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# 2,2′-Diphenyl-1-picrylhydrazyl Radical-Scavenging Active Components from Adlay (*Coix lachryma-jobi* L. Var. *ma-yuen* Stapf) Hulls

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An activity-directed fractionation and purification process was used to identify the antioxidative components of adlay hulls. Hulls of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) were extracted with methanol and then separated into water, 1-butanol, ethyl acetate, and hexane fractions. The 1-butanol-soluble fraction exhibited greater capacity to scavenge 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals when compared with fractions soluble in water, ethyl acetate, and hexane phases. The 1-butanol fraction was then subjected to separation and purification using Diaion HP-20 chromatography, silica gel chromatography, and HPLC. Six compounds showing strong antioxidant activity were identified by spectroscopic methods ($^1$H NMR, $^{13}$C NMR, IR, and MS) and by comparison with authentic samples to be coniferyl alcohol (**1**), syringic acid (**2**), ferulic acid (**3**), syringaresinol (**4**), 4-ketopinoresinol (**5**), and a new lignan, mayuenolide (**6**).

**KEYWORDS: Adlay hulls; free radical; antioxidant activity; active components**

## INTRODUCTION

Adlay (soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been consumed both as an herbal medicine and as a nourishing food. Adlay has been widely used as a diuretic, stomachic, analgesic, and antispasmodic agent from ancient times. Adlay is also effective in treating verrucae caused by the human papilloma virus and other tumorous diseases. The action of adlay against many kinds of disease can be attributed to various components with pharmacologically different activities. Recently, a number of pharmacologically and physiologically interesting substances have been isolated from the different parts of adlay, including antiinflammatory (*1*), antitumor (*2*), hypoglycemic (*3*), antimicrobial (*4*), and ovulatory-active (*5*) agents.

Reactive oxygen species (ROS) and oxygen free radicals play important roles, both beneficial and detrimental, in aerobic life (*6*). Excess ROS have been implicated in a variety of pathophysiological phenomena, such as inflammation, aging, atherosclerosis, cancer, rheumatoid arthritis, hepatotoxicity, and reperfusion injury (*7*). Methanolic extracts from adlay seed have been reported to have a moderate antioxidant effect (*8*−*10*). Our previous study demonstrated that the methanolic extract from adlay hulls exhibited greater antioxidant capacity than the other parts of adlay seed, including testa, bran, and polished adlay (*11*). In addition, adlay hull had long been used in the folk medicine of China as a nourishing food to regulate female endocrine system. There are some commercial products of functional food in Taiwan and Japan in which adlay hull is added as an ingredient for some unknown reason.

The adlay hull methanol extract is a strong scavenger of reactive oxygen species that inhibits free radical-generating enzymes, blocks tumor promoter-generated oxidative processes in neutrophile-like leukocytes, exhibits a cytoprotective effect on cultured cells exposed to *tert*-butyl hydroperoxide, and obstructs the growth and viability of cancer cells, that is, U937 leukemia cells, through apoptosis (*11*). However, the specific compounds responsible for the antioxidant activity of the adlay hull methanol extract remain unknown. This study was designed to identify the antioxidant constituents in the adlay hull methanol extract, to elucidate their chemical structures, and to compare their antioxidant capacities.

## MATERIALS AND METHOD

**General Procedures.** IR spectra were recorded on a Perkin-Elmer 983 G infrared spectrometer. $^1$H NMR and $^{13}$C NMR spectra were

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obtained on Bruker AM-300 and Bruker AMX-500 instruments, COSY and HMBC spectra were obtained on a Bruker AMX-500 instrument and recorded using standard pulse sequences. MS analysis was taken on a JEOL JMS-HX300 mass spectrometer. The measurement of melting points was performed with a Yanaco MP-S3 micro melting point apparatus (Yanagimoto Co., Kyoto, Japan). Ultraviolet absorption spectra of the purified active fractions were recorded on a U-2000 spectrophotometer (Hitachi) in methanol. Thin-layer chromatography was performed on silica gel $60F_{254}$ TLC plates (Merck, Darmstadt, Germany), with compounds visualized by spraying with 10% (v/v) $H_2SO_4$ in an ethanol solution. Diaion HP-20 ion-exchange resin (Mitsubishi, Japan) and silica gel (~230−400 $\mu$m) (Macherey-Nagel, Germany) were used for column chromatography. High performance liquid chromatography (HPLC) was performed with a GBCLC-1440 instrument and a GBCLC-1240 RI detector (GBC Scientific Equipment, Australia). A 10 × 250-mm-i.d., 7-$\mu$m, Lichrosorb Si-60 column (Merck, Darmstadt, Germany) was used for analysis. α,α-Diphenyl-β-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St. Louis, MO). All solvents used for chromatographic isolation were of analytical grade and purchased from Tedia Co. (Fairfield, OH).

