Phenolic compounds from the stem bark of *Erythrina orientalis* and their cytotoxic and antioxidant activities

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ABSTRACT

Two prenylated pterocarpsans, phaseollin (1), shinpterocarpin (2) together with flavonoids, 4'-O-methyl licoflavanone (3), alpinumisoflavone (4), 8-prenyl-daidzein (5), have been isolated from the stem bark of *Erythrina orientalis*. The structures of these compounds were determined based on UV, IR, HRESIMS, 1D and 2D NMR data. Compounds 1–5 were evaluated for their cytotoxic properties against P-388 cells, their IC_{50} values 2.55, 2.43, 17.98, 4.31, and 5.82 µg/mL, respectively. Compounds 1, and 5 exhibited very high antioxidant activity against DPPH radical scavenging.

Keywords: Pterocarp, Flavonoid, *Erythrina orientalis*, Cytotoxic, Antioxidant.

INTRODUCTION

*Erythrina* is a large genus of Leguminosae family consisting of about 120 species and distributed in the tropical and subtropical region, including Indonesia [1]. The phytochemical studies of *Erythrina* have known that these plants producing alkaloids [2,3], flavonoids [4,5], pterocarps [6,7], stilbenes [8], and arylbenzofurans [9], which are the active compounds. *Erythrina orientalis* known local name as “Dadap”. Decoction of the bark or leaves of *Erythrina orientalis* has been used by Indonesian people as a traditional medicine of malaria [10]. In continuation of our research for phenolic compound in this medicinal plant, we report the isolation of prenylated pterocarpsans, phaseollin (1), shinpterocarpin (2) together with three flavonoids, 4'-O-methyl licoflavanone (3), alpinumisoflavone (4), 8-prenyl-daidzein (5), from the methanol extract of the stem bark of *Erythrina orientalis*. The cytotoxic activity against murine leukemia P-388 cells and antioxidant activity against DPPH radical scavenging of the isolated compounds 1–5 are also briefly described.

MATERIALS AND METHODS

**General**

UV and IR spectra were measured with a Shimadzu 1800 and Perkin Elmer Spectrum One FTIR spectrometer, respectively. 1H and 13C NMR spectra were recorded with a Agilent 500 spectrometer operating at 500 (1H) and 125 (13C) MHz in CDCl₃ or acetone-d₆ using TMS as the internal standard. Mass spectra were obtained from a Waters LCT Premier XE. Vacuum liquid chromatography (VLC) and radial chromatography were carried out using Si gel 60 GF₅₄ and Si gel 60 PF₅₄, for TLC analysis, pre-coated silica gel plates (Merck Kieselgel 60 GF 254, 0.25 mm
thickness) were used. Solvents used for extraction and preparative chromatography were of technical grade and distilled before used. Solvent used for purification was pro analysis grade.

**Plant material**

The stem bark of *E. orientalis* were collected from Kunir Kidul Village, Kabupaten Lumajang, East Java, Indonesia. The species was identified at the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia and a voucher specimen had been deposited at the Herbarium Bogorienses.

**Extraction and isolation**

The pulverized stem bark of *E. orientalis* (2.5 kg) were macerated with MeOH two times at room temperature, and then concentrated under reduced pressure. The residue was suspended in water and partitioned sequentially with *n*-hexane (56 g) and EtOAc (18 g). The EtOAc extract was fractionated on silica gel by VLC eluting with mixtures *n*-hexane-EtOAc (9:1, 4:1, 7:3, 1:1, and 3:7) to give four major fractions A-D. Fraction A (1.0 g), purified using radial chromatography eluted with a mixtures of *n*-hexane-diisopropylether (9:1, 4:1, and 7:3) to give compounds 1 (30.2 mg) and 2 (9.6 mg). Fraction B (1.8 g) was purified using radial chromatography eluted with a mixtures of *n*-hexane-CHCl₃ (3:7, 1:1, and 7:3) yielded compound 3 (50 mg). Fraction C (6.8 g) was fractionated on silica gel by VLC eluting with a mixtures of *n*-hexane-acetone (9:1, and 4:1) to give three subfractions C₁-C₃. Subfractions C₁ purified using radial chromatography eluted with a mixtures of *n*-hexane-acetone (9:1, and 4:1) yielded compound 4 (9 mg). Using the same methodology on fraction C₃ afforded compound 5 (12 mg).

