

Cytotoxicity activity oil of *Anguilla marmorata* (Q.) Gaimard<sup>1,\*</sup>Jamaluddin, <sup>1</sup>Fadriani, <sup>1</sup>Ihwan and <sup>2</sup>Herwin<sup>1</sup>Department of Pharmacy, Mathematics and Natural Science of Faculty, Tadulako University, Palu, Central Sulawesi, Indonesia<sup>2</sup>Department of Microbiology, Faculty of Pharmacy, Universitas Muslim, 90245 Makassar, Indonesia**Article history:**

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**DOI:**[https://doi.org/10.26656/fr.2017.7\(2\).785](https://doi.org/10.26656/fr.2017.7(2).785)**Abstract**

Eel fish (*Anguilla marmorata* (Q.) Gaimard) contains unsaturated fatty acids such as omega-3 and omega-6. Furthermore, EPA and DHA are forms of omega-3 and are known to treat various diseases such as atherosclerosis, cancer, inflammation, heart disease, stroke, lupus, hypertension, impaired growth and intelligence, diabetes, and act as antifungal agents. This study aimed to determine the cytotoxic activity of eel fish oil extract against *Artemia salina* leach shrimp larvae using the *brine shrimp lethality test* (BSLT) method. It also aimed to determine the lethal concentration 50 (LC<sub>50</sub>) value of the oil, which is the concentration that could cause the death of 50% of the test animal population. The cytotoxicity testing was carried out using the larvae of *Artemia salina* leach shrimp larvae aged 48 hrs and eel fish oil extract at concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm with five repetitions. Furthermore, the cytotoxic effect was observed by calculating the number of shrimp larvae mortality, while the probit analysis was used to determine the LC<sub>50</sub> value of the oil. The results showed that the eel fish oil extract had the highest bioactivity against *Artemia salina* leach shrimp larvae at concentrations of 200, 250, 300, 350, 400, 450, and 500 ppm, respectively, and its LC<sub>50</sub> value was 232.6802 ppm, which is <1000 ppm. Therefore, it was concluded that the oil is toxic to *Artemia salina* leach shrimp larvae and has the potential to be further investigated as an anticancer agent.

**1. Introduction**

Cancer is one of the leading causes of death in the world and is a complex disease involving the growth of abnormal or unusual cells known as malignant tumours. According to Mangan (2013), cancer cells would continue to divide even when not required by the body, leading to a buildup of new cells. The accumulation of these cells destroys normal tissue and interferes with the functioning of the diseased organs.

The latest report by the International Agency for Research on Cancer, World Health Organization (WHO), estimated that there were 18.1 million new cases and 9.6 million deaths in 2018. It was also estimated that cancer would be the number one cause of death in the world at the end of this century. This estimate was obtained after data on 36 types of the disease from 185 countries worldwide was analyzed. Some types of cancer include lung, colorectal, stomach, liver, and breast cancer (Ramadhan and Kurniawati, 2020).

The American Cancer Society provides updates on the incidence of colorectal cancer based on several data. In 2020, approximately 147,950 people were diagnosed with the disease, and 53,200 deaths occurred, including 17,930 cases and 3,640 deaths in individuals under 50 years of age (Siegel, 2020). Every year the number of cancer sufferers in the world increases by 6.25 million, and two-thirds of the patients are in developing countries, including Indonesia. Data from the Ministry of Health shows that the number of cancer sufferers in Indonesia reached 6% of the population. Furthermore, based on Riskesdas data, the prevalence of tumour/cancer in the country showed an increase from 1.4 per 1000 population in 2013 to 1.79 per 1000 population in 2018. The highest prevalence was in DI Yogyakarta province at 4.86 per 1000 population, followed by West Sumatra at 2.4779 per 1000 population and Gorontalo at 2.44 per 1000 population (Ministry of Health, 2019).

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Current cancer treatments, such as chemotherapy, radiotherapy, and surgery, induce unintended side effects that endanger the health and well-being of patients (Razanto *et al.*, 2014). Therefore, in the last six years, the National Cancer Institute (NCI) has recorded some health documents and facts about nutraceuticals in cancer treatment. Great efforts have also been devoted to research on the cytotoxic effects of nutraceutical active components on the proliferation of cancer cells, reduction of metastasis, inhibition of apoptosis, and reduction of necrosis and malignant growth rates at an early stage.

