in a decreasing order were totarol (IC_{50} = 33.7 μg/mL), ferruginol (IC_{50} = 48.0 μg/mL) and α-pinene (IC_{50} > 2000 μg/mL). Hence, we deduced that the phenolic diterpene compounds were mainly responsible for the radical scavenging. The results are also in congruency with the conclusions of several other reports [2g-2i]. When the DPPH free radical scavenging capabilities of the seed essential oil were compared with those of leaf oils of different provenances from Taiwan, such as cinnamon (*Cinnamomum osmophloeum*), with IC_{50} values ranging from 33.4 to 708.5 μg/mL [3a], the seed essential oil was within the same range. The threshold concentration also compared favorably with the IC_{50} values of 460 μg/mL for the leaf oil of black seed oil (*Nigella sativa*) [3b], 460 μg/mL for the flower oil of oregano (*Origanum vulgare*) [3c], and 500 μg/mL for the leaf oil of turmeric (*Curcuma zedoaria*) [3d].

The seed essential oil of *C. formosana* was tested against three Gram-positive and five Gram-negative bacteria, as well as two fungi. The results, presented in Table 2, show medium to strong growth suppression against all ten microbes studied. The most sensitive were *Bacillus cereus*, *Staphylococcus aureus*, *S. epidermidis*, and *Candida*

Table 1: Chemical composition of the seed essential oil of *Calocedrus formosana*.

Compound ID	KI [a]	Conc. (%)	Identification [b]
Tricyclene	927	0.4	KI, MS, ST
α-Pinene	939	63.8	KI, MS, ST
α-Fenchene	953	0.4	KI, MS, ST
Camphene	954	0.8	KI, MS, ST
β-Pinene	979	1.0	KI, MS, ST
β-Myrcene	991	2.9	KI, MS, ST
α-Terpinene	1017	0.5	KI, MS, ST
p-Cymene	1025	0.4	KI, MS, ST
Limonene	1029	2.9	KI, MS, ST
(*E*)-β-Ocimene	1050	0.4	KI, MS, ST
exo-Fenchol	1122	0.3	KI, MS, ST
α-Campholenal	1126	0.6	KI, MS, ST
trans-Pinocarveol	1139	0.2	KI, MS, ST
Camphor	1146	0.5	KI, MS, ST
Camphene hydrate	1150	0.3	KI, MS, ST
iso-Pulegol	1150	0.6	KI, MS, ST
trans-3-Pinanone	1163	0.1	KI, MS, ST
Borneol	1169	0.8	KI, MS, ST
Terpinen-4-ol	1177	0.4	KI, MS, ST
α-Terpineol	1189	0.2	KI, MS, ST
4-Methylene-isophorone	1218	0.4	KI, MS, ST
Bornyl acetate	1289	0.5	KI, MS, ST
n-Nonadecane	1900	2.2	KI, MS, ST
Pimaradiene	1950	0.4	KI, MS, ST
Abietatriene	2057	0.2	KI, MS, ST
Totarol	2314	9.9	KI, MS, ST
Ferruginol	2332	8.9	KI, MS, ST
Compound identified			
Monoterpene hydrocarbons		73.5	
Oxygenated monoterpenes		4.9	
Sesquiterpene hydrocarbons		0.0	
Oxygenated sesquiterpenes		0.0	
Diterpene hydrocarbons		0.7	
Oxygenated diterpenes		18.8	
Others		2.2	
Yield (mL/100g)		3.38 ± 0.03	

[a] Retention index on a DB-5 column with reference to *n*-alkanes [4].

[b] MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds.

albicans, with inhibition zones of 40- 56 mm and MIC values of 31.25- 125 μg/mL, respectively. The essential oil demonstrated stronger growth suppression of Gram-positive bacteria than Gram-negative bacteria and fungi.

These observations are similar to those reported [5a-5h]. In comparison with the antimicrobial activity of the essential oils from *Metasequioa glyptostroboides* [5c], *Litsea kostermansii* [5d], *L. nakaii* [5e], *L. laevigata* [5f], *Cinnamomum subavenium* [5g] and *Machilus pseudolongifolia* [5h], the antimicrobial activity of the seed essential oil of *C. formosana* was superior. The results validated the excellent antimicrobial activity of *C. formosana* seed essential oil.

