*Jurnal Biologi Tropis*

Original Research Paper

# Antioxidant Activity of Crude Protein of *Xestospongia* Sponge from Spermonde Sulawesi

**Tahirah Hasan[1], Mega Sari Juane Sofiana[2*], Apriansyah[2], Lucky Hartanti[3], Desriani Lestari[4], Ikha Safitri[2], Syarif Irwan Nurdiansyah[2]**

[1]Department of Chemistry, Faculty of Mathemathics and Natural Science, Islamic University of Makassar, Makassar, Indonesia;

[2]Department of Marine Science, Faculty of Mathemathics and Natural Science, Tanjungpura University, Pontianak, Indonesia;

[3]Department of Food Technology, Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia

[4]Faculty of Medicine, Tanjungpura University, Pontianak, Indonesia

**Article History**

Received : October 21th, 2022

Revised : November 20th, 2022

Accepted : December 01th, 2022

*Corresponding Author:
Mega Sari Juane Sofiana,
Tanjungpura University,
Pontianak, Indonesia;
Email:
msofiana@marine.untan.ac.id

**Abstract:** *Xestospongia* sp. is a barrel sponge belonging to the phylum porifera of the class Demospongia. This sponge abundant in coral reefs ecosystem. It is a filter feeder where the filtering of nutrients available in the environment occurs in the pores along the body. Various microorganisms in the seawater were exposed to sponges, so that they can produce various biological activities. This study aims to determine the antioxidant activity of crude protein from sponge *Xestospongia* sp from Spermonde waters of Sulawesi Island. The protein of the sponge was extracted using ammonium sulfate. Then, the protein was determined by the Lowry method in each fraction. Furthermore, the antioxidant activity was carried out by the DPPH method. The crude protein extracts in various fractions produced intermediate antioxidant activity produced from each fraction. They are fraction 1 (0-30%) of 81.31 ppm, fraction 2 (30-60%) was 180.22 ppm, and fraction 3 (60–90%) was 93.97 ppm, while crude protein was 128.33 ppm.The ascorbic acid as a positive control has antioxidant 2.98 ppm.

**Keywords:** antioxidant, Spermonde, protein, sponge, *Xestospongia*.

## Introduction

The marine sponges are known as the source of bioactivecompounds. These organism arethe most diverse benthos in coral reefs ecosystem (Hadi et al., 2016). Sponges are sessile and filter-feeding animals (Girard et al., 2021). These characteristics are considered the sponges as bioindicators of the health ecosystem (Pawar & Al-Tawaha, 2017). Sponges play an important role in facilitating nutrients in the ocean. They convert dissolved organic matter (DOM) and particulate organic matter (POM) from benthic producers (e.g., algae and corals) into particulate detritus. The nutrients could be consumed by higher trophic levels such as fishes (de Goeij et al., 2017; Rix et al., 2018).

Demospongiae dominates the type of sponge in the ocean up to 75-90%. The sponge has an asymmetrical shape and variation size (a few millimeters to 2 m). It is dominated by brightly colored ones such as orange, red, yellow, green and purple sponges are not uncommon. These bright colors are caused by the pigments in their dermal cells (Hickman et al., 2008). The demosponges have varied bioactive secondary metabolites. They have antiviral, antitumor, antimicrobial, antifouling, and antiplasmodial activities (Esposito et al., 2022). Marine sponges in South East Asia have ninety-five metabolites, such as alkaloids, sterols, terpenoids, quinones (Nyan et al., 2019).

Sponges produce metabolites such as peptides and lipids. The metabolites have biological activities that have potential in pharmaceutical applications. Several demosponge fatty acids showed antimycobacterial and antifungal activity, antimalarial. The glycolipids exhibit anti-inflammatory, antitumor, anticomplement and

immunomodulatory properties (Koutsouveli et al., 2021). Many bioactive peptides have been extracted from sponges. It has been discovered in 11 species of sponge (Akbarian et al., 2022). The peptides have been developed as promising therapeutic in medical treatments. It is caused by highly specific action, less interferes with a normal metabolism and less likely to induce an immune response (Muheem et al., 2016).