**Phaseollin (1):** Pale yellow solid: UV (MeOH) λₘₐₓ nm (log ε) 210 (4.66), 280 (3.96); IR (KBr) νₘₐₓ (cm⁻¹) 3433, 3043, 1628, 1444, 1213; HR-ESI-MS m/z 321.1126 [M+H]^+ (calcd for C₁₉H₁₉O₂; 321.1127); ¹H NMR (500 MHz, CDCl₃) δH (ppm): 7.41 (1H, d, J = 8.5 Hz, H-1), 6.55 (1H, dd, J = 8.5, 2.0 Hz, H-2), 6.41 (1H, d, J = 2.0 Hz, H-4), 4.22 (1H, dd, J = 11.0, 5.0 Hz, H-6a), 3.60 (1H, t like, J = 11.0 Hz, H-6b), 3.48 (1H, m, H-6a), 6.95 (1H, d, J = 8.0 Hz, H-7), 6.34 (1H, d, J = 8.0 Hz, H-8), 5.48 (1H, d, J = 7.0 Hz, H-11a), 5.57 (1H, d, J = 10.0 Hz, H-3'), 6.51 (1H, d, J = 10.0 Hz, H-4'), 1.46 (3H, s, H-5'), 1.40 (3H, s, H-6'); ¹³C NMR (125 MHz, CDCl₃) δC (ppm): 132.3 (C-1), 109.7 (C-2), 157.1 (C-3), 103.6 (C-4), 156.7 (C-4a), 66.6 (C-6), 39.7 (C-6a), 119.1 (C-6b), 123.8 (C-7), 106.2 (C-8), 153.7 (C-9), 106.2 (C-10), 112.6 (C-11a), 78.7 (C-11a), 78.7 (C-11b), 76.1 (C-12'), 129.7 (C-13'), 116.5 (C-14'), 27.8 (C-15'), 27.7 (C-16').

Further support for structure 1 were also obtained by HMQC and HMBC spectra. The spectra data of compound 1 were comparison with phaseollin from *Erythrina subumbraens* [11].

**Shinpterocarpin (2):** Pale yellow solid: HR-ESI-MS m/z 323.1276 [M+H]^+ (calcd for C₁₉H₁₉O₂; 323.1283); ¹H NMR (500 MHz, acetone-d₆) δH (ppm): 7.04 (1H, d, J = 8.0 Hz, H-1), 6.28 (1H, d, J = 8.0 Hz, H-2), 4.25 (1H, dd, J = 9.5, 5.0 Hz, H-6a), 3.60 (1H, t like, J = 9.5 Hz, H-6b), 3.54 (1H, m, H-6a), 7.34 (1H, d, J = 8.0 Hz, H-7), 6.56 (1H, dd, J = 8.0, 2.5 Hz, H-8), 6.37 (1H, d, J = 2.5 Hz, H-10), 5.52 (1H, d, J = 6.5 Hz, H-11a), 5.64 (1H, d, J = 9.5 Hz, H-3'), 6.45 (1H, d, J = 9.5 Hz, H-4'), 1.38 (3H, s, H-5'), 1.35 (3H, s, H-6'); ¹³C NMR (125 MHz, acetone-d₆) δC (ppm): 124.9 (C-1), 120.9 (C-2), 154.4 (C-3), 106.5 (C-4), 156.3 (C-4a), 67.0 (C-6), 40.5 (C-6a), 106.5 (C-6b), 130.0 (C-7), 112.6 (C-8), 159.6 (C-9), 103.9 (C-10), 157.6 (C-10a), 79.7 (C-11a), 121.6 (C-11b), 76.1 (C-12'), 129.7 (C-13'), 116.5 (C-14'), 27.8 (C-15'), 27.7 (C-16'). Further support for structure 2 were also obtained by HMQC and HMBC spectra. The spectra data of compound 2 were comparison with shinpterocarpin from *Erythrina sacleuxii* [12].

