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Identification of Cytotoxic Constituent of Indonesian Sponge *Kaliapsis* sp. (Bowerbank)

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Abstract: Identification of cytotoxic constituent of Indonesian sponge *Kaliapsis* sp. has been conducted. The structure identification was judged based on the spectroscopic data, namely, ultraviolet, MS, one and two-dimensional ^{1}H-NMR and ^{13}C-NMR methods. The cytotoxic constituent was identified as 1-(tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-methyl pyrimidine-2,4(1H,3H)-dione. This constituent hasn't been isolated from sponges as natural product.

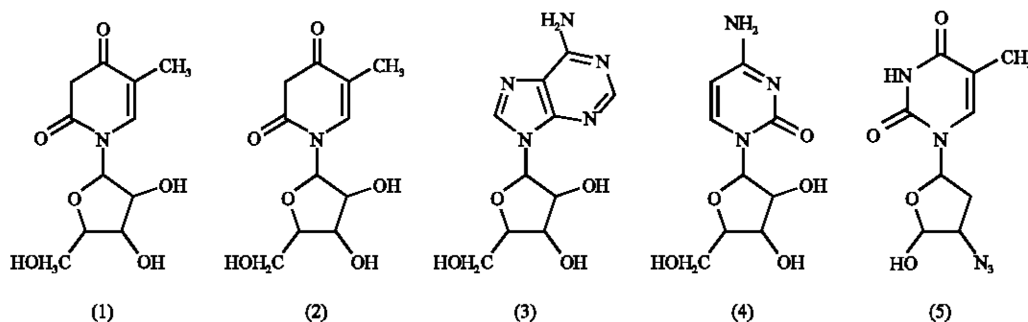
Key words: Structure identification, *Kaliapsis* sp. sponge, cytotoxic constituent

INTRODUCTION

Sponge is a multicellular organism, spineless and porous (Castro and Huber, 2003). Bergmann and Feeney (1951) isolated strong tumor inhibitor nucleoside compounds spongotimidine (1) and spongouridine (2) isolated from Caribean sponge *Cryptotethia crypta* (Tethylida). Following this finding, sponge become potential source for marine bioactive secondary metabolites. This compounds led the researchers to synthesized the analogs, namely, Ara-A (3, Vidarabine®, Vidarabin Thilo®) and Ara-C (4, Cytarabine, Alexan®, Udicil®), which were found enhancing the antiviral activity (Kijjoa and Sawangwong, 2004; Proksch *et al*., 2003). Further development of the analogs, the 3'-azido-3' deoxythymidine (5, AZT, Zidovudin) is currently being used to treat cancer and AIDS (Acquired Immune Deficiency Syndrome) (Müller *et al*., 2004; García *et al*., 2007; Newman and Cragg, 2004).

Kaliapsis sp. sponge is Lithistide family (Hooper and Soest, 2002). It is a unique family containing various functional groups of natural products. The sponge genera from this family are famous for their spineless characteristics and for their ability to produce various bioactive metabolites, such as, poliketide, cyclic peptide, alkaloid, pigments and sterols. Sponges in this family are also known for their various cytotoxic constituents. Sponge in the family of Theonellidae the genus of *Discodermia* contained an anticancer *Discodermolide* (6). This compound was also an immunosuppressant which has been tested in phase I clinical evaluation I (Newman and Cragg, 2004; Bewley and Faulkner, 1998).

We collected *Kaliapsis* sp. sponge in the sea around Menjangan island, West Bali, Indonesia. Earlier research showed that the ethanolic extract of this sponge and the isolate were cytotoxic active on Myeloma cell having IC_{50} 0.18 μg mL^{-1} (Setyowati *et al*., 2007). Now we are reporting the identity of the isolate (isolate 1).



