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Airlanggon A, a new isoprenylated benzoic acid fromthe roots of *Erythrinasu bumbrans* and their antioxidant and antimalarial activities

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ABSTRACT

*This research was aimed to determine the antioxidant and antimalarial activities from a new isoprenylated benzoic acid, airlanggon A (**1**) isolated from the roots of Erythrina subumbranstogether with known compounds, methyl 4,6-dihydroxy-2,5-dimethyl benzoic acid **(2)** and 8-hydroxy-7,4'-dimethoxy isoflavone (**3**) from the stem barkof E. subumbrans. The structure of three compounds were elucidated by UV, IR, 1D and 2D NMR, and HR-ESI-MS spectra. Compounds **1-3** were evaluated for their antimalarial and antioxidant properties against P. falciparum and DPPH radical scavenging activities.Compounds **1**–**3** showing their antimalarial activity against P. falciparum with IC_{50} of 1.98; 1.67 and 1.64 µg/mL and antioxidant properties against DPPH, showing their $IC_{50}$of 266.48 ; 34.61 and 22.33 µg/mL respectively.The results indicate that 8-hydroxy-7,4'-dimethoxy isoflavone (**3**) slightly more active thanmethyl 4,6-dihydroxy-2,5-dimethyl benzoic acid (**2**)and airlanggon A (**1**) showed moderate activities.*

Keywords: Airlanggon A, Methyl isoprenyl benzoic acid, *Erythrina subumbrans*, Antioxidant, Antimalarial

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INTRODUCTION

The genus *Erythrina* (Euphorbiaceae) comprises more than 100 species that are widely distributed in tropical and subtropical regions worldwide. The evergreen plants of *Erythrina* occur in almost every part of Indonesia, from Sumatra to Irian and the plants is commonly known as "dadap". Many of these species are used indigenously as traditional medicines to treat various diseases, such as infection, cough, malarial, inflammation, and, asthma. This genus has been shown to produce a number of phenolic compounds, particularly alkaloids, flavonoids, pterocarpans and stilbenoids[1,2,3,4]. In continuation of our phytochemical work of Indonesian tropical plants aiming to find new antimalarial and antioxidant compounds from *Erythrina subumbrans*, we report the isolation and structure elucidation of isoprenylated benzoic acid derivatives, airlanggon A **(1)**from the methanol extract of the root of *E. subumbrans*, together withmethyl 3,6-dimethyl-2,4-dihydroxy benzoic acid **(2)** and 8-hydroxy-7,4'-dimethoxy isoflavone **(3)** from theextract of stem bark of *E. subumbrans*. The antimalarial and antioxidant properties of compounds **1**–**3** against *P. falciparum* and DPPH radical scavenging are also briefly described.

MATERIALS AND METHODS

General experimental procedures

UV spectra was measured with a Shimadzu 1700. ^{1}H and ^{13}C NMR spectra were recorded with a Agilent 500 spectrometer operating at 500 (^{1}H) and 125 (^{13}C) MHz, using residual and deuterated solvent peaks (δ_H 7.26 and δ_C 77.0, respectively) as reference standards.Mass spectra were obtained with a Waters LCT Premeir XE ESI-TOF mass spectrometer. Vacuum liquid chromatography (VLC) and column chromatography were carried out using Si

gel 60 G, and for TLC analysis, precoated Si gel 60 F254 plates were used. Solvents used for extraction and separation were of technical grades that were distilled before use.

Plants material

The stem bark and roots of *E. subumbrans* were collected in April 2013 fromBotani Garden, Pasuruan. East Java, Indonesia. The species were identified at the Herbarium Bogorienses, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

Extraction and isolation of phenolic compounds

The dried root of *E. subumbrans* (2.0 kg) was macerated in methanol at room temperature two times, and the methanol extract was evaporated under reduced pressure to give a dark brown residue(90 g).Furthermore, the methanol extract were partitioned with n-hexane and ethylacetate.The ethylacetate extract (18 g)was separated by vacuum liquid chromatography on silica gel.Elution with n-hexane-ethylacetate mixture with increasing amount of ethylacetate (90:10, 80:20; 70:30; 50:50 and 20:80) gave three fractions A-C.On TLC analysis, fraction B (460 mg) showed three major spots.Purification of this fraction using planar radial chromatography, and eluting with n hexane-chloroform (from 8:2 to 1:1) yielded compound **1** (48 mg).

