Phenolic Compounds from The Stem Bark Erythrina Orientalis and Detection of Antimalaria Activity by ELISA

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Phenolic Compounds from The Stem Bark *Erythrina Orientalis* and Detection of Antimalaria Activity by *ELISA*

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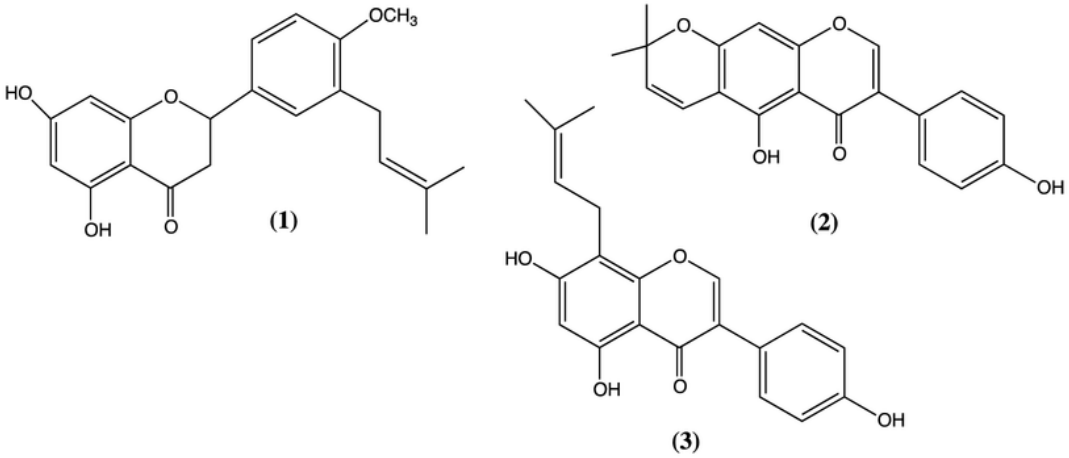
Abstract. *Erythrina orientalis* has local name "Dadap". This plant has known producing alkaloids, flavonoids, pterocarpans, stilbenes, and arylbenzofurans which are active compounds.Three prenylated flavonoids, 8-prenyl-daidzein (**1**), alpinumisoflavone (**2**) and 4'-O-methyl licoflavanone (**3**) had been isolated from the stem bark of *Erythrina Orientalis*. The structures were elucidated on the basis of spectroscopic data,which are IR, UV, MS, and NMR 1D (^{1}H-NMR and ^{13}C-NMR) and 2D (COSY, HMQC, and HMBC).



Compounds **1-3** were evaluated for their antimalaria with *ELISA* methods, which showed percentage inhibition values of74.46%, 62.06%, and 0% respectively. Based on the inhibition value showed 4'-O-methyl licoflavanone (**1**) exhibit very high antimalaria activity compared to compounds **(2)** and **(3)**.

Keywords: Pterocarpan, Flavonoid, *Erythrina Orientalis,* Antimalaria

1. Introduction

Malaria remains world's one of the most devastating human parasitic infection affecting more than 500 million people and causing about 1–3 million deaths each year. This disease has been found as endemic in all region of Indonesia. Recently, chloroquine and artemisinin have used as antimalaria drug and showed resistance against *Plasmodium* parasites in Indonesia[1]. *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 been known that these plants producing alkaloids [2,3], flavonoids [4,5], pterocarpans [6,7], stilbenes [8], and

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arylbenzofurans [9], which are active compounds. *Erythrina orientalis* known local name as "Dadap". 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 of phenolic compound in this medicinal plant, we reported the isolation of prenylated flavonoids, 4'-O-methyl licoflavanone (**1**), alpinumisoflavone (**2**) and8-prenyl-daidzein (**3**), from the ethyl acetate extract of the stem bark of *Erythrina orientalis*. The antimalaria properties of compounds **1-3** against *Plasmodium palcifarum* were analysed using *ELISA* method.

