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A Reviews on Use of Sea Cucumber as a Treatment for Oral Cancer

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ABSTRACT

Introduction: Cancer is an abnormality in body tissue cells that continues to grow and develop uncontrollably. One type of cancer that is dangerous and can cause death is oral cancer. The content of sea cucumbers is useful as an anti-virus, help the growth of new cells, and as an anti-cancer.

Objective: The purpose of this literature is to look based on literature, the treatment of oral cavity cancer using sea cucumbers as herbs.

Methods: Scientific evidence and clinical cases are taken from the literature to support this review and information about sea cucumber herbs as an alternative treatment for oral cancer. A systematic review of the literature is carried out by searching for related articles. The article search begin on April 22nd, 2020, with keywords; "Oral cancer, sea cucumbers". The following searching database is: google.

Results: It is using sea cucumbers or *Holothuria atra* as an ingredient to be assessed for its anti-cancer properties. This study used 20kg of sea cucumbers, which were then extracted by the maceration method with 96% alcohol for 24 hours, then filtered with Whatman paper and repeated up to 6 times at room temperature. Triterpene glycosides are a type of saponin in sea cucumbers whose structure contains a sugar group. Triterpene glycoside compounds are compounds that can inhibit the mechanism of division and trigger apoptosis of cancer cells. The effects of cytotoxic on three types of sea cucumbers such as *H.scabra, H.parva, and H. leucospilota* are then extracted using organic extracts namely, n-Hexane , ethyl acetate and methanol by using several organs in specimens such as body wall , gonads , intestine tract , respiratory tree , coelomic fluid and cuverien tubules.

Conclusion: Bioactive compounds isolated from sea cucumbers for use as anti-cancer agents have attracted the attention of cancer researchers because of their natural origin and long history as nutritious foods. The detailed mechanism of anti-cancer activity of sea cucumber compounds is still unclear, and a comprehensive study identifying this mechanism is still needed. Sea cucumber extract has a promising future to be developed as a functional food.

Keywords: Sea cucumber, Oral cancer, Holothurians, Herb, Triterpene glycosides

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INTRODUCTION

Sea cucumbers are one of the marine products that are quite economical because of its high nutritional content, easy to find, and has long been a consumption ingredient among the community, is also trusted with its efficacy in infecting diseases.Indonesia is the biggest sea cucumber exporting country in the world. However, only a few Indonesian people understand the benefits of sea cucumbers. The content of sea cucumbers is useful as an anti-virus, anti-microbial, anti-inflammatory, beauty, enhance immunity, help the growth of new cells, and as an anti-cancer. [1,2]

Sea cucumbers have health benefits and can be an ingredient in making drugs. Janakiram (2015) states that sea cucumber extracts contain bioactive compounds as antioxidants such as triterpene glycosides as inhibitors of degenerative diseases due to free radicals such as heart disease and cancer. [3,4,5,6]

Cancer is one of the leading cause of death worldwide. Data taken from Ministry of Health Republic of Indonesia mentioned that about 10 million deaths was caused by cancer. More than 60% of new cases and about 70% of deaths caused of cancer worldwide occurs annually in Africa, Asia, Central and South America. It is estimated that cancer cases will increase by 0.5 million cases annually (WHO)[7,8]

Cancer is an abnormality in body tissue cells that continues to grow and develop uncontrollably. One type of cancer that is dangerous and can cause death is oral cancer. This type of cancer occurs in the head and neck that includes the lips, labial and buccal mucosa, 2/3 anterior to the tongue, retromolar pad, floor of the mouth, gingiva, and hard palate. Up to 70% of oral cancers show red or white patches that remain in the mouth at the beginning.[9,10,11,12,13]

To treat this disease, it has been usually done is the conventional way treatment, such as chemotherapy, radiotherapy, surgery, or the combinations of those therapies. But as widely known, conventional cancer therapies are giving adverse side effects, with the healing rate that is not commensurate with the side effects. Chemotherapy, for example, having side effects such as decreased in blood cell count, multiple infections, anemia, nosebleed, alopecia, skin dryness, nausea, dehydration, hypotension, constipation, diarrhea, and nervous system disorders. Whilst radiotherapy having side effects such as nausea, white decreased in leukocytes count, multiple

infections/inflammations, sunburn like effect, fatigue, mouth and throat pain, severe diarrhea, and alopecia.[14,415,16]
So it needs an alternative method to treat cancer with minimum effect, such as natural treatment. Natural treatment uses natural materials derived from clinically tested extracts from animals or plants. This kind of treatment able to detoxify blood tissue and stimulate the immune system to jointly fight the cancer cells. The use of natural compounds in this type of cancer treatment appear from several approaches, based on the concept from the research that cancer was reversible; concept of treatment by inhibiting cancer growth by eliminating carcinogens and environments that support the genetic mutation of cancer cell growth; concept of cancer cells aging by inhibiting the growth of cancer cells in order to eradicates the opportunity to develop, then the cancer cells will aging and death; concept of strengthening other healthy cells around the cancer cell to form a cell defense that can withstand the development of cancer cells.[17,18,19,20,21]
Sea cucumber is classified in Echinodermata phylum Holothuridea class 3. Most species of sea cucumbers are having high economic value as a food source with also some promising biological activities. Demand for sea cucumbers in global market for food and pharmaceutical use increases significantly and has been recognized as a traditional treatment in Chinese and Malaysian literatures for hypertension, asthma, rheumatism, wounds and burns, and impotence. The benefits from sea cucumbers as medicinal compounds can be attributed to the presence of several bioactive compounds, most notably triterpen glycoside.[22,23,24,25]
So the purpose of this literature is to look based on literature, the treatment of oral cavity cancer using sea cucumbers as herbs.