Maceration of the stem bark (3.0 kg) with methanol followed by partition with n-hexane and ethylacetate gave 38 grethylacetate extract. Separation of this extractusing gradient elution ofn-hexane-ethylacetate mixturegave three fractions A-C. Separation of fraction C(630 mg) by vacuum liquid chromatography using n-hexane-ethylacetate mixture (9:1 to 7:3),followed by purification using radial chromatography with n hexane-chloroform (7:3 to3:7) gave compound **2** (35 mg).Further purification of fraction Aby radial chromatography with n hexane-chloroform (9:1 and 3:2) gave compound**3** (8 mg).

Antimalarial activity test

Antimalarial properties of the isolated compounds **1-3** against *Plasmodium palcifarum* was obtained from the Institute of Tropical Diseases, Universitas Airlangga, Surabaya, Indonesia. In vitro antimalarial activity against *Plasmodium palcifarum* was carried out according to a modified method of Trager and Jensen using RPMI 1640 medium with10% O+ serum[5,6]. The antimalarial activity of three phenolic compounds and chloroquine (positive control) were measured in triplicate. Fresh red blood cells was used as a negative control. The active compound was dissolved in DMSO and diluted with RPMI 1640 medium to prepare a series of concentration. Parasitaemia was evaluated after 48 by Giemsa stain and the average percentace suppression of parasitaemia was calculated by following equation[8]:

$$\%\ average\ suppression\ = \frac{100\ \times\ (\%\ average\ in\ control - \%\ average\ in\ active\ compound)}{\%\ average\ parasitemia\ in\ control}$$

The influence of the active compound on the growth of parasites were expressed by the 50% inhibitory concentrations (IC_{50}) which were determined using linier regression analysis.

DPPH scavenging activity test

The antioxidant activities of the isolated compounds **1-3** against DPPH (2,2-diphenyl-1-pikrihidrazil) radical measured by UV spectrometer at λ 517 nm as described previously [7,8].The inhibition percentage (%) of radical scavenging activity was calculated using the following equation: Inhibition (%) = $(A_o - A_s / A_{o)} \times 100$

Where A_o is the absorbance of the control reaction (containing all reagents except the test compound), and A_s is the absorbance of the test compound.

Statistical analysis

Statistical analysis was performed using One-way analysis of variance (ANOVA) and followed by least squaredifference. Results were expressed as mean±SD from three replications. The P values < 0.01 were considered significant.

RESULTS AND DISCUSSION

Extraction of the dried milled roots of *E. subumbrans*using methanol, and then methanol extract were partitioned with n-hexane and ethylacetate yielded 18 gr ethylacetate extract (18 g).Separation by vacuum liquid chromatography on silica gel and radial chromatography gave namely airlanggon A (**1**). Exctraction and separation compounds from the stem bark resultedmethyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (**2**), and 8-hydroxy-7,4'-dimethoxy isoflavone (**3**) as can be seen on Figure 1.

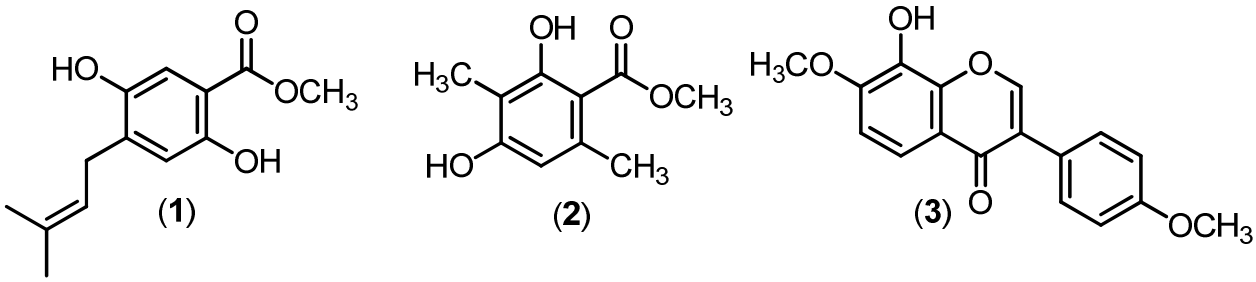

Figure 1. Structures of phenolic compounds

Airlanggon A(**1**), white solid, ^{1}H NMR (500 MHz, $CDCl_3$), and ^{13}C NMR (125 MHz, $CDCl_3$) data,see Table 1; UV (MeOH) λ_{maks} nm (log ε) :224 (4.86), and 287 nm (3.95). HR-ESI-MS: m/z $[M-H]^-$ calcd.for $C_{13}H_{15}O_4$ 235.0970, found 235,0971.

