Anticancer Activity of 3-Hydroxystigmastan-5(6)-en (β-Sitosterol) Compound from Salacca Edulis Reinw Variety Bongkok in MCF-7 and T47D Cell Line

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***Abstract*—Discovery of two compounds in ethylacetate extract of snake fruits (*Salacca edulis* Reinw) variety Bongkok were pyrolle-2,4-dicarboxylic acid-methyl ester and 3-hydroxystigmastan-5(6)-en (β-sitosterol) compounds. In a previous study, two new compounds were observed an antioxidant activity by 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging activity. Pyrolle-2,4-dicarboxylic acid-methyl ester posses cytotoxic activity against MCF7 and T47D cell line. 3-hydroxystigmastan-5(6)-en (β-sitosterol) from snake fruit ethyl acetate extract- potential as anticancer still remains unknown and will be observed in his study. The cytotoxic assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2- (4-sulfophenyl)-2H-tetrazolium) (MTS) method in MCF and T47D cell lines to determine the IC_{50} of the β-sitosterol from snake fruit extract. Cytotoxic assay revealed that the (β-sitosterol) compound of snake fruit (*Salacca edulis* Reinw) inhibits the proliferation and viability of MCF7-breast cancer line- (IC_{50}= 45.414 μg/mL) and T47D -breast cancer stem cell line- (IC_{50} = 1.1942 μg/mL). The result obtained from the current study demonstrated that the β-sitosterol from snake fruit (*Salacca edulis* Renw.) extract exhibited *in vitro* cytotoxicity against MCF7 (breast cancer cell line) and T47D (breast cancer stem cell line).**

***Index Terms*—anticancer, *salacca edulis*, β-sitosterol, breast cancer.**

I. Introduction

Cancer is one of the most common life threatening diseases worldwide [1]. Radiotherapy, chemotherapy and surgery, oncolytic viruses have reported as a promising treatments for cancer [2]. Cancer prevention by using a dietary or natural substance has a potential as an approach to reduce the increasing incidence of cancer. Novel chemotherapeutic agents, especially from natural substance could become an alternative to be anticancer agents [3]. Most of fruits contain antioxidant property including ascorbic acids, amino acids, β-carotene, lycopene, melanoidin, certain organic acids, reducing agents, peptides, phospatides, polyphenols, tannins, tocopherols, polyphenols and flavonoids [4], [5].

The snake fruit (*Salacca edulis* Reinw) is known in Java, Sumatera, and other island as snake fruit and known has antioxidant properties [6]. Phytochemical screening of snake fruit variety Bongkok indicate that fruit containing flavonoid, alkaloid, terpenoid, tannin and quinones while saponin was not found [7]. Alkaloid, coumarins and flavonoid are anticancer properties that reported found in some species of plant [8]. In a previous study, two new compounds including 3-hydroxystigmastan-5(6)-en (β-sitosterol) and pyrolle-2,4-dicarboxylic acid-methyl ester were isolated from snake fruit var. Bongkok. That two compounds can be seen in Fig. 1. and Fig. 2. [9] Both of compounds were isolated from an ethyl acetate extract of snake fruit cv. Bongkok. The ethyl acetate was fractionated by VLC into eight major and repeated purification of the fraction using a flash chromatoraphic technique yielded compound 1 and 2. Compound 1 was isolated as a white crystal. Compound 2 was isolated as an amorphous orange solid [10].

That two compounds were observed has an antioxidant activity by 2,2-diphenylpicrylhydrazyl (DPPH) free radical scavenging activity in previous studies. Antioxidant compound found in some fruits could be used to maintain the immune system, slower the aging process, overcoming the stress response, and degenerative diseases prevention such as cancer, heart

Manscript received January 23, 2015; revised May 3, 2015.

doi: 10.12720/joaat.2.2.129-133

failure, brain dysfunction and cataracts [11]. Pyrolle-2,4-dicarboxylic acid-methyl ester was observed has anticancer activity in breast cancer cell. β-sitosterol constitute the largest class of natural products and are a rich reservoir of candidate compounds for drug discovery. Recent efforts into the research and development of anti-cancer drugs derived from natural products have led to the identification of variety of terpenoids [12]. The snake fruit β-sitosterol-terpenoid from *S.edulis*- potential of snake fruit cv.Bongkok as anticancer agents still remain unknown. The aim of this research is to observe the β-sitosterol potential as anticancer agent using MCF7 (breast cancer cell line) and T47D (breast cancer stem cell).

