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Protein Content of Sea Urchins (*Tripneustes Gratilla*) and The Potential of Its Shell as An Antibacterial

Martha Kaihena[1*], Deford C Birahy[2], Maria Nindatu[1], Bustomi[2], Richard A Matulessy[3]

[1]*Biology Study Program, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon 97233, Maluku, Indonesia.*
[2]*Biotechnology Study Program, IPB University, Bogor 16680, West Java, Indonesia.*
[3]*Geology Study Program, Faculty of Engineering, Pattimura University, Ambon 97233, Maluku, Indonesia.*

**Corresponding author's E-mail: marthakaihena5@gmail.com*

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Abstract

Sea urchins are often used as a food ingredient by taking their gonads. Sea urchins are also a potential fishery resource that greatly benefits human life. The study aimed to determine the gonad protein levels of sea urchins (Tripneustes gratilla) in different aquatic environments. The variables observed in this study were sea urchin gonadal index, gonadal index, protein content analysis, and antibacterial sea urchin shell methanol extract against E. coli, Salmonella sp., and S. aureus. The results showed that the average gonadal index for sea urchins (Tripneustes gratilla) on these two substrates was still relatively low, 1.46 for sandy substrates and 1.72 for rocky substrates. The gonad protein content of sea urchins can be seen on a sandy substrate and overgrown with seagrass. The average percentage of gonad protein content in sea urchins is 17.3%, while on a rocky substrate, the average percentage of protein content is 16.5%. The results of the T-test showed that the average results for the gonad protein content of sea urchins on these two substrates were not significantly different. Concluded that the gonad protein content of rocky sea urchins had a higher value of 17.3 with a gonadal index of 1.72 compared to the sandy substrate with a gonadal protein content of 16.5 with a gonadal index of 1.46. The highest content of essential amino acids in rocky locations was found in the type of amino acid leucine, with a concentration value of 8.59. Antibacterial methanol extract of Tripneustes gratilla sea urchin shell against E. coli, salmonella sp., and S. aureus showed the diameter of the inhibition zone formed on the agar media; in this case, the bacterial inactivation unit ranged from 1.84 mm/g – 2.65 mm/g.

Keywords: *Sea urchins, gonads, protein, shell, antibacterial.*

1. Introduction

Protein is a food substance that is very important for the body, so it functions as a building and regulatory substance that contains the elements C, H, O, and N, which do not have metal protein types such as iron and copper (Triwahyuni, 2018). Almost all human protein sources generally come from mammals such as chickens, cows, and pigs. This causes concern from most consumers based on religious and health reasons. Sea urchins, often referred to as sea urchins, are a group of animals often found in the coastal areas of Indonesia. People use sea urchins as a food ingredient by taking the gonads (Banudi et al, 2021). So far, the international market for sea urchins is quite high. For example, in France and other European countries, the gonad production rate for sea urchins is more than 500 tons per Month—Japan, as much as 20,000 tons per Month (Sun and Chiang, 2015). In addition, sea urchins are also a potential fishery resource that has great benefits for human life.

Sea urchins in Indonesia, especially in Maluku, where people work as fishermen, are currently not used commercially. Its utilization is only as additional animal feed and a side dish, especially for coastal communities. According to information obtained by the people of Negeri Hulaliu, they often consume sea urchins as a substitute for side dishes. the people of Hulaliu Country have a unique designation for this animal: tailor lau (sea egg). In general, people often serve sea urchins by frying them. But another small part can consume it raw by adding a little lemon juice. Sea urchins are found in large numbers on

the coast of Negeri Hulaliu. Suryanti & A'in (2013) stated that sea urchins are found in many coral reef ecosystems because the abundance of these species populations is important for coral reefs as a counterweight. However, Diyzel et al. (2022) stated that sea urchins generally live in coral reefs, grasses, and sand. In the location, the habitat of Hulaliu Country is the habitat of sea urchins with coral reef conditions with lots of algae growing and sandy substrate conditions and lots of seagrasses growing. What is the protein content of the gonads of sea urchins (*Tripneustes gratilla*) in different aquatic environments? Knowing the gonad protein levels of sea urchins (*Tripneustes gratilla*) in different aquatic environments.