However, to ascertain the source compounds of the antimicrobial activity of *C. formosana* seed essential oil, the main components were individually tested for antimicrobial activity. Results indicated that the active compounds were totarol and ferruginol. Various studies support the argument that these compounds are highly active in suppressing microbial growth [5a,5b].

The seed essential oil was also tested against two white rot fungi (*Trametes versicolor, Phanerochaete chrysosporium*) and two brown rot fungi (*Phaeolus schweinitzii, Lenzites sulphureus*). The anti-wood-decay fungal indices presented in Table 3 clearly demonstrate the excellent anti-wood-decay fungal activity of the seed essential oil of *C. formosana*. Growth of *T. versicolor, Phane. Chrysosporium, Phaeo. schweintizii* and *L. sulphureus* were completely inhibited at concentrations of 100, 75, 75, and 50 μg/mL, respectively. The anti-wood-decay fungal activity of the seed essential oil of *C. formosana* was superior to the essential oils of *Chamaecyparis formosensis* [6a] and *Cryptomeria japonica* [6b].

In order to ascertain the source compounds of the *C. formosana* seed essential oil, we also tested the anti-wood-decay fungal activities of its major componentS. The results indicated that the sources of activity were also totarol and ferruginol. The IC_{50} values of the two compounds against the four decay fungi were 18 and 58 μg/mL against *T. versicolor*; 26 and 42 μg/mL against *Phane. chrysosporium*; 28 and 33 μg/mL against *Phaeo. shweinitzii*; and 20 and 28 μg/mL against *L. sulphureu*, respectively. At a 50 μg/mL concentration, totarol showed total growth inhibition against all the white-rot and brown-rot fungi tested, while ferruginol at 50 μg/mL could partially inhibit white-rot and brown-rot fungi. The results agree with those of Rudman [6c] and Chang *et al.* [6d]. Thus, the excellent wood-decay-fungi inhibitive activities exhibited by *C. formosana* seed essential oil could be attributed to the presence of compounds such as totarol and ferruginol.

Table 2: Antimicrobial activity of the seed essential oil of *C. formosana*.

Microbial species	*C. formosana*		Compounds						Antibiotics		
			1	2	3	4	5	6	Tetracycline (30 μg/disk)	Gentamicine (10 μg/disk)	Nystatine (30 μg/disk)
	IZ	MIC	MIC	MIC	MIC	MIC	MIC	MIC	IZ	IZ	IZ
Bacillus cereus	56 ± 0.8	31.25	>1000	>1000	>1000	>1000	1.95	15.625	22 ± 0.8	-	nt
Staphylococcus aureus	48 ± 0.4	31.25	>1000	>1000	>1000	>1000	1.95	62.5	21 ± 0.4	-	nt
Staphylococcus epidermidis	52 ± 0.4	31.25	>1000	>1000	>1000	>1000	1.95	15.625	34 ± 0.4	-	nt
Escherichia coli	32 ± 0.8	375	>1000	>1000	>1000	>1000	125	250	-	22 ± 0.8	nt
Enterobacter aerogenes	32 ± 0.8	375	>1000	>1000	>1000	>1000	125	250	10 ± 0.4	-	nt
Klebsiella pneumoniae	29 ± 0.4	500	>1000	>1000	>1000	>1000	250	375	-	21 ± 0.8	nt
Pseudomonas aeruginosa	32 ± 0.8	375	>1000	>1000	>1000	>1000	125	250	-	12 ± 0.8	nt
Vibrio parahaemolyticus	29 ± 0.4	500	>1000	>1000	>1000	>1000	250	375	-	13 ± 0.8	nt
Aspergillus niger	28 ± 0.4	500	>1000	>1000	>1000	>1000	375	375	nt	nt	17 ± 0.8
Candida albicans	40 ± 0.4	125	>1000	>1000	>1000	>1000	15.625	62.5	nt	nt	19 ± 0.8

[a] Inhibition zone diameter (mm), including diameter of sterile disk 6 mm; values are given as mean ± SD. [b] Minimum inhibitory concentration values as μg/mL. [c] 1. α-Pinene (≥ 98.5%), 2. β-Myrcene (≥ 98.5%), 3. Limonene (≥ 98.5%), 4. *n*-Nonadecane (≥ 98%), 5. Totarol (≥ 98%), 6. Ferruginol (100%), Compounds 1 to 5 were purchased from the Fluka Co. (Milwaukee, USA), whereas compound 6 was from an isolate of Ho *et al.*'s study of *Cryptomeria japonica* essential oil [6e]. Essential oil tested at 15 μL/disc for bacteria and 30 μL/disc for fungi.(-), Inactive; (7-14), moderately active; (>14), highly active; nt, not tested.

