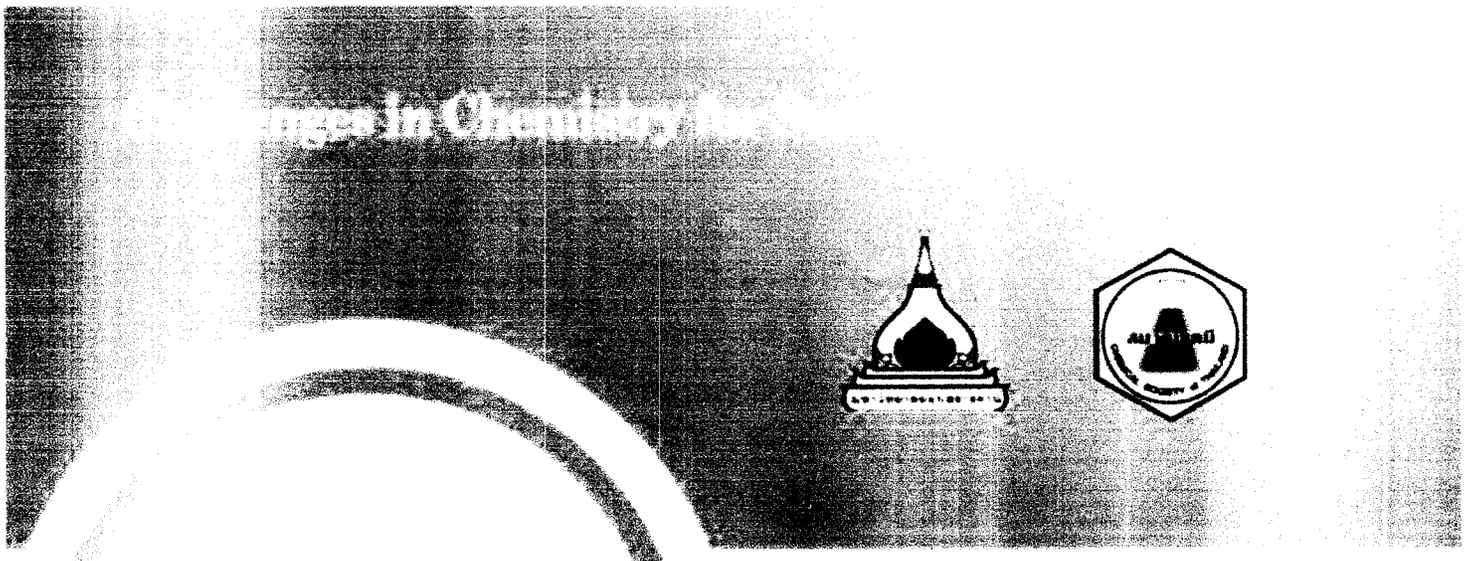

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LIST OF CONTENT

Biological / Biophysical Chemistry and Chemical Biology

Anti cancer activity of ampelopsin H from stem bark of hopea odorata

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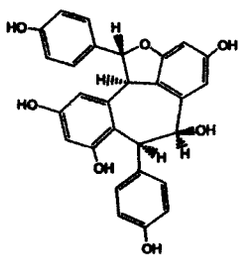
Abstract: Ampelopsin H was isolated from the stem bark of Hopea odorata, Dipterocarpaceae family. The structure of this compound was elucidated by NMR spectroscopy, including 1D and 2 D NMR. The invitro cytotoxicity of ampelopsin H against human cancer cell lines indicated that this compound had cytotoxic effect against HeLa, Raji, and Myeloma cell with the IC_{50} values of 129.718; 91.075; and 165.959□g/ml respectively and had no cytotoxic effect against Vero human cell (IC50 305.652□g/ml). The antiproliferative mechanism toward these human cancer cell lines showed that ampelopsin H influenced on cell cycle progression by defered doubling time cell. Also, this compound was able to induce apoptosis by influenced the expression of p53.

Introduction

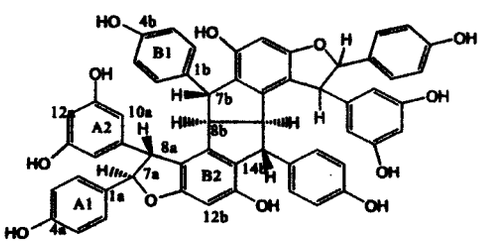
Dipterocarpaceae is a large family found in Indonesia. There is about sixteen genera and six hundred species [1] and nine genera of which found in Indonesia extending from Aceh to Papua with the greatest population in Kalimantan. Hopea is one of genera largely found in Indonesia, at least about one hundred species [2,3].

