

## Anticancer and Antioxidant Activities from Sea Cucumber (*Stichopus variegatus*) Flour Dried Vacuum Oven

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### ABSTRACT

The anticancer and antioxidant activities from sea cucumber (*Stichopus variegatus*) flour dried vacuum oven have not been widely reported. This study aimed to determine *in vitro* inhibition of enzyme and water extracts from sea cucumber flour against colon cancer cells WiDr, breast cancer cells T47D, and normal cells Vero, then the apoptotic induction. Sea cucumber flour was made by vacuum oven. It was water extracted and hydrolyzed by digestive enzyme gradually. The water extract (SV-WE) and enzyme hydrolysis (SV-EE) were tested for their antioxidant activities using DPPH method. Samples of SV-WE and SV-EE were tested for their anticancer activities using MTT assays. Antioxidant activity of SV-EE at  $1.67 \pm 0.05$  mg/mL was significantly different from SV-WE at  $2.30 \pm 0.30$  mg/mL. SV-EE and SV-WE have anticancer activity against WiDr cells at  $13.01 \pm 2.75$   $\mu$ g/mL and  $69.37 \pm 24.25$   $\mu$ g/mL, respectively. The IC<sub>50</sub> value of apoptotic ability of cell inductions from SV-EE was  $64.9 \pm 1.63\%$ . SV-WE and SV-EE showed higher anticancer activity against WiDr cells and T47D cells. Gamma sea cucumber flour from Indonesia could be utilized as a potential ingredient in functional foods.

**Keywords:** Anticancer, antioxidants, enzyme hydrolysis (SV-EE), *stichopus variegatus*, water extract (SV-WE)

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### INTRODUCTION

Functional food is a natural food or minimally processed foods that are safe, and have benefits for human health. Functional food can be made from the animal source, such as sea cucumbers that are usually consumed to prevent degenerative diseases. Sea cucumbers are marine invertebrates

of the phylum *Echinodermata* abundantly found in the world. In Asian folk medicine, sea cucumbers are mainly used for an antibacterial and antifungal, anticoagulant, antihypertensive, and immune system booster (Bordbar, Anwar, & Nzamid, 2011). The potency of them were as a therapeutic properties and medicinal benefits because of their bioactive compounds, such as peptide, phenolic, glycoprotein, glycosaminoglycans, and the others (Bordbar et al., 2011).

Wijesinghea, Perumal, Effendy and Charles (2013) noted that natural products had been used in health management, such as in the prevention and treatment of a cancer. One of the functional properties of sea cucumbers was in alternative treatment for cancer (Bandgar & Gawande, 2010). For the cancer evidence, the solution for eliminating the cancer tissues was the pharmacy, surgery, and chemotherapy (Dong, Dong, Tian, Wang, & Zhang, 2006). In contrast, Sondhi et al. (2010) stated that the pharmaceutical drug for the cancer sometime had a short clinical life, especially serious side effects. Therefore, we do need an anticancer that is perfectly safe and effective.

Ogushi, Yoshie-stark and Suzuki (2006) reported that *Stichopus japonicus* extract could inhibited and reduced the growth of a cancer cells through an apoptosis induction process. Previous research showed that *Stichopus japonicas* hydrolysate obtained from the hydrolysis process using enzymes from its digestive tract had a hydroxyl radical scavenging activity with the highest

activity on protein molecular weight fractions between 1 and 5 kDa (Pan, Yao, Zhou, & Wu, 2012).

This research examined the potential of anticancer and antioxidant activities of water-soluble and enzyme extracts from sea cucumber (*Stichopus variegatus*) flour. In this study, sea cucumber was dried into flour using a vacuum oven. The information of enzyme hydrolysis and water extracts from sea cucumber flour have not been widely reported as an anticancer potential against WiDr colon cell line and T47D breast cell line, plus the ability of apoptosis induction.

## **MATERIALS AND METHODS**

### **Flour Material Preparation**

The processing of sea cucumber flour dried vacuum oven was done according to Ridhowati, Chasanah, Syah and Zakaria's (2018) method. The fresh flesh of sea cucumber was washed with seawater after removing the part of internal organs. The fresh flesh of sea cucumber was cut, dried with vacuum oven (50°C, 65 cmHg, 4 h), and milled to obtain 60-mesh flour. Then, all samples were homogenized and kept in frozen at -20°C until used for analysis.