**4'-O-Methyl licoflavone (3):** Pale yellow solid: UV (MeOH) λₘₐₓ nm (log ε) 203 (4.62), 226 sh (4.43), 288 (4.25), 332 sh (3.78); (MeOH+NaOH) 204 (4.67), 218 sh (4.48), 323 (4.41); (MeOH+AlCl₃) 204 (4.67), 223 (4.54), 310 (4.34), 368 (3.54); (AlCl₃+HCl) 204 (4.67), 223 (4.54), 309 (4.34), 368 (3.54); (NaOAc) 204 (4.68); 225 sh (4.43); 228 (4.16), 322 (4.10); HR-ESI-MS m/z 355.1460 [M+H]^+ (calcd for C₁₉H₁₉O₂; 355.1462); ¹H NMR (500 MHz, CDCl₃) δH (ppm): 5.33 (1H, dd, J = 13.0, 3.0 Hz, H-2), 3.11 (1H, dd, J = 17.0, 13.0 Hz, H-3), 2.77 (1H, dd, J = 17.0, 3.0 Hz, H-3a), 5.97 (1H, d, J = 1.8 Hz, H-6), 5.99 (1H, d, J = 1.8 Hz, H-8), 7.19 (1H, d, J = 2.0 Hz, H-2'), 6.87 (1H, d, J = 8.0 Hz, H-5'), 7.25 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 3.33 (1H, d, J = 7.0 Hz, H-1'), 5.29 (1H, t like, J = 7.0 Hz, H-2'), 1.74 (3H, s, H-4'), 1.70 (3H, s, H-5'); 3.85 (3H, s, 4'-OCH₃), 12.06 (1H, s, 5'-OH); ¹³C NMR (125 MHz, CDCl₃) δC (ppm): 79.3 (C-2'), 43.1 (C-3'), 196.4 (C-4'), 103.1 (C-4a), 164.3 (C-5), 95.5 (C-6), 164.8 (C-7), 96.6 (C-8a), 129.9 (C-1'), 127.6 (C-2'), 130.8 (C-3'), 157.8 (C-4'), 110.3 (C-5'), 125.1 (C-6'), 28.5 (C-1'''), 121.9 (C-2'''), 133.0 (C-3'''), 25.8 (C-4''), 17.8 (C-5''), 55.5 (4'-OCH₃). Further support for structure 3 were also obtained by HMBC and HMBC spectra. The spectra data of compound 3 were comparison with 4'-O-methyl licoflavone from *Macaranga trichocarpa* [13].
Alpinumisoflavone (4): Pale yellow solid: HR-ESI-MS m/z 337.1082 [M+H]^+ (calcld for C_{30}H_{25}O_{3}: 337.1076); \(^1\)H NMR (500 MHz, acetone-d$_6$) $\delta$ (ppm): 8.17 (1H, s, H-2), 6.36 (1H, s, H-8), 7.45 (2H, d, J = 8.5, H-3'/5'), 5.76 (1H, d, J = 10.0), 6.67 (1H, d, J = 10.0), 1.46 (6H, s, H-5'/6'), 13.42 (1H, s, 5-OH); \(^1^3\)C NMR (125 MHz, acetone-d$_6$) $\delta$ (ppm): 154.4 (C-2), 124.1 (C-3), 181.8 (C-4), 106.0 (C-4a), 157.7 (C-5), 106.7 (C-6), 160.2 (C-7), 95.4 (C-8), 158.1 (C-8a), 122.9 (C-1'), 131.1 (C-2'/6'), 116.0 (C-3'/5'), 158.5 (C-4'), 78.8 (C-2''), 129.4 (C-3''), 115.7 (C-4''), 28.4 (C-5'/6''). Further support for structure 4 were also obtained by HMQC and HMBC spectra. The spectra data of compound 4 were comparison with alpinumisoflavone from *Erythrina fusca* [14].

