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

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ABSTRACT

*Two prenylated pterocarpans, 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: Pterocarpan, 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], pterocarpans [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 pterocarpans, phaseollin (**1**), shinpterocarpin (**2**) together with three flavonoids, 4'-*O*-methyl licoflavanone (**3**), alpinumisoflavone (**4**), 8-prenyldaidzein (**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. ^{1}H and ^{13}C NMR spectra were recorded with a Agilent 500 spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz in $CDCl_3$ or acetone-d_6 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 GF_{254} and Si gel 60 PF_{254}, 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 putification 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 Bogorienses, 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 of 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-$CHCl_3$ (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 C_1-C_3. Subfractions C_1 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 C_3 afforded compound **5** (12 mg).

Phaseollin (1): Pale yellow solid: UV (MeOH) λ_{max} nm (log ε) 210 (4.66), 280 (3.96); IR (KBr) ν_{max} (cm^{-1}) 3433, 3043, 1628, 1444, 1213; HR-ESI-MS *m/z* 321.1126 $[M-H]^-$ (calcd for $C_{20}H_{17}O_4$: 321.1127); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (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-6α), 3.60 (1H, t like, *J* = 11.0 Hz, H-6β), 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'); ^{13}C NMR (125 MHz, $CDCl_3$) δ_H (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), 155.4 (C-10a), 78.7 (C-11a), 112.6 (C-11b), 76.1 (C-2'), 129.7 (C-3'), 116.5 (C-4'), 27.8 (C-5'), 27.7 (C-6'). 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 subumbrans* [11].

Shinpterocarpin (2): Pale yellow solid: HR-ESI-MS *m/z* 323.1276 $[M+H]^+$ (calcd for $C_{20}H_{19}O_4$: 323.1283); ^{1}H NMR (500 MHz, acetone-d_6) δ_H (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-6α), 3.60 (1H, t like, *J* = 9.5 Hz, H-6β), 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'); ^{13}C NMR (125 MHz, acetone-d_6) δ_H (ppm): 124.9 (C-1), 109.0 (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), 133.0 (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-2'), 130.4 (C-3'), 117.0 (C-4'), 28.0 (C-5'), , 27.9 (C-6'). 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 licoflavanone (3): Pale yellow solid: UV (MeOH) λ_{maks} (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+$AlCl_3$) 204 (4.67), 223 (4.54). 310 (4.34), 368 (3.54); ($AlCl_3$+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 $C_{21}H_{23}O_5$: 355.1462); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H (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_{ax}), 2.77 (1H, dd, *J* = 17,0, 3,0 Hz, H-3_{eq}), 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'-OCH_3), 12.06 (1H, s, 5-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_H (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-8), 163.4 (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'-OCH_3). Further support for structure **3** were also obtained by HMQC and HMBC spectra. The spectra data of compound **3** were comparison with 4'-*O*-methyl licoflavanone from *Macaranga trichocarpa* [13].

Alpinumisoflavone (4): Pale yellow solid: HR-ESI-MS *m/z* 337.1082 $[M+H]^+$ (calcd for $C_{20}H_{17}O_5$: 337.1076); ^1H NMR (500 MHz. acetone-d_6) δ_H (ppm): 8.17 (1H, s, H-2), 6.36 (1H, s, H-8), 7.45 (2H, d, *J* = 8.5, H-2'/6'), 6.90 (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); ^{13}C NMR (125 MHz. acetone-d_6) δ_H (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 $[M-H]^-$ (calcd for $C_{20}H_{17}O_4$: 321.1127); ^1H NMR (500 MHz. acetone-d_6) δ_H (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'); ^{13}C NMR (125 MHz. acetone-d_6) δ_H (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].