Table 3: Anti-wood-decay fungal indices of seed essential oil from *C. formosana*.

Dosage (μg/mL)	Antifungal index (%)			
	Trametes versicolor	*Phanerochaete chrysosporium*	*Phaeolus schweintizii*	*Lenzites sulphureus*
12.5	36 ± 3.3	28 ± 3.3	32 ± 3.3	38 ± 3.3
25	58 ± 6.6	58 ± 3.3	53 ± 3.3	82 ± 3.3
50	81 ± 6.6	82 ± 3.3	86 ± 6.6	100 ± 0
75	92 ± 6.6	100 ± 0	100 ± 0	100 ± 0
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0

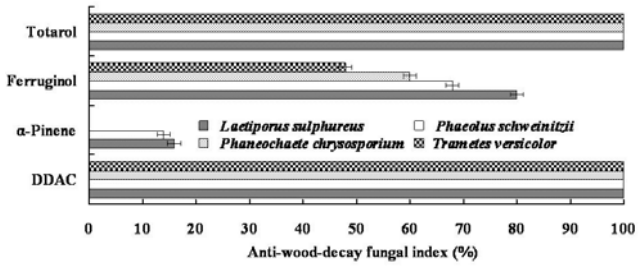


Figure 1: Anti-wood-decay fungal indices of the three main compounds (50 μg/mL) of the seed essential oil of *C. formosana*.

Experimental

Plant materials: Fresh seeds of *C. formosana* were collected in October 2009 from Chilan Mt in northeast Taiwan (Yilan County, elevation 850 m, N 24º 40′ 50″, 121º 39′ 10″). The samples were compared with specimen no. ou 5886 from the Herbarium of National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH- 010) was deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the seed essential oil: Seeds of *C. formosana* (1 Kg) were placed in a round-bottom flask and hydrodistilled for 8 h with 3 L of distilled water. The essential oil obtained was dried with anhydrous sodium sulfate. The oil yield and all test data are the average of triplicate analyses.

Essential oil analysis and component identification: The experimental conditions for GC analysis of the essential oil were similar to those reported earlier [5h]. Identification of the oil constituents was based on comparisons of retention index (RI) [7a], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [4,7b], respectively.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test: The method of Ho *et al*. [6e] was used for DPPH assay in this study. Fifty μL of various dilutions of the oils were mixed with 5 mL of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was determined at 517 nm using a Jasco 7800 spectrophotometer. Tests were carried out in triplicate, and ascorbic acid was used as a positive control.

Antimicrobial activity [8]. Discs containing 15 μL and 30 μL of the oil dissolved in dimethylsulfoxide (DMSO) were placed on the inoculated plates with test microorganisms. Growth inhibition zones (including disc diameter of 6 mm) were measured after 24 h and 48 h of incubation at 37°C and 24°C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria, and nystatin for fungi were used as positive controls. Microbial strains were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. The microbial strains included 5 Gram-negative bacteria: *Escherichia coli* (IFO 3301), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (IFO 3080), and *Vibrio parahaemolyticus* (ATCC 17803); 3 Gram-positive bacteria: *B. cereus* (ATCC 11778), *S. aureus* (ATCC 6538P), and *S. epidermidis* (ATCC 12228); 1 fungus: *A. niger* (ATCC 16404) and 1 yeast: *C. albicans* (ATCC 10231). Minimum inhibitory concentration (MIC) values were measured by the microdilution broth susceptibility assay recommended by NCCLS [9] and as reported earlier [5h].

