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The Biological Activity of Batissa violacea celebensis Marten 1897 Extract towards the Proliferation of Lymphocyte Cells and Cancer Cells

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Abstract:

Marine molluscs and their bioactive compounds are of particular relevance to the growing pool of nutraceutical resources under global investigation. Pokea clam (*Batissa violacea celebensis* Marten 1897) is of bivalve from the *Batissa violacea celebensis* is one type of clam that lives in freshwater. *Corbiculidae* family originating from Pohara River, Southeast Sulawesi Province Indonesia. This study aims to conduct an exploration of lymphocyte cell proliferation activity, and identify the anti-proliferative even of Pokea clam extract in some A549 cancer cell lines, Hepatocarcinoma, and MCF-7 using *in vitro* method. The lymphocyte cells proliferation test and anti-proliferation test used the MTT reduction method using several concentrations, namely 10 µg/ml, 100 µg/ml, 500 µg/ml, and 1000 µg/ml. The result showed that the best result occurred at 1000 µg/ml. The three Pokea clam extracts, at the highest concentration of 1000 µg/ml, showed the increase in lymphocyte cells proliferation as immunostimulator. It is in line with the result in the antiproliferation activity in A549 cancer cells, Hepatocarcinoma, and MCF-7. From the activity, it can correlate with the dosage level (*dose-response relationship*). This extract sample had never been reported, including its potential biological function as a new immunostimulating and anti-cancer agent. This information may guide future health/nutraceutical clam product development.

Key-words: Pokea clam; Proliferation; Lymphocyte cells; Cancer cell line; *Biological activity*

Introduction

Currently, the demand for obtaining safe and non-toxic substances that can increase the cellular and humoral immune responses towards cancer is increasing. Immunostimulants, hemocyanins, glycoproteins (oxygen-carrier proteins), are widely found in Mollusks, and they are proven to be able to strongly trigger the cellular and Th1 humoral response towards certain cancer and have no toxic effects; therefore, they are ideal for long-term treatment[1]

In the past few years, opportunistic infection or complication frequently causes immune pressure related to stress that is difficult to be treated using antibiotics and it continues to be a challenge in the clinical sector[2]. Besides, most immunomodulatory medications are not appropriate for chronic use or prevention. Hence, the increased interest in identifying a new immunomodulator for improving the mechanism of non-specific host defense[2,3]

Cancer is a disease marked by the excessive, abnormal, and uncontrollable cell growth of body tissues (proliferation). The cancer cells penetrate the surrounding tissues (invasive) and continue to spread (metastasis). Cancer is classified into a degenerative disease that potentially causes mortality[4]. In some Asian countries, including Indonesia, cancer becomes the second cause of death after a heart attack and the mortality rate of cancer tends to increase annually. In Indonesia, the prevalence of cancer is quite

high; 4.3% per 1,000 residents suffers from cancer. In 2025, it was estimated that the new case for cancer in Indonesia increased by 49%[5].

Clam, among other mollusks, has been exploited for its bioactive properties[6-12]. Freshwater clam, *Batissa violeacea*, or usually known as Pokea clam, is a bivalve type from the *Corbiculidae* family is the edible clam mollusk, especially the clam found in Pohara River, Konawe Regency, Southeast Sulawesi[13].

Batissa violacea celebensis is one type of clam that lives in freshwater. In general, bivalves or better known as shellfish is a group of animals with no vertebrae. Pokea shell is classified by Kingdom: Animalia, Phylum: Molusca, Class: Bivalvia, Order: Eulamellibranchia, Family: Corbiculidae, Genus: Batissa, Species: *Batissa violacea celebensis* (Martens, 1897), and area name: Pokea Shells. Pokea shell is a type of bivalves that lives on the bottom of the water and has two shells that can open and close. The shell on the dorsal part is thick, and the ventral part is thin. The top of the eggplant is called the umbo and is the oldest part of the shell. A circular line around the umbo shows the growth of the shell. The mantle in the pelecypoda is shaped as a thin, wide tissue, covering the whole body located under the shell[13].