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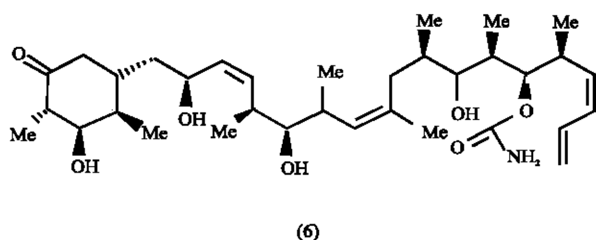

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MATERIALS AND METHODS

Materials: *Kaliapsis* sp. sponge was collected on 15 October 2004 in the sea around Menjangan island, West Bali National Park, 20 m under sea level.

General instrument: Infrared spectrophotometer (IR) (FTIR 8201 PC Shimadzu, Ultraviolet Spectrometer (UV) (Milton Roy 3000), mass spectrometer EIMS (Electron Impact Mass Spectroscopy) of INCOS 50 (Finigan MT). Spektrometer Resonance Magnetic Nuclear (NMR) 500 MHz (Jeol), operating at radiofrequency of 500 MHz.

Isolation procedure: The bioactivity guided extraction, fractionation and isolation of the active isolate were conducted based on standard procedure as reported previously (Setyowati *et al*., 2007; Houssen and Jaspars, 2005).

Cytotoxicity evaluation: The cytotoxic evaluation was performed following an established standard procedure (Doyle and Griffiths, 1998).

RESULTS AND DISCUSSION

Isolate 1, transparent colorless needle crystals, MP (uncorrected) 132-134°C. The isolate was soluble in methanol and DMSO but water insoluble. TLC detection on Cerium (IV) sulfate showed brown color after 110°C heating for 10 min.

Isolate 1 showed infra red absorption at v 3500-3200 cm^{-1} indicating the hydroxide (OH stretching) or amide (NH stretching) functional groups (Silverstein and Webster, 1998; Jenie *et al*., 2006). The isolate contained aliphatic alcohol as shown on the infra red absorption of aliphatic alcohol v 1200 and 1000 cm^{-1}. The absorption at v 2931.6 and 2839 cm^{-1} showed the methyl and methylene functional groups. It was supported also by the infra red absorption at v 1477.4, 1434.9 and 1400.2 cm^{-1}. The absorption at v 1662.5 cm^{-1} was the characteristics of carbonyl (-C = O) functional group of an amide and the absorption of v 1272.9 cm^{-1} was due to the occurrence of ether linkage –C-O-C-. Therefore, the isolate 1 contained -NH-CO-H, $-CH_3$, CH_2, R-OH and –C-O-C functional groups.

The ^{1}H-NMR spectra showed that the isolate 1 (Fig. 1) showed the chemical shift of methyl (s), at δ_H 1.8 ppm (3H), two metilen protons at δ_H 3.8 and 2.4 ppm and one methin proton of secondary alcohol at δ_H 3.9 ppm.

From the spectra, it was clear the occurrence of proton at δ_H 11 ppm for amide proton (-CONH).

The ^{1}H-^{1}H Cosy spectrum (Fig. 2), showed the proton-proton correlations of isolate 1. This spectrum