Anti-wood-decay fungal assays: The method of Su *et al.* [10] was adopted. The fungi used were *T. versicolor* (BCRC 35253), *Phane. chrysosporium* (BCRC 36200), *Phaeo. schweinitzii* (BCRC 35365) and *L. sulphureus* (BCRC 35305). Microbial strains were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. Anti-wood-decay fungal assays were carried out in triplicate and the data were averaged. Different concentrations of the essential oil (12.5~100 μg/mL) were added to sterilized potato dextrose agar (PDA). The test plates were incubated at 27ºC. When the mycelium of the fungi reached the edge of the control plate, the anti-wood-decay fungal index was calculated as follows: Anti-wood-decay fungal index (%)= (1–Da/Db) X 100, where Da is the diameter of the growth zone in the experimental dish (cm) and Db is the diameter of the growth zone in the control dish (cm). DDAC (didecyl dimethyl ammonium chloride) was used as a positive control.

References

[1] Kuo PC. (**1995**) *The Precious 5 Conifers of Taiwan*. Chinese Forestry Association, Taipei, Taiwan.

[2] (a) Chang HT, Cheng SS, Chang ST, Su YC, Tasi KH, Chen WJ. (**2003**) Mosquitocidal activity of leaf essential oil and its components from *Calocedrus formosana*. *Quarterly Journal of Chinese Forestry*, ***36***, 73-79; (b) Chao KP, Hua KF, Hsu HY, Su YC, Chang ST. (**2005**) Anti-inflammatory activity of sugiol, a diterpene isolated from *Calocedrus formosana* bark. *Planta Medica*, ***71***, 300-305; (c) Cheng SS, Wu CL, Chang HT, Kao YT, Chang ST. (**2004**) Antitermitic and antifungal activities of essential oil of

Calocedrus formosana leaf and its composition. *Journal of Chemical Ecology*, ***30***, 1957-1967; (d) Chang HT, Cheng YH, Wua CL, Chang ST, Chang TT, Su YC. (**2008**) Antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* Florin leaf against plant pathogenic fungi. *Bioresource Technology*, ***99***, 6266-6270; (e) Yen TB, Chang HT, Hsieh CC, Chang ST. (**2008**) Antifungal properties of ethanolic extract and its active compounds from *Calocedrus macrolepis* var. *formosana* (Florin) heartwood. *Bioresource Technology*, ***99***, 4871-4877; (f) Wang SY, Wu JH, Cheng SS, Lo CP, Chang HN, Shyur LF, Chang ST. (**2004**) Antioxidant activity of extracts from *Calocedrus formosana* leaf, bark, and heartwood. *Journal of Wood Science*, ***50***, 422-426; (g) Wang SY, Wu JH, Shyur LF, Kuo YH, Chang ST. (**2002**) Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. *Holzforschung*, ***56***, 487-492; (h) Halliwell B. (**1997**) Antioxidants and human diseases: A general introduction. *Nutrition Reviews*, ***55***, S44-S52; (i) Haraguchi H, Ishikawa H, Kubo I. (**1997**) Antioxidative action of diterpenoids from *Podocarpus nagi*. *Planta Medica*, ***63***, 213-215.

[3] (a) Chen IY. (**2003**) *The antioxidative properties of leaf essential oils of Cinnamomum osmophloeum*. MS Thesis, Dept. of Food Tech., Natl. Taiwan Univ., Taipei, Taiwan; (b) Burits M, Bucar F. (**2000**) Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research*, ***14***, 323-328; (c) Kulisic T, Radonic A, Katalinic V, Milos M. (**2004**) Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, ***85***, 633-640; (d) Mau JL, Lai EYC, Wang NP, Chen CC, Chang CH, Chyau CC. (**2003**) Composition and antioxidant activity of the essential oil from *Curcuma zedoaria*. *Food Chemistry*, ***82***, 583-591.

[4] Van den Dool H, Kratz PD. (**1963**) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography*, ***11*,** 463-471.