**Plant Material.** Adlay was purchased from a local farmer who planted Taichung Shuenyu no. 4 (TCS4) of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf in Taichung, Taiwan, in March 1997 and harvested it in July of the same year. After the harvest, the seeds were dried at ambient temperature with ventilation and dehulled by a grinder. The samples were divided into hull, testa, and dehulled adlay by gently blowing using an electric fan. The dehulled adlay was separated into bran and polished adlay. All the materials, including hull, testa, bran, and polished adlay were blended in powder form, and screened through a 20-mesh sieve (aperture, 0.94 mm).

**Preparation of the Methanolic Extracts from the Different Parts of Adlay Seed.** To prepare a small amount of the methanolic extracts from the different parts of adlay seed for selecting the greatest DPPH-scavenging material, the following procedure was used. Each sample powder (100 g) was extracted with 1 L of methanol stirred on a stirring plate at room temperature for 24 h. Contents were filtered through no. 1 filter paper (Whatman Inc., Hillsboro, OR). The filtrate was concentrated to dryness in vacuo to obtain methanolic extract and stored at −20 °C. The methanolic extracts from different parts of adlay seeds were named as AHM (hull), ATM (testa), ABM (bran) and PAM (polished adlay).

**Extraction and Isolation of Antioxidant Compounds from Adlay Hull Methanol Extract. Figure 1** shows the scheme for the preparation of antioxidant compounds from adlay hulls. To prepare a large amount of the methanolic extracts from adlay hull for isolation and purification of antioxidants with DPPH-scavenging activity, the following procedure was used. The sample powder (5 kg) was extracted three times with 15 L of methanol at room temperature for 2 weeks (5 day for each time). To minimize methanol consumption in the large alday hull methanol extraction, we prolonged the extraction time to replace the methanol of use. The plant material was filtered off, and the methanolic extracts were combined and concentrated under reduced pressure by a rotatory vacuum evaporator. The obtained dry extract (AHM, 65.6 g) was suspended in 650 mL of $H_2O$, followed by an extraction with same the volumes of *n*-hexane, ethyl acetate, and 1-butanol, yielding four subfractions denoted as the AHM-P1 (*n*-hexane fraction), AHM-P2 (ethyl acetate fraction), AHM-P3 (1-butanol fraction), and AHM-P4 (water fraction). AHM-P3 (21.3 g) was dissolved in 100 mL of methanol, then subjected to column chromatography on Diaion HP-20 resin (8 × 100 cm i.d.) and eluted by a $H_2O$/MeOH/EtOAc gradient with monitoring at 280 and 340 nm to afford fractions D1 (~50−70% MeOH/$H_2O$), D2 (~70−100% MeOH/$H_2O$), D3 (100% MeOH ~ 30% EtOAc/MeOH), D4 (~30−50% EtOAc/MeOH), D5 (~50−100% EtOAc/MeOH), and D6 (100% EtOAc). The greatest DPPH-active fraction D2 was chromatographed on a silica gel column using a $CHCl_2$/MeOH gradient system to afford four subfractions, including D2-I (2−5% $CHCl_2$/MeOH), D2-II (5−10% $CHCl_2$/MeOH), D2-III (~10−20% $CHCl_2$/MeOH), D2-IV (~20−100% $CHCl_2$/MeOH). Fraction D2-II was further purified by HPLC on a Lichrosorb Si-60 column at 2 mL/min, using 30% EtOAc/$CHCl_2$ as the eluent to yield compound **1** (17 mg).