Airlanggon A (**1**) was obtained as white solid, had a formula $C_{13}H_{15}O_4$ as determined by HR-ESI-MS (m/z 235,0971$[M-H]^-$, calcd. 235.0970) suggesting that **1** is a methyl benzoic acid derivative with one isoprenyl group and two hydroxyl groups.The UV spectrum showed absorption maxima(λ_{max}224, and 287 nm)typical of a methyl benzoic acid chromophore. The ^{1}H NMR (Table 1) spectrum of **1**,the presence of two singlet aromatic proton signals at δ_H7.56(1H, *s*, H-6) and 6.39 (1H, *s*, H-3) suggest that compound **1** is a typical for a methyl benzoic acid derivative with three substituents[9].In the ^{13}C NMR spectrum (Table 1), of **1**showed 13 carbon signals consistent with methyl isoprenylated benzoic acid structure, and two carbon signals at δ_C 52.0 and 170.4 were assigned to a methoxyl and carbonyl carbon from methyl benzoic acid structure. These spectroscopic data, therefore, suggested that **1** is a methyl benzoic acid containing C_5-side chain. Furthermore, the presence of other two oxyaryl signals (δ_C 160.9 and 162.1) indicated that the methyl benzoic acid have two hydroxyl groups. The side chain was deduced to be a isoprenyl group from the observation in the ^{1}H NMR spectrum (Table 1) of two methyl singlets (δ_H 1.76 and 1.77), one methylene signals (δ_H 3.27), and one methin vinyl signals (δ_H 5.28). The presence of a chelated –OH group at δ_H 10.79 to give the position of hydroxyl at C-2. In the aromatic region of ^{1}H NMR spectrum, two singlet signals at δ_H6.39 and 7.56 suggested that the position protons at H-3 and H-6. The presence of long range correlations in the HMBC spectrum of **1** between the proton signal aromatic at H-3 (δ_H 6.39) with four quarternary carbon signals at δ_C 105.4 (C-1), 162.1 (C-2), 103,4 (C-4), and 160.9 (C-5) unambiguously placed the isoprenyl and hydroxyl groups at C-3 and C-4. The placement of isoprenyl group at C-3 suggested the correlation methylene signal (δ_H 3.27) with three quarternary carbon signals at δ_C 160.9 (C-5), 119.2 (C-4), 135.0 (C-3'), and two tertiery carbon signals at δ_C 103.4 (C-4), 121.6 (C-3'). Therefore, compound **1**, trivially named airlanggon A was elucidated as methyl 4-isoprenyl-2,5-dihydroxy-benzoic acid. Other HMBC correlations consistent with the structure **1** are shown in Table 1 and Figure 2. To our knowledge, compound **1** was the first example of methyl benzoic acid in the genus *Erythrina* with one isoprenyl side chain.

Table-1.NMR spectroscopic data of airlanggon A (1) in $CDCl_3$

No	δ_H (mult, *J* Hz)	δ_C	HMBC (H ⟺ C)
1	-	105.4	-
2	-	162.1	-
3	6.39 (*s*)	103.4	C-1, C-2, C-4, C-5
4	-	119.2	-
5	-	160.9	-
6	7.56 (*s*)	131.1	C-2, C-4, C-1', C=O
1'	3.27 (*d*, 7,0)	28.9	C-3, C-4, C-5, C-2', C-3'
2'	5.28 (*tm*, 7,5)	121.6	C-4', C-5'
3'	-	135.0	-
4'	1.77 (*s*)	25.8	C-2', C-3', C-5'
5'	1.76 (*s*)	17.9	C-2', C-3', C-4'
C=O	-	170.4	-
2-OH	10.79 (*s*)	-	C-1, C-2, C-3
OCH_3	3.91 (*s*)	52.0	C=O