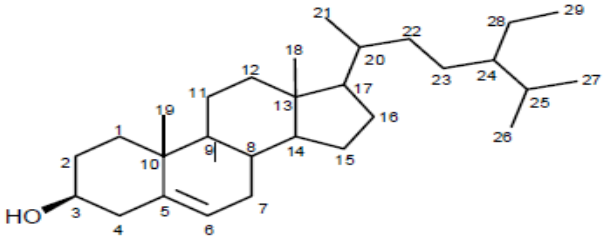

Figure 1. 3-hydroxystigmastan-5(6)-en (β-sitosterol)

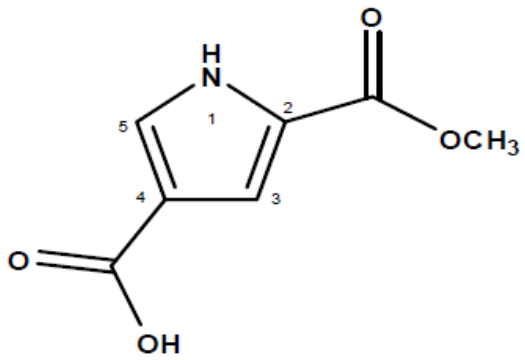

Figure 2. Pyrolle-2,4-dicarboxylic acid-methyl ester

II. Material and Method

A. Plant Material

The snake fruit (*Salacca edulis* Reinw.) cv. Bongkok was collected from Conggeang a sub district of Sumedang West Java, Indonesia and identified by Herbarium Bandungense, Institute Teknologi Bandung, Indonesia. 3-hydroxystigmastan-5(6)-en (β-sitosterol)was isolated from ethyl acetate extract of snake fruit (*Salacca edulis* Reinw) as described in the previous research [10].

B. Cell Culture

The cancer line that used for anticancer analysis including cell line of MCF7 and T47D. The MCF7 cells were grown and maintained in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% FBS (invitrogen), 100U/mL. T47D cells were grown and maintained in Dulbeco modified Eagle's medium supplemented with 10% FBS (invitrogen), 100 U/mL penicilin (Invitrogen),100 µg/mL streptomycin (Invitrogen). Both of culture incubated at 37 ℃ in a humidified atmosphere and 5% CO_2) [13], [14]. Confluent cancer cell was harvested using Tripsin EDTA. The cell was counted using haemocytometer to make standard curve of cell number.

C. Cytotoxic Asay Using MTS Method

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay (Promega, Madison, WI, USA) with an optimized reagent containing resazurin converted to fluorescent resorufin by viable cells that absorb 490nm light wavelength. [15] The cells were seeded in 96-well plate (5 x 10^3 cells per well) in 100 µL medium and incubated for 24 hours at 37 ℃ in a humidified atmosphere and 5% CO_2. The medium then washed and supplemented with 90µL new medium and 10µL β-sitosterol of snake fruit extract at various concentrations (100 µg/mL, 50 µg/mL, 25 µg/mL, 12,5 µg/mL, 6,25 µg/mL and 3,125 µg/mL) and incubated for 24-48 hours. Untreated cells served as the negative control. MTS was added to each well at 1:5 MTS medium ratio. The plate was incubated in 5% CO_2 at 37 ℃ incubator for 2-4 hours. Absorbance was measured at 490 nm on microplate reader. The data are presented as the percentage of viable cells (%) and were analyzed by calculating the median inhibitory concentration (IC_{50}) using Probit Analysis (SPSS 20) [13].

III. Result

The cytotoxic assay was performed to confirm the β-sitosterol of snake fruit as anticancer agent using MCF7 and T47D cell line. Table I. shows the effect of various concentrations of β-sitosterol from snake fruit toward the number of breast cancer cell line. From Table I we know that the concentration has a negative corelation to the number of both MCF7 and T47D cancer cell viability which mean the higher β-sitosterol concentration the strongest the anticancer activity of the β-sitosterol. Table II shows the viability of MCF 7 and T47D cells treated with β-sitosterol extract of snake fruit decreased in concentration manner; higher extract concentration exhibited stronger anticancer activity.