Bioactive peptides from *Xestospongia* have been reported. A novel cytotoxic peptide JENPVLSLVNGMF has been extracted from marine sponge *Xestospongia testudinaria*. It has potent anticancer drugs (Quah et al., 2018). A few secondary metabolites (alkaloids, sterol, and polyketide) have been isolated from *Xestospongia testudinaria, Xestospongia exigua, Xestospongia muta and Xestospongia wiedemayeri*. The compounds exhibit anticancer activity (Fristiohady et al., 2021). *Xestospongia testudinaria* extract has shown neuroprotective effects through its antioxidant and anti-inflammatory mechanisms Spermonde waters, South Sulawesi have diverse sponges, including *Xestospongia*. Therefore, the sponges also have potential as peptides bioactive, such as antioxidant activity.

## Materials and Methods

### Extraction and Fractination of Protein from *Xestospongia* sponge

Crude protein fractionation from sponge *Xestospongia* using ammonium sulfate at saturation level (0-90%) as the method used by Bollag *et al.* (1996). The *Xestospongia* from the Spermonde of South Sulawesi, which has been cleaned, was chopped to a size of 0.5 $cm^2$. Five hundred gram of sample was added 500 mL of buffer solvent A, then mashed using a blender. The supernatant was filtered into filtrate and residue. The filtrate was frozen and thawed 2-3 times, then centrifuged at 6,000 rpm 4$^o$C for 30 minutes. Fractionation was carried out on the resulting supernatant (crude protein extract). Fractionation was carried out using ammonium sulfate solution at various levels of saturation (0-30%; 30-60%; and 60-90%). Furthermore, the samples centrifuged at 10,000 rpm with a temperature of 4$^o$C for 30 minute. The residue is then added with 5 mL of buffer B into a cellophane bag and dialysed in a volume of buffer C solution while stirring with a magnetic stirrer. The dialysis process is stopped when there is no more color change from buffer C.

### Determination of Protein Content

The protein content in each fraction was determined by the Lowry method, using bovine serum albumin (BSA) as the protein standard. The preparation of the sample solution was carried out by mixing 4 mL of protein fraction solution and 5.5 mL of Lowry B reagent, shaken until homogeneous and then left for 10-15 minutes. Furthermore, 0.5 mL of Lowry A reagent was added to the solution, shaken until homogeneous, then left for 30 minutes. The absorbance of the sample solution was measured using an UV-Vis spectrophotometer, where the protein content could be determined through a linear regression equation.

### Antioxidant Activity

The antioxidant activity test was carried out by scavenging DPPH free radicals by protein extracts contained in the sample of *Xestospongia*. The protein fraction (dialysate) produced in several concentrations (ppm) measured the absorbance using a UV-Visible spectrophotometer at a wavelength of 517 nm. Ascorbic acid as a standard.

$$\%\ Antioxidant\ activity = \frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} x100\%$$

## Results and Discussion

### Extraction of Protein

Exploration of active compounds from marine natural products is currently increasing rapidly. Sponges are one of the targets to obtain active compounds that have unique and important structures and activities. The habitat of sponges are coral reefs ecosytem. Sponges as filter feeders filtrate the nutrient through the pores of the sponge around their bodies (Trani et al., 2021). It makes sponges very strong in interacting with

microorganisms in their aquatic environment (Riisgard & Larsen, 2022). One of the sponge that grows in the Spermonde waters is *Xestospongia*, from the Demospongia family which dominates the phylum porifera in marine waters.

Recently, research on secondary metabolites of the sponge *Xestospongia* has been reported (Sadarun et al., 2022). They found 4 sterol compounds from the ethanol extract of *Xestospongia* from the waters of Southeast Sulawesi. Protein fraction of *Niphates* from Spermonde of South Sulawesi exhibit antioxidant and antibacterial activities (Warsidah et al., 2020) The importance of protein in the body's metabolic system through vital enzymatic reactions has led to many efforts to find protein sources. Extraction and fractionation of crude protein produced from samples of *Xestospongia* sponges, can be shown in Table 1.