8-Prenyldaizein (5): Pale yellow solid: HR-ESI-MS m/z 321.1120 [M-H] (calcld for C_{30}H_{23}O_{3}: 321.1127); \(^1\)H NMR (500 MHz, acetone-d$_6$) $\delta$ (ppm): 8.23 (1H, s, H-2), 6.36 (1H, s, H-8), 7.92 (1H, d, J = 9.0, H-5), 7.03 (1H, d, J = 9.0, H-6), 7.48 (2H, d, J = 9.0, H-2'/6'), 6.88 (2H, d, J = 9.0, H-3'/5'), 3.57 (1H, d, J = 7.0 Hz, H-1''), 5.28 (1H, tm, J = 6.8 Hz, H-2'''), 1.83 (3H, s, H-4''), 1.66 (3H, s, H-5''); \(^1^3\)C NMR (125 MHz, acetone-d$_6$) $\delta$ (ppm): 153.2 (C-2), 124.7 (C-3), 176.1 (C-4), 118.8 (C-4a), 125.3 (C-5), 114.8 (C-6), 160.1 (C-7), 116.3 (C-8), 156.6 (C-8a), 124.5 (C-1'), 131.0 (C-2'/6'), 115.8 (C-3'/5'), 158.1 (C-4'), 22.6 (C-1''), 122.5 (C-2''), 132.5 (C-3''), 17.9 (C-4''), 25.8 (C-5''). Further support for structure 5 were also obtained by HMQC and HMBC spectra. The spectra data of compound 5 were comparison with 8-prenyldaizein from *Erythrina fusca* [14].

Cytotoxicity assay: Cytotoxic properties of the isolated compounds 1–5 against murine leukemia P-388 cells were evaluated according to the method of MTT assay as described previously [15,16]. The cytotoxicity assay was performed against murine leukemia P-388 cells were grown in RPMI 1640 medium containing 10% fetal bovine serum, 2 mg mL$^{-1}$ sodium carbonate, 100 μg mL$^{-1}$ penicillin sodium salt, and 100 μg mL$^{-1}$ penicillin streptomycin sulfate. The cells were harvested at the log phase of growth, and then seeded into 96-well plates (1 x 10^4 cells/well). After 24 h incubation at 37 °C and 5% CO$_2$ to allow cell attachment, the cultures were exposed to the test compounds 1–5 were dissolved in DMSO at various concentrations and incubated for 48 h followed by MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay at 540 nm with artonin E as a positive control.

DPPH scavenging: Determination of the antioxidant activity of the isolated performed using reagent DPPH (2,2-diphenyl-1-pikirhizdrazil) was measured by UV spectrometer at λ = 517 nm [17,18]. Determination of antioxidant activity was done by dissolving a compounds assay with methanol, then added solution of 0.1 M buffer acetate (pH 5.5) and added DPPH radical solution of 5.10^−4 M. Determination of the inhibition of isolated compounds against DPPH radical was observed using a spectrometer at λ = 517 nm after incubation for 30 min at 20°C and ascorbic acis as positive control.
RESULTS AND DISCUSSION

Two prenylated pterocarpan, phaseollin (1), shipterocarpin (2), together three prenylated flavonoids, 4′-O-methyl licoflavonone (3), alpinumisoflavone (4), and 8-prenyl daidzein (5) have been isolated from the stem bark of *E. orientalis*. Their structures were elucidated with extensive by UV, IR, HRESIMS, 1D and 2D NMR spectrum.

