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Protein Isolation from Sponge *Niphates* sp. as an Antibacterial and Antioxidant

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ABSTRACT

Research on the antioxidant and antibacterial activity of the isolated protein of *Niphates* sp. sponge origin Spermonde aquatic of South Sulawesi had been conducted. Determination of antimicrobial activity was based on the formation of inhibition zones/cleared areas surrounding the isolated protein for two tested bacteria, i.e., *Bacillus subtilis* and *Escherichia coli*. The isolated protein from this sponge has the highest antimicrobial activity in *E. coli* bacterial assay by 14.21 mm zone of inhibition, while the inhibition zone of *B. subtilis* was 12.05 mm. After extra time for 24 hours of incubation, the medium of *B. subtilis* showed turbidity in the clear spot before. This indicated that the isolated protein covered the bacteriostatic ability against the tested bacteria. The antioxidant activity assay by DPPH free radical scavenging effect of isolated protein with some levels of saturation was determined. Following the test results indicated the antioxidant activity of isolated protein with scavenging effect against free radical DPPH possessed IC_{50} values from crude protein extract of 5.05 µg/mL, protein fraction 0 – 30% of 203.71 µg/mL, protein fraction 31 – 50% of 163.75 µg/mL, protein fraction 51 – 70% of 111.31 µg/mL, and protein fraction 71 – 90% amounted to 590.03 µg/mL. The results indicated that the sponge material's protein was potential as an antioxidant and antimicrobial, which can then be further research to several other biologic activities.

Keywords: *Niphates* sp., *Bacillus subtilis*, *Escherichia coli,* Antioxidant, Antibacterial

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INTRODUCTION

Sponge in the waters is one of the reservoir animals for marine microbes that reached 60% of sponge's total biomass. The microbial symbionts produce active compounds to respond to extreme environmental conditions through the body's defense mechanisms. The sponge can associate with many different microorganisms such as cyanobacteria, heterotrophic bacteria (Hentschel et al., 2002). Sea sponge has a densely of microorganisms potentially benefitted as a pharmacological activity (Hentschel et al., 2001). The sponge and bacteria interact with commensalism symbiosis, which produces bioactive compounds (Roy et al., 2000). Microbial metabolites associate with marine invertebrates has structural similarities to their host (Proksch et al., 2002; Putri et al., 2015; Thiel and Imhoff, 2003).

Some of the activities shown by microbes associated sponges, among others, were *Microascus* strain K14 and *Monochaetia* strain 193A20 shows antimicrobial activity. *Penicilliumbrocae* associated sponge *Zyzzya* sp. shows antimicrobial activity against methicillin-resistant *Staphylococcus aureus*. The cytotoxic activity was demonstrated by fungi *Gymnascela dankaliensis* associated sponge *Halichondria japonica* (Bugni and Ireland, 2004). Some of the compounds produced from the sponges, especially those in Indonesia's waters, among others were compound sesquiterpenoids type bisabolen, curcuphenol, and curcudiol resulting from *Axynissa* sp. have an activity inhibiting the synthesis of protein kinase on testing the anticancer *in vitro* (Hertiani et al., 2008). Also, Barrangamida compound, a new cyclic polypeptide, is active compounds produced by *Theonella swinhoei* sponge origin Spermonde waters of South Sulawesi (Roy et al., 2000). Cytotoxic activity against cervical cancer of *Cinachyrella* sp. sponge origin of the Situbondo coast has been reported (Nurhayati et al., 2015). *Niphates* sp. sponge is one of the sponges growing in Spermonde waters South Sulawesi. There are no reports or the scientific study of this sponge species' activity, and it becomes the basis for testing some of the biological activity of this sponge protein. The antimicrobial and antioxidant activity of a sponge protein is an early indication of the potential natural product for further testing, such as anticancer and some other important biological activities. This study was an effort to determine the antimicrobial and antioxidant activity of *Niphates* sp. sponge protein origin Spermonde waters of South Sulawesi.

MATERIALS AND METHODS

Sample preparation

Samples taken from the sponge reefs in Spermonde waters of South Sulawesi was cleaned, then milled while added buffer A (0.1 M Tris-HCl pH 8.3, 2 M NaCl, 0.01 M $CaCl_2$, 1% B-mercaptoethanol, Triton X-100 0.5%) and then stored in the refrigerator at 4 °C overnight). The suspension of *Niphates* sp. was conducted through the filtration using the Buchner funnel. After, the filtrate was freeze and thawed for 2-3 times. Next, it was centrifuged 30 min. at 6000 rpm and 4 °C, then the obtained supernatant kept in a refrigerator before subjecting to further analysis.