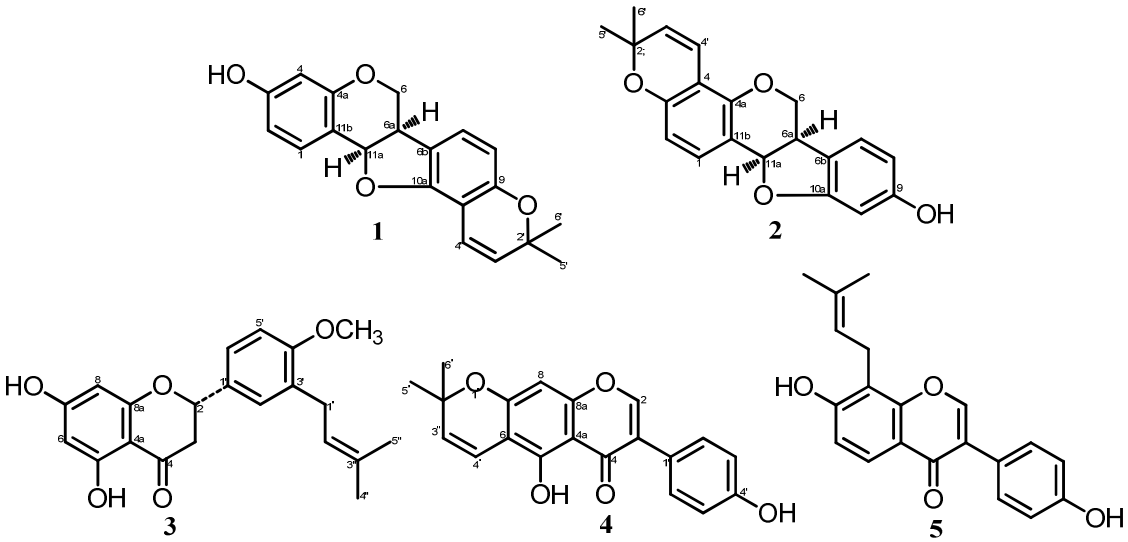

Fig. 1. Structures of phenolic compounds

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 (1×10^4 cells/well). After 24 h incubation at 37 ºC 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-pikrihidrazil) 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 pterocarpans, phaseollin (**1**), shinpterocarpin (**2**), together three prenylated flavonoids, 4'-*O*-methyl licoflavanone (**3**), alpinumisoflavone (**4**), and 8-prenyldaidzein (**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 $C_{20}H_{17}O_4$, suggesting that **1** is a prenylated pterocarpans. 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-6α), 3.60 (1H, t, *J* = 11.0 Hz, H-6β), 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* = 8.5 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 pterocarpan 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 ^{13}C NMR spectrum of 1 showed 20 carbon signals consistent to prenylated pterocarpan. Based on ^1H and ^{13}C NMR spectra, the placement 2,2-dimethylpyrano ring were fused at C-3 and C-4 or C-9 and C-10. The placement 2,2-dimethylpyrano ring was determined with HMQC and HMBC spectra. In the HMBC spectrum showed correlations between an aromatic proton signal at δ_H 6.34 (H-8) with three aromatic quarternary carbon atoms [δ_C 119.1 (C-6b), 153.7 (C-9), 106.2 (C-10)], and correlation proton singlet δ_H 6.51 (H-4') with three quaternary and one methine carbon atoms [δ_C 153.7 (C-9), 106.2 (C-10), 155.4 (C-10a), 129.7 (C-3')] consequently these correlations correspond to the 2,2-dimethylpyrano ring was fused at C-9 and C-10.

HR-ESI-MS, 1D and 2D NMR data, compound **1** was identified as phaseollin [16].

Shinpterocarpin (**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 $C_{20}H_{17}O_4$, suggesting that **2** is a prenylated pterocarpans. 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-6β), and 3.54 (1H, m, H-6a) characteristic for pterocarpan 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 ^{13}C NMR spectra, compound **2** and **1** are isomeric. From HMQC and HMBC spectra of **2** consistent with the shinpterocarpin structure. The spectra data of compound **2** were compared with shinpterocarpin from *Erythrina sacleuxii* [17].

4'-*O*-methyl licoflavanone (**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 $C_{21}H_{23}O_5$, 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-3_{ax}), and 2.77 (1H, dd, *J* = 17.1, 3.0 Hz, H-3_{eq}) confirmed for the flavanone structure. In the ^1H NMR spectrum of **3** showed ABX system at 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 spectra 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 ^{13}C 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 HMQC and HMBC spectra. The one bond and two/three bonds ^1H-^{13}C correlations found in the HMQC 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 δ_H 3.85 with one oxyaryl signals δ_C 157,8 and between two proton signal of a aromatic group at δ_H 7.19 and 7.25 with one oxyaryl signals δ_C 157,8. The prenyl group at C-3'showed correlations between the methylene signal at δ_H 3.33 with a oxyaryl carbon at δ_C 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 $C_{20}H_{17}O_5$, suggesting that **4** is a prenylated flavonoid. The ^{1}H NMR spectra of **4** showed singlet proton deshielding signals at δ_H 8.17 characteristic for H-2 of the isoflavone structure. The ^{1}H 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 δ_H 13.42 and a singlet signal aromatic proton at δ_H 6.36 characteristic for 5-OH and H-6 or H-8 in the ring A of isoflavonoid structure. In addition, the ^{1}H and ^{13}C NMR patterns of **4** exhibited the presence of a 2,2-dimethylpyrano ring due to the presence of a *gem*-dimethyl resonance [δ_H 1.46 (6H, s, H-5'' and H-6''), δ_C 28.4 (C-5'' and C-6'')], an olefinic group [δ_H 5.76 (1H, d, J = 10.0 Hz, H-3'') and 6.67 (1H,d, J = 10 Hz, H-4''), δ_C 129.4 (C-3'') and 115.7 (C-4'')], and an oxygenated quaternary carbon (δ_C 78.8). Based on ^{1}H and ^{13}C 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 δ_H 13.42 and three quaternary carbon signals at δ_C 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-Prenyldaizein (**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 $C_{20}H_{17}O_4$, suggesting that **5** is a prenylated flavonoid. The ^{1}H NMR spectra of **5** showed singlet proton signals at δ_H 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 δ_H 6.88 and 7.48 (each 2H), assignable to the signals of a phydroxyphenyl group in the ring B. The signal of a pair of doublets (J = 9.0 Hz) in the ring A at δ_H 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 HMQC and HMBC spectra. The spectra data of compound **5** were compared with 8-prenyldaizein from *Erythrina fusca* [19].

The result of compounds **1**–**5** were evaluated for their antioxidant activity against DPPH radical scavenging, showing their IC_{50} 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.

Table 1 Cytotoxic and antioxidant activities of phenolic compounds

Compound	Cytotoxic IC_{50} (µg/mL)	DPPH scavenging IC_{50} (µM)
Phaseollin (**1**)	2.55	241.9
Shinpterocarpin (**2**)	2.43	909.8
4'-*O*-Methyl licoflavanone (**3**)	17.98	648.1
Alpinumisoflavone (**4**)	4.31	708.5
8-Prenyldaidzein (**5**).	5.82	174.2
Artonin E	1.33	-
Ascorbic acid	-	329.0

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

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-prenyldaidzein (**5**).

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