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ORIGINAL ARTICLE

Antioxidative catechol lignans/neolignans isolated from defatted kernel of *Jatropha curcas*

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Abstract *Jatropha curcas* L. has been cultivated in many countries for biodiesel production, and defatted kernel is generated in huge quantities as a by-product in the production. This study investigated the phenolic content and antioxidative activity of the defatted kernel of Jatropha. Four extracts, EtOAc-organic, EtOAc-water, MeOH-organic, and MeOH–water fractions, were tested for phenolic content and antioxidative activity using the Folin–Ciocalteu method and the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method, respectively. The MeOH-organic fraction showed a high content of phenolic compounds and exhibited a relatively strong antioxidative activity. Eight compounds were isolated from the MeOH-organic fraction, and identified as a catechol-type lignan (3,3′- bisdemethylpinoresinol), five neolignans (isoamericanol A, americanol A, 9′-*O*-methylisoamericanol A, 9′-*O*-methylamericanol A, and isoamericanin A), and two sesquineolignans (isoprincepin, princepin). The antioxidative activities of 3,3′-bisdemethylpinoresinol (IC_{50}: 16.0 μM), isoprincepin (9.12 μM), and princepin (11.7 μM), which have two catechol groups were higher than that of Trolox (21.5 μM). The isoamericanol A inhibited the oxidation of biodiesel. These results indicate that the defatted kernel of Jatropha is a source of natural antioxidants that would applicable for stabilizers in Jatropha biodiesel industries.

Keywords Antioxidative activity · Biodiesel fuel · Catechol · DPPH · *Jatropha curcas*

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Introduction

Jatropha (*Jatropha curcas* L.), which is native to tropical Latin America, is an oil-bearing plant belonging to Euphorbiaceae [1]. The shrub is cultivated in tropical and subtropical regions throughout Asia, Africa, and Latin America. This plant is hardy, easy to propagate, drought tolerant, high in oil content, grows rapidly, and adapts to wide climatic conditions [2, 3]. Jatropha does not compete with food crops for land and water, and the non-edible oil from Jatropha is favored for biodiesel production. The seed kernels of the plant contain 40–60 % oil, which can be transesterified to produce biodiesel. The oil is also utilized for the production of soap and cosmetics. However, the seed oil contains phorbol esters that are toxic to humans and animals; edible and nutritional applications are impossible [4–7].

Although many studies have been investigated biodiesel production from Jatropha [8, 9] and the toxicity of phorbol esters, the chemical constituents and biological activities of Jatropha are not fully known [10]. In this study, we isolated and identified a catechol-type lignan, five neolignans, and two sesquineolignans from the defatted kernel of Jatropha and investigated their antioxidative activities and oxidation inhibition tests of biodiesel.

Materials and methods

General experimental procedures

Column chromatography was conducted on an FMI pump system (Yamazen) with a column of Merck silica gel 60 (230–400 mesh, ASTM). Analytical thin-layer chromatography (TLC) and preparative TLC were performed

using plates precoated with Merck silica gel 60 F_{254} (thicknesses of 0.25 and 0.50 mm, respectively). Analytical high-performance liquid chromatography (HPLC) was carried out on a Jasco PU-980 equipped with a Jasco UV-970 intelligent UV/Vis detector (280 nm) and Spectra Manager (ver.1.53) using a reversed-phase column (TSK-GEL ODS-80Ts, 250 × 4.6 mm i.d., Tosoh). Preparative HPLC was done using the same system with a different column size (300 × 7.8 mm i.d.). Chiral HPLC was performed on a Jasco PU2089 with Jasco OR-990 using chiral OD-H (250 × 4.6 mm i.d., Daisel). Optical rotation was recorded on a Jasco P-1010 polarimeter. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra and two-dimensional NMR spectra were obtained on a JEOL JNM ECA 600 FT-NMR spectrometer (600 MHz) using tetramethylsilane as an internal standard. Electron-impact (EI, 70 eV) and fast-atom bombardment (FAB) mass spectrometry (MS) were performed using a JEOL JMS-SX102A mass spectrometer. High-resolution time of flight mass spectrometry (HRTOFMS) was performed with a Waters Xevo G2-XS.

Plant material

The kernels of Jatropha (356 g) were harvested at the farm of the Faculty of Agriculture, Chiang Mai University and treated with an oil expeller to remove the Jatropha oil. A voucher specimen (EK625) was deposited in the Faculty of Agriculture, Kagawa University.

Extraction and isolation

The resulting expelled material (278 g) was crushed with a blender and extracted in Soxhlet extractors with *n*-hexane, ethyl acetate, and methanol, successively. The ethyl acetate (3.08 g) and methanol extracts (12.6 g) were concentrated in vacuo, and the residues were partitioned between ethyl acetate and water to give four fractions (Fig. 1): EtOAc-organic (1.78 g), EtOAc-water (0.915 g), MeOH-organic (0.758 g), and MeOH–water fractions (11.6 g).

The MeOH-organic fraction was fractionated on a silica gel column (dichloromethane/methanol = 100:0 to 0:100 as a stepwise elution, column size 550 × 10 mm i.d.) to afford twelve fractions (Frs. 1–12). Fr. 7 (163 mg) was separated by preparative TLC (dichloromethane/methanol = 9:1) to give two fractions (Frs. 7-1 and 7-2). Fr. 7-1 was purified by preparative HPLC (50 % methanol, 1.0 mL/min) to afford compounds **1** (23.7 mg), **2** (17.4 mg), and **3** (6.7 mg). Fr. 4 (43.5 mg) was purified by preparative HPLC (60 % methanol, 0.8 mL/min) to give compounds **4** (6.0 mg), **5** (1.9 mg), and **6** (1.2 mg). Fr 7-2 was further separated by preparative HPLC (50 % methanol, 1.0 mL/min) to afford compounds **7** (6.0 mg) and **8** (2.7 mg). Enantiomeric excess (e.e.) of compounds **1–3** were calculated from chiral HPLC.