Apart from plants, nutraceutical treatment could also be obtained from animals such as fish, which is one of the animals that have the potential to be developed as natural medicinal ingredients. Fish contains very good and prospective protein, fat, vitamins, and minerals. Moreover, the fat it contains is generally polyunsaturated fatty acids, such as omega-3.

Natural fatty acids include omega-3, linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), while the more dominant types in fish oil are DHA and EPA (Panagan *et al.*, 2011). Quality fish oil is fish oil that is rich in fatty acids that are beneficial for health. Omega-3 is one of the essential unsaturated fatty acids for the body, and EPA and DHA are its most dominant types. Therefore, several studies examining the role of either of the oils in treating various dangerous diseases have been carried out. Additionally, Wibawa *et al.* (2006) cited, their importance as inhibitors of atherosclerosis, cancer, inflammation, heart disease, stroke, lupus, hypertension, growth and intelligence disorders, diabetes, and antifungal agents.

Polyunsaturated fatty acids (PUFA) omega-3 could weaken cell growth and induce apoptosis in various types of cancer cells, such as the colon, pancreas, prostate, and breast cancer cells. The metabolic effect could also decrease angiogenesis, suppress endothelial cell proliferation, decrease small blood vessel density, and tumour growth (Wendel and Heller, 2009). A study by Yang *et al.* (2014) recommended administering omega-3 fatty acids together with chemotherapy drugs as the oil could also be used to increase tumour radiosensitivity. In cancer patients with cachexia, pro-inflammatory status could be modulated by suppressing the cause of inflammation and releasing pro-inflammatory mediators such as cytokines and prostaglandins through preparations derived from fish oil, thereby enabling anabolic effects. The mechanism underlying the effects is the incorporation of omega-3 fatty acids into biological membranes, which leads to the modulation of the expression of proteins involved in cell

cycle regulation and apoptosis, such as Bcl-2, Bax, and c-Myc.

In Sulawesi, fishes such as eels with a high content of omega-3 have been shown to possess the potential to weaken cancer cells. According to Ndobe (2010), rivers in Central Sulawesi generally contain eel populations, Poso Lake, and the estuary of Palu Bay. The high level of consumption of eels cannot be separated from their high nutritional content. They include sufficient amounts of protein, vitamins, fatty acids, and microelements. The vitamin content in eels includes vitamins A, B1, and B2, as well as microelements such as Zn. In addition to vitamins and microelements, fish also contain unsaturated fatty acids needed by the body, including omega fatty acids, which are useful for the development of children's brain cells. The number of unsaturated fatty acids in the fish is shown to be several times that of beef, while the number of the microelement Zn is also nine times more than the amount in cow's milk (Ndobe, 2010). Eel marrow extract also contains several types of beneficial ingredients, such as DHA, and EPA. These compounds are unsaturated fatty acids that could reduce blood fat in the human body (Roy, 2013).

Several studies related to the analysis of the fatty acid content of freshwater fish have been carried out. An example is a study conducted by Jamaluddin *et al.* (2018) on the nutritional content of eel, which discovered that the Palu river eel and Poso lake contain multiple unsaturated fatty acids. They include Polyunsaturated fatty acids (PUFA), omega-3, and omega-6, which are essential fatty acids that cannot be synthesized by the body and have great health benefits. PUFAs (linoleic and linolenic fatty acids) play an important role in fat transport and metabolism, immune function, and the maintenance of cell membrane function and integrity. Moreover, Natural fatty acids including omega-3 fatty acids are linolenic acid (C18:3,w-3), EPA (C20:5,w-3), DHA (C22:6,w-3), while omega-6 is linoleic acid (C18:2,w-6) and arachidonic acid or ARA (C20:4,w-6) while the more dominant in fish oil are DHA, ARA and EPA (Panagan *et al.*, 2012).