METHOD AND MATERIAL

Scientific evidence and clinical cases are taken from the literature to support this review and information about sea cucumber herbs as an alternative treatment for oral cancer.

LITERATURE FINDING

A systematic review of the literature is carried out by searching for articles related to the title of this article. The article search begins on April 22, 2020, with keywords; "Oral cancer, sea cucumbers". The following searching database is: google.

DISCUSSION

1. Sea Cucumber

Sea cucumbers or *holothurians (Holorhurioidea, Echinoderms)* are one of the specific and easily recognizable groups of sea biota. Sea cucumber, in general, is cylindrical shape extending from the tip of the mouth towards the anus (orally-aborally). The mouth is located anteriorly at the tip, and the anus is at the posterior end. As with echinoderm in general, the sea cucumber's body is a "pentamerous radial symmetry" with a horizontal axis. However, the shape of the symmetry is modified by an upright plate (dorsoventral plane), appearing as "bilateral symmetry." Like other Echinoderms, besides the symmetry radial, other characteristics are the shape of the skeleton and the presence of a watervascular system.[7]
Sea cucumbers have an elastic, and its body comes with varied shapes, such as rounded, cylindrical, rectangular, or elongated round like a snake. The mouth is located at the anterior end, while the anus is at the posterior end. Length according to the type and length, ranging from 3 cm to 150 cm. Sea cucumber body shape is a taxonomic characteristic at the level of the nation (order) and tribe (family), specifically for the tribes of the nation *Aspidochirotida*.[6,7,26]
Sea cucumbers, in general, have a dull skin color, such as gray, brown, full green, or black. The ventral side is usually lighter than the backside, such as white, yellow, pink, or red. Some types of sea cucumbers have skin with patches or stripes.[2,7]
Around its mouth, the term tube was changed to tentacles. The number of tentacles varies from 10 to 30, usually a multiple of five. Tentacles in each individual are generally the same. Sea cucumber tentacle forms vary, such as the shape of the shield (peltate), dendritic form (dendritic), pinnate form (pinnate) (Figure 1). also the shape of the digits (digitate) and the shape of the guarding fingers (peltate-digitate). The number and form of tentacles are taxonomic characteristics in the classification of sea cucumbers at the nation and tribe level.[27,28,30]

2. Classification of Sea Cucumber (Identification)

Taxonomically, the classification of sea cucumbers is: [7]
Phylum: *Echinoderms*
Subfilum: *Echinozoa*
Class: *Holothuroidea*
Subclass: *Aspidochirotacea*
Order: *Aspidochirotide*
Family: *Holothuriidae*
Genus: *Holothuria, Muelleria, Stichopus*

Table 1: Classification of Sea Cucumber (Identification)

Family	Order	Genus	Species	Local's name
Aspidochirotida	Holothuriidae	Actinopyga	*A. miliaris*	Teripang lotong
			A. lecanora	Teripang batu
			A. echinites	Teripang batu
			A. mauritiana	Teripang bilalo
		Holothuria	*H. scabra*	Teripang pasir, teripang hitam
			H. nobilis	Teripang susuan putih
			H. fuscogilva	Teripang susuan putih
			H. atra	Teripang dada merah
			H. edulis	Teripang keling
			H. coluber	Teripang tali jangkar
			H. leucospilota	Teripang hitam
			H. pervicas	Teripang karang
			H. fuscocinirea	Teripang karang
			H. gyrifer	Teripang karang
			H. hilla	Teripang karang
			H. impatiens	Teripang karang
			H. pardalis	Teripang karang
		Bohadschiaa	*B. argus*	Teripang mata kucing
			B. graeffei	
			B. marmorata	Teripang getah putih
	Stichopodiae	Stichopus	*S. chloronotus*	Teripang belimbing
			S. horrens	
			S. variegates	Teripang kasur
		Thelenota	*T. ananas*	Teripang nenas
			T. anax	

3. Oral Cancer

Oral cancer is malignant neoplasia that appears on the lips or oral cavity. Traditionally defined as squamous cell carcinoma (SCCs), because in the tooth area, 90% of cancers are histologically originated in squamous cells. It has varying degrees of differentiation and a tendency for lymph node metastases.Oral cancer is two to three times more common in men than women in most ethnic groups. In reports worldwide, cancers from all regions of the oral cavity and pharynx are grouped together and collectively represent the sixth most common cancer in the world. According to the latest report from the International Agency for Research on Cancer (IARC) for oral cancer (ICD-10 code C00-08: Lips, Oral Cavity) which includes lips, tongue, gingiva, the floor of the mouth, parotid glands and saliva, the annual incidence is higher around the world, which is more than 300,000 diagnosed cases, and annual deaths are around 145,000. The table shows the incidence and mortality for oral cancer according to the World Health Organization (WHO) region, and which shows the most critical figures are the WHO Southeast Asia (SEARO) and WHO European regions (EURO). Specifically by region, which is characterized by a high incidence of oral cancer found in South and Southeast Asia (Sri Lanka, India, Pakistan, and Taiwan), the West (France) and Eastern Europe (Hungary, Slovakia, and Slovenia), Latin America and the Caribbean (Brazil, Uruguay, and Puerto Rico) and the Pacific region (Papua New Guinea and Melanesia). The events also vary according to the human development index of the United Nations Development Program (UNDP). According to this index, the incidence is higher in countries with better development indicators. [8,27,28,29,31]