3,3′-Bisdemethylpinoresinol (**1**). Pale brown oil. $[\alpha]_D^{20}$ +7.08 (c 0.456, EtOH). (+) 22 % e.e. FAB-MS *m/z*: 331 $[M + H]^+$. HRTOFMS: *m/z* 353.1061 $[M + Na]^+$ (calcd for $C_{18}H_{18}O_6Na$ 353.1001).

Isoamericanol A (**2**). Pale brown oil. $[\alpha]_D^{20}$ −3.89 (c 0.368, EtOH). (−) 12 % e.e. FAB-MS *m/z*: 331 $[M + H]^+$. HRTOFMS: *m/z* 353.1000 $[M + Na]^+$ (calcd for $C_{18}H_{18}O_6Na$ 353.1001).

Americanol A (**3**). Pale brown oil. $[\alpha]_D^{20}$ +21.5 (c 0.140, EtOH). (+) 18 % e.e. FAB-MS *m/z*: 331 $[M + H]^+$. HRTOFMS: *m/z* 353.1018 $[M + Na]^+$ (calcd for $C_{18}H_{18}O_6Na$ 353.1001).

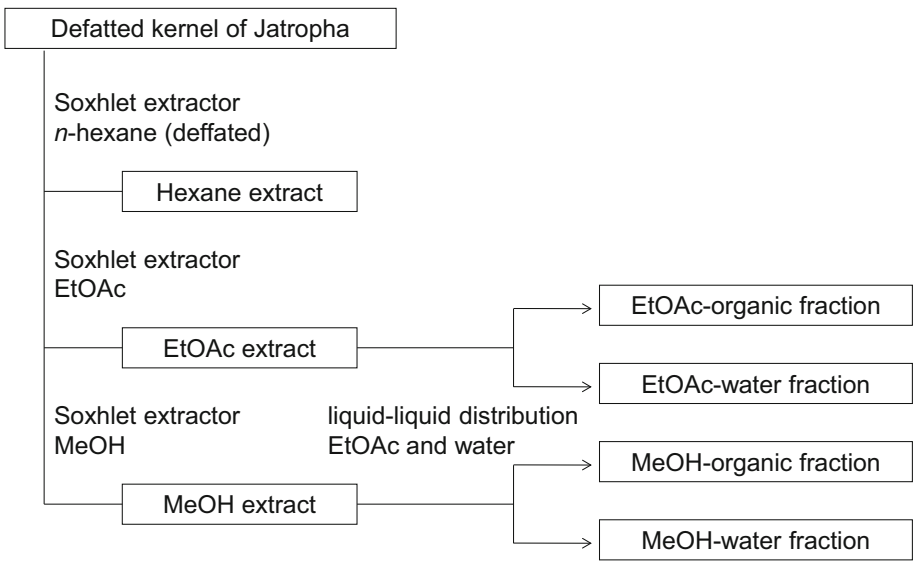

Fig. 1 Solvent extraction from the defatted kernel of Jatropha

9′-*O*-Methylisoamericanol A (**4**). Pale brown oil. $[\alpha]_D^{20}$ +18.6 (c 0.0192, EtOH). EI-MS *m/z* (rel. int.): 344 $[M]^+$ (52), 180 (58), 148 (61), 123 (68), 91 (100). HRTOFMS: *m/z* 367.1187 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$ 367.1158).

9′-*O*-Methylamericanol A (**5**). Pale brown oil. $[\alpha]_D^{20}$ +29.2 (c 0.084, EtOH). EI-MS *m/z* (rel. int.): 344 $[M]^+$ (100), 180 (35), 148 (86), 123 (82), 91 (56). HRTOFMS: *m/z* 367.1194 $[M + Na]^+$ (calcd for $C_{19}H_{20}O_6Na$ 367.1158).

Isoamericanin A (**6**). Pale brown oil. $[\alpha]_D^{20}$ +78.8 (c 0.0032, EtOH). EI-MS *m/z* (rel. int.): 328 $[M]^+$ (100), 166 (30), 148 (33), 123 (48), 110 (33). HRTOFMS: *m/z* 351.0847 $[M + Na]^+$ (calcd for $C_{18}H_{16}O_6Na$ 351.0845).

Isoprincepin (**7**). Pale brown oil. $[\alpha]_D^{20}$ +74.2 (c 0.0160, EtOH). FAB-MS *m/z*: 495 $[M + H]^+$. HRTOFMS: *m/z* 517.1534 $[M + Na]^+$ (calcd for $C_{27}H_{26}O_9Na$ 517.1475).

Princepin (**8**). Pale brown oil. $[\alpha]_D^{20}$ +55.7 (c 0.0080, EtOH). FAB-MS *m/z*: 495 $[M + H]^+$. HRTOFMS: *m/z* 517.1537 $[M + Na]^+$ (calcd for $C_{27}H_{26}O_9Na$ 517.1475).

Synthesis of isoamericanol A

Isoamericanol A was synthesized by radical coupling reaction of caffeyl alcohol with silver carbonate (Ag_2CO_3) [11]. Caffeyl alcohol (708.6 mg, 4.26 mmonl) was dissolved in acetone–toluene (50 mL, 1:2, v/v), and Ag_2CO_3 (1.403 g) was added to the solution at room temperature. After stirring for 17 h, the solid was filtered off, and the solvent was evaporated under reduced pressure to give solid residue. Purification by HPLC (Cholester, 250 × 20 mm i.d., Nacalai Tesque) yielded isoamericanol A as a 7′-8′*trans*-isomer (309.6 mg, 0.94 mmol, 44.1 %).