Fractionation with ammonium sulfate

Protein crude extract was fractionated by using ammonium sulfate at saturation levels (0-90%) (Bollag et al., 1996). Ammonium sulfate precipitation process performed by the saturation level of 0-30%; 30-50%; 50-70%, and 70-90%. Protein crude extracts in which certain volume was added with ammonium sulfate at a certain level of saturation while stirring with a magnetic stirrer until completely

dissolved and left overnight at 4 °C. It centrifuged 6000 rpm, 4 °C for 30 minutes to separate the precipitation. Further, the precipitated protein obtained dissolved in a solution of Tris-HCI buffer pH 8.3.

Dialysis

This step was carried out by using a cellophane membrane (Sigma D 0655). Size 10 cm dialysis membrane was boiled in a solution of 2.0% (w/v) Na-bicarbonate and 10 mM EDTA for 10 minutes (repeat 2-3 times). According to (Plummer, 1979), dialysis is conducted for three hours in a cold room; buffer solution is replaced every 1 hour. Precipitates of each ammonium sulfate saturation level fractionation results were dialyzed in several buffers, i.e., buffer B (0.1 M Tris-HCl pH 8.3, 0.2 M NaCl, 0.01 M $CaCl_2$), then dialyzed with buffer C (Tris HCI 0.01 M pH 8.3, 0.2 M NaCl, 0.01 M $CaCl_2$). Each protein fraction was included in a cellophane bag and then put in a beaker containing a buffer B, and stirred. This process continued by replacing the buffer B with buffer C. The dialysis is continued until colorless.

Antibacterial activity assay

The Kirby-Bauer method was used for antimicrobial activity identification (Bauer et al., 1966). *Bacillus subtilis* (ATTC 25950) and *E. coli* (ATTC NCTC 25 922 12241), used as bio-indicators, were rejuvenated on NA medium in the tilted tube for 1x24 hours at a temperature of 25 °C. Colonies that grow in tilted agar were taken, homogenized with 0.9% NaCl and measured turbidity is equivalent to the standard Mac Parland. The bacterial suspension was poured into a medium that has been prepared and allowed to solidify. Each paper discs was dropped with 200 mL of crude extract and the fractions of proteins with a saturation level of 30%, 50%, 70%, and 90%, and then continue by incubation at 37 °C for 1x24 hours then observed and measured the zone by using a sliding ruler stated in units of mm. Observations continued for 1x24 hours.

Antioxidant activity assay

Antioxidant activity assay was done through the scavenging effect against the free radical DPPH. Protein fractions were made in several concentrations (ppm), and then absorbance was measured with a visible spectrophotometer at a wavelength of 517 nm, and ascorbic acid was used as a standard.

RESULT AND DISCUSSION

Measurements results of protein concentration of *Niphates* sp. sponge protein using bovine serum albumin (BSA) as standard is presented in Table 1.

Table 1. The concentration ratio of protein level on *Niphates* sp. extract with ammonium sulfate saturation

Extracts	Absorbance (y)	Protein concentration (x)	Protein level ((x)xdf mg/L)
Crude protein extract	0.342	0.179	30.609
Protein fraction 0 – 30%	0.224	0.159	17.808
Protein fraction 31 – 50%	0.220	0.057	6.270
Protein fraction 51 – 70%	0.180	0.015	1.350
Protein fraction 71 – 90%	0.207	0.129	13.351

The analysis of levels Niphates sponge protein indicates the protein content of each fraction is different from each faction. This suggests the protein is precipitated from each fraction is a different protein. The protein is precipitated by different solubility in water.

Antimicrobial activity

In the present study, *in vitro* antimicrobial activity of the protein isolated from *Niphates* sp. sponge showed that their potential against *Bacillus subtilis* and *Escherichia coli*. The observation was conducted based on the clear zone shown after treatment; a larger clear zone may indicate more potent a compound used as antibacterial (Dewi et al., 2020a; Warsidah et al., 2020). Dialysate obtained from the dialysis was tested for antibacterial activity using *Bacillus subtilis* dan *Escherichia coli* as a bio-indicator of pathogenic to humans. Based on the observations of the inhibition diagram tested zone of antibacterial activity against *B. Subtilis*, the crude extract and the fractions of proