研究報告

柳杉 (*Cryptomeria japonica* D. Don) 各部位精油組成分及抗氧化活性之探討

何振隆 [1,2] 王益眞 [3] 游琇婷 [4] 游漢明 [5] 蘇裕昌 [6*]

【摘要】本研究探討以水蒸餾法及頂空間氣相層析 (Headspace-GC) 等二方法萃取柳杉 (*Cryptomeria japonica* D. Don)各部位精油，分析其組成化合物及收率，並評估各精油之抗氧化活性。由水蒸餾法取得之葉、心材、邊材、枝條及樹皮精油，分別鑑定出 53、44、50、87 及 60 個化合物，而以 Headspace-GC取得之各部位揮發性化合物，分別鑑定出 38、35、46、61 及 46 個化合物，且其主成分及收率均與水蒸餾法所得之精油相若。葉精油主要組成分爲 kaurene (19.1%)、α-pinene (16.5%)、elemol (16.3%)等。心材精油主要組成分爲 1-epi-cubenol (18.9%)、δ-cadinene (17.2%)、cubenol (14.9%)等。邊材精油主要組成分爲 cubebol (19.5%)、cubenol (13.3%)、1-*epi*-cubenol (12.5%) 及 ferruginol (10.8%)等。樹皮精油主要組成分爲 camphor (46.5%)、α-pinene (16.2%) 等。柳杉枝條精油主要組成分爲α-eudesmol (25.2%)、γ-eudesmol (11. 8%) 等。柳杉葉、心材、邊材及枝條之精油均以倍半萜含氧化合物量爲最多，而樹皮爲以單萜含氧化合物爲主。柳杉各部位之抗氧化能力依序爲邊材＞枝條＞心材＞樹皮＞葉，於邊材部位精油成分中，以TLC分離各區段後，證實 ferruginol 有最佳之抗氧化活性，推論柳杉邊材精油，可做爲天然抗氧化劑之應用。

【關鍵詞】抗氧化活性、柳杉、精油、鐵鏽醇、頂空間採樣氣相層析儀

Research paper

Compositions and antioxidant activities of essential oils of different tissues from *Cryptomeria japonica* D. Don

Chen-Lung Ho[1,2] Eugene I-Chen Wang[3] Hsiu-Ting Yu[4] Han-Ming Yu[5] Yu-Chang Su [6*]

1. 林業試驗所木材纖維組副研究員
Associate Scientist, Division of Wood Cellulose, Taiwan Forestry Research Institute.
2. 國立中興大學森林學系博士班研究生
Graduate Student, Dept. of Forestry, National Chung Hsing University.
3. 林業試驗所木材纖維組研究員
Senior Scientisi, Division of Wood Cellulose, Taiwan Forestry Research Institute.
4. 中國醫藥大學藥用化妝品學系
Department of Cosmeceutics, China Medical University.
5. 林業試驗所六龜研究中心副研究員
Associate Scientist, Liouguei Research Center, Taiwan Forestry Research Institute.
6. 國立中興大學森林學系教授，通訊作者
Professor, Dept. of Forestry, National Chung Hsing University, Corresponding Author.

【**Abstract**】Both hydrodistillation and headspace-GC methods were applied to extract essential oils from leaves, heartwood, sapwood, twig, and bark of *Cryptomeria japonica* D. Don and their chemical compositions determination and oil yields were analyzed. The antioxidant activities of these oils were also evaluated. The hydrodistillated leaves, heartwood, sapwood, twig, and bark essential oils contained 53, 44, 50, 87, and 60 identified compounds, respectively. Whereas the headspace methods separated from those parts 38, 35, 46, 61 and 46 identified compounds, respectively. Both methods showed similar main components and yields. The main composition of leaf essential were kaurene (19.1%), α-pinene (16.5%) and elemol (16.3%). Its heartwood oil had 1-epi-cubenol (18.9%), δ-cadinene (17.2%), andcubenol (14.9%) as the main ingredients; the sapwood oil contained cubebol (19.5%), cubenol (13.3%), 1-epi-cubenol (12.5%) and ferruginol (10.8%) as the main components; the bark oil, however, contained camphor (46.5%) and α-pinene (16.2%) etc.; and α-eudesmol (25.2%), γ-eudesmol (11.8%) in the twig oil. Essential oils from the leaves, heartwood, sapwood, and twigs of the species were predominately oxygenated sesquiterpenes, while the bark oil was mostly oxygenated monoterpenes. The antioxidant activities of these oils in a decreasing order was sapwood > twig > heartwood > bark > leaves. After TLC fractionation of the sapwood oil, we found that ferruginol processed the highest antioxidant activity, and may be applicable as a natural antioxidant.

【**Key words**】antioxidant activity, *Cryptomeria japonica*, essential oil, ferruginol, headspace-GC

I. INTRODUCTION

Sugi, *Cryptomeria japonica* D. Don (*Taxodiaceae*), originally grows in Japan and is one of its most representative plantation tree species. It is a fast growth tree with the good wood workability. Due to its excellent wood properties, it is well received in Japan and other Asian countries. The sugi wood is reputed to have various bioactivities such as antifungal (Morita *et al*., 1991, 1997; Kofujita *et al*., 2001, 2006; Cheng *et al*., 2005; Matsushita *et al*., 2006; Cha *et al*., 2007); antibacterial (Matsushita *et al*., 2006; Cha *et al*., 2007; Li et al., 2008), antitermitic (Yatagi *et al*. 1991; Sogabe *et al*., 2000a, b; Cheng and Chang, 2002); antimite (Morita *et al*., 1991; Morita and Yatagi, 1994), antifeedant (Chen *et al*., 2001a, b; Kashiwagi *et al*., 2007), repellent (Morisawa *et al*., 2002), anti-ulcer (Matsunaga *et al*., 2000), insecticidal (Wang *et al*., 2006; Cheng *et al*., 2009). However, to the best of our knowledge, there is rare reported on the antioxidant activities of the essential oils from this specie. In our current study, the volatile components in the different parts of sugi, including leaf, heartwood, sapwood, twigs and bark were collected by using hydrodistillation and headspace-GC (HS-GC) methods. Following, GC-FID and GC-MS analysis, the composition of volatile compounds were evaluated. Furthermore, to determine the essential oil yields, a multiple headspace extraction (MHE) method was employed. Finally, the antioxidative activity of essential oils were also study in this study assay. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

II. EXPERIMENTALS

(I) Materials

Sugi logs of 50 years old were harvested

from a stand in Chilan Mountain, in northeastern part of Taiwan. The leaves were collected separately, and the stem divided on the site into heartwood, sapwood, twigs and bark. Materials for samples were then shipped to the Taipei lab of Taiwan Forestry Research Institute for extracting the essential oils and conducting subsequent works.

