

**ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT NATUNA MARINE SPONGE (*PORIFERA*) WITH DPPH METHODE**

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Received: 23 October 2020, Revised and Accepted: 02 March 2021

**ABSTRACT**

**Objective:** Natuna sponge is a marine animal that contains a wide range of secondary metabolites that can be utilized in the field of pharmacy. The research aims to test the phytochemical content and antioxidant activity of the Natuna methanol marine sponge extract. Sample extraction is done by the maceration method with methanol solvent.

**Methods:** Phytochemical tests include alkaloid testing, flavonoids, steroids, saponins, and tannins. Test antioxidant activity using the immersion DPPH method (1,1-diphenyl-2-picryl hydroxyl) measured absorption at a wavelength of 517 nm.

**Results:** The phytochemical test results showing the sponge of Natuna contain secondary metabolites including alkaloids, flavonoids, steroids, saponins, and tannins.

**Conclusion:** This Natuna sponge methanol extract has an antioxidant activity that is very strong with an  $IC_{50}$  value of 52.91 ppm, and the comparator used is Vitamin C with an  $IC_{50}$  value of 43,51 ppm.

**Keywords:** Antioxidants, DPPH, Marine sponge, Phytochemical tests.

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**INTRODUCTION**

Indonesian waters, especially the Riau Islands, have abundant marine biota wealth, their use has so far been focused on fish as a food product. One type of marine biota that can be used as medicine is a sponge. Not many studies about the benefits of marine life typical of the Riau Islands are used as medicinal ingredients, especially to ward off free radicals, namely antioxidants.

Free radicals contained in the human body have the potential to inactivate various enzymes, oxidize fats, and disrupt the body's DNA so that cell mutations occur which is the beginning of cancer [1]. Free radicals can be resisted by giving antioxidants [2].

So far, people only know the source of antioxidants from plants, such as the fruits, ginger, and others [3]. Meanwhile, the source of antioxidants from marine animals is not widely known [4]. However, along with the development of technology and science, the use of antioxidants derived from marine biota is also growing, one of them by utilizing sea sponges.

Sponges from tropical Indonesian waters have very significant potential as bioactive compounds to be further developed into commodities of high economic value [5]. Sponges contain the most extensive bioactive compounds such as antibacterial activity which were isolated from *Agelas clathrodes* [6], antioxidant activity of *Callyspongia* sp. [7], and *Lamellodysidea* sp. [8] (Fig. 1).

Although there have been studies of antioxidant activity in sponges with maceration extraction methods and it is proven that sponges have high antioxidant activity with  $IC_{50}$  41.21 ppm [7,9,10], this test was carried out on sea sponges originating from the Thousand Islands with a different species from the sea sponge in Natuna. Giving rise to the thought that it is necessary to test the antioxidant activity of the Natuna marine sponge which is rich in marine biota as well as to find new medicinal ingredients.

This study aims to determine the  $IC_{50}$  value of the methanol extract of the Natuna sea sponge and test the activity of secondary metabolites contained therein.

**METHODS****Tools and materials**

The tools used include glass tools for extraction, UV-visible Spectrophotometer (Shimadzu 265). Materials used include sponges obtained from Natuna waters, Riau Islands, DPPH, Vitamin C, methanol, Liebermann-Burchard reagents, Dragendorff, Mayer, and Bouchard at.

**The way of research***Extraction*

Fresh samples of 4 kg sponges that have been washed, drained, chopped, and then macerated with methanol until completely submerged in brown glass bottles and stored in a light-protected place for 3 × 5 days while occasionally stirring and filtered with filter paper. The methanol macerate is combined and concentrated with a rotary evaporator until a thick extract is formed then weighed to obtain 99.74 g [11,12].

*Phytochemical test extract [13]**Alkaloid test*

As much as, 4 ml of sponge extract was put into a test tube then added 2 ml of chloroform and 5 ml of 10% ammonia and then added 10 drops of 2M sulfuric acid to clarify the separation of the formation of 2 different phases. The upper part of the formed phase is taken, and then, a Mayer reagent is added. The presence of alkaloids is characterized by the formation of red deposits.

*Flavonoid test*

Sponge extract is taken as much as 1 ml added with enough magnesium powder and 10 drops of concentrated hydrochloric acid. The presence of flavonoids is characterized by the formation of reddish-black, yellow, or orange.

**Steroid test**

As much as, 1 ml of sponge extract added 2 ml of chloroform and then shake it. Then, the filtrate was added with anhydrous acetic acid and 2 drops of concentrated sulfuric acid. A positive reaction is shown in the red color change in the first solution which turns blue and green.