The GLOBOCAN grouping shows that crude oil levels and age-standardized incidence rates (worldwide) are higher in more developed regions, but mortality rates are higher in less developed regions, which shows social inequality.[6,8]

Table 2: GLOBOCAN cancer incidence and mortality, all ages, both sexes by population

	Incidence			
Population	Numbers	Crude rate	ASR (W)	Accumulative risk
WHO African Region (AFRO)	13,484	1.5	2.7	0.30
WHO Americas Region (PAHO)	49,200	5.2	4.1	0.48
WHO East Mediterranean Region (EMRO)	20,681	3.3	4.6	0.52
WHO Europe Region (EURO)	65,933	7.3	4.6	0.53
WHO South-East Asia Region (SEARO)	103,464	5.6	6.4	0.73
WHO Western Pacific Region (WPRO)	47,524	2.6	2.0	0.22
UNDP Very High Human Development	92,338	8.0	4.8	0.54
UNDP Low Human Development	40,954	3.1	5.2	0.59
GLOBOCAN More Developed Regions*	100,823	8.1	4.7	0.54
GLOBOCAN Less Developed Regions*	199,550	3.4	3.7	0.42
	Mortality			
	Numbers	Crude rate	ASR (W)	Accumulative risk
WHO African Region (AFRO)	8,530	1.0	1.8	0.20
WHO Americas Region (PAHO)	12,803	1.3	1.0	0.12
WHO East Mediterranean Region (EMRO)	10,997	1.8	2.5	0.30
WHO Europe Region (EURO)	25,202	2.8	1.7	0.19
WHO South-East Asia Region (SEARO)	65,734	3.5	4.1	0.48
WHO Western Pacific Region (WPRO)	22,068	1.2	0.9	0.09
UNDP Very High Human Development	26,970	2.3	1.2	0.14
UNDP Low Human Development	25,238	1.9	3.3	0.39
GLOBOCAN More Developed Regions*	33,313	2.7	1.4	0.16
GLOBOCAN Less Developed Regions*	11,2040	1.9	2.1	0.24

4. Correlation of Sea Cucumbers and Cancer

Table 3: Literature findings related to Sea Cucumbers and Cancer

No.	Author and title	Year	Conclusion and result
1.	Putram NM, et al. *Anti-cancer Activity from Active Fraction of Sea Cucumber* JPHPI 2017, volume 20 number 1	2017	The active fraction of sea cucumber *Holothuria atra*, which has anti-cancer activity, is the water-methanol fraction with an IC50 value of 14.27 µg / mL against HeLa cells and 14.33 µg / mL in MCF-7 cells.
2.	Souhaly JW, Rahayu S *Cytotoxic Activities of Sea Cucumber (Bohadschia argus) Extract Against T47D Cells* AIP Conference Proceedings	2019	Sea cucumber protein migrates at 55-80 kDa. Water and methanol extract of sea cucumber *Bohadschia argus* has potential as an anti-cancer therapy because it inhibits the growth of T47D cells.
3.	Mashjoor S, Yousefzadi M *Cytotoxic Effect of Three Persian Gulf Species of Holothurians* Iranian Journal of Veterinary Research	2018	Data illustrates that toxicity depends on concentration but the highest BSA for M extracts from *H. leucospilota* (CT) cuvierian tubular organs (up to 95% at 1000 µg / ml, LC50 = 616.4 µg / ml) and respiratory tree (RT) organs H. parva (up to 86% at 1000 µg / ml, LC50 = 607.2 µg / ml). Based on cell lines, more effective extracts are noted for the E fraction of CT organs from *H. leucospilota* (up to 85% at 250 ug / ml, LC50 = 37.25 ug / ml) against MCF-7 and for extract E from the intestinal tract (IT) H. parva organ (up to 80% at 250 ug / ml, LC50 = 46.25 ug / ml) against HeLa cells. This variation indicates that the possibility of cytotoxic compounds in the fraction is selective toxicity to cell lines
4.	Satari MH, et al. *Anti-cancer Potency of Black Sea Cucumber (Holothuria Atra) from Mentawai Island, Indonesia* Padjajaran Journal of Dentistry; 29(1)	2017	Black sea cucumber extract has a strong cytotoxic effect on SP-C1 cancer cells; therefore, black sea cucumber extract has the potential to be developed as a new source of cancer drugs.
5.	Ridhowati S, et al. *Anti-cancer and Antioxidant Activities from Sea Cucumber (stichopagus variegatus) Flour Dried Vacuum Oven* Pertanika J; 41(3)	2018	The findings of this study determine the potential of a new anti-cancer of sea cucumber flour (*Stichopus variegatus*), which is dried in a vacuum oven. The presence of biological activity in Stichopus variegatus extract powder indicates that their consumption can be beneficial to health. Besides, these findings can facilitate awareness about the potential anti-cancer properties of *Stichopus variegatus* and foster future development of anti-cancer therapy on an industrial scale. In addition, the enzyme fraction of Stichopus variegatus flour extract can be further developed as a complementary cancer treatment, functional food ingredient, and nutraceutical.
6.	Soltani M et al. *Hemolytic and Cytotoxic Properties of Saponim Purified from Holothuria leucospilota Sea Cucumber*	2014	The ethanol fraction of 80% saponins isolated from *H. leucospilota* showed hemolytic activity and was promising as an anti-cancer candidate.
7.	Adrian TE, Collin P *The Anti-Cancer Effect of Fronoside A* Marine Drugs	2018	Frondoside A has a strong anti-cancer effect