Isoamericanol A. colorless solid. FAB-MS *m/z*: 331 $[M + H]^+$. ^{1}H NMR (CD_3OD): δ 3.47 (1H, dd, $J = 12.2$, 4.6 Hz, H-9b), 3.67 (1H, dd, $J = 12.4$, 2.4 Hz, H-9a), 3.99 (1H, ddd, $J = 7.8$, 4.9, 2.8 Hz, H-8), 4.18 (2H, dd, $J = 5.2$, 1.4 Hz, H-9′), 4.80 (1H, d, $J = 8.2$ Hz, H-7), 6.20 (1H, dt, $J = 15.8$, 5.9 Hz, H-8′), 6.49 (1H, d, $J = 15.8$ Hz, H-7′), 6.76 (1H, dd, $J = 8.0$, 1.9 Hz, H-6), 6.80 (1H, d, $J = 7.9$ Hz, H-5), 6.86 (1H, d, $J = 1.7$ Hz, H-2), 6.89 (1H, d, $J = 8.6$ Hz, H-5′), 6.92 (1H, dd, $J = 8.6$, 2.0 Hz, H-6′), 6.96 (1H, d, $J = 1.7$ Hz, H-2′). ^{13}C NMR (CD_3OD): δ 62.3 (C-9), 63.9 (C-9′), 77.8 (C-7), 80.2 (C-8), 115.7 (C-2), 115.7 (C-2′), 116.5 (C-5), 118.1 (C-5′), 120.6 (C-6), 121.0 (C-6′), 129.7 (C-1), 131.5 (C-7′), 128.3 (C-8′), 132.2 (C-1′), 144.7 (C-3′), 145.4 (C-4′), 146.8 (C-3), 147.3 (C-4).

Antioxidative activity

The antioxidative activities of the isolated compounds were assayed for the scavenging of stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals according to the method reported in the literature [12]. The compounds and trolox were dissolved in methanol to make a 50 ppm solution. Each of the test sample solutions (0, 30, 60, 90, 120, and 150 µl) was added into a mixture (0.9 mL) of 0.4 mM DPPH solution, 20 % methanol aqueous solution, and 0.2 M MES (2-(*N*-morpholino) ethanesulfonic acid) buffer solution (1:1:1). The resulting mixtures were shaken on a vortex mixer and allowed to stand for 20 min, and the absorbance of the remaining DPPH was determined with ultraviolet (UV)-visible spectrophotometer at 520 nm. The percentage of DPPH radical inhibition by each sample was calculated from the following equation:

$$\text{DPPH radical scavenging } (\%) = (1 - A/A_0) \times 100$$

where A_0 is the absorbance of the mixture without a sample and A is the absorbance of the mixture with a sample after 20 min. The inhibitory concentration that results in 50 % scavenging of DPPH radical (IC_{50}) was estimated based on the plot of inhibition versus final concentration of the test samples.

Total phenolic content

The amount of total soluble phenolic compounds was determined using the Folin–Ciocalteu method [13]. The isolated compounds were dissolved in methanol to make a 100-ppm solution. Each of the test solutions (0.2 mL) was added to a mixture of 0.2 mL of the Folin–Ciocalteu reagent diluted twofold with water and 0.2 mL of 10 % (w/v) sodium carbonate solution. The resulting mixtures were shaken vigorously and left at room temperature for 30 min. Color development at 760 nm was determined with UV–visible spectrophotometer. The calibration curve was obtained from various concentrations of gallic acid. The phenolic content was expressed as the weight of total gallic acid equivalents in the sample (µg/mg).

Oxidation inhibition test of salad oil

The MeOH-organic fraction was applied in the oxidation inhibition test of a commercially available salad oil. Canola oil (10.0 mL) was added to the fraction (1.0 mg) or α-tocopherol (1.0 mg) as positive control. The oils were heated at a constant temperature of 180 °C. After cooling to room temperature, the acid value (AV) and thiobarbituric acid value (TBA) of the oxidized oil were checked using an AV-CHECK (Advantec) and TBA testing paper (SIBATA) according to the instructions, respectively.

Oxidation inhibition test of biodiesel

The synthesized isoamericanol A was examined in the oxidation inhibition of a biodiesel (C-FUEL,

Revointernational INC, Japan), which was made from waste of edible oils. The biodiesel (10 ml) was added to the isoamericanol A (5.0 mg) or α-tocopherol (5.0 mg) as positive control. The biodiesels were heated at a constant temperature at 180 °C. After cooling, the AV and TBA were checked using the method described above.

Results and discussion

Jatropha has been cultivated in many countries for biodiesel production, which produces huge quantities of defatted kernel as a by-product. However, the biological activities and chemical constituents of the seed kernel have not been fully clarified. We investigated the phenolic content and antioxidative activity of defatted Jatropha kernel. The defatted kernel was crushed with a blender and extracted in Soxhlet extractors with *n*-hexane, ethyl acetate, and methanol, successively (Fig. 1). Ethyl acetate and methanol extracts in Soxhlet extractors usually contain high polar compounds. These compounds reduce antioxidant activity, and make it difficult separation and purification. The ethyl acetate and methanol extracts were concentrated in vacuo, and the residues were partitioned between ethyl acetate and water to remove water-soluble substances. Four extracts (Fig. 1), EtOAc-organic, EtOAc–water, MeOH-organic, and MeOH–water fractions, were analyzed for phenolic content and antioxidative activity using the Folin–Ciocalteu method and the DPPH method, respectively (Table 1). The results showed that the MeOH-organic fraction showed a high content of phenolic compounds (255 μg/mg) and exhibited a relatively strong antioxidative activity (IC_{50} 4.62 μg/mL).