**Antioxidant activity test with DPPH method [14,15]****Preparation of DPPH solutions**

A 50 ppm DPPH solution was prepared by weighing 5 mg of DPPH dissolved with 100 ml of absolute methanol in a volumetric flask [14].

**Preparation of sample solutions**

A 500 ppm stock solution is made by weighing 5 mg of sponge methanol extract and dissolving it with absolute methanol while stirring and homogeneous, and then, the volume is sufficient to 10 ml and subsequently made variations of the concentration of 10 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm [14].

**Making comparative solutions**

100 ppm stock solution is made by weighing as much as 1 mg of Vitamin C and then dissolved with absolute methanol while stirring and homogenized, and then, the volume is sufficient to 10 ml. Furthermore, variations in the concentration of 2 ppm, 4 ppm, 6 ppm, and 8 ppm were made [14].

**Measurement of sponge antioxidant power**

The test was carried out by piping 0.5 ml of sample solution from various concentrations (10 ppm, 50 ppm, 100 ppm, 150 ppm, and 200 ppm), then each added 3.5 ml of DPPH, and then Divortex and incubated at 37°C in a dark room. Its absorbance was measured at a wavelength of 517 nm [14].

**Measurement of the antioxidant power of Vitamin C comparison samples**

Tests carried out by pipette 0.5 ml of Vitamin C solution of various concentrations (2 ppm, 4 ppm, 6 ppm, and 8 ppm), then each added 3.5 ml of DPPH, and then Divortex and incubated at 37°C in a dark room. Its absorbance was measured at a wavelength of 517 nm [14].

**Data analysis**

The antioxidant activity of each sample and the comparative antioxidant Vitamin C are expressed by percent inhibition, which is calculated by the formula, and then, the data are analyzed using a linear regression equation using the Microsoft Excel program.

$$\% \text{inhibition} = \frac{(A \text{ blanko} - A \text{ sample})}{A \text{ blanko}} \times 100\%$$



Fig. 1: Natuna sea sponge

**RESULTS AND DISCUSSION****Results of making sponge extracts**

Sea sponge samples were taken from the Natuna Waters of the Riau Islands precisely Kelarik Village, Bunguran Utara District, Natuna Island, Riau Islands, at a depth of ±15 m below sea level. Then, the sponge is cleaned using seawater and running water. Samples were immediately put into bottles and soaked with methanol.

The weight of the wet sponge is 4050 g and then extracted with methanol solvent and obtained as much as 99.74 g of thick extract. Hence, we get a yield of sponge extract of 2.46%.

Methanol solvents are used because methanol solvents are solvents that successfully bind the highest bioactive compounds [16]. Based on Harborne (1984) states that methanol can bind all compounds, both polar to non-polar. Widyawati [17] confirms that methanol can extract phytochemical compounds in greater amounts.

**Phytochemical test results of sponge extracts**

The obtained sponge extract then tested the phytochemical content to determine the class of secondary metabolite compounds contained in it. The complete results are shown in Table 1. Phytochemical testing was carried out to determine the content of compounds contained in the extract. The compounds identified include alkaloids, flavonoids, steroids, saponins, and tannins.

The results of phytochemical testing are by previous studies where sea sponges contain secondary metabolites such as alkaloids, flavonoids, and steroids [7,8].

**Antioxidant activity test results with DPPH method**

Antioxidant activity testing is carried out by the DPPH method because this method is simple, easy, fast, and sensitive and only requires a small sample [15]. Antioxidant compounds will react with DPPH radicals through a hydrogen atom donation mechanism and cause color decay from purple to yellow [18].

In this study, the process refers to the procedure of Brand-Wiliams *et al.*, 1995 and Handayani, 2014 with some modifications. Where the measurement of absorbance of the sample on a UV-visible spectrophotometer with a wavelength of 517 nm with a sample volume used of 0.5 mL and a DPPH of 3.5 mL. Where the concentration of samples used was 50, 100, 150, and 200 ppm, while the comparative concentrations were 2, 4, 6, and 8 ppm. Where the comparison used, as positive control is Vitamin C.

Measurement of DPPH test results using a UV-visible spectrophotometer and the value of % inhibition of each concentration ( $IC_{50}$ ). The  $IC_{50}$  value of the sponge methanol extract was 52.91 ppm. While the  $IC_{50}$  value of the Vitamin C comparison is 43.51 ppm, this means that the antioxidant power of sea sponge extract and Vitamin C is almost the same based on the  $IC_{50}$  value. These results are shown in Table 2.

Based on Table 2, we can get a graph that can be seen in Fig. 2.