### ***Stichopus Variegatus*' Flour Water Extraction (SV-WE)**

The extraction was established based on the method published by Farshadpour et al. (2014) with slight modification. A water extract was prepared from *Stichopus variegatus* flour (5 g) then mixed in distilled water (50 mL), then sonicated for 30 min at

4°C with sonicator (Branson 8510, Danbury, USA). After that, it was separated at 5,031 g for 30 min at 4°C. The supernatant was collected. The collected water extracts were freeze-dried and stored at -20°C until analysis.

### ***Stichopus Variegatus*' Flour Enzyme Hydrolyzed (SV-EE)**

The enzyme extraction was accorded by the method of Perez-Vega, Leticia, Jose and Blanca (2013) with some modification. The digestion of sea cucumber flour was conducted with gastrointestinal enzymes simultaneously. Sea cucumber flour (8 g) was homogenized with 160-mL distilled water in waterbath shaker at 98°C for 20 min. An aqueous solution of flour was initially hydrolyzed with pepsin (10 U/g; P7000-25G, Sigma-Aldrich St Louis, MO, USA) for 120 min at 37°C and pH 2.0. After pepsin hydrolysis, pH was raised to 7.5 with 1 M NaOH, trypsin (0.4 Unit/g, T4799-5G, Sigma- Aldrich), and chymotrypsin (100 Unit/g, C4129-250mgr, Sigma-Aldrich) were added, then continuously incubated at 37°C for 120 min. After hydrolysis, enzymes were inactivated by heating at 85°C for 20 min, followed by cooling to room temperature. Then, sea cucumber hydrolysate was centrifuged at 9820 g at 4°C for 45 min to separate insoluble and soluble fractions. Finally, the soluble phase recovery was freeze-dried and preserved at -20°C until used for further analysis.

### **Chemical Composition**

Approximate chemical composition of SV-EE and SV-WE were determined according to the AOAC (2005) method, such as moisture, ash, crude lipid, and crude protein. The content of amino acids was also conducted according to AOAC (2005) using high-performance liquid chromatography, and expressed as a percentage of amino acids content.

### **DPPH Scavenging Assay**

Determination of antioxidant activity using DPPH Scavenging Assay was based on Blois method (1958) with slight modification. The antioxidant activity test was performed by DPPH method based on the sample's ability to reduce the stable-free radical of DPPH (1,1,1-diphenyl-2-picrylhydrazyl). All measurements were conducted in triplicate.

### **Total Phenols**

Determination of total phenol was assessed by using Folin-Ciocalteu reagent, as described by Anesini, Graciela and Rosana (2008). All determinations were performed in triplicate. The total phenol count of the sample was based on the results of plotting the absorbance values on the standard curve.

### **Cell Culture**

Human colon cancer cell line (WiDr) and breast cancer cell line (T47D) were grown in RPMI 1640 (Gibco, Invitrogen Corporation) media that contains 10% (v/v) FBS (Qualified, Gibco, Invitrogen), and 1000 mg mL streptomycin penicillin

antibiotics 100 µg/mL (Gibco, Invitrogen Corporation), while Vero cells (ATCC CCL 81) were grown in Dulbecco's Modified Eagle's Medium (D-MEM), Phosphate Buffer Saline (PBS) and incubated at 37°C with a 5% CO<sub>2</sub> flow. Anticancer activity was tested using MTT assay. The WiDr, T47D, and Vero cells were distributed into 96 well plates (Nunc) with a total of 5000 cells per well and incubated with both single application and combination samples using DMSO solvent for 24 h on CO<sub>2</sub> (Heraceus). At the end of incubation, the sample was added 100 MTT (Sigma) in RPMI media (Gibco) for WiDr cells, T47D cells, and also in D-MEM media for Vero cells. The plates were incubated for 4 h at 37°C until formazan crystals (see under inverted microscope (Zeiss)). The living cells reacted with the MTT until purple color was formed. After 4 h, the MTT reaction was discontinued by adding 100 µL 10% SDS reagent at each well, then incubated overnight at room temperature by covered with aluminum foil. Absorption was read by ELISA reader (Bio-Rad) at 595 nm wavelength. The data was obtained in the form of absorbance of each well converted into viability cell percentage. It was calculated using searching linear regression equation, and calculated concentration of IC<sub>50</sub> that is concentration causing death 50% of cell population so that it can be a known potency of anticancer activity.