(II) Methods

a. Extraction of essential oils and compositional and yield determinations

(a) Hydrodistillation

A kilogram each of the leaf, heartwood, sapwood, bark and twigs was placed in a round-bottom flask and added with 3 L of distilled water. The water was heated to boil and refluxed for 8 h. The essential oil layer above the water layer was separated and added with anhydrous sodium sulfate to dewater. The essential oils obtained were placed in specimen bottles and the yields determined. Each test was repeated three times and the data were averaged.

(b) GC and GC-MS Analysis

A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness, J&W Scientific) and a FID detector was used for the quantitative determination of oil components. Oven temperature was programmed as follows: 50℃ for 2 min, rising to 250℃ at 5 ℃/min. Injector temperature: 270℃. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250℃, split ratio: 1:10. One μL sample was injected. Identification of the oil components was based on their retention indices and mass spectra, obtained from GC/MS analysis on a Hewlett-Packard HP 6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μm film thickness, J&W Scientific). The GC analysis parameters were the ones listed above and the MS was operating (full scan mode: scan time: 0.3 s, mass range was *m/z* 30-500) in the EI mode at 70 eV. All test data are the average of triplicate analyses.

(c) HS-GC analysis

The leaf, heartwood, sapwood, bark and twigs were cut into small pieces with scissors or a handsaw just prior to the headspace sampling. Each sample (20 mg) was filled into a 20 ml vial respectively, and then the vials were hermetically sealed with a PTFE-coated rubber septum and an aluminum cap. A Perkin Elemer Headspace Turbomatrix 40 unit connected to a Hewlett-Packard HP6890 GC was used for the analysis. The Headspace analysis programs and conditions were as follows: The vial oven temperature was 100℃ for each analysis as transfer line (110℃), and the needle temperature was 110℃. Treatment in oven with a shaker lasted 50 min. Pressurization time was 3.0 min; the thermostaiting time was 50 min; and the injection volume was 10 μl. The GC and GC-MS analysis programs used were the same as the above section. Each test was repeated three times and the data were averaged (Ho *et al*., 2008).

(d) Oil yields determined by MHE method

The total amount of oil in each sample was determined by HS-GC. Calibration curves were made with different quantities of different tissues essential oils previously obtained by hydrodistillation. A special quantitative method, MHE, was used. According to Kolb (1985) and Ho *et al*., (2008), the matrix effect can be eliminated by using the MHE method. The total area of each oil volume was calculated according to the following equation:

$\Sigma A = A_1^2/(A_1 - A_2)$ .....(a)

Where: ΣA is the total area; A_1 is the first area value; A_2 is the second area volume from 2 successive chromatograms.

(e) Identification of the components

Identification of the leaf essential oil chemical constituents was based on comparisons of the peaks Retention indices (RI) (Van den Dool and Kratz, 1963), their retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra and literature (Massada, 1976; Adams, 2001).

b. Determination of antioxidative activity

(a) DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capability test

The method of Cuendet *et al*. (1997), Kirby and Schmidt (1997), Burits *et al*. (2001) and Ho *et al*. (2008) 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 triplicates, and ascorbic acid was used as a positive control.

(b) TLC analysis and determination of the DPPH staining method

After activating a 20×20 cm TLC plate, we dotted 3 μl of sapwood essential oil on the plate and developed the plate using a solution of hexane : ethyl acetate=95:5. The plate was dried and observed under a UV light, and the color changes of the separated components were recorded. And then, the plate was immersed in the DPPH solution for 10 s. After drying, parts of the separated components would turn from purple to yellow, which was deduced to be the portions having free radical scavenging capabilities. GC-MS was then applied to the active separations to identify the compounds (Choi *et al*., 2002).

III. RESULTS AND DISCUSSION

(I) Yields of leaf, heartwood, sapwood, bark and twigs essential oils

a. Oil yields by the hydrodistillation method

The yields of essential oils from leaf, heartwood, sapwood, bark and twigs after hydrodistillation of *C. japonica*. were 3.93 ± 0.09, 0.90 ± 0.05, 0.11 ± 0.01, 0.68 ± 0.03, and 0.31 ± 0.02 ml/100 g of o.d. materials, respectively. Leaf had the highest essential oil content and sapwood had the least.

b. Oil yields by the HS-GC method

The medium values of the total area corresponding to each volume of the leaf, heartwood, sapwood, bark, and twigs oil, submitted to the multiple headspace extraction on HS-GC (Table 1), were calculated by means of the previously described equation. The leaf, heartwood, sapwood, bark, and twigs oil calibration curves obtain from those values corresponds to a simple regression equation of the form $y = a + bx$. The equation of leaf oil was a = -21.64, b = 17942, and $r^2 = 0.9971$; the equation of heartwood oil was a = -30.789, b = 4396.2, and $r^2 = 0.9980$; the equation of sapwood oil was a = -0.2625, b = 1085.8, and $r^2 = 0.9998$; the equation of bark oil was a = -7.385, b = 2276.8, and $r^2 = 0.9989$; the equation of twigs oil was a = -20.307, b = 1429.9, and $r^2 = 0.9910$.

Table 2 shows the peak area values corresponding to different quantities of plant material (leaf, heartwood, sapwood, bark, and twigs) submitted to the MHE on the HS-GC and by extrapolating of the area values of the leaf, heartwood,

sapwood, bark, and twigs oils calibration curve, we obtained three values for the leaf, heartwood, sapwood, bark, and twigs oils of respectively of 3.92 ± 0.04, 0.91 ± 0.02, 0.12 ± 0.01, 0.68 ± 0.02, and 0.30 ± 0.02 ml/100 g of o.d. materials. These results were nearly identical to the oil yield results of the hydrodistillation method which mentioned in the previous section. Thus, our results indicated that HS-GC method can provide reliable essential oil yields from various plant materials which are comparable to those obtained by the hydrodistillation method (Table 3).

Table 1. The values of the total area corresponding to each quantity of leaf, heartwood, sapwood, bark and twig oils subjected to MHE on HS-GC

Leaf		Heartwood		Sapwood		Bark		Twig	
Oil (μl)	Area	Oil (μl)	Area	Oil (μl)	Area	Oil (μl)	Area	Oil (μl)	Area
0.3	5012.3 ± 18.2	0.1	396.7 ± 5.7	0.1	105.9 ± 2.6	0.1	213.8 ± 3.5	0.1	128.3 ± 2.1
0.6	10623.2 ± 26.5	0.2	803.9 ± 6.0	0.2	216.4 ± 2.8	0.2	451.3 ± 5.3	0.2	261.8 ± 3.5
0.9	16036.7 ± 20.3	0.3	1266.0 ± 6.5	0.3	328.9 ± 2.6	0.3	678.9 ± 3.8	0.3	388.9 ± 3.6
1.2	22316.3 ± 18.5	0.4	1757.0 ± 10.9	0.4	438.1 ± 3.5	0.4	900.3 ± 5.6	0.4	531.3 ± 3.8
1.5	27635.3 ± 28.6	0.5	2231.6 ± 11.3	0.5	536.9 ±2.9	0.5	1102.4 ± 6.8	0.5	660.2 ± 5.8
1.8	31256.7 ± 23.9	0.6	2561.9 ± 12.6	0.6	652.2 ± 3.9	0.6	1383.0 ± 8.1	0.6	890.2 ± 8.2