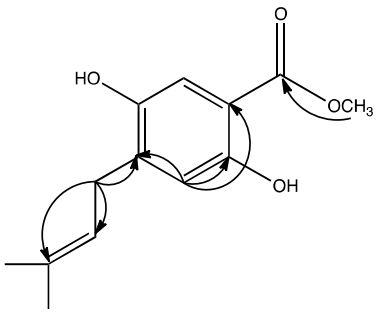

Figure 2.The significant HMBC correlations of 1

Methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (**2**) was isolated as a pale yellow solid. UV (MeOH) λ_{maks} nm (log ε) : 220 (4.93), and 290 (4.09). HR-ESI-MS: m/z $[M-H]^-$ calcd.for $C_{10}H_{11}O_4$ 195.0657, found 195.0704. ^{1}H NMR (500 MHz in $CDCl_3$), δ_H ppm: 12.05 (1H, *br*, *s*, 2-OH), 6.22 (1H, *s*, H-5), 3.93 (3H, *s*, OCH_3), 2.47 (3H, *s*, 6-CH_3), and 2.07 (3H, *s*, 3-CH_3). ^{13}C NMR (125 MHz in $CDCl_3$), δ_C ppm: 172.7 (C=O), 163.1 (C-4), 164.4 (C-2), 158.2 (C-4), 140.3 (C-6), 110.5 (C-5), 108.3 (C-3), 104.9 (C-1), 51.7 (OCH_3), 24.0 (6-CH_3), and 24.0 (3-CH_3).

The HRESIMS spectrum of **2** showed a quasimolecular ion $[M-H]^-$ (m/z 195.0704) consistent with a molecular formula $C_{10}H_{11}O_4$ (calculated m/z 195,0657, Δ 4,7 mDa), suggesting that **2**is a methyl benzoic acid derivative containing two methyl and two hydroxyl groups[10].The presence of a singlet signal of aromatic proton at δ_H 6.22 and a singlet signal of methoxyl group at δ_H 3.93 ppm suggest that compound **2** is a typical for a methyl benzoic acid derivative with four substituents. The presence of one downfield signals at δ_H 12.05 ppm was assignable to 2-OH strongly hydrogen-bonded intramolecularly to the carbonyl group of methyl benzoic.Two singlet signals of methyl groups, assignable to the signals at ring methyl benzoic. Furthermore, the presence of two oxyaryl signals (δ_C 163.1 and 158.2), and of two methyl signals (δ_C 24.0 and 7.5), indicated that the methyl benzoic acid have two hydroxyl and two methyl groups (Table 2).The presence of long range correlations in the HMBC spectrum of **2** between the proton signal aromatic at H-5 (δ_H 6.22) with two quarternary carbon signals at δ_C 104.9 (C-1), 163.1 (C-2) and one methyl carbon signal δ_C24.0 (6-CH_3),unambiguously placed the aromatic proton at H-5. The placement of methyl group at C-3 suggested the correlation methyl signal (δ_H2.07) with three quarternary carbon signals at δ_C 163.1 (C-2), 158.2 (C-4), and δ_C 108.3 (C-3). Therefore, compound **2**, trivially named isoclavatolor known as methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid[10].Other HMBC correlations consistent with the structure **2** are shown in Table 2 and Figure 3.

Table-2.NMR spectroscopic data of methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (2) in $CDCl_3$

No	δ_H (mult, *J* Hz)	δ_C	HMBC (H C)
1	-	104.9	-
2	-	163.1	-
3	-	108.3	-
4	-	158.2	-
5	6.22 (*s*)	110.5	C-1, C-2, 6-CH_3
6	-	140.3	-
C=O	-	172.7	-
3-CH_3	2.07 (*s*)	7.5	C-2, C-3, C-4
6-CH_3	2.47 (*s*)	24.0	C-1, C-5, C-6
2-OH	12.05 (*s*)	-	C-1, C-2, C-3
4-OH	5.10 (*s*)	-	C-3, C-4, C-5
OCH_3	3.93 (*s*)	51.7	C=O