### **Cell Growth Inhibitory Assay**

The cytotoxicity of the SV-WE and SV-EE against the tumor cells was assessed via a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. WiDr and T47D cells were seeded ( $1.0 \times 10^5$  cells/mL) together. Both WiDr and T47D cells were seeded with various concentrations (2, 10, 50, 250, and 1250 µg/mL) then incubated for 48 h prior to MTT treatment. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide stock solution (50 µL; 2 mg/mL in PBS) was added to each well to achieve a total reaction volume of 200 µL. After 4 h of incubation, MTT reaction was stopped by 10% SDS, 100 µL in each well then incubated overnight. The formazan crystals in each well were dissolved in dimethyl sulfoxide. The amount of purple formazan was assessed by measuring the absorbance at 595 nm using an ELISA reader (Sunrise; Tecan Co.Ltd., Australia).

### **Detection of Apoptotic using Flow Cytometric Analysis**

Differentiation of apoptotic and necrotic cells using FITC Annexin V apoptosis Detection Kit II (BD Biosciences) based on Elmore (2007) methods. WiDr cancer cells with a final density of  $7 \times 10^5$  in a 6-well microculture were incubated for 12 h in CO<sub>2</sub> (37°C, 5% CO<sub>2</sub> flowing). The extracts were added to cells according to IC<sub>50</sub> concentrations for 24 h. Later, 100 µL of extracts were resuspended in 5-µL FITC Annexin and 5-µL PI, then incubated for

15 min, in dark room at 20 to 25°C. Typical histogram of apoptotic and necrotic cells was performed using FACSCalibur (Becton-Dickinson) flow cytometer.

### **Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

The protein profile was characterized by SDS-PAGE (Sodium Dodecyl Sulphate Poliacrylamide Gel Electrophoresis) according to Laemmli (1970) method. A 10- $\mu$ L protein sample plus 10- $\mu$ L RSB (Reducing Sample Buffer), inserted into the microtube, was then heated to 100°C for 2 min. Electrophoresis is carried out at 60 mA, 220 V for 3.5 h. Protein bands were stained by silver nitrate 0.1% (w/v) (Sigma Aldrich). Analysis of molecular weight (MW) was carried out by using protein Marker (Sigma-Aldrich) as standard. The scanned protein bands were plotted into the linear regression equation curve to obtain the MW protein values in each sample.

### **FTIR Analysis**

Fourier Transform Infrared Spectroscopy (FTIR) spectrometer (Model Equinox 55, Bruker, Ettlingen, Germany) was used to identify functional groups or side chains of a material, with the range of 400–4,000  $\text{cm}^{-1}$  then automatic signal gain were collected in 32 scans at a resolution of 4  $\text{cm}^{-1}$  at 25°C. A 2-mg sample was added with KBr powder up to 200 mg. Previously, KBr powder was heated at 105°C overnight. Samples that have been mixed with KBr are then pounded using agate mortar until

homogeneous. The samples were placed onto the crystal cell. The data was obtained using the curve of the relationship between wave number (x axis) and absorbance (y-axis). The frequency of the obtained wave numbers is then determined by the type of bond and functional group compared with the secondary data of FTIR.

### **Statistical Analysis**

The value data are expressed as mean  $\pm$  standard deviation with three replications. One-Way ANOVA were analyzed using SAS 9.1.3.Portable program.

## **RESULTS**

### **The Profile of SV-WE and SV-EE**

The organisms contained high protein and low fat have been associated with good health (Bordbar et al., 2011). Ridhowati et al. (2018) reported that the fat content of SV-WE and SV-EE were  $0.57 \pm 0.66\%$  and  $0.45 \pm 0.02\%$  of total dry weight, respectively. The protein content of SV-EE and SV-WE were  $37.09 \pm 0.06\%$  and  $5.73 \pm 0.01\%$  of total dry weight, respectively (Ridhowati et al., 2018). The contents of amino acid profiles were different between SV-EE and SV-WE. Moreover, all amino acid contents from SV-EE and SV-WE have decreased from sea cucumber flour, as shown in Table 1. The differences of protein content and amino acid profile were influenced by the type of sea cucumbers, the extraction process, handling and drying process into a product.