Table 2. Area values corresponding to different quantity of leaf, heartwood, sapwood, bark and twig subjected to MHE on HS-GC

Leaf		Heartwood		Sapwood		Bark		Twig	
Leaf (mg)	Area	Heartwood (mg)	Area	Sapwood (mg)	Area	Bark (mg)	Area	Twig (mg)	Area
10	6985.3 ± 13.1	10	376.3 ± 2.4	30	37.8 ± 2.8	10	151.2 ± 3.9	20	70.9 ± 2.8
20	14296.7 ± 15.2	20	768.3 ± 5.7	45	60.0 ± 2.9	20	310.1 ± 4.8	40	146.8 ± 3.9
30	20965.4 ± 18.6	30	1185.7 ± 10.6	60	77.8 ± 2.6	30	451.3 ± 4.6	60	238.9 ± 4.2
40	27956.9 ± 25.3	40	1538.7 ± 12.8	75	89.2 ± 2.8	40	601.4 ± 5.8	80	300.8 ± 4.8

(II) Compositional and content analyses of essential oils from various tissues

a. Composition of leaf essential oil

A total of 53 compounds were identified from the hydrodistilllated leaf oil of the samples. The main components were kaurene (19.1%), α -pinene (16.5%), elemol (16.3%), δ-3-carene (9.5%), α-eudesmol (8.9%), and γ-eudesmol (5.4%). Furthermore, when the identified compounds were grouped as monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, oxygenated sesquiterpene, diterpene hydrocarbon, and oxygenated diterpene; these 6 groups had relative areal percentages in decreasing order of oxygenated sesquiterpenes (40.1%), monoterpene hydrocarbons (34.8%), diterpene hydrocarbons (18.1%), sesquiterpene hydrocarbons (2.8%), oxygenated monoterpenes (2.3%) etc. From the HS-GC analysis of leaf essential oil, a total of 38 compounds were identified and again with kaurene (18.8%), α-pinene (16.8%), elemol (15.8%), δ-3-carene (9.9%), α-eudesmol (8.6%), and γ-eudesmol (5.2%) as the main ingredients. Similarly,

oxygenated sesquiterpenes predominated the leaf oil as well.

The leaf oil composition of sugi had been studied by Uchida (1916) and found to be mainly α-pinene, dipentene, cadinene, and α-cryptomerene. Nakatsuka *et al*. (1957) obtained hydrodistillated sugi leaf oil and found α-pinene, camphene, dipentene, p-cymene, 4-terpineol, cadinene, α-podocarpene, and α-cryptomerene predominated; Shieh *et al*. (1981) separated 38 compounds with β-eudesmol, α-eudesmol, and elemol as the main ingredients. Lee and Lin (1986) found the leaf essential oil to contain mainly (-)-kaurene, elemol, and sabinene etc.; Cheng *et al*. (2005) determined only 20 compounds with *ent*-kaur-16-ene, valencene, α-gurjunene predominated; in 2009, Cheng *et al*. again determined leaf essential oils of Japanese cedar from different age classes to contain mainly 16-kaurene, elemol, α-eudesmol, (+) β-eudesmol, and sabinene etc. for a total of 30 compounds.

Comparing with the literatures, more components were identified in this study, and the composition of essential oil seems to different with the literatures. It might due to originate from differences in the provenances, tree ages, habitats or methods of extraction.

b. Heartwood oil composition

Forty-fore compounds were identified from the heartwood essential oil, with 1-epi-cubenol (18.9%), δ-cadinene (17.2%), cubenol (14.9%), α-muurolene (9.1%), α-eudesmol (5.6%), and trans-calamenene (5.3%) as the main ingredients. And we group according to the scheme described above, oxygenated sesquiterpenes again made up the largest fraction (52.1%), followed by sesquiterpene hydrocarbons (40.7%), oxygenated diterpenes (5.4%), and oxygenated monoterpenes (1.2%), and diterpene hydrocarbons (0.5%), There was no presence of monoterpene hydrocarbon. On the other hand, HS-GC identified a total of 35 compounds with mostly similar main components of 1-epi-cubenol (18.6%), δ-cadinene (17.3%), cubenol (14.8%), α-muurolene (9.3%), α-eudesmol (5.7%), and trans-calamenene (5.3%) etc., and revealed identical fractionoal group distribution.

Comparing our results with those reported in the literature, Nagahama *et al*. (1996a, b; 1998; 2000; 2001; 2002) noted that the heartwood oil of sugi contained mainly sandaracopimarinol, ferruginol, cubebol, and δ-cadinene etc., somewhat different from out results. Cheng *et al*. (2005) determined from their heartwood samples mostly δ-cadinene, isoledene, and γ-muurolene. Twenty-six compounds were identified in their report. The number of compounds identified in our report was greater than these reports while the compositions also varied. The marked differences among the results were the possible causes included the factors mentioned above.

c. Sapwood oil composition

Fifty compounds were identified from the hydrodistilllated sapwood oil. The main components were cubebol (19.5%), cubenol (13.3%), 1-epi-cubenol (12.5%), and ferruginol (10.8%) etc. Integrating the areas of the 6 compound fractions, oxygenated sesquiterpenes made up the most (61.4%), followed in decreasing order by sesquiterpene hydrocarbons (17.9%), oxygenated diterpenes (16.9%), oxygenated monoterpenes (1.4%), diterpene hydrocarbons (0.8%), and monoterpene hydrocarbons (0.1%). Analysis of the sapwood sample by the HS-GC found a total of 46 compounds, mostly cubebol (20.5%), cubenol (13.2%), 1-*epi*-cubenol (12.3%), and ferruginol (8.9%) etc. Their group distribution

was also dominated by oxygenated sesquiterpenes as described above.

Literature reports concerning the sapwood of Japanese cedar were limited. Nagahama *et al.* (1995) found sandaracopimarinol and ferruginol etc. as its main components. Cheng *et al*. (2005), on the other hand. detected diterpenes of sclarene and cupressene as its main components. The marked differences among the results were the possible causes included the factors mentioned above.

d. Bark oil composition

The hydrodistillated bark oil contained 87 identified compounds, its main components included camphor (46.5%), α-pinene (16.2%), and δ-3-carene (7.5%) etc. Integrating the peak areas of the 6 groups suggested that oxygenated monoterpenes composed of the major fraction (50.9%), it was followed by monoterpene hydrocarbons (27.7%), sesquiterpene hydrocarbons (7.7%), oxygenated sesquiterpenes (6.8%), oxygenated diterpenes (2.0%), and diterpene hydrocarbons (1.0%). Whereas the HS-GC analysis identified 61 compounds also with camphor (48.4%), α-pinene (17.1%), and δ-3-carene (8.6%) etc predominated. Grouping of the chemicals also indicated a preponderance of oxygenated monoterpenes.