Based on many research, it had been reported that terpenoid, phenylpropanoid, flavanoid, benzofuran derivative, fenolic acid, and oligostilbenoid were compounds commonly found in some species of Dipterocarpaceae. Some of oligostilbenoid exhibited the interesting biological activity, such as resveratrol. It is the first compound found and isolated in 1977 as phytoalexin, antimicrobial product from the leaves of Vitis vinifera plant as reaction against infection or another physiologic stimulus [4]. The other research also indicated that resveratrol had chemo preventive activity against cancer cells [5]. Many other biological activity of oligostilbenoid had been reported, such as ε-viniferin and copaliferol A exhibiting antimicrobial activity against some micro-organism [6]. Some of these oligostilbenoid also showed cytotoxic activity against certainly strain, for example (-)-ampelopsin A and hopeaphenol had cytotoxic activity against KB epidermoid carcinoma cell [7-10], while malibatol A and malibatol B had cytotoxic activity against CEM SS cell in antiviral test.

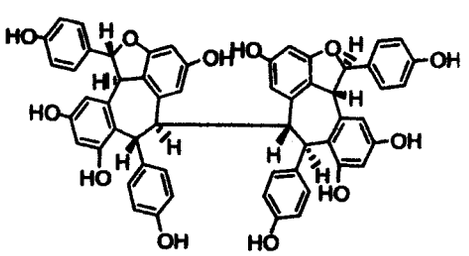
We have been isolated a number of oligostilbenoid from steam bark of Hopea odorata [12]. The elucidation structure by NMR spectroscopy, including ID and 2D NMR showed that these compounds were Balanocarpol (1), Ampelopsin H (2), Hopeafenol (3), and Hemlesyanol C (4) (Figure 1). This paper reports the cytotoxic activity, anti proliferative effect, and how the anti proliferative mechanism of Ampelopsin H that have been isolated from steam bark of H.odorata against HeLa S3, and Raji, human cancer cell lines, and Vero.



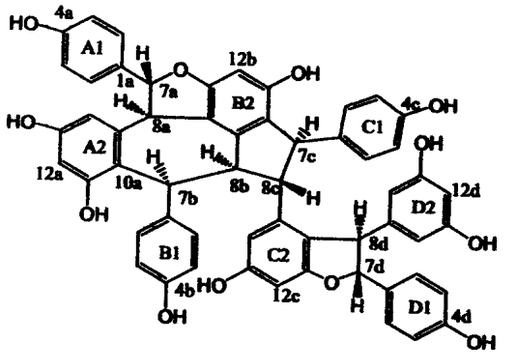
Balanocarpol (1)



Ampelopsin H (2)



Hopeafenol (3)



Hemlesyanol (4)

Figure 1. Compounds have been isolated from H. odorata : Balanocarpol (1), Ampelopsin H (2), Hopeafenol (3), and Hemlesyanol (4)

Materials and Methods

Materials : Ampelopsin H has been isolated from stem bark of H. odorata (from garden of attempt of Dramaga and Jasinga, Bogor at June 2007.

In-vitro cytotoxicity test : The in-vitro cytotoxicity test was investigated using 96 wells plate with cell density $2x10^4$ cells per ml for HeLa S3 and Raji human cancer cell lines, and $1x10^4$ cells per ml for vero cell. Into each well was added with 100 µl cells in culture medium (87.5% RPMI 10,4 g/L; 2% penstrep; and 10% FBS) and was then incubated in CO_2 incubator for 12-24 hours at 37^0C. Each sample was dissolved in culture medium containing 0.05% DMSO, and 100 µl of each sample in the different concentrations was added into each well in triplicate and was then incubated in CO_2 incubator for 12-24 hours at 37°C. MTT solution (10 µl per 100 al medium) was added to all wells of an assay, and plates were incubated for 4 hours at 37°C in CO_2 incubator. As much as 100µl formazon (10% SDS and 0, 01 N chloride acid) was added into each well and mixed on a shaker for 5 minutes. The wells were incubated in the dark room for 12-24 hours at room temperature. The absorbance was measured using multiwell scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly proportional to the number of living cells. So the dead cell could be calculated to determine LC_{50}.

