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Research Article

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Antimalarial and antioxidant activity of phenolic compounds isolated from *Erythrina crista-galli* L.

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ABSTRACT

*Threephenolic compounds, phaseollidin (**1**), sandwicensin (**2**), and lonchocarpol A (**3**) had been isolated from the stem bark of Erythrina crista-galli. Their structures were established on the basis of spectroscopic evidence. Compounds **1**–**3** were evaluated for their antimalarial properties against Plasmodium palcifarum, showing their IC_{50} were 1.66, 1.83, and 1.69 µg/mL.The antioxidant activity of **1**–**3** against 2,2-diphenyl-1-picrylhydrazyl (DPPH), showing their IC_{50} were 209.4,305.6,and 1115.7 µM, respectively.*

Keywords: *Erythrina crista-galli*, Phenolic compound, Antimalarial, Antioxidant

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INTRODUCTION

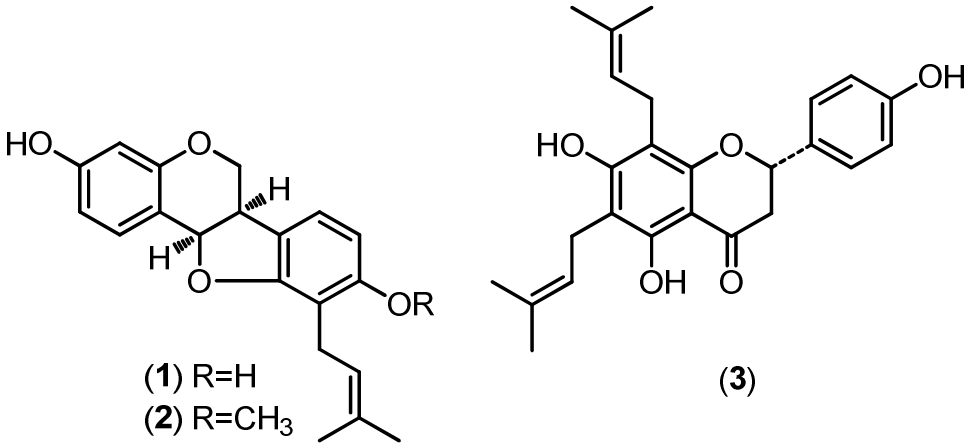
Malaria is a major cause of death in the word than any parasitic infection, especially in tropical developing countries. This disease has been found endemic in all of region in Indonesia. Recently, chloroquine and artemisinin have used as antimalarial drug and showed resistance against *Plasmodium* parasites in Indonesia [1]. *Erythrina*belongs to the family of Leguminosae. This plant has been shown to produce a number of pterocarpan, flavonoid, and alkaloid compounds that showed activity as anticancer, antioxidant, antiviral and antimalaria[2,3,4,5,6]. Decoction of the bark or leaves of *Erythrina crista-galli*(local name: DadapMerah),has been used in the Indonesian people as a traditional medicine on malaria. In continuation of these chemical investigations, we have examined*Erythrina crista-galli* and succeeded in isolating three phenolic compounds, with namely phaseollidin (**1**), sandwicensin (**2**), and lonchocarpol A (**3**). This paper discusses the structure elucidation of the three phenolic compounds. Also, antimalarial properties of compounds **1**-**3** against *Plasmodium palcifarum*and antioxidant properties against DPPHare briefly described.

EXPERIMENTAL SECTION

The stem barkof *E.crista-galli* were collected from near campus, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. The plant was identified at the Herbarium Bogoriense, Bogor Botanical Garden, Bogor, Indonesia, and a voucher specimen had been deposited at the herbarium. The tree bark was cleaned, air dried under the shade, cut into small pieces and milled.

The dried and powder of the stem bark *E.crista-galli*(2.0 kg) was macerated with methanol two times at room temperature, and then concentrated under reduced pressure. The residue was suspended in water (9:1) and partitioned sequentially with *n*-hexane and EtOAc (3 g). The crude EtOAc was then fractionated using vacuum liquid chromatography on silica gel eluting with mixtures of *n*-hexane-EtOAc (9:1, 4:1, and 7:3) to give three major fractions A-C. Fraction B (250 mg) was separated with radial chromatography and eluting with mixtures of *n*-hexane-EtOAc (9:1, and 4:1) gave sandwicensin (36 mg), and lonchocarpol A (4 mg). Furthermore, fraction C (400

mg) was purified using radial chromatography eluted with mixtures of*n*-hexane-$CHCl_3$ (7:3, to chloroform) yielded phaseollidin (52 mg)



Phaseollidin(**1**), yellow 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), 281 (3.93), and 287 nm (3.95). HRESI-MS: m/z $[M+H]^+$calcd.for $C_{20}H_{21}O_4$ 325.1472, found 325.1440.