2. Material 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 an 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 GF254 and Si gel 60 PF254, for TLC analysis, pre-coated silica gel plates (Merck Kiesel gel 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 Purwodadi, Kabupaten Pasuruan , 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 stem bark of *E. orientalis* (3.0 kg) was macerated with MeOH two times at room temperature and concentrated to dryness on a rotary evaporator under reduced pressure. The residu 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 of *n*-hexane-EtOAc (9:1, 4:1, 7:3, 1:1, and 3:7) to give four major fractions A-D. Fractions B-C were separated further to isolate flavonoids compounds. 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 **1**(50 mg). Fraction C (6.8 g) was fractionated on silica gel byVLC eluting with a mixtures of *n*-hexane-acetone (9:1, and 4:1) to give three sub fractions C_1-C_3. Sub fractions C_1 purified using radial chromatography eluted with a mixtures of *n*-hexane-acetone (9:1, and 4:1) yielded compound **2**(9 mg). Using the same methodology on fraction C_3 afforded compound **3**(12 mg).

4'-*O*-Methyl licoflavanone (1): 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 (500MHz, $CDCl_3$) 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-3ax), 2.77 (1H, dd,*J* = 17,0, 3,0 Hz, H-3eq), 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, tlike, *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 **1** was also obtained by HMQC and HMBC spectra. The spectra data of compound **1** was compared with 4'-*O*-methyl licoflavanone from *Macaranga trichocarpa* [11].

Alpinumisoflavone (2): Pale yellow solid: HR-ESI-MS *m/z* 337.1082 $[M+H]^+$ (calcd for $C_{20}H_{17}O_5$: 337.1076); ^{1}H-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 **2** was also obtained by HMQC and HMBC spectra. The spectra data of compound **2**was compared with alpinumisoflavone from *Erythrina fusca*[12].

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); ^{1}H-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 **3**was obtained by HMQC and HMBC spectra. The spectra data of compound **3** was compared with 8-prenyldaizein from *Erythrina fusca* [12].

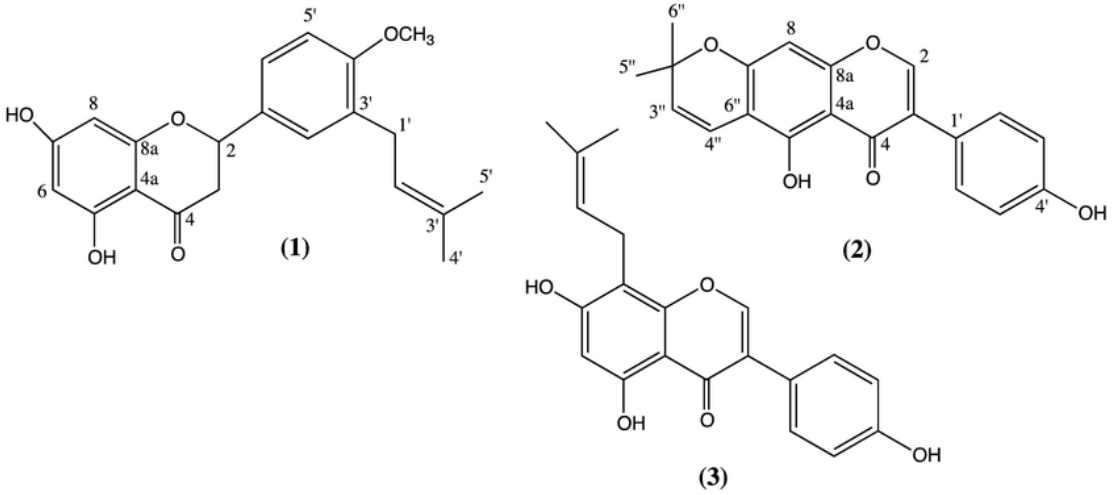

Figure 1. Structures of phenolic compounds

Antimalaria assay

In vitro antimalarial activity against *Plasmodium palcifarum* was carried out according to a modified method of *ELISA* using PBS liquid (Buffer Phosphat) and 19G7 antibody using *EZ-Link Sulfo-NHS-LC*and 2-(4-hidroksibenzena) (HABA). The isolated compounds **1**–**3**have been inserted into the well of micro enzyme-linked immune sorbent assay (ELISA) with the addition of 100 µL19G7 antibodies were incubated for 1 h at 37 ° C. After incubation, to the *ELISA* well was added 100 µl peroxide

and100 µl TMB (3,3',5,5'-tetrametilbenzidin) and then incubated for 20 min at rom temperature. The isolated performed using reagent H_2SO_4 2N was measured by UV spectrometer at λ 450 nm[17].