Compounds suspected of having anti-cancer activity need to be tested first on experimental animals. One method that is widely used in this cytotoxicity test is the brine shrimp lethality test (BSLT). This is because apart from being fast and cheap, this test is considered quite easy to carry out. Several bioactive compounds that have been studied and isolated showed a correlation to a specific anti-cancer test. Therefore, when a compound is found to be toxic, further research could be carried out to isolate the toxic compound as an alternative anti-cancer drug (Harmita, 2008).

Toxicity testing is a test method used to determine the toxic level of a compound. The cytotoxicity test with the BSLT method is often associated as a reference or basis for anticancer testing, therefore, the test in the study by Purwanto *et al.* (2015) was carried out on the shrimp larvae using the BSLT method. This testing method was also used in the research by Antonius *et al.* (2018) to search for anti-cancer compounds.

The higher the level of toxicity of a compound, with a smaller LC<sub>50</sub> value, the more potential the compound is to be used in anticancer treatment. From the research by Wibawa *et al.* (2006) on the toxicity test of mackerel oil extract using the BSLT method, it was stated that the fish oil extract provided toxic (cytotoxic) properties to *Artemia salina* leach shrimp larvae cells. Meanwhile, an extract of natural ingredients that has an LC<sub>50</sub> value in the toxicity test has the potential as an anticancer agent. The LC<sub>50</sub> value could be calculated by probit analysis.

Based on the description above, it could be seen that the eel contains fatty acids, omega-3 and PUFA, which have the potential as anti-cancer agents. Therefore, it was necessary to research the cytotoxicity test of the eel using the BSLT method in order to determine the cytotoxicity of cells (cytotoxic) of eel fish oil extract using *Artemia salina* leach shrimp larvae as a bioindicator.

## 2. Materials and methods

### 2.1 Materials

The tools used were dropper, ruler/ruler, scissors, hotplate, thermometer, stirring rod, tube rack, blender, scalpel, porcelain cup, water bath, tweezers, measuring cup (Iwaki Pyrex®), glass chemical (Iwaki Pyrex®), and volumetric flasks (Iwaki Pyrex®). Furthermore, soxhlet apparatus, oven, aquarium, 10 mL pycnometer, Buchi rotary evaporator, 40-watt light bulb, vial, analytical balance (Ohaus), aerator, and several glassware commonly used in laboratory research were also used. The main ingredients used are eel fish (*Anguilla marmorata* (Q) Gaimard) in the silver eel phase, diethyl ether, *Artemia salina* egg hatching media, mineral water and crystal salt, tween 80, span 80, distilled water, 70% ethanol, aluminium foil, universal pH paper, and filter paper.

### 2.2 Processing of sample

#### 2.2.1 Sampling technique

The sampling technique used was purposive sampling and was carried out by selecting a sample among the population following the inclusion criteria, such as weight, size, and location (Hardani *et al.*, 2020). Moreover, the eels selected were those weight 200-600

grams and length 30-60 cm. Finally, the location of the selection was Lake Poso, Central Sulawesi.

#### 2.2.2 Sample preparation

The sample preparation stage began with washing the samples to get rid of blood and mucus, then cutting them into small sizes. Furthermore, the samples were dried using an oven at 60°C for 24 hrs and when dry, they were blended to powdered form, and then stored at room temperature (26°C) in a container (Jamaluddin *et al.*, 2019).

#### 2.2.3 Extraction of sample

A total of 50 g of the sample was put into a fat sleeve, and then 150 mL of diethyl ether was added to a round bottom flask. Moreover, the extraction process was carried out at 60°C for 5 hrs. The mixture of fat and solvent in a round bottom flask was evaporated until all the solvent evaporated. The evaporated extract was then put into an oven at 105°C for 1 hr. Furthermore, the fat was cooled in a desiccator for 30 mins and the round bottom flask containing fat was weighed until a constant weight, which is the weight of the material that would not decrease or remain after being put in the oven, was achieved (Handayani, 2020).

### 2.3 Extracted crude oil purification

Approximately 3% bentonite was added to the extracted crude oil and then the mixture was homogenized using a magnetic heater stirrer at a temperature of 29°C for 20 mins. Furthermore, the homogenized mixture was then centrifuged at 6500 rpm and at a temperature of 10°C for 10 mins to obtain the pure oil.