**Table 1**. The volume and content of protein from protein fraction of *Xestospongia*

| Protein fraction | Volume of dialysate (mL) | Protein content (ppm) | Total Protein (mg) |
|---|---|---|---|
| Crude protein | 626 | 22.56 | 14.122,56 |
| Fraction 1 (0-30%) | 42 | 0,79 | 33,18 |
| Fraction 2 (30-60%) | 72 | 2,72 | 295,84 |
| Fraction 3 (60-90%) | 55 | 1,12 | 61,6 |

**Protein Content of *Xestospongia***

Each protein fraction produced a different volume of dialysate, as well as the protein content of each fraction. The difference in the volume of dialysate indicated a difference in the solubility of the sponge protein in water. The higher the solubility of protein in water, the precipitation will be longer and the resulting dialysate is also smaller. Fraction 2, which is the level of saturation of ammonium sulfate, gives the total amount of protein compared to fractions 1 and 3, which is 295.84 g.

Proteins have different solubility in water, from the category of very soluble to insoluble (Grossmann et al., 2019). All proteins are insoluble in organic solvents such as chloroform, n-hexane, ether and benzene. Protein is a macromolecule that is quite unstable. Thus, it is very easily influenced by physical and chemical factors and eventually undergoes denaturation or damage. Denatured proteins will cause changes, either decreasing or increasing their biological activity. The addition of ammonium sulfate salt to protein samples will cause protein deposition (Bruno et al., 2013).

The peptides and proteins are very important in supporting the body's biological activities. Therefore, these two macromolecules have great potential to be developed as drugs for the therapy of a disease. Hence, peptides and proteins can be applied in efforts to establish a diagnosis (Joseph et al., 2017). However, the safety, effectiveness of use, quality of materials and several physicochemical factors of these macromolecules still need to be reviewed in more detail.

**Antioxidant Activity of Crude Protein**

One of the biological activities tested on the crude protein fractions of the *Xestospongia* sponge was antioxidant activity, using the DPPH method. Free radicals are unstable electrons, very reactive. They try to get electron pairs by attracting or capturing free electrons from biological macromolecules in the surrounding environment, such as lipids, proteins, and deoxyribose nucleic acid (DNA) (Phaniendra et al., 2015). Free radicals that are formed can be neutralized by antioxidant compounds either produced by the body or through the intake of antioxidants from outside that are consumed or used either orally or topically. The body has the ability to fight free radicals that are formed, but if the free radical attack is intensive and the amount exceeds the body's ability to detoxify the effects of these free radicals, the body's antioxidant defense system will decrease and ultimately cause oxidative stress conditions (Butnariu & Samfira, 2012) . The measured antioxidant activity states the amount of active ingredient that can reduce 50% of DPPH free radicals, or also expressed as Inhibitory Concentration 50 ($IC_{50}$).

The protein fraction of *Xestospongia* exhibits antioxidant activity ($IC_{50}$) produced

from each fraction is fraction 1 (0-30%) of 81.31 ppm, fraction 2 (30 – 60%) of 180.22 ppm, and fraction 3 (60 – 90%) of 93.97 ppm. The crude protein has antioxidant activity with $IC_{50}$ of 128.33 ppm. Ascorbic acid as a positive control was 2.98 ppm. The protein fraction 1 had higher antioxidant activity. However, the value of $IC_{50}$ of the protein fraction 1 is smaller than the vitamin C (positive control). The difference is not only caused by different protein levels, but also probably due to the amino acid composition of the protein constituents in each fraction. The mechanism of antioxidant activity in the sample against exposure to DPPH free radicals will depend on the conformation of the antioxidant compound content (Sofiana et al., 2020). In the antioxidant mechanism, the number of hydroxyl (OH) groups and their locations in the aromatic rings of structure compound play an important in antioxidant work (Al-Mamary, 2021). The protein fraction of the *Xestospongia* has antioxidant activity in the category of strong and medium. It is indicating the fractions can **be used** by the body to capture exposure to free radicals in the environment.

## Conclusions

The research in antioxidant activity of *Xestospongia* from Spermonde South Sulawesi, it can be concluded that: a) Each protein fraction has a different protein content. They are 22.56 ppm (crude protein extract), 0.79 ppm (fraction I), 2.72 ppm (fraction 2) and 1.12 ppm (fraction 3), b) The antioxidant activity ($IC_{50}$) of protein fractions 1 and 3 are 81.31 ppm and 93.97 ppm, respectively. It was classified as a strong antioxidant. While, the protein fraction 2 has weak antioxidant potential ($IC_{50}$ of 180.22 ppm)

## Acknowledgment

We want to express our deepest appreciation to everyone who helped us with this project, especially my research group. We are grateful for their support and guidance.