In the first study, conducted by Nurul Mutia (2017), it is using sea cucumbers or *Holothuria atra* as an ingredient to be assessed for its anti-cancer properties. This study used 20kg of sea cucumbers, which were then extracted by the maceration method with 96% alcohol for 24 hours, then filtered with Whatman paper and repeated up to 6 times at room temperature. Then evaporate using a rotary evaporator to dry. Then phytochemical tests are performed to see the bioactive components contained therein, such as alkaloid, flavonoid, phenolic, steroid, triterpenoid, and saponin tests. It also carried out cytotoxic tests.[1,16]

In the saponin test, there are many glycoside triterpenes found in the walls of *H.atra*'s body, and there are also several types of glycoside triterpenes detected that have anti-cancer activities.[2,15]

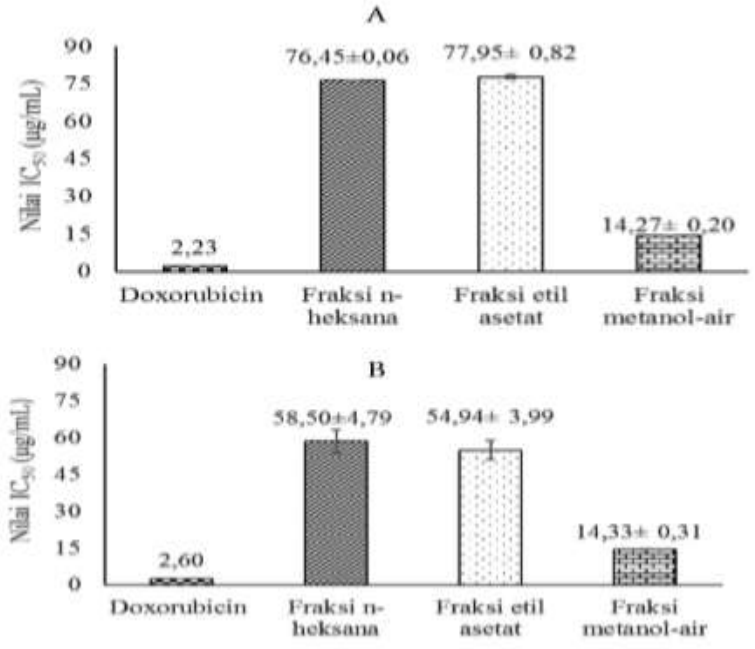
Fractionation was carried out to separate the compounds in the crude extract of *H.atra* based on its level of polarity using n-hexane (non-polar) solvent, ethyl acetate (semi-polar), and methanol-water (polar). The results of the n-hexane fraction, the ethyl acetate fraction, and the H. sea cucumber methanol-water fraction are shown in table 4.

Table 4: Yield yields of fractions on *H.atra* extract

Sampel	Persentase rendemen (%)	Rf	Warna	Senyawa
Fraksi n-heksana	17,73	0,78;0,87;0,91	Biru dan kuning	Steroid/triterpenoid dan fenol
Fraksi etil asetat	26,70	0,51;0,91	Biru keunguan	Steroid/triterpenoid
Fraksi metanol-air	75,64	0,25;0,84	Biru	Steroid/triterpenoid

Cytotoxic test results showed that the methanol-water fraction was more toxic to HeLa cells and MCF-7 cells compared to the n-hexane and ethyl acetate fractions. The n-hexane sea cucumber fraction Holothuria fuscocinerea had an IC50 value of 162.76 µg / mL, the ethyl acetate fraction had an IC50 value of 143.96 µg / mL, and the methanol-air fraction was 38.58 µg / mL on HeLa cells (Bambang 2013). Nimah et al. (2012) reported that cytotoxic test results of sea cucumber sand (*H. scabra*) in the n-hexane fraction had an IC50 value of 62.86 µg / mL, ethyl acetate fraction of 43.56 µg / mL and methanol-water fraction of 18.85 µg / mL. Cytotoxic activity in the high methanol-water fraction is suspected because it contains triterpenes glycoside compounds that are soluble in polar solvents. Triterpene glycosides are a type of saponin in sea cucumbers whose structure contains a sugar group. Triterpene glycoside compounds are compounds that can inhibit the mechanism of division and trigger apoptosis of cancer cells.[1,17]

Table 5: IC50 values of Doxorubicin, hexane fraction, ethyl acetate fraction and methanol-water fraction on HeLa (A) and MCF-7 (B) cells



So the active fraction of sea cucumber *Holothuria atra* which has anti-cancer activity is the water-methanol fraction with an IC50 value of 14.27 µg / mL against HeLa cells and 14.33 µg / mL in MCF-7.1 cells.[18]

The second study by Jantje (2019), it is using sea cucumbers with *bohadschia argus*type. Specimens are extracted using two types of extraction, namely by treating water and using methanol, which will then be tested for cytotoxic activity against cancer cells. 100 grams of sea cucumbers are then extracted and divided into four concentration groups of 50ug / ml, 100ug / ml, 250ug / ml, and 500ug / ml.[3,19]

