

ANTIOXIDATIVE LIGNANS FROM PHYTOCHEMICAL EXTRACT OF *CALOCEDRUS FORMOSANA* FLORIN

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Antiradical and antioxidant activities of ethanolic extract and its fractions from *Calocedrus formosana* Florin heartwood were investigated, and active compounds were identified by spectral analyses. Among extract and three fractions, the ethyl acetate soluble fraction had a high total phenolic content and exhibited the desirable reducing power, antiradical activity, and antioxidant activity. The compounds haplomyrfofin, O-ethyl- α -conidendral, matairesinol, 7,8-dehydro-4-O-methyl-thujaplicatin, and 5-methoxy-isosalicifoline were isolated from antioxidative activity guided fractionation of the ethyl acetate soluble fraction. Haplomyrfofin and O-ethyl- α -conidendral were for the first time isolated and identified from *C. formosana*. Matairesinol and 5-methoxy-isosalicifoline exhibited superior DPPH radical scavenging ability and antioxidant activity, as evaluated by TEAC assay. Results obtained in the present study revealed that ethyl acetate soluble fraction from *C. formosana* heartwood extract and its active compounds, matairesinol and 5-methoxy-isosalicifoline, exhibit antioxidant potential as natural nutraceuticals.

Keywords: *Calocedrus formosana*; Antioxidant activity; Lignan; Matairesinol; 5-Methoxy-isosalicifoline

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INTRODUCTION

Natural products present in numerous plants are of great potential as nutraceuticals or pharmaceuticals (Clark 1996; Chan *et al.* 2007; Mittal *et al.* 2009; Garzón *et al.* 2010). Kuti and Konuru (2004) reported that antioxidant activity of leaf extract from *Cnidioscolus* spp. were highly correlated with total phenolic contents, and *Cnidioscolus* spp. leaves are recommended to be the source of natural antioxidant supplements. Many studies also have revealed that phenolic constituents such as flavonoids, lignans, *etc.*, from higher plants possess marvelous antiradical and antioxidant activities (Sultana *et al.* 2007; Singh *et al.* 2009). Leaf extract (EGb 761) from *Ginkgo biloba* contains bioactive constituents, mainly flavonoids and terpenoids, and is one of the famous commercial complementary nutraceuticals with antiradical, antioxidant, antiplatelet, and anti-aging effects (Chan *et al.* 2007). Using natural nutraceuticals and alternative medicines to benefit human health is an increasing trend throughout the globe (Holst and Williamson 2008; Joseph *et al.* 2009).

Species of *Calocedrus* belonging to *Cupressaceae* are living fossil plants and are distributed in eastern Asia and western North America; only four species are recognized in this genus (Chen *et al.* 2009). *Calocedrus formosana* (Taiwan incense cedar) is one of

the precious endemic woods in Taiwan. Ichikawa (1932) first reported that the main constituents of *C. formosana* heartwood oil were acidic components. Part constituents, including terpenoids, lignans, and steroids, have been isolated and identified from *C. formosana* wood, bark, and leaf (Lin *et al.* 1956; Kuo *et al.* 1975; Fang *et al.* 1985, 1990; Chiang *et al.* 2003; Hsieh *et al.* 2006). Bioactivities of natural products from *C. formosana* were reported in recent years, including antitumor, antioxidant, anti-inflammatory, antibacterial, antitermitic, and antifungal activities (Chao *et al.* 2005; Cheng *et al.* 2004; Chiang *et al.* 2003; Wang *et al.* 2004; Hsieh *et al.* 2006).

In the present study, the content of antioxidants of ethanolic extract from *C. formosana* heartwood and its fractions were analyzed, and the antiradical and antioxidant activities of ethanolic extract and its fractions were evaluated. In addition, the antioxidative lignans were isolated and identified from active fraction by chromatographic and spectroscopic methods.

EXPERIMENTAL

Plant Materials and Extraction

The heartwood of *Calocedrus formosana* Florin was collected from 36-year-old trees at the Experimental Forest of National Taiwan University (located in Nan-Tou county, Taiwan). The species was identified, and a voucher specimen (CF92) was deposited in National Taiwan University. Specimens were extracted twice with 95% ethanol at ambient temperature. Then, the crude extract (200 g) was further separated into an *n*-hexane-soluble fraction (HEXF, 42.5%), an ethyl acetate-soluble fraction (EAF, 41.9%), and a water-soluble fraction (WF, 12.4%) by liquid-liquid partition. Antioxidant activities of three fractions were evaluated to screen the active one for further separation. The active fraction, EAF, was coated onto silica gel (Silica-60, Merck 7734); the eluted solvents were *n*-hexane, ethyl acetate, and ethanol with different proportions. EAF (43.7 g) was separated into 11 subfractions by open column chromatography (CC) and thin layer chromatography (TLC). In order to obtain pure active compounds, high performance liquid chromatography (HPLC) was used to isolate the compounds from the active subfractions based on an activity-guided fractionation procedure.

HPLC Analysis

Active subfractions were analyzed by HPLC (Hitachi L-2130) fitted with a C18 column (Phenomenex, Luna 5u, 250 x 10 mm). The gradient mobile phase consisted of water (A) and acetonitrile (B). The flow rate was 3 mL/min. The elution program involved a linear gradient from 40% B in A for 0 to 15 min, 40 to 55% B in A by 35 min, and 55 to 100% B in A by 30 min followed by 5 min of equilibrium with 100% B. The elution profile was detected by UV detector at 254 nm. Isolated and purified compounds (ca. 10 to 25 mg) were obtained for spectral analyses and antioxidant assays.

Spectral Analyses

Structures of compounds were identified by spectroscopic methods including NMR (Nuclear magnetic resonance spectroscopy, Bruker Avance 500 MHz), FTIR

(Fourier transform infrared spectroscopy, Bio-rad FTS-40), UV/VIS (Ultraviolet and visible absorption spectroscopy, Jasco V-550), and MS (Mass spectroscopy, Finnigan MAT-958).

Determination of Total Phenolics Content

The total phenolics contents in tested specimens were determined according to the Folin-Ciocalteu procedure and expressed as milligrams per gram gallic acid equivalents (GAE). The Folin-Ciocalteu phenol reagent (0.5 mL) was mixed with 0.5 mL of a diluted sample solution. After an interval of 5 min, 1 mL of 10% aqueous sodium carbonate was added to the vial, and the mixture was allowed to stand for 8 min at ambient temperature; then the mixture was centrifuged at 12,000 rpm for 10 minutes. The absorbance of the solution was read at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample. The R-squared value of the calibration curve of gallic acid ($y = 27.637x - 0.1362$) was 0.9962.

DPPH Free Radical Scavenging Assay

Different concentrations of specimens (0.5 mL) were added to an ethanol solution of 1 mL of 0.15 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) in test tubes. The mixture was shaken and left to stand for 30 min at room temperature. The absorbance of the solution was then measured at 517 nm with a spectrophotometer (Hitachi U-3900). IC_{50} values are the concentrations of compounds required for a 50% decrease of the DPPH radicals (Bamoniri *et al.* 2010).

Reducing Power Assay

The reducing power of specimen was determined according to the method of Kapoor *et al.* (2009). Specimen (0.5 mL) was mixed with phosphate buffer (0.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 mL, 1%). The mixture was incubated at 50°C for 20 min. After cooling by ice water, 0.5 mL trichloroacetic acid (10%) was added to the mixture, and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (0.5 mL) was mixed with distilled 0.5 mL water and 0.1 mL $FeCl_3$ (0.1%). The absorbance of the solution was measured at 700 nm.

Total Antioxidant Activity

The ability of the specimen to scavenge $ABTS^{\cdot+}$ radical cation was compared to trolox standard (Garzón *et al.* 2010). The $ABTS^{\cdot+}$ radical cation was generated by mixing 7 mM $ABTS^{\cdot+}$ stock solution with 2.45 mM potassium persulfate and incubating for 12 to 16 h in the dark at room temperature until the reaction was complete and the absorbance was stable.

The absorbance of the $ABTS^{\cdot+}$ solution was diluted to 0.70 (± 0.02) with water at room temperature, then 1.485 mL was mixed with 15 μ L of the specimens, and the absorbance was measured at 734 nm after 6 min. All experiments were repeated three times. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and specimen to determine the trolox equivalent antioxidant concentration (TEAC).

Statistical Analyses

Mean values ($n \geq 3$) and standard deviations of specimens examined were calculated. The data were grouped by Scheffe's test of the SAS system (at a level of significance of $p < 0.05$).