## References

Akbarian, M., Khani, A., Eghbalpour, S., & Uversky, V. N. (2022). Bioactive Peptides: Synthesis, Sources, Applications, and Proposed Mechanisms of Action. *International Journal of Molecular Sciences*, *23*(3). https://doi.org/10.3390/ijms23031445

Al-Mamary, M. A. (2021). *Antioxidant Activity: The Presence and Impact of Hydroxyl Groups in Small Molecules of Natural and Synthetic Origin* (Z. M. E.-V. Waisundara (ed.); p. Ch. 13). IntechOpen. https://doi.org/10.5772/intechopen.95616

Bollag, D. M., BOLLAG, 오|DANIEL M, Rozycki, M. D., & Edelstein, S. J. (1996). *Protein Methods*. Wiley. https://books.google.co.id/books?id=4QZrAAAAMAAJ

Bruno, B. J., Miller, G. D., & Lim, C. S. (2013). Basics and recent advances in peptide and protein drug delivery. *Therapeutic Delivery*, *4*(11), 1443–1467. https://doi.org/10.4155/tde.13.104

Butnariu, M., & Samfira, I. (2012). Free Radicals and Oxidative Stress. *Journal of Bioequivalence & Bioavailability*, *04*(03). https://doi.org/10.4172/jbb.10000e13

de Goeij, J. M., Lesser, M. P., & Pawlik, J. R. (2017). *Nutrient Fluxes and Ecological Functions of Coral Reef Sponges in a Changing Ocean BT - Climate Change, Ocean Acidification and Sponges: Impacts Across Multiple Levels of Organization* (J. L. Carballo & J. J. Bell (eds.); pp. 373–410). Springer International Publishing. https://doi.org/10.1007/978-3-319-59008-0_8

Esposito, R., Federico, S., Bertolino, M., Zupo, V., & Costantini, M. (2022). Marine Demospongiae: A Challenging Treasure of Bioactive Compounds. *Marine Drugs*, *20*(4), 1–22. https://doi.org/10.3390/md20040244

Fristiohady, A., Ibrahim, P. S., & Pascayantri, A. (2021). Aktivitas Antikanker dari Spons Laut Genus Xestospongia. *Pharmacy Medical*, *4*(1), 11–19.

Girard, E. B., Fuchs, A., Kaliwoda, M., Lasut, M., Ploetz, E., Schmahl, W. W., & Wörheide, G. (2021). Sponges as bioindicators for microparticulate pollutants? *Environmental Pollution*, *268*. https://doi.org/10.1016/j.envpol.2020.115851

Grossmann, L., Hinrichs, J., & Weiss, J. (2019). Solubility and aggregation behavior of protein fractions from the heterotrophically cultivated microalga Chlorella protothecoides. *Food Research International*, *116*, 283–290. https://doi.org/https://doi.org/10.1016/j.foodres.2018.08.037

Hadi, T. A., Suharsono, -, Hadiyanto, -, Budiyanto, A., & Wentao, N. (2016). the Morphological and Species Diversity of Sponges on Coral Reef Ecosystems in the Lembeh Strait, Bitung. *Marine Research in Indonesia*, *40*(2), 65–77. https://doi.org/10.14203/mri.v40i2.45

Hickman, Cl. P., Roberts, L. S., Keen, S. L., Larson., A., L'Anson, H., & Eisenhour, D. J. (2008). Integrated Principles of Zoology. In *McGraw-Hill Companies, Inc.* (Fourteenth).

Joseph, M., Trinh, H. M., & Mitra, A. K. (2017). Chapter 7 - Peptide and Protein-Based Therapeutic Agents∗. In A. K. Mitra, K. Cholkar, & A. B. T.-E. N. for D. Mandal Drug Delivery and Medical Devices (Eds.), *Micro and Nano Technologies* (pp. 145–167). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-42978-8.00007-3

Koutsouveli, V., Balgoma, D., Checa, A., Hedeland, M., Riesgo, A., & Cárdenas, P. (2021). Oogenesis and lipid metabolism in the deep-sea sponge *Phakellia ventilabrum*: an histological, lipidomic and transcriptomic approach. In *ResearchSquare Preprint*.

