

The antibacterial and anticancer test of cyclomulberochromen compounds from *Artocarpus altilis*

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Abstract

One flavonoid compound, cyclomulberochromen (1) has been isolated from the wood branch of *Artocarpus altilis*. This compound has been physically and spectroscopically determined and tested for bioactivity against *Bacillus subtilis* and *Escherichia coli*, it showed strong activity and in cytotoxic test using murine leukemia P-388 cells, was very active.

Keywords: *A. altilis*, bioactivity, cytotoxic, cyclomulberochromen.

Introduction

Artocarpus is a plant that is a source of various chemical compounds with diverse activities for the benefit of health. Previously some compounds have successfully been isolated from *Artocarpus*. Artonin E has been isolated from *A. rotunda* and *A. rigida*^{1,2}. Oxyresveratrol has been isolated from *A. dadah*³ and it has shown antiplasmodial activity and also cytotoxicity towards P-388 leukemia cells. Artocarpin and cudraflavone were isolated from *A. heterophyllus* and they were also active against P-388 leukemia cells⁴, Artonin O has also been isolated from *A. rigida*⁵ and it has very good antibacterial activity, in addition, flavonoid compounds from *Artocarpus* plants have also been tested for anti-inflammatory activity^{6,7}, antioxidative⁸ and antiplatelet⁹.

A. altilis is one of the species of *Artocarpus*, native to Indonesia, which includes fruit-producing plants that are edible and have therapeutic properties such as toothache, melting blood, heart, kidney and inflammatory drugs¹⁰. From this plant various prenylated flavonoid compounds have been isolated which have also been tested for some biological activities. For example, artocarpin and cycloartocarpin were tested for antitubercular and antimalarial activity¹¹, isocycloartobioxanthone was active *in vitro* against *S. aureus* and antioxidative activities¹², chalcone has moderate cytotoxicity against SPC-A-1, SW-480 and SMMC-7721 human cancer cells¹³.

In this work, we report the isolation and structure determination of cyclomulberochromen compounds from branch wood of *A. altilis* which was obtained from Banjar Negara Village, Wonosobo District, Tanggamus Regency, Lampung Province, Indonesia and its structure was completely determined by UV-Vis, IR and NMR spectroscopy; antibacterial test is against *E. coli* and *B. subtilis* and cytotoxic test using P-388 murine leukemia cells.

Material and Methods

Plant Material: The branch woods of *A. altilis* were collected from Banjar Negara village, Tanggamus, Lampung and were identified in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia and a voucher specimen has been deposited at the herbarium.

Bioactivity test: The bioactivity test performed was cytotoxicity test of compound (1) based on the method of Alley et al¹⁴ and was conducted at Laboratory of Natural Product Chemistry, Department of Chemistry, Bandung Institute of Technology, Bandung, Indonesia and antibacterial activity test has been performed using agar diffusion method¹⁵⁻¹⁷.

General Experimental Procedures: Thin layer chromatography (TLC) analysis was carried out on pre-coated Si-gel plates (Merck Kieselgel 60 F254) and the UV lamp of Spectraline, ENF-240 C/F model was used to see spot in TLC. Vacuum Liquid Chromatography (VLC) and chromatography column (CC) were carried out using Merck Si-gel 60. Melting point was determined on a Fisher Johns micro-melting point apparatus and was uncorrected. UV-Vis and IR spectra were measured with Beckman DU-7000 and Varian 2000 FTIR spectrophotometer respectively. ¹H-NMR spectrum was recorded on JEOL ECA 500 spectrometer, operating at 500.00 MHz and ¹³C-NMR operating at 125 MHz.

Isolation and Purification of the Compounds: 1.5 kg of branch wood *A. altilis* which was mashed, maceration was carried out using 9.2 L of methanol solvent for 3x24 hours. The maceration results were then filtered and concentrated obtained 36 grams of extract. The maceration extract was fractionated using KCV, using Merck 60 Silica Gel adsorbent and eluted with a mixture of ethyl acetate / *n*-hexane which gradually increased their polarity. The results of KCV produced four main fractions (A-D), fraction A 2.7gram, B 11gram, C 4.1 gram and D 6.95 gram. The fraction B was further VLC and subsequently in CC repeatedly using the same adsorbent and eluent to give compound (1), in the form of yellow needle crystal (30 mg), melting point 289-291°C.

Results and Discussion

Spectrometry Analysis: The UV-Vis spectrum of compound (1) is shown in figure 1, it can be seen that the compound gives maximum absorption at λ_{max} . 207nm, 231 nm, 294 nm and 368 nm in methanol solvents.

In addition of NaOH as a shift reagent, there was a shift in band I from λ_{max} 368 nm to 412 nm, an addition of 44 nm indicating that there was a free hydroxyl group at position C4' ring B (Figure 2).

Data from the infrared spectrum of compound (1) (Figure 3) at wave number 3412 cm^{-1} has a broad peak which is a stretching vibration of a hydroxyl group to another atom that can form hydrogen bonds. At wave number 2974 cm^{-1} and

2926 cm^{-1} , it is an indication of the presence of aliphatic H groups. The absorption peak at wave number 1654 cm^{-1} indicates the presence of a carbonyl group (C = O) which conjugates with C = C. The absorption peak at the area of 1550-1469 cm^{-1} provides an indication of the presence of an aromatic ring. This is reinforced by the presence of aromatic C-H absorption at 862-588 cm^{-1} wave number. UV and IR spectra of compound (1) are similar to those reported for cycloartocarpine compounds.⁴

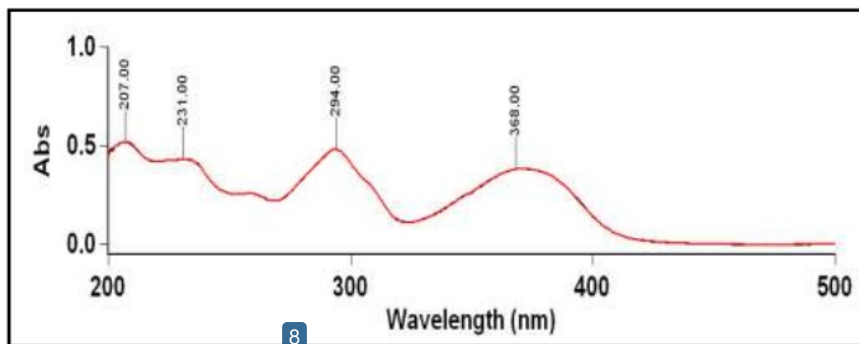


Figure 1: UV spectrum of compound (1) in MeOH

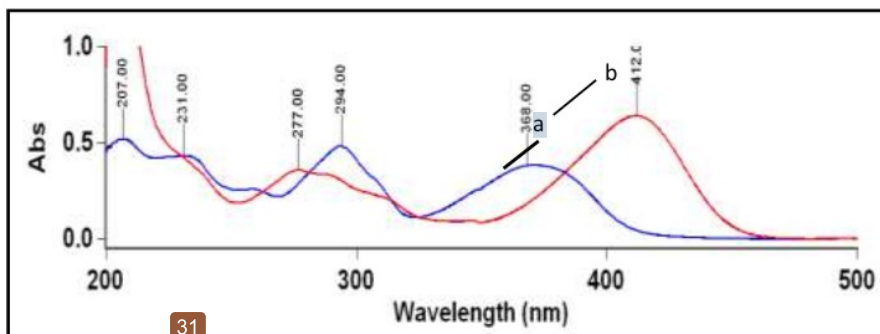


Figure 2: UV spectrum of compound (1) in (a) MeOH, (b) MeOH + NaOH.

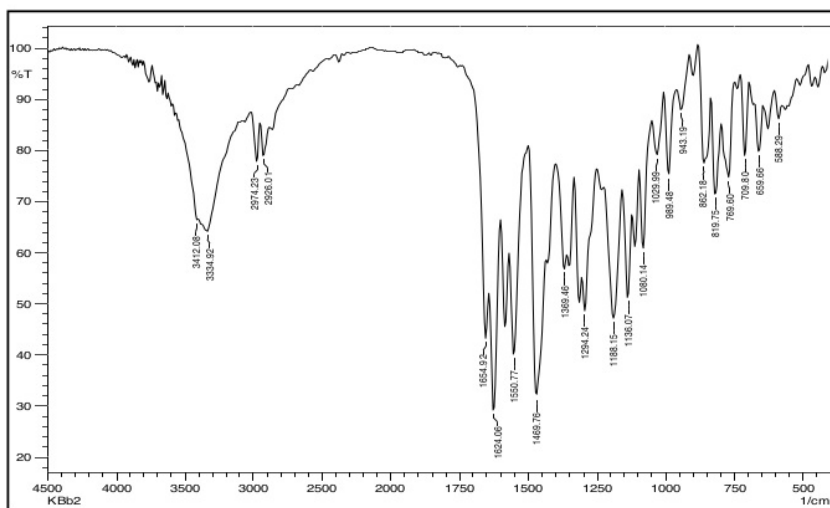


Figure 3: IR spectrum of compounds (1)

18

The $^1\text{H-NMR}$ spectrum of compound (1) (Figure 4) showed the presence of 2,2-dimethylch¹⁷en groups which are characteristic indicated by δ 6.63 (d, $J = 10$ Hz) and 5.7 ppm (d, $J = 10$ Hz) of vinyl protons and the presence of six singlet protons at 28.5 ppm. In addition there was a cyclization of the hydroxy group at C2' with methylene gro⁵ C9, to produce ring D which was shown with δ 6.14 (d, $J = 9.4$ Hz), 5.4 ppm (d, $J = 9.4$ Hz) and two methyl groups on vin¹ carbon at δ 1.9 and 1.7 Hz. In addition, there were δ 7.6 (d, $J = 8.6$ Hz), 6.5 (d, $J = 8.6$ and 2.06 Hz) and 6.3 ppm (d, $J = 1.6$ Hz) derived from ABX type proton aromatic benzene trisubstitution on the ring B. At δ 6.4 ppm singlet⁴ me from proton C8 at ring A from the carbon frame of the compound (1).

The $^{13}\text{C-NMR}$ spectrum of compound (1) showed there were 25 C atoms (Figure 5 and Table 1). Five of them were derived from prenyl carbon that forms pyranocyclic, between OH at C2' with C9, at δ 70.6, 122.4, 139.9, 18.7 and

25.9 ppm; also there were five carbons from prenyl which were cyclized to form γ,γ -dimethyl piran at δ 116, 129.5, 79.1 and two carbons at δ 28.5 ppm. In addition there were aromatic carbon atoms, two of which bind to OH groups at δ 157.7 and 159.6 ppm, four carbons bind to oxygen at δ 160.4, 157.5, 165.8 and 157.4; four carbon atoms bind to hydrogen at δ 96, 105.02, 111.15 and 126.4 ppm; other aromatic carbons were tertiary carbon at δ 106.5, 106.3, 110.4 and 108.4 ppm and one carbonyl carbon at δ 179.6 ppm.

All protons were correlated with carbon based on the HMQC spectrum of compounds (1) whose position were strengthened³² the HMBC spectrum. An important correlation of the HMBC spectrum of com³⁰nd (1) can be seen in figure 6. The NMR spectrum data of compound (1) are compared with $^1\text{H-}$ and $^{13}\text{C-NMR}$ data from cudraflavon A (cyclomulberochromen, isocyclomorusin) (Table 1).

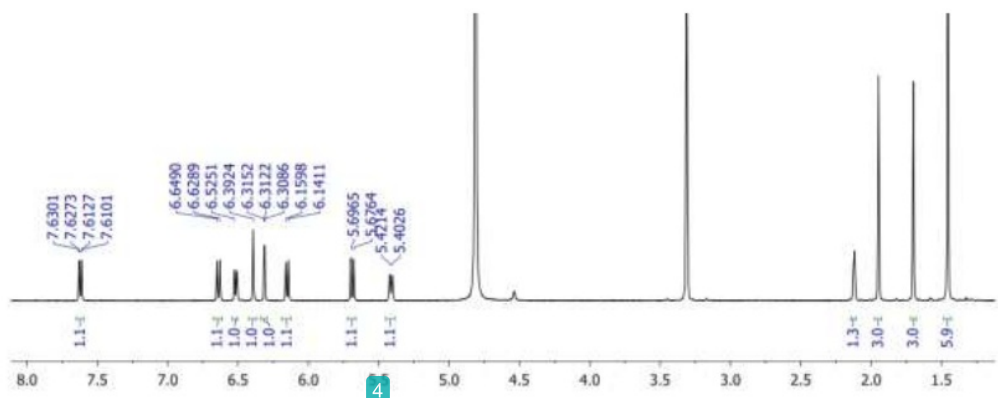


Figure 4: $^1\text{H-NMR}$ spectrum of compounds (1)

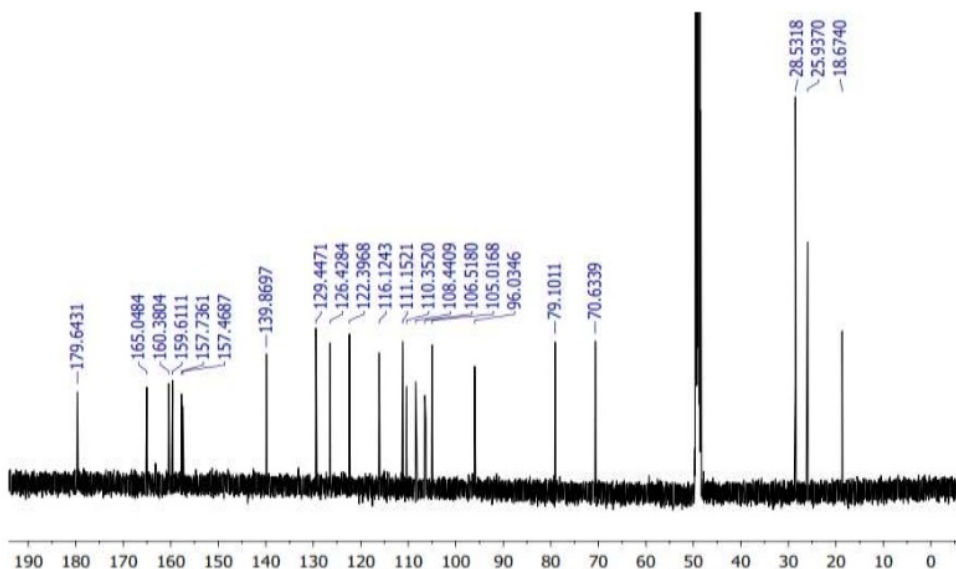


Figure 5: $^{13}\text{C-NMR}$ spectrum of compounds (1)

Table 1
Comparison of data spectrum ^{13}C -, ^1H -NMR from compound (1) in CD_3OD solvent with cudraflavon A compound in $\text{Me}_2\text{CO D}_6$ solvent¹⁸

Nu C	^{13}C -NMR, δ (ppm)		^1H -NMR, δ (ppm)	
	Compound (1)	Cudraflavon A	Compound (1)	Cudraflavon A
2	165	157.9		
3	110.4	106.2		
4	179.6	178.0		
4a	106.3	104.8		
5	157.7	155.8		
6	106.5	108.3		
7	160.4	163.9		
8	96	95.4	6.4 (s)	6.28 (s)
8a	157.5	163.9	5	1
9	70.6	69.2	6.14 (d, $J = 9.4$ Hz)	6.13 (d, $J = 9.4$ Hz)
10	122.4	121.0	5.4 (d, $J = 9.4$ Hz)	5.36 (d, $J = 9.4$ Hz)
11	139.9	138.7		
12	18.7	18.7	1.9 (s)	1.88 (s)
13	25.9	17.9	1.7 (s) 20	1.61 (1)
14	116	114.5	6.63 (d, $J = 10$ Hz)	6.60 (d, $J = 10$ Hz)
15	129.5	128.8	5.7 (d, $J = 10$ Hz)	5.53 (d, $J = 10.1$ Hz)
16	79.1	78.3		
17	28.5	27.7	1.45 (s)	1.37 (s)
18	28.5	27.6	1.45 (s)	1.37 (s)
1'	108.4	110.2		
2'	157.4	156.1	1	12
3'	105	103.7	6.3 (d, $J = 1.6$ Hz)	6.37 (d, $J = 2.3$ Hz)
4'	159.6	158.8		
5'	111.15	110.2	6.5 (dd, $J = 8.6; 2.06$ Hz)	6.51 (dd, $J = 8.6; 2.3$ Hz)
6'	126.4	125.4	7.62 (d, $J = 8.6$ Hz)	7.55 (d, $J = 8.4$ Hz) 13.03 (5' -OH)

9 Based on the spectroscopic data of compound (1) and compared to the literature, the structure of the compound (1) is found to have same structure as cyclomulberochromen (synonym: cudraflavon A, isocyclomorucin).

14 **Antibacterial Activity Test:** The compound (1) tested antibacterial activity using gram positive bacteria *B. subtilis* and gram-negative bacteria *E. coli* (Figures 7 and 8). The method used in this antibacterial test is the agar diffusion method by observing antibacterial activity based on inhibition zone diameter / inhibition.

The bacterial inhibition distribution is based on the inhibitory zone diameter for ampicillin from *S. aureus*: sensitive / strong (inhibition zone more than 29 mm), intermediate (21-28 mm inhibition zone) and resistant (inhibition zone less than 20 mm), while from other organisms are sensitive / strong (inhibition zone more than 14 mm), intermediates (12-13 mm inhibition zone) and resistant (inhibition zone less than 11 mm)¹⁵, taken on average, strong category: greater than 21 mm, intermediates 16.5-20 mm and resistance smaller than 15 mm.

The sample concentration in this study was made in three concentration variations, namely 0.5; 0.4; 0.3 mg / disk.

Likewise, positive controls and negative controls are made in the same three variations as the sample. The positive control used for *E. coli* bacteria is chloramphenicol and *B. subtilis* used amoxicillin. While negative controls were solvents used, namely acetone p.a. The results of the antibacterial bioactivity test against *B. subtilis* and *E. coli* of compound (1) can be seen in table 2.

11 Antibacterial bioactivity test showed that compound (1) had antibacterial activity against *B. subtilis* and *E. coli* bacteria in the strong category at a concentration of 0.5 mg/disk.

Anticancer Activity Test: Cytotoxicity test using murine leukemia P-388 cells, has IC_{50} : 1.87 μg / mL (Table 3 and Figure 9). The determination of IC_{50} is processed from the data of table 3 using origin 8.5 program as shown in figure 9. This data shows that compound (1) has high cytotoxicity activity.

Compound (1) shows strong antibacterial activity against *B. subtilis*, *E. coli* and high cytotoxicity in P-388 murine leukemia cells, possibly because there is a hydroxy group at C4 and a piranoflavone ring next to A ring which is linear with carbon xanthenes framework.

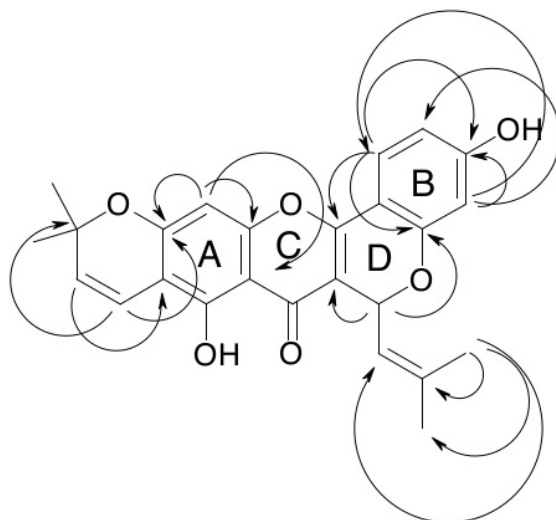


Figure 6: Some important HMBC correlations of compounds (1)

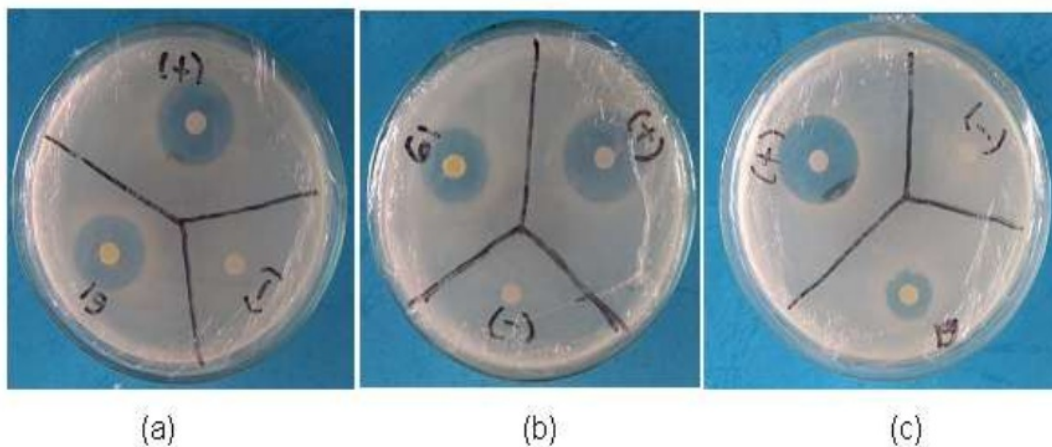


Figure 7: Test results of antibacterial activity of compound (1) against *B. subtilis*, positive control of Amoxycillin, G1 = KBB2 = compound (1), concentration (a) 0.5; (b) 0.4, (c) 0.3 mg/disk

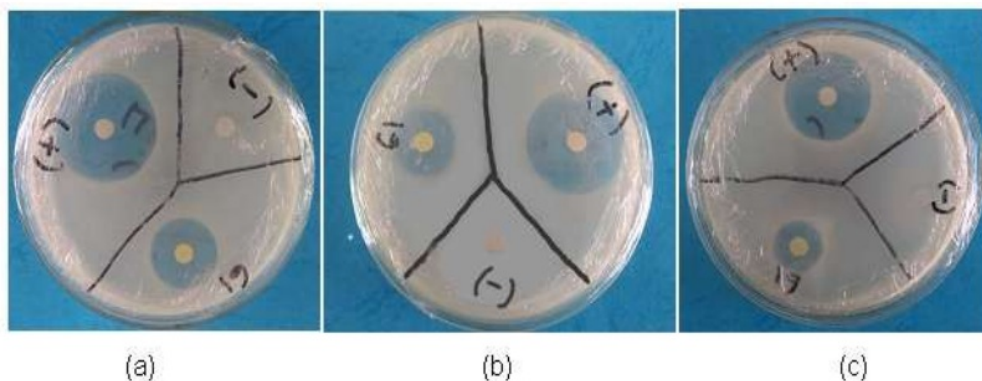


Figure 8: Test results for antibacterial activity of compounds (1) against *E. coli*, positive control of Chloramphenicol, G1 = KBB2 = compound (1), concentration (a) 0.5; (b) 0.4, (c) 0.3 mg/disk

Table 2
Diameter zone of inhibition of compound (1) against bacteria *B. subtilis* and *E. coli*