RESULTS AND DISCUSSION

Plant extracts with high phenolic phytochemicals exhibit superior antioxidant potency as the sources of nutraceuticals (Sultana *et al.* 2007; Enayat and Banerjee 2009). The total phenolic contents in the heartwood extract and its fractions of *C. formosana* were expressed as gallic acid equivalents (GAE) as shown in Table 1. Total phenolic contents of the specimens fell within the range of 161.4 to 238.5 mg of GAE/g, except for the *n*-hexane soluble fraction (HEXF, 33.3 mg of GAE/g). Heartwood extract (187.1 mg of GAE/g) and ethyl acetate soluble fraction (EAF, 238.5 mg of GAE/g) contained more phenolic constituents.

Table 1. Antioxidant Content and Antioxidative Activities of Ethanolic Extract of *Calocedrus formosana* Heartwood and Its Fractions

| Specimen | Total Phenolic Content (mg of GAE/g) | DPPH IC ₅₀ (µg/mL) | TEAC at 0.5 mg/mL (mM of trolox) |
|----------|--------------------------------------|-------------------------------|-----------------------------------|
| Extract | 187.1 ± 2.2 | 19.2 ± 0.4 | 0.67 ± 0.01 |
| HEXF | 33.3 ± 0.2 | > 100 | 0.09 ± 0.01 |
| EAF | 238.5 ± 3.1 | 10.0 ± 0.3 | 0.82 ± 0.06 |
| WF | 161.4 ± 2.3 | 24.9 ± 0.4 | 0.41 ± 0.04 |
| Catechin | ND | 2.3 ± 0.1 | 2.89 ± 0.07 * |

HEXF: *n*-hexane soluble fraction; EAF: ethyl acetate soluble fraction; WF: water soluble fraction; ND: not detected; * At the concentration of 1 mM

The free radical scavenging activities of specimens were assessed by DPPH assay (Table 1). Except for the HEXF, most test samples showed a superior scavenging activity against the DPPH radicals. The IC₅₀ values of EAF and heartwood extract were 10.0 and 19.2 µg/mL in DPPH assay, respectively. The TEAC assay was used to evaluate the antioxidant activity of specimens. EAF shows the same trend in TEAC and DPPH assays; it exhibited the highest antioxidant capacity (0.82 mM of trolox at the concentration of 0.5 mg/mL) among three fractions.

Reductive potentials of specimens were determined by reducing power assay as shown in Fig. 1. The reducing power of the specimen increased with increasing concentration; EAF could reduce the higher amount of Fe³⁺ ions than those of the other specimens with statistical significance. Based on the evaluations of antioxidant contents

and activities of *C. formosana* heartwood extract and its fractions, these results revealed that EAF is of great interest to be a potential source of natural antioxidants.

Activity-guided fractionation of the ethyl acetate soluble fraction led to the isolation of five lignan compounds, as shown in Fig. 2, including haplomyrfofin (**1**), *O*-ethyl- α -conidendral (**2**), matairesinol (**3**), 7,8-dehydro-4-*O*-methyl-thujaplicatin (**4**), 5-methoxy-isosalicifoline (**5**).

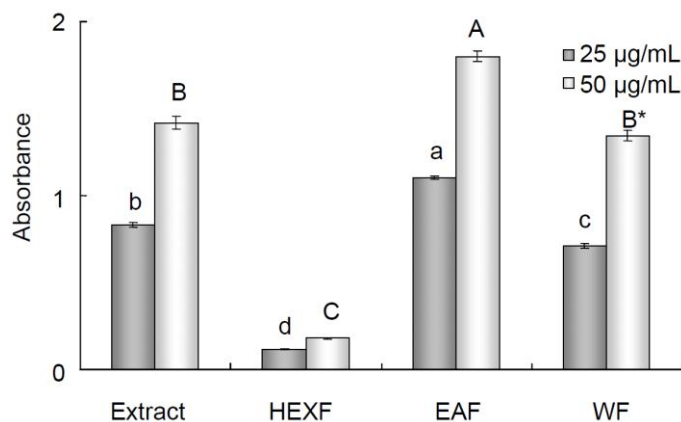


Fig. 1. Reducing power of the ethanolic extract and its fractions from the heartwood of *C. formosana* (HEXF: *n*-hexane soluble fraction; EAF: ethyl acetate soluble fraction; WF: water soluble fraction; * Different letters are statistically different, $p < 0.05$).

