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Lethality Test of Some Varieties of Sea Cucumber Extract Against Artemia Salina, as Natural Food Additive in Meat-Based Functional Food

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A B S T R A C T

Cancer is the main cause of death in the world with the number of sufferers in 2008 as many as 12.7 million with a death rate of 7.6 million people. In Indonesia, it is estimated that there are more than 1 million cancer patients in 2010. One of the ways to prevent cancer is primary prevention by adjusting the pattern and type of food consumed. Functional food introduced by bioactive components from sea cucumbers is an alternative for cancer prevention. The specific objective of this research was to produce meat-based functional foods containing anti-cancer compounds derived from sea cucumbers that can prevent cancer. The preliminary tests conducted were the sea cucumber extract lethality test against *Artemia salina*, namely the Brine Shrimp Lethality Test (BSLT), and the total bacteria of beef meatballs. The results of the observation of the lethality of sea cucumber extract against *Artemia salina* and total bacteria of the meatball showed that the sea cucumber extract had high lethality against *Artemia salina* and was able to reduce the total bacteria in meatballs during the storage period. The lethality of sea cucumber extract was strongly influenced or stimulated by heating treatment. Based on the BSLT test, of the five types of sea cucumbers, two of them (*Holothuria similis* and *Pearsonothuria graeffei*) have high lethality. Sea cucumber extract was also able to inhibit both bacterial and fungal growth in meatballs during storage at room temperature.

1. INTRODUCTION

1.1. Research Background

Cancer is the leading cause of death in developed countries and the second leading cause of death in developing countries. In 2008 there were 12.7 million cancer patients worldwide (56% of whom were in developing countries) and resulted in the death of 7.6 million people (64% in developing countries) [1]. The prevalence of cancer sufferers in Indonesia reaches 4.3 people per 1,000 population. With a population of 237.6 million in 2010, cancer sufferers in Indonesia are estimated at 1.02 million [2].

Various ways to prevent cancer that can be done include primary, secondary, and tertiary prevention, in which diet and type of food regulation are types of primary prevention. Cancer treatment can be done by, among others, surgery, chemotherapy, radiation, immunotherapy, and gene therapy. In recent decades, the concept of functional foods has offered new and practical approaches to achieving optimal health by promoting the use of natural products with physiological benefits thereby reducing the risk of various chronic diseases. Most of the functional foods that are therapeutic agents come from natural sources, especially,

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terrestrial food plants and marine species. Due to the rich marine biodiversity, marine organisms are a source of nutritious food, a source of active biological components, especially bioactive peptides, as well as antimicrobial agents, anti-inflammatory and anticancer agents, one of which is a sea cucumber.

Given the medical and health benefits, sea cucumbers have the potential bioactive peptides as a natural food preservative to be introduced into meat-based foods that are very popular in the community such as meatballs, sausages, and corned beef so that they become functional foods that can prevent and treat cancer.

1.2. Literature Review

Sea cucumbers are marine invertebrates that have a body shape like cucumber which are members of echinoderms, commonly known as teripang, or gamat, have long been utilized in the food and folk medicine systems of Asia and Middle East communities [3] and [4], which has important economic value because it contains high nutrients and is empirically believed to be a food ingredient has properties as a medicinal ingredient (Nutraceutical) [5]. According to Ref. [6], the health benefits obtained after consuming sea cucumbers are vitality, because sea cucumbers contain bioactive compounds including terpenoids,

saponins, essential amino acids, glycosaminoglycans, chondroitin sulfates, sulfate polysaccharides, sterols, lectins, phenols, and peptides.

1.3. Research Objective

The research aimed to examine the lethality of some varieties of sea cucumber against *artemia salina*, as a natural food additive in meat-based functional food, and the organoleptic value of meatball as added with sea cucumber powder.

2. MATERIALS AND METHODS

2.1. Materials

The basic material used in this research were five varieties of sea cucumber (*Holothuria* Sp) purchased by the fishermen of the crew of the village at Kuta-Lombok Island and Bungin Island, The ingredients used to make the meatballs were beef thigh purchased at the Kebon Roek market, STPP, tapioca flour, ice cubes, garlic, fried shallots, salt, and pepper. While the materials for the total bacterial count were: meatball samples, NA (nutrient agar), distilled water, 70% alcohol, aluminum foil, sterile physiological NaCl, and label paper.