Cytotoxic activity in *B.argus* extract is carried out in vitro against breast cancer cells in humans (T47D), to determine cytotoxic activity based on IC50 values as an effective dose.[18,19]

Table 6: Percentage of visibility of T47D cells after treatment

Extract	50 µg/mL	100 µg/mL	250 µg/Ml	500 µg/mL	IC_{50}
Water	80.60%	83.80%	72.44%	40.61%	480.4
Methanol	79.34%	71.75%	13.15%	6.86%	146

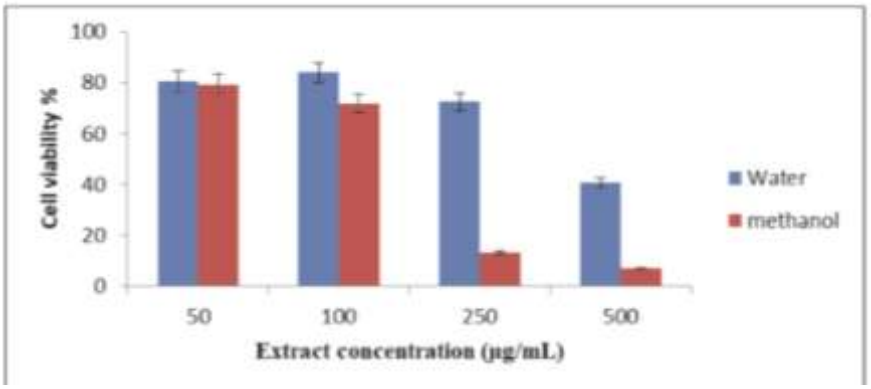


Figure 1: Graphic visibility of T47D cells after treatment

Based on the results, quantitative calculations indicate that there is a significant difference between the IC50 value of *B.argus* extract with methanol and water. It happens because methanol can dissolve more components, polar and non-polar. While in water can only dissolve polar components only.[1,9]

So in this study also concluded that extracts from sea cucumbers in this case *B.argus* species have potential as a therapy to fight cancer cells. This study also supports the argument that sea cucumber herbs can be used to fight cancer cells because of the content contained therein, although it is still in a limit that still needs to be examined further.[32,33,34]

In the next article by Mashjoor (2019), investigating the effects of cytotoxic on three types of sea cucumbers such as *H.scabra, H.parva,* and *H. leucospilota* are then extracted using organic extracts namely, n-Hexane (nH), ethyl acetate (E) and methanol (M) by using several organs in specimens such as body wall (BW), gonads (G), intestine tract (IT), respiratory tree (RT), coelomic fluid (CF) and cuverien tubules (CT). Cytotoxic potential in all three fractions was assessed using the model; brine shrimp (artemia salina) lethality assay (BSA) and tetrazolium-based colorimetric assay (MTT) in human cancer cells (MCF-7) and healthy cells (HeLa).[4,20,35,36]

This study also concluded that the toxicity depends on the high concentration used, but BSA showed high results for extract M in the CT organ *H. leucospilota*. Based on cell lines, extracts were seen to be more effective in the E fraction of the CT *H. leucospilota* organ against MCF-7. So this showsthat the species of Holothuria or sea cucumber can be used as a material in the manufacture of drugs.[11]

Satari (2017), in his article, also said the same thing about sea cucumbers (*Holothuria atra*) as potential anti-cancer drugs. The cytotoxic activity test on condensed ethanol extract and three black sea cucumber fractions resulted in a calculation of the percentage of viability as shown in Table 6.[9]

Cytotoxic test of ethanol extract and fractionation of black sea cucumber was carried out with three treatments. In the sample concentration of 100 µg / mL, the highest percentage of eligibility was in the administration of a hexane fraction of 118.00% on the second iteration, and the lowest percentage of eligibility was on the administration of ethanol extract 7% on the second iteration. IC50 values were obtained by probit analysis of the SP-C1 cell viability percentage, resulting in an IC50 value of 12,167, meaning that black sea cucumber ethanol extract has a potent cytotoxic effect (12,167 <IC50 <100) against SP-C1 cancer cells. The increased concentration of black sea cucumber extract resulted in a decrease in the percentage of SP-C1 cell viability, as shown in Table 7.[9,37,38]

Table 7: Percentage of SP-C1 viability tested with black sea cucumber extract and fraction

Sample 100 µg / mL	Cell viability percentage		
	Repetition I	Repetition II	Repetition III
Ethanol extracts	12.00	7.00	17.50
Hexane fraction	108.50	118.00	117.50
Ethyl acetate fraction	98.50	95.00	114.00
Butanol fraction	99.50	105.00	93.50

Table 8: Average cell absorbance measured using ELISA reader at a wavelength of 550 nm.

