International Journal of Nutrition and Food Sciences
2016; 5(5): 330-336
http://www.sciencepublishinggroup.com/j/ijnfs
doi: 10.11648/j.ijnfs.20160505.13
ISSN: 2327-2694 (Print); ISSN: 2327-2716 (Online)

Immune Nutrient Content of Sea Urchin (*Diadema setosum*) Gonads

Wa Ode Salma[1], Sitti Wahyuni[2, *], Ilyas Yusuf[3], La Ode Muhammad Yasir Haya[4], Irawan Yusuf[5], Suryani As´ad[6]

[1]Department of Nutrition, Faculty of Medicine, Halu Oleo University, Kendari, Indonesia

[2]Hasanuddin University Medical Research Centre, Makassar, Indonesia

[3]Pharmachology Laboratory, Pharmacy Academy of Bina Husada Foundation, Kendari, Indonesia

[4]Marine Science Department, Fisheries and Marine Science Faculty, Halu Oleo University, Kendari, Indonesia

[5]Physiology Department, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

[6]Department of Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

Email address:

sittiwahyuni@gmail.com (S. Wahyuni)

[*]Corresponding author

To cite this article:

Wa Ode Salma, Sitti Wahyuni, Ilyas Yusuf, La Ode Muhammad Yasir Haya, Irawan Yusuf, Suryani As´ad. Immune Nutrient Content of Sea Urchin (*Diadema setosum*) Gonads. *International Journal of Nutrition and Food Sciences.* Vol. 5, No. 5, 2016, pp. 330-336.
doi: 10.11648/j.ijnfs.20160505.13

Received: July 14, 2016; **Accepted**: July 26, 2016; **Published**: August 29, 2016

Abstract: Over population of marine sea urchins (*Diadema setosum*) may disrupt the growth of *Crustose* coralline causing the reduction of coral reefs growth. Consumption of *Diadema setosum* gonads as the alternative food may assists to preserve the balance of coral reefs ecosystem. The Objective of this study was to measure and evaluate the nutrient content of the gonad of *Diadema setosum.* Mass spectrophotometer was used to measure vitamin and albumin contents, Kjeldahl methods for protein content, and Atomization method for trace elements (Fe, Mg, and Zn) content. The presence of active compounds such as steroids, amino acids and antioxidants were identified by thin-layer chromatograph (TLC). Protein, albumin, vitamin A, vitamin E and trace elements (Fe, Mg, and Zn) were found in the gonad of *Diadema setosum*. Vitamin E (23.47 mg) was the highest nutrient content compared to other nutrient elements. The extracts of the gonad of *Diadema setosum* were found to have steroid, amino acids and antioxidant compounds. Overall, nutrient contents and active compounds in the gonad of *Diadema setosum* are essential components needed for immune system, therefore besides its potency as alternative food source, gonad of *Diadema setosum* has potency to become the source of immune-nutrient.

Keywords: *Diadema setosum,* Gonad, Immune, Nutrient

1. Introduction

Population of *sea urchin (Diadema setosum)* is abundance in Indonesia, especially in the Wakatobi National Marine Park, South Sulawesi Province. The park has coral triangle consisting of approximately 50,000 ha of coral reefs [1]. *Crustose coralline,* a food source for *Diadema setosum*, belongs to algae family that produce calcium carbonate, animportant component for the formation of coral reefs structure. Wildlife Conservation Society [2] reported that over population of marine sea urchins potentially decreases the population of *Crustose coralline*. The reduction of coral reefs also decreases the population of sea otter [3], the animal which is known play a role in the conservation of coastal ecosystem. Therefore, the population of sea urchins have to be controlled to prevent ecosystem damage in the sea [4].

One method for controlling the over population of marine *sea urchins* is by starting to consume it as an alternative for nutritional source. The body fluids of sea urchins has been reported to have antitoxic effect on microbes [5]. In addition, gonad of *Diadema setosum* contains essential amino acids, *β*-carotene and dochosahecaenoat (DHA) [6]. Chen et al. in his analysis found that *Diadema setosum* gonads contains 80%

polyunsaturated fatty acids (PUFA) among other, eicosapentaenoic acid (EPA), arachidonic acid (AA) and natural carotenoids which contain antioxidant substances, such as echinenone, β-carotene and fucoxathine [7]. In Chili, Japan, California Alaska, New Zealand, *Diadema setosum* gonads has been used as a common staple food. In Indonesia, people living in coastal area of South East Sulawesi province consume the gonad as food supplement to improve body stamina. Until now there is no any comprehensive study has addressing the immune nutrient content of the gonad of *Diadema setosum* yet. This study was aimed to measure nutrient and trace element contents of *Diadema setosum* gonads, as well as to detect its active compound in its extract.