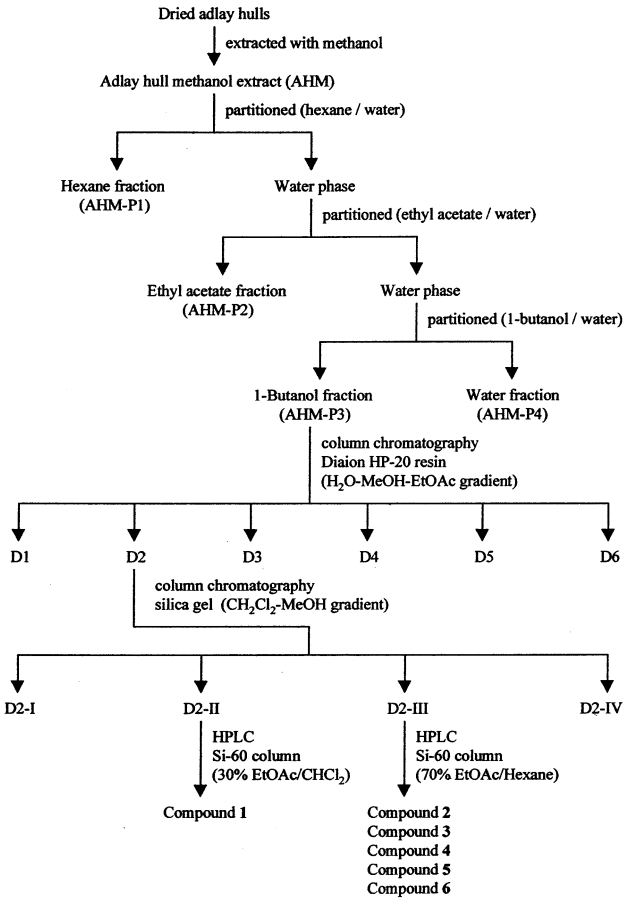


**Figure 1.** Scheme for preparation of antioxidant fraction and compounds from adlay hulls.

Fraction D2-III was further purified by HPLC using 70% EtOAc/hexane as the eluent to obtain compounds **2** (45 mg), **3** (18 mg), **4** (27.5 mg), **5** (123 mg), and **6** (11.5 mg). Each compound was collected using manual labor and concentrated at 40 °C under reduced pressure and check for purity by TLC and HPLC.

**Structural Determination of Isolated Compounds.** Coniferyl alcohol (**1**): amorphous powder [identical with the literature values (*12*)]. Syringic acid (**2**): colorless crystals. mp ~203−204 °C (lit. mp ~203−205 °C) [identical with the literature values (*13*)]. Ferulic acid (**3**): colorless crystals. mp ~174−175 °C (lit. mp ~178−179 °C) [identical with the literature values (*14*)]. Syringaresinol (**4**): colorless crystals. mp ~168−170 °C [(identical with the literature values (*15*)]. 4-Ketopinoresinol (**5**): amorphous powder [identical with the literature values (*16*)]. Mayuenolide (**6**): light yellow crystals. mp = ~215−217 °C. $[\alpha]^{25}_D$= −25.5°. EI-MS *m*/*z* (%) 402 ($M^+$, 10), 368 (5), 143 (100), 115 (65). HR-EIMS *m*/*z*: 402.1321 (calcd. for $C_{21}H_{22}O_8$, 402.1315). UV $\lambda_{max}$ (MeOH) nm (log $\epsilon$): 280 (3.1). IR $\nu_{Max}$ $cm^{-1}$ (KBr): 3416, 1772, 1619, 1520, 1215. $^1H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 3.18 (m, 1H, H-1), 3.42 (dd, $J$ = 9.1, 3.8 Hz, 1H, H-5), 3.88 (s, 9H, $-OCH_3$ × 3), 4.00 (dd, $J$ = 9.5, 4.5 Hz, 1H, H-$8_{(a)}$), 4.30 (dd, $J$ = 9.5, 6.8 Hz, 1H, H-$8_{(eq)}$), 5.29 (d, $J$ = 3.8 Hz, 1H, H-2), 5.32 (d, $J$ = 3.8 Hz, 1H, H-6), 6.47 (s, 2H, H-2′, H-6′), 6.83 (1H, d, $J$ = 8.1 Hz, 1H, H-5″), 6.86 (dd, $J$ = 8.1, 1.8 Hz, 1H, H-6″), 6.87 (1H, d, $J$ = 1.8 Hz, H-2″). $^{13}C$ NMR ($CDCl_3$, 300 MHz) $\delta$ 50.18 (C-1), 53.15 (C-5), 56.00 ($-OCH_3$), 72.68 (C-8), 83.35 (C-6), 84.71 (C-2), 101.87 (C-2′, C-6′), 108.06 (C-2″), 114.38 (C-5″), 117.97 (C-6″), 130.37 (C-1′), 134.96 (C-1″), 145.31 (C-3″), 146.69 (C-3′, C-5′), 147.37 (C-4′, C-4″), 176.90 (C-4). This compound was synthesized by Ralph et al. (*17*), but has been isolated from a natural source for the first time in this study.