The shells and gonads of sea urchins (*Tripneustes gratilla*) are known to have high economic value (Chen dan Hwang, 2014). The gonads can be used as an alternative food source because they contain 28 kinds of amino acids, B complex vitamins, vitamin A, minerals, and omega-3 and omega-6 fatty acids (Archana et al., 2016). At the same time, shells have the potential as anticancer, antitumor, and antimicrobial (Kresnamurti and Budiarti, 2021). Sea urchins have a hard shell and a symmetrical five-sided interior (Salazar, 2018). The shells of certain types of sea urchins are coated with a stable black liquid pigment. This liquid can be used as a street and leather coloring. The shells of sea urchins are also of interest as jewelry items, while the organs from the rest of the sea urchin processing are usually in the form of shells and internal organs (offal), which can be further processed into fertilizer. Sea urchin shells contain active compounds that are toxic. The content in sea urchin shells has been known so far to be polyhydroxy and asteroids A and B (Johnstone, 2015). It is estimated that the poison in the shell and thorns can also be used as a medicinal ingredient. As an antimicrobial, sea urchin shells contain bioactive compounds, including serotonin, glycosides, steroids, cholinergic substances, and bradykinin-like substances (Kresnamurti et al., 2021).

2. Materials And Methods

Sea urchin gonad index calculation

The sampling of sea urchins in the State of Hulaliu randomly consisted of 5 individuals of the same size. Samples were stored in a cool box filled with seawater and brought to the laboratory for analysis.

Gonad index

Fresh sea urchins were weighed for each individual and their respective gonads using a weighing scale. Measurement of the value of gonad weight or gonad index of sea urchins was carried out in research with the equation:

$$GSI = \frac{\text{gonad weight (g)}}{\text{body weight (g)}} \times 100$$

Protein content analysis procedure

Determining protein levels can be done with the kjeldahl method, the protein test is divided into several stages: samples were weighed as much as 1-2 g, put into the destruction flask, added 5 g of a mixture of natrium sulfate and mercury oxide (20:1), concentrated sulfuric acid was added to the digestion flask, the destruction flask was heated initially at 200-250ºc until the solution is no longer smoky, then it is heated at a temperature of 300-400ºc until the solution in the digestion flask becomes clear. The destruction flask was rinsed with distilled water and heated at a temperature until the solution became clear. The destruction flask was transferred to the destruction apparatus and diluted with sufficient distilled water to a 45% naoh solution until the solution was alkaline (alkaline). Tested with paper in the destruction flask, added litmus. The erlenmeyer is placed at the cooling end of the distillation apparatus, with the condenser end position having to be immersed in the holding solution (5% boric acid). Distillation is carried out until the volume of the solution in the distillation flask 2/3 has evaporated, or the solution that comes out of the distillation cooler is no longer alkaline (tested with litmus paper). Titration was carried out with 0.1 n hcl until it reached the equivalence point (grayish color), and the number of ml of 0.1 n hcl used was recorded.

Analysis of amino acid content using High-Performance Liquid Chromatography (HPLC) method

Analysis of amino acid content by acid

About 1 g of fat-free sea urchin gonads were refluxed at 110ºC for 12 hours with 4 M concentrated H_2SO_4. The solution was neutralized by adding $Ba(OH)_2$ slowly. Then the solution was filtered to the mark of 50 mL in a measuring flask.

Analysis of amino acid content by alkaline hydrolysis

As much as 1 g of fat-free sea urchin gonads were refluxed at 110ºC for 12 hours with 4 M concentrated $Ba(OH)_2$. The solution was neutralized by slowly adding H_2SO_4. Then the solution was filtered to the mark of 50 mL in a measuring flask.

Separation of amino acid components by HPLC

Take 20 µL of each acid and base hydrolyzed sample, add 100 µL of OPA (o-phthalaldehyde) solution, stir for 5 minutes, then add 20 µL of HPLC injectors to the injector. Observe the types and levels of amino acids from the results of HPLC analysis that appear on the recorder. The column used was Microsob-MV-100-5C18 with a column length of 250 x 4.6 mm x mobile phase, A=Methyl hydroxide:50 nM sodium acetate: Tetra hyro furans (2:29:2) pH 6.8 and B = 65% metal hydroxide while the detector used is fluorescent.