The MeOH-organic fraction was applied in the oxidation inhibition test of canola oil. The AV values of the MeOH-organic fraction and α-tocopherol were lower than that of control (Fig. 2). After 3 days, the AV value of the fraction was lower than that of tocopherol. Similarly, the TBA value of the MeOH-organic fraction and α-tocopherol was lower than that of control (Fig. 3). The effect of MeOH-organic fraction and α-tocopherol was the same in the short reaction time.

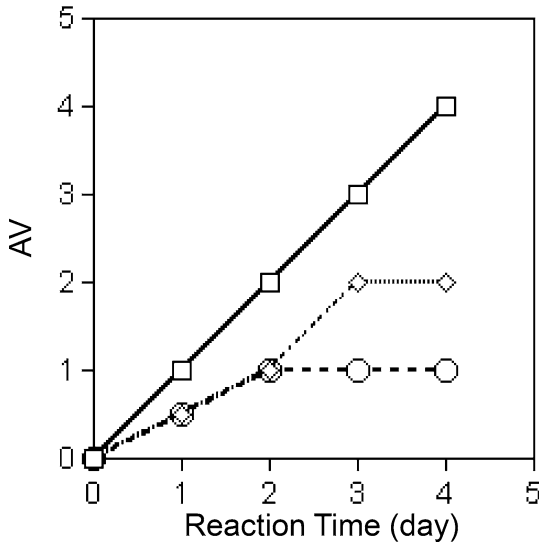

Fig. 2 Effects of the MeOH-organic fraction on AV (acid value) of the oxidized oil. Control (*square*), α-tocopherol (*diamond*), and the MeOH-organic fraction (*circle*)

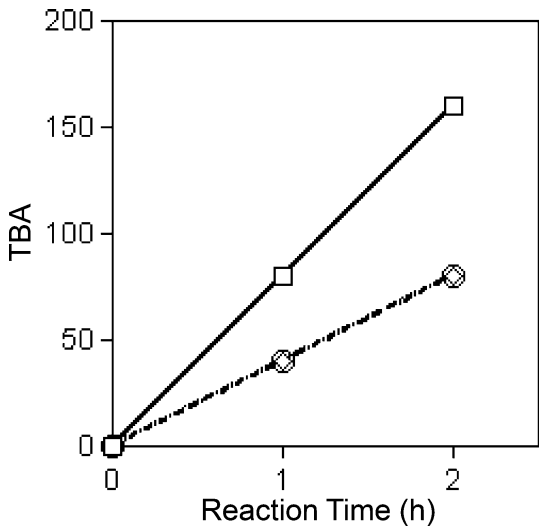

Fig. 3 Effects of the MeOH-organic fraction on TBA (thiobarbituric acid value) of the oxidized oil. Control (*square*), α-tocopherol (*diamond*), and the MeOH-organic fraction (*circle*)

Table 1 Antioxidant activities and total phenol contents of four extracts from defatted kernel of *Jatropha curcas*

	DPPH method IC_{50} (μM)	Folin–Ciocalteu method (μg/mg)
EtOAc-organic fraction	>10	54
EtOAc-water fraction	>10	30
MeOH-organic fraction	4.62	255
MeOH–water fraction	>10	76
Trolox	5.38	204

The MeOH-organic fraction was subjected to silica-gel column chromatography, TLC, and HPLC, to lead the isolation of eight compounds (Fig. 4) [14]. The enantiomeric excess (e.e.) values of the main compounds (**1–3**) were measured with chiral HPLC. Compound **1** had a molecular weight of *m/z* 331 $[M + H]^+$ on FAB-MS, and was assigned the molecular formula $C_{18}H_{18}O_6Na$ from its molecular ion $[M + Na]^+$ peak at *m/z* 353.1061 in the HRTOFMS. The ^1H and ^{13}C NMR spectra of compound **1**