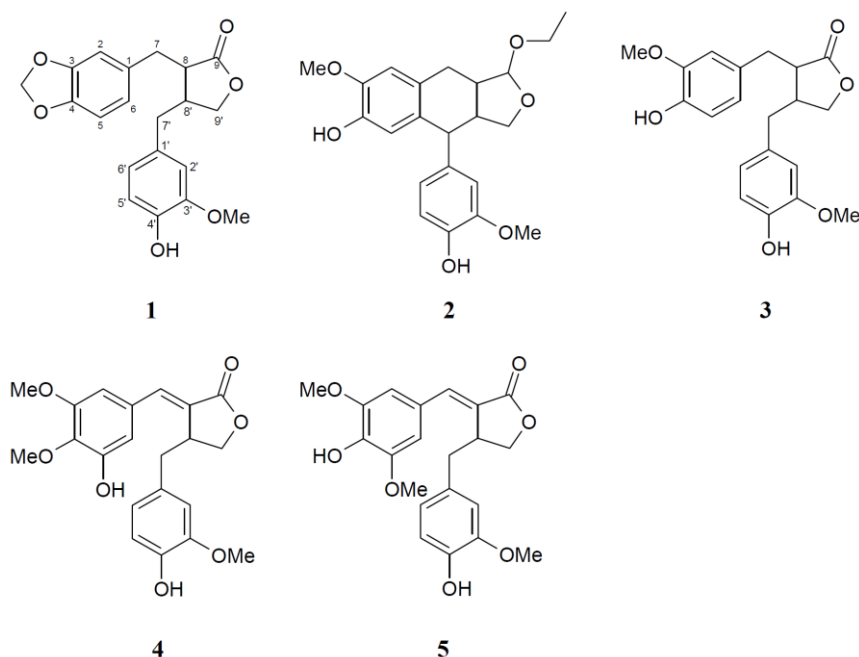


Fig. 2. Lignans isolated from the heartwood extract of *C. formosana*. (1: Haplomyrfofin; 2: *O*-ethyl- α -conidendral; 3: Matairesinol; 4: 7,8-Dehydro-4-*O*-methyl-thujaplicatin; 5: 5-Methoxy-isosalicifoline)

Spectral data of five lignan compounds are listed below:

Haplomyrfofin (**1**): colorless solid; $C_{20}H_{20}O_6$, HREI mass: 356.1252 (theor. 356.1254); UV (MeOH) λ_{\max} nm: 218, 242, 296; IR (KBr) ν_{\max} cm^{-1} : 3452, 2960, 2919, 2854, 1765, 1607, 1512, 1443, 1369, 1259, 1032, 929, 808; 1H NMR (500 MHz, $CDCl_3$): δ 2.49 (1H, m, H-8'), 2.51 (H, m, H-7'a), 2.53 (H, m, H-8), 2.56 (H, m, H-7'b), 2.84 (H, dd, $J=14.10, 7.15$ Hz, H-7a), 2.95 (H, dd, $J=14.10, 5.15$ Hz, H-7b), 3.82 (3H, s, C-3'-OMe), 3.84 (1H, dd, $J=9.30, 7.40$ Hz, H-9'a), 4.12 (H, dd, $J=9.30, 7.20$ Hz, H-9'b), 5.50 (1H, br s, C-4'-OH), 5.91 and 5.92 (2H, d, $J=1.40$ Hz, OCH_2O), 6.44 (1H, d, $J=1.80$ Hz, H-2'), 6.51 (1H, dd, $J=8.00, 1.80$ Hz, H-6'), 6.56 (1H, dd, $J=1.80, 7.80$ Hz, H-6), 6.57 (1H, d, $J=1.80$ Hz, H-2), 6.70 (1H, d, $J=7.80$ Hz, H-5), 6.78 (1H, d, $J=8.00$ Hz, H-5'); ^{13}C NMR (δ_c , $CDCl_3$, 125 MHz): 34.7 (C-7), 38.3 (C-7'), 41.3 (C-8'), 46.4 (C-8), 55.8 (C-3'-OMe), 71.2 (C-9'), 100.9 (OCH_2O), 109.4 (C-2), 108.2 (C-5), 111.0 (C-2'), 114.5 (C-5'), 121.2 (C-6'), 122.2 (C-6), 129.7 (C-1'), 131.4 (C-1), 144.4 (C-4'), 146.4 (C-4), 146.6 (C-3'), 147.9 (C-3), 178.5 (C-9).