3. Results and Discussion

Classification and Morphology of Sea Urchins (*Tripneustes gratilla*)

Classification of sea urchins (*Tripneustes gratilla*), according to Clark & Rowe (1971), is as follows:

Kingdom : Animalia

Filum : Echinodermata

Subclass : Echinoidae

Class : Echinonidae

Ordo : Cidaroidea

Family : Tripneustesidae

Genus : *Tripneustes*

Species : *Tripneustes gratilla*

Figure 1. *Tripneustes gratilla*

The internal organs of sea urchins are enclosed in a hard shell. Most sea urchins have a rounded body with five symmetrical fold lines divided into five ambulacral areas (Brink, 2020). Five inter-ambulacral areas separate the ambulacral areas. In each ambulacral region, tubular legs are near the mouth on the underside (Khanna, 2005). The lower half of the sea urchin's body is the oral surface, which is called the peristome. While the middle part upwards is the aboral surface called the period which contains the anus, genital opening, and madreporite (Piryaei et al, 2018)

Sea urchin (*Tripneustes gratilla*) Gonad Index

The gonad index of sea urchins (*Tripneustes gratilla*) can be seen in table 1. The T-test analysis of the gonadal index values of the sea urchins showed that the gonadal index results for sea urchins (*Tripnesutes gratilla*) were significantly different between the gonads on sandy and rocky substrates.

Table 1. Average gonadal index of individual sea urchins (*Tripneustes gratilla*).

Substrate	Test					Average (X±SD)
	1	2	3	4	5	
Sandy	1.3	1.8	1.7	1.3	1.2	1.46±0.11[a]
Rocky	1.9	2.0	1.6	1.3	1.8	1.72±0.34[b]

Based on table 1, then obtained a graph of the average gonadal index of sea urchins (*Tripneustes gratilla*) from two different locations, which can be seen in Figure 2.

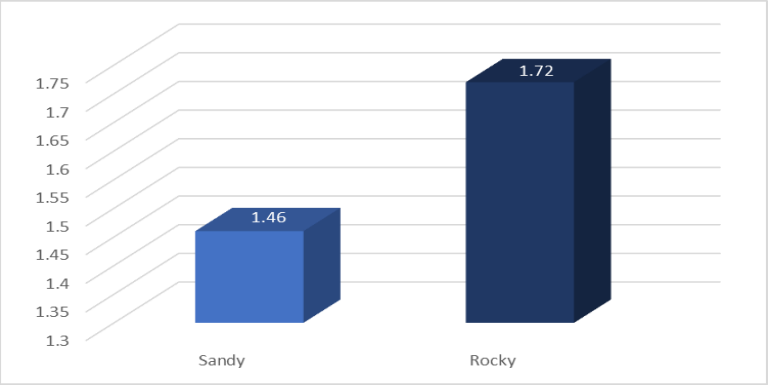

Figure 2. Graph of the average gonadal index of sea urchins (*Tripneustes gratilla*).

Data for the gonadal index of sea urchins (*Tripneustes gratilla*) from sandy substrates with an average total gonadal index of 1.46 ± 0.11 and 1.72 ± 0.34 for the porous substrate. Sea urchin total gonadal index data were analyzed using the T-test. The F value was less than 0.05, so the mean total sea urchin total gonadal index data were significantly different, which means that these two substrates have a different effect on the resulting gonadal index.

Protein Levels in Sea Urchins (*Tripneustes gratilla*)

The gonad protein levels of sea urchins (*Tripneustes gratilla*) of the two substrates showed that the gonadal protein levels of the two substrates were not significantly different.

Table 2. The average gonadal protein content of sea urchins (*Tripneustes gratilla*).

Substrate	Test					Average (X±SD)
	1	2	3	4	5	
Sandy	17.6	17.8	18.3	16.3	16.8	17.3±0.13[a]
Rocky	16.3	17.4	16.9	15.5	17.8	16.5±0.11[b]

Based on Table 2, then obtained a graph of the average protein content in sea urchins from 2 different substrates, which can be seen in Figure 3 as follows:

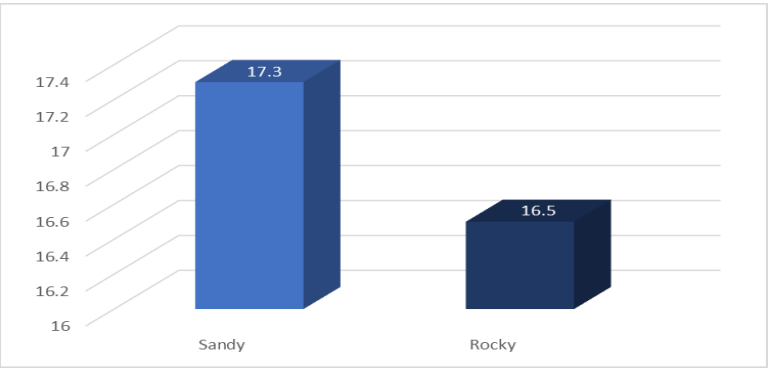

Figure 3. Graph the average gonadal protein content of sea urchins (*Tripneustes gratilla*) on two substrates.

The mean protein content of sea urchin gonads on the sandy substrate was 17.3 ± 0.13, and on the rocky substrate was 16.5 ± 0.11. The total protein content of sea urchins was analyzed using the T-test. The F value was greater than 0.05, so the average total protein content data in sea urchin gonads did not differ significantly. The two substrates had the same effect on the resulting protein content.

Amino Acid Content in Sea Urchin Gonads (*Tripneustes gratilla*)

Analysis of amino acid content used the HPLC method, acid hydrolysis was carried out for 12 hours at 110ºC using H_2SO_4, while alkaline hydrolysis used $Ba(OH)_2$ 4 M, which was carried out for 24 hours at 110ºC with a sample weight of 1 g each, After obtaining the chromatogram of amino acid results (appendix 5). The concentration of amino acids is calculated. The results of the gonadal amino acid concentrations of sea urchins (*Tripneustes gratilla*) can be seen in table 3.

Table 3. Results of gonadal amino acid concentrations of sea urchins (*Tripneustes gratilla*) using the HPLC method.

No	Amino Acid	Sandy	Rocky
		mg/100g Protein	mg/100g Protein
1	**Threonine***	5.32	4.89
2	**Valin***	2.46	5.00
3	**Phenylalanine ***	0.00	0.00
4	**Isoleucine ***	0.00	2.56
5	**Leusin ***	4.56	8.59
6	**Arginine**	0.00	4.30
7	**Alain**	14.40	15.56
8	**Tyrosin**	4.26	5.00
9	**Aspartate**	0.00	2.58
10	**Serin**	6.48	15.64
11	**Glisten**	0.00	0.00
12	**Glutamate**	23.35	18.74

Description: * (essential amino acid)

After obtaining the results of the amino acid concentrations of the two research locations, further calculations were carried out to determine the Essential Amino Acid Index, Nutrition index, and Protein Efficiency Ratio of the gonads of sea urchins (*Tripneustes gratilla*).

Table 4. Calculation results of the Essential Amino Acid Index (EAAI), Nutrition Index (NI), and Protein Efficiency Ratio (PER) of the gonads of sea urchins (*Tripneustes gratilla*) in two study locations.

Protein Quality	Sandy	Rocky
EAAI	4.92	6.34
NI	0.63	0.85
PER	1.15	2.90

Sea urchin shell extract (*Tripneustes gratilla*) on sandy substrate

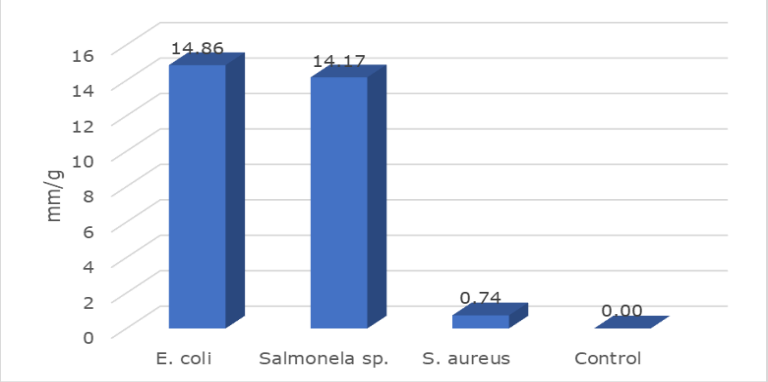

Figure 4. Diagram of the antibacterial activity of sea urchin shells (*Tripneustes gratilla*) on sandy substrate.