2. Materials and Methods

2.1. Sampling Location

Diadema setosum were collected from Wakatobi National Marine Park, Wakatobi District Southeast Province, Indonesia. The collection was done in the morning and during the night and only collected *Diadema setosum* that located 2-3 meters under the sea level and has weight between 60-100 gr. The gonads then separated from sea urchin body, placed in plastic bags (Figure 1) and stored in thermos bottles/cooling containers which had been filled before with dry ice. Freeze conditions was maintained during transportation from Wakatobi District to Makassar and until the gonads were processed in Pharmacy Faculty, Hasanuddin University and in Makassar Health Laboratory, Indonesia. Figure 1 shows the sea urchins and the gonads.

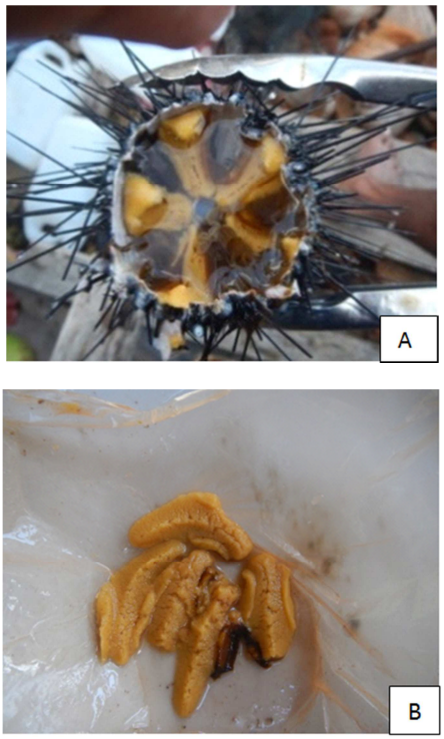

Figure 1. *Diadema setosum collected from Wakatobi National Marine Park of Wakatobi District, Southeast Province, Indonesia. (A) in cross section, (B) the gonads.*

2.2. Measurement of Protein, Vitamin and Trace Elements Contents

Analysis of protein and vitamin contents in the gonad of *Diadema setosum* was done at Makassar Health Laboratory. The laboratory facility and technique had been certified by Ministry of Health (No. 14118280/LHU/BBLK-MKS/XII/2014). Protein and albumin content of the gonad of *Diadema setosum* were measured by using Kjeldahl method (titrimetric), vitamin A, vitamin E content were analyzed by ultra violet (UV)/ Vis spectrophotometer [8] and the active compounds were detective by thin layer chromatography (TLC) [9].

2.2.1. Protein Content

A half gr of *Diadema setosum* gonads which had been finely ground, 1 gr of Selenium and 25 ml of H_2SO_4 were place in digestion flask and was stirred until mixed. After settling for 24 hours, the mixture was heated by using hotplate which was placed in the 350°C acid container for 1 hour until white smoke emitted and the color of the extract became clear. After the temperature of the extract reached room temperature, the extract then diluted with distilled water until its volume reached 200 ml. Five drops of phenolphthalein 0.1% indicators was added to the extract solution and then NaOH solutions were dropped in until its pH became alkalis. The solution then distillated with boric acid 1% until the volume reached 100 ml. Distillate then was poured into the 500 ml Erlenmeyer, added with 3 drops of indicator Conway and titrated with 0.1 N HCL until the color became purple. The protein content expressed as percent (%) based on the titration volumes.

2.2.2. Albumin Content

For standard albumin, distilled water was use to prepare albumin in concentration 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ L. Four ml of each albumin concentration was filled into volumetrick flask, added with biuret reagent until the volume reached 10 ml and then incubated for 10 min at 37°C. The absorption of albumin for each concentration was measured by spectrophotometer UV/Vis with 575 nm wavelength. The albumin uptake from all concentration was used as a calibration curve. To meansure albumin content, five gr of *Diadema setosum* gonads that had been finely macerated were mixed with 50 ml of distilled water. After 2 hours, the solution then filtered. Four ml of filtered solutions then put into a volumetric flasks, added with biuret reagent until its volume reached 10 ml and then incubated for 10 min at 37°C. The absorption albumin was measured by Spetrofotometric UV-Vis with 575 nm wavelength. The albumin content of *Diadema setosum* gonads was measured by comparing its absorsption with the one in the calibration curve.