Pertinent literature included a study by Yatagai et al. (2002) who found the inner bark of sugi containing mostly α-pinene (16.18~51.14%), D-limonene (7.42~13.35%), 3-carene (6.57~13.16%), and δ-cadinene (3.73~8.65%); Cheng *et al*. (2005) detected 3-carene. and limonene in the bark of the tree. The disparaging bark oil compositions might be the results of the aforementioned factors.

e. Twig oil composition

A total of 60 compounds were detected from the hydro-distillated branch oil of the tree. In decreasing order of presence, there were α-eudesmol (25.2%), γ-eudesmol (11.8%), elemol (8.7%), cryptomerione (7.5%), and bisabolatrien-1-ol-4-one (5.8%) etc. By integrating the peak areas of the 6 groups of compounds, oxygenated sesquiterpenes showed preponderant presence (73.7%), followed by oxygenated diterpenes (11.1%), sesquiterpene hydrocarbons (7.3%), oxygenated monoterpenes (0.5%), monoterpene hydrocarbons (0.4%), and diterpene hydrocarbons (0.1%). Similarly, 46 compounds were identified from the HS-GC branch oil run. There were α-eudesmol (25.6%), γ-eudesmol (11.8%), elemol (8.5%), cryptomerione (7.6%), and bisabolatrien-1-ol-4-one (5.7%) etc. The HS-GC analysis of the twig sample produced an identical chemical grouping with oxygenated sesquiterpenes made up the largest fraction. This is the first report on the composition of Japanese cedar twig oil.

The above yield values and compositions indicate that hydrodistillation and the HS-GC methods gave comparable leaf, heartwood, sapwood, bark and twig oils yields. When the composition of these oils was compared, however, minor components obtained by hydrodistillation (content < 0.1%) could not be detected by the HS-GC. The major reason was probably due to the small size of the specimens used, as the former used ca. 1 kg of sample, while HS-GC only took 20~50 mg. Overall, the HS-GC yielded main components and compound groups similar to those of the hydrodistillation results. The methodology proved that HS-GC can be an effective method for an essential oil compositional analysis; furthermore, it requires only a minute amount of specimen and a long period of distillation is not needed (Ho et al., 2008).

Table 3. Chemical compositions and yields of essential oils obtained from *C. japonica* leaf, heartwood, sapwood, bark and twig by hydrodistillation extraction and headspace methods

Peak no.	Consituents	K.I.[a]	Concentration(%)										Identification [d]
			Leaf		Heartwood		Sapwood		Bark		Twig		
			HD [b]	HS [c]	HD	HS	HD	HS	HD	HS	HD	HS	
1	tricyclene	927	0.1	0.1	—	—	—	—	te)	0.1	—f)	—	MS, KI, ST
2	α-thujene	930	0.1	0.2	—	—	—	—	t	—	—	—	MS, KI, ST
3	α-pinene	939	16.5	16.8	—	—	0.1	0.1	16.2	17.1	0.2	0.3	MS, KI, ST
4	camphene	954	1.0	1.1	—	—	—	—	0.8	0.9	—	—	MS, KI, ST
5	sabinene	975	1.2	1.3	—	—	—	—	0.2	0.2	—	—	MS, KI, ST
6	β-pinene	979	0.9	0.9	—	—	—	—	0.4	0.4	—	—	MS, KI, ST
7	β-myrcene	991	1.4	1.6	—	—	—	—	0.3	0.4	t	—	MS, KI, ST
8	α-terpinene	1017	0.3	0.4	—	—	—	—	t	—	—	—	MS, KI, ST
9	*p*-cymene	1025	0.2	0.2	—	—	—	—	0.3	0.3	—	—	MS, KI, ST
10	limonene	1029	2.3	2.5	—	—	—	—	1.4	1.4	t	—	MS, KI, ST
11	δ-3-carene	1031	9.5	9.9	—	—	t	0.1	7.5	8.6	0.1	0.1	MS, KI
12	1,8-cineole	1031	—	—	t	0.1	—	—	0.8	0.8	t	—	MS, KI, ST
13	*trans*-β-ocimene	1050	t	—	—	—	—	—	t	—	—	—	MS, KI, ST
14	γ-terpinene	1060	0.5	0.6	—	—	—	—	t	—	—	—	MS, KI, ST
15	*m*-cymenene	1085	0.1	—	—	—	—	—	0.1	0.2	—	—	MS, KI
16	terpinolene	1089	0.8	1.0	—	—	—	—	0.1	0.1	t	—	MS, KI, ST
17	*p*-cymenene	1091	t	—	—	—	—	—	0.3	0.3	t	—	MS, KI, ST
18	linalool	1097	0.3	0.4	t	0.1	—	—	t	—	—	—	MS, KI, ST
19	*exo*-fenchol	1122	—	—	—	—	—	—	0.1	0.1	—	—	MS, KI
20	camphor	1146	t	—	1.1	1.3	1.2	1.5	46.5	48.4	0.4	0.4	MS, KI, ST
21	camphene hydrate	1150	—	—	—	—	—	—	0.1	0.1	—	—	MS, KI
22	*trans*-pinocamphone	1163	—	—	—	—	—	—	0.1	0.2	—	—	MS, KI
23	pinocarvone	1165	—	—	—	—	—	—	0.2	0.2	—	—	MS, KI
24	borneol	1169	—	—	—	—	—	—	0.4	0.4	—	—	MS, KI, ST
25	4-terpineol	1177	1.3	1.4	t	—	0.1	0.1	0.6	0.5	t	—	MS, KI, ST
26	α-terpineol	1189	0.2	0.3	t	—	0.1	0.1	1.7	1.6	0.1	0.1	MS, KI, ST
27	verbenone	1205	—	—	—	—	—	—	0.1	0.2	—	—	MS, KI
28	4-methylene-isophorone	1218	—	—	—	—	—	—	0.3	0.2	—	—	MS, KI
29	carvacrol, methyl ether	1245	—	—	—	—	—	—	t	—	—	—	MS, KI
30	carvotanacetone	1247	—	—	—	—	—	—	t	—	—	—	MS, KI
31	piperitone	1253	—	—	—	—	—	—	t	—	—	—	MS, KI
32	isobornyl acetate	1286	0.5	0.5	—	—	—	—	0.1	0.1	—	—	MS, KI
33	safrole	1287	—	—	t	—	0.2	0.3	t	—	—	—	MS, KI
34	carvacrol	1299	—	—	—	—	—	—	t	—	t	—	MS, KI, ST
35	δ-elemene	1338	t	—	—	—	—	—	t	—	t	—	MS, KI
36	α-cubebene	1351	t	—	0.3	0.2	0.2	0.2	0.3	0.3	0.1	0.1	MS, KI
37	α-copaene	1377	t	—	0.1	0.1	t	0.1	0.3	0.2	t	0.1	MS, KI
38	β-cubebene	1388	0.1	t	0.3	0.3	0.2	0.2	0.2	0.1	0.1	0.2	MS, KI
39	β-elemene	1391	0.1	t	0.3	0.3	0.3	0.2	0.2	0.1	0.1	0.1	MS, KI
40	α-cedrene	1412	0.1	0.1	0.1	t	0.1	0.1	0.4	0.3	0.3	0.4	MS, KI, ST
41	β-caryophyllene	1419	t	—	0.1	—	0.2	0.2	0.2	0.2	0.1	0.2	MS, KI, ST
42	β-cedrene	1421	0.1	—	t	—	t	—	0.1	—	0.1	0.1	MS, KI
43	*cis*-thujopsene	1431	0.1	0.1	—	—	—	—	—	—	—	—	MS, KI
44	β-copaene	1432	t	—	t	—	t	—	0.4	0.4	t	—	MS, KI
45	β-gurjunene	1434	—	—	t	—	—	—	t	—	t	—	MS, KI
46	*cis*-muurola-3,5-diene	1450	—	—	0.6	0.6	0.8	0.9	0.1	—	0.2	0.2	MS, KI
47	α-humulene	1455	—	—	0.7	0.7	0.3	0.4	0.1	—	0.2	0.3	MS, KI
48	*trans*-β-farnesene	1457	0.1	0.1	—	—	—	—	—	—	—	—	MS, KI
49	*allo*-aromadendrene	1460	t	—	t	—	—	—	0.1	—	t	—	MS, KI, ST
50	*trans*-cadina-1(6),4-diene	1477	0.1	—	1.9	2.0	3.8	4.1	0.4	0.3	1.3	1.2	MS, KI
51	γ-gurjunene	1477	—	—	—	—	—	—	1.1	1.1	—	—	MS, KI
52	γ-muurolene	1480	0.1	0.1	0.3	0.4	—	—	—	—	—	—	MS, KI
53	α-curcumene	1481	—	—	0.1	0.1	0.1	—	t	—	0.1	0.1	MS, KI
54	germacrened	1485	—	—	—	—	—	—	0.2	0.2	0.2	0.3	MS, KI
55	β-selinene	1490		—	0.2	0.3	—	—	—	—	—	—	MS, KI
56	*trans*-muurola-4(14),5-diene	1494	0.1	—	1.2	1.3	1.4	1.5	0.3	0.3	0.3	0.4	MS, KI
57	*epi*-cubebol	1494	—	—	4.3	4.7	3.6	3.7	—	—	—	—	MS, KI
58	α-muurolene	1500	0.3	0.4	9.1	9.3	3.4	3.5	1.5	1.3	1.4	1.4	MS, KI
59	β-bisabolene	1506	0.1	0.1	0.2	0.2	0.1	0.2	t	—	0.2	0.2	MS, KI
60	γ-cadinene	1514	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.1	—	MS, KI