Black sea cucumber (extracts and fractions) 100 µg / mL	SP-C1 culture	
	Absorbance average*	Viability average
Ethanol extracts	12.00	12.167
N-Hexane fraction	108.50	114.667
Ethyl acetate fraction	98.50	102.500
Butanol fraction	99.50	99.500

Then Ridhowati (2018), anti-cancer and antioxidant activity of the vacuum oven dried sea cucumber flour (*Stichopus variegatus*) has not been widely reported. This study aims to determine in vitro the inhibition of enzyme extracts and water from sea cucumber flour on WiDr colon cancer cells, T47D breast cancer cells, and normal Vero cells, the induction of apoptosis. Sea cucumber flour is made with a vacuum oven. The water is extracted and hydrolyzed by digestive enzymes gradually. Water extract (SV-WE) and hydrolysis enzyme (SV-EE) were tested for their antioxidant activity using the DPPH method. The SV-WE and SV-EE samples were tested for anti-cancer activity using the MTT test. The antioxidant activity of SV-EE at 1.67 ± 0.05 mg / mL was significantly different from SV-WE at 2.30 ± 0.30 mg / mL. SV-EE and SV-WE had anti-cancer activity against WiDr cells, respectively, at 13.01 ± 2.75 µg / mL and 69.37 ± 24.25 µg / mL. The IC50 value of cell induction apoptosis ability from SV-EE is 64.9 ± 1.63%. SV-WE and SV-EE show higher anti-cancer activity against WiDr cells and T47D cells. Gamma sea cucumber flour from Indonesia can be used as a potential ingredient in functional food. Then, this research determines the potential of new anti-cancer of sea cucumber flour (*Stichopus variegatus*) which is dried by vacuum oven.[10,33,34,38,39]

Similarly, in Soltani (2014), sea cucumber body wall is dried and made into raw powder and saponin isolated using various solvents. Raw saponins were further purified by column chromatography using HP-20 resin. Foam test, Thin Layer Chromatography (TLC), hemolytic test, and Fourier Transform Infrared Spectroscopy (FTIR) confirm the presence of saponins. Cytotoxicity was analyzed using 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) tests on A549 cells, human lung cancer cell lines. Foam test, hemolytic test, and TLC supported the presence of saponin compounds in the 80% H. leucospilota ethanol fraction. The infrared (IR) spectrum of the extract shows hydroxyl (-OH), alkyl (C-H), ether (C-O), and ester (-C = O) absorption characteristics of triterpenoid saponins. Absorption of C-O-C shows the relationship of glycosides with sapogenin. Raw saponins extracted from sea cucumbers are cytotoxic to A549 cells. Ethanol fraction of 80% saponin isolated from *H. leucospilota* shows hemolytic activity and is promising as an anti-cancer candidate.[11,40,41,42,43]

Then Adrian (2018), who examined the content of Fronoside A in sea cucumbers, revealed that Frondoside A has a strong anti-cancer effect on all types of solid malignancies, lymphomas, and leukemias investigated to date. Frondoside A causes growth inhibition, induces inhibition of migration, invasion, and metastasis, and inhibits angiogenesis. The effects of frondoside A are mediated by inhibition of PAK1 and possibly other mechanisms. Frondoside A potentiates the effect of conventional therapeutic agents, such as paclitaxel, cisplatin, and gemcitabine, in several different types of cancer. During a relatively wide range of therapies in experimental animals, frondoside A is well tolerated and appears to have no toxicity to bone marrow, liver, kidney, or other tissues, and does not affect body weight. Frondoside A can be directly produced from waste streams from specific sea cucumber processing; However, it can also be produced from skin cell cultures from source organisms or perhaps by chemical synthesis. Frondoside A can be useful in the treatment of various malignancies either as a single agent or in combination with other therapies.[44,45,46,47,48]

CONCLUSION

Bioactive compounds isolated from sea cucumbers for use as anti-cancer agents have attracted the attention of cancer researchers because of their natural origin and long history as nutritious foods. Sea cucumbers contain many marine-derived agents that have the potential to inhibit the growth of several different types of human tumor cells, as shown in in vitro studies, in vivo murine models, and studies in humans. Some secondary metabolites derived from sea cucumbers exhibit anti-cancer properties through various

mechanisms including cytotoxicity, induction of apoptosis, cell cycle arrest, reduction of tumor growth, suppression of invasion, and tumor cell metastasis, inhibition of angiogenesis, and decreased drug resistance. However, the detailed mechanism of anti-cancer activity of sea cucumber compounds is still unclear, and a comprehensive study identifying this mechanism is still needed.[5,49,50,51]

In general, the potential anti-cancer activity of bioactive compounds isolated from sea cucumbers offers promising hope for the treatment and prevention of cancer in humans.[5,52,]

It has been explained in the three articles above that bioactive compounds in sea cucumbers have the potential for anti-cancer activity with results that support each other, so it can be said that sea cucumber herbs can be made.[1,2,51]

As in Navenaa (2015), sea cucumber extract has a promising future to be developed as a functional food. This extract can be a potential candidate for cancer prevention and treatment. The composition and dosage of sea cucumber extract must be standardized for human clinical use, and analysis of individual agents must be carried out to determine the potential benefits for the prevention and treatment of inflammatory diseases and cancer.[6,52,53,54,55]