The clam can survive in both clean or polluted environments. If it lives in polluted water, it will have a specific defense system, including fighting against toxic and carcinogenic substances. Clam contains glycoproteins that play a role as an immunomodulator substance with high activity[14].

The use of an extract in medication can provide a risk to decrease the body's immune system. The effect of the extract in decreasing the body's immune system is unexpected in treating cancer[15].

In the past several decades, several studies on exploring the potency of marine biomass had been widely conducted. Biofunctional peptides are protein fragments of 2 to 20 amino acid residues, and they are correlated with some potential physiological functions, such as immunomodulators, anti microbes, antithrombotics, agonist opioids or antagonists, and anti-hypertension activity. Further, the anticancer activity of biopeptides from marine organisms, such as sponges, Ascidia, mollusks, and soft-shell clams, is isolated[16].

These days, the natural compounds extracted from marine mollusks are, at least, the four anticancer agents with different structures in the clinical trial at the class of polypeptides, terpene, steroids, and peptides. Marine biofunctional peptides can be made using one of the three methods, such as solvent extraction, enzymatic hydrolysis, and microbial fermentation of dietary protein[17].

The first thing to do is evaluating the in vitro inhibition effect of HSS in the growth and the activity of MMP-2 and MMP-9 in the human A549 cell line of lung carcinoma and NCI-H292, then verifying *in vitro* inhibition effect by using the A549 xenograft mouse model. The result of the observation, especially after *in vivo* verification, provides scientific evidence that HSS can be promising anticancer therapeutic agents after the result is reproduced in the clinical trial[18].

Investigated the cytotoxicity effect from three species of surf clam originating from New Zealand (NZ), namely Diamond clam, Storm clam, and Tuatua clam. Each of them is tested in several types of cancer, such as A549, Hep G2, MIA Paca-2, and WiDr. The cytotoxicity effect is collected from the four fraction types (ethanol, Petroleum ether, ethyl acetate, and water) collected from the three species of clam; it showed that the most significant result is seen from WiDr cells (Kolon)[12].

Most current studies are focused on natural sources with an effect that can modulate the immune system and it, further, can be used for decreasing the risk of several diseases, such as decreasing the inflammatory reaction up to cancer.

Due to the explanation above, it is important to surely know the effect of giving a certain extract/fraction from natural substances against the immune response through lymphocyte cell proliferation activity that can also inhibit the cancer cell proliferation. Therefore, this study aimed to conduct an exploration of lymphocyte cell proliferation activity and to identify the anti-proliferative activity of Pokea clam extract (endemic fresh water clam) in some A549 cancer cell lines, Hepatocarcinoma, and MCF-7.

Materials and Method

Materials. RPMI-1640 media (Gibco, the United States), sodium bicarbonate (Sigma-Aldrich, German), fetal bovine serum (FBS) (Gibco, the United States), pen-strep (Sigma-Aldrich, German), sodium dodecyl sulfate (SDS) (Merck, German), Tween 80 (Merck, German), MTT (3 - (4,5 - dimethylthiazole-2-il)-2.5-diphenyltetrazolium bromide) (Merck, German), Tris-buffered ammonium chloride (Merck, German).

Laboratory animals. The laboratory animal used in this study was a Sprague Dawley mouse at 8-12 weeks old with a weight of 250-350g collected from *Laboratorium Hewan Pusat Studi Satwa Primata*/PSSP (Animal Laboratory, Primate Research Center), IPB.

Sampling

Pokea clam was collected from Pohara River in Besu village, Pondoala sub-district, Konawe Regency, Southeast Sulawesi, exactly at the following coordinates: S= 03°56'15.5" and E= 122°24'42.6"

with around 9 meters in depth using a fish trap known as wire basket (*tangge*)[19]. Pokea clam was soaked in water for a day, then it was washed and the shell was separated from the clam; then, the clam was dried under the sunlight until the water level reached around 6%. After it was dried, Pokea clam meat was ground using a grinding machine.