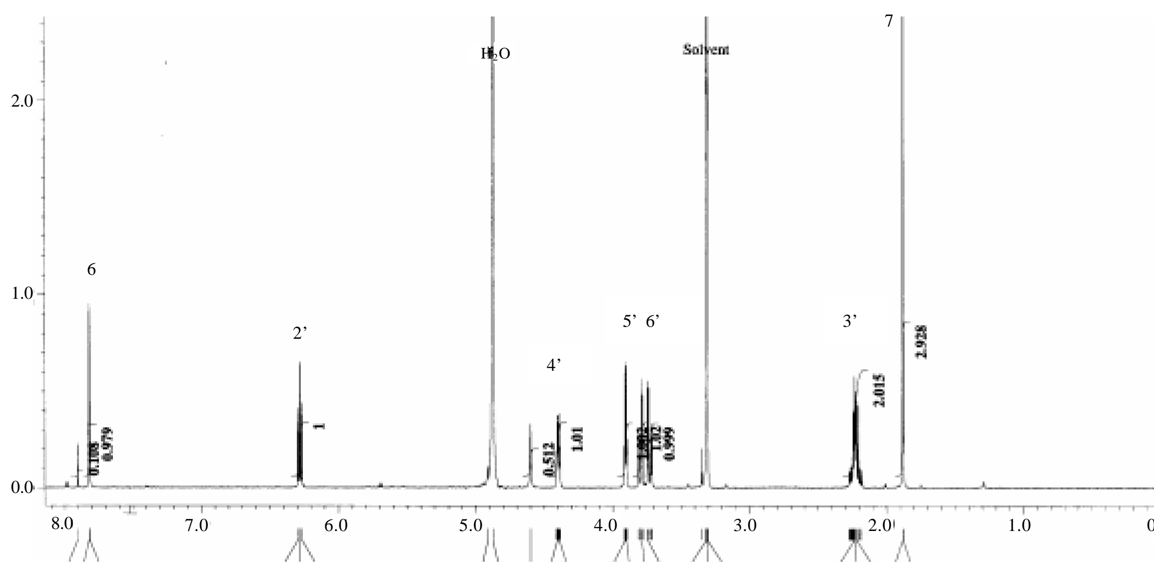

Fig. 1: The ^{1}H-NMR spectra of isolate 1 in CD_3OD

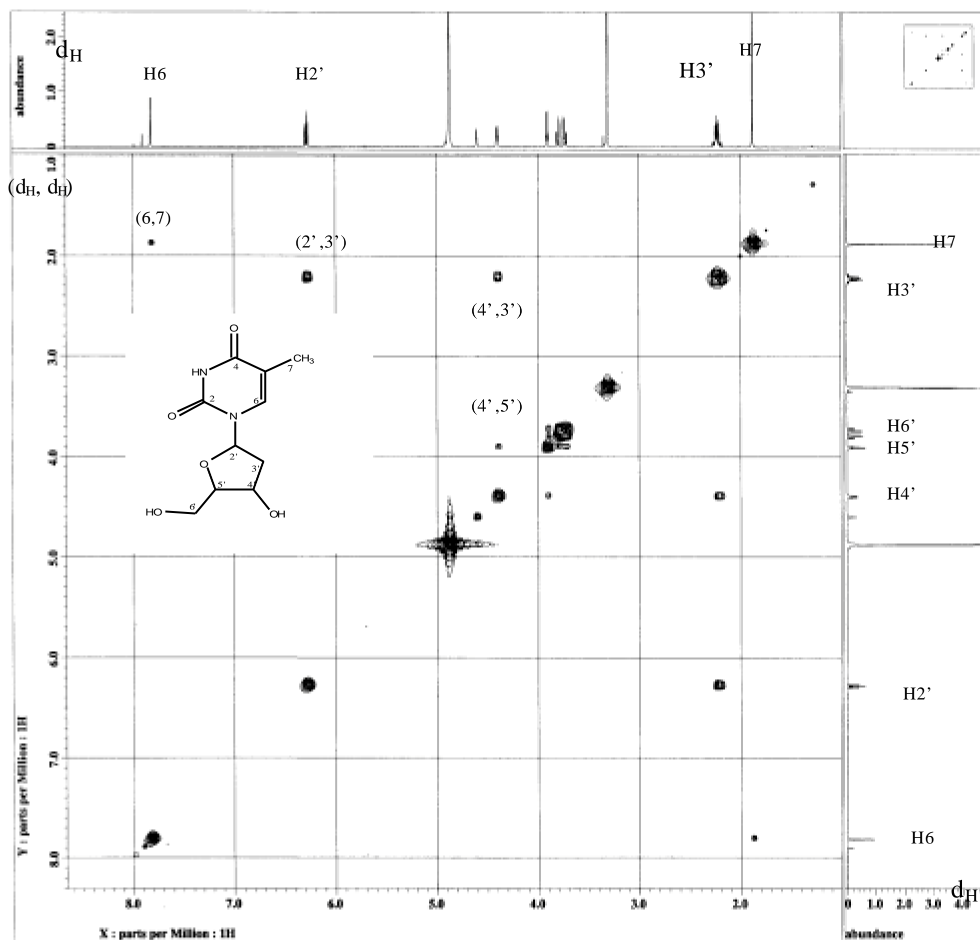

Fig. 2: ^{1}H-^{1}H COSY spectra of isolate 1

confirmed the proton chemical shifts (δ_H) ppm of 7.8 (s), 1.8 (s), 6.4 (t, J = 1.35 Hz), 2.4 (m), 4.4 (m), 3.9 (q, J = 7.3 Hz) and 3.8 (dd, J = 6.1, 7.3 Hz), assigned for protons at C-6, 7, 2', 3', 4', 5' and 6', respectively.

Figure 3, showed the ^{13}C-NMR spectra indicating that isolate 1 had 10 carbon atoms. Two carbons was in a very downfield position, δ_C 166.5 and 152.5 ppm were assigned as the carbonyl carbons. The methyl peak was showed at δ_C 12.5 ppm. The APT (Attached Proton Test) showed the number of $-CH_3$, $-CH_2$, -CH and -C (quaternary carbon) of isolate 1 and this isolate consisted of one methyl ($-CH_3$), two methylenes ($-CH_2$) and five methines (-CH) and two quaternary carbons. Table 1 showed the chemical shifts assignment of the ^{13}C signals.

Correlation of protons and carbon signals as showed at Table 2, were confirmed by the HMQC (Heteronuclear Multiple Quantum Coherence) spectrum. Long-range proton-carbon correlation was judged by the HMBC (Heteronuclear Multiple Bond Coherence) spectrum. This spectrum showed the correlation of C$\underline{H}_3$ correlated to CO (C-4), $\underline{H}$-6 to (C-2'), O$\underline{H}$ to C-4'. Figure 4 and Table 2, showed long-range correlation of ^{1}H-^{13}C correlation of isolate 1 resulted from the HMBC spectrum.

Table 1: The ^{13}C-NMR assignment of isolate 1 in CD_3OD

Assignment	Type of carbon	δ_C (ppm)
4	C	166.5
2	C	152.5
6	CH	138.3
5	C	111.6
5'	CH	88.9
2'	CH	86.3
4'	CH	72.3
6'	CH_2	62.9
3'	CH_2	40.0
7	CH_3	12.5