2.2.3. Vitamin A Content

For standard, petroleum benzene was used to dilute beta carotene into 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L concentrations. Absorption of beta carotene was measured by

spectrophotometer with 450 nm wavelength. To measure beta carotene content, ten gr of refined *Diadema setosum* gonads finely macerated with 100 ml acetone solvent. After evaporation process, the powder *Diadema setosum* gonads was mixed with 5 ml of 15% KOH solution and allowed to stand for 24 hours. The extraction process was carried out by mixing *Diadema setosum* gonads with 20 ml KOH in diethyl ether and shake gently by separated funnel tubes. This process was repeated a few times until the solution became colorless. The absorption of beta carotene *Diadem*a setosum gonads was measured by spectrophotometer at a 450 nm wavelength and compared with the standard curve.

2.2.4. Vitamin E Content

For the calibration curve, alpha tocopherol diluted by diethyl ether in concentration 0, 2, 4, 6, 12, 16, 20 mg/L. The absorption of alpha tocopherol was measured by spectrophotometer at 299 nm wavelength. Ten gr of refined *Diadema setosum* gonads finely macerated with 100 ml acetone solvent. After evaporation, the left powder was mixed with 5 ml of 15% KOH solution and allowed to stand for 24 hours. The extraction process was carried out by mixing *Diadema setosum* gonad with 20 ml KOH in diethyl ether and shaked gently by separated funnel tubes. The process was repeated until the solution becomes colerless. Absorption of *alpha tocopherol* was measured by spectrophotometer 299 wavelength. The content of vitamin E and the levels of alpha tocopherol in *Diadema setosum* gonads was calculated by compering its absorption with absorption in the standard curve.

2.2.5. Trace Element Content

Determination of trace elements such as minerals zinc (Zn), iron (Fe), magnesium (Mg) was performed using the methods developed before [10]. For details, 10 µl HNO_3 was mixed with 2 gr *Diadema setosum* gonads then heated until the solution became clear. The solution then filtered by polycarbonate filter paper (pore size 0.40 - 0.45 µm), which had been rinsed before with sterile distilled water. Filtered solution then aspirated into the Atomic Absorbance Spectrophotometric (AAS) with 307.59, 371.99 and 285.21 nm wavelength absorbance for Zn, Fe, and Mg, respectively.

2.3. Extraction of Diadema setosum Gonads and Detection of Active Compound

2.3.1. Extraction

The extraction process of *Diadema setosum* gonads was conducted in Biopharmacy and Phytopharmacy laboratories, Faculty of Pharmacy, University of Hasanuddin, Makassar, Indonesia. The extraction process was done by using a suitable solvent acetone as described previously [7, 11]. For detail, to produce 500 gr dry gonad, a 2500 gr *Diadema setosum* gonads were dried by freeze dry's for 24 hours at 108°C. The process of extraction was done by mixing 2500 ml acetone with dry gonads and then stored for 24 hours in the dark room. The extraction procedure was repeated several times to produce a translucent white materials. To obtain an extract that does not contain acetone, the extract was evaporated in 45°C rotating tool. As much 54.14 gr extract was obtained from 2500 gr gonad.

2.3.2. Detection of Active Compound

Active substance in the gonad *Diadema setosum* were detected using the well-made designed prosedures done previously in severel studies [9, 12] with some modifications. 1 gr extract gonad of *Diadema setosum* was diluted in 1 ml methanol and was speckled to the thin- layer chromatography (TLC) plates, and then eluted with an eluent containing n-hexane and ethyl acetate in a ratio of 2: 1. The presence or absence of active substances such as antioxidants, steroids, amino acids was determined by using TLC plates. TLC that had sprayed before with reagents, heated at 110°C and observed by using UV light 254 / 366 nm. Steroid compound was detected by spraying Liebermann-Burchard solution containing 5 ml of acetic sulfuric acid 97% and 45 ml ethanol. Steroid compounds were also detected by spraying solution containing 25 gr Antimony (III) Chloride ($SbCI_3$) in 75 ml Chloroform. Steroid compounds were characterized when blue-green stain was seen on the TLC. Ninhydrin solutions 1% (0.1 gr ninhydrin dissolved in 100 ml of ethanol) spray was used to detect amino acid compounds which is on TLC was marked with red stains. The active substance of antioxidants detected by spraying 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) 2% solutions (DPPH 0.2 gr dissolved in 100 ml of methanol). Antioxidant activity was marked by yellow stains with purple background.