TABLE I. Effect of Beta-Sitosterol in Various Concentrations toward Number of Cells of Breast Cancer Cell Lines (Data were Expressed as Means, Standard Deviation, Duncan Post Hoc Test)

Samples (Concentrations) of β-sitosterol	Type of breast cancer	
	MCF7	T47D
FBS (β-sitosterol 0)	5968 ±184 ^{e}	5433 ±338 ^{e}
DMSO (β-sitosterol 0)	6161 ±325 ^{e}	5680 ±379 ^{e}
Starving (β-sitosterol 0)	5841 ±279 ^{e}	5311 ±401 ^{e}
β-sitosterol 100 µg/ml	1070 ±40 ^{a}	0.00 ±0.00 ^{a}
β-sitosterol 50 µg/ml	3894 ±179 ^{b}	0.00 ±0.00 ^{a}
β-sitosterol 25 µg/ml	4385 ±103 ^{c}	559 ±133 ^{b}
β-sitosterol 12.5 µg/ml	4562 ±258 ^{c}	1126 ±40 ^{c}
β-sitosterol 6.25 µg/ml	4925 ±163 ^{d}	1375 ±86 ^{c}
β-sitosterol 3.125 µg/ml	5100 ±220 ^{d}	2406 ±43 ^{d}

Table III shows that the terpenoid could inhibit the cell line, the higher the concentration the stronger the viability inhibition activity. The Table IV shows that β-sitosterol from snake fruit extract has IC_{50} 45,414 μg/mL in MCF7 cell line and 1,1942 μg/mL in T47D cell line.

TABLE II. EFFECT OF BETA-SITOSTEROL IN VARIOUS CONCENTRATIONS TOWARD VIABILITY OF CELLS OF BREAST CANCER CELL LINES (DATA WERE EXPRESSED AS MEANS, STANDARD DEVIATION, DUNCAN POST HOC TEST)

Samples (Concentrations) of β-sitosterol	Type of breast cancer	
	MCF7	T47D
FBS (β-sitosterol 0)	95.44 ±0.31 [e]	95.67 ±0.71 [f]
DMSO (β-sitosterol 0)	100.00 ±0.00 [f]	100.00 ±0.00 [g]
Starving (β-sitosterol 0)	94.82 ±0.83 [e]	93.47 ±0.88 [f]
β-sitosterol 100 μg/ml	17.43 ±1.53 [a]	0.00 ±0.00 [a]
β-sitosterol 50 μg/ml	63.26 ±2.29 [b]	0.00 ±0.00 [a]
β-sitosterol 25 μg/ml	71.27 ±2.57 [c]	9.99 ±3.11 [b]
β-sitosterol 12.5 μg/ml	74.08 ±2.65 [c]	19.91 ±1.91 [c]
β-sitosterol 6.25 μg/ml	80.01 ±1.95 [d]	24.34 ±3.08 [d]
β-sitosterol 3.125 μg/ml	82.84 ±2.73 [d]	42.47 ±2.27 [e]

TABLE III. EFFECT OF BETA-SITOSTEROL IN VARIOUS CONCENTRATIONS TO INHIBITE OF CELLS OF BREAST CANCER CELL LINES (DATA WERE EXPRESSED AS MEANS, STANDARD DEVIATION, DUNCAN POST HOC TEST)

Samples (Concentrations) of β-sitosterol	Type of breast cancer	
	MCF7	T47D
FBS (β-sitosterol 0)	4.56 ±0.31 [b]	4.33 ±0.71 [b]
DMSO (β-sitosterol 0)	0.00 ±0.00 [a]	0.00 ±0.00 [a]
Starving (β-sitosterol 0)	5.18 ±0.83 [b]	6.53 ±0.88 [b]
β-sitosterol 100 μg/ml	82.57 ±1.53 [f]	100.00 ±0.00 [g]
β-sitosterol 50 μg/ml	36.74 ±2.29 [e]	100.00 ±0.00 [g]
β-sitosterol 25 μg/ml	28.73 ±2.57 [d]	90.01 ±3.11 [f]
β-sitosterol 12.5 μg/ml	25,92 ±2.65 [d]	80.09 ±1.91 [e]
β-sitosterol 6.25 μg/ml	19.99 ±1.95 [c]	75.66 ±3.08 [d]
β-sitosterol 3.125 μg/ml	17.16 ±2.73 [c]	57.53 ±2.27 [c]