Doubling time test: The cell was fasted (distarvation) for 24 hours in culture medium containing 0.5% FBS. Then they were grown in multiple dishes containing compound in low concentration ($\leq LC_{50}$). The sampling was done at 24, 48 and 72 hours. The living cell in each well was measured using multi well scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly proportional to the number of living cells. The curve between the number of living cell and incubation time indicated how the anti proliferative effect. The difference of doubling time was measured from the slope of log the number of cell versus incubation time curve.

Determination of Apoptosis: The determination of induction apoptosis was investigated by DNA cellular coloration using ethidium bromide/acrydine orange and observed on fluorescence microscope. The living cell give bright green colour and the apoptosis cell give orange colour. Futhermore the expression of p53 was determination by immunohistochemical analysis [15].

Results and Discussion

The cytotoxic activity of the Ampelopsin H against HeLa S3, Raji, and Vero cell measured as LC_{50} were provided in Table 1.

Table 1: LC_{50} of Ampelopsin H against HeLa S3, Raji, Myeloma, and Vero cell

Cell Lines	LC_{50} (□g/mL)	Note
HeLa S3	129.71	Active
Raji	34.69	Very Active
Myeloma	165.95	Less Active
Vero	305.65	Less Active

HeLa-S3, a continuous cell line that living as adherent cell, is a cell derivate of ephythell cell of human cervix cancer [16]. While Raji cell is a lymphoblast cell found by R.J.V Pulvertaft (1963) from Burkitt's lymphoma at the left of upper jaw of negro boy oldest 11 years [17].

Table 1 showed that the highest cytotoxic activity of Ampelopsin H is on Raji cell.

Based on doubling time test showed that Ampelopsin H reduce the growing of HeLa S3 (Figure 2), Raji (Figure 3), and Myeloma (Figure 4) cell compared with non treatment cell (control). It also showed that the slope curve of log number of cell versus incubation time with Ampelopsin H treatment smaller than control. It proved that Ampelopsin H caused a doubling time deferment. The doubling time value of HeLa S3, Raji, and Myeloma cell with Ampelopsin H treatment have been provided in Table 2. It mean that Ampelopsin H inhibit proliferate cell through cell cycle progression as well as transduction signal.

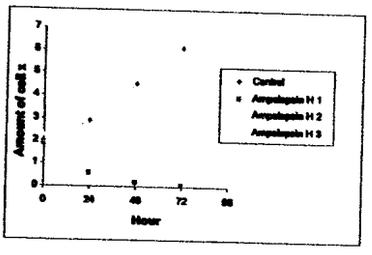


Figure 2. The growing profile of Hela S3 cell without treatment (control), and with Ampelopsin H (concentration 135, 67.5, and 33.75 ag/mL).

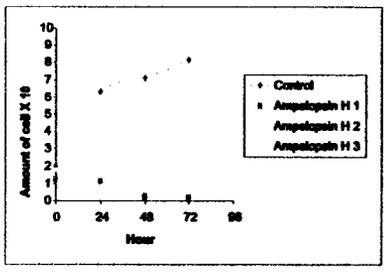


Figure 3. The growing profile of Raji cell without treatment (control), and with Ampelopsin H (concentration 90, 45, and 22.5 ag/mL).

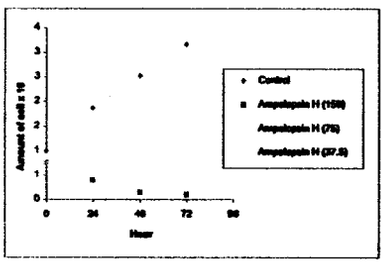


Figure 4. The growing profile of Myeloma cell without treatment (control), and with Ampelopsin H (concentration 150, 75, and 37.5 ag/mL).