Concentration $\mu\text{g}/\text{disk}$	Diameter zone of inhibition, mm			
	<i>B. subtilis</i>		<i>E. coli</i>	
	Control + Amoxicillin	Compound (1)	Control + Chloramphenicol	Compound (1)
500	25	22	34	22
400	24	20	32	18
300	22	16	29	17

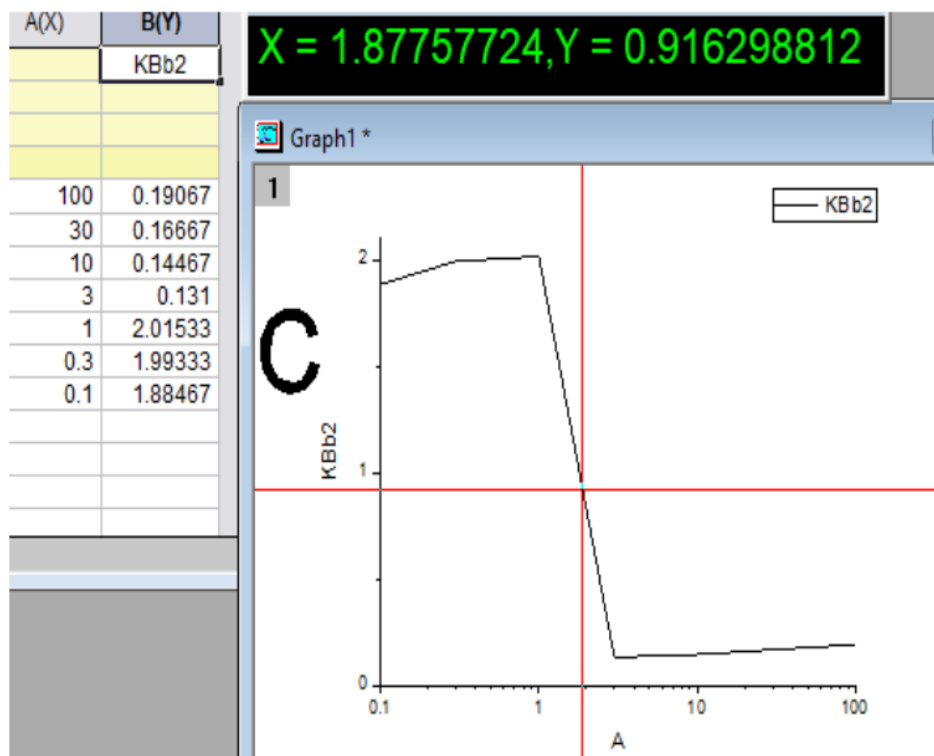


Figure 9: Graph of determination of IC_{50} compound (1) in cytotoxicity test using P-388 murine leukemia cells

Table 3
Test results of compound (1) using P-388 leukemia cells, at wavelength 540 nm

Concentration ppm	Optical density compound (1)			Average
100	0.19	0.188	0.194	0.190667
30	0.168	0.167	0.165	0.166667
10	0.144	0.144	0.146	0.144667
3	0.132	0.129	0.132	0.131
1	1.928	2.138	1.98	2.015333
0.3	2.015	2.146	1.819	1.993333
0.1	2.155	1.878	1.621	1.884667

Conclusion

From *A. altilis* branch wood, cyclomultichromen compounds have been isolated having strong antibacterial activity against *B. subtilis* and *E. coli*. It was also found to have high cytotoxicity against P-388 murine leukemia cells with IC₅₀ value of 1.87 µg/ml.

Acknowledgement

The authors are grateful to the Directorate of Research and Community Services, the Directorate General of Higher Education and the Ministry of Research, Technology and Higher Education, Republic of Indonesia, who provided funds for this project to be undertaken through Penelitian Berbasis Kompetensi Scheme 2018 with contract number 384/UN26.21/PN/2018, 12 February 2018.

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(Received 08th May 2019, accepted 10th July 2019)

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