### 2.4 Density test and specific gravity of fish oil extract

The density of the eel fish oil extract was obtained using the pycnometer method by first weighing the empty pycnometer and then the pycnometer containing the fish oil. Moreover, the density of the fish oil substance was its mass divided by the volume of the pycnometer, with 3 repetitions. While the density of the fish oil was the density obtained from previous measurements divided by the density of distilled water at 25°C (room temperature).

### 2.5 Testing process

#### 2.5.1 Artificial seawater

The artificial seawater was manufactured by dissolving 20 g of salt in 880 mL of distilled water, and then the pH of the water was measured until it reached 7-8.5. The water was then filtered using filter paper.

### 2.5.2 *Artemia salina* leach shrimp larvae hatching

The hatching was carried out by soaking 100 mg of shrimp eggs into 500 mL of artificial seawater, which was illuminated with a 40-watt lamp, in order that the hatching temperature was maintained at 26°C. Moreover, the eggs were left for 48 hrs until they hatched into mature nauplii that were ready for use. Finally, the larvae were taken with a pipette and put into a petri dish containing artificial seawater (Wibawa et al., 2006).

### 2.5.3 Control setup

The negative controls were made, and the first was saltwater without adding the extract, while the second was made by adding 80% span and tween respectively, without adding the extract. Finally, 5 mL of artificial seawater and 10 *Artemia salina* leach shrimp larvae were added to the vial (Wibawa et al., 2006).

### 2.5.4 Preparation of sample solution

The eel fish oil extract was weighed and was 0.0848 g/10 mL. Then the extract was emulsified using 0.0848 g of 80% span and 0.254 g of 80% tween as an emulsifier. The manufacture of the emulsion began with the division of 2 phases, where the aqueous phase consisted of water and 80% span, while the oil phase consisted of eel extract and 80% tween, each of which was stirred first using a magnetic stirrer for 10 mins. Then the water phase was added to the oil phase and stirred again for 10 mins. Once the emulsion was formed, it was put in a 10 mL volumetric flask as a stock solution. Before the testing, orientation was conducted to determine the concentration levels used in this study. The tested extracts were made in concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm.

The fish oil extract cytotoxicity test was carried out by adding 10 healthy 48-hour-old (actively active) *Artemia salina* leach shrimp larvae selected at random, into a vial using a dropper. In addition, 1 mL of artificial seawater was added and the mixture was put into a diluted vial. From the stock solution of 10,000 ppm, dilutions were carried out to make concentrations of 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 ppm. It was carried out by pipetting from the stock solution into vials of 25, 50, 75, 100, 125, 150, 200, and 250 L that already contained *Artemia salina* leach shrimp larvae, using a micropipette. Then, 5 mL of seawater was added to the mixture. The vial was then placed under a lamp for 24 hrs, and after that period, the number of live larvae was counted with the help of a flashlight. Furthermore, the LC<sub>50</sub> value was obtained from the treatment and control. The controls were used to compare the causes of death of the larvae. Finally, the data obtained from the

observations were calculated using probit analysis to obtain the LC<sub>50</sub> value.

### 2.5.5 Observation of toxicity to shrimp larvae

Observation of potential bioactivity was carried out based on the Lethal Concentration value of 50% (LC<sub>50</sub>), which is the value that indicates the concentration of toxic substances that could cause the death of up to 50% of an organism. When the LC<sub>50</sub> is < 1000 ppm, the extract is very toxic and has the potential to contain anticancer bioactive compounds.

### 2.6 Data analysis

The analytical technique used to test the mortality of the shrimp larvae was based on the lethal concentration value of 50% (LC value), which was determined through probit analysis using Microsoft excel. Moreover, probit analysis is used to perform stimulation with random number generators. For various distributions, you could subset, merge and rearrange, transform or use database query techniques easily. Finally, the effectiveness of the extract against the leach shrimp larvae was expressed in LC<sub>50</sub> (ppm) 24 hrs after treatment (Tekha and Akkas, 2016).

## 3. Results and discussion

The sample used in this study was eel silver phase oil. Eels are often found in the waters of Central Sulawesi and have been proven to contain EPA and DHA, which could overcome dangerous diseases in the body, including inhibiting atherosclerosis, cancer, inflammation, heart disease, stroke, lupus, hypertension, growth and intelligence disorders, diabetes, and fungal growth (Wibawa et al., 2006).