Sandwicensin (**2**), yellow 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) :223 (4.60), 285 (3.84), and 287 nm (3.89). HRESI-MS: m/z $[M+H]^+$ 339.1642 calcd.for $C_{21}H_{23}O_4$, found 339.1596.

Lonchocarpol A (**3**), yellow solid, ^{1}H NMR (500 MHz, $CDCl_3$), and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2;UV (MeOH) λ_{maks} nm (log ε): 236 (3.95), 293 (4.14), and325 sh (3.60) nm.

Antimalarial properties of the isolated compounds **1-3** against *Plasmodium palcifarum* were 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 [7]. The antimalarial activity of three phenolic compounds and chloroquine (positive control) were measured in triplicate. Fresh red blood cells were used as a negative control. The active compound was dissolved in DMSO and diluted with RPMI 1640 medium to prepare a series of concentrations. Parasitaemia was evaluated after 48 by Giemsa stain and the average percentage suppression of parasitaemia was calculated by following equation[8]:

$$\% \text{ average suppression} = \frac{100 \text{ x } (\% \text{ average in control} - \% \text{ average in active compound})}{\% \text{ average parasitaemia in control}}$$

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

The antioxidant activities of of the isolated compounds **1-3**against DPPH (2,2-diphenyl-1-pikrihidrazil) radical were measured by UV spectrometer at λ 517 nm as described previously [9,10,11].

RESULTS AND DISCUSSION

Extraction of the dried milled tree bark of *E.crista-galli*with methanol gave a fraction which was separated by vacuum liquid chromatography and radial chromatography to give namely phaseollidin (**1**), sandwicensin (**2**), and lonchocarpol A (**3**). The structures of three phenolic compounds were identified by UV, HR-ESI-MS, 1D and 2D NMR spectra. Phaseollidin (**1**) was obtained as yellow solid with the molecular formula $C_{20}H_{21}O_4$ as determined by HR-ESI-MS (m/z 325.1472 $[M+H]^+$, calcd. 325.1440) indicating eleven degrees of unsaturation. The ^{1}H NMR (Table 1) spectrum of **1**,showed the presence of four peculiar aliphatic protons at δ_H 5.30 (1H, *d, J* = 6.8 Hz, H-11a), 4.09 (1H, *dd, J* = 10.9 and 5.1 Hz, H-6), 3.47 (1H, *t, J* = 10.9 Hz, H-6), and 3.34 ppm (1H, *m*, H-6a), suggest that compound **1** is a typical for a pterocarpan. The ^{1}H NMR spectrum of **1**showed two ABX coupling aromatic systems, one is δ_H 7.22 (1H, *d, J* = 8.4 Hz, H-1); 6.46 (1H, *dd, J* = 8.4 and 2.4 Hz, H-3), and 6.30(1H, *d, J* = 2.4 Hz, H-4), and

another is δ_H 6.75 (1H, *d*, *J* = 8.0 Hz, H-7) and 6.25 ((1H, *d*, *J* = 8.0 Hz, H-8) indicating the presence of trisubstituted pterocarpan skeleton. This compound showed one isoprenyl group assignable to a 3-methyl-2-buten-1-yl group at δ_H 5.37 (1H, *t*, *J* = 7.2 Hz, H-2'), 3.12 (2H, *d*, *J* = 7.1 Hz, H-1'), 1.63 (3H, *s*, H-4') and 1.53 (3H, *s*, H-5'). Based on ^{1}H NMR spectrum suggests that isoprenyl group is either at C-4 or C-10 of the pterocarpan skeleton. The ^{13}C NMR (Table 1) spectrum of **1**showed 20 carbon signals consistent toisoprenylatedpterocarpan structure. Furthermore, the presence of other fouroxyaryl signals (δ_C 158.5, 158.0, 156.3, and 155.5) indicated that the oxyaryl signals atC-3, C-4a, C-9. and C-10a is a derivative of isoprenylatedpterocarpan. The placement isoprenyl group at C-4 or C-10 was determined with HMQC and HMBC spectra. In the HMBC spectrum showed correlations between a proton signal at δ_H3.12 with two oxyaril at δ_C 155.5 (C-9), and 155.8 (C-10a) unambiguously placed the isoprenyl group at C-10. The 1D NOE spectrum, irradiation proton signal at δ_H 5.30 ppm (H-11a)gave increasing proton signals at δ_H 7.22 (H-1), and 3,47 ppm (H-6a) suggest that of **1** is *cis* from isoprenylatedpterocarpan structure. From the above UV, HR-ESI-MS, 1D and 2D NMR data, compound **1** was identified as (6aR, 11aR)-3,9-dihydroxy-10-isoprenyl pterocarpan named as phase ollidin [12].