Magadmi, R., Borouk, K., Youssef, D. T. A., Shaala, L. A., Alrafiah, A. R., Shaik, R. A., & Alharthi, S. E. (2022). Neuroprotective Effect of Red Sea Marine Sponge *Xestospongia testudinaria* Extract Using In Vitro and In Vivo Diabetic Peripheral Neuropathy Models. *Pharmaceuticals*, *15*(11), 1309. https://doi.org/10.3390/ph15111309

Muheem, A., Shakeel, F., Jahangir, M. A., Anwar, M., Mallick, N., Jain, G. K., Warsi, M. H., & Ahmad, F. J. (2016). A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives. *Saudi Pharmaceutical Journal : SPJ : The Official Publication of the Saudi Pharmaceutical Society*, *24*(4), 413–428. https://doi.org/10.1016/j.jsps.2014.06.004

Nyan, L. K., Abas, F., Maulidiani, Ahmed, M., Wei, L. S., Ismail, I. S., & Shaari, K. (2019). Chemical constituents and biological activities of south east asia marine sponges: A review. *Pertanika Journal of Science and Technology*, *27*(2), 953–983.

Pawar, P. R., & Al-Tawaha, A. R. M. S. (2017). Marine Sponges As Bioindicator Species of Environmental Stress At Uran (Navi Mumbai), West Coast of India. *American-Eurasian Journal of Sustainable Agriculture*, *11*(3), 29–37.

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free radicals: properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry : IJCB*, *30*(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0

Quah, Y., Mohd Ismail, N. I., Ooi, J. L. S., Affendi, Y. A., Abd Manan, F., Wong, F.-C., & Chai, T.-T. (2018). Identification of Novel Cytotoxic Peptide KENPVLSLVNGMF from Marine Sponge Xestospongia testudinaria, with Characterization of Stability in Human Serum. *International Journal of Peptide Research and Therapeutics*, *24*(1), 189–199. https://doi.org/10.1007/s10989-017-9604-6

Riisgard, H. U., & Larsen, P. S. (2022). Actual and Model-Predicted Growth of Sponges—With a Bioenergetic Comparison to Other Filter-Feeders. *Journal of Marine Science and Engineering*, *10*(5). https://doi.org/10.3390/jmse10050607

Rix, L., De Goeij, J. M., Van Oevelen, D., Struck, U., Al-Horani, F. A., Wild, C., & Naumann, M. S. (2018). Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. *Marine Ecology Progress Series*, *589*(March), 85–96. https://doi.org/10.3354/meps12443

Sadarun, B., Wahyuni, Malaka, M. H., Fristiohady, A., Yodha, A. W. M., Rahmatika, N. S., Islami, Z. S.,

Nurjayadin, M., Sabandar, C. W., Darmawan, A., Sundowo, A., Rosandi, A. R., & Sahidin, I. (2022). Biological activities of Steroids and Extracts from Xestospongia sp. growing in Southeast Sulawesi (Indonesia). *Research Journal of Pharmacy and Technology*, *15*(4), 1487–1493. https://doi.org/10.52711/0974-360X.2022.00247

Sofiana, M. S. J., Aritonang, A. B., Safitri, I., Helena, S., Nurdiansyah, S. I., Risko, Fadly, D., & Warsidah. (2020). Proximate, Phytochemicals, Total phenolic content and antioxidant activity of ethanolic extract of Eucheuma spinosum seaweed. *Systematic Reviews in Pharmacy*, *11*(8), 228–232. https://doi.org/10.31838/srp.2020.8.34

Trani, R., Corriero, G., de Pinto, M. C., Mercurio, M., Pazzani, C., Pierri, C., Scrascia, M., & Longo, C. (2021). Filtering activity and nutrient release by the keratose sponge sarcotragus spinosulus schmidt, 1862 (Porifera, demospongiae) at the laboratory scale. *Journal of Marine Science and Engineering*, *9*(2), 1–16. https://doi.org/10.3390/jmse9020178

Warsidah, Masrianih, Sofiana, M. S. J., Safitri, I., Sapar, A., Aritonang, A. B., Muttalib, Y. S., & Fadly, D. (2020). Protein isolation from sponge niphates sp. as an antibacterial and antioxidant. *Systematic Reviews in Pharmacy*, *11*(9), 518–521. https://doi.org/10.31838/srp.2020.9.76