Table 1  
Amino acid composition of *Stichopus variegatus* (%w/w protein, dry weight)

Amino acid (% w/w)	SV-Flour*	SV-EE	SV-WE
Acid amino group			
Aspartic acid	2.59 ± 0.01	3.06 ± 0.01	0.43± 0.01
Glutamic acid	4.71 ± 0.01	4.99± 0.01	0.96± 0.01
Polar amino group			
Serine	0.93 ± 0.01	1.76± 0.01	0.17± 0.02
Glycine	4.99 ± 0.01	5.90± 0.01	0.30± 0.01
Threonine	1.31 ± 0.02	2.14± 0.02	0.24± 0.01
Basic amino group			
Arginine	2.33 ± 0.02	2.91± 0.01	0.27± 0.02
Lysine	5.79 ± 0.01	1.28± 0.01	0.21± 0.03
Histidine	0.29 ± 0.02	0.47± 0.02	0.06± 0.02
Hydrophobic amino group			
Alanine	2.61 ± 0.02	2.48± 0.01	0.25± 0.03
Methionine	0.51 ± 0.02	0.96± 0.02	0.13± 0.01
Valine	1.10 ± 0.03	1.28± 0.01	0.15± 0.01
Phenylalanine	0.86 ± 0.02	1.07± 0.02	0.14± 0.01
Isoleucine	0.86 ± 0.03	1.01± 0.01	0.11± 0.01
Leucine	1.16 ± 0.02	1.64± 0.02	0.19± 0.02
Proline	2.61 ± 0.01	2.64± 0.03	0.18± 0.01

Source: Ridhowati et al. (2018)

### The Antioxidant Activity and Total Phenol of SV-EW and SV-WE

Table 2 shows that the IC<sub>50</sub> value of SV-EE and SV-WE have the ability to scavenge DPPH free radicals at 1.67 ± 0.05 mg/mL and 2.30 ± 0.30 mg/mL, respectively. Both of them were failed to capture 50% of DPPH

radical even though in high concentrations, compared to ascorbic acid that showed 10.59 ± 0.07 µg/mL on IC<sub>50</sub> value as the control. In contrast, the highest total phenol contained of 10.90 ± 0.14 mgGAE/g in the SV-WE was significantly different to 10.55 ± 0.07 mgGAE/g in the SV-EE.

Table 2  
Total phenolic contents, DPPH scavenging capacities and yields of *Stichopus variegatus* flour

Samples	DPPH scavenging capacity (IC <sub>50</sub> = mg/mL)*	Total Phenols (mgGAE/g)**	Yields (%)
SV-WE***	2.30 ± 0.30a	10.90 ± 0.14a	3.33 ± 0.18a
SV-EE***	1.67 ± 0.05b	10.55 ± 0.07b	1.12 ± 0.83b
Ascorbic acid	0.01059 ± 0.00007		

Note. \*Data expressed as IC<sub>50</sub> (the concentration of extract that required for scavenging of 50% DPPH) in mg extract/mL DPPH, mean ± SD (n = 3); \*\*Data expressed as gallic acid equivalents (GAE) mg/g extract, mean ± SD (n = 3); \*\*\* SV-EE fraction (*Stichopus variegatus*' flour enzyme hydrolyzed) and SV-WE fraction (*Stichopus variegatus*' flour water extraction)

**The Anticancer Activity of SV-EE and SV-WE**

Evaluations of sea cucumber *Stichopus variegatus* for potential anticancer activity on WiDr and T47D cells were determined the growth inhibitory effects of both SV-EE and SV-WE fractions using the MTT assay. The IC<sub>50</sub> values against WiDr cells of SV-EE and SV-WE were 13.01 ± 2.75 µg/mL and 69.37 ± 24.25 µg/mL, respectively. The SV-EE was strongly inhibited WiDr proliferation in a dose-dependent manner (Figure 1). While, the IC<sub>50</sub> value of anticancer activity

against T47D cells of SV-WE and SV-EE were 219.10 ± 22.17 µg/mL and 157.92 ± 99.91 µg/mL, respectively (Figure 2). Moreover, SV-EE showed cell proliferation were inhibited more than 82% at IC<sub>50</sub> value. Otherwise, both of them were more effective against WiDr cell lines than T47D cell lines. Then, the anticancer effect of them on cell viability in normal (Vero) cells was tested as well (Figure 3). Wherein, the IC<sub>50</sub> value against Vero cells for SV-WE and SV-EE was 1053.49 ± 90.26 µg/mL and 634.22 ± 32.75 µg/mL, respectively.