Sea urchin shell extract (*Tripneustes gratilla*) on sandy substrate

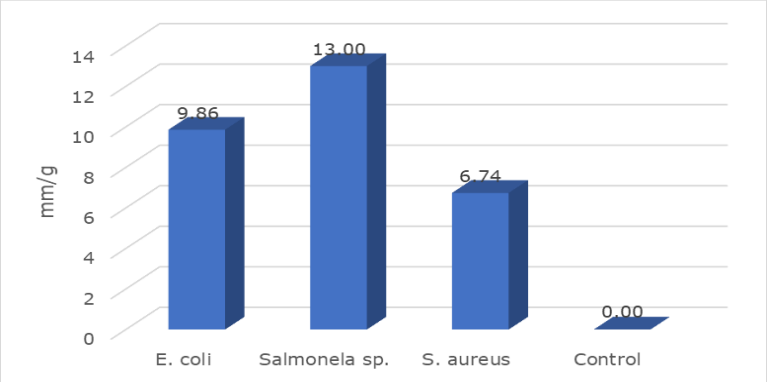

Figure 5. Diagram of the antibacterial activity of sea urchin shells (*Tripneustes gratilla*) on hard substrate.

Discussion

The average gonadal index value of sea urchins (*Tripneustes gratilla*) of the two substrates is significantly different where the gonadal index value of sea urchins (*Tripnesutes gratilla*) on rocky substrates is greater than that of sandy substrates, Rocha et al. (2019) reported that temperature and length day is negatively correlated with the gonadal index (gonad-temp), meaning that larger gonadal sizes are found when water temperature and day length are low, as is the case with environmental factor data (temperature, DO, pH, salinity). It is suspected that the study locations were almost the same, so environmental factors did not affect the gonadal index (Lekatompessy et al, 2023). Other studies, such as Vatilingon et al. (2005), explain that food composition and frequency of eating also affect gonadal growth and nutrient content of the gonads. Li et al (2021) stated that brown algae are a group of macroalgae known as a rich source of bioactive compounds. The nutritional content contained in brown algae includes protein, minerals, and vitamins (André et al, 2021). According to Kumar et al (2015), Kumar and Sahoo (2017), brown algae has a protein content of 3-15%. According to Morais et al (2020), brown algae (Laminaria sp) could increase gonad weight gain due to the effectiveness of digestion, so gonad growth is better than artificial feed. In contrast to seagrass, which only contains protein and carbohydrates (Kannah et al, 2020).

Based on the results of these studies, it can be said that the gonads of sea urchins on a rocky substrate have a higher index value, because the food supply from the habitat where they live is very good and nutritious, in this case, brown algae. The average gonadal index for sea urchins (*Tripneustes gratilla*) on these two substrates is still relatively small, 1.46 for sandy substrates and 1.72 for rocky substrates. The small gonad index value for sea urchins is due to the weight of the gonads being too small compared to the animal's total weight. Gonads when entering the gametogenesis/mature phase, nutrient storage cells serve as energy providers for gametogenesis. According to Nagarajan et al statement (2021), the nutrient content of feed ingredients includes water, minerals, protein, fat, and carbohydrates. At the end of the gametogenesis/mature phase, the cells that contain nutrients in the gonads decrease in number and size and even disappear completely, and this reduces the size and weight of the gonads, which in turn causes the gonad index to be small as well as causing a lack of gonad quality (nutrient content) (Tremellen, 2016). So it is suspected that the gonads used in this study have not yet entered the gametogenesis/mature phase (Rocha et al, 2019).

The gonad protein content of sea urchins can be seen on sandy substrates and overgrown with seagrass beds. The average percentage of gonad protein content in sea urchins is 17.3%, while on rocky substrates, the average percentage of protein content is 16.5%. The results of the T-test showed that the average results for the gonad protein content of sea urchins on these two substrates were not significantly different. Challener (2013) found the gonad protein content of sea urchins from the *Tripneustes gratilla* species had a protein content of 15.43%, while Afifudin et al. 2014 described the gonad protein of sea urchins Diadema setosum 12.60%, Echinothrix calamari 11.40 %, and E. diadema 13.20%. When compared with the research results obtained, it can be seen that the gonadal protein levels of sea urchins on both substrates on the coast of Negeri Hulaliu are still quite high. This is because the rocky substrate, which is the sea urchin footing area, contains a lot of macro algae (brown algae), which are food for the sea urchins. The same goes for sandy substrates. It is a habitat for sea urchins and seagrasses. Seagrass is also food for the survival of sea urchins. Rengasamy et al. (2013) explained that seagrass is a potential plant that has high antioxidants and good nutritional value.