Sampling was carried out in the Poso lake area, Central Sulawesi, Indonesia because the estuary of the Poso River to Lake Poso is a habitat for eels. According to Krismono and Putri (2012), these two water bodies have a fairly high potential for eels, as adults caught from Lake Poso to the sea range from 0-323 fishes per month or an average of 138 fishes per month. The type of eel selected was adult *Anguilla marmorata* (Q.) Gaimard, because they have more fatty acid content, weigh 200-600 g, and are of lengths 30-60 cm.

Eels in the silver phase have a high fatty acid content, therefore, 3 kg of the fish could produce as much as 200 mL of oil (Faoziyah, 2018; Jamaluddin et al., 2019). The sample was heated using an oven at a temperature of 60°C for 24 hrs to remove moisture, and its dry weight was determined. Additionally, the heating could extend the sample's storage time in order that it is not overgrown with microorganisms. The dried sample

was then mashed into a coarse powder using a blender to expand the surface because the greater the surface contact of the sample particles with the solvent, the more penetration of the solvent into the sample (Husni, 2018).

In this test, the components separated by extraction are solids from a solid-liquid system (soxhletation). Moreover, the method is the most effective and efficient way to determine the oil or fat content in the material, because the solvent used could be recovered and the time it uses is relatively short. The principle of fat analysis using the soxhlet method is to extract fat from the material using an organic solvent. A lipid is defined as an organic compound that occurs in nature and is insoluble in water, however, soluble in non-polar solvents (Fitriana and Fitri, 2019).

The soxhletation extraction process was carried out using diethyl ether which is a non-polar solvent, therefore, it was suitable for extracting the fish oil or fat which is also non-polar. In addition, the solvent used was based on the results of previous research. Jamaluddin *et al.* (2021) found that the soxhletation method for eels using the diethyl ether solvent and a temperature of 60°C for 5 hrs was the most optimal. Therefore, the temperature used for extraction in this study was 60°C for 5 hrs. The solvent was then separated from the extract by evaporation until it was almost completely evaporated or was almost considered non-existent. This was following the research by Nurjanah *et al.* (2014), which stated that the difference in temperature ratio had a significant effect on the percentage of fish oil yield from catfish skin. High temperatures cause damage to the cell wall, leading to its easy breakage, including easy penetration by oil or fat. Therefore, the oil would easily come out and increase the level of extracted fish oil (Wildan, 2012).

From Table 1, the per cent (%) yield of eel fish oil was 73.3462%. Moreover, yield is the ratio of the dry weight of a product to the weight of the raw material (Senduk *et al.*, 2020). Nurhayati *et al.* (2009) reported that the high yield value indicates a large number of contained bioactive components. Moreover, Budiyanto (2015) reported that the higher the yield of the extract, the higher the content of substances that are attracted to the raw material. Therefore, the yield value serves to determine the effectiveness of the extraction technique used for the number of samples needed to produce a certain amount of extract.

Table 1. Yield of eel fish oil

Temperature (°C)	Sample Weight (g)	Solvent	Empty Pumpkin Weight (g)	Pumpkin Weight + Oil (g)	Yield (%)
60	50	Diethyl ether	120.108	152.4633	73.3462

The fish oil obtained from the extraction procedure needs to be purified to be used optimally. This is because crude oil from industrial sampling and extraction still contains some impurities, both dissolved and insoluble. Impurity components include water, free fatty acids that react to form coloured compounds that could give a bad colour to the oil, and free metals such as Fe, which could accelerate the colour change of the fish oil (Rashid, 2003). These impurity components could cause a decrease in the quality of the oil, including the presence of an unwanted dark colour (Andhiarto and Wijaya, 2018).

Table 2 shows that the average weight loss of the fish oil that has been purified with 3% bentonite was 4.3178 g from 36.6731 g of extracted samples. Moreover, the resulting colour was clearer than before the purification, and this proves bentonite has the potential as an adsorbent. This is following the research results of Andhiarto and Wijaya (2018), that purification of the best-laying fish oil at a concentration of 3% produces fish oil with better quality characteristics.