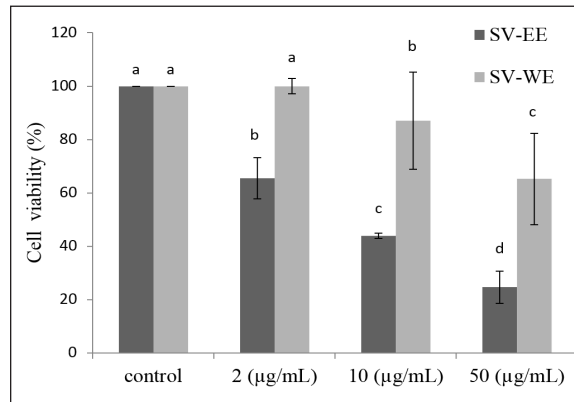


Figure 1. Effect of SV-EE (*Stichopus variegatus*' flour enzyme hydrolyzed) and SV-WE (*Stichopus variegatus*' flour water extraction) on cell viability in WiDr cells. Mean ± SD of three determinations as data

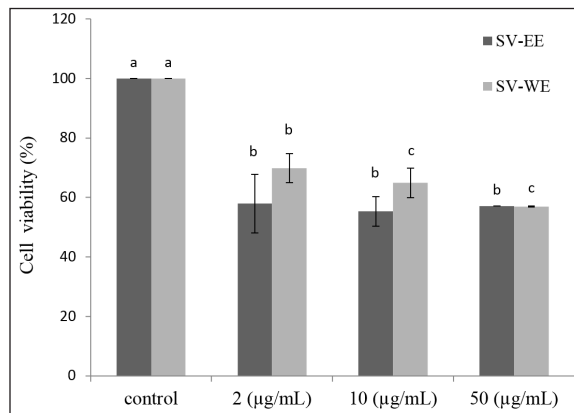


Figure 2. Effect of SV-EE (*Stichopus variegatus*' flour enzyme hydrolyzed) and SV-WE (*Stichopus variegatus*' flour water extraction) on T47D cell viability. Mean ± SD of three data

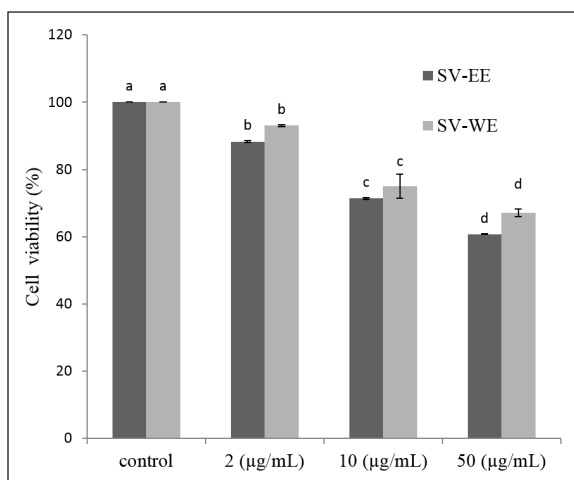


Figure 3. Effect of SV-EE (*Stichopus variegatus*' flour enzyme hydrolyzed) and SV-WE (*Stichopus variegatus*' flour water extraction) on Vero cell viability. Mean  $\pm$  SD of three determinations as data

## DISCUSSION

The content of amino acid was different between SV-EE and SV-WE (Table 1). Nevertheless, both of them have higher hydrophobic amino acid components than polar amino acid group. The quality of marine animals can be affected significantly by free amino acid composition. Lysine, glycine, and hydrophobic amino acids, such as alanine and methionine, may function as immunomodulator and anticancer (Bordbar et al., 2011). Amino acid components of sea cucumbers are involved in the regulation of immune system (Qin et al., 2008). In addition, amino acid content is different because of the variation of organisms, marine geography, species, age and physiological condition (Capillas, Moral, Morales, & Montero, 2002).