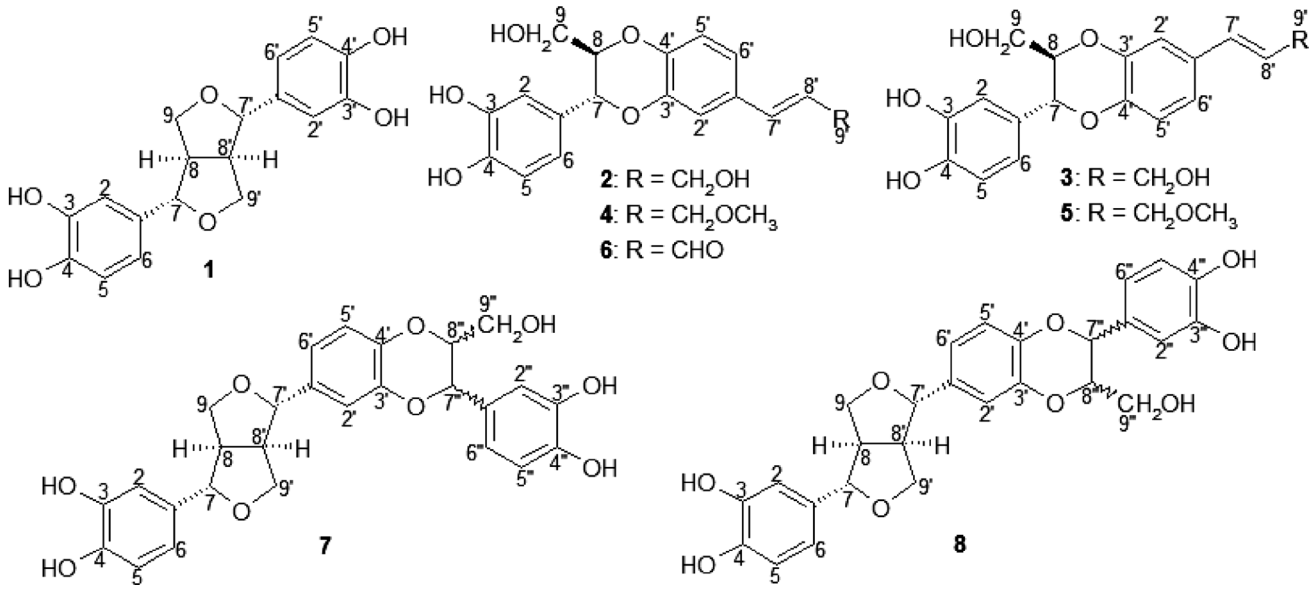

Fig. 4 Compounds isolated and identified from the defatted kernel of Jatropha. (**1**) 3,3′-Bisdemethylpinoresinol, (**2**) isoamericanol A, (**3**) americanol A, (**4**) 9′-*O*-methylisoamericanol A, (**5**) 9′-*O*-methylamericanol A, (**6**) isoamrricanol A, (**7**) isoprincepin, and (**8**) princepin

were similar to those of sesamin, except that the proton and carbon signals of the methylenedioxy moiety were not observed (Tables 2, 4). The spectra showed the presence of 1,3,4-trisubstituted phenyl groups at δ 6.72 (*d*, $J = 1.9$ Hz), 6.67 (*d*, $J = 8.0$ Hz) and 6.58 (*dd*, $J = 8.2$, 1.9 Hz), oxygen-bearing methine protons at δ 4.52, methylene protons at δ 4.07, 3.67, and methine protons at δ 2.93. In COSY spectra, the methine protons at δ 2.93 (H-8, 8′) were coupled to the oxygen-bearing methine protons δ 4.52 (H-7, 7′) and the methylene protons at δ 4.07, 3.67 (H-9a, 9′a, 9′b, 9′b). In HMBC spectra, the oxygen-bearing methine protons at δ 4.52 (H-7, 7′) were correlated with phenolic carbons at δ 113.6 (C-2, 2′) and δ 116.9 (C-6, 6′). Compound **1** was identified as the catechol-type furofuran lignan, (+)-3,3′- bisdemethylpinoresinol ((+) 22 % e.e.). This compound has been reported as a metabolite of (+)-sesamin in rat liver homogenate [15, 16]; this is the first report in which optically active **1** was isolated directly from a plant. (+)-Sesamin itself has no antioxidative activity, but the methylenedioxyphenyl moieties in (+)-sesamin were changed to catechol moieties by cytochrome P-450 enzymes. The metabolite exerts protective actions against oxidative damage in the liver.

Compound **2** had a molecular weight of *m/z* 331 $[M + H]^+$ on FAB-MS, and was assigned the molecular formula $C_{18}H_{18}O_6Na$ from its molecular ion $[M + Na]^+$ peak at *m/z* 353.1000 in the HRTOFMS. The ^{13}C NMR and DEPT 135 spectra indicated 18 carbon signals comprising six quaternary carbons, ten methine carbons, and two methylene carbons (Table 4). The ^{1}H NMR spectra (Table 2) indicated that the presence of a *trans*-double bond at δ 6.49 (d, $J = 15.8$ Hz) and 6.20 (dt, $J = 15.8$, 5.7 Hz), two 1,3,4-trisubstituted benzene rings at δ 6.76 (dd, $J = 8.2$. 2.1 Hz), 6.80 (d, $J = 7.9$ Hz), and 6.86 (d, $J = 1.7$ Hz); δ 6.89 (d, $J = 8.6$ Hz), 6.92 (dd, $J = 8.6$, 2.1 Hz), and 6.95 (d, $J = 1.7$ Hz), and two methine protons involving a 1,4-benzodioxane ring at δ 3.99 (ddd, $J = 7.8$, 4.9, 2.8 Hz) and 4.80 (d, $J = 8.2$ Hz) (Table 2). In COSY spectra, the *trans*-double bond proton at δ 6.20 (H-8′) linked to hydroxylmethylene protons at δ 4.18 (2H, H-9′). The methine proton at δ 3.99 (H-8) was coupled with the methine proton at δ 4.80 (H-7), and methylene protons at δ 3.47 (H-9b) and 3.66 (H-9a). In HMBC spectra, correlations between δ 3.99 (H-8) and δ 145.5 (C-4′), δ 4.80 (H-7) and δ 144.7 (C-3′), and δ 4.80 (H-7) and δ 115.7 (C-2), 120.6 (C-6) were observed. A large coupling constant (8.2 Hz) between H-7 and H-8 indicated the *trans* relationship on H-7/H-8. From the literatures [17, 18], compound **2** was identified as (−)-isoamericanol A. The specific rotations and enantiomeric excess of almost all isolated compounds exhibited plus values; however, the specific rotation and enantiomeric excess of isoamericanol A showed −3.89 and (−) 12 % e.e., respectively.