61	cubebol	1515	—	—	—	—	19.5	20.5	2.1	2.1	4.5	4.3	MS, KI
62	δ-cadinene	1523	1.3	1.5	17.2	17.3	—	—	—	—	—	—	MS, KI
63	*trans*-calamenene	1529	—	—	5.3	5.3	4.5	4.6	1.1	0.9	1.5	1.5	MS, KI
64	*trans*-cadina-1(2),4-diene	1535	t	—	0.9	1.0	1.6	1.6	0.3	0.2	0.6	0.5	MS, KI
65	α-cadinene	1539	0.1	—	—		—	—	—	—	—	—	MS, KI
66	α-calacorene	1546	—	—	0.7	0.8	0.6	0.7	0.3	0.2	0.4	0.4	MS, KI
67	selina-3,7(11)-diene	1547	—	—	0.7	0.7	—	—	—	—	—	—	MS, KI
68	elemol	1550	16.3	15.8	0.6	0.5	0.6	0.7	0.5	0.5	8.7	8.5	MS, KI
69	*cis*-muurol-5-en-4-α-ol	1561	—	—	0.5	0.5	—	—	—	—	—	—	MS, KI
70	(e)-nerolidol	1563	—	—	—		0.1	t	0.2	0.1	—	—	MS, KI
71	β-calacorene	1566	—	—	0.2	0.2	—	—	—	—	—	—	MS, KI
72	spathulenol	1578	—	—	0.2	0.3	—	—	—	—	—	—	MS, KI
73	caryophyllene oxide	1583	—	—	0.3	0.3	0.1	0.1	t	—	0.1	—	MS, KI
74	gleenol	1587	—	—	2.4	2.6	1.8	1.9	0.2	0.1	0.4	0.4	MS, KI
75	salvial-4(14)-en-1-one	1595	—	—	—	—	—	—	0.1	—	—	—	MS, KI
76	guaiol	1601		—	0.1	—	—	—	—	—	—	—	MS, KI
77	β-oplopenone	1608	0.1	t	—	—	—	—	t	—	—	—	MS, KI
78	humulene epoxide II	1608	—	—	0.5	0.5	—	—	—	—	—	—	MS, KI
79	*epi*-cedrol	1619	3.2	3.8	—	—	0.3	0.3	0.1	0.1	0.7	0.8	MS, KI
80	1,10-di-*epi*-cubenol	1619	—	—	—	—	—	—	—	—	—	—	MS, KI
81	10-*epi*-γ-eudesmol	1624	—	—	—	—	—	—	—	—	0.2	0.2	MS, KI
82	1-*epi*-cubenol	1629	—	—	18.9	18.6	12.5	12.3	—	—	3.3	3.2	MS, KI
83	γ-eudesmol	1632	5.4	5.2	—	—	1.0	0.9	0.9	0.8	11.8	11.8	MS, KI
84	τ-cadinol	1640	0.6	0.6	—	—	—	—	—	—	—	—	MS, KI
85	τ-muurolol	1642	0.7	0.7	—	—	—	—	—	—	—	—	MS, KI
86	cubenol	1647	—	—	14.9	14.8	13.3	13.2	0.7	0.6	4.0	4.0	MS, KI
87	α-eudesmol	1654	8.9	8.6	5.6	5.7	3.7	3.8	1.5	1.3	25.2	25.6	MS, KI
88	selin-11-en-4-α-ol	1660	—	—	1.5	1.5	1.2	1.3	—	—	—	—	MS, KI
89	*trans*-calamenen-10-ol	1669	0.2	0.4	—	—	0.1	0.1	—	—	0.3	0.2	MS, KI
90	8,9-epoxide-cadalene	1676	0.3	0.3	—	—	0.2	0.2	0.1	0.1	—	—	MS, KI
91	5-*neo*-cedranol	1685	0.5	0.4	—	—	—	—	—	—	—	—	MS, KI
92	eudesm-7(11)-en-4-ol	1700	—	—	—	—	0.4	0.4	t	—	0.9	0.8	MS, KI
93	cryptomerione	1725	—	—	2.1	1.9	2.2	2.0	0.2	0.2	7.5	7.6	MS, KI
94	8-isopropyl-2,5-dimethyl -5,6,7,8-tetrahydro-1-nap hthalenol	1801	—	—	0.2	0.2	0.1	0.2	t	—	0.4	0.4	MS, KI
95	bisabolatrien-1-ol-4-one	1914	—	—	—	—	0.7	0.8	0.1	—	5.8	5.7	MS, KI
96	biformen	1927	3.9	3.6	—	—	—	—	0.1	—	t	—	MS, KI
97	2,4b-dimethyl-8-methyl ene-2-vinyl-1,2,3,4,4a,4b, 5,6,7,8,8a,9-dodecahydro phenanthrene	1942	—	—	—	—	—	—	0.2	0.2	— 0.1	— 0.1	MS, KI
98	isophyllocladene	1967	—	—	—	—	t	—	0.5	0.5			MS, KI
99	kaurene	2043	19.1	18.8	—	—	—	—	t	—	—	—	MS, KI
100	abietatriene	2057	—	—	—	—	0.1	0.1	0.2	0.1	—	—	MS, KI
101	abietadiene	2088	—	—	0.5	0.4	0.7	0.6	—	—	—	—	MS, KI
102	sandaracopimarinal	2185	—	—	0.9	0.7	1.4	1.3	0.4	0.3	1.4	1.3	MS, KI
103	phyllocladanol	2210	—	—	0.7	0.5	1.2	1.1	0.3	0.2	1.8	1.7	MS, KI
104	M.W.=286	2220	—	—	—	—	—	—	0.3	0.3	—	—	
105	sandaracopimarinol	2270	—	—	1.4	1.3	3.7	3.3	—	—	3.4	3.2	MS, KI
106	ferruginol	2332	—	—	2.4	2.3	10.8	8.9	1.0	0.8	4.5	4.3	MS, KI, ST
	Identifiedcompound (%)		99.1	99.8	99.9	99.8	98.7	98.2	96.2	97.2	93.1	92.7	
	Monoterpenehydrocarbons		34.8	36.6	0.0	0.0	0.1	0.2	27.7	29.8	0.4	0.4	
	Oxygenatedmonoterpenes		2.3	2.5	1.2	1.4	1.4	1.7	50.9	52.8	0.5	0.5	
	Sesquiterpenehydrocarbons		2.8	2.5	40.7	41.1	17.9	18.6	7.7	6.5	7.3	7.5	
	Oxygenatedsesquiterpenes		36.2	35.8	52.1	52.1	61.4	62.2	6.8	6.0	73.7	73.7	
	Diterpenehydrocarbons		23.0	22.4	0.5	0.4	0.8	0.7	1.0	0.7	0.1	0.1	
	Oxygenatedditerpenes		t	t	5.4	4.9	16.9	14.5	2.0	1.6	11.1	10.5	
	Others		t	t	t	t	0.2	0.3	0.1	t	t	t	
	Yield (ml/100g)		3.93 ± 0.09	3.92 ± 0.04	0.90 ± 0.05	0.91 ± 0.02	0.11 ± 0.01	0.12 ± 0.01	0.68 ± 0.03	0.68 ± 0.02	0.31 ± 0.02	0.30 ± 0.02	