[5] (a) Becerra J, Flores C, Mena J, Aqueveque P, Alarcón J, Bittner M, Hernández V, Hoeneisen M, Ruiz E, Silva M. (**2002**) Antifungal and antibacterial activity of diterpenes isolated from wood extractables of Chilean Podocarpaceae. *Boletín de la Sociedad Chilena de Química*, ***47***, 151-157; (b) Solís C, Becerra J, Flores C, Robledo J, Silva M. (**2004**) Antibacterial and antifungal terpenes from *Pilgerodendron uviferum* (D. Don) Florin. *Journal of the Chilean Chemical Society*, ***49***, 157-161; (c) Bajpai VK, Sharif MA, Choi UK, Lee JH, Kang SC. (**2009**) Chemical composition, antibacterial and antioxidant activities of leaf essential oil and extracts of *Metasequioa glyptostroboides* Miki ex Hu. *Food and Chemical Toxicology*, ***47***, 1876-1883; (d) Ho CL, Wang EIC, Hsu KP, Lee PY, Su YC. (**2009**) Composition and antimicrobial activities of the leaf essential oil of *Litsea kostermansii* from Taiwan. *Natural Product Communications*, ***4***, 1123-1126; (e) Ho CL, Wang EIC, Lee PY, Su YC. (**2009**) Composition and antimicrobial activities of the leaf essential oil of *Litsea nakaii* from Taiwan. *Natural Product Communications*, ***4***, 865-868; (f) Arif, MM, Raj SM, Jirovetz L, Shafi MP. (**2008**) Composition and antimicrobial analysis of the essential oil of *Litsea laevigata* Nees. (Lauraceae). *Natural Product Communications*, ***3***, 1069-1072; (g) Ho CL, Wang EIC, Wei XT, Lu SY, Su YC. (**2008**) Composition and bioactivities of the leaf essential oils of *Cinnamomum subavenium* Miq. from Taiwan. *Journal of Essential Oil Research*, ***20***, 328-334; (h) Ho CL, Liao PC, Wang EIC, Dong WZ, Su YC. (**2010**) Composition and antimicrobial and anti-wood-decay fungal activities of the leaf essential oils of *Machilus pseudolongifolia* from Taiwan. *Natural Product Communications*, ***5***, 1143-1146.

[6] (a) Wang SY, Wu CL, Chu FH, Chien SC, Kuo YH, Shyur LF, Chang ST. (**2005**) Chemical composition and antifungal activities of essential oil isolated from *Chamaecyparis formosensis* Matsum. wood. *Holzforschung,* ***59***, 295-299; (b) Cheng SS, Lin HY, Chang ST. (**2005**) Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cryptomeria japonica*). *Journal of Agricultural and Food Chemistry*, ***53***, 614-619; (c) Rudman, P. (**1965**) The causes of natural durability in timber. Pt. XVII. The causes of decay and termite resistance in *Callitris columellaris* F. Muell. *Holzforschung*, ***19***, 52-57; (d) Chang ST, Wang SY, Wu CL, Su YC, Kuo YH. (**1999**) Antifungal compounds in the ethyl acetate soluble fraction of the extractives of Taiwania (*Taiwania cryptomerioides* Hayata) heartwood. *Holzforschung*, ***53***, 487-490; (e) Ho CL, Wang EIC, Yu HT, Yu HM, Su YC. (2010) Compositions and antioxidant activities of essential oils of different tissues from *Cryptomeria japonica* D. Don. *Quarterly Journal of Chinese Forestry*, ***32***, 63-76.

[7] (a) Adams RP. (**2001**) *Identification of Essential Oil Components by Gas Chromatography/Quadruple Mass Spectroscopy*, Allured, Carol Stream, IL; (b) Massada Y. (**1976**) *Analysis of Essential Oil by Gas Chromatography and Spectrometry*, Wiley, New York.

[8] Baron EJ, Finegold SM. (**1990**) Methods for testing antimicrobial effectiveness. In: *Diagnostic Microbiology*. Stephanie M. (Ed.). Baltimore, Mosby. 171-194.

[9] NCCLS (**1999**) *National Committee for Clinical Laboratory Standards*. Performance standards for antimicrobial susceptibility testing. 9th International Supplement, Wayne PA., M100-S9.

[10] Su YC, Ho CL, Wang EIC, Chang ST. (**2006**) Antifungal activities and chemical compositions of essential oils from leaves of four Eucalypts. *Taiwan Journal of Forest Science*, ***21***, 49-61.

Review/Account

Natural Product Communications
2011
Volume 6, Number 1

Contents

Continued Overleaf