O-ethyl- α -conidendral (**2**): white solid; $C_{22}H_{26}O_6$, HREI mass: 386.1723 (theor. 386.1724); UV (MeOH) λ_{\max} nm: 218, 234, 295; IR (KBr) ν_{\max} cm^{-1} : 3284, 2935, 2841, 1722, 1638, 1583, 1511, 1454, 1431, 1367, 1252, 1211, 1113, 986; 1H NMR (500 MHz, $CDCl_3$): δ 1.18 (3H, t, $J=7.05$ Hz, $-OCH_2CH_3$), 2.06 (1H, m, H-8), 2.50 (H, m, H-8), 2.84 (1H, dd, $J=14.75, 5.00$ Hz, H-7a), 3.05 (H, t, $J=14.75$ Hz, H-7b), 3.45 (1H, m, $-OCH_2CH_3$), 3.53 (1H, dd, $J=9.85, 8.45$ Hz, H-9'a), 3.61 (1H, d, $J=11.35$ Hz, H-7'), 3.76 (1H, m, $-OCH_2CH_3$), 3.79 (3H, s, C-3-OMe), 3.84 (3H, s, C-3'-OMe), 3.87 (1H, m, H-9'b), 5.09 (1H, d, $J=4.60$ Hz, H-9'b), 5.32 (1H, br s, C-4'-OH), 5.51 (1H, br s, C-4-OH), 6.33 (1H, s, H-5), 6.54 (1H, s, H-2), 6.62 (1H, br s, H-2'), 6.64 (1H, dd, $J=8.05, 1.60$ Hz, H-6), 6.81 (1H, d, $J=8.05$ Hz, H-5'); ^{13}C NMR (δ_c , $CDCl_3$, 125 MHz): 15.2 (C9- OCH_2CH_3), 29.2 (C-7), 45.9 (C-8), 46.1 (C-8'), 50.9 (C-7'), 55.9 (C-3-OMe), 55.9 (C-3'-OMe), 63.0 (C9- OCH_2CH_3), 72.2 (C-9'), 103.8 (C-9), 110.5 (C-2'), 111.1 (C-2), 114.2 (C-5'), 115.0 (C-5), 121.6 (C-6'), 128.2 (C-1), 132.8 (C-6), 136.4 (C-1'), 143.5 (C-4), 144.4 (C-4'), 145.0 (C-3), 146.7 (C-3'). The ethyl group in *O*-ethyl- α -conidendral is possibly derived from ethanol used for extraction.

Matairesinol (**3**): white solid; $C_{20}H_{22}O_6$, HREI mass: 358.1408 (theor. 356.1411); UV (MeOH) λ_{\max} nm: 217.5, 234, 285.5; IR (KBr) ν_{\max} cm^{-1} : 3434, 2937, 2846, 1758, 1606, 1516, 1452, 1371, 1274, 1123, 1031; 1H NMR (500 MHz, $CDCl_3$): δ 2.46 (1H, m, H-8'), 2.52 (H, m, H-7'a), 2.54 (H, m, H-8), 2.58 (H, m, H-7'b), 2.85 (H, dd, $J=14.10, 7.05$ Hz, H-7a), 2.93 (H, dd, $J=14.10, 5.20$ Hz, H-7b), 3.80 (6H, s, OMe), 3.86 (1H, dd, $J=9.15, 7.35$ Hz, H-9'a), 4.13 (H, dd, $J=8.95, 7.35$ Hz, H-9'b), 5.53 (1H, br s, C-4'-OH), 5.55 (1H, br s, C-4-OH), 6.39 (1H, d, $J=1.80$ Hz, H-2'), 6.49 (1H, dd, $J=8.00, 1.80$ Hz, H-6'), 6.57 (1H, d, $J=1.80$ Hz, H-6), 6.59 (1H, s, H-2), 6.78 (1H, d, $J=8.00$ Hz, H-5'), 6.79 (1H, d, $J=7.80$ Hz, H-5); ^{13}C NMR (δ_c , $CDCl_3$, 125 MHz): 34.5 (C-7), 38.3 (C-7'), 41.0 (C-8'), 46.5 (C-8), 55.7 (C-3'-OMe), 55.8 (C-3-OMe), 71.3 (C-9'), 110.9 (C-2'), 111.4 (C-2), 114.0 (C-5'), 114.4 (C-5), 121.3 (C-6'), 122.0 (C-6), 129.5 (C-1'), 129.7 (C-1), 144.3 (C-4'), 144.5 (C-4), 146.6 (C-3'), 146.7 (C-3), 178.8 (C-9).