Extraction Procedure

The extraction method used in this study was a multistep extraction[13]. It aimed to extract the components in the clam based on the polarity level, so unidentified characteristics of the bioactive component can be extracted optimally in one of the solvents that are used. Extraction was performed using a maceration method with a shaker for 24 hours. The solvents used here were methanol, ethyl acetate, and hexane p.a with a sample to solvent ratio of 1: 2 (b/v).

In Vitro Immunostimulant Test

Preparing the Medium of Cell Culture and the Sample of Pokea Clam Extract

The medium for cell culture and cell maintenance used RPMI-1640 by dissolving the powder in deionized water until reaching a volume of 1 (one) liter. Then, it continued by adding 2 grams of $NaHCO_3$, 10 ml of Glutamin 2 mM, and Penicillin-Streptomycin antibiotics of 0.2%; after that, the cold sterilization using a sterile membrane of 0.22 µm. If it is used as a growth medium, the 10% sterile FBS should be added to the medium composition[20].

Lymphocyte Cell Isolation

The SD mouse was anesthetized using ketamin to xylazine ratio of 1: 1 of 100 µL using the intraperitoneal method; then, the neck dislocation was performed (The recommendation of Ethical Approval Number: 820/UN4.6.4.5.31/PP36/2020 by the Ethical Committee of Medical Research, Faculty of Medicine, Universitas Hasanuddin). Further, the skin of the belly part was opened and its peritoneal sheath was cleaned using 70% alcohol. The spleen was taken and put onto a Petri dish with a diameter of 50 mm containing 10 mL of RPMI medium. The RPMI medium was pumped into the spleen until the lymphocytes also exited along with the medium. The cell suspension was put in a 10 mL centrifuge tube and it was centrifuged at 4 °C with 1.500 rpm for 10 minutes. The collected pellets were suspended in 5 mL of Tris-buffered ammonium chloride for lysing the erythrocytes. The cells were mixed until homogenous and set aside at room temperature for 15 minutes or until the color changed into yellow. Furthermore, the RPMI medium was added until reaching 10 mL and centrifuged at 4°C with 1.500 rpm for 10 minutes; then the supernatant was discarded. The collected pellets were cleaned twice using the RPMI medium for eliminating the available erythrocytes to obtain the white pellets. Afterward, the pellets containing lymphocyte cells were suspended using the complete RPMI medium. The cells were calculated using a hemocytometer to obtain 1.5 x 10^6 cells/mL. The activity of lymphocyte cells was ready to be tested and cultured in the CO_2 incubator at 37°C.

The Proliferation Test of Lymphocyte Cells using MTT Reduction Method

100 µL of lymphocyte cells (1.5 x10^6 cells/mL) was distributed to the 96-well microplates based on the treatment group and incubated for 24 hours in the CO_2 incubator in a stream element of 5% CO_2 at 37°C. After incubation, 10 µL/well of the tested sample was added with each concentration and re-incubated for 48 hours in an incubator in a stream element of 5% CO_2 at 37°C. After 48-hour incubation, each well was added with 10µL of solvent and 5mg/mL of MTT. Further, it was re-incubated for 4 hours at 37°C. The living cells would react with MTT to form formazan crystals. The reaction with MTT was stopped by adding 10% SDS solvent into 50µL 0.01N hydrochloric acid to each well. Then, the absorbance value was measured using a microplate reader with a wavelength of 550nm. The absorbance data was then converted into stimulation index (SI) data of lymphocyte cell proliferation based on the following equation.

$$\frac{Abs\ (sampel - kontrol\ medium)}{Abs\ (kontrol\ normal - kontrol\ medium)}$$

Anti-proliferative Activity Test[4]