REFERENCES

1. Putran NM, Setyaningsih I, Tarman K, Nursid M. Anticancer activity from active fraction of sea cucumber. JPIP 2017; 20(1): 53-62.
2. Yahya IB, Anagnostou F. FDI World Dental Federation. 2018.
3. Souhaly JW, Rahayu A. Cytotoxic Activities of Sea Cucumber (Bohadschia argus) Extract Against T47d Cells. AIP Confrence.2019.
4. Mashjoor S, yousefzadi M. Cytotoxic Effect of Three Persian Gulf Species of Holothurians. IJVR 2019; 20(1).
5. Wargasetia TL, Widodo. Mechanism of Cancer Cell Killing by Sea Cucumber-Derived Compounds. Springer 2017.
6. Janakiram NB, Mohammed A, Rao CV. Sea Cucumber Metabolites as Potent Anti-Cancer Agents. Marine Drugs 2015 ; 13.
7. Darsono, Prapto. General Introduction to sea cucumber (Holothrians). Oseana; 23(1).
8. Rivera, Caesar. Review Article : Essentials of Oral Cancer. IntJClinExpPathol 2015; 8(9).
9. Satari MH,et al. Anti-cancer Potency of Black Sea Cucumber (*Holothuria Atra*) from Mentawai Island, Indonesia. Padjajaran Journal of Dentristry 2017; 29(1)
10. Ridhowati S. Anticancer and Antioxidant Activities from Sea Cucumber (Stichous variegatus) Flour Dried Vacuum Oven
11. Soltani M,et al. Hemolytic and Cytotoxic Properties of Saponin Purified from Holothurian Leucospilota Sea Cucumber. Reports of Biochemistry& Molecular Biologi 2014; 3(1).
12. Adrian TE, Collin P. The Anti-Cancer Effect of Fronoside A. Marine Drugs 2018.
13. Wijaya FA. 2015. Anticancer activity of compounds of secondary metabolite Holothuria atra against cancer cells T47D and WiDr. Purwokerto (ID): Sudirman University.
14. Janakiram N.B, et al. Improved innate immune responses by Frondanol® A5, a sea cucumber extract, prevent intestinal tumorigenesis. Cancer Prev. Res. 2015, 8, 327–337.
15. Hu S,et al. Eicosapentaenoic acid-enriched phosphatidylcholine isolated from Cucumaria frondosa exhibits anti-hyperglycemic effects via activating phosphoinositide 3-kinase/protein kinase B signal pathway. J. Biosci. Bioeng. 2014, 117, 457–463.
16. Huldani IP, Massi MN, Idris I, Bukhari A, Widodo ADW, Achmad H. Research Reviews on Effect of Exercise on DAMPís, HMGB1, Proinflammatory Cytokines and Leukocytes. *Systematic Reviews in Pharmacy*, 2020; 11(4): 306-12.
17. Mariño R, et al. Oral cancer screening practices of oral health professionals in Australia. BMC Oral Health; 17.2017.
18. Mupparapu M, Shanti RM. Evaluation and Staging of Oral Cancer. Dent Clin North Am 2018; 62: 47–58.
19. Curry JM,et al. Tumor microenvironment in head and neck squamous cell carcinoma. Semin Oncol 2014; 41: 217–234.
20. Aminin DL,et al. Anticancer Activity of Sea Cucumber Triterpene Glycosides. Marine drugs 2015.
21. Park JI,et al. Relationships between chemical structures and functions of triterpene glycosides isolated from sea cucumbers. Front. Chem. 2014, 2, 1–14.
22. Guo Y,et al. Systems pharmacology-based drug discovery for marine resources: An example using sea cucumber (Holothurians). J.Ethnopharmacol. 2015, 165, 61–72.
23. Pangestuti R,Arifin, Z. Medicinal and health benefit effects of functional sea cucumbers. J. Trad. Complim. Med. 2017.
24. Al Shemaili J,et al. Frondoside A enhances the antiproliferative effects of gemcitabine in pancreatic cancer. Eur. J. Cancer 2014, 50, 1391–1398.
25. Achmad, H. Khairunnisa, P. Mardiana, Karni Aulia, A. (2018). Potentially Of Extracted Papua's Anthill (*Myrmecodia pendans*) As Antitumor To Emphasis The Expression Of Vascular Endothelial Growth Factor Cell Burkitt's Lymphoma Cancer, Asian Journal. of Microbiol. Biotech. Env Sc. © Global Science Publications pp. 108-112, Vol. 20, No. (1). ISSN-0972-3005.
26. Seydi E, et al.Selective toxicity of Persian gulf sea cucumber (Holothuria parva) and sponge (Haliclona oculata) methanolic extracts on liver mitochondria isolated from an animal model of hepatocellular carcinoma. Hepat Mon 2015. 15(12)
27. Suzery M, Cahyono B. Cytotoxic effects of sea cucumber extracts using BSLT and MTT methods. Journal of Indonesian Pharmacy. 2014, 22 (3): 84-88.
28. Elfidasari D,et al. Identifikasi Jenis Teripang Genus Holothuria Asal Perairan Sekitar Kepulauan Seribu