Phaseollin (1) was isolated as pale yellow solid, and its UV spectrum exhibited absorption maxima (210, and 280 nm) typical for a pterocarpan [7]. The IR spectrum indicated absorptions for hydroxyl (3433 cm⁻¹), aromatic (1628, 1444 cm⁻¹), and C-O-C ether (1213 cm⁻¹) groups. The HRESIMS spectrum showed a quasimolecular ion [M+H]⁺ at *m/z* 321.1126 consistent to the molecular formula C₁₇H₁₂O₄, suggesting that 1 is a prenylated pterocarpan. The ¹H NMR spectra of 1, the presence of four aliphatic protons at δ H 5.48 (1H, d, J = 7.0 Hz, H-11a), 4.22 (1H, dd, J = 11.0 and 5.0 Hz, H-6a), 3.60 (1H, t, J = 11.0 Hz, H-6b), and 3.48 (1H, m, H-6a) suggest that compound 1 is a typical for a pterocarpan [7]. The ¹H NMR spectrum of 1 showed two aromatic regions, one is ABX system at δ H 7.41 (1H, d, J = 8.5 Hz, H-1); 6.55 (1H, dd, J = 5.0 and 2.0 Hz, H-2), and 6.41 (1H, d, J = 2.0 Hz, H-4), and another is δ H 6.95 (1H, d, J = 8.0 Hz, H-7) and 6.34 ((1H, d, J = 8.0 Hz, H-8) indicating the presence of trisubstituted pterocarpian structure. The ¹H NMR spectrum displayed characteristic signals attributable to a 2,2-dimethylpyrano ring [δ H 6.51 (1H, d, J = 10.0 Hz, H-4'), 5.57 (1H, d, J = 10.0 Hz, H-3'), 1.43 (3H, s, H-5') and 1.40 (3H, s, H-6')]. The ¹³C NMR spectrum of 1 showed 20 carbon signals consistent to prenylated pterocarpan. Based on ¹H and ¹³C NMR spectra, the placement 2,2-dimethylpyrano ring was determined with HMOC and HMBC spectra. In the HMBC spectrum showed correlations between a proton signal of a methoxyl group and prenyl group were obtained from the HMQC and HMBC spectra. The one bond and two/three functionalities are at C-5, C-7 and C-4' of the flavanone skeleton. The placement of one methine carbon atoms [δ C 196.4 (C-6b), 153.7 (C-9), 106.2 (C-10)], and correlation proton singlet [δ H 5.33 (1H, dd, J = 17.1, 3.0 Hz, H-3′α)] confirmed for the flavanone structure. In the HRESIMS spectrum showed a quasimolecular ion [M+H]⁺ at *m/z* 209, 226, 288 and 323 sh nm) typical for a flavanone structure [13]. The HRESIMS spectrum showed a quasimolecular ion [M+H]⁺ at *m/z* 355.1460 consistent to the molecular formula C₁₇H₁₂O₄, suggesting that 3 is a prenylated flavanone with contain one methoxyl group. The ¹H NMR spectra of 3 showed three doublet-doublet proton signals at δ H 5.33 (1H, dd, J = 13.0, 3.0 Hz, H-2), 3.11 (1H, dd, J = 17.0, 13.0 Hz, H-3α), and 2.77 (1H, dd, J = 17.1, 3.0 Hz, H-3′α) confirmed for the flavanone structure. In the ¹H NMR spectrum of 3 showed ABX aromatic system at δ H 7.34 (1H, d, J = 8.0 Hz, H-7), 6.56 (1H, dd, J = 8.0, 2.5 Hz, H-8), 6.37 (1H, d, J = 2.5 Hz, H-10), a pair of doublets of aromatic (J = 8.0 Hz) at δ H 7.04 (H-1), 6.28 (H-2), and one 2,2-dimethylpyrano ring at δ H 6.45 (1H, d, J = 9.5 Hz, H-4'), 5.64 (1H, d, J = 9.5 Hz, H-3'), 1.38 (3H, s, H-5') and 1.35 (3H, s, H-6'). Based on ¹H and ¹³C NMR spectra, compound 2 and 1 are isomeric. From HMOC and HMBC spectra of 2 consistent with the shipterocarpin structure. The spectra data of compound 2 were compared with shipterocarpin from *Erythrina sacleuxii* [17].

Shipterocarpin (2) was isolated as pale yellow solid, and the HRESIMS spectrum showed a quasimolecular ion [M+H]⁺ at *m/z* 323.1276 consistent to the molecular formula C₁₇H₁₂O₄, suggesting 2 is a prenylated pterocarpan. The ¹H NMR spectra of 2, the proton signals at δ H 5.52 (1H, d, J = 6.5 Hz, H-11a), 4.25 (1H, dd, J = 9.5, 5.0 Hz, H-6α), 3.60 (1H, t, J = 9.5 Hz, H-6b), and 3.54 (1H, m, H-6a) characteristic for pterocarpian structure [7]. The ¹H NMR spectrum, proton signals showed an ABX aromatic system at δ H 7.34 (1H, d, J = 8.0 Hz, H-7), 6.56 (1H, dd, J = 8.0, 2.5 Hz, H-8), 6.37 (1H, d, J = 2.5 Hz, H-10), a pair of doublets of aromatic (J = 8.0 Hz) at δ H 7.04 (H-1), 6.28 (H-2), and one 2,2-dimethylpyrano ring at δ H 6.45 (1H, d, J = 9.5 Hz, H-4'), 5.64 (1H, d, J = 9.5 Hz, H-3'), 1.38 (3H, s, H-5') and 1.35 (3H, s, H-6'). Based on ¹H and ¹³C NMR spectra, compound 2 and 1 are isomeric. From HMOC and HMBC spectra of 2 consistent with the shipterocarpin structure. The spectra data of compound 2 were compared with shipterocarpin from *Erythrina sacleuxii* [17].