Based on Table 2, both of them were failed to capture 50% of DPPH radical even though in high concentrations, compared to

ascorbic acid that showed  $10.59 \pm 0.07$   $\mu\text{g}/\text{mL}$  on  $\text{IC}_{50}$  value as the control. The results of this study was almost the same  $\text{IC}_{50}$  value as with Althunibat's study, the water and organic extract of sea cucumber *Stichopus chloronotus* research that have the ability to capture a DPPH radical at  $2.13 \pm 0.05$   $\text{mg}/\text{mL}$  and  $>10$  (Althunibat et al., 2009). In this research, the contribution of total phenol as antioxidant properties was low. According to Zheng and Wang (2003), the capacity of free radical scavenging for phenolic compounds was fluctuated depending on the chemical species and the concentration. In this case, there is no correlation existed between radical scavenging capacity and total phenolic content. The scavenging activity of DPPH radical suggested that from other components, such as salts, sugars, glutathione and peptides (Zhong, Khan, & Shahidi, 2007). However, the phenolic compounds could contribute to



the antioxidant activity of sea cucumber (Zhong et al., 2007). Esmat, Said, Soliman, El-Masry and Badiea (2012) stated that sea cucumbers were rich in phenolic compounds, such as phytoplankton and particles derived from the degradation of marine macroalgae, suspected as a source of phenolic active compounds that accumulated in the sea cucumber body. Polyphenols are the main antioxidants known as free radical terminators. These compounds can neutralize free radicals by acting as a rapid donor from hydrogen to radical atom.

Toxicity evaluation in Vero cells showed less cell deaths at the tested concentration. For the Vero cells, SV-EE was higher in  $IC_{50}$  value than SV-WE. The strong inhibitory activity on SV-WE was due to the antiproliferative hydrophilic compounds, triterpene glycosides (Adrian & Collins, 2005). Potential anticancer activity was demonstrated by the ability of the compound

material capable of inducing apoptosis. In this study, the cytotoxic effect of SV-EE was investigated in the WiDr cell line. The promising cytotoxic effect showed by SV-EE; it was further continued to evaluate on cell apoptosis induction against WiDr cells. Percentage of apoptotic cells treated with SV-EE fraction was  $64.9 \pm 1.63\%$  (Figure 4).

It is proposed that the effect of apoptosis induction in this study could be associated with bioactive compound in SV-EE. It was known that hydrophobic and positively charged amino acids existing in peptide C-terminal are contributed to the peptide inhibitory properties. The same pattern almost happened in positively charged amino acids (Arg, Lys), although it is not comparable with those of  $IC_{50}$  values (Forghani et al., 2012). The SV-EE has low molecular weight protein fractions. In fact, the position of those amino acids in the peptide sequence is more crucial than

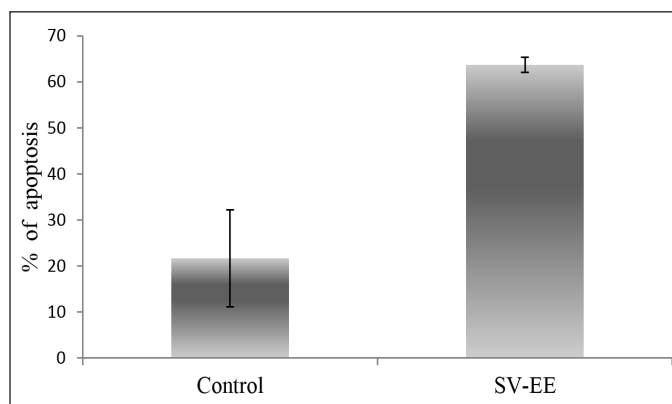


Figure 4. The WiDr cells were subjected to flow cytometric analysis using FITC Annexin V apoptosis detection kit I. The apoptosis induced by SV-EE (*Stichopus variegatus*' flour enzyme hydrolyzed) fraction at the concentration  $IC_{50}$  value are shown.

their total amounts (Forghani et al., 2012). It is suspected that a group of hydrophobic amino acids play a role in SV-EE to induce cell apoptosis (Lee, Kim, Lee, Kim, & Lee, 2004), as seen in Table 1.