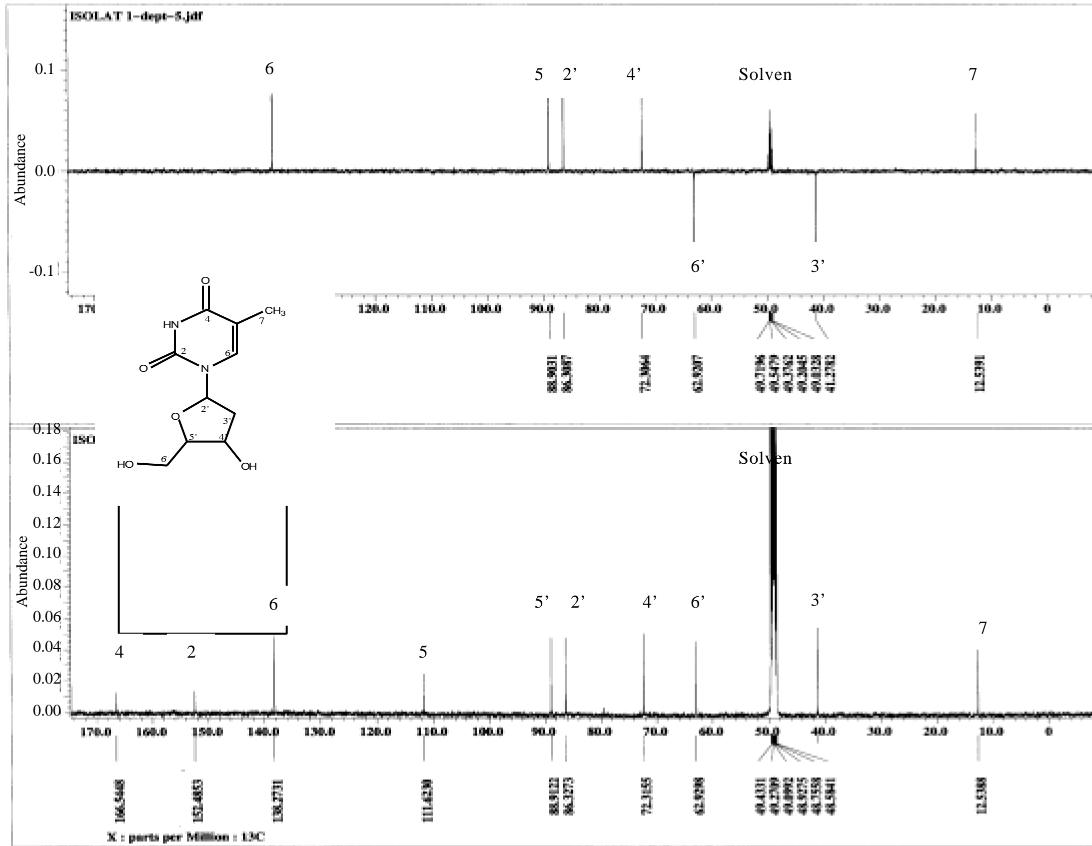

Fig. 3: ^{13}C-NMR and APT of isolate 1 in CD_3OD

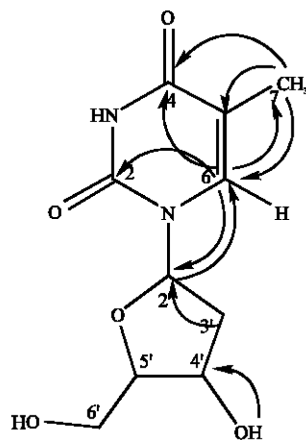

Fig. 4: HMBC long-range correlation of isolate 1

The mass spectrum (EI-MS) (Fig. 5), showed that the isolate 1 had molecular weight of 242 for chemical formula of $C_{10}H_{14}O_5N_2$ based on its molecular ion peak of m/z 242.

Table 2: HMBC long-range correlation of isolate 1

Nomor carbon	^1H (δ_H) ppm, J (Hz)	^{13}C (δ_C) ppm
1	-	-
2	-	152.5
3	-	-
4	-	166.5
5	-	111.6
6	7.8 (s)	138.3
7	1.8 (s)	12.5
1’	-	-
2’	6.4 (t, J = 1.35 Hz)	86.3
3’	2.4 (m)	40.0
4’	4.4 (m)	72.3
5’	3.9 (q, J = 7.3 Hz)	88.9
6’	3.8 (dd, J = 6.1, 7.3 Hz)	62.9

The EI-MS spectrum of isolate 1 showed the ion fragments at m/z 242 (3%), 207 (3%), 150 (3%), 126 (41%), 117 (100%, base peak), 99 (32%), 97 (11%), 73 (47%), 69 (18%), 43 (49%), 41 (15%). Figure 6 showed the structure of isolate 1 and Fig. 7 showed fragmentation pattern of isolate 1.