4′-O-methyl licoflavonone (3) was obtained as yellow solid, and its UV spectra exhibited absorption maxima (203, 226, 288 and 323 sh nm) typical for a flavanone structure [13]. The HRESIMS spectrum showed a quasimolecular ion [M+H]⁺ at *m/z* 355.1460 consistent to the molecular formula C₁₇H₁₂O₄, suggesting that 3 is a prenylated flavanone with contain one methoxyl group. The ¹H NMR spectra of 3 showed three doublet-doublet proton signals at δ H 5.33 (1H, dd, J = 13.0, 3.0 Hz, H-2), 3.11 (1H, dd, J = 17.0, 13.0 Hz, H-3α), and 2.77 (1H, dd, J = 17.1, 3.0 Hz, H-3′α) confirmed for the flavanone structure. In the ¹H NMR spectrum of 3 showed ABX aromatic system at δ H 7.19 (d, J = 2.0 Hz, H-2′), 6.87 (d, J = 8.0 Hz, H-5′), 7.25 (dd, J = 8.0, 2.0 Hz, H-6′) characteristic for aromatic in the ring B. The presence of the proton signals of a pair of doublets (J = 1.8 Hz) in the aromatic region at δ H 5.97 and 5.99 ppm, characteristic for H-6 and H-8 in the ring A. Furthermore, in the ¹H NMR spectrum showed one isoprenyl group assignable to a 3-methyl-2-buten-1-yl group at δ H 5.29 (1H, t, J = 7.0 Hz, H-2''), 3.33 (2H, d, J = 7.0 Hz, H-1′′), 1.74 (3H, s, H-4′′), 1.70 (3H, s, H-5′′), and one methoxyl group at δ H 3.85 ppm. The presence of a chelated hydroxyl group (δ H 12.06, 5-OH) suggested that methoxyl group (δ H 3.85) at C-7 or C-4′. In the ¹³C NMR spectrum, the presence of four oxyaryl (δ C 164.8, 164.3, 163.4 and 157.8) and a carbonyl carbon signal at δ C 196.4 suggested that the oxygenates functionalities are at C-5, C-7 and C-4′ of the flavanone skeleton. The placement of methoxyl group and prenyl group were obtained from the HMOC and HMBC spectra. The one bond and two/three bonds ¹H-¹³C correlations found in the HMOC and HMBC spectra of compound 3 unambiguously placed the methoxyl group at C-4′ was observed. In the HMBC spectrum showed correlations between a proton signal of a
methoxyl group at δH 3.85 with one oxyaryl signals δC 157.8 and between two proton signal of a aromatic group at δH 7.19 and 7.25 with one oxyaryl signals δC 157.8. The prenyl group at C-3 showed correlations between the methylene signal at δH 3.33 with an oxyaryl carbon at δC 157.8 (C-4’). Compound 3, trivially named 4’-O-methyl licoflavanone, was identified as 4’-O-methyl-3’-prenylnaringenin. Further support for the structure 3 was also obtained from the comparison of the NMR data with those reported for 4’-O-methyl licoflavanone from Macaranga trichocarpa [18].