The higher component of hydrophobic amino acid peptide would penetrate deeper into the hydrophobic core of the cell membrane, thereby causing strong activity forming pores or channels in the cell membrane of cancer. The greater anticancer activity is higher always accompanied with the hydrophobic amino acid content (Huang, Xiao-Fei, & Hong-Ye, 2011). The hydrophobicity of protein or peptide is very important for accessibility to the target hydrophobic, and increases the affinity and reactivity of the peptide with membrane of living cells (Huang et al., 2011).

In this research, SDS-PAGE was conducted to identify the possible molecular weight proteins that may be involved in promoting apoptosis cells in this study. The potential biological activities of SV-EE and SV-WE as a functional food were also supported by the results of electrophoresis

SDS-PAGE. The SV-EE has low molecular weight protein fractions of 21, 19, and 9 kDa compared to SV-WE protein that has varying molecular weights between 279 and 9 kDa (Figure 5). The result of this study was similar to the hydrolysis of *Stichopus horrens* with alcalase because the hydrolysis process of sea cucumbers occurred in almost all proteins with large molecular weight to produce polypeptide under 20 kDa (Forghani et al., 2012).

The electrophoretic patterns of protein have shown that SV-EE and SV-WE fraction mostly contained peptides with molecular weight in the range 279 until 9 kDa. Our results were in agreement with those of several previous studies done on different sources, such as Atlantic cod viscera (Aspmo, Horn, & Eijsink, 2005), which revealed high efficiency of alcalase for protein cleavage leading to production of small peptides. The absence of high molecular bands above 100 kDa indicated that gastrointestinal enzyme is able to degrade protein to smaller molecules with molecular weight below 21 kDa. This study

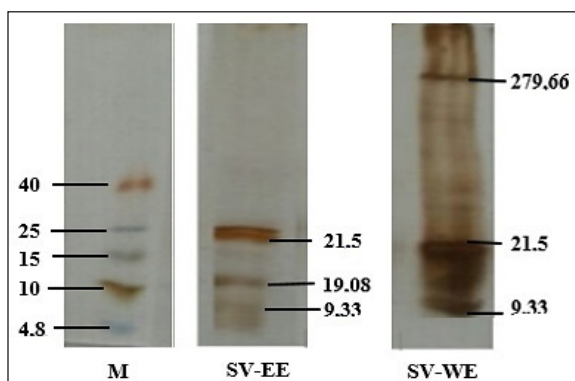


Figure 5. Peptide profile of SV-EE) and SV-WE fractions; M (marker), SV-EE (*Stichopus variegatus*' flour enzyme hydrolyzed) fraction, and SV-WE (*Stichopus variegatus*' flour water extraction) fraction

proposed that the fraction molecules of SV-EE and SV-WE with molecular weight 9 kDa are defined as non-digestible food ingredients.

In this research, the electrophoretic patterns and FTIR spectra were as supporting data. FTIR spectra of both SV-EE and SV-WE from sea cucumber flour are presented in Figure 6. Their FTIR spectra were similar to those of other sea cucumber, *Stichopus japonicus* (Zhu et al., 2012). The SV-EE using gastrointestinal enzyme was found to be the most efficient for degrading protein of *Stichopus variegatus* because of the cleavage of nearly all proteins in the high molecular weight region and generation of polypeptides, SV-EE fraction. The result of SV-EE was supported the FT-IR spectra

analysis and MTT assays compared to SV-WE. The major protein compound of sea cucumber was collagen that consisted of 100 kDa  $\alpha$ -chain with the molecular weight of and mucopolysaccharides (Saito, Kunisaki, Urano, & Kimura, 2002). The saponin of *Holothuria leucospilota* extract showed several functional groups, namely hydroxyl (OH), alkyl (CH), and ester ( $-C = O$ ) (Soltani, Parivar, Baharara, & Kerachian, 2014). These functional groups have anticancer activity against A549 cancer cell lines (Soltani et al., 2014).