Based on the results of research on the gonadal amino acids of sea urchins *Tripneustes gratilla* on a sandy substrate, there are 9 types of amino acids consisting of 5 types of non-essential amino acids and 4 types of essential amino acids. and 3 essential amino acids. Karnila et al (2022) explains that differences in the chemical composition of the gonads of sea urchins can be influenced by the waters where biota live. In rocky locations, there are brown algae and seagrass, which are a favorite food for sea urchins (*Tripneustes gratilla*) and play an important role in the growth of sea urchins (*Tripneustes gratilla*) (Seymor et al. 2013 in Silahooy et al. 2013). The study results showed that the highest essential amino acid content was found in the type of amino acid leucine with a concentration value of 8.59. The amino acid leucine stimulates brain function, can also increase muscle energy levels, helps reduce excessive blood sugar levels, and helps heal bones, muscle tissue, and skin (especially to accelerate post-operative wound healing). Leucine also functions in maintaining the immune system (Ananieva et al, 2016). For this reason, it can be said that the gonads of sea urchins (*Tripneustes gratilla*) are good for consumption by the community.

The protein quality of sea urchins (*Tripneustes gratilla*) in this study was in the form of 'Essential Amino Acid Index' (EAAI) values (Affidun et al, 2014), 'Nutritional Index (NI) values , and 'Protein Efficiency Ratio' values (PER). The EAAI value is the comparison between the content of essential amino acids in the gonads of sea urchins (*Tripneustes gratilla*) and chicken eggs (Dinh et al, 2023). In contrast, the PER value is the ratio of the increase in human body weight (g) and the weight of protein (g) eaten by sea urchins. The gonads of sea urchins (*Tripneustes gratilla*) on rocky substrates have higher EAAI, NI and PER values when compared to sandy substrates (Lekatompessy et al, 2023). This is due to the location's amount and concentration of essential amino acids. Rockier/larger than sandy locations. The inhibition test results of the methanol extract of *Tripneustes gratilla* sea urchin shells against *E. coli*, *Salmonella* sp., and *S. aureus* bacteria showed the diameter of the inhibition zone formed on the agar medium; in this case, the bacterial inactivation unit ranged from 1.84 mm/g – 2.65 mm/g (Figure 5). The inhibition of Tripneustes gratilla shell extract can be determined by the diameter of the inhibition zone. Gheorghita et al (2022) stated that there were 3 categories of inhibition areas of active substances based on the diameter of the inhibition zone. Namely, for the weak category, the diameter of the inhibition zone was <5 mm/g, Moderate 5-10, and Strong 10-20 mm/g. The results showed that the antibacterial properties of the extract were in the weak category for S. aureus, the inhibition zone diameter was <5mm/g, and strong for *E. coli* and *Salmonella* sp. The diameter of the inhibition zone was 14.86 for *E. coli* and 14.17 for *Salmonella* sp. The bioactive compound from the methanol extract of sea urchin shells of the type Salmacis virgulate, namely polyhydroxy naphthoquinone, is known to have the potential as an antibacterial and antioxidant (Hou et al, 2020; Kresnamurti et al, 2021). From research, sea urchin shell extract can react with gram-negative and gram-positive bacteria. This proves that the bioactive compounds dissolved in the methanol solvent are not strong enough as antibacterial agents.

4. Conclusion

Based on the results of our research, it can be said that sea urchins (*Tripneustes gratilla*) have significant benefits for the future, where sea urchin gonads can be used as an alternative food source at a low price as a substitute for animal protein containing amino acids which are The body needs to increase the body's immunity including the amino acids threonine, valine, phenylalanine, isoleucine, leucine, arginine, alanine, tyrosine, aspartate, serine, glycine, and glutamate. In addition, the sea urchin shell can be utilized by the health and beauty industry as an antibacterial base because it has broad antibacterial capabilities, killing the growth of gram-positive and gram-negative bacteria.