Table 2. Results of Refining eel (*Anguilla marmorata* (Q) Gaimard) fish oil

	Before Purification (a)	After Purification (b)	$\Delta$ (a-b)
Weight (g)	36.6731	32.3553	4.3178
Volume (mL)	43.246	38.154	5.0917

After purification, the density of the extract was tested to determine its specific gravity. Moreover, specific gravity is a quantity that expresses the ratio between the mass (g) and volume (mL), thus, its unit is g/mL. While density is the ratio between the density of the sample and the density of distilled water, therefore it has no units (Januarti, 2016). Determination of the specific gravity of the oil was carried out using the pycnometer method. Moreover, the density of the oil used was its density obtained from previous measurements divided by the density of distilled water at 25°C (room temperature). The purpose of determining the density in this study was to determine the specific gravity of the extract. Meanwhile, the purpose of testing the density of the species was to determine the specific gravity of the extract, and because it could facilitate weighing at the time of testing.

This study is the first time where the density of eel fish oil was ascertained using the pycnometer method. Therefore, the finding was a novelty that has not been discovered in previous studies. It could be seen in Table 3 that the results of the density test and the specific

gravity were obtained at 0.848 g/mL. Therefore, the weight of this type is quite low compared to the specific gravity of fish oil in general. According to Defandi (2015), the specific gravity of tuna fish oil extract at room temperature is 0.88 g/mL. Finally, different specific gravity values could be caused by differences in

Table 3. Test results of density and specific gravity of eel fish oil extract using a pycnometer

Volume of fish oil pycnometer	Empty pycnometer	Empty pycnometer + Sample	Average RJ	Average BJ
	16.48	24.90	0.848 g/mL	0.848
10.0 mL	16.02	24.63		
	16.17	24.60		

the samples used.

RJ: specific weight, BJ: specific gravity

The cytotoxicity test was carried out using the BSLT method and was intended to determine the potential of the fish oil extract as a toxic agent. Moreover, the parameter used to indicate the toxicity of the extract was its LC<sub>50</sub> value, with regard to the mortality of *Artemia salina* leach shrimp larvae. The LC<sub>50</sub> value is a value that indicates the concentration of a test substance that could cause the death of 50% of the population of test animals after 24 hrs of treatment. Therefore, by determining the value, the initial screening of the active compound would take place relatively quickly and at a relatively low cost. This is because only extracts or compounds that have anticancer activity based on the BSLT method could then be confirmed for their anticancer effects on cancer cell cultures (Harli and Safitri, 2016). When the LC<sub>50</sub> value using the BSLT method on the extract is toxic, the extract could be developed as an anticancer drug (Carballo and India, 2002).

Research with *Artemia salina* leach shrimp larvae has been used by the Purdue Cancer Center, Purdue University at Lafayette to ascertain the toxicity of plant-active compounds in general and not specific for anticancer substances. However, the significant relationship between toxic samples to the larvae showed that it could be used for cytotoxic tests. Research using *Artemia salina* leach shrimp larvae has several advantages, including being fast, cheap, easy, and simple.

The process of hatching the *Artemia salina* leach shrimp larvae eggs was carried out by immersing the eggs in artificial seawater. Meanwhile, the initial step in preparing shrimp larvae is making artificial seawater by adding 20 grams of salt in 880 mL of water. This is because when the level of salt is high, the eggs would not hatch. Another factor that affects the growth of *Artemia salina* leach shrimp larvae is temperature, therefore, the temperature in this study was 26°C. At temperatures of 25-28°C, hatchability is more because this range of

temperatures is optimal. Below 25°C cysts would hatch more slowly, while above 33°C, the metabolism of the cyst is irreversibly stopped. Moreover, the effect of more extreme temperatures on the hatching media is that most cysts would not hatch (Bahari et al., 2014).