This study also showed that functional groups were based on spectra analysis. Therefore, SV-EE and SV-WE could inhibit the proliferation of WiDr and T47D cancer cell lines. In this study, sea cucumbers were

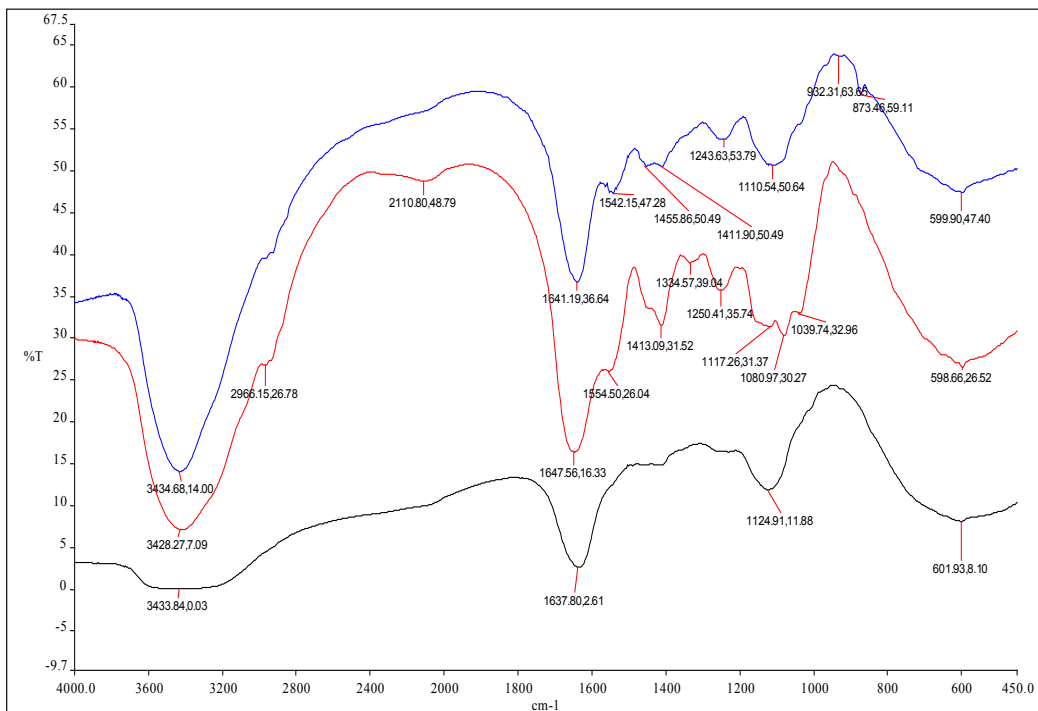


Figure 6. FTIR spectra of black line (sea cucumber flour), red line (SV-EE, *Stichopus variegatus*' flour enzyme hydrolyzed fraction), and blue line (SV-WE, *Stichopus variegatus*' flour water extraction fraction)

caught alive, immediately cleaned, frozen ( $-20^{\circ}\text{C}$ ) and dried using vacuum oven at  $50^{\circ}\text{C}$  for 4 h, which then caused chemical degradation of the tissues. Sea cucumbers that were being processed after post-mortem then dried in the oven at  $60^{\circ}\text{C}$  for 36 h did not show any tissue degradation (Chang-Lee, Price, & Lampila, 1989).

The results of this study indicated that sea cucumber (*Stichopus variegatus*) flour drying a vacuum oven can be hoped as a functional ingredient. The properties of the sea cucumber flour increased the interaction with components of cancer cells and enhanced anticancer activity (Guadalupe, Armando, & Josafat, 2012). The sea cucumber flour has great potential as an anticancer functional ingredient for cereals, soup, flavor, or biscuits although the flour drying was using a vacuum oven.

## CONCLUSION

The findings of this study determined for the new anticancer potential of sea cucumber (*Stichopus variegatus*) flour dried with vacuum oven. The presence of biological activity in the flour of *Stichopus variegatus* extract showed that their consumption could be advantage for healthy. In addition, these findings may facilitate awareness about the potential anticancer properties of *Stichopus variegatus* and help future developments of anticancer therapeutics on industrial scale. In addition, the enzyme-extract fraction of *Stichopus variegatus* flour could be further developed as a complementary cancer remedy, ingredients functional food, and nutraceutical.

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