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References:

Ananieva EA, Powell JD, Hutson SM. 2016. Leucine metabolism in T cell activation: mTOR signaling and beyond. Advances in nutrition 7(4):798S-805S. DOI: https://doi.org/10.3945/an.115.011221

André R, Pacheco R, Bourbon M, Serralheiro ML. 2021. Brown algae potential as a functional food against hypercholesterolemia. Foods 10(2):234. https://doi.org/10.3390/foods10020234

Archana A, Babu KR. 2016. Nutrient composition and antioxidant activity of gonads of sea urchin Stomopneustes variolaris. Food chemistry 197:597-602. DOI: https://doi.org/10.1016/j.foodchem.2015.11.003

Banudi L, Koro S, Anasiru MA, Nurmiaty N. 2021. The Effect of the Provision of Bagea Enriched with Sea Urchin Gonads on Weight Gain in Toddlers of the Bajo Ethnic. *Indonesian Journal of Public Health Nutrition* 2(1):44-52. DOI: https://doi.org/10.7454/ijphn.v2i1.5343

Brink M. 2020. Genetic studies for sustainable aquaculture of the sea urchin, *Tripneustes gratilla*. [Dissertation]. Stellenbosch Central, Stellenbosch University. [South Africa]

Challener RC. 2013. An investigation of the sublethal effects of carbon dioxide on the common sea urchin Lytechinus variegatus and of the carbonate chemistry of its nearshore habitat. The University of Alabama at Birmingham

Chen YC, Hwang DF. 2014. Evaluation of antioxidant properties and biofunctions of polar, nonpolar, and water-soluble fractions extracted from gonad and body wall of the sea urchin *Tripneustes gratilla. Fisheries science* 80:1311-1321. DOI: https://doi.org/10.1007/s12562-014-0808-9

Clark AM, Rowe FWE. 1971. Monograph of Shallow Water Indo West Pacific Echinoderms. Brithis Museum, London.

Dinh TKH, Nguyen PH, Phuong DL, Dang TPL, Quan PM, Dao TKD, Long PQ. 2023. Component and Content of Lipid Classes and Phospholipid Molecular Species of Eggs and Body of the Vietnamese Sea Urchin *Tripneustes gratilla.* Molecules 28(9):3721. DOI: https://doi.org/10.3390/molecules28093721

Ditzel P, König S, Musembi P, Peters MK. 2022. Correlation between coral reef condition and the diversity and abundance of fishes and sea urchins on an East African coral reef. In *Oceans* 3(1):1-14. DOI: https://doi.org/10.3390/oceans3010001

Gheorghita D, Grosu E, Robu A, Ditu LM, Deleanu IM, Gradisteanu Pircalabioru G, Antoniac VI. 2022. EsseNtial oils as antimicrobial active substances in wound dressings. *Materials* 15(19):6923. DOI: https://doi.org/10.3390/ma15196923

Hou Y, Carne A, McConnell M, Mros S, Vasileva EA, Mishchenko NP, Bekhit AEDA. 2020. PHNQ from Evechinus chloroticus Sea Urchin Supplemented with Calcium Promotes Mineralization in Saos-2 Human Bone Cell Line. Marine Drugs 18(7):373. https://doi.org/10.3390/md18070373

Johnstone CR. 2015. *Investigation of coelomic fluid from the sea urchin E. chloroticus*. [Dissertation]. University of Otago, 362 Leith Street. [New Zealand]

Karnila R, Iriani D, Shaarani SM, Yunus AA, Salma R. 2022. Nutritional characteristics of sea urchin (*Diadema setosum*) in Bungus, West Sumatera Province. In IOP Conference Series: Earth and Environmental Science 1118(1):012037. DOI: https://doi.org/10.1088/1755-1315/1118/1/012037

Khanna DR. 2005. Biology of Echinodermata. Discovery Publishing House.

