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ISOLATION OF ASTILBIN FROM LEAVES OF *CRATOXYLUM ARBORESCENS*

(Pemencilan Komponen Astilbin dari Daun *Cratoxylum arborescens*)

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Abstract

Phytochemical studies was conducted on the leaves of *Cratoxylum arborescens* that has been collected from Post Brooke, Gua Musang, Kelantan, Malaysia. Traditionally, latex of the stem bark of *C. arborescens* is being used for the treatment of wound. Extraction of leaves of *C. arborescens* using organic solvents followed by purification using standard procedure of purification yielded known compound, astilbin. This compound was identified by NMR spectral data using various 2D-techniques and comparison with the literature data. Reports showed that this compound has a unique immunosuppressive activity, a selective inhibition against activated T lymphocytes. This characteristic of astilbin is beneficial for the treatment of human immune diseases.

Keywords: guttiferae, *cratoxylum arborescens*, astilbin, leaves, NMR

Abstrak

Kajian fitokimia yang dijalankan ke atas daun *Cratoxylum arborescens* yang dikutip dari Post Brooke, Gua Musang, Kelantan, Malaysia. Secara tradisional, lateks kulit batang *C. arborescens* digunakan untuk mengubat luka. Pengekstrakan ke atas daun *C. arborescens* menggunakan pelarut organik diikuti dengan kaedah lazim penulenan menghasilkan sebatian yang telah dikenal pasti, astilbin. Komponen ini dikenal pasti menggunakan data spektrum RMN dengan teknik variasi 2D dan perbandingan dengan data literatur. Laporan menunjukkan komponen ini menunjukkan aktiviti immunosupresif yang unik, perencatan selektif terhadap limfosit T diaktifkan. Ciri astilbin ini adalah bermanfaat untuk merawat penyakit berkenaan sistem imun.

Kata kunci: guttiferae, *cratoxylum arborescens*, astilbin, daun, RMN

Introduction

Cratoxylum arborescens is known locally as Geronggang. This genus belongs to the family of Guttiferae. It is an emergent tree up to 60 m tall and diameter of about 120 cm. The stem has yellow latex and the leaves are opposite, simple, penni-veined, glabrous and venation is inconspicuous. Flowers are about 8 mm in diameter, white-pink-red and placed in panicles. The fruits are about 8 mm long red-brown-black dehiscent capsule, with many small winged seeds. This plant is normally found in undisturbed to slightly mixed dipterocarp, sub-montane forests up to 1000 m altitude. Geronggang is mostly growing on alluvial sites and along rivers, but also found on ridges. In secondary forests, this plant is usually presents as pre-disturbance remnant tree [1]. A number of studies have already been conducted on *C. arborescens*, where the compound 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone was isolated from stem bark [2], while 1,3,8-trihydroxy-2,4-dimethoxyxanthone, 1,7-dihydroxy-2,8-dimethoxyxanthone, 1,3,7-trihydroxy-6-methoxy-4,5-diisoprenylxanthone, euxanthone, friedelin, friedelinol, methoxyemodin, betulinic acid, lup-20(29)-ene-3,30-diol, 3β-hydroxylup-20(29)-en-30-oic acid, 3,4-dihydroxybenzoic acid, eucryphin, astilbin, and isoastilbin from leaves and twigs of *C. arborescens* collected from Narathiwat province, Thailand [3,

4]. Astilbin has also been isolated from the leaves of Chinese folk medicine, *Engelhardia chrysolepis* [5]. Astilbin from stem bark of *Dimorphandra mollis* collected in Rio Claro, Sao Paulo State, Brazil showed insecticidal activity against confined bees [6].

Experimental

Plant Material

The leaves of *Cratoxylum arborescens* was collected at Post Brooke, Gua Musang, Kelantan, Malaysia. The voucher specimen of *C. arborescens* (SK 1932/11) is deposited at Herbarium UPM (UPM), Serdang, Selangor, Malaysia.

Extraction and Isolation

The leaves of *C. arborescens* (GGB, 569.3 g) was dried in oven at 50^{o}C, ground and extracted using solvent extraction at room temperature for five days. The powdered leaves was extracted using methanol (crude extract 45.6 g) and then fractionated with hexane with ratio 1:1. 1.3 g of hexane fraction (GGBA) and 36.8 g of methanol fraction (GGBC) yielded. Chemical components from methanol fraction was separated using vacuum liquid chromatography (VLC). A mixture of solvent ethyl acetate and methanol from 100% ethyl acetate to 100% methanol have been used as mobile phase and 12 fractions were collected. Vial 5-6 from VLC of GGBC have selected for further purification using column chromatography and 148 vials were collected.

Fractions 106-114 were combined for further purification using column chromatography with an internal diameter x length of column is 1.0 cm x 50.0 cm and 27 fractions were collected. The compound was eluted with solvent mixture of ethyl acetate and methanol. The combined fractions was evaporated and gave a greenish crystal (181 mg). The structure of the purified compound was analyzed using Nuclear Magnetic Resonance (NMR) and Infrared (IR) spectrometer.

Astilbin (1)

Greenish needles (181 mg). IR cm^{-1}: 3321 (OH), 2944, 2833, 1648, 1412, 1113 and 1020. ^{1}H NMR (600 MHz, CD_3COCD_3) δ: 5.17 (d, *J*=10.8Hz, H-2), 4.67 (d, *J*=10.8Hz, H-3), 11.92 (s, 5-OH), 5.96 (dd, *J*=1.8, 13.5Hz, H-6, H-8), 9.98 (brs, 7-OH), 7.07 (d, *J*=1.8Hz, H-2'), 6.86 (d, *J*=8.4Hz, H-5'), 6.89 (dd, *J*=1.8, 7.8Hz, H-6'), 4.08 (s, H-1"), 3.55 (s, H-2"), 3.79 (d, *J*=3.0Hz, 2"-OH), 3.64-3.65 (m, H-3"), 3.97 (d, *J*=3.6Hz, 3"-OH), 3.32 (ddd, *J*=3.0, 9.3Hz, H-4"), 3.82 (brs, 4"-OH), 4.20 (dd, *J*=6.0, 9.6Hz, H-5") and 1.13 (d, *J*=6.6Hz, H-6"). ^{13}C NMR (125 MHz, CD_3COCD_3) δ: 195.2 (C-4, C=O), 166.9 (C-5), 164.4 (C-7), 162.8 (C-9), 146.0 (C-3'), 145.2 (C-4'), 128.1 (C-1'), 119.7 (C-6'), 115.2 (C-5'), 114.5 (C-2'), 101.5 (C-10), 100.5 (C-1"), 96.2 (C-6), 95.1 (C-8), 82.5 (C-2), 76.4 (C-3), 72.6 (C-4"), 71.3 (C-3"), 70.6 (C-2"), 69.0 (C-5") and 17.1 (CH_3, C-6").

Results and Discussion

^{1}H NMR showed 5 types of coupling patterns i.e. singlet (s), doublet (d), doublet of doublets (dd), doublet of doublet of doublet (ddd) and multiplet (m). Singlet was shows for H-1" (δ 4.06), two hydroxyl group that attached to C-5 (δ 11.92) and C-7 (δ 9.98) in aglycone structure and one hydroxyl group at C-4" (δ 3.82) in rhamnose structure. There are eight of doublet patterns at chemical shift 1.13 (d, *J*=6.6Hz, 6"-CH_3, neighbour with H-5"), 3.79 (d, *J*=3.0Hz, 2"-OH, neighbour with H-2"), 3.97 (d, *J*=3.6Hz, 3"-OH, neighbour with H-3"), 4.67 (d, *J*=10.8Hz, H-3, neighbour with H-2), 5.17 (d, *J*=10.8Hz, H-2, neighbour with H-3), 6.86 (d, *J*=8.4Hz, H-5', neighbour with H-6'), 7.07 (d, *J*=1.8Hz, H-2', neighbour with H-6') and 8.25 (d, *J*=15.6Hz, 3'-OH, 4'-OH, between them). There are three doublet of doublet patterns. *J* coupling value revealed the proton neighbour is ortho, meta or para coupling. The pattern at δ 5.96 (dd, H-6, H-8) with *J*=13.5Hz revealed ortho coupling with H-7, J_{meta}=1.8Hz between H-6 with H-8. While the dd pattern at δ 6.90 (dd, H-6') revealed J_{ortho}= 7.8Hz with H-5' and J_{meta}=1.8Hz with H-2'. Chemical shift 4.20 (dd, H-5") revealed J_{ortho}=9.6Hz between H-5' with H-6' and para coupling with H-2'. The only one ddd pattern was observed at δ 3.32 (H-4") revealed J_{ortho}=9.3Hz (coupling with H-5" and H-3") and J_{meta}=3.0 Hz (coupling with H-2"). One multiplet pattern is corresponding to H-3" at chemical shift 3.64-3.65. This proton has ortho coupling with H-2" and H-4" and meta coupling with H-1" and H-5".

The spectral data of ^{1}H NMR was supported by a ^{1}H-^{1}H COSY NMR data. COSY is correlated spectroscopy. It indicates which protons are coupling with other proton. The data allowed us to identify a four correlations between six proton. The first correlation could be seen between proton methyl at δ 1.13 (d, *J*=6.6Hz, 6"-CH_3) with proton

attached to C-5" (δ 4.20 (dd, *J*=6.0, 9.6Hz). The other correlation is between δ 3.32 (ddd, *J*=3.0, 9.3Hz, H-4") with δ 3.64-3.65 (m, H-3"), δ 3.32 (ddd, *J*=3.0, 9.3Hz, H-4") with δ 4.20 (dd, *J*=6.0, 9.6Hz, H-5") and δ 4.67 (d, *J*=10.8Hz, H-3) with δ 5.17 (d, *J*=10.8Hz, H-2). There are *J* coupling J_{eq-eq} =1.8Hz between H-4" with H-3" and J_{ax-eq} =3.0Hz between H-3" with H-2". The four correlations were showed in Figure 1.

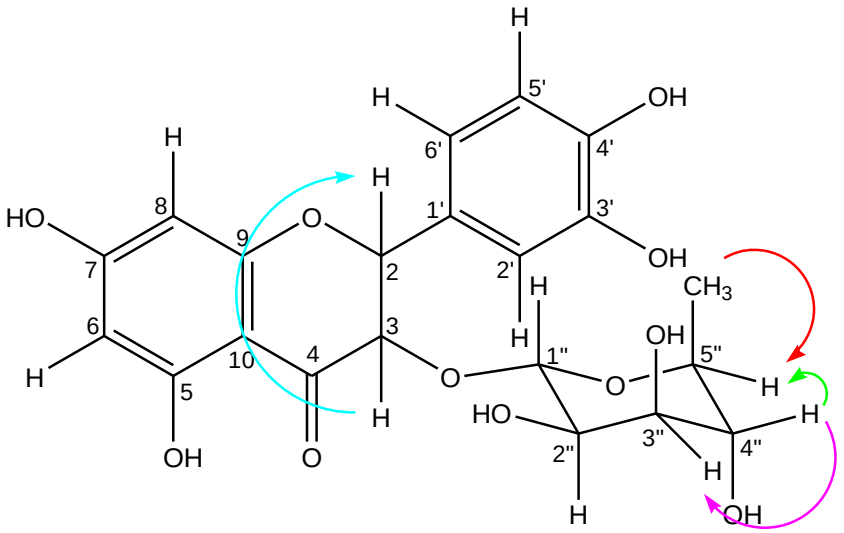

Figure 1. ^{1}H-^{1}H COSY connectivity of GGBC 4

^{13}C-APT is one of the method that showed a carbon attached to the proton, where -CH_3 and CH peaks appear phased in one direction and -CH_2- peaks appear in opposite phase. Quaternary C are seen but may be quite small in size depending on the length of relaxation allowed. There are 21 peaks of carbon for this GGBC 4 compound. From those peaks, 12 peaks of carbon were revealed a correlation with proton in ^{1}H-^{13}C HSQC NMR. The correlation of those peaks are 17.1 (CH_3, C-6") with (1.13 (d, *J*=6.6Hz, 6"-CH_3), 70.6 (C-2") with 3.55 (s, H-2"), 71.3 (C-3") with 3.64-3.65 (m, H-3"), 100.5 (C-1") with 4.08 (s, H-1"), 69.0 (C-5") with 4.20 (dd, *J*=6.0, 9.6Hz, H-5"), 76.4 (C-3) with 4.67 (d, *J*=10.8Hz, H-3), 82.5 (C-2) with 5.17 (d, *J*=10.8Hz, H-2), 96.2 (C-6) and 95.1 (C-8) with 5.96 (dd, *J*=1.8, 13.5Hz, H-6, H-8), 115.2 (C-5') with 6.86 (d, *J*=8.4Hz, H-5'), 119.7 (C-6') with 6.89 (dd, *J*=1.8, 7.8Hz, H-6'), 114.5 (C-2') with 7.07 (d, *J*=1.8Hz, H-2') and 72.6 (C-4") with 3.32 (ddd, *J*=3.0, 9.3Hz, H-4").

Table 1. Correlation of HSQC, HMBC and COSY NMR of GGBC 4

δ_H	HSQC (δ_{H-C})	HMBC (δ_{H-C})	COSY (δ_{H-H})
5.17 (H-2)	82.5 (C-2)	C-2, C-3, C-1', C-2', C-6', C-9, C-4	H-3
4.67 (H-3)	76.4 (C-3)	C-2, C-3, C-4, C-1', C-1"	H-2
5.96 (H-6)	96.2 (C-6)	C-4, C-5, C-6, C-7, C-8, C-9, C-10	
5.96 (H-8)	95.1 (C-8)	C-4, C-5, C-6, C-7, C-8, C-9, C-10	
7.07 (H-2')	114.5 (C-2')	C-2, C-3', C-6'	
6.86 (H-5')	115.2 (C-5')	C-1', C-3'	
6.90 (H-6')	119.7 (C-6')	C-2, C-2', C-3'	
4.08 (H-1")	100.5 (C-1")	C-3, C-1", C-2", C-5"	H-2"
3.55 (H-2")	70.6 (C-2")	C-3", C-4"	H-1"
3.64-3.65 (H-3")	71.3 (C-3")	C-2", C-4"	H-4"
3.32 (H-4")	72.6 (C-4")	C-5", C-3", C-6"	H-3", H-5"
4.20 (H-5")	69.0 (C-5")		H-4", H-6"
1.13 (H-6")	17.1 (C-6")	C-6", C-5", C-4"	H-5"

The spectral data of ^{1}H-^{13}C HMBC NMR was revealed 11 correlations. HMBC is heteronuclear multiple bond correlation. This eleven correlations were showed in Figure 2. The correlations of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC of GGBC 4 were showed in Table 1. These NMR data was compared to the astilbin reported data [5, 6, 7, 8]. Table 2 was showed a synchronize data of GGBC 4 with astilbin.

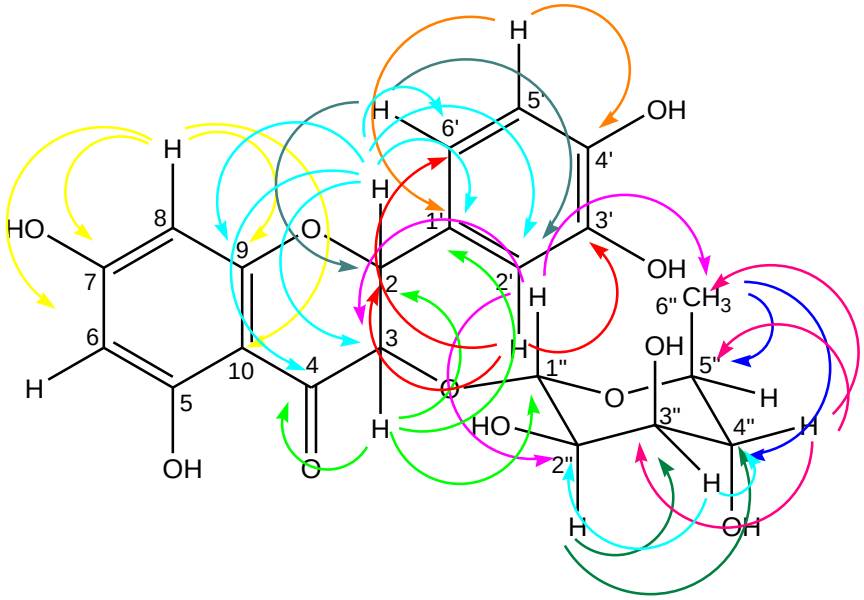

Figure 2. Heteronuclear multiple bond (^{1}H-^{13}C HMBC) connectivity of GGBC 4

Combination of all spectral data of ^{1}H, ^{13}C-APT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC NMR and IR spectrogram could determined the compound GGBC 4 was consistent to astilbin as structured in Figure 3 [5, 6].

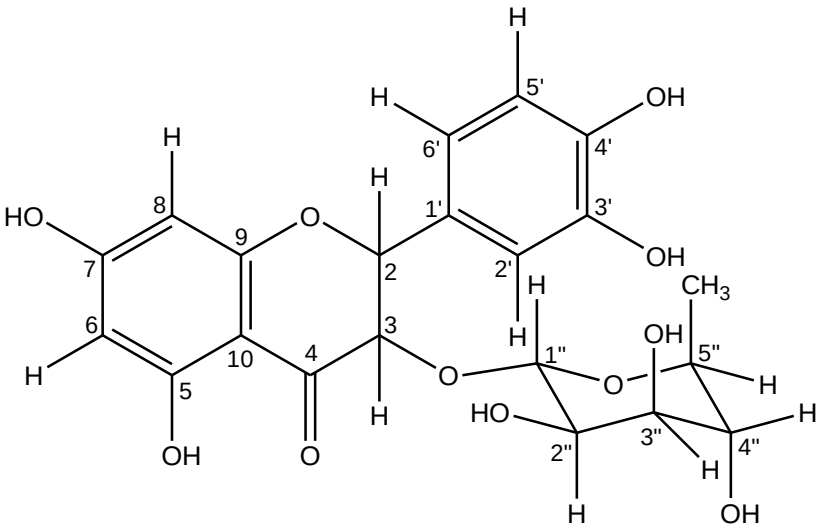

Figure 3. Astilbin

Table 2. ^{1}H and ^{13}C NMR spectral data of GGBC 4 * and astilbin **

Position of carbon and proton	Chemical shift, δ (ppm) carbon GGBC 4 *	Chemical shift, δ (ppm) carbon Astilbin **	Chemical shift, δ (ppm) proton GGBC 4 *	Chemical shift, δ (ppm) proton Astilbin **
Aglycone				
2	82.5	84.8	5.17 (d, *J*=10.8Hz)	5.06 (d, *J*=10.0Hz)
3	76.4	79.4	4.67 (d, *J*=10.8Hz)	4.57 (d, *J*=10.0Hz)
4	195.2	196.7		
5	166.9	166.3	11.92 (s, OH)	
6	96.2	98.2	5.96 (dd, *J*=1.8, 13.5Hz)	5.89 (d, *J*=2.1Hz)
7	164.4	169.7	9.98 (brs, OH)	
8	95.0	97.2	5.96 (dd, *J*=1.8, 13.5Hz)	5.91 (d, *J*=2.1Hz)
9	162.9	164.9		
10	101.5	103.2		
1’	128.1	130.0		
2’	114.5	117.1	7.07 (d, *J*=1.8Hz)	6.95 (d, *J*=1.8Hz)
3’	146.0	147.3	8.25 (d, *J*=15.6Hz, OH)	
4’	145.2	148.1	8.25 (d, *J*=15.6Hz, OH)	
5’	115.2	116.3	6.86 (d, *J*=8.4Hz)	6.80 (d, *J*=8.2Hz)
6’	119.7	121.3	6.89 (dd, *J*=1.8, 7.8Hz)	6.84 (dd, *J*=1.8,8.2Hz)
Rhamnose				
1”	100.5	102.9	4.08 (s)	4.04 (brs)
2”	70.6	72.6	3.79 (d, *J*=3.0Hz, OH), 3.55 (s)	3.53 (brd, *J*=3.3Hz)
3”	71.3	72.9	3.97 (d, *J*=3.6Hz, OH), 3.64-3.65 (m)	3.64 (dd, *J*=3.3,9.6Hz)
4”	72.6	74.6	3.82 (OH), 3.32 (ddd, *J*=3.0, 9.3Hz)	3.32 (t, *J*=9.6Hz)
5”	69.0	71.3	4.20 (dd, *J*=6.0, 9.6Hz)	4.26 (m)
6”	17.1	18.6	1.13 (d, *J*=6.6Hz)	1.18 (d, *J*=6.2Hz)

Reference Astilbin : Guo et al. 2007.

* Recorded in CD_3COCD_3 at 600 MHz, ** Recorded in $CDOD_3$ at 300MHz. Chemical shift, δ values in ppm and coupling constant (*J*) values in Hz. Splitting patterns: s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublet; brd, broad doublet; t, triplet; m, multiplets.

Conclusion

The identification of compound GGBC 4 only using Nuclear Magnetic Resonance (NMR). It is ^{1}H, ^{13}C-APT, ^{1}H-^{13}C HSQC, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC NMR. Even though only one technique, the compound could be defined as known compound astilbin, a flavonoid glycosides. Therefore, NMR is the most essential tools in structural identification beside other chromatography. NMR spectroscopy also a very useful method in various fields of pharmaceutical sciences like pharmaceutical analysis, medicinal chemistry, natural product chemistry and pharmaceutical technology.

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