The Preparation of A549 Cells, Hepatocarcinoma, and MCF-7

Then, sub-culture was applied to the cells that had grown in a confluent manner. The medium of cells was discarded and PBS of 10mL was added to clean the flask from the remaining medium, then the PBS was discarded. 5mL of Trypsin (0.125%) was added into a flask and incubated at 37°C for 5 minutes to release the cells from the growing area. The cells that had been released from the growing area were put into a 15mL-tube, then they were centrifuged of 500gr for 5 minutes and the supernatants were

$$Proliferation\ (SI) = \frac{\text{Absorbance (Sample} - \text{medium control)}}{\text{Absorbance (normal control} - \text{medium control)}}$$

discarded. The cell calculation was performed using a Hemocytometer, the cells were prepared based on the needs for a test. The cells were re-incubated in an incubator at 37°C and CO_2 5%.

Cell Calculation

50 ⌈l of cell solvent was added into 50 ⌈l of Trypan blue and then it flowed to a hemocytometer; after that, it was observed and the living cells were calculated (it did not absorb color) from two big boxes. The collected result was calculated using the following formula: cell per mL + mean calculated cells x dilution factor 10^4.

MTT Test

The cell line that had been grown in the T25 flask was sub-cultured, and then the cell was grown in the 96-well tissue culture plate with a total of 5000 cells/well and incubated for 24 hours in the growing medium at 37°C and CO_2 5%. The bioactive compound of 100 ⌈l/well at each concentration was added; the cells without any treatment were included as the control cells and they were re-incubated for 48 hours. The MTT compound was added and incubated for 4 hours at 37°C and CO_2 5%. The supernatant cells were discarded and the formazan crystal that had been formed was diluted with 96% ethanol. The Optic Density (OD) reading was performed using the microplate reader at a wavelength of 565nm.

Statistical Analysis

The collected data were analyzed using statistical analysis, namely, analysis of variance (ANOVA test), and it was continued to use Duncan's Multiple Range Test to see if there was no significant difference ($p<0.05$).

Result and Discussion

Rendement (yield) is the percentage of the weight of a part that can be used compared to the weight of the whole material. The part that is commonly used by people as food material is the meat. Based on the result of the rendement for Pokea clam meat, it obtained 27.8% on average. This rendement value is useful for knowing the economical value of a product or substance. If the rendement value of a product or substance increases, the economical value also increases and the usage can be more effective.

One of the parameters to see the immunomodulatory activity of a component was the capability to stimulate the lymphocyte cell proliferation. The proliferation process in lymphocyte cells was the maturation process and cell production through cell division or mitosis. The effect of Pokea clam extract on the lymphocyte cells proliferation of the mouse spleen at 4 different concentrations can be seen in Figure 1. It was tested to identify the comparison and the effect of Pokea clam extract on the lymphocyte proliferation of the mouse spleen using in vitro method.

The activity of these proliferated T and B lymphocyte cells can be measured using the Stimulation Index (SI) value. Mitogen was used for triggering non-specific proliferation of lymphocyte cells; in this case, the mitogenic activity of lipopolysaccharides (LPS) and Concanavalin A (Con A) was used as the control for B and T cell stimulation.

The data in figure 1 shows that Pokea clam can stimulate lymphocyte cells. The increased Stimulation Index (SI) of the three Pokea clam extracts was 3% to 63%. The ethanol extract at a concentration of 1000 µg/ml had a Stimulation Index (SI) value of 1.63 (an increase of 63%); the ethyl acetate extract at a concentration of 1000 µg/ml had a Stimulation Index (SI) value of 1.26 (an increase of 26%) exceeding the mitogenic activity of LPS with an SI value of 1.19 (an increase by 19%). The higher the extract concentration added into a culture, the higher the active component content. It can cause increased lymphocyte cell proliferation. All activities resulted from a concentration of 1000 ppm tended to increase the lymphocyte cell proliferation (immunostimulators) and no one had a characteristic that suppressed the lymphocyte cells. This shows that at a certain concentration, (the relatively higher concentration) it can provide a better stimulation that can be correlated with the higher concentration of a compound that contributes to the lymphocyte cells proliferation, so the activity of increasing proliferation is also higher[21].