Compound **3** had a molecular weight of *m/z* 331 $[M + H]^+$ on FAB-MS, and was assigned the molecular formula $C_{18}H_{18}O_6Na$ from its molecular ion $[M + Na]^+$ peak at *m/z* 353.1018 in the HRTOFMS. The ^{1}H and ^{13}C NMR spectra of compound **3** were very similar to those of isoamericanol A (Tables 2, 4). Waibel et al. reported that the method to distinguish americanol-type and isoamericanol-type compound [19]. The chemical shift difference between H-2′ and H-5′ of americanol-type was larger than

Table 2 ^{1}H NMR spectral data of compounds **1–4** (δ, mult, J in Hz)

Proton	1	2	3	4
2	6.72 (d, 1.9)	6.86 (d, 1.7)	6.85 (d, 1.7)	6.85 (d, 1.7)
5	6.67 (d, 8.0)	6.80 (d, 7.9)	6.80 (d, 7.9)	6.80 (d, 8.2)
6	6.58 (dd, 8.2, 1.9)	6.76 (dd, 8.2, 2.1)	6.76 (dd, 8.2, 1.4)	6.76 (dd, 8.3, 2.1)
7	4.52 (d, 4.4)	4.80 (d, 8.2)	4.80 (d, 8.2)	4.80 (d, 8.3)
8	2.93 (m)	3.99 (ddd, 7.8, 4.9, 2.8)	3.99 (ddd, 7.5, 4.6, 2.7)	3.99 (ddd, 7.8, 4.9, 2.7)
9a	4.07 (dd, 8.8, 6.9)	3.66 (dd, 12.4, 2.4)	3.67 (dd, 12.0, 2.4)	3.67 (dd, 12.4, 2.4)
9b	3.67 (dd, 9.1, 3.5)	3.47 (dd, 12.2, 4.6)	3.48 (dd, 12.2, 4.6)	3.47 (dd, 12.2, 4.6)
2′	6.72 (d, 1.9)	6.95 (d, 1.7)	7.02 (d, 1.7)	6.97 (d, 2.0)
5′	6.67 (d, 8.0)	6.89 (d, 8.6)	6.82 (d, 8.6)	6.89 (d, 8.2)
6′	6.58 (dd, 8.2, 1.9)	6.92 (dd, 8.6, 2.1)	6.90 (dd, 8.2, 1.7)	6.93 (dd, 8.4, 1.9)
7′	4.52 (d, 4.4)	6.49 (d, 15.8)	6.50 (d, 15.8)	6.51 (d, 15.8)
8′	2.93 (m)	6.20 (dt, 15.8, 5.7)	6.21 (dt, 15.8, 5.8)	6.15 (dt, 15.8, 6.4)
9′a	4.07 (dd, 8.8, 6.9)	4.18 (dd, 5.2, 1.4)	4.19 (d, 5.8)	4.05 (dd, 6.2, 1.4)
9′b	3.67 (dd, 9.1, 3.5)	4.18 (dd, 5.2, 1.4)	4.19 (d, 5.8)	4.05 (dd, 6.2, 1.4)
OMe				3.34 (s)

Spectral were recorded in CD_3OD at 600 Mz

that of isoamericanol-type. A comparative large difference of 0.2 ppm is observed between H-2′ and H-5′ in the spectrum of compound 3. Correlations between δ 3.99 (H-8) and δ 145.0 (C-3′), and δ 4.80 (H-7) and δ 145.2 (C-4′) were observed in HMBC spectra, and compound **3** was identified as (+)-americanol A [(+) 18 % e.e.]. The neolignans containing 1,4-benzodioxane rings have choline acetyltransferase and neurotrophic activities [17, 18].

Compounds **4**, **5,** and **6** were determined as 9′-*O*-methylisoamericanol A, 9′-*O*-methylamericanol A, and isoamericanin A, respectively [20, 21].

Compound **7** had a molecular weight of *m/z* 495 $[M + H]^+$ on FAB-MS, and was assigned the molecular formula $C_{27}H_{26}O_9Na$ from its molecular ion $[M + Na]^+$ peak at *m/z* 517.1534 in the HRTOFMS. The ^{13}C NMR and DEPT 135 spectra indicated 27 carbon signals comprising nine quaternary carbons, 15 methine carbons, and three methylene carbons (Table 4). The ^{1}H NMR spectra (Table 3) indicated the presence of three 1,3,4-trisubstituted aromatic rings at δ 6.87 (dd, $J = 8.4$, 1.9 Hz), 6.92 (*m*) and 6.93 (d, $J = 8.2$); δ 6.76 (dd, $J = 8.1$, 1.9 Hz), 6.80 (d, $J = 7.9$ Hz), and 6.85 (d, $J = 2.1$ Hz); δ 6.68 (dd, $J = 8.2$, 2.1 Hz), 6.73 (d, $J = 7.9$ Hz), and 6.80 (d, $J = 2.1$ Hz), a furofuran ring at δ 3.08 (2H, *m*), 3.81 (2H, *m*), 4.21 (2H, *m*), 4.62 (d, $J = 3.8$ Hz), and 4.69 (d, $J = 4.1$ Hz), and two methine protons involving a 1,4-benzodioxane ring at δ 3.98 (ddd, $J = 7.8$, 4.9, 2.7 Hz) and 4.80 (d, $J = 8.2$ Hz), which suggested that the compound was sesquineolignan comprising pinoresinol-type and 1,4-benzodioxan-type moieties [22]. In COSY spectra (Fig. 5), the methine proton at δ 3.98 (H-8″) was coupled with the methine proton at δ 4.80 (H-7″) and methylene protons at δ 3.47 (H-9″b) and 3.66 (H-9″a). A small difference of 0.01 ppm is observed between H-2′ and H-5′, which indicated isoamericanol A type [19]. In HMBC spectra (Fig. 5), correlations between δ 3.98 (H-8″) and δ 144.5 (C-4′), and δ 4.80 (H-7″) and δ 145.3 (C-3′) were observed. Further proton–aromatic carbon correlations between δ

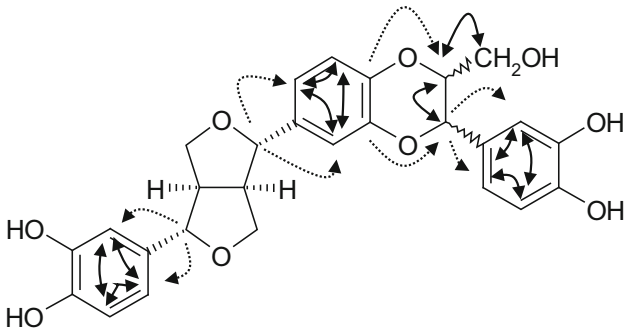

Fig. 5 Important COSY (*double-headed arrows*) and HMBC (*dotted arrows*) correlations of isoprincepin (**7**)