2.2. Research methods

2.2.1. Sea Cucumber Extraction

Dry samples of five varieties of sea cucumbers were weighed including *Holothuria similis* (127.95 grams), *Pearsonothuria graeffei* (121.00 grams), *Thelenota anax* (183.00 grams), *Holothuria fuscogilva* (161.00 grams), and *Bohadschia marmorata* (225, 00 grams) and each variety was extracted by ordinary maceration. The dried samples of sea cucumbers were cut into small pieces and 100 to 200 ml of methanol from the soaking samples were added to the blender [7] and [8]. Once smooth, the sample was put into a 1000 ml beaker glass and then macerated with methanol for 24 hours. The obtained filtrate was then filtered using Whatman filter paper no.1. The filtrate obtained was put into the evaporating flask, then evaporated using a vacuum rotary evaporator at a temperature of 40 °C at 90 rpm so that it thickens into a crude extract. After that, the crude extract was weighed and stored in a dark bottle.

2.2.2. Test for the lethality of the active compounds of sea cucumber extract [9]

The concentration of sea cucumber extract solution was made as follows. As much as 0.5 g of each crude extract from 5 varieties of sea cucumbers, namely *Holothuria similis*, *Pearsonothuria graeffei*, *Thelenota anax*, *Holothuria fuscogilva*, and *Bohadschia marmorata*, were diluted using methanol up to 50 ml. The solution obtained from each type was called the mother liquor with a concentration value of 10,000 µg / ml, then each mother liquor was diluted to several final concentrations in vials, namely 1000 µg / ml, 750 µg / ml, 500 µg / ml, 250 µg / ml, 100 µg / ml, 75 µg / ml, 50 µg / ml, 25 µg / ml, and 10 µg / ml. Furthermore, as much as 0.5 ml of each concentration was put into a vial bottle and as a control, a vial was provided which contained methanol with the same volume of 0.5 ml. One concentration series was carried out three times (triple). All crude extract and control solutions in the vial bottles were allowed to dry at room temperature for approximately 24 hours.

2.2.3. Hatching Artemia salina eggs [9]

Artemia salina eggs (0.2 g / 1000 ml seawater) were placed in a container (aquarium) filled with seawater. During hatching, seawater was given an aerator to circulate air in the aquarium. The eggs start hatching after 24 hours. The eggs hatch and move actively at the age of 36 to 48 hours. The 48-hour age of *Artemia* known as *Artemia salina nauplii* was used in the BSLT test.

2.2.4. Brine Shrimp Lethality Test (BSLT) [9]

A total of 10 *Artemia salina nauplii* were piped into each vial bottle containing crude extract with various concentrations and controls, then added seawater until the final volume was 5 ml. The final concentrations in the vial after the addition of seawater are 1000 µg / ml, 750 µg / ml, 500 µg / ml, 250 µg / ml, 100 µg / ml, 75 µg / ml, 50 µg / ml, 25 µg / ml, and 10 µg / ml. Then the vial was closed with a rubber cap and left for 24 hours. Deaths at each concentration were recorded and compared with controls, then calculated using the formula:

$$\% \text{ Lethality} = \frac{\sum \text{Mortality of every Concentration} - \sum \text{Mortality of Control}}{\sum \text{Early Larvae}} \times 100\%$$

Calculation of LC50 was done using Ms. Excel. An extract is said to be active if the obtained LC50 value = 1000 µg / ml.

2.2.5. Meat-based food (meatballs processing)

According to Ref. [10] and [11], the working steps of processing meatballs were as follows: 1. Fresh beef cut into small pieces and then ground together with all other ingredients (ice, salt, STPP, tapioca flour, sea cucumber extract, garlic, fried shallots, and pepper; 2. Let the dough stand for 30 minutes in the refrigerator; 3. The dough was formed into a round shape and then boiled at a temperature of 65°C until it arises on the surface, then boiled until cooked at a temperature of 100°C.

Table 1. Meatballs Formula

INGREDIENT	1 st	2 nd	3 rd
	Formula %	Formula %	Formula %
Sea cucumber flour	0	2	4
Fresh beef	54	52	50
Tapioca flour	20	20	20
Salt	1.5	1.5	1.5
Garlic	2	2	2
Fried shallots	1.5	1.5	1.5
Pepper	0.5	0.5	0.5
STPP	0.5	0.5	0.5
Ice Cube	20	20	20
Total	100	100	100

2.3. Data analysis

2.3.1. Observed variables

The variables observed in this study were the proliferation of cancer cells in various treatments and total bacteria in meatballs as the main variable and visual observation of meatballs as supporting variables.

2.3.2. Total bacterial count procedure

Total bacteria count in beef meatball samples using the TPC (Total Plate Count) method [12]. To report the results of the

microbiological analysis as Standard Plate Count (SPC), the following procedure was carried out [13]:

- 1) The number of colonies that meet the counting requirements is between 25 and 250.
- 2) Several colonies that are joined into one large colony, can be counted as one colony.
- 3) A row of colonies that is visible as one solid line can be counted as one colony.