The pH of the water in the rearing media was found to be suitable for hatching, with a value of 7, which was in the optimum range of 7–8.5. Moreover, at this pH, the hatching enzymes worked optimally, while at <7, adult *Artemia salina* cannot grow optimally, and the growth of nauplii decreases (Bahari et al., 2014). Sufficient light was produced using artificial light with the help of lamps, while food supplies and oxygen were also available, therefore in the hatching process, the media was given air using an aerator that produces air bubbles. The aerator functions as a supplier of oxygen and keeps the eggs from settling. The hatchery was lit to stimulate hatching and an aerator was also installed to provide oxygen to the eggs that would hatch into larvae. Moreover, good larvae would swim towards a bright room because they are phototropic. Finally, the larvae would be ready for use in testing after 48 hrs because at that age it grows very fast, therefore, it is assumed as abnormal cell growth such as cancer cells (Harli, 2016).

Sample testing was carried out by inserting the larvae into a vial that had been diluted from the stock solution. The dilution was carried out according to the concentration that has been determined from the results of the previous orientation. The purpose of the orientation was to determine the concentration range that would be used in the test. The vial containing the larvae and extract was placed under a lamp. After 24 hrs the number of live larvae was counted with the help of a flashlight. Furthermore, the LC<sub>50</sub> value was obtained from the treatment and control. The controls were used to compare the causes of death of the larvae. The data obtained from the observations were calculated using probit analysis to obtain the LC<sub>50</sub> of the extract. This is because this analysis could estimate the effective dose by determining the concentration of death (Kumalasari, 2015).

Figure 1 shows the toxicity test graph for eel fish oil extract using the BSLT LC<sub>50</sub> method above, the equation  $Y = 6.7722x - 11.028$  was obtained, therefore, the LC<sub>50</sub> value was 232.6802 g/mL. Moreover, the LC<sub>50</sub> value was obtained from the results of the straight-line equation using the Microsoft office excel application. The results of this study were still classified as very toxic because they were below the value of <1000 g/mL (Table 4). In this study, it was found that the oil extract had acute toxicity potential. This is related to the compounds contained in the extract, which at certain levels have the

Table 4. The results of the toxicity test of eel fish oil extract using the BSLT method

Concentration (ppm)	Concentration Log (X) (ppm)	Probit (Y)	Percentage of Deaths (%)	LC <sub>50</sub> (ppm)	Regression Equation
50	1.699	0.00	0	232.68	$y = 6.772x - 11.028$
100	2.000	3.59	8		
150	2.176	3.82	12		
200	2.301	4.36	26		
250	2.398	5.15	56		
300	2.477	5.05	52		
350	2.544	5.64	74		
400	2.602	6.75	96		
450	2.653	6.75	96		
500	2.699	8.09	100		

potential for acute toxicity and could cause the death of the *Artemia salina* larvae. According to Meyer *et al.* (1982), an extract is said to be toxic to *Artemia salina* leach shrimp larvae if it has an LC<sub>50</sub> value <1000 µg/ml, and is said to be non-toxic if the LC<sub>50</sub> value is >1000 g/ml. Several studies have shown that fish oil, such as unsaturated fatty acids, has acted as an anticancer drug which can be proven by using the BSLT method, but this method has drawbacks such as the results of observations that cannot show abnormalities in the development of test animals, further research is needed to see the effect of compounds contained in the sample.

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## Probit Analysis and LC<sub>50</sub> Calculation

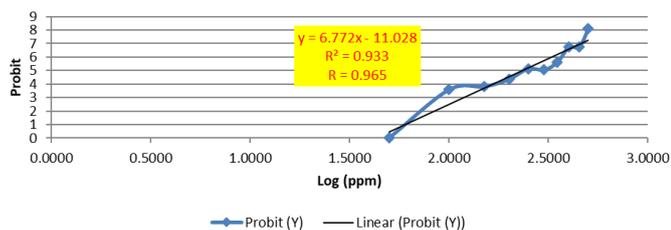


Figure 1. Graph of toxicity test of eel fish oil extract using the BSLT LC<sub>50</sub> method

## 4. Conclusion

Based on the results of this study it could be concluded that the oil extract of eel fish had toxic activity against *Artemia salina* leach shrimp larvae and the extract also had a high toxicity activity with a value of 232.6802 g/mL, therefore, it was included in the category of very toxic, and has the potential to be developed as raw materials or alternative products in anticancer treatment.

## Conflict of interest

The authors declare no conflict of interest.

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