3. Result and Discussion

The gonad of *Diadema setosum* contains vitamin A, vitamin E, Albumin, protein, Iron, Magnesium and Zink. Vitamin E is the highest nutrient content found in the gonad. Table 1 shown the nutrient content per 100 gr *Diadema setosum* gonads.

Table 1. *Nutrient content in the gonad of Diadema setosum.*

No	Nutrient	Unit	Content per 100 gr	Method
1.	Vitamin E	mg	23.47	Spectrophotometric
2.	Vitamin A	mg	1.79	Spectrophotometric
3.	Albumin	gr	1.240	Spectrophotometric
4.	Protein	gr	11.03	Kjeldahl method (Titrimetric)
5	Iron	mg	0.96	Atomization
6.	Magnesium	mg	1.90	Atomization
7	Zinc	mg	0.022	Atomization

The results of the identification of active compounds in the extract *Diadema setosum* gonads can be seen through the

spots on the chromatogram that was sprayed with various solutions as seen in figure 2. Steroid compounds characterized by blue-green stain, amino acid compounds by red stain, and antioxidant by yellow and purple stain.

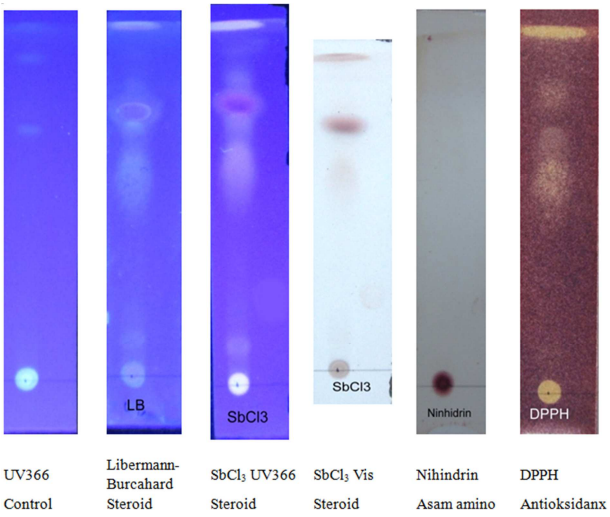

Figure 2. *Active compound in the extract of Diadema setosuma gonads.*

Optimal immune system is required to prevent and eliminate pathogen invasion to the body and to control when the pathogen eventually escapes into the body. To provide an optimal and eficient immune response, the composition of the diet should contain enough vitamin A, vitamin C and vitamin E and the trace element Se, Zn, Fe [13]. Trace elements play an important role in the physical barrier, cellular and humoral immunity [14] while albumin is required to distribute the trace element to various organs and body tissues [15]. In the analysis performed on the gonad of *Diadema setosum* here, protein, albumin, vitamin A, vitamin E contents were identified and the trace element Zinc, Ferron, Magnesium were detected.

Vitamin E was an antioxidant, which serves to neutralize free radicals, prevent lipid oxidation dan maligancy [16]. Vitamin E deficiency can cause neurological disorders, fat malabsorption, formation of atherosclerotic plaques and arthritis [17]. Diet of vitamin E can restore cellular homeostasis balance, protection against the effects of oxidative stress, the development of degenerative diseases, and aging process [18]. In this analysis, a high vitamin E (23.47 mg) in 100 gr *Diadema setosum* gonads was found. In order to be used as antioxidants, fatty acids is required as a transporter of vitamin E to the network [17]. Interestingly, Chen et al. [7] reported that poly unsaturated fatty acid (PUFA) is high in *Diadema setosum* gonads.

In this study, 1.79 mg or equal to 2983.93 IU vitamin A was detected in 100 gr *Diadema setosum* gonads. Vitamin A plays a role in the regulation of immune function [19]. visual function [20], and the growth and the maintenance of epithelial tissue [21], Deficiency of vitamin A in infants and preschool children can causing them to become susceptible to tractus respiratory infections [22] Supplementation vitamin A has showed a decrease of mortality and the incidence of infectious diseases in infants and children [23]. To be know, vitamin A in 100 gr *Diadema setosum* gonads is four times higher than vitamin A found in 100 gr of mackerel fish that is only 727 IU [24].