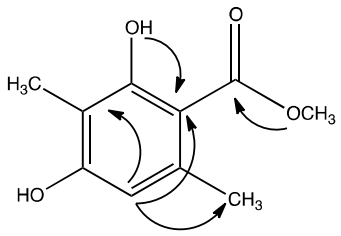

Figure 3.The significant HMBC correlations of 2

8-Hydroxy-7,4'-dimethoxy isoflavone (**3**) was isolated as a pale yellow solid. ^{1}H NMR (500 MHz, acetone-d6) δ_H (ppm): 8.00 (1H, *s*, H-3), 7.99 (1H, *d*, *J* = 9.7 Hz, H-5), 7.51 (2H, *d*, *J* = 9.7 Hz, H-2'/6'), 7.06 (1H, *d*, *J* = 9.7 Hz, H-

6), 6.99 (2H, *d*, *J* = 9.7 Hz, H-3'/5'), 6.25 (1H, *br*, *s*, 8-OH), 4.10 (3H, *s*, 7-OCH_3), and 3.86 (3H, *s*, 4'-OCH_3). HR-ESI-MS: *m/z* [M^+] calcd for $C_{17}H_{13}O_5$: 297.0763; found 297.0761.

The presence of a singlet signal of aromatic proton at δ_H8.00 ppm suggest that compound **3** is a typical for a isoflavone[11].The HRESIMS spectrum of **3** showed a quasimolecular ion [M-H]$^-$ (m/z 297.0763) consistent with a molecular formula $C_{10}H_{11}O_4$ (calculated m/z 297.0761), suggesting that **3** is a isoflavone containing two methoxyl and one hydroxyl groups. The presence of the proton signals of a pair of doublets (*J* = 9.7 Hz) in the aromatic region at δ_H 7.51 and 6.99 (each 2H), and a pair of doublets (*J* = 9.7 Hz) in the A ring at δ_H 7.99 and 7.06 assignable to the signals of *p*-hydroxyphenyl group at B ring, H-5 and H-6. Two singlet signals of methoxyl groups, indicated that the methoxyl groups at C-7 and C-4'. From the HR-ESI-MS, ^{1}H NMR spectra, compound **3** was identified as 8-hydroxy-7,4'-dimethoxy isoflavone[12].

Compound **1-3** isolated from *E.subumbrans* were assessed for their antimalarial activity against *Plasmodium falciparum*and radical scavenging against DPPH. The result are presented in Table 3.

Table 3. Antimalarial and antioxidant activities of isolated compounds

Compound	Antimalarial (µg/mL)	DPPH (µg/mL)
Airlanggon A	1.98	266.48
Methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid	1.67	34.61
8-Hydroxy-7,4'-dimethoxy isoflavone	1.64	22.33
Chloroquine	1.02	-
Ascorbic acid	-	57.87

Compounds **1**–**3** were evaluated for their antimalarial properties against *Plasmodium palcifarum*by Tragger and Jensen methods, showing their IC_{50} were 1.98; 1.67 and 1.64 µg/mL, respectively (chloroquine as a positive control, IC_{50} 1.02 µg/mL). These antimalarial data suggested that the compound **1-3** showed moderate activity against *Plasmodium falciparum*. Compounds **1**–**3** were evaluated for their antioxidant properties against DPPH radical, showing their IC_{50} were 266.48 ; 34.61 and 22.33 µg/mL, respectively (ascorbic acid as a positive control, IC_{50}57.87 µg/mL).Based on the results of experiments assay on antioxidant activity of methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (**2**), and 8-hydroxy-7,4'-dimethoxy isoflavone (**3**) showed very high activities than ascorbic acid, and airlanggon A (**1**) was inactive.

CONCLUSION

Threephenolic compounds, airlanggon A (**1**), together with two known compounds, methyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (**2**), and 8-hydroxy-7,4'-dimethoxy isoflavone (**3**) have been isolated from *E.subumbrans*,a species belongs to the family Leguminosae. The antimalarial activityof **1-3** against *P. falciparum*showed moderate activitiesand antioxidant activity of compounds **1-3**were evaluated against DPPHwhich showed that 8-hydroxy-7,4'-dimethoxy isoflavone (**3**)andmethyl 3,6-dimethyl-2,4-dihydroxy benzoic acid (**2**)more active than ascorbic acid, and airlanggon A was inactive.

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