Table 3 ^{1}H NMR spectral data of compounds **5–8** (δ, mult, J in Hz)

Proton	**5**	**6**	**7**	**8**
2	6.84 (d, 2.1)	6.86 (d, 2.1)	6.80 (d, 2.1)	6.80 (d, 1.7)
5	6.80 (d, 7.9)	6.81 (d, 7.9)	6.73 (d, 7.9)	6.74 (d, 8.2)
6	6.76 (dd, 7.9, 2.0)	6.78 (dd, 8.1, 1.9)	6.68 (dd, 8.2, 2.1)	6.69 (dd, 8.1, 1.9)
7	4.81 (d, 7.9)	4.80 (d, 7.6)	4.62 (d, 3.8)	4.64 (d, 4.5)
8	3.99 (ddd, 7.6, 4.6, 2.9)	4.08 (ddd, 7.8, 4.7, 2.8)	3.08 (m)	3.10 (m)
9a	3.67 (dd, 12.2, 2.6)	3.71 (12.5, 2.6)	4.21 (m)	4.22 (m)
9b	3.48 (dd, 12.2, 4.7)	3.49 (12.4, 4.5)	3.81 (m)	3.83 (m)
2′	7.04 (d, 2.0)	7.26 (d, 2.1)	6.92 (m)	6.98 (m)
5′	6.83 (d, 8.3)	7.03 (d, 8.2)	6.93 (d, 8.2)	6.86 (m)
6′	6.92 (dd, 8.3, 2.1)	7.24 (dd, 8.2, 2.1)	6.87 (dd, 8.4, 1.9)	6.86 (m)
7′	6.53 (d, 15.8)	7.58 (d, 15.8)	4.69 (d, 4.1)	4.71 (d, 4.1)
8′	6.16 (dt, 15.8, 6.2)	6.64 (dt,15.8, 7.9)	3.08 (m)	3.10 (m)
9′a	4.06 (dd, 6.2, 1.3)		4.21 (m)	4.22 (m)
9′b	4.06 (dd, 6.2, 1.3)		3.81 (m)	3.83 (m)
2″			6.85 (d, 2.1)	6.84 (d, 1.7)
5″			6.80 (d, 7.9)	6.80 (d, 7.9)
6″			6.76 (dd, 8.1, 1.9)	6.75 (dd, 8.2, 2.1)
7″			4.80 (d, 8.2)	4.79 (d, 7.9)
8″			3.98 (ddd, 7.9, 4.8, 2.7)	3.99 (ddd, 7.7, 4.8, 2.7)
9″a			3.66 (dd, 12.4, 2.7)	3.67 (dd, 12.4, 2.7)
9″b			3.47 (dd, 12.2, 4.6)	3.48 (dd, 12.2, 4.6)
OMe	3.36 (s)			
CHO		9.59 (d, 7.6)		

Spectral were recorded in CD_3OD at 600 Mz

4.62 (H-7) and δ 114.5 (C-2), 118.9 (C-6); between δ 4.69 (H-7′) and δ 115.8 (C-2′), 120.3 (C-6′); and between δ 4.80 (H-7″) and δ 115.6 (C-2″), 120.4 (C-6″) were observed. From the literature, compound **7** was identified as isoprincepin [19, 22].

Compound **8** had a molecular weight of m/z 495 $[M + H]^+$ on FAB-MS, and was assigned the molecular formula $C_{27}H_{26}O_9Na$ from its molecular ion $[M + Na]^+$ peak at m/z 517.1537 in the HRTOFMS. The ^{1}H and ^{13}C NMR spectra were very similar to those of isoprincepin (Tables 3, 4). A comparative large difference of 0.12 ppm is observed between H-2′ and H-5′, which indicated americanol A type. Compound **8** was identified as princepin from the literature [19]. Princepin showed potent inhibitory effects on estrogen-enhanced cell proliferation [23].

The eight compounds were examined for antioxidative activity and phenolic content using the DPPH method and the Folin–Ciocalteu method, respectively (Table 5). All of them exhibited strong or moderate antioxidative activity compared with the known antioxidant, trolox. Isoprincepin exhibited the highest antioxidative activity among the isolated compounds. The antioxidative activities of compounds **1–8** were weakly correlated with total phenol content in this study. The Folin–Ciocalteu assay has been used to measure total phenolic contents in plant extracts; however, the reaction mechanism was not specific to phenolic compounds [24].

The antioxidative activity of 9′-*O*-methylisoamericanol, which contains three hydroxyl groups, was higher than those of isoamericanol A and americanol A, which contain four hydroxyl groups, thus the number of hydroxyl groups is not correlated closely with the antioxidative activity. The antioxidative activities of 3,3′-bisdemethylpinoresinol, isoprincepin, and princepin, which have two catechol groups were higher than those of compounds with one catechol group, thus the number of catechol groups is more important for the antioxidative activity. Bendary et al. reported that the *ortho* position of two hydroxyl group was more active in structure–antioxidant relationship than *para* position and *meta* position [25]. It can be concluded that the catechol lignans/neolignans are the major contributors to the antioxidative property of Jatropha.