Berdasarkan Perbedaan Morfologi. Jurnal Al Azhar Indonesia.2012; 1(3).
29. Achmad, H. (2018). Apoptosis Induction (Caspase-3, -9) and Human Tongue Squamous Cell Carcinoma VEGF Angiogenesis Inhibition using Flavonoids's Ethyl Acetate Fraction of Papua Ant Hill (*Myrmecodia pendans*) SP-C "), Journal of International Dental and Medical Research. ISSN 1309-100X. Volume 11 Number (1). pp. 276-284.
30. Pangestuti R,Arifin Z. Medicinal and Health Benefit Effects of Functional Sea Cucumber. JTCM.2017.
31. Soltani M,et al. Heolytic and Cytotoxic Properties of Saponin Purified from Holothurian Leucospilota Sea Cucumber. RMB. 2014;3(1).
32. Aminin, D. Holothuria Triterpene Glycosides as Modulator of Cellular Functions. Ecronian.2019.
33. Silchenko AS,et al. Fallaxosides B1 and D3, triterpene Glycosides with Novel Skeleton Types of Aglycones from the Sea Cucumber Cucumaria Fallax. Tetrahedrom.2017.
34. Achmad H , Sherly Horax, Sri Ramadhany, Irene Edith Rieuwpassa, Melyanti Sari, Hendrastuti Handayani, Marhamah F. Singgih, Sumintarti Sugiharto. (2019). Anti-Cancer and Anti-Proliferation Activity of Ethyl Acetate Extract From Ant Nest (Myrmecodia pendans) in Burkitt's Lymphoma Cancer Cells. Pesquisa Brasileira em Odontopediatria e Clinica Integrada. ISSN 1519-0501, 19 (1): e4325.
35. Yun CW, Kim HJ, Lee SH. Therapeutic Application of Diverse Marine-derived Natural Products in Cancer Thrapy. Anticancer Research.2019
36. Pangestuti R, Arifin Z. Medicinal and Health Benefit Effect of Functional Sea Cucumber. JTCM. 2017.
37. Esmat AY,et al. Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (Holothuria atra) against thioacetamide intoxication in rats. Nutrition.2013;29(1), 258-267.
38. Soltani M,et al.Hemolytic and cytotoxic properties of saponin purified from Holothuria leucospilota sea cucumber. Reports of Biochemistry and Molecular Biology.2014;3(1), 43.
39. Rivera C. Essential of Oral Cancer. IntJClinExp. 2015;8(9)
40. Huldani IP, Massi MN, Idris I, Bukhari A, Widodo ADW, Achmad H. Research Reviews on Effect of Exercise on DAMPís, HMGB1, Proinflammatory Cytokines and Leukocytes. *Systematic Reviews in Pharmacy*, 2020; 11(4): 306-12.
41. Edefonti V, et al. Vitamin E intake from natural sources and head and neck cancer risk: a pooled analysis in the International Head and Neck Cancer Epidemiology consortium. Br J Cancer 2015. 113(1):182–192.
42. American Cancer Society. About Oral Cavity and Oropharyngeal Cancer. American Cancer Society.2018
43. Ridhowati S, Chasanah E, Syah D, Zakaria, F.A study on the nutrient substances of sea cucumber Stichopus variegatus flour using vacuum oven. International Food Research Journal.2018;25(4), 1419-1426. G
44. Wijesinghea WAJP., Perumal R., Effendy MAW,Charles SV. Anticancer activity and mediation of apoptosis in human HL-60 leukaemia cells by edible sea cucumber (Holothuria edulis) extract. Food Chemistry.201; 139:326–331.
45. Achmad H, Sri Ramadhany, Philip Eric Suryajaya. (2019). Streptococcus Colonial Growth of Dental Plaque Inhibition Using Flavonoid Extract of Ants Nest (*Myrmecodia pendans*): An in Vitro Study. Pesquisa Brasileira em Odontopediatria e Clinica Integrada. ISSN 1519-0501, 19 (1): e4250.
46. Brocklehurst P, Kujan O, O'Malley LA, Ogden G, Shepherd S, Glenny AM. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev 2013; 11.
47. Elfidasari D, Noriko N, Wulandari N, Perdan AT. Identification of the type of sea cucumber Genus Holothuria from around the Thousand islands based on morphological differences. Al Azhar Journal. 2012; 1 (3): 140-6.
48. Uinarni H, Tanjung C, Huldani APP, Mashuri BIS, Wahyudi H, Zuhair A, Tarius AG, Wiryawan W, Dewi RK, Ramadhany YF, Achmad H. The Importance of Ultrasound Findings in Children with Acute Abdominal Pain to Prevent Unnecessary Surgery. *Systematic Reviews in Pharmacy*, 2020; 11(4): 377-83.
49. Ministry of health Republic of Indonesia. Data Center and Health Information. Stop Cancer. Infodatin-Cancer. 2015. P. 3.
50. Food and drug supervisory bodies. IONI: Indonesian national drug Informatorium. Jakarta: The Food and Drug Supervisory agency. 2008.
51. Bordbar S, Anwar F, Saari N. High-value components and bioactives from sea cucumbers for functional foods - A review. Marine Drugs 2011;9(10):1761–805.
52. Ahmad H. Horax S, Ramadhany S, et.al. (2019). Resistivity of Ant Nest (*Myrmecodia pendans)* On Ethanol Fraction Burkitt's Lymphoma Cancer Cells (Invitro) Through Interleukin 8 Angiogenesis Obstacles (Il-8). Journal of International Dental and Medical Research ,. ISSN 1309-100X. Vol 12 No. (2) pp.516-523.
53. Mehmet A, Huseyin S, Bekir T, Yilmaz E, Sevim K. Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. Int J of Food Science and Technology 2011;46:500–8.
54. Wen J, Hu C, Fan S. Chemical composition and nutritional quality of sea cucumbers. J of the Science of Food and Agriculture 2010;90:2469– 74.
55. Achmad H, Ramadhany YF, Ramadhany S. (2019). Resistivity of Protein Kinase-B (Akt), Nf-Kb Transduction Obstacles, and Apoptosis Induction (Caspace -3, -9) as Anti-Proliferation and Anti-Cancer of Burkitt's Lymphoma Using Flavonoid Fraction Of Ethyl Acetate From Ant Nest (*Myrmecodia pendans*). The 3rd International Conference On Science. Journal of Physics: Conference Series. p.1341