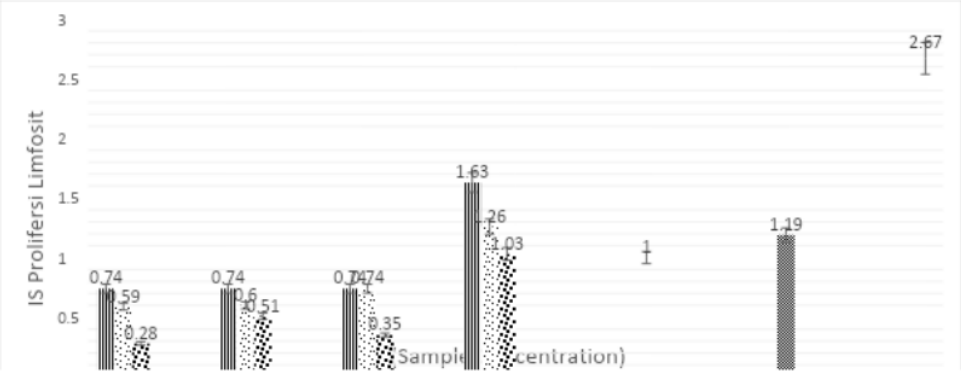

Figure 1. The Stimulation Index for the lymphocyte cell proliferation of Pokea clam extract (ethanol extract (EE), ethyl acetate extract (EEA), N-Hexane extract (ECH), K- = the control of RPMI 1640 medium, LPS = mitogenic activity of lipopolysaccharides, Con A = mitogenic activity of Concanavalin A. (*Error bar adalah standard error*)

The Capability of Clam Extract in Inhibiting the Proliferation of A549 Cancer Cells

The bioactivity testing of clam extract against several routes of cancer cells using the MTT method. The inhibiting capacity of clam extract was tested on A549 cancer cells, Hepatocarcinoma, and MCF-7. The A549 cancer cells were from the lung carcinoma of a 58-year man with a morphology that appeared similar to epithelial cells. Hepatocarcinoma or usually known as *Hepatocellular carcinoma* (HCC) is a type of liver cancer; liver cancer can begin from the hepar (hepatocellular cancer) or from other organs, such as colons, that spread to the liver (metastatic liver cancer), and the MCF-7 cell is one of the breast cancer cells; the cells are taken from a 69-year Caucasian woman's breast tissue with O type and positive Rh in the form adherent cells. The three types of cells are, morphologically, the epithelial cells with a polygonal shape that adheres to monolayer cells.

Giving clam extract with multistep dosage showed potency in the form of a decline in tumor cells that marked the anti-proliferation activity of clam extract to those three cancer cells. Freshwater clam (*Corbicula fluminea* Muller) and its active compound, namely FME (Freshwater clam extracted by methanol then partitioned off by ethyl acetate), significantly decreased the viability of cells in the three types of cancer cells out of the five tested human cancer cells based on the level of dosage[22]. The inhibiting activity of A549 cancer cells growth can be seen in Table 1 below:

Table 1. Antiproliferative activity of ethanol extract (EE), ethyl acetate extract (EEA) and Hexana extract (ECH) of Pokea shellfish against cancer cells A549

Antiproliferative activity A549 (%)			
Concentration (⌈g/mL)	Etanol	Etil Asetat	N-Hexana
	Mean ± Stdev	Mean ± Stdev	Mean ± Stdev
K1000	0.385 ± 0.062 [a]	0.372 ± 0.032 [c]	0.396 ± 0.030 [b]
K500	0.420 ± 0.049 [a]	0.404 ± 0.017 [bc]	0.419 ± 0.033 [ab]
K100	0.423 ± 0.039 [a]	0.431 ± 0.010 [b]	0.424 ± 0.035 [ab]
K10	0.491 ± 0.024 [a]	0.491 ± 0.029 [a]	0.463 ± 0.039 [a]
Control	0.477 ± 0.004 [a]	0.477 ± 0.004 [a]	0.477 ± 0.004 [a]
P-value	0.052	0.000**	0.050*

