1.2.2

By JHONS SUWANDI

WORD COUNT	2611	TIME SUBMITTED	27-SEP-2019 03:55PM
		PAPER ID	50709213

Two Flavan Derivatives Isolated from *Artocarpus dadah* Grown in Lampung, Indonesia

INTRODUCTION

We have reported that from the root skin of *A. dadah* has been isolated morusin, a flavonoid compound, which showed high activity against murine leukemia cancer cell, P-388 with IC_{50} value of 3.1 µg/mL[1]. Furthermore from the root wood of *A. dadah*, oxyresveratrol has also succesfully been isolated which showed high activity on cytotoxicity test against murine leukemia cancer cell, P-388 as well as *in vitro* and *in vivo* antimalarial test[2,3].

In this paper we reported the isolation and structural determination of two compounds of flavan derivatives from root bark of *A. dadah*, afzelecin-3-O-α-L-ramnoside (**1**) and chatecin (**2**) based on physical properties and spectroscopies of UV-VIS, IR and 1D and 2D of ^{1}H- and ^{13}C NMR. However, these two compounds did not showed good activity in cytotoxicity test against murine leukemia cancer cell, P-388.

EXPERIMENTAL

Thin layer chromatography analysis was carried out on pre-coated Si-gel plates (Merck Kieselgel 60 F254, 0.25 mm) and the UV lamp of Spectroline, ENF-240 C/F Model was used to see the spot in thin layer chromatography. VLC was carried out using Merck Si-gel 60. Melting points were deter-mined on a Fisher Johns micro-melting point apparatus and are uncorrected. UV-VIS and IR spectra were measured with Beckman DU-7000 and Varian 2000 IR spectrophotometers respectively. ^{1}H NMR spectrum was recorded with a JEOL ECA 500 spectrometer, operating at 500.00 MHz, ^{13}C NMR NMR spectrum was recorded with a JEOL ECA 500 spectrometer operating at 125.00 MHz; DEPT-135, HMQC and HMBC spectra were also recorded with a JEOL ECA 500 spectrometer.

The samples of root bark of *A. dadah* were collected from Purwoasri Village, North Metro, Lampung. The plant was identified by the staff at the Herbarium Bogoriense, Research Centre for Biology, Indonesia Institute of Sciences Bogor, Indonesia and a voucher specimen has been deposited at the herbarium.

Extraction and isolation: 2.4 kg of root bark powdered of *A. dadah* was macerated for 24 h with 18 L methanol, in every maceration 200 g of samples was used and was perfor-med 3 times. The methanol extract was filtered off then evaporated with vacuum rotary evaporator at 45-50 ºC with rotation rate of 120-150 rpm. The concentrated extract of methanol was added with NaCl 1 % by 20 % volume based on the methanol extract and then partitioned with dichloromethane-ethyl acetate 20 %. The partition result was evaporated with vacuum rotary evaporator at 30-40 ºC with rotation rate of 120-150 rpm and the extract obtained was 151.28 g. The extract was partitioned by vacuum liquid chromatography with adsorbent of silica gel using eluent of methanol/dichloromethane by increasing the eluent polarity from 0-100 %. Based on the chromatogram obtained by thin layer chromatography, there were 4 main fractions, A-D fractions. B fraction (2.1675 g) and C fraction (47 g) were further fracinated with VLC using eluent of EtOAc/hexane. C fraction produced 8 fractions (C 1-8).

After few steps of VLC, column chromatography and flash chromatography using eluent of n-hexane, dichloromethane and EtOAc with variety of concentrations, the fractions with the same R_f on thin layer chromatography from main fraction B and result of fractionation C (C1) were combined and further purified using column chromatography and flash chromatography.

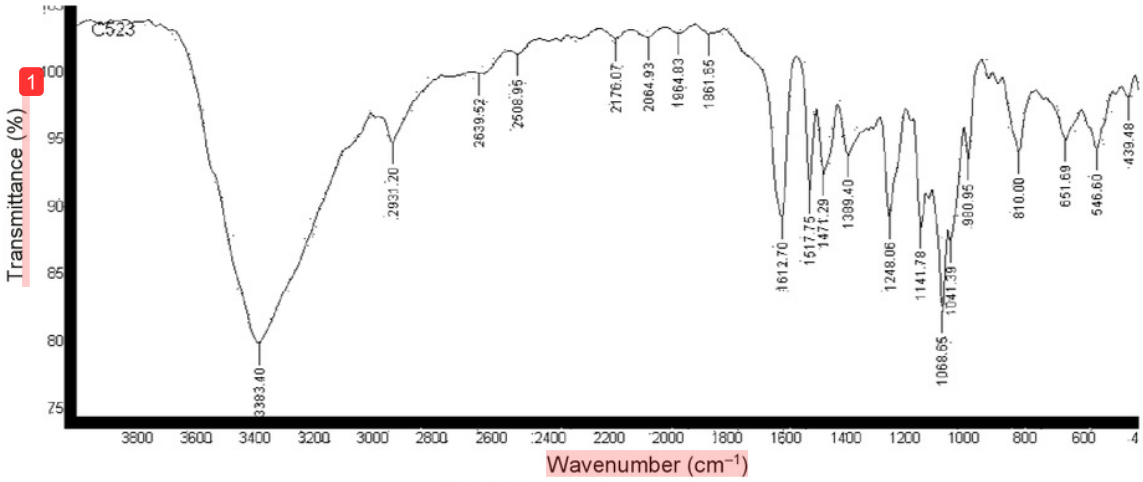

Fig. 1. IR Spectrum of compound **1**

From this combined fractions, yellow brown crystal was obtained and it was known as morusin[2], 25 mg, m.p. 118-123 ºC (crystallized in dichloromethane-*n*-hexane). From C5 fraction, after few steps of chromatography was obtained compound **1**, white amorphous crystal 14.6 g, m.p. 162-167 ºC (crystallized in EtOAc-*n*-hexane). The thin layer chromato-graphy analysis of compound **1** showed one spot with R_f of 0.25 using eluent of acetone/dichloromethane 50 %, R_f 0.48 using eluent of acetone/EtOAc 20 % and R_f 0.58 using eluent acetone/EtOAc 40 %.

By applying a different method, 1.5 kg of root bark sample was macerated with methanol/water 90 % 3 × 24 h and it was repeated 4 times. The filtrate was then evaporated with vacuum rotary evaporator to produce methanol extract. The methanol extract was partitioned using EtOAc and the result of the partitioned was evaporated again with vacuum rotary evaporator to produce EtOAc extract 25 g. The EtOAc extract was partitioned with VLC using eluent of methanol/dichloromethane (0-20 %) and produced 10 main fractions. 2.75 g of fraction 8 was parti-tioned few times by chromatography and produced compound **2** as white amorphous cystral 10 mg, melting point 153-153 ºC. The thin layer chromatography analysis of compound **2** using three eluent systems, one spot was obtained with R_f 0.33 using eluent EtOAc/*n*-hexane 70 %, R_f 0.53 using eluent EtOAc/ dichloromethane 70 % and R_f 0.69 using eluent EtOAc 100 %.

Structure determination: The structure of pure compound was determined based on physical data of melting point and spectroscopy techniques of UV-VIS, IR and NMR as well as test with some specific reagents.

Bioactivity test on the pure compound: The bioactivity test performed was cytotoxicity test of compound **1** and **2** based on the method of Alley *et al.*[4].

RESULTS AND DISCUSSION

Spectrophotometry analysis of compound 1: The infra-red spectrum of **1** (Fig. 1) showed that there is wide band in the region of 3500-3300 cm^{-1}, which indicated the stretching vibration of hydroxyl group that can form hydrogen bond.

The absorption peak at 2931 cm^{-1} indicated the presence of C-H alipihatic. The peaks at 1612, 1517 and 1471 cm^{-1} were indication the presence of C=C aromatic which was supported by the presence of C-H aromatic at 900-600 cm^{-1}. The absorption peak at 1389 and 1248 cm^{-1} indicated the bond stretch of C-O from alcohol. Based on the IR data (Fig. 1) the compound **1** is predicted to have phenolic frame.

The UV-Vis spectrum of compound **1** is shown in Fig. 2. Based on this spectrum, there are maximum wavelength with of 274, 226 (shoulder) and 206 nm in metanol using λ_{max} concentration of 0.0001 g/0.01 L (0.1 mg/10 mL). By the addition of NaOH, the was batochromic shift to longer λ_{max} 285, 244 and 209 nm were observed. This shift strengthen the theory that in compound **1** has unconjugated phenolic group[5].

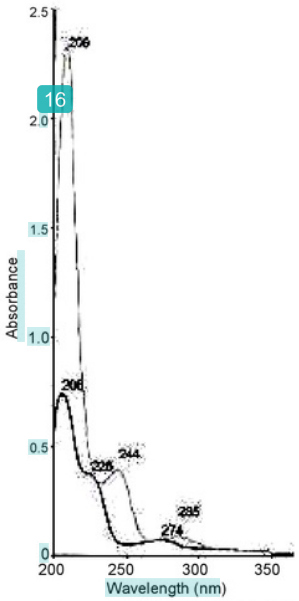

Fig. 2. UV spectrum of compound **1** (a) in MeOH, (b) in MeOH + NaOH

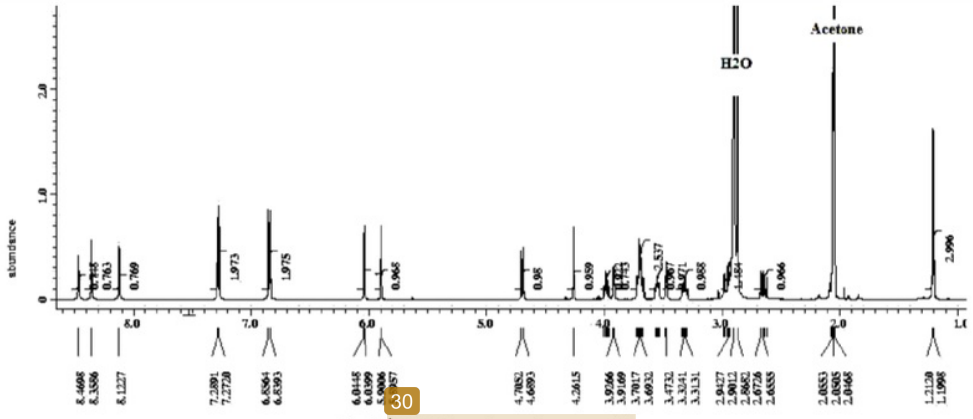

Fig. 3. ^{1}H NMR spectrum of compound **1**

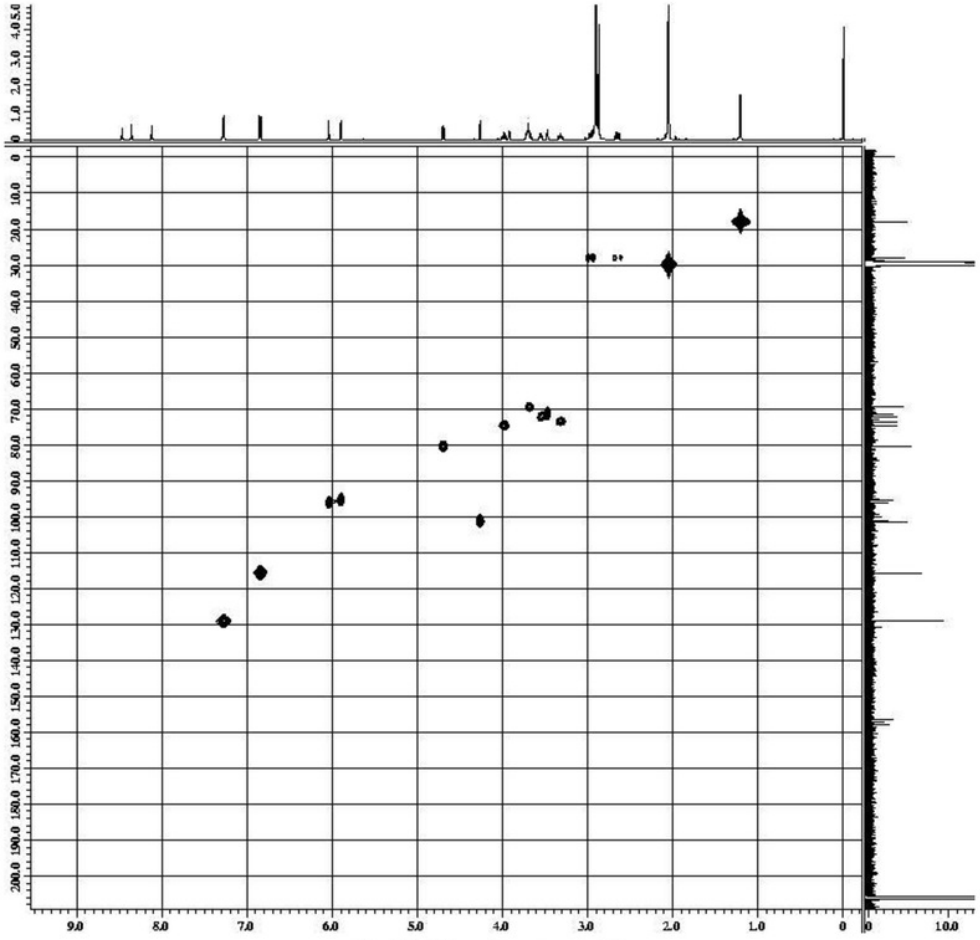

Fig. 4 HMQC spectrum compound 1

The ^{1}H NMR spectrum of **1** (Fig. 3) showed signals at δ (ppm): 5.90 (1H, d, J = 2.5 Hz); 6.04 (1H, d, J = 2.5 Hz); 6.845 (2H, d, J = 8.5 Hz); 7.275 (1H, d, J = 8.5 Hz) due to the aromatic protons. The proton signals of aromatic OH were observed as singlet at 8.12; 8.36; and 8.47 ppm. The aliphatic proton signals were observed at 2.665 (1H, q, J = 8.5 and 18.5 Hz); 2.92 (1H, q, J = 5.5 dan 20 Hz); 3.92 (1H, t, J = 4.85 Hz); and 4.695 (1H, d, J = 8.00 Hz) ppm. Using HMQC (Fig. 4) and *dept with decouple* (Fig. 5) data, the first two protons were bound to one methylene carbon, while two other protons of

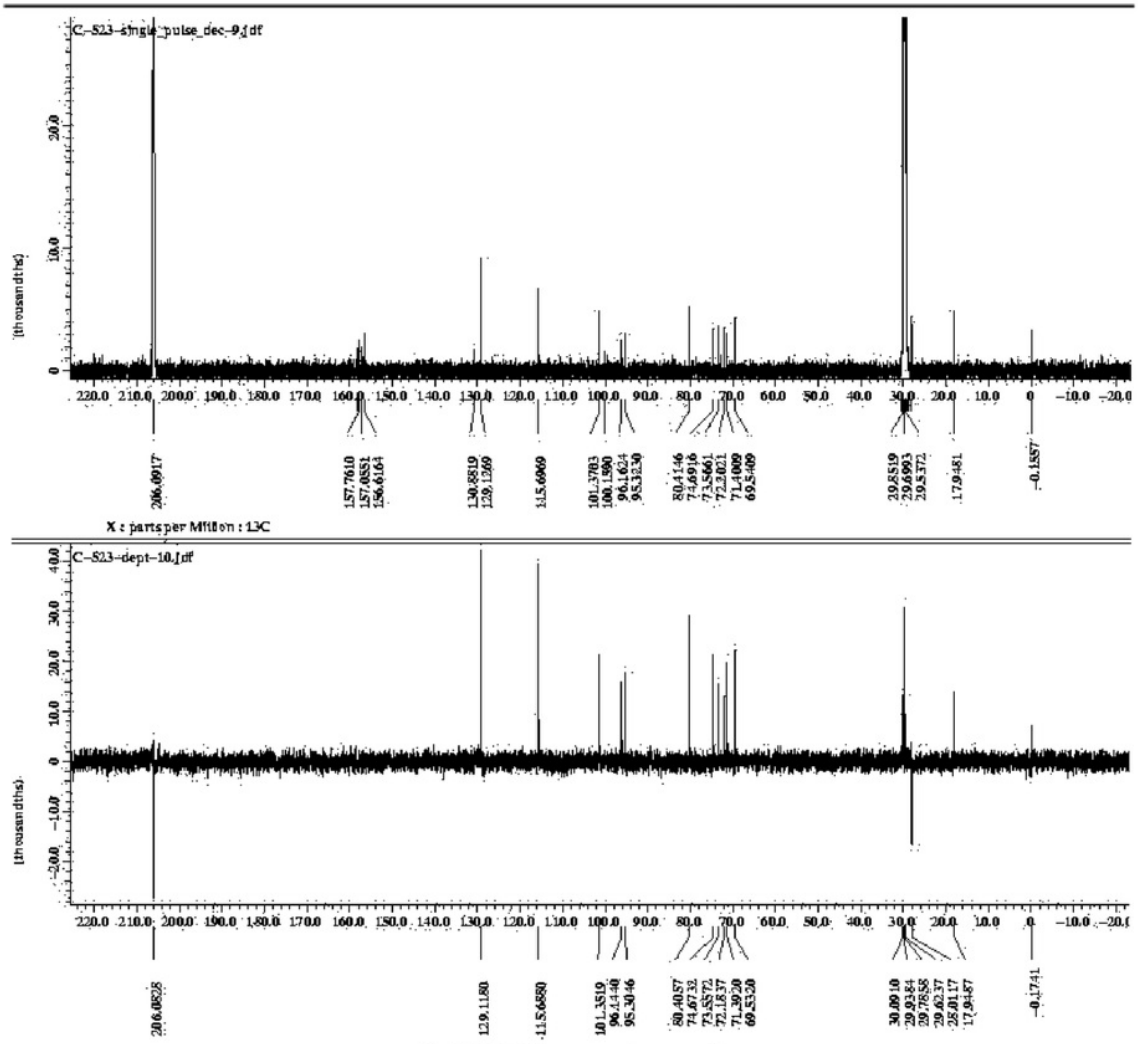

Fig. 5 DEPT-135 spectrum of compound **1**

each methyne where the carbon is bound to oxygen indicated substituted pirane p[illegible]n. The same methyne protons were also observed at 3.315 (1H, m); 3.47 (1H, t, J = 8.5 Hz); 3.54 (1H, m); 3.69 (1H, m); 4.2[illegible]1H, s) ppm, one proton methyl signal was also observed at 1.20 ppm (3H, d, J = 6.0 Hz). This signal is perhaps due to the presence of one sugar group. Based on the DEPT spectrum, there were six carbon signals lost at δ (ppm) 158.02; 157.76; 157.06; 156.61; 130.88; and 100.16 ppm. The first four signals were from aromatic carbon substi-tuted by oxygen, while the other two were from aromatic carbon substituted by carbon.

Based on the HMBC spectrum of **1** (Fig. 6) which showed the correlation of protons with carbon in the molecule resulted the deduction tha[illegible]ompound **1** is afzelecin-3-O-α-L-ramnoside. The data comparison of ^{1}H and ^{13}C NMR of **1** with data in the literature[6] is shown in Table-1. Afzelecin-3-O-α-L-ramnoside has also been found and previously reported by Su *et al.*[7] from the stem and twig bark of *A. dadah* by Achmad *et al.*[6] and from stem and root barks of *A. reticulatus* and (+)-afzelecin 3-ramnoside by Drewes and Taylor[8] from *Cassipourea gerrardii*.

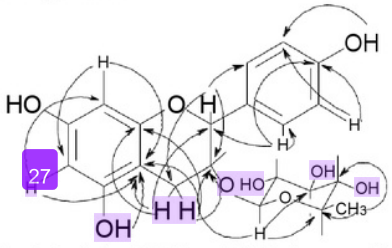

Fig. 6. Corelation of H-C from HMBC of compound **1**

Spectrophotometry analysis of compound 2: The infra-red spectrum of **2** (Fig. 7) showed that there is wide band in the region of 3500-3200 cm^{-1} which indicated the stretching vibration of hydroxyl group that can form hydrogen bond.

TABLE-1
THE COMPARISON OF ^{1}H- AND ^{13}C-NMR DATA OF COMPOUND **1** WITH AFZELECIN-3-O-α-L- RAMNOSIDE[6]

Afzelecin-3-O-α-L-ramnoside δ (ppm) in literature[6]		C	Compound **1** (acetone d_6) δ (ppm)	
^{1}H-NMR	^{13}C-NMR		^{1}H-NMR	^{13}C-NMR
4.65 (1H. d. *J* = 8.1; 16.1 Hz)	81.14	2	4.695 (1H. m)	80.41
3.92 (1H. ddd. *J* = 5.9; 7.4; 8.5 Hz)	76.20	3	3.92 (1H. d. *J* = 4.85 Hz)	74.69
2.63 (1H. dd. *J* = 8.5; 16.1 Hz)	28.26	4	2.665 (1H. dd. *J* = 8.5; 18.5 Hz)	28.03
2.90 (1H. dd. *J* = 5.9; 16.1 Hz)			2.92 (1H. dd. *J* = 5.50; 20.0 Hz)	
	100.68	4a		100.16
	157.57	5		157.06
5.93 (1H. d. *J* = 2.5 Hz)	96.38	6	6.04 (1H. d. *J* = 2.5 Hz)	96.16
	157.97	7		157.76
5.85 (1H. d. *J* = 2.4 Hz)	95.45	8	5.90 (1H. d. *J* = 2.5 Hz)	95.32
	156.92	8a		156.62
	131.22	1′		130.88
7.21 (1H.tt. *J* = 1.7; 2.8; 8.9 Hz)	129.40	2′	7.28 (1H.d. *J* = 8.5 Hz)	129.13
6.78 (1H. tt. *J* = 2.0; 2.8; 8.9 Hz)	116.07	3′	6.845 (1H. d. *J* = 8.5 Hz)	115.70
	158.53	4′		158.02
6.78 (1H. tt. *J* = 2.0; 2.8; 8.9 Hz)	116.07	5′	6.845 (1H. d. *J* = 8.5 Hz)	115.70
7.21 (1H.tt. *J* = 1.7; 2.8; 8.9 Hz)	129.4	6′	7.28 (1H.d. *J* = 8.5 Hz)	129.40
4.24 (1H. d. *J* = 1.8 Hz)	102.26	1″	4.26 (1H. s)	101.37
3.47 (1H. dd. *J* = 1.7; 3.1 Hz)	71.97	2″	3.51 (1H. t. *J* = 8.5 Hz)	71.40
3.56 (1H. dd. *J* = 3.4; 9.5 Hz)	72.24	3″	3.54 (1H. m)	72.2
3.30 (1H. dd. *J* = 3.4; 9.5 Hz)	73.93	4″	3.315 (1H. m)	73.57
3.69 (1H. m)	70.32	5″	3.69 (1H. m)	69.54
1.23 (3H. d. *J* = 6.1 Hz)	17.91	6″	1.20 (3H. d. *J* = 6.0 Hz)	17.95

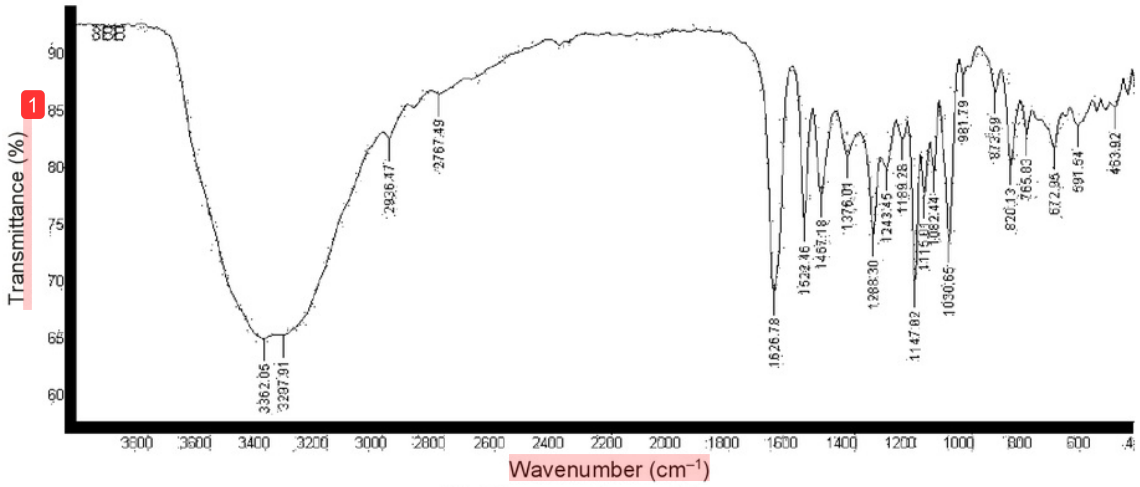

Fig. 7 IR spectrum of compound 2

The absorption peak at 2936 cm^{-1} and 2767 cm^{-1} indicated the presence of C-H alipihatic. The peaks at 1626, 1522 and 1467 cm^{-1} were indication the presence of C=C aromatic which was supported by the presence of C-H aromatic at 900-600 cm^{-1}. The absorption peak at 1376, 1288 and 1243 cm^{-1} indi-cated the bond stretching of C-O from alcohol. Based on the IR data in Fig. 7 the compound **2** is predicted to have phenolic frame.

Based on the UV-VIS spectrum of compound **2** (Fig. 8), there are maximum wavelenght with max at 280, 229 (shoulder) and 205 nm in methanol with concentration of 0.0001 g/0.01 L (0.1 mg/10 mL). In addition with NaOH, there was batochromic shift to longer maks 290, 243 and 208 nm. This shift strengthen the theory that in compound **2** has unconjugated phenolic group[5].

The ^{1}H NMR spectrum of 1 (Fig. 9) shown signals at δ (ppm): 5.96 (1H, d, *J* = 1.85 Hz); 5.815 (1H, d, *J* = 2.45 Hz); 6.83 (1H, d, *J* = 1.85 Hz); 6.73 (1H, d, *J* = 8.6 Hz); 6.70 (1H, d, *J* = 1.8; 7.95 Hz) due to the aromatic protons. The proton signals of aromatic OH were observed as singlet at 8.15; 7.96; and 7.85 p The aliphatic proton signals were observed at 2.465 (1H, dd, *J* = 8.5; 16.12 Hz); 3.92 (2H, m); 4.50 (1H, d,

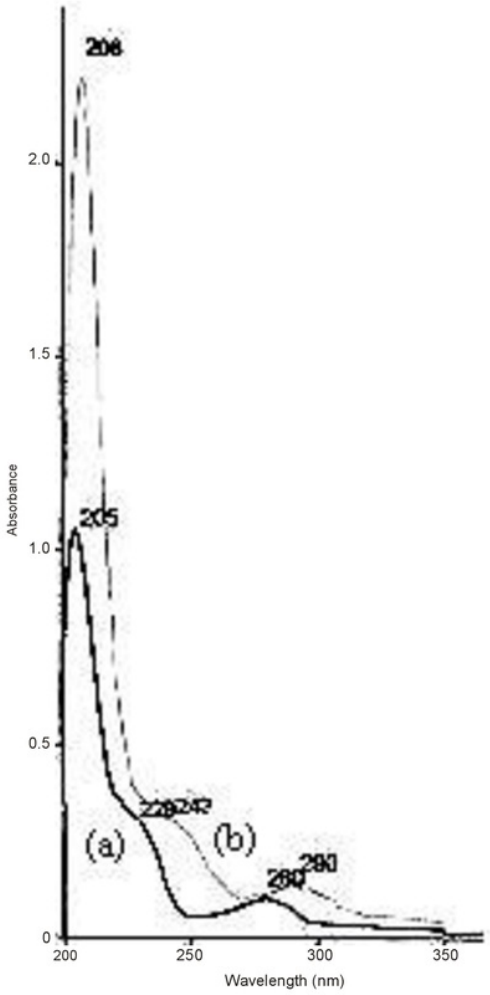

Fig. 8. UV-VIS spectrum of compound **2** (a) in MeOH, (b) in MeOH + NaOH

J = 7,5 Hz) ppm. Using HMQC (Fig. 10) and DEPT with decouple, the first two protons were bound to one methylene carbon, while two other protons of each methyne where the carbon is bound to oxygen indicated substituted pirane proton. Based on the DEPT spectrum, there were six carbon signals lost at δ (ppm) 157.58; 157.06; 156.78; 145.50; 132.04; dan 100.48 ppm. The first four signals were from aromatic carbon substituted by oxygen, while the other two were from aromatic carbon substituted by carbon.

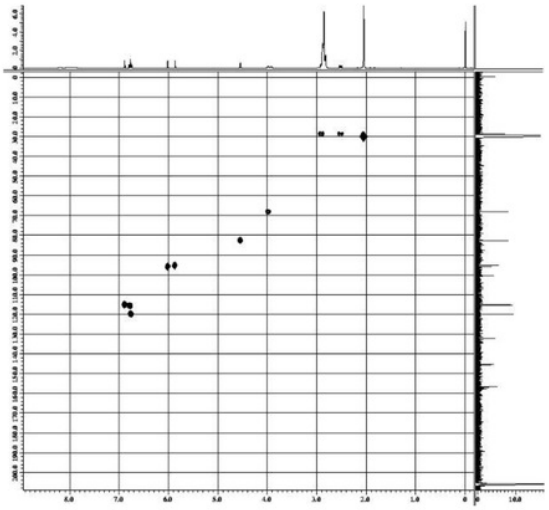

Fig. 10. HMQC spectrum of compound **2**

Based on the HMBC spectrum of **2** (Fig. 11), which showed the correlation of protons with carbon in the molecule resulted the deduction that compound **2** is chatecin. This deduction is strengthen with COSY data (Fig. 12), the correlation of H-H in **2**, from δ (ppm): 2.465 to 2,865 and 3.92; 3.92 to 2.465 and 4.50; as well as 6.70 to 6.73 and 6.83. The data comparison of ^{1}H and ^{13}C NMR of **2** with data in the literature[6] is shown in Table-2. Besides isolated from Artocarpus

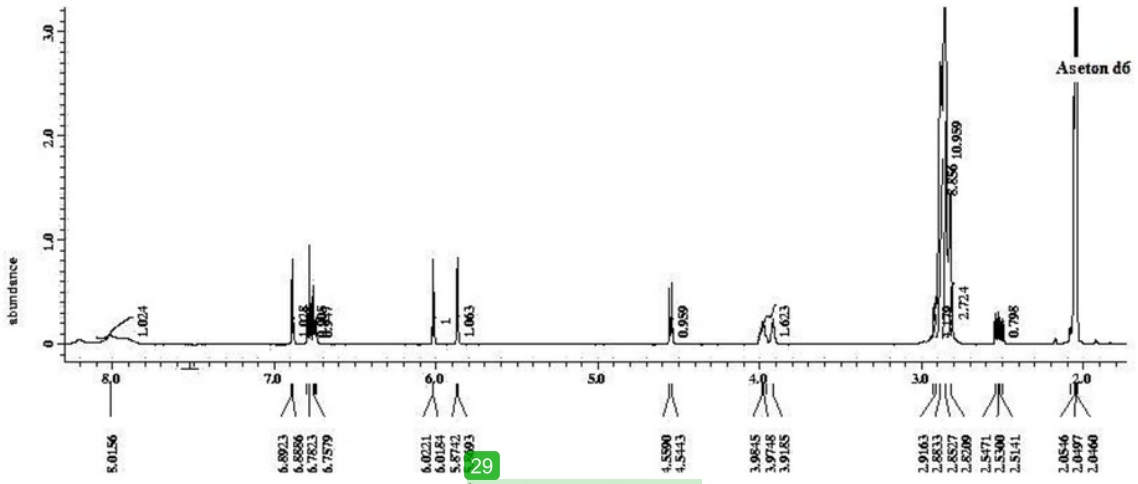

Fig. 9. ^{1}H NMR spectrum of compound **2**

TABLE-2
COMPARISON OF ^{1}H- AND ^{13}C-NMR DATA OF COMPOUND **2** AND CHATECIN[6]

Chatecin[6] (CD_3OD), δ (ppm)		C	Compound **2**, (acetone d_6), δ (ppm)	
^{1}H-NMR	^{13}C-NMR		^{1}H-NMR	^{13}C-NMR
4.56 (1H. d. *J* = 7.5 Hz)	82.88	2	4.50 (1H. d. *J* = 7.5 Hz)	82.59
3.97 (m)	68.83		3.92 (1H. m)	68.17
(1H. dd. *J* = 8.0; 16.1 Hz. 4β)	28.53	4	2.465 (dd. *J* = 8.5; 16.12 Hz)	28.71
2.84 (1H. dd. *J* = 5.4; 16.1 Hz. 4β)			2.865 (1H. d. j = 5.5 Hz)	
	100.84	4a		100.48
	157.59	5		157.06
5.91 (1H. d. *J* = 2.4 Hz)	96.31	6	5.96 (1H. d. *J* = 1.85 Hz)	95.90
	157.86	7		157.658
5.84 (1H. d. *J* = 2.4 Hz)	95.52	8	5.815 (1H. d. *J* = 2.45 Hz)	95.25
	156.93	8a		156.78
	132.25	1'		132.04
6.83 (1H. d. *J* = 2.0 Hz)	115.28	2'	6.83 (1H. d. *J* = 1.85 Hz)	115.09
	146.24	3'		145.53
	146.17	4'		145.46
5 (1H. d. *J* = 8.1 Hz)	116.10		6.73 (1H. d. *J* = 8.6 Hz)	115.54
6.71 (1H. dd. *J* = 2.2; 8.2 Hz)	120.04	6'	6.70 (1H. dd. *J* = 1.8; 7.95 Hz)	119.92

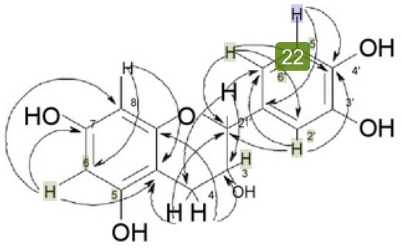

Fig. 11. Correlation of H-C from HMBC data of compound **2**

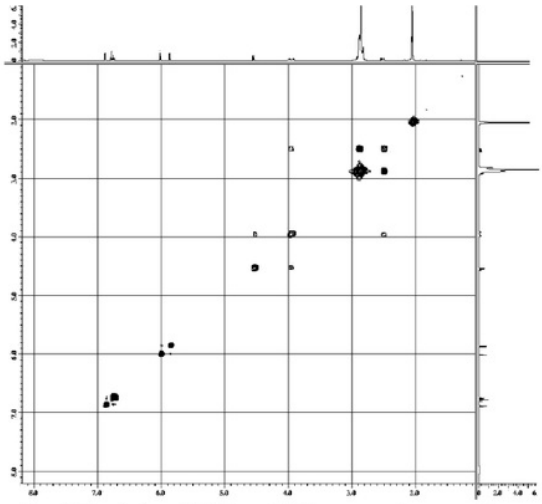

Fig. 12. Correlation of H-H from COSY spectrum of compound **2**

plants, chatecin has also been isolated from other plants, such as from *Acacia catechu*[9], *Centaurea maculosa*, Lam.[10,11] and from *Paullinia cupana* var. sorbilis)[12].

Bioactivity assay: The cytotoxicity test using murine leukemia cancer cell P-388 showed that compounds **1** and **2** were not active with IC_{50} > 100 μg/mL.

1.2.2

ORIGINALITY REPORT

22%

SIMILARITY INDEX

PRIMARY SOURCES

1	www.orientjchem.org Internet	73 words — 2%
2	mitizane.ll.chiba-u.jp Internet	59 words — 2%
3	www.aensiweb.com Internet	41 words — 1%
4	ccsenet.org Internet	39 words — 1%
5	Philip Magnus, Lewis Gazzard, Lindsay Hobson, Andrew H. Payne, Trevor J. Rainey, Neil Westlund, Vince Lynch. "Synthesis of the Kopsia alkaloids (±)-pauciflorine B, (±)-lahadinine B, (±)-kopsidasine, (±)-kopsidasine-N-oxide, (±)-kopsijasminilam and (±)-11-methoxykopsilongine", Tetrahedron, 2002 Crossref	35 words — 1%
6	www.faqs.org Internet	33 words — 1%
7	archive.org Internet	32 words — 1%
8	scholar.lib.vt.edu Internet	31 words — 1%
9	www.google.it Internet	30 words — 1%

10	ijcc.iregway.com Internet	29 words — 1%
11	www.ijppsjournal.com Internet	28 words — 1%
12	Wimal Herath, Daneel Ferreira, Ikhlas A. Khan. "Microbial metabolism. Part 7: Curcumin", Natural Product Research, 2007 Crossref	25 words — 1%
13	www.thieme-connect.com Internet	24 words — 1%
14	B. Wang, W. Wang, H. Zhang, D. Shan, K. Nimkar, O. Gudmundsson, S. Gangwar, T. Siahaan, R.T. Borchardt. "Synthesis and evaluation of the physicochemical properties of esterase-sensitive cyclic prodrugs of opioid peptides using coumarinic acid and phenylpropionic acid linkers", The Journal of Peptide Research, 2008 Crossref	18 words — 1%
15	www.lp.itb.ac.id Internet	15 words — 1%
16	mro.massey.ac.nz Internet	13 words — < 1%
17	Ojika, M.. "Aplyronine A, a potent antitumor macrolide of marine origin, and the congeners aplyronines B and C: isolation, structures, and bioactivities", Tetrahedron, 20070409 Crossref	12 words — < 1%
18	www.google.de Internet	12 words — < 1%
19	thieme-connect.de Internet	11 words — < 1%
20	www.mdpi.com Internet	

		10 words — < 1%
21	www.patentgenius.com Internet	10 words — < 1%
22	road.unimol.it Internet	9 words — < 1%
23	www.mdpi.org Internet	9 words — < 1%
24	library.mifaff.go.kr Internet	9 words — < 1%
25	jocpr.com Internet	8 words — < 1%
26	uweb.cas.usf.edu Internet	8 words — < 1%
27	www.coursehero.com Internet	8 words — < 1%
28	J Vasse. "Chiral biomimetic NADH models in the benzo[b]-1,6-naphthyridine series. A novel class of stable, reactive and highly enantioselective NADH mimics", Tetrahedron, 2003 Crossref	6 words — < 1%
29	Noviany Noviany, Arif Nurhidayat, Sutopo Hadi, Tati Suhartati, Muhammad Aziz, Neny Purwitasari, Iman Subasman. " Sesbagrandiflorain A and B: isolation of two new 2-arylbenzofurans from the stem bark of ", Natural Product Research, 2018 Crossref	6 words — < 1%
30	JI Zhiqing, Wu Wenjun, Yang Hua, Shi Baojun, Wang Mingan. " Four novel insecticidal sesquiterpene esters from ", Natural Product Research, 2007 Crossref	6 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	OFF		