Kannah RY, Kavitha S, Gunasekaran M, Kumar G, Banu JR, Zhen G. 2020. Biohydrogen production from seagrass via novel energetically efficient ozone coupled rotor stator homogenization. *International Journal of Hydrogen Energy* 45(10):5881-5889. DOI: https://doi.org/10.1016/j.ijhydene.2019.04.151

Kresnamurti A, Budiarti F. 2021. The Effectiveness of Sea Urchin Extract (Echinometra matthaei) for Wound Healing on Deep Second-Degree in White Rats (*Rattus norvegicus*) Wistar. Journal of Tropical Pharmacy and Chemistry 5(3):194-202. DOI: https://doi.org/10.25026/jtpc.v5i3.281

Kumar S, Sahoo D, Levine I. 2015. Assessment of nutritional value in a brown seaweed *Sargassum wightii* and their seasonal variations. Algal Research 9:117-125. DOI: https://doi.org/10.1016/j.algal.2015.02.024

Kumar S, Sahoo D. 2017. A comprehensive analysis of alginate content and biochemical composition of leftover pulp from brown seaweed *Sargassum wightii*. Algal research 23:233-239. DOI: https://doi.org/10.1016/j.algal.2017.02.003

Lekatompessy VC, Marhendra APW, Kurniawan N. 2023. Accumulation of Microplastics in the Digestive Tract and Gonads and its Effects on Gonad Quality of Sea Urchins Tripneustes gratilla. Biotropika: Journal of Tropical Biology 11(1):53-63. DOI: https://doi.org/10.21776/ub.biotropika.2023.011.01.07

Li Y, Zheng Y, Zhang Y, Yang Y, Wang P, Imre B, Wang D. 2021. Brown algae carbohydrates: Structures, pharmaceutical properties, and research challenges. Marine Drugs 19(11):620. DOI: https://doi.org/10.3390/md19110620

Salazar A. 2018. *Advanced Chordate Zoology*. Scientific e-Resources.

Morais T, Inácio A, Coutinho T, Ministro M, Cotas J, Pereira L, Bahcevandziev K. 2020. Seaweed potential in the animal feed: A review. *Journal of Marine Science and Engineering* 8(8):559. DOI: https://doi.org/10.3390/jmse8080559

Nagarajan D, Varjani S, Lee DJ, Chang JS. 2021. Sustainable aquaculture and animal feed from microalgae–nutritive value and techno-functional components. *Renewable and Sustainable Energy Reviews* 150:111549. DOI: https://doi.org/10.1016/j.rser.2021.111549

Piryaei F, Ghavam Mostafavi P, Shahbazzadeh D, Pooshang Bagheri K. 2018. Description on anatomy and histology of Echinometra mathaei (Echinoidea: Camarodonta: Echinometidae), the Persian Gulf sea urchin. Sustainable Aquaculture and Health Management Journal 4(2):1-27. DOI: https://doi.org/10.29252/ijaah.4.2.1

Rengasamy RRK, Radjassegarin A, Perumal A. 2013. Seagrasses as potential source of medicinal food ingredients: Nutritional analysis and multivariate approach. *Biomedicine & Preventive Nutrition* 3(4):375-380. DOI: https://doi.org/10.1016/j.bionut.2013.06.011

Rocha F, Baião LF, Moutinho S, Reis B, Oliveira A, Arenas F, Valente LM. 2019. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of Paracentrotus lividus along the North Atlantic coast of Portugal. Scientific Reports 9(1):2994. DOI: https://doi.org/10.1038/s41598-019-39912-w

Silahooy V, Tohal AH, Hakim IL, Silahooy WI. 2013. Spatial distribution of *Tripneustes gratilla* on Ambon Island. The Journal of Tropical Science 3(3): 177-181. DOI: https://doi.org/10.11594/jtls.03.03.06

Sun J, Chiang FS. 2015. Use and exploitation of sea urchins. *Echinoderm aquaculture*, 25-45.

Suryanti, A'in C. 2013. Differences in the abundance of sea urchins (sea urchin) on different substrates in Legon Boyo Karimunjawa Jepara: Proceeding SEMNAS III Fishery and Marine Products FPIK. Diponegoro University, Semarang, 02 November 2013. [Indonesian].

Tremellen K. 2016. Gut Endotoxin Leading to a Decline IN Gonadal function (GELDING)-a novel theory for the development of late onset hypogonadism in obese men. Basic and Clinical Andrology 26:1-13. DOI: https://doi.org/10.1186/s12610-016-0034-7

Vaitillingon D, Solofonirina R, Jangoux M. 2005. Reprodutive cycle of edible ecinoderms from the southwestern indian ocean *Tripneustes gratila* L. (Ehinoidea, Echinodermata). Western Indian Ocean J, Mar, Sci 4(1): 47-6. DOI: https://doi.org/10.4314/wiojms.v4i1.28473