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Research Article

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Antioxidant activity of two isomeric benzoxepin derivatives from the stem bark of *Bauhinia aculeata* L.

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ABSTRACT

*Two benzooxepin types isolated from the stem bark of Bauhinia aculeata L. (Fabaceae) have been identified as bauhiniastin 4 (**1**) and pacharin (**2**). The structure of both compounds have been elucidated based on its spectroscopic data, including UV, 1D and 2D NMR, and HREISMS spectra. Compounds **1** – **2** were evaluated for their radical scavenging against 2,2-diphenyl-1-picrylhydrazyl (DPPH), showing their IC_{50} were 32.7, and 1495.2 μM, respectively. The results indicate that as bauhiniastin 4 (**1**) slightly more active than ascorbic acid (62.8 μM).*

Keywords: Benzooxepin, *Bauhinia aculeata L.*, Elucidation structure, Antioxidant

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INTRODUCTION

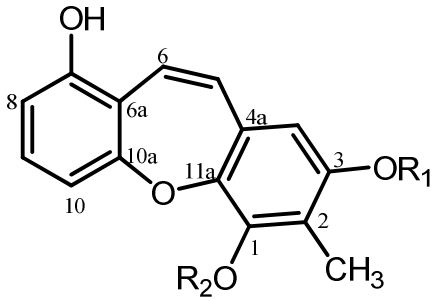
Free radicals are molecules containing one or more unpaired electrons that are highly reactive, unstable, and paramagnetic which can cause damage to the system of DNA, proteins and lipids. Oxidant formed in the pathological process most of them from natural biological process involving compound of oxygen reactive (reactive oxygen species, ROS). Types of reactive oxygen compounds such as hydroxyl radical, peroxide, superoxide, hydroperoxide, ozone, and diatomic oxygen. The antioxidant compounds are compounds that can reduce or neutralize free radicals [1,2,3].

The genus *Bauhinia* is a large genus of the Fabaceae comprises more than 300 species distributed in tropical and subtropical regions. This genus has been shown to produce a number of phenolic compounds, particularly flavonoids and stilbenoids [4,5]. The phenolic compounds from this plants showed activity as an antioxidant, anticancer, antibacterial, anti-inflammatory, antimalarial and antifungal [6,7,8]. In continuation of our phytochemical work of Indonesian tropical plants aiming to find new antioxidant compounds another *Bauhinia* species, *B. acuelata*. In this paper, we report the isolation of benzoxepin derivatives, bauhiniastin 4 (**1**) and pacharin (**2**), from the methanol extract of the leaves of *B. acuelata*. The antioxidant properties of compounds **1**–**2** against DPPH is also briefly described.

EXPERIMENTAL SECTION

The stem bark of *B. acuelata* were collected from Bogor Botanical Garden, Bogor, Indonesia. The dried stem bark of *B. acuelata* (2.5 kg) were macerated in methanol at room temprature three times , and the methanol extract was evaporated under reduced pressure to give a dark brown residue (130 g). Furthermore, the methanol extract were partition with n-heksana and ethyl acetate. The ethyl acetate extract (40 g) was separated by vacuum liquid chromatography on silica gel. Elution with n hexane-ethyl acetate mixture containing increasing amount of ethyl acetate (90:10, 80:20; 50:50 and 30:70) to give four fraction A-D. On TLC analysis, fraction B (150 mg) showed two major spots on purification of this fraction using planar radial chromatography, and using *n* hexane-chloroform

(from 1:1 and 7:3) to yielded compound 1 (15 mg). Further purification of fraction C (420 mg) by radial chromatography with n hexane- ethyl acetate 9:1, and 8:2, and to give compound 2 (10 mg).



(**1**) $R_1 = OH$; $R_2 = CH_3$
(**2**) $R_1 = CH_3$; $R_2 = OH$

Bauhiniastatin 4 (**1**), pale white solid, UV (MeOH) λ_{maks} nm (log ε): 223 (4.46), 236 (4.33) and 312 nm (4.22), (MeOH + NaOH) λ_{maks} nm (log ε): 224 (4.52), and 336 (4.04). HRESIMS: *m/z* $[M-H]^-$ calcd for $[M-H]^-$ $C_{16}H_{13}O_4$ 269.0814 found 269.0814. ^{1}H NMR (400 MHz, acetone-d6): see Table 1. ^{13}C NMR (100 MHz, acetone-d6): see Table 1.

Pacharin 4 (**2**), pale white solid, UV (MeOH) λ_{maks} nm (log ε): 221.5 (4.36), 234 (4.28) and 309.5 nm (4.06), (MeOH + NaOH) λ_{maks} nm (log ε): 224 (4.52), and 336 (4.04). HRESIMS: *m/z* $[M-H]^-$ calcd for $[M-H]^-$ $C_{16}H_{13}O_4$ 269.0814 found 269.0816. ^{1}H NMR (400 MHz, acetone-d6): see Table 1. ^{13}C NMR (100 MHz, acetone-d6): see Table 1.

DPPH scavenging activity test: Determination of the antioxidant activity of the isolated performed using reagent DPPH (2,2-diphenyl-1-pikrihidrazil) using methods of reduction of free radicals as measured by UV spectrometer at λ 517 nm [9,10]. Determination of antioxidant activity done by the 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.

RESULTS AND DISCUSSION

Two isomeric benzoxepin, bauhiniastin 4 (**1**) and pacharin (**2**). were isolated from the stem bark of *Bauhinia aculeata* L. The structure of both compounds have been elucidated based on its spectroscopic data, including UV, 1D and 2D NMR, and HREIMS spectrum.

Bauhiniastatin 4 (1) was obtained as pale white solid, showed a quasimolecular ion $[M-H]^-$ at *m/z* 269.0814 consistent to the molecular formula $C_{16}H_{13}O_4$, supported by the presence of 16 carbon signals in its ^{13}C NMR (see Table1) spectrum with double bond equivalent DBE = 10. The UV spectrum of **1** exhibited absorption maxima at λ_{max} 223, 236, and 312 nm, and showed a bathocromic shift on addition of NaOH solution. In the ^{1}H-NMR (see Table1) spectrum, the presence of a pair of doublets ppm (J = 11.5 Hz) at δ_H 6.70 and 7.00 assignable to the signals of a benzoxepin structure[9]. The presence of four signals of aromatic proton at δ_H 6.32 (*s*), 6.71 (*dd*, *J*= 8.1 and 1.0 Hz), 7.01 (*dd*, *J* = 8.1 Hz), and 7.12 ppm (*t*, *J* = 8.1 Hz) indicated that the benzoxepin have four substituents. Furthermore, by the observation of four substituents of **1** in the ^{1}H-NMR spectrum showed at δ_H 6.32 (*s*), 6.71 (*dd*, *J*= 8.1 and 1.0 Hz), 7.01 (*dd*, *J* = 8.1 Hz), and 7.12 ppm (*t*, *J* = 8.1 Hz) indicated that the benzoxepin containing an two hidroxyl -OH groups, one methyl -CH_3 and a methoxyl –OCH_3 groups. Based on NMR spectra and mass spectra of comfirm the assignment of structure **1**. From HMBC spectra, correlation aromatic proton singlet δ_H 6.32 with three quaternary carbon signal at δ_C 113.4 (C-2), 128.1 (C-6), and 138.6 (4a) suggested that the proton aromatic was unambiguously located at C-4. Furthermore, by an NOEs correlation between hydroxyl group at C-3 with hydroxyl group at C-7. Other HMBC correlations consistent with the structure **1** are shown in Table 1.

Table 1. NMR spectroscopic data of bauhiniastatin 4 (1)

	Bauhinistatin 4		
No.C	δ_H (mult, *J* Hz)	δ_C	HMBC ($^1H \Leftrightarrow {}^{13}C$)
1	-	154.8	-
2	-	113.4	-
3	-	147.0	-
4	6.32 (*s*)	100.7	C-2, C-4a, C-11a
4a	-	138.6	-
5	7.00 (*d*, 11.5)	124.2	C-4a, C-6, C-6a, C-11a
6	6.70 (*d*, 11.5)	128.8	C-4, C-6a
6a	-	118.5	-
7	-	159.2	-
8	6.71 (*dd*, 8.1,1.0)	111.7	C-6a
9	7.12 (*t*, 8.1)	129.7	C-7, C-10a
10	7.01 (*d*, 8.1)	112.5	C-6a, C-8, C-10a
10a	-	155.2	-
11	-	-	-
11a	-	128.1	-
3-OH	7.93 (*s*)	-	C-1, C-2, C-3
7-OH	8.85 (*s*)	-	-
2-CH_3	2.09 (*s*)	8.3	C-1, C-2, C-3
1-OCH_3	3.79 (*s*)	55.2	C-1

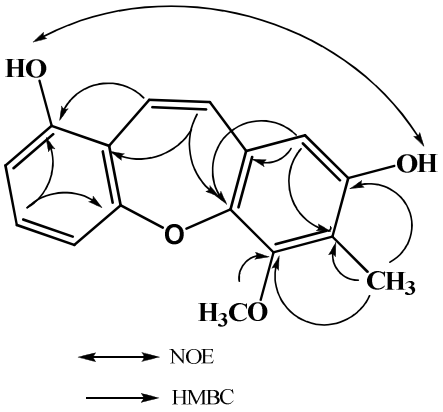


Figure 1. Significant HMBC and NOEs correlation for 1

Pacharin (2) was obtained also as pale white solid, has the molecular formula $C_{16}H_{13}O_4$, deduced from the $[M-H]^-$ ion at *m/z* 269.0816. Compound **2** was almost identical with compound **1** in all UV, ^1H and ^{13}C NMR spectrum. The ^1H NMR spectrum signals (Table 2) at exhibiting one set of *cis* vinylic system at δ_H 6.63 (*d*, *J*=11.5 Hz) and 6.98 (*d*, *J*=11.5 Hz). Three of four aromatic protons showed an ABX system at δ_H 6.70 (*dd*, *J*= 8.1 and 1.0 Hz), 6.79 (*d*, *J* = 8.1 Hz), and 7.14 ppm (*t*, *J* = 8.1 Hz), and other aromatic proton singlet at δ_H 6.47. Compound **2** showed one methyl group at δ_H 2.13 and one methoxyl group at δ_H 3.95 ppm. Based on NMR spectra and mass spectra, the structure of **2** is isomeric compound **1**. From HMQC and HMBC spectra, consistent with the pacharin structure [11].

Table 2. NMR spectroscopic data of pacharin (2)

	Pacharin		
No.C	δ_H (mult, *J* Hz)	δ_C	HMBC ($^1H \Leftrightarrow {}^{13}C$)
1	-	143.3	-
2	-	119.2	-
3	-	151.0	-
4	6.47 (*s*)	108.9	C-1, C-2, C-6, C-11a
4a	-	129.7	-
5	6.98 (*d*, 11.5)	124.4	C-4a, C-7, C-10a
6	6.63 (*d*, 11.5)	128.5	C-4, C-4a, C-6a
6a	-	118.7	-
7	-	155.1	-
8	6.70 (*dd*, 8.1; 1.0)	111.3	C-6a, C-10
9	7.14 (*t*, 8.1)	129.6	C-7, C-10a
10	6.79 (*d*, 8.1)	112.7	C-6a, C-8, C-10a
10a	-	159.8	-
11	-	-	-
11a	-	152.2	-
2-CH_3	2.13 (*s*)	8.5	C-2, C-3
3-OCH_3	3.95 (*s*)	60.7	C-3

The antioxidant activity of bauhiniastatin 4 and pacharin at different concentration (500, 250, 125, 62.5, 31.25, 15.625, and 7.81 ppm) were evaluated against the DPPH radical scavenging. The compound bauhiniastatin 4 and pacharin can reduce free radicals at a concentration 8.79 and 402.4 ppm against DPPH radical scavenging. The IC_{50} values of bauhiniastatin 4 and pacharin showed radical scavenging activity with IC_{50} values 32.7 and 1495.2 µM, respectively. Ascorbic acid as positive control have IC_{50} 62.8 µM. Based on the results of experiments assay the antioxidant activity of bauhiniastatin 4 (IC_{50} 32.7 µM) more than ascorbic acid (IC_{50} 62.8 µM). It is different with pacharin (IC_{50} 1495.2 µM) compound showed inactive. The data antioxidant activity of both isolated compounds showed the presence of hydroxyl groups at C-3 in bauhiniastatin 4 to increasing antioxidant activity, while the presence of a methoxy group at C-3 lower antioxidant activity.

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