Note: Numbers followed by different letters in one column indicate a significant difference; **)Significant at the 1% level; *) Significant at 5% level

The data in Table 1 shows the antiproliferation against cancer cells in three groups of extract. The type of solvent affected the inhibition of proliferation. The inhibition of A549 lung cancer cells using the

clam extracts was ethyl acetate > Hexane extract > Ethanol extract. The higher inhibiting value was 22.01% at a concentration of 1000 µg/ml with ethyl acetate extract. Ethyl acetate of *Corbicula fluminea* extract had an antioxidant capacity and it is effective to inhibit the proliferation of human QBC939 cholangiocarcinoma cells with in vitro method[21]. The statistical analysis using the analysis of variance against the treatment of ethyl acetate extract and N-Hexane extract in A549 cells at a concentration of 1000 µg/ml showed a significant difference (P<0.05) among the treatment groups that were compared, while the treatment of ethanol extract in A549 cells did not show a significant difference. It can be assumed that the extracted bioactive component in ethanol solvent has a low value.

Capacity of Clam Extract in Inhibiting the Proliferation Cell of Hepatocarcinoma and MCF-7

The inhibiting activity of Hepatocellular carcinoma growth is sown in Table 2, Furthermore, the Inhibition of Hepatocellular carcinoma Proliferation with N-Hexane extract had the highest value of 29.25% at 1000µg/ml of the Hexane extract of clam. The statistical analysis was carried out, using the analysis of variance against the N-Hexane extract in the Hepatocellular carcinoma, at a concentration of 1000 µg/ml. It further showed that there was a significant difference ($P < 0.05$) among the treatment groups being compared. The inhibiting activity of MCF-7 growth is shown in Table 2, Furthermore, the inhibition of MCF-7 cancer cell proliferation with the N-Hexane extract of clam had the highest value of 8.71% at 500µg/ml and (Table 3). Also, the ethanol extract showed less inhibition effect on MCF-7 cancer cells. Moreover, this result was in line with the statistical analysis, which stated that there was no significant difference ($P > 0.05$).

Table 3. Antiproliferative activity of N-Hexana extract (ECH), of Pokea shellfish against cancer cells MCF-7 and Hepatoma

Concentration (∫g/mL)	MCF-7		Hepatoma	
	Mean	± Stdev	Mean	± Stdev
K1000	0.469	± 0.046[a]	0.376	± 0.100[c]
K500	0.468	± 0.041[a]	0.446	± 0.100[bc]
K100	0.502	± 0.022[a]	0.542	± 0.083[ab]
K10	0.497	± 0.011[a]	0.623	± 0.061[a]
Control	0.513	± 0.005[a]	0.531	± 0.004[ab]
P-value	0.317		0.026*	

Note: Numbers followed by different letters in one column indicate significant difference; *) Significant at 5% level

The inhibiting activity of Hepatocellular carcinoma growth is show N-Hexane extract in the Hepatocellular carcinoma, at a concentration of 1000 µg/ml. It further showed that there was a significant difference ($P < 0.05$) among the treatment groups being compared. Providing the N-Hexane extract of Pokea clam with a multistep dosage, produced an effect in the form of a decrease in the number of cancer cells, which showed the availability of antiproliferation activity in the extract, against the Hepatocellular carcinoma. Similar result was also stated in a study[12], which showed that the A549 and Hep cells at a concentration of 1000 µg/ml had a lower antiproliferation percentage, compared to the WiDr (colon cancer cells) and MIA PaCa-2 (pancreatic cancer cells) cell types.