[a)] Kovats index on a DB-5 column in reference to *n*-alkanes (Van den Dool and Kratz,1963). [b)] HD：Hydrodistillation extraction. [c)] HS：Headspace method. [d)] MS：NIST and Wiley libraries spectra and literature, KI：Kovats index, ST：Authentic standardcompounds. [e)] trace：<0.05%. [f)] not detected

(III) Antioxidant activities of the oils fom various tree parts

a. DPPH free radical scavenging capability

Essential oils from various tissues of *C. japonica* exhibited different DPPH free radical scavenging capacities and IC_{50} values as shown in Fig. 1 and Table 4. The sapwood oil possessed the best radical scavenging capability (IC_{50} =113μg mL^{-1}), followed by twig, heartwood, and bark oils. The leaf oil had the poorest antioxidant activity. Comparing the antioxidant activity (IC_{50} values) of the sugi heartwood, sapwood, and twig oils were superior than the leaf essential oil of black cumin (*Nigella sativa*) (Burits and Bucar, 2000), flower oil of oregano (*Origanum vulgare*) (Khaled *et al*. 2002) and leaf oil of white turmeric (*Curcuma zedoaria*) (Yildirim *et al*, 2001), which have IC_{50} values of 460, 500, and 6000 μg mL^{-1}, respectively. Furthermore, a comparison of the 4 sugi stem part oils with the IC_{50} values of the leaf oils of different *Cinnamomum osmophloeum* clone strains which ranged from 33.38 to 708.55 μg mL^{-1} (Chen, 2003) were within the same range. Thus, the stem oils of sugi can be considered to possess comparable antioxidant activities as the leaf oils of *C. osmophloeum*.

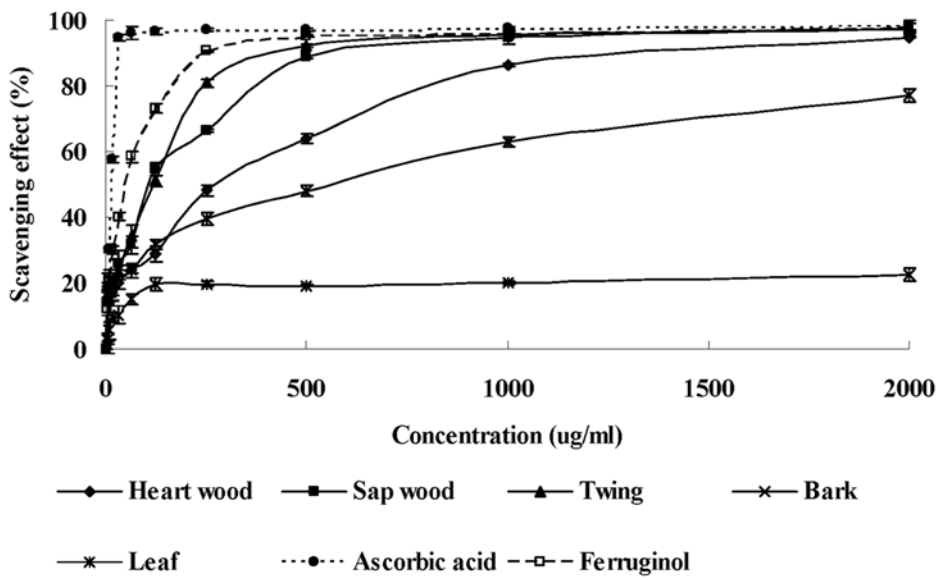

Fig. 1. DPPH free radical scavenging effects of essential oils from C. japonica leaf, heartwood, sapwood, bark and twig.