Table 2: The equation curve of log number of cell vs time and the value of doubling time of HeLa S3, Raji, and Myeloma cell

Ampl (□g/mL)	Equation curve of log number of cell vs time	Slope	Doubling time (hour)
A. HeLa			
0	y = 0.0069x + 4.3048	0.0069	43.087
67.5	y=-0.0004x + 4.3571	-0.0004	-
135	y= -0.0186x+ 4.2381	-0.0186	-
33.75	y= 0.0027x + 4.3680	0.0027	110.852
B. Raji			
0	y = 0.0078x + 4.4337	0.0078	21.577
90	y =-0.0127x + 4.1532	-0.0127	-
45	y = -0.0084x+ 4.6828	-0.0084	-
22.5	y = 0.0026x + 4.5345	0.0026	-
C. Myeloma			
0	y = 0.0068x + 4.4098	0.0068	36.946
150	y =-0.0132x + 3.8796	-0,0132	-
75	y = -0.008x + 3.9407	-0.0023	-
37.5	y = 0.0029x + 4.0146	0.0029	768.087

The ethidium bromide/acrydine orange coloration indicated that Ampelopsin H also inhibits cell through apoptosis mechanism in HeLa S3 (Figure 5), Raji (Figure 6), and Myeloma cell (Figure 7).

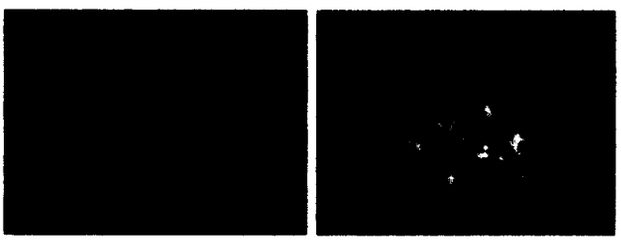


Figure 5. The acridine orange coloration of : (A) HeLa S3 cell without Ampelopsin H, and (B) HeLa S3 cell with 135 ag/mL Ampelopsin S3.

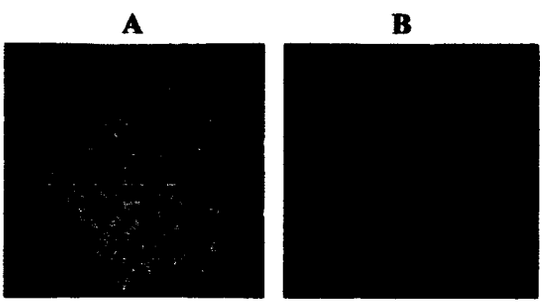


Figure 6. The acridine orange coloration of : (A) Raji cell without Ampelopsin H, and (B) Raji cell with 34.69 ag/mL Ampelopsin H.

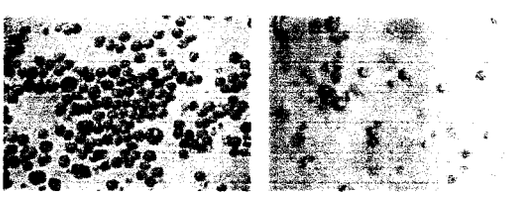


Figure 7. The acridine orange coloration of : (A) Myeloma cell without Ampelopsin H, and (B) Myeloma cell 100 ag/mL Ampelopsin H.

The effect of Ampelopsin toward the DNA change of Raji cells were indicated in Figure 6b. The living cell with intact nucleus gives the bright green color, while the apoptosis cell gives orange color. The Figure show that Ampelopsin H treatment cause cell become apoptosis therefore give orange color, and also indicate the morphology of apoptosis characteristic as cell become small, membrane bleeding, and nucleus fragmentation.

As we know that HeLa S3 cell is immortal cell because there is E6 protein that degrades p53, and E7 that degrades pRb. Therefore cell lost the growing cell protein. Until now there is no information about Ampelopsin H activity can repress E6 and E7 expression. Based on some anticancer compound like curcumin, it is possible that the antiproliferative activity of Ampelopsin H is initiated by bounding between this compound and protein. Therefore it cause the protein was recognize by proteosome that it degrade this protein. Furthermore, the expression of p53 was analyzed by immunohistochemical. It show that HeLa cell without treatment has a blue colour . It's mean that there are no expression of p53. The other way, HeLa S3 cell by adding Ampelopsin H show a brown colour, that it's mean that there was expression of p53.

Conclusions

In this paper we conclusion that Ampelopsin H isolated from the steam bark of H. odorata have cytotoxic effect against HeLa S3, Raji, Myeloma cell lines, and relatively no toxic effect to Vero cell . This compound gives the highest cytotoxic effect against Raji cell. It also have antiproliferative effect by induced apoptosis toward HeLa S3, Raji and Myeloma cell. This compound was able to induce apoptosis by influence the expression of p53 in HeLa cell.

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