Cytotoxicity evaluation showed that isolate 1 inhibit the growth of HeLa, Raji, Myeloma and T47D cells

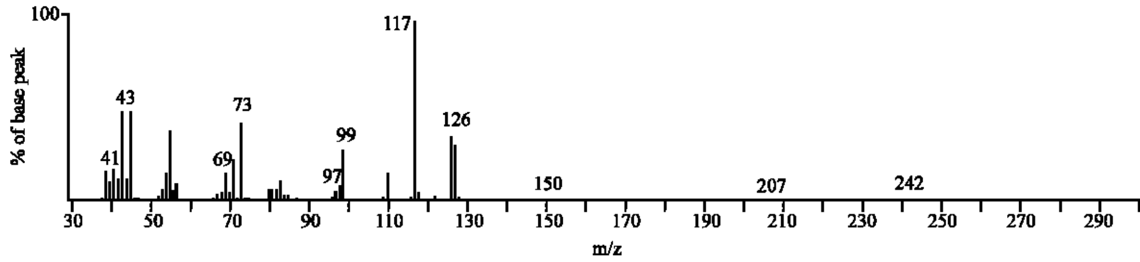

Fig. 5: EI-MS spectrum of isolate 1

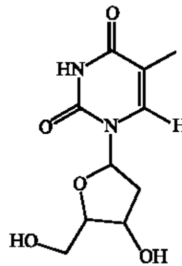

Fig. 6: Structure of isolate 1, 1-(tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-methylpyrimidine-2,4(1H,3H)-dione

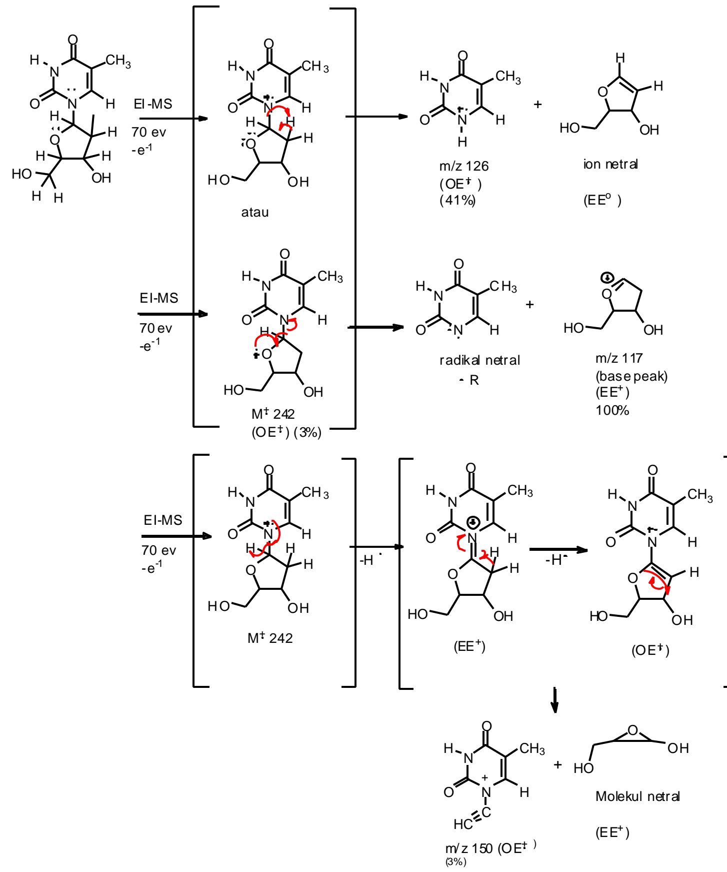

Fig. 7: Splitting of fragmentation pattern of isolate 1 isolated from *Kaliapsis* sp. sponge