Alpinumisoflavone (4) was isolated as pale yellow solid and the HRESIMS spectrum showed a quasimolecular ion [M+H]+ at m/z 337.1082 consistent to the molecular formula C29H29O9, suggesting that 4 is a prenylated flavonoid. The 1H NMR spectra of 4 showed singlet proton deshielding signals at δH 8.17 characteristic for H-2 of the isoflavone structure. The 1H NMR spectrum also displayed a pair of doublets (J = 8.5 Hz), each integrating for two protons, at δ 6.90 and 7.45 assignable to the signals of a para-hydroxyphenyl group in the ring B. The presence of a chelated –OH group at δH 13.42 and a singlet signal aromatic proton at δH 6.36 characteristic for 5-OH and H-6 or H-8 in the ring A of isoflavonoid structure. In addition, the 1H and 13C NMR patterns of 4 exhibited the presence of a 2,2-dimethylpyrano ring due to the presence of a gem-dimethyl resonance [δH 1.46 (6H, d, H-5” and H-6”), δC 28.4 (C-5” and C-6”)], an olefinic group [δH 5.76 (1H, d, J = 10.0 Hz, H-3’’), 6.67 (1H,d, J = 10 Hz, H-4’’), δC 129.4 (C-3’’) and 115.7 (C-4’’)], and an oxygenated quaternary carbon (δC 78.8). Based on 1H and 13C NMR spectra, the placement 2,2-dimethylpyrano ring were fused at C-6 and C-7 or C-7 and C-8. The location of the 2,2-dimethylpyrano unit at C-6 and C-7 determined on the basis of the HMBC correlations observed. The presence of long-range correlations in the HMBC spectrum of 4 between the proton signal of a chelated 5-OH group at δH 13.42 and three quaternary carbon signals at δC 106.0 (C-4a), 157.7 (C-5), 106.7 (C-6) unambiguously placed the 2,2-dimethylpyrano fused at C-6 and C-7. Further support for the structure 4 was also obtained from the comparison of the NMR data with those reported for alpinum isoflavone from Erythrina fusca [19].

8-Prenyldaidzein (5) was isolated as yellow solid and the HRESIMS spectrum showed a quasimolecular ion [M+H]+ at m/z 321.1120 consistent to the molecular formula C26H25O9, suggesting that 5 is a prenylated flavonoid. The 1H NMR spectra of 5 showed singlet proton signals at δH 8.23 characteristic for H-2 of the isoflavone structure. The presence of the proton signals of a pair of doublets (J = 9.0 Hz) in the aromatic region at δH 6.88 and 7.48 (each 2H), assignable to the signals of a phenoxyphenyl group in the ring B. The signal of a pair of doublets (J = 9.0 Hz) in the ring A at δH 7.03 (H-6) and 7.92 (H-5), suggested that the prenyl group attached at C-8. Further support for structure 5 were also obtained by HMOC and HMBC spectra. The spectra data of compound 5 were compared with 8-prenyldaidzein from Erythrina fusca [19].

The result of compounds 1–5 were evaluated for their antioxidant activity against DPPH radical scavenging, showing their IC50 values were 241.9, 909.8, 648.1, 708.5, and 174.2 µM, respectively. Based on the results of antioxidant activity of phaseollin (1), and 8-prenyldaidzein (5) more active than ascorbic acid.

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<th>Table 1 Cytotoxic and antioxidant activities of phenolic compounds</th>
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<td>Compound</td>
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<td>Phaseollin (1)</td>
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<td>8-Prenyldaidzein (5)</td>
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CONCLUSION

Maceration of dried and powdered stem bark of E. orientalis in methanol at room temperature yielded a brown extract. Methanol extract was suspended in water and partitioned sequentially with n-hexane, and then ethyl acetate. Fractionation of the ethyl acetate extract by VLC on silica gel gave four major fractions, A-D and then purified with radial chromatography yielded phaseollin (1), shinpterocarpin (2), 4’-O-methyl licoflavanone (3), alpinumisoflavone (4), and 8-prenyldaidzein (5). Their structures were elucidated based on UV, IR, HR-ESI-MS, 1D and 2D NMR data by comparing spectoscopic data with reported value. All of isolated compounds 1-5 were assessed for their cytotoxicity activity against murine leukemia P-388 and antioxidant activity against DPPH radical are shown in

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Table 1. Compounds 1–5 were evaluated for their cytotoxicity activity against murine leukemia P-388, showing their IC$_{50}$ values were 2.55, 2.43, 17.98, 4.31, and 5.82 µg/mL, respectively. The results of cytotoxic activity against murine leukemia cells P-388 showed that skeleton of pterokarpan (1-2) > isoflavone (4-5) > flavanone (3). The results indicate that compounds 1, 2, 4, and 5 showed moderate cytotoxicity, and compound 3 was inactive [19]. The presence of pirano group on shinpterocarpin (2) tends to be more active than phaseollin (1). The presence of pirano, and hydroxyl (C-5) group on alpinumisoflavone (4) more active than the presence of isoprenyl (C-8), and hydroxyl (C-7) group on 8-preneylaidzein (5).

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REFERENCES