**Determination of the Scavenging Effect on DPPH Radicals.** This method was modified from that of Shimada et al. (*18*). For the modified procedure, a 400 $\mu$M solution of DPPH was prepared in 100% methanol. Instead of reading samples spectrophotometrically, the assay was

**Table 1.** $EC_{50}$ of the Methanolic Extracts and Subfractions from Different Parts of Adlay Seed in Scavenging DPPH Radicals[a]

| test sample | $EC_{50}$ ($\mu$g/mL) |
|---|---|
| Methanolic Extracts from Different Parts of Adlay Seed | |
| AHM | $81.3 \pm 3.3^{e}$ |
| ATM | $566 \pm 15^{d}$ |
| ABM | $795 \pm 18^{c}$ |
| PAM | $1707 \pm 28^{b}$ |
| Subfractions of AHM by Solvent Partition | |
| AHM-P1 | >500 |
| AHM-P2 | $102.5 \pm 1.5^{c}$ |
| AHM-P3 | $85.1 \pm 3.2^{d}$ |
| AHM-P4 | $275.3 \pm 2.4^{b}$ |
| Subfractions of Diaion HP-20 Resin Column Chromatography from AHM-P3 | |
| AHM-P3-D1 | $62.5 \pm 1.2^{c}$ |
| AHM-P3-D2 | $38.3 \pm 0.5^{d}$ |
| AHM-P3-D3 | $58.1 \pm 3.2^{c}$ |
| AHM-P3-D4 | $500 \pm 2.4^{b}$ |
| AHM-P3-D5 | inactive |
| AHM-P3-D6 | inactive |

[a] The reaction was started by addition of DPPH (final concentration = 300 $\mu$M) to test sample containing methanol solution. The decrease of absorbance of DPPH was recorded spectrophotometrically 90 min after mixing at 517 nm. $EC_{50}$ were mean ± standard deviation of three replicates. Values in each column with different superscripts are significantly ($p < 0.05$) different by analysis of variance with multiple comparisons.

performed in a microplate. To a well in a 96-well flat-bottom EIA microtitration plate were added 50 $\mu$L of sample (final concentration of use: AHM and its subfration, ~0−500 $\mu$g/mL; ATM, ABM, and PAM, ~0−2000 $\mu$g/mL; pure compound, ~0−250 $\mu$g/mL) and 150 $\mu$L of DPPH solution. The final concentration of DPPH solution was 300 $\mu$M. After thorough mixing, the solutions were kept in the dark for 90 min. Thereafter, the absorbency of the samples was measured using an Optimax automated microplate reader (Molecular Devices, CA) at 517 nm against methanol without DPPH as the blank reference. Each sample was quadruplicated in the test, and the values were averaged. For the determination of $EC_{50}$ (the efficient concentration of antioxidant decreasing initial DPPH concentration by 50%), each sample was measured at seven different concentrations in the DPPH test. The $EC_{50}$ was obtained by interpolation from linear regression analysis. We collected the $EC_{50}$ values of each sample from three replicates, then we obtained mean ± standard deviation values for the results of the $EC_{50}$ assay (**Tables 1** and **2**).

**Statistical Analysis.** All data were expressed as mean ± standard deviation. Analysis of variance was performed by ANOVA procedures. Duncan's new multiple-range test was used to determine the difference of means, and $p < 0.05$ was considered to be statistically significant.

## RESULTS

**DPPH Radical-Scavenging Activity of the Different Part of Adlay Seed.** Free radical scavenging is generally the accepted mechanism for antioxidants' inhibiting lipid oxidation. The model of scavenging stable DPPH radicals can be used to evaluate the antioxidative activities in a relatively short time, as compared to other methods, and it has been used extensively to predict the antioxidant activities of various chemicals (*19*−*25*). As shown in **Table 1**, the scavenging activity order of the different part of adlay seed was adlay hull methanol extract (AHM) > adlay testa methanol extract (ATM) > adlay bran methanol extract (ABM) > polished adlay (PAM) ($p < 0.05$). AHM possesses the strongest capacity to scavenge DPPH radicals, as compared to the others.