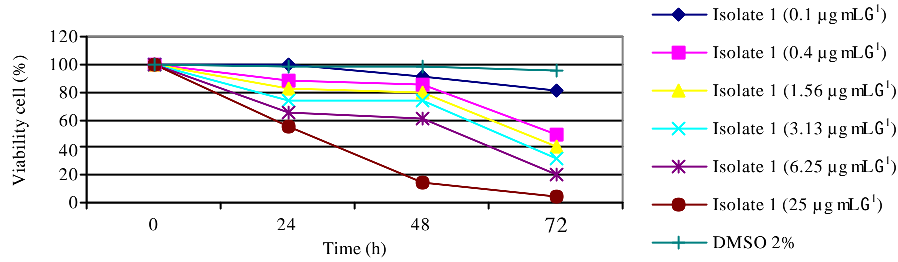

Fig. 8a: Percentage of living HeLa cells vs. isolate 1 concentration

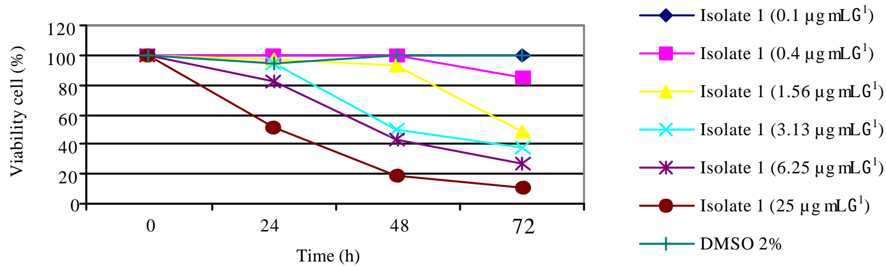

Fig. 8b: Percentage of living Raji cells vs. isolate 1 concentration

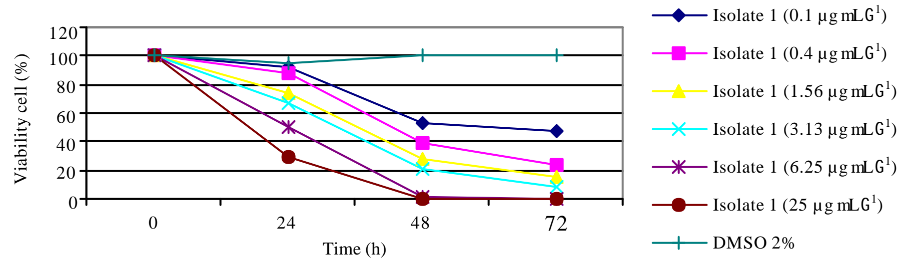

Fig. 8c: Percentage of living Myeloma cells vs. isolate 1 concentration

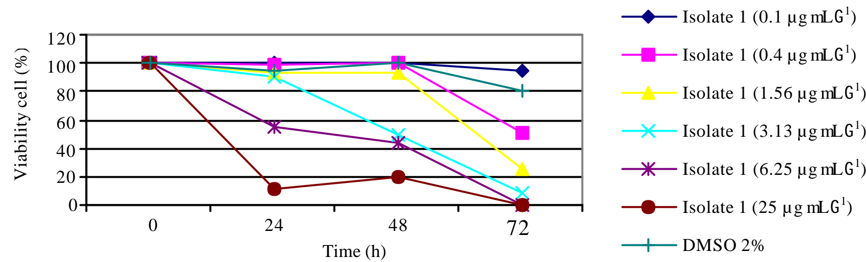

Fig. 8d: Percentage of living T47D cells vs. isolate 1 concentration

in vitro. The isolate 1 decreased the percentage of living cells of HeLa (Fig. 8a), Raji (Fig. 8b), Myeloma (Fig. 8c) and T47D (Fig. 8d). It was likely a dose dependent since the more extracts in the cells, the more decreasing of the percentage of living cells.

It was showed that the cell growth for HeLa, Myeloma and Raji cells, in media control being increased up to 72 h however, for T47D cells being increased up to 48 h, then showed the cell death starting fro 72 h. It was likely that the nutrition in the media was not sufficient for

cell growth. The IC_{50} *in vitro* cytotoxicity of isolate 1 was 0.18, 7.9, 6.9 and 5.8 $\mu g\ mL^{-1}$ on Myeloma, T47D, HeLa and Raji cells, respectively.

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