7,8-Dehydro-4-*O*-methyl-thujaplicatin (**4**): colorless solid; C₂₁H₂₂O₇, HREI mass: 386.1373 (theor. 386.1360); UV (MeOH) λ_{max} nm: 211.5, 237, 317.5; IR (KBr) ν_{max} cm⁻¹: 3433, 2937, 2846, 1758, 1606, 1516, 1452, 1432, 1372, 1274, 1153, 1031; ¹H NMR (500 MHz, CDCl₃): δ 2.58 (H, dd, *J*=14.20, 3.95 Hz, H-7'a), 3.01 (H, dd, *J*=14.10, 4.10 Hz, H-7'b), 3.77 (H, m, H-8'), 3.87 (3H, s, C-3-OMe), 3.88 (3H, s, C-4-OMe), 3.95 (3H, s, C-3'-OMe), 4.24 (1H, dd, *J*=9.00, 6.6 Hz, H-9'a), 4.28 (H, dd, *J*=9.00, 1.80 Hz, H-9'b), 5.50 (1H, br s, C-4'-OH), 5.86 (1H, br s, C-5-OH), 6.60 (1H, d, *J*=1.80 Hz, H-2), 6.68 (1H, d, *J*=7.90 Hz, H-6'), 6.69 (1H, s, H-2'), 6.82 (1H, d, *J*=7.90 Hz, H-5'), 6.95 (1H, d, *J*=1.80 Hz, H-6), 7.45 (1H, s, H-7); ¹³C NMR (δ_c, CDCl₃, 125 MHz): 37.6 (C-7'), 40.1 (C-8'), 55.9 (C-3'-OMe), 56.0 (C-3-OMe), 61.1 (C-4-OMe), 69.8 (C-9'), 107.6 (C-2), 108.6 (C-6), 111.3 (C-2'), 114.5 (C-5'), 121.5 (C-6'), 127.7 (C-1), 129.8 (C-8), 129.8 (C-1'), 137.0 (C-4), 137.1 (C-7), 144.6 (C-4'), 146.7 (C-3'), 149.5 (C-5), 152.3 (C-3), 172.5 (C-9).

5-Methoxy-isosalicifoline (**5**): light yellowish solid; C₂₁H₂₂O₇, HREI mass: 386.1368 (theor. 386.1360); UV (MeOH) λ_{max} nm: 216, 243, 346; IR (KBr) ν_{max} cm⁻¹: 3414, 2935, 2845, 1738, 1641, 1604, 1516, 1456, 1428, 1357, 1210, 1117, 1033; ¹H NMR (500 MHz, CDCl₃): δ 2.62 (H, dd, *J*=14.55, 4.25 Hz, H-7'a), 3.05 (H, dd, *J*=14.55, 4.05 Hz, H-7'b), 3.84 (H, m, H-8'), 3.81 (3H, s, C-5-OMe), 3.87 (6H, s, C-3-OMe, C-3'-OMe), 4.22-4.27 (2H, m, H-9'), 5.62 (1H, br s, C-4'-OH), 5.93 (1H, br s, C-4-OH), 6.60 (1H, br s, H-2'), 6.65 (1H, d, *J*=8.20 Hz, H-6'), 6.80 (2H, s, H-2, H-6), 6.82 (1H, d, *J*=8.20 Hz, H-5'), 7.47 (1H, s, H-7); ¹³C NMR (δ_c, CDCl₃, 125 MHz): 37.3 (C-7'), 39.4 (C-8'), 55.8 (C-5-OMe), 56.4 (C-3-OMe), 56.4 (C-3'-OMe), 69.7 (C-9'), 107.3 (C-2), 107.3 (C-6), 111.5 (C-2'), 114.6 (C-5'), 121.0 (C-6'), 125.3 (C-1'), 125.8 (C-1), 129.5 (C-8), 137.0 (C-4), 137.7 (C-7), 144.6 (C-4'), 146.6 (C-5), 147.2 (C-3), 147.2 (C-3'), 172.6 (C-9).

The compounds haplomyrfofin, matairesinol, 7,8-dehydro-4-*O*-methyl-thujaplicatin, and 5-methoxy-isosalicifoline belong to the dibenzylbutyrolactone type of lignan; compound *O*-ethyl- α -conidendral is the aryltetralin-type lignan. Among these lignans, haplomyrfofin and *O*-ethyl- α -conidendral are isolated and identified for the first time from *Calocedrus formosana*. Lignans occur commonly in the higher plants, and they have been reported to possess a variety of biological properties, including antioxidant activities (Willfor *et al.* 2006; Yamauchi *et al.* 2006).