Sandwicensin (**2**) was isolated as a yellow solid, and its UV spectrum exhibited absorption maxima λ_{maks} (nm) (log ε): 223 (4.60), 285 (3.84), and 287 nm (3.89), that showed a typical for a pterocarpan. From HR-ESI-mass spectra (m/z 339.1642 $[M+H]^+$, calcd. for $C_{21}H_{23}O_4$, 325.1440) indicating that**2** is methylated from compound **1**. The ^{1}H and ^{13}C NMR (see Table 1) spectrum of **2** very similar with compound **1**, but there is one methoxyl groups attached to the aromatic core from compound 2 that proton signal at δ_H3.81and the carbon signal at 55.9. Thus compound 2 was 3-*O*-methyl or 9-*O*-methyl phaseollidin. The placement of a methoxy group in the C-3or C-9 confirmed by the HMQC and HMBC spectra. The HMBC spectrum showed a correlation between the proton signals at δ_H3.81with carbon oxyaril signal at δ_C 155.8. Furthermore, the correlation between aromatic proton signals at δ_H7.02 (*d*, 8.1, H-7) with oxyaril carbon signal at δ_C 155.8 indicated that the methoxyl group attached at C-9. From the above UV, HR-ESI-MS, 1D and 2D NMR data, compound **2** was identified as (6aR, 11aR)-3-hydroxy-9-methoxy-10-isoprenyl pterocarpan named as sandwicensin [12].

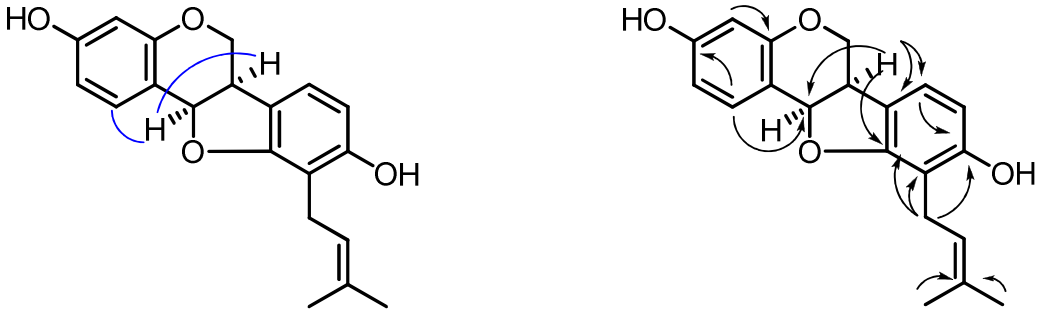


Figure 1. The significant NOE and HMBC correlations of 1

Table 1. NMR spectroscopic data of phaseollidin (1)and sandwicensin(2) in $CDCl_3$

No.C	**1**,δ_H (mult, *J* Hz)	**1**,δ_C	**2**,δ_H (mult, *J* Hz)	**2**,δ_C
1	7.22 (*d*, 8.4)	132.0	7.41 (*d*, 8.4)	132.3
2	6.46 (*dd*, 8.4; 2.4)	109.7	6.58 (*dd*, 8.4; 2.4)	109.9
3	-	158.0	-	157.5
4	6.30 (*d*, 2.4)	103.2	6.44 (*d*, 2.4)	103.1
4a	-	156.3	-	156.5
6	3.47 (*t*, 10.9) 4.09 (*dd*,10.9; 5.1)	66.3	3.66 (*t*,11.0) 4.23 (*dd*, 11.0; 5.1)	66.5
6a	3.34 (*m*)	39.9	3.52 (*m*)	40.0
6b	-	118.0	-	119.4
7	6.75 (*d*, 8.0)	121.6	7.02 (*d*, 8.1)	121.6
8	6.25 (*d*,8.0)	107.5	6.43 (*d*,8.1)	103.5
9	-	155.8	-	158.5
10	-	111.2	-	112.6
10a	-	158.5	-	158.4
11a	5.30 (*d*, 6.8)	77.9	5.46 (*d*, 6.8)	78.0
11b	-	112.0	-	113.3
1'	3.12 (*d*, 7.1)	22.7	3.31 (*d*, 7.2)	22.9
2'	5.37 (*t*, 7.2)	122.0	5.26 (*t*, 7.2)	122.3
3'	-	131.9	-	132.3
4'	1.63 (*s*)	17.5	1.78 (*s*)	17.8
5'	1.53 (*s*)	25.5	1.68 (*s*)	25.8
9-OCH_3	-	-	3.81 (*s*)	56.0