Albumin was a component of body fluid that function to prevent the plasma fluid move out from capillaries, therefore it is widely used as parameters of nutritional status [25]. In addition, albumin is a major antioxidant in plasma which provide protection against oxidative stress [26]. Malnutrition and inflammation can suppress albumin formation, as shown in cancer patients who have poor nutritional status. Cancer survival have shown to have positive association with the high serum albumin level in the serum [27]. In this study 1.24 gr albumin in 100 gr the gonad *of Diadema setosum.* Although it is only a half of albumin obtained fom 100 ml snakehead fish / canna striata, 2.17 gr [28], the albumin the gonad of *Diadema setosum* may add the one taken from another source.

Magnesium plays an important role in the metabolisms of glucose [29], the development of bone structure [30], physical endurance and metabolism of muscle cells [31], regulation of blood pressure [32] and nerve impulses [33]. Magnesium is also required for the synthesis of proteins, DNA and RNA [34]. In a meta-analysis of seven prospective studies with a total of 241,378 participants, it was reported that the addition of magnesium in the diet reduced 8% of hemorrhagic stroke risks [35]. In addition, Guerrero-Romero and Rodríguez-Morán [36] in seven cohort studies involved 24,388 participant has shown that magnesium intake reduced risk of diabetes type 2. There was also a five controlled trials that showed the effectiveness of the magnesium in reducing glycemia in high risk subjects. In this study, it was found 1.90 mg magnesium in 100 gr of *Diadema setosum* gonads. As comparison, one chicken eggs contain 6 mg magnesium [37]. Although magnesium in the gonad of *Diatema setosum* is low but it is potential as addition of trace elements that may taken from another sources to meet the daily requirement.

The content of iron we found in 100 gr of *Diadema setosum* gonads was 0.96 mg wich is equivalent to the iron content in 100 gr of salmon fish, 1.06 mg [38]. Iron is required for the manufacture of hemoglobin (Hb) which is function as transporter of oxygen to the tissues [39]. In addition, iron also plays a role as a co-factor of several enzymes involved in the metabolic processes of the body [40]. Deficiency of iron due to poor nutrition, resulting in the decreasing productivity and impairment of psychomotor [41]. In 29 systematic reviews which investigated 8182 articles, it was reported that anemia in pregnant women gave a risk to have a low birthweight (RR: 1.31; 95% CI: 1.13-1.51), preterm delivery (RR: 1.63; 95% CI: 1.33-2.01), perinatal mortality (RR: 1.51; 95% CI: 1.30-1.76), and neonatal death (RR: 2.72; 95% CI: 1.19-6:25) on pregnancy outcomes [42].

Zink (Zn) is component of proteins, hormones, enzymes and cell signaling [43], it was needed for wound healing and tissue recovery after infections [44]. Zn also important in the growth and development of children [45]. Deficiency of Zn

can cause damage of epithelial lining in the gastrointestinal and respiratory tractus [46]. Two meta-analysis studies reported that administration of Zn supplements reduced the incidence of pneumonia in children under five years [47, 48]. Daily 15 mg suplement of Zn for 8 weeks in malnutrition children reduced the prolong and severity of diarrhea and it was followed by body weight gaining [49]. In this study, 0.022 mg Zn was detected in 100 gr of *Diadema setosum* gonads. Although the amount is low, it may add Zn that is taken from other sources.

4. Conclusion

Overall, nutrient content in the gonad of *Diadema setosum* is complete and meet requirements to be used as an alternative food source. The use of *Diadema setosum* gonads as a food source can indirectly save coral reefs in the ocean. The amount of vitamin E and vitamin A are higher compared to the ones found in mackerel, salmon, sardines, snakehead fish and eggs, indicate that the gonad of *Diadema setosum* is sufficient to become a new source of nutrient to boost the immune response as well as to regulate the inflammation. In our knowledge, the analysis done here is the most complete study investigate the immune nutrient components of *Diadema setosum* gonads. However, since we did not measure the effect of *Diadema setosum* gonads consumption, its mechanisms on boosting and regulating the immune responses is still unclear. Further study need to be done, either in vitro or in vivo.

Acknowledgment

We thank to Hamsina and Nurul, chemical and food analyst from Balai Besar Laboratorium Kesehatan Makassar for helping us in the laboratory analysis. Thank also to Prof. Gemini Alam and Abdul Rahim from Pharmacy Faculty, University of Hasanuddin for valuable discussions on extraction methods and the detection of active substance. High appreciation to Tarzan, and Bina Laut Foundation Indonesia (YBLI) Southeast Sulawesi for helping us in collecting sea urchin. This study was financially supported by the RisbinIptekdok projects, Ministry of Health and Ministry of High Education-Research and Technology Indonesia.

Contribution

Waode Salma and Sitti Wahyuni contributed equally in the study

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