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Based on the  $IC_{50}$  calculation results show that the Natuna sea sponge methanol extract has an  $IC_{50}$  value of 52.91 ppm, the smaller the

Table 1: Phytochemical test of Natuna sea sponge extract

Compound	Sponges extract	Indicator
Alkaloid	+	Red sediment
Flavonoid	+	The color turns reddish-black
Steroid	+	The color turns green
Saponin	+	Formed stable foam
Tannin	+	Greenish black/yellow color change

+: Contains detected compounds

Table 2: IC<sub>50</sub> values of the Natuna sea sponge extract

Concentration (ppm)	% Inhibition	IC <sub>50</sub> (ppm)
50	42	52.91
100	75	
150	87,8	
200	96	

Table 3: IC<sub>50</sub> values of Vitamin C

Concentration (ppm)	% Inhibition	IC <sub>50</sub> (ppm)
2	9.6	43.51
4	9.9	
6	12.5	
8	15.34	

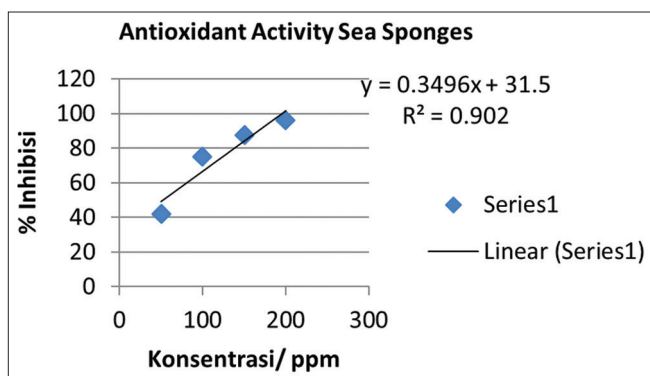


Fig. 2: Percentage curve of inhibition of sea sponge

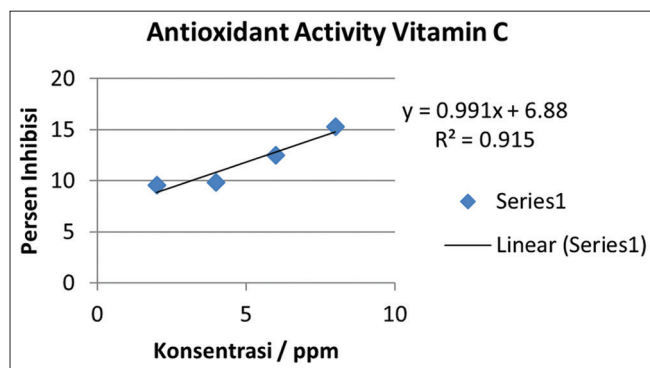


Fig. 3: Curve percentage curve of Vitamin C

IC<sub>50</sub> value and the higher its antioxidant activity [18]. Specifically, a compound is said to be a very strong antioxidant when IC<sub>50</sub> <50 ppm, strong if the IC<sub>50</sub> value is 50–100 ppm, while if IC<sub>50</sub> is 100–150 ppm and is said to be weak when the IC<sub>50</sub> value is above 150 ppm [8,19].

According to the above classification, the Natuna sea sponge methanol extract is classified as a powerful antioxidant, because the IC<sub>50</sub> value <50 ppm is equal to 52.91 ppm.

Based on the research results of compounds that have potential as antioxidants in sea sponge methanol extracts are alkaloids, flavonoids, and saponins. Where alkaloids, especially indole, can stop the reaction of free radical chain compounds efficiently. Other alkaloid compounds which are antioxidants are quinolone which can act as a radical, hydroxy, and silencer melatonin which play an important role in protecting cells from the effects of radiation and drug toxicity.

The next compound that has the potential as an antioxidant is a flavonoid which is a polyphenol compound can donate hydrogen atoms to free radical compounds, and then, the antioxidant activity of polyphenol compounds can be produced in free radical neutralization reactions or at the cessation of chain reactions that occur [20].

## CONCLUSION

The secondary metabolite compounds found in the Natuna sea sponge extract based on phytochemical tests are alkaloids, flavonoids, steroids, saponins, and tannins.

Natuna sea sponge extract has a very strong antioxidant activity, with an IC<sub>50</sub> value of 52.91 ppm.

## ACKNOWLEDGMENT

The author would like to thank all those who helped to complete the research. Thank you very much to the Directorate General for Strengthening Research and Development of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia, for funding this research through the Beginner Lecturer Research Grant for the 2020 Budget Year.

## AUTHOR'S CONTRIBUTIONS

All the authors have contributed equally to this research work.

## CONFLICTS OF INTEREST

There were no conflicts of interest or financial support among the authors.

## AUTHOR'S FUNDING

The experiment was funded by the Department of Pharmacy, Mitra Bunda Health Institute.

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