Lonchocarpol A (**3**) was isolated as a yellow solid, and its UV spectrum exhibited absorption maxima λ_{maks} nm (log ε): 236 (3.95), 293 (4.14), and325 sh (3.60) a typical for a flavanone structure. The ^{1}H NMR (Table 2) spectrum of **3** showed three doublet of doublets proton signals at δ_H 5.33 (1H, dd, *J* = 12.8, 3.0 Hz, H-2), 3.05 (1H, dd, *J* = 17.1, 12.8 Hz, H-3$_{ax}$), and 2.80 (1H, dd, *J* = 17.1, 3.0 Hz, H-3$_{eq}$) that confirmed for the flavanone skeleton in **3**. The presence in the aromatic region of proton signals of a pair of doublets (*J* = 8.4 Hz) at δ_H 7.33 and 6.89 (each 2H), assignable to the signals of a *p*-hydroxyphenyl group at ring B. In the absence of aromatic proton at A ring showed two isoprenyl groups at C-6 and C-8. The Placement of two side chain isoprenyl groups at C-6 and C-8 can be suggested by HMBQ and HMBC spectra. Based on 1D and 2D NMR data, compound **3** was identified as 6,8-diisoprenyl naringenin named as lonchocarpol A [13].

Table 2. NMR spectroscopic data of lonchocarpol A(3) in $CDCl_3$

No.C	**3**,δ_H (mult, *J* Hz)	**3**,δ_C	HMBC
1	-	-	-
2	5.33 (*dd*, 12.8; 3.0)	78.6	C-1', C-2', C-6'
3	3.05 (*dd*, 17.1; 12.8) 2.80 (*dd*, 17.1; 2.4)	43.6	C-2. C-4
4	-	196.9	-
4a	-	102.8	-
5	-	159.4	-
6	-	107.3	-
7	-	162.3	-
8	-	106.3	-
8a	-	158.0	-
1'	-	131.2	-
2'/6'	7.33 (*d*, 8.4)	128.0	C-2, C-4'
3'/5'	6.89 (*d*, 8.4)	115.4	C-1', C-4'
4'	-	156.2	-
1''	3.35 (*d*, 7.4)	22.0	C-5, C-6, C-7, C-2', C-3'
2''	5.25 (*t*, 7.7)	121.8	C-4'', C-5''
3''	-	134.7	-
4''	1.83 (*s*)	17.9	C-2', C-3', C-5'
5''	1.76 (*s*)	25.8	C-2', C-3', C-4'
1'''	3.31 (*d*, 7.1)	21.3	C-7, C-8, C-8a, C-2'', C-3''
2'''	5.21 (*t*, 7.9)	121.7	C-4''', C-5'''
3'''	-	133.9	-
4'''	1.71 (*s*)	17.8	C-2'', C-3'', C-5''
5'''	1.60 (*s*)	25.6	C-2'', C-3'', C-4''
5-OH	12.3 (*s*)	-	C-4a, C-5, C-6

Compound **1**-**3** isolated from the tree bark of *E.crista-galli* were assessed for their antimalarial activity against *Plasmodium falciparum*. The results are presented in Table 3. Compounds **1**–**3** were evaluated for their antimalarial properties against *Plasmodium palcifarum*, showing their IC_{50} were 1.66, 1.83, and 1.69 µg/mL, respectively (chloroquine as a positive control, IC_{50} 1.02 µg/mL). These antimalarial data suggested that the compound **1-3** showed very high activity against *Plasmodium palcifarum.*

Table 3. Antimalarialand DPPH scavenging assay of 1-3

Compound	**Antimalarial IC_{50} (µg/mL)**	**DPPH scavenging IC_{50} (µM)**
Phaseollidin	1.66	209.4
Sandwicensin	1.83	305.6
Lonchocarpol A	1.69	1115.7
Chloroquine	1.02	-
Ascorbic acid	-	241.3

On antioxidant activity evaluation against DPPH radical, compounds **1-3** exhibited IC_{50} values of 209.4, 305.6,and 1115.7 µM, respectively (ascorbic acid as a positive control, IC_{50} 241.3µM). Based on the results of experiments assay the antioxidant activity of phaseollidin(IC_{50}209.4 µM) showed very high activity than ascorbic acid as a positive control (IC_{50}241.3µM).It is different with lonchocarpol A(IC_{50}1115.7 µM) showedinactive activity.The structure-activity relationship of compounds **1**–**2** against DPPH radical suggested that the presence of hydroxyl group at C-9 on phaseollidin (**1**) play role to increase activity than the methoxyl group at C-9 on sandwicensin (**2**).

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