3. Results and Discussion

Three prenylated flavonoids, 4'-*O*-methyllicoflavanone (**1**), alpinumisoflavone (**2**), and 8-prenyldaidzein (**3**) 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. 4'-*O*-methyl licoflavanone (**1**) 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 quasi-molecular ion $[M+H]^+$at *m/z* 355.1460 consistent to the molecular formula $C_{21}H_{23}O_5$, suggesting that **1** is a prenylated flavanone with contain one methoxyl group. The ^{1}H-NMR spectra of **1** 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-3ax), and 2.77 (1H, dd, J = 17.1, 3.0Hz, H-3eq) confirmed for the flavanone structure. In the ^{1}H-NMR spectrum of **1**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 thering 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 and5.99 ppm, characteristic for H-6 and H-8 in the ring A. Furthermore, in the ^{1}H-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 (δH 3.85) at C-7 or C-4'. In the ^{13}C-NMRspectrum, 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/threebonds ^{1}H-^{13}C correlations found in the HMQC and HMBC spectra of compound **1** unambiguously placed themethoxyl 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 signals of a aromatic group atδ_H 7.19 and 7.25 with one oxyaryl signals dC 157.8.

The prenyl group at C-3'showed correlations between them ethylene signal at δ_H 3.33 with a oxyaryl carbon at δ_C 157.8 (C-4'). Compound **1**, trivially named 4'-*O*-methyl licoflavanone, was identified as 4;-*O*-methyl-3'-prenyl naringenin. Further support for the structure **1**was alsoobtained from the comparison of the NMR data with those reported for 4'-*O*-methyl licoflavanone from *Macaranga trichocarpa* [15].

Alpinumisoflavone (**1**) 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 **1**is a prenylated flavonoid.The ^{1}H-NMR spectra of **1**showed singlet proton deshielding signals at δ_H 8.17 characteristic for H-2 of their isoflavone structure. The ^{1}H-NMR spectrum also displayed a pair of doublets (J = 8.5 Hz), each integrating for two protons, at δ_H 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 orH-8 in the ring A of isoflavonoid structure. In addition, the ^{1}H and ^{13}C-NMR patterns of **1**exhibited the presence ofa 2,2-dimethyl pyrano ring due to the presence of a *gem*-dimethyl resonance [δ_H 1.46 (6H, s, H-5'' and H-6''), δ_C28.4 (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''), δC129.4 (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-dimethyl phyrano 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 **1** 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-dimethyl pyrano fused at C-6 and C-7. Further support for the structure **1** was also obtained from the comparison of the NMR data with those reported for alpinum isoflavone from *Erythrina fusca* [16].

8-Prenyldaizein (**3**) 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 **3**is a prenylated flavonoid. The ^{1}H-NMR spectra of **3** 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 δH 6.88 and 7.48 (each 2H), assignable to the signals of a *p*-hydroxy phenyl group in the ring B. The signal of a pair of doublets (J = 9.0 Hz) inthe 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 **3** was also obtained by HMQC and HMBC spectra. The spectra data of compound **3** was compared with 8-prenyldaizein from *Erythrina fusca* [16]. The result of compounds **1–3** were evaluated for their antimalaria with *ELISA* methods, which showed percentage inhibition values 74.46%,62.06%, and 0 % respectively. Compounds **1** exhibited very high antimalaria activity.

Table-1. Antimalaria activities of flavonoids compounds

Compound	% inhibition antimalaria (*ELISA* method) dosage 100 µg/ml
4'-*O*-methyl licoflavanone (**1**)	74.46%
Alpinumisoflavone (**2**)	62.06%
8-Prenyldaizein (**3**)	na %

4. 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 fraction B-C purified with radial chromatography yielded flavonoids compounds 4'-*O*-methyl licoflavanone (**1**), alpinum isoflavone (**2**), and 8-prenyldaidzein (**3**). 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 **1-3** were assessed for their malaria activity against showed in Table-1. Compounds **1–3** were evaluated for their antimalarial activity against *Plasmodium palcifarum* was carried out according to a modified method of *ELISA*, showing their percentage inhibition values74.46%, 62.06%, and 0 % respectively. The results of antimalaria activity against *Plasmodium palcifarum* showed that compound **1** is more active than two other flavonoid compounds. Compounds **1** exhibited very high antimalaria activity, while compound **3** was inactive.

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