TABLE IV. THE IC_{50} OF BETA-SITOSTEROL IN MCF7 AND T47D CELL LINES FOR 24 HOURS INCUBATION

Samples	IC_{50} (μg/ml)	
	MCF7	T47 D
β-sitosterol	45.414	1.1942

IV. DISCUSSION

There is a convincing evidence that fruits and vegetables are playing beneficial role in the prevention and even treatment of different diseases [16], [6]. Plants maybe an alternative to currently used anticancer agents, because they are rich of bioactive chemicals and most of them free from adverse effects [17]. Terpenoids are the largest group of phytochemicals, traditionally used for medicinal purposes in India and China, are currently being explored as anticancer agents in clinical trials. A large number of terpenoids exhibit cytotoxicity against a variety of tumor cells and cancer preventive as well as anticancer efficacy in preclinical animal models [18]. Snake fruits (*Salacca edulis* Reinw) have high concentrations of bioactive compound including terpenoid.

Identification of cytotoxic compounds led the development of anticancer therapeutics for several decades. Cytotoxic agents could induced damaged to the cells, especially to DNA, triggers apoptosis through two signaling mechanisms, the activation and release of mitochondrial pro-apoptotic proteins known as caspases under the control of Bcl-2 protein family or upregulated expression of pro-apoptotic receptors on cancer cells, whose subsequent interaction with their ligands activates apoptotic signaling pathway [19]. Cytotoxic assay revealed that the terpenoid extract of snake fruit (*Salacca edulis* Reinw) inhibit the proliferation and viability of MCF7-breast cancer line- (IC_{50}= 45.414 μg/mL) and T47D -breast cancer stem cell line- (IC_{50} = 1.1942 μg/mL). There is an increasing trend in the inhibitory activity of the extract in relation to its increasing concentration. That IC_{50} value shows terpenoid from snake fruit extract has a potential become anticancer agent against MCF7 (breast cancer cell line) and T47D (breast cancer stem cell line).

Some plant extracts or phytochemical have been found to be effective against cancer cells, which are resistant to conventional chemotherapy agents [17], [20]. 3-hydroxystigmastan-5(6)-en (β-sitosterol)-terpenoid from snake fruit extract- belong to phytosterol. Plant sterol or pytosterols are structurally similar to cholesterol and exist in several forms in plants. Phytosterol have been shown to reduce blood cholesterol levels [20]-[24]. In addition to their-lowering cholesterol actions, mounting evidence suggests that phytosterols possess anti-cancer effect [25] against cancer of the lung, [26] stomach, [27] ovary [28] and estrogen-dependent human breast cancer [29], [25]. Patra,*et al* (2010) confirmed that β-sitosterol reduced carcinogen-induce cancer of the colon. It also shows anti-inflammatory, anti-pyretic, antiarthritic, anti ucler, insulin releasing and oestrogenic lowering property [30]. In our study, the cytotoxic effect of 5(6)-en (β-sitosterol)-terpenoid from snake fruit extract exhibited potent cytotoxic activity against MCF7 (breast cancer cell line) and T47D (breast cancer stem cell line).

V. CONCLUSION

The result obtained from the current study demonstrated that the β-sitosterol from snake fruit (*Salacca edulis* Renw.) extract exhibited *in vitro* cytotoxicity against MCF7 (breast cancer cell line) and T47D (breast cancer stem cell line).

ACKNOWLEDGMENT

We gratefully acknowledge the financial support of the Directorate General of Higher Education, National Ministry of Republic Indonesia for research grant of Hibah Kompetensi 2014

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Dr Afrianti L.H. has received research funding from the National Strategic Grant Batch II of from 2009 to 2011 and Competence Grant 2014 to 2016 of the Ministry of Education (DP2M) Indonesia.

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