The antiradical activities of the lignan compounds are shown in Table 2; the activity of lignans decreased in the following order: haplomyrfofin > matairesinol > 5-methoxy-isosalicifoline > 7,8-dehydro-4-*O*-methyl-thujaplicatin > *O*-ethyl- α -conidendral in terms of the DPPH assay. Haplomyrfofin and matairesinol exhibited a superior ability to scavenge DPPH radicals (with IC₅₀ values of 7.9 and 9.9 μ M, respectively), and the capacity of these compounds was comparable to that of catechin (positive control, IC₅₀ = 8.6 μ M) without the statistical significant difference (*p* < 0.05). 5-Methoxy-isosalicifoline also had a good antiradical activity with an IC₅₀ value of 12.2 μ M. Antioxidant activities of isolated lignans were evaluated as the trolox equivalent antioxidant concentration (TEAC); TEAC values ranged between 1.25 and 1.92 mM Trolox. 5-Methoxy-isosalicifoline had the highest TEAC value (1.92 mM trolox), followed by matairesinol

(1.69 mM trolox). Haplomyrfofin exhibited the high antiradical activity and did not show the higher TEAC values. Based on the above results, matairesinol and 5-methoxy-isosalicifoline had better antiradical and antioxidant activities among these lignans. As for the effects of structure of compound on its antiradical and antioxidant activities, both matairesinol and 5-methoxy-isosalicifoline possess the dibenzylbutyrolactone skeleton with hydroxyl group at C4 and C4' positions.

Table 2. Antiradical and Antioxidant Activities of Lignan Compounds

| Compounds | DPPH IC ₅₀ (μM) * | TEAC at 1 mM (mM of trolox) * |
|--------------------------------------|---------------------------------|----------------------------------|
| Haplomyrfofin | 7.9 ± 0.3 ^e | 1.25 ± 0.04 ^D |
| O-ethyl-α-conidendral | 31.9 ± 0.2 ^a | 1.49 ± 0.04 ^C |
| Matairesinol | 9.9 ± 0.8 ^d | 1.69 ± 0.02 ^B |
| 7,8-Dehydro-4-O-methyl-thujaplicatin | 26.3 ± 1.0 ^b | 1.48 ± 0.01 ^C |
| 5-Methoxy-isosalicifoline | 12.2 ± 0.1 ^c | 1.92 ± 0.03 ^B |
| Catechin (Positive control) | 8.6 ± 0.1 ^{de} | 2.75 ± 0.04 ^A |

* Mean value of three replicates ± standard deviation; numbers followed by different superscript letters are statistically different ($p < 0.05$).

Multiple antioxidant products from forest bioresources, including leaf, fruit, stem, etc., have been investigated for applications in the food, pharmaceutical, and other industries (Sultana *et al.* 2007; Chan *et al.* 2007; Ebringerova *et al.* 2008; Kang *et al.* 2011; Luis *et al.* 2012). Antioxidant extract and compounds from *C. formosana* are worth further research and trial for complementary and alternative nutraceuticals.

CONCLUSIONS

1. In the total antioxidant assay, *C. formosana* heartwood extract showed good performance with a TEAC value of 0.67 mM of trolox at a concentration of 0.5 mg/mL; among three fractions of extract, ethyl acetate soluble fraction possesses highest antioxidant activity (0.82 mM of trolox) than the other fractions.
2. Five lignans, including haplomyrfofin, O-ethyl-α-conidendral, matairesinol, 7,8-dehydro-4-O-methyl-thujaplicatin, and 5-methoxy-isosalicifoline were isolated and identified from ethyl acetate soluble fraction. Among these compounds, haplomyrfofin and O-ethyl-α-conidendral were first found from *C. formosana*. Among these lignans, matairesinol, and 5-methoxy-isosalicifoline had preferable antiradical and antioxidant activities.
3. Based on the evaluations of antioxidant contents and activities of ethyl acetate soluble fraction and isolated lignans from *C. formosana* heartwood extract, it was revealed that the ethyl acetate soluble fraction, matairesinol, and 5-methoxy-isosalicifoline are of great interest to be a potential source of natural antioxidants.

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