b. TLC analysis and determination of the DPPH staining method

The DPPH assay suggested that sapwood oil of sugi possessed the best free radical scavenging capacity. In order to further understand the responsible constituents in the oil contributing to the performance, thin layer chromatography (TLC) separation of the sugi twig oil were performed using hexane : ethyl acetate = 95 : 5 as the developing solution. A UV lamp was then used to ascertain the positions of the component spots (Fig. 2). The TLC plates were

then immersed in the DPPH solution whence the active compounds reacted to become yellow colored. By measuring the b* values of the spots, their DPPH scavenging capabilities could be determined (Choi *et al*., 2002). The active isolates were examined using GC-MS to establish their identities. The results indicated that the fraction 3 (R_f = 0.28) of the sapwood oil of sugi reacted instantly upon immersing in the DPPH solution and produced a b* value of 6.56. As noted by Choi *et al*. (2002), the fast reaction speed of DPPH color rendering reaction implied a high free radical scavenging capacity. The GC-MS results indicated that the isolate was mainly ferruginol (Fig. 2). Ferruginol were separated from fraction 3 using high performance liqluid chromatography (HPLC) (LiChrosporb Si-60 column (250 mm × 10 mm, 7 μm), mobile phase Hexane: Ethyl acetate = 90: 10) and was again tested for the DPPH assay, the IC_{50} value was 48 μg/ml. The result was in agreement with those of Wang *et al*. (2002) who found that among the abietane-type diterpenes, ferruginol passed the strongest DPPH free radical scavenging capacity. We concluded that ferruginol is the principal compounds which contributed to the antioxidative of the sapwood oil of Japanese cedar.

Table 4. IC_{50} of *C. japonica* leaf, heartwood, sapwood, bark, twig oils and ferruginol in scavenging DPPH free radical.

Sample	IC_{50} (μg/ml)
Leaf	>2000
Heartwood	327
Sapwood	113
Bark	580
Twig	124
Ferruginol	48

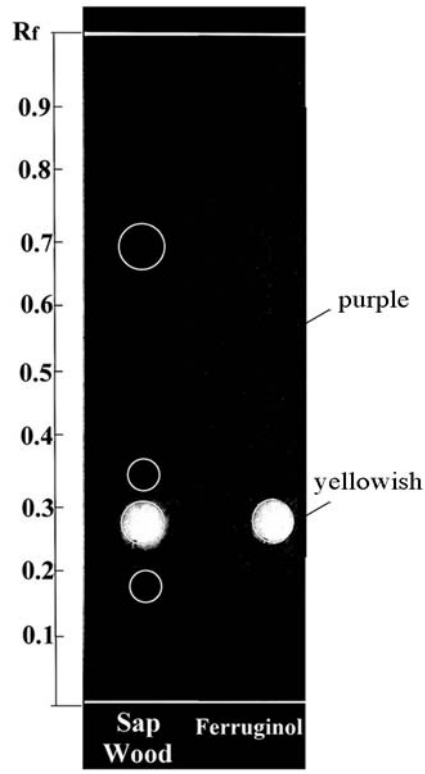

Fig. 2. Active compound identification by GC-MS chromatogram of the fraction separated by means of TLC dot-blot and the DPPH staining method of sapwood oil of C. *japonica*.

IV. CONCLUSIONS

In this study, we extracted the volatile oils of the leaf, heartwood, sapwood, twigs and bark of *C. japonica* separately using hydrodistillation and HS-GC methods. The leaf provided the highest essential oil yield and that of the sapwood the least. As for the compositions, the main composition of leaf oil were kaurene (19.1%)、α-pinene (16.5%)、elemol (16.3%); the heartwood oil consisted mostly 1-epi-cubenol (18.4%), δ-cadinene (17.2%), cubenol (14.9%) etc.; the sapwood oil was mostly cubebol (19.5%), cubenol (13.3%), 1-*epi*-cubenol (12.5%), and ferruginol (10.8%) etc.; the twig oil was mostly α-eudesmol

(25.15%), and γ-eudesmol (11.78%) etc., and the bark oil consisted of camphor (46.45%), and α-pinene (16.17%) etc. The leaf, heartwood, sapwood, and twig oils of the species composed mainly of oxygenated sesquiterpenes. The HS-GC produced results nearly identical to those of the hydrodistillation oils. As for the antioxidant activities of the essential oils from various tissues of the tree, the ranking was sapwood > twig > heartwood > bark > leaf. After TLC fractionation of the sapwood oil, we found that ferruginol had the best antioxidant activity, and may be applicable as a natural antioxidant.

V. ACKNOWLEDGMENTS

The Author would like to acknowledge financial support from the Council of Agriculture, Executive Yuan. Taipei, Taiwan. (Contract/grant number: 97AS-12.2.2 -FB-e3.)

VI. REFERENCE

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

Burits, M. and F. Bucar (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14(5): 323-328.

Burits, M., K. Asres and F. Bucar (2001) The antioxidant activity of the essential oils of *Artemisia afra, Artemisia abyssinica* and *Juniperus procera*. Phytotherapy Res. 15: 103-108.

Cha, J.D., M.R. Jeong, S.I. Moon, B.S. Kil, S. I. Yun, K.Y. Lee, and Y.H. Song (2007) Chemical composition and antimicrobial activity of essential oil of *Cryptomeria japonica*. Phytotherapy Res. 21: 295-299.

Chen, I.Y. (2003) The antioxidative properties of leaf essential oils of *Cinnamomum osmophloeum*. MS Thesis, Dept. of Food Tech., Natl. Taiwan Univ., Taipei, Taiwan. 88 pp.

Chen, X.H., C.S. Kim, T. Kashiwagi, S. Tebayashi and M. Horiike (2001a) Antifeedants against *Acusta despesta* from the Japanese cedar, *Cryptomeria japonica*. Z. Naturforschg. 59c: 249-252.

Chen, X.H., C.S. Kim, T. Kashiwagi, S. Tebayashi and M. Horiike (2001b) Antifeedants against *Acusta despesta* from the Japanese cedar, *Cryptomeria japonica* II. Biosci. Biotechnol. Biochem. 65: 1434-1437.

Cheng, S.S. and S.T. Chang (2002) Antitermitic activity of essential oils from *Cryptomeria japonica*. Q. J. Chin. For. 35: 193-199.

Cheng, S.S., H.Y. Lin and S.T. Chang (2005) Chemical composition and antifungal activity of essential oils from different tissues of Japanese cedar (*Cyptomeria japonica*). J. Agric. Food Chem. 53: 614-619.

Cheng, S.S., M.T. Chua, E.H. Chang, C.G.. Huang, W.J. Chen, and S.T. Chang (2009) Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at different ages. Bioresour. Technol. 100: 465-470.

Choi, C.W., S.C. Kim, S.S. Hwang, B.K. Choi, H.J. Ahn, M.Y. Lee, S.H. Park, and S.K. Kim (2002) Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163: 1161-1168.

Cuendet, M., K. Hostettmann and O. Potterat (1997) Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helv. Chim. Acta. 80: 1144-1152.

Ho, C.L., E.I.C. Wang, X.T. Wei, S.Y. Lu and

Y.C. Su (2008) Composition and bioactivities of the leaf essential oils of *Cinnamomum subavenium* Miq. from Taiwan. J. Essent Oil Res. 20: 328-34.