**DPPH Radical-Scavenging Activity of the Subfractions from Adlay Hull Methanol Extracts.** To isolate the component responsible for the antioxidative activity, AHM was further partitioned to obtain four subfractions, including AHM-P1 (*n*-hexane fraction), AHM-P2 (ethyl acetate fraction), AHM-P3 (1-butanol fraction), and AHM-P4 (water fraction). The DPPH radical-scavenging activities of the fractions were measured, and the data is shown in **Table 1**. AHM-P1 showed no activity under the conditions used, but the other three fractions showed antioxidant activity. AHM-P2 and AHM-P3 showed much stronger activity than AHM-P4. The efficiency of AHM-P2 was less than that of the AHM-P3 ($p < 0.05$). AHM-P3 was further fractionated using Diaion HP-20 resin column chromatography with stepwise gradient elution, affording six fractions (AHM-P3-D1−AHM-P3-D6), and a thorough DPPH test was conducted with these six fractions. The result is shown in **Table 1**. The scavenging capacity on DPPH radical was found to follow the order: AHM-P3-D2 > AHM-P3-D3 > AHM-P3-D1. AHM-P3-D4, AHM-P3-D5, and AHM-P3-D6 showed no activity under the conditions used. TLC showed the presence of several compounds in fraction AHM-P3-D2. Therefore, this fraction was subjected to another round of fractionation to obtain six compounds with strong antioxidant activity from subfractions AHM-P3-D2-II and AHM-P3-D2-III. Compound **1** was isolated from fraction AHM-P3-D2-II by HPLC using 30% EtOAc/$CH_2Cl_2$ as the eluent. Compounds **2**−**6** were purified from AHM-P3-D2-III by HPLC using 30% EtOAc/$CH_2Cl_2$ as the eluent. **Figure 2** show the HPLC chromatograms of fraction AHM-P3-D2-III.

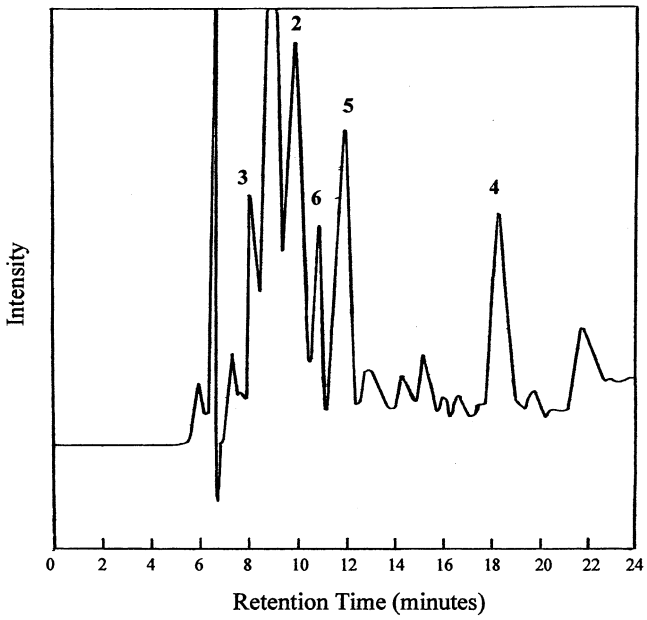


**Figure 2.** HPLC chromatograms of fraction AHM-P3-D2-III from adlay hull methanol extract. **2**, syringic acid; **3**, ferulic acid; **4**, syringaresinol; **5**, 4-ketopinoresinol; **6**, mayuenolide.

**Identification of the Isolated Compounds from AHM-P3-D2.** Compounds **1**−**5** were identified as coniferyl alcohol (**1**) (*12*), syringic acid (**2**) (*13*), ferulic acid (**3**) (*14*), syringaresinol (**4**) (*15*) and 4-ketopinoresinol (**5**) (*16*) by comparison of analytical data (IR, NMR, and MS) with those of authentic compounds. Their structures are shown in **Figure 3**.

Compound **6** had a molecular formula of $C_{21}H_{22}O_7$ from its HR-EIMS. Its IR spectrum showed hydroxy and ester carbonyl bond (3416 and 1772 $cm^{-1}$) absorbances, and the UV spectrum revealed the presence of an oxygenated aromatic ring (280 nm). The $^1H$ NMR spectral data of compound **6** showed two oxygenated methine protons [$\delta_H$ 5.32 (1H, d, $J = 3.8$ Hz), 5.29

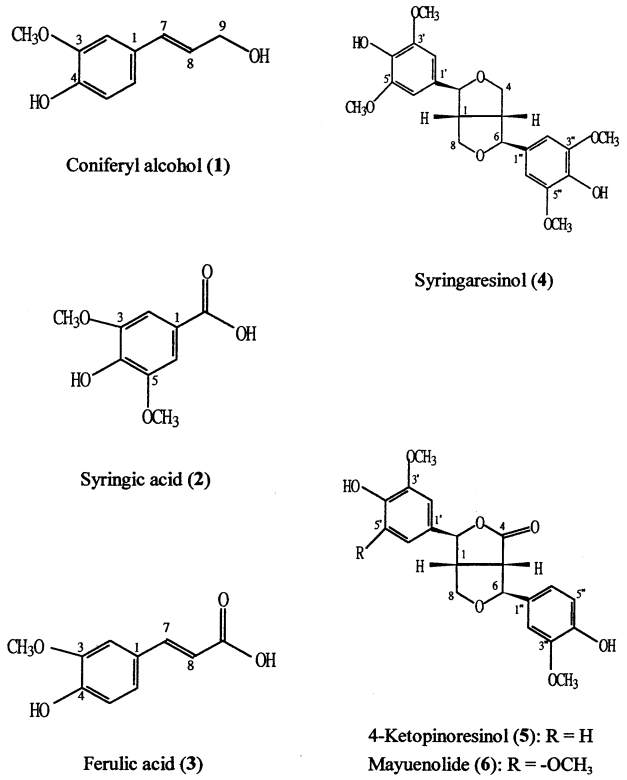


**Figure 3.** Structure of antioxidants isolated from adlay hulls.

(1H, d, $J$ = 3.8 Hz)], oxygenated methylene protons [$\delta_H$ 4.00 (1H, dd, $J$ = 9.5, 4.5 Hz), 4.30 (1H, dd, $J$ = 9.5, 6.8 Hz)], a 1,3,4,5-tetrasubstituted benzene ring [$\delta_H$ 6.47 (2H, s)], and a 1,3,4-trisubstituted benzene ring [$\delta_H$ 6.87 (1H, d, $J$ = 1.8 Hz), 6.83 (1H, dd, $J$ = 8.1, 1.8 Hz), 6.86 (1H, d, $J$ = 8.1 Hz)]. Its $^{13}$C NMR spectroscopic data revealed a lactone carbonyl carbon ($\delta_C$ 176.9), an oxygenated methylene ($\delta_C$ 72.68), two oxygenated methine ($\delta_C$ 83.35 and 84.71), and carbon signals within the downfield region, indicating the presence of two benzene groups. From these observations, compound **6** was assumed to be a lignan of the 3,7-dioxabicyclo[3.3.0]octane type. From the $^1$H-$^1$H COSY and HMBC spectra, two partial structures [−O−CHCHCOO − and −$CH_2$CHCH−O−] were revealed, and could be assigned at positions C-4, C-5, C-6, and C-1, C-2, C-8 of the 3,7-dioxabicyclo[3.3.0]octane framework. From analysis of the coupling pattern, it was concluded that the two aryl groups were syringyl (4-hydroxy-3,5-dimethoxyphenyl) and guaiacyl (4-hydroxy-3-methoxyphenyl) moieties, respectively. In the HMBC spectrum of compound **6**, the proton signal at $\delta_H$ 5.29 (H-2) correlated with the carbon signals at $\delta_C$ 130.37 (C-1′), 101.87 (C-2′ and C-6′), 176.9 (C-4), and 72.68 (C-8). The signal at $\delta$ 5.32 (H-6) correlated with the carbon signals at $\delta$ 134.96 (C-1″), 117.97 (C-6″), 176.9 (C-4), and 72.68 (C-8) (**Figure 4**). Thus, the syringyl and guaiacyl groups were assigned at positions C-2 and C-6, respectively. The coupling constants of H-2 ($J$ = 3.8 Hz) and H-6 ($J$ = 3.8 Hz) indicated that both were axial protons. Therefore, compound **6** was 2$\beta$-syringyl-4-oxo-6$\beta$-guaiacyl-3,7-dioxabicyclo[3.3.0]octane. Its structure is shown in **Figure 3**. This compound is a radical cross-coupling product formed by oxidative coupling of ferulate with syringyl units that was described by Ralph et al. in 1995 (*17*), but no physical data has been reported. Compound **6** had not previously been found as a naturally occurring substance. We isolated this compound for the first time from hulls of adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf), and named it mayuenolide.

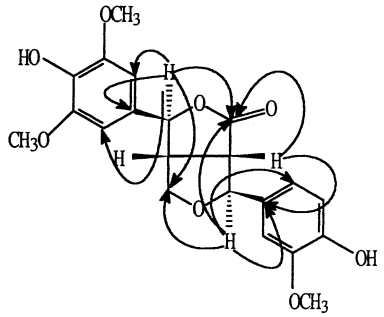

**Figure 4.** Selected carbon–proton long-range connectivities observed in HMBC spectrum of compound **6** (mayuenolide).

**Table 2.** $EC_{50}$ of Active Components from Fraction AHM-P3-D2 in Scavenging DPPH Radicals[a]

| active components | $EC_{50}$ $\mu$g/mL | $EC_{50}$ $\mu$M |
|---|---|---|
| Positive Control | | |
| BHT | 14.1 ± 3.3 | 64.1 |
| BHA | 17.0 ± 2.9 | 94.4 |
| α-tocopherol | 30.8 ± 5.4 | 71.5 |
| Antioxidant from AHM-P3-D2 | | |
| (a) phenolic alcohol | | |
| coniferyl alcohol | 15.6 ± 2.4 | 86.7 |
| (b) phenolic acid | | |
| syringic acid | 6.8 ± 1.2 | 34.3 |
| ferulic acid | 12.4 ± 3.2 | 63.9 |
| (c) lignan | | |
| syringaresinol | 24.6 ± 3.1 | 58.9 |
| 4-ketopinoresinol | 52.7 ± 4.6 | 141 |
| mayuenolide | 46.3 ± 3.8 | 115 |

[a] The reaction was started by addition of DPPH (final concentration = 300 $\mu$M) to the test sample containing methanol solution. The decrease in absorbance of DPPH was recorded spectrophotometrically at 517 nm 90 min after mixing. $EC_{50}$ was mean ± standard deviation of three replicates.

**DPPH Radical-Scavenging Activity of the Isolated Compounds.** The six purified compounds demonstrated DPPH free radical scavenging activity following the order of syringic acid (**2**) > syringaresinol (**4**) > ferulic acid (**3**) > coniferyl alcohol (**1**) > mayuenolide (**6**) > 4-ketopinoresinol (**5**) (**Table 2**). Compounds **1**−**6** were phenolic antioxidants. 4-Ketopinoresinol was isolated from the seed of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf by a research group in Japan (*16*), and we have now found it to exist in seed hulls. Except for 4-ketopinoresinol, the these purified antioxidants were found to exist in the seed of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf for the first time by this study. These antioxidants were divided into phenolic alcohols, phenolic acids, and lignans (**Table 2**). Syringic acid, syringaresinol, ferulic acid, and coniferyl alcohol showed a strong effect in DPPH radical scavenging, with $EC_{50}$ values of 34.34, 58.85, 63.91, and 86.67 $\mu$M, respectively, and the antioxidant capacity of these adlay constituents compared favorably with the standards BHA, BHT, and α-tocopherol. However, 4-ketopinoresinol and mayuenolide showed weaker inhibition than the above compounds.

## DISCUSSION

Methanol was chosen for extraction in this study because it has wide solubility properties for low molecular and moderately polar substances, including the antioxidant-active phenolic compounds. Our previous study showed that methanolic extracts of adlay hull (AHM) displayed multiple antioxidant effects in scavenging superoxide anions, hydrogen peroxides, and hydroxy

radicals (*11*). In this experiment, we also found that, for scavenging DPPH radicals, AHM possesses the strongest capacityof the methanolic extracts from the other parts of adlay seed. DPPH is a free radical compound that has been used extensively to predict the antioxidant activities of various chemicals (*19*−*23*). Several studies have shown many plant extracts and phytochemicals to have a significant correlation between DPPH scavenging activity and inhibition of lipid peroxidation in isolated hepatocytes treated with *tert*-butyl hydroperoxide (*24*, *25*).

The seed hull, which covers the seed, plays the major role in the physical and chemical defense systems of the seed. Antioxidant compounds in plants, for example, tocopherols, carotenoids, and other phenolic compounds, are effective in the protection against oxidative damage toward membranes that contain polyunsaturated fatty acids. Therefore, many plants were investigated as sources of natural antioxidants; a great variety of compounds have been isolated, many of which are phenoic compounds (*19*−*20*). The antioxidative activity of phenolic compounds is generally ascribed to their hydroxyl groups, but that is not the only factor in determining the potency of their activities. In this experiment, we isolated six compounds with DPPH radical-scavenging activity, that is, coniferyl alcohol, syringic acid, ferulic acid, syringaresinol, 4-ketopinoresinol, and mayuenolide, from adlay hulls.

Syringic acid is a naturally occurring phenolic compound with antioxidant capacity. The *para* substitution allows the phenoxy radical of syringic acid to be delocalized across the entire molecule and, therefore, stabilized. The *ortho* substitution with the electron donor methoxy group is also a factor that increases the stability of the phenoxy radical and therefore increases its antioxidative efficiency (*26*). In this study, syringic acid showed the strongest DPPH radical scavenging activity, with an $EC_{50}$ of 34.34 $\mu$M. Syringic acid inhibits the peroxidatic activity of human ferricyanohemoglobin (*27*). Hirota et al. (*28*) found that syringic acid was one of the major antioxidants existing in soybean miso. Syringaresinol is a lignan of ($\alpha-\gamma$) double-cyclized type with two syringyl groups. It was a potent antioxidant of DPPH scavenging in our study and also inhibits lipid autoxidation (*29*), and LDL oxidation induced by $Cu^{2+}$ (*30*).

In the case of ferulic acid, because of its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance-stabilized phenoxy radical, which accounts for its potent antioxidant potential (*31*). The *ortho* substitution with the electron donor methoxy group is also a factor that increases the stability of the phenoxy radical and, therefore, increases its antioxidative efficiency (*32*). In this study, ferulic acid showed the stronger DPPH radical scavenging activity, with an $EC_{50}$ of 63.9 $\mu$M, as compared to the standards BHA, BHT, and $\alpha$-tocopherol. Ferulic acid was also effective on the lipid peroxidation system of liposomes induced by ferrous ion (*33*). In addition, ferulic acid also inhibits benzoyl peroxide, phorbol-12-myristate-13-acetate, and mezerein-induced superoxide anion radical production in both in vivo and in vitro conditions (*34*, *35*). By virtue of effectively scavenging deleterious radicals, ferulic acid may serve an important antioxidant function in preserving physiological integrity of cells exposed to oxidative degradation (*31*), and by the same mechanism, ferulic acid may protect against various inflammatory diseases (*31*, *36*, *37*). Both ferulic acid and coniferyl alcohol possess a guaiacyl (4-hydroxy-3-methoxyphenyl) moiety and an extended side chain conjugation. Therefore, coniferyl alcohol also is a potent radical scavenger. In addition, coniferyl alcohol has almost the same activity as BHA in inhibition of lipid peroxidation (*29*).

Both 4-ketopinoresinol and mayuenolide are lignans of the ($\alpha-\gamma$) double cyclized type, with 4-ketopinoresinol having two guaiacyl groups and mayuenolide having a syringyl and a guaiacyl group, respectively. The difference in the antioxidative activities between these two lignans might be attributed to the extent of methoxyl substitution in their structures. Brand-Williams et al. (*38*) reported that interaction of a potential antioxidant with DPPH radical depends on its structural conformation. Certain compounds react very rapidly with the DPPH radical, reducing the number of DPPH radical molecules corresponding to the number of available hydroxyl group. However, for the majority of the compounds tested, the mechanism is more complex.

In summary, the DPPH-directed fractionation and identification study has resulted in the identification of six phenolic compounds from the hulls of adlay that all showed strong free radical scavenging activity. This has furthered our understanding of the antioxidant activity of adlay hulls and also has implications in the food industry for use as a natural antioxidant. Further studies on the physiological functions of these antioxidants are required.

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