The defatted kernel of Jatropha is generated as a by-product in tropical countries; however, the supply of the defatted kernel was limited for our studies. To obtain enough amount of the antioxidant of Jatropha, isoamericanol A was selected. Isoamericanol A was prepared by

Table 4 ^{13}C NMR spectral data of compounds **1–8**

Carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	132.2	129.7	129.7	129.5	129.5	128.4	133.9	133.9
2	113.6	115.7	115.7	115.7	115.8	115.7	114.5	114.5
3	144.6	146.8	146.8	146.7	146.7	146.9	146.5	146.5
4	145.1	147.3	147.3	147.2	147.2	147.5	146.1	146.2
5	115.3	116.5	116.5	116.3	116.4	116.6	116.3	116.3
6	116.9	120.6	120.6	120.4	120.4	120.6	118.9	118.9
7	84.9	77.8	77.9	77.6	77.8	77.7	87.5	87.5
8	53.5	80.2	80.1	80.1	80.1	80.6	55.3	55.4
9	70.7	62.3	62.3	62.1	62.2	62.1	72.7	72.7
1′	132.2	132.2	132.3	131.7	131.9	129.3	135.7	135.8
2′	113.6	115.7	115.8	115.5	115.6	118.8	115.8	115.9
3′	144.6	144.7	145.0	144.3	145.0	145.9	145.3	144.9
4′	145.1	145.5	145.2	145.3	145.3	148.3	144.5	144.9
5′	115.3	118.1	118.1	118.0	118.0	118.8	118.0	118.0
6′	116.9	121.0	120.9	121.0	120.9	124.1	120.3	120.1
7′	84.9	131.5	131.6	124.9	124.9	155.4	87.1	87.1
8′	53.5	128.3	128.3	133.7	133.8	128.4	55.5	55.5
9′	70.7	63.9	63.9	74.2	74.3		72.7	72.7
1″							129.6	129.6
2″							115.6	115.6
3″							146.7	146.7
4″							147.2	147.2
5″							116.4	116.4
6″							120.4	120.4
7″							77.7	77.6
8″							80.0	80.0
9″							62.2	62.2
Me				58.0	58.1			
CHO						196.2		

Spectral were recorded in CD_3OD at 150 Mz

Table 5 Antioxidant activities and total phenol contents of compounds **1–8** and trolox

	DPPH method IC_{50} (μM)	Folin–Ciocalteu method (μg/mg)
3,3′-Bisdemethylpinoresinol (**1**)	16.0	300
Isoamericanol A (**2**)	19.7	250
Americanol A (**3**)	18.9	214
9′-*O*-Methylisoamericanol A (**4**)	18.3	240
9′-*O*-Methylamericanol A (**5**)	29.6	164
Isoamericanin A (**6**)	24.6	201
Isoprincepin (**7**)	9.12	301
Princepin (**8**)	11.7	270
Trolox	21.5	204

radical coupling reaction of caffeyl alcohol with silver carbonate [11]. The *trans*- and *cis*-mixture were purified with HPLC, and the *trans*-isomer was examined in the oxidation inhibition of the biodiesel (Fig. 6). The biodiesel without an antioxidant was discolored under the condition at 1 day, and the AV value of negative control reached the maximum value. The AV values of the isoamericanol A and α-tocopherol were lower than that of the control for 4 days, and the AV values of isoamericanol A and the positive control were almost the same. The results

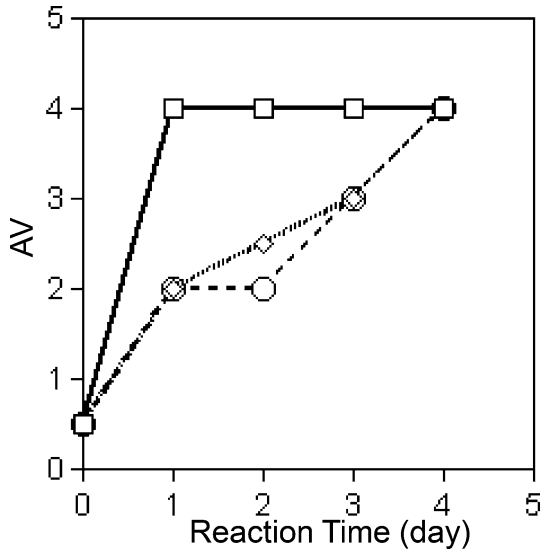

Fig. 6 Effects of isoamericanol A on AV (acid value) of the biodiesel. Control (*square*), α-tocopherol (*diamond*), and isoamericanol A (*circle*)

indicated that isoamericanol A inhibited the oxidation of biodiesel. The TBA value of biodiesel was too high, and the effects of isoamericanol and α-tocopherol on TBA value could not been measured.

In most cases, the residues generated during the extraction of oil from oilseeds contain bioactive substances, such as phenolic compounds, flavonoids, and lignans [26]. These compounds could be used as natural antioxidants for the protection of fats and oils against oxidative damage. Tocopherols from soybean and palm are widely applied antioxidants for the stabilization of edible fats and oils. The extraction of such antioxidants from the residues of oilseeds can be considered to contribute to the added value of these wastes. It might be difficult to use the antioxidants from residues of Jatropha oil for edible fats and oil products; however, the catechol lignans/neolignans from Jatropha wastes would be applicable as stabilizers of Jatropha biodiesel [27]. The average quantity of Jatropha oil obtained per 100 g of kernel was ca 30 ml, and 3,3′-bis-demethylpinoresinol (**1**, 6.66 mg), isoamericanol A (**2**, 4.89 mg), americanol A (**3**, 1.89 mg), 9′-*O*-methylisoamericanol A (**4**, 1.7 mg), 9′-*O*-methylamericanol A (**5**, 0.53 mg), isoamericanin A (**6**, 0.34 mg), isoprincepin (7, 1.7 mg), and princepin (**8**, 0.76 mg) were prepared from the 100 g of kernel in this study. The yield of antioxidants would be sufficient to inhibit oxidation of the Jatropha oil. Further study is needed to more efficiently recover and utilize the antioxidants in Jatropha biodiesel industries.

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