3. RESULT AND DISCUSSION

3.1. Lethality test

The BSLT test results for the five types of sea cucumbers are listed in the following table, the extracts of the five types of dried sea cucumbers were tested at various concentrations, ranging from 10 µg / mL to 1000 µg / mL.

Table 2. BSLT (*Artemia salina*) test results

No.	Varieties of Sea Cucumber	Average of Deaths (%)	Lethal Concentration (µg / ml)
1	<i>Holothuria similis</i>	56.30	25.00
2	<i>Pearsonothuria graeffei</i>	48.89	70.31
3	<i>Thelenota anax</i>	26.67	937.50
4	<i>Holothuria fuscogilva</i>	32.59	661.76
5	<i>Bohadschia marmorata</i>	48.52	357.14

The results of the analysis of the BSLT test with the help of Ms. Excel show that the LC50 value of crude extract from the five types of sea cucumbers studied varies as shown in Table 2. Of the five types of sea cucumber, *Holothuria similis* produced the most active crude extract (25.00 µg / ml), while *Thelenota anax* produced the crude extract with the lowest activity namely 937.50 µg / ml. This means that the crude extract of the five types of sea cucumbers was active against BSLT because the resulting LC50 value was less than 1000 µg / ml [14]. Two of the five types of sea cucumbers (*Holothuria similis* and *Pearsonothuria graeffei*) have a crude extract activeness value which can be said to be very active because it ranges from the standard value of activeness from the American National Cancer Institute (NCI) which states the standard for the effectiveness of bioactive components to fight cancer cells is = 30 µg / ml.

3.2. Total Bacteria Count of Beef Meatball

The results of the total bacteria count of beef meatball given the addition of sea cucumber extract at various levels can be seen in Table 4. It can be seen that the folded extract can effectively reduce the bacterial content of meatballs during the storage period.

Based on the observations, it was evident that the higher the level of sea cucumber extract, the lower the content of the meatball bacteria. If it is related to the effectiveness of the extract against the inhibition of cancer cells, it turns out that the heating treatment (autoclave in the extraction and heating in the meatball ripening process) strengthens the killing power of both cancer cells and bacterial cells. Meanwhile, for the second day of

storage, the total bacterial colonies continued to show an increase, this was more due to contamination during room temperature storage without packaging.

Table 4. Total bacteria count of meatball in various treatments (Log CFU mL⁻¹)

Sea Cucumber Flour (%)	Storage Period (day)		
	0	1	2
0 (Control)	3.11	5.42	8.07
2	2.95	5.42	7.89
4	2.75	5.36	7.89
Average	2.94	5.40	7.96

Ref. [12], stated that anti-bacterial work is influenced by several things, namely antibacterial concentration, bacterial species, number of bacteria, environmental pH, and including temperature. During the observation, no fungus grew. This shows that teripang extract can inhibit fungal growth during storage.

3.3. Visual Observation of Beef Meatballs

Physical observations of beef meatballs with the addition of sea cucumber extract can be seen in Table 5. Physical observations include smell, texture, and formation of mucus during storage at room temperature (28 °C).

Table 5. Visual observation results of meatballs with added sea cucumber flour

TREATMENTS (%)	Storage Period (day)			
	0	1	2	
0% (Control)				
Aroma	Normal	Appear	Acid	Rotten Odor
Texture	Chewy	Start to Softened	to Crumbling Easily	
Mucus	-	+		++
2%				
Aroma	Normal	Appear	Acid	Rotten Odor
Texture	Chewy	Start to Softened	to Crumbling Easily	
Mucus	-	+		++
4%				
Aroma	Normal	Appear	Acid	Rotten Odor
Texture	Chewy	Start to Softened	to Crumbling Easily	
Mucus	-	+		++

Description: (-) There was no mucus
 (+) There was mucus (A little)
 (++) There was mucus (More)

4. CONCLUSION

The results of observing the effectiveness of sea cucumber extract on the growth of cancer cells and total meatball bacteria showed that the sea cucumber extract was able to inhibit the growth of cancer cells and was able to reduce the total bacteria in meatballs during processing. The effectiveness of sea cucumber

extract is greatly influenced or stimulated by heating treatment. Based on the BSLT test, of the five types of sea cucumbers, two of them (*Holothuria similis* and *Pearsonothuria graeffei*) have high activity. Sea cucumber extract was also able to inhibit both bacterial and fungal growth on meatballs during storage at room temperature. The extraction method needs to be refined by avoiding the use of materials that are less environmentally friendly, especially such as chloroform, for example, considering the extract to be applied to functional foods. The content of organic acids or active compounds in sea cucumber extract needs to be isolated and purified. The content of active compounds in sea cucumber extract needs to be tested clinically on experimental animals for its effect on the growth of cancer cells.

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