The inhibiting activity of MCF-7 growth is show this result was in line with the statistical analysis, which stated that there was no significant difference ($P > 0.05$). Similar observation was conducted[22], which indicated that the breast cancer cells were not affected by the treatment of *Corbicula fluminea* extract at a concentration of ≤ 250 µg/mL. A similar process was also conducted in a study[20], which stated that there is no significant difference in MCF-7 cells. The results of the study on New Zealand surf clam extract (storm shell and Tua-tua with ethyl acetate and petroleum ether solvents at a concentration of 500 g/mL also showed insignificant results. However, the different antiproliferative activities in those extracts were caused by the various membrane receptors and cancer cell lines[4] or that lower concentrations are simply ineffective, thereby promoting cell growth, and resulting in treatment resistance in the cells. Another possible reason might be that at lower concentrations, the cells activate pro-survival pathways, which are shut down at higher concentrations[12].

The results indicate that clam extract exhibited great suppression on cell viability especially in the A-549, and Hepatoma cell lines at 500 and especially 1000 µg/mL. The results also show that Pokea clam extracts exhibited a broad spectrum of cytotoxicity against different cancer cells under identical conditions.

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The result of active compound identification showed that the crude extract of dried Pokea clam in the non-polar (hexane), contained all active components that were analyzed, except for that of the peptide. In the semi-polar (ethyl acetate), no saponins, carbohydrates, peptides, and free amino acids were discovered. Also, in the polar (ethanol), only phenol and peptides were not identified. The purified extract is potentially more effective at targeting and eliminating cancer cells, than the unrefined extract[12].

Therefore, it was assumed that the different types of this bioactive component were due to the various polarity of each solvent[23], that there was a strong correlation between phenolic content and anti-proliferative activity[24].

Flavonoids and triterpenoids are known to have a vital role in chemoprevention and chemotherapy for cancer. These compounds have an antioxidant activity with a capability of Reactive Oxygen Species (ROS), inhibiting the enzyme involved in the ROS formation, and blocking the cellular and extracellular oxidizing agents[25,26]. Besides that, flavonoids played a role in the modulation route of cancer proliferation, cell cycle, apoptosis induction, and angiogenesis inhibition[25, 27].

Reported that[28] the cytotoxicity source was potential, due to the high antioxidant capacity and the total phenolic content. Moreover, [28]stated that the cytotoxicity of crude extract was possibly caused by the higher potential antioxidant or the synergic effect of some bioactive compounds in the product. The cytotoxicity activity likely affects and disturbs the fundamental mechanism, which is related to cell growth, mitosis activity, differentiation, and functions[27]. Also, these compounds were reported to have antioxidant activity, with a capacity to scavenge in Reactive Oxygen Species (ROS)[25,26].

Flavonoids also had a potent anti-cancer mechanism, with that of the abnormal tyrosine kinase inhibitors. Tyrosine kinase was the enzyme playing a role in signal transduction (cell signaling), by regulating the continuation of the cell cycle & transformation, transcription regulation, proliferation, differentiation, and apoptosis. However, Oncogene FES transforms the kinase activity and cause the formation of leukemia[26].

The strong cytotoxicity activity from some active compounds above the cell line, which were derived from cancer, were active in the same targeted enzymes[27]. Therefore, this study showed that Pokea clam extract tended to induce the apoptosis derived from cancer cells. Moreover it is assumed that the inhibition of cancer cells was due to flavonoids and phenol contained in the clam extract, which in turn affects the cell cycle, causing non-proliferation. Also, cancer cells in the proliferation cycle were sensitive to the effects of anti-tumor compounds. Generally, the cytotoxicity compounds likely function, by damaging the enzymes or substrates, which are related to synthetic DNA[28]. Therefore, the bioactive compounds involved in the clam are inhibited in the parent cells or synthetic DNA.

Conclusion:

From the three Pokea clam extracts, the highest concentration of 1000 µg/ml showed an increase in lymphocyte cell proliferation (immunostimulator). Also, the lower concentration suppressed lymphocyte cells, which was in line with the result of the antiproliferation activity in A549, Hepatocarcinoma, and MCF-7, which also correlated with the dosage level (dose-response relationship). Therefore, the Pokea clam extract was confirmed to possess the potential substance, which enables it to be a functional food.

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