Kashiwagi, T., B. Wu, K. Iyota, X.H. Chen, S. Tebayashi and C.S. Kim (2007) Antifeedants against *Locusta migratoria* from the Japanese Cedar, *Cryptomeria japonica*. Biosci. Biotechnol. Biochem. 71: 966-970.

Khaled, F., H. Ahmed and F. Amr (2002) Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chem. 79: 331-336.

Kirby, A.J. and R.J. Schmidt (1997) The antioxidant activity of Chinese herbs for eczema and of placebo herbs. J. Ethnopharmacol. 56: 103-108.

Kofujita, H., Y. Fujino, T. Sasaki, M. Hasebe, M. Ota and K. Suzuki (2001) Antifungal activity of the bark of *Cryptomeria japonica* and its relevant components. Mokuzai Gakkaishi. 47:479-486.

Kofujita, H., Y. Fujino, M. Ota and K. Takahashi (2006) Antifungal diterpenes from the bark of *Cryptomeria japonica* D. Don. Holzforschung. 60: 20-23.

Kolb, B. (1985) Quantitative aspects of equilibrium and dynamic headspace gas-chromatography with capillary columns. Abs. Pap. Americ. Chem. Soc. 790: 73.

Lee, C.L. and H.Y. Lin (1986) Study on the essential oil of tree leaf 1. The leaf oil constituents of *Cryptomeria japonica*. Bull. Exp. For. Natl. Taiwan Univ. 169: 1-7.

Li, W.H., S.T. Chang, S.C. Chang and H.T. Chang (2008) Isolation of antibacterial diterpenoids from *Cryptomeria japonica* bark. Nat. Prod. Res. 22: 1085-1093.

Massada, Y. (1976) Analysis of Essential Oil by Gas Chromatography and Spectrometry. Wiley, New York. 340 pp.

Matsunaga, T., C. Hasegawa, T. Kawasuji, H. Suzuki, H. Saito, T. Sagioka, R. Takahashi, H. Tsukamoto, T. Morikawa and T. Akiyama (2000) Isolation of the antiulcer compound in essential oil from the leaf of *Cryptomeria japonica*. Biol. Pharm. Bull. 23: 595-598.

Matsushita, Y., Y. Hwang, K. Sugamoto and T. Matsu (2006) Antimicrobial activity of heartwood components of sugi (*Cryptomeria japonica*) against several fungi and bacteria. J. Wood Sci. 52: 552-556.

Morisawa, J., C.S. Kim, T. Kashiwagi, S. Tebayashi and M. Horiike (2002) Repellents in the Japanese cedar, *Cryptomeria japonica*, against the pill-bug, *Armadillidium vulgare*. Biosci. Biotechnol. Biochem. 66: 2424-2428.

Morita, S., M. Yatagai and T. Ohira (1991) Antimite and antifungi activities of the hexane extractives from yakusugi bogwood. Mokuzai Gakkaishi. 37: 352-357.

Morita, S. and M. Yatagai (1994) Antimite components of the hexane extractives from domaiboku yakusugi (*Cryptomeria japonica*). Mokuzai Gakkaishi. 40: 996-1002.

Morita, S., T. Hidaka and M. Yatagai (1997) Antifungal compounds of the extractives of yakusugi (*Crypromeria japonica* D. Don). Wood Preservation. 23: 11-19.

Nagahama, S., M. Tazaki, H. Nomura, K. Nishimura and M. Tajima (1995) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) II. Components of same clone from different habitats. Mokuzai Gakkaishi. 42: 330-333.

Nagahama, S., M. Tazaki, T. Sanetika, H. Nomura, K. Nishimura and M. Tajima (1996a) Terpenoids of the wood oil of sugi (*Cryptomeria*

japonica) III. Components of yakusugi. Mokuzai Gakkaishi. 42: 1121-1126.

Nagahama, S., M. Tazaki, H. Nomura, K. Nishimura, M. Tajima and Y. Iwasita (1996b) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) IV. Components of yabukuguri. Mokuzai Gakkaishi. 42: 1127-1133.

Nagahama, S., M. Tazaki, T. Sanetika, K. Nishimura and M. Tajima (1998) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) V. Components of form ayasugi. Mokuzai Gakkaishi. 44: 282-286.

Nagahama, S., T. Iwaoka and T. Ashitani (2000) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) VI. Components of elite clones kenkuma-3, kenkoyu-3 and kenaira-14. Mokuzai Gakkaishi. 46: 225-230.

Nagahama, S., T. Tukamoto, N. Torii, T. Sonoda and T. Yamanobe (2001) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) VII. Components of elite clones minamatasho-4, and five others. Mokuzai Gakkaishi. 47: 487-492.

Nagahama, S., H. Fujii, T. Sonoda and M., Sasaki (2002) Terpenoids of the wood oil of sugi (*Cryptomeria japonica*) VIII. Components of kenkunisaki-5 and five others elite clones. Mokuzai Gakkaishi. 48: 380-386.

Nakatsuka, T., Y. Hirose and M. Matsuda (1957) Terpenoids II. Composition of the essential oil from the leaf of "sugi" (*Cryptomeria japonica* D. Don). Mokuzai Gakkaishi. 3: 206-208.

Shieh, B., Y. Iizuka and Y. Matsubara (1981) Monoterpenoid and sesquiterpenoid constituents of the essential oil of sugi (*Cryptomeria japonica* D. Don.). Agric. Biol. Chem. 45: 1493-1495.

Sogabe, A., K. Kinjo, F. Abe, T. Yamauchi and S. Yaga (2000a) Termiticidal substances from the wood and bark of *Wikstroemia retusa* A. Gray. Mokuzai Gakkaishi. 46: 47-53.

Sogabe, A., K. Kinjo, F. Abe, T. Yamauchi and S. Yaga (2000b) Termiticidal substances from the heartwood of *Cryptomeria japonica* D. Don. Mokuzai Gakkaishi. 46: 124-131.

Uchida, S. (1916) The essential oil of sugi (*Cryptomeria japonica*) leaf. J. Am. Chem. Soc. 38: 687-698.

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

Wang, S.Y., J.H. Wu, L.F. Shyur, Y.H. Kuo and S.T. Chang (2002). Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung 56: 487-492.

Wang, S.Y., W.C. Lai, F.H. Chu, C.T. Lin, S.Y. Shen and S.T. Chang (2006). Essential oil from the leaf of *Cryptomeria japonica* acts as a silverfish (*Lepisma saccharina*) repellent and insecticide. J. Wood Sci. 52: 522-526.

Yatagai, M., Y. Miyazaki and T. Ohira (1991) Extractives from yakusugi bogwood and their termicidal activity and growth regulation effects on plant seeds. Mokuzai Gakkaishi. 37: 352-357.

Yatagai, M., H. Makihara and K. Oba (2002) Volatile components of Japanese cedar cultivars as repellents related to resistance to *Cryptomeria* bark borer. J. Wood Sci. 48: 51-55.

Yildirim, A., A. Mavi and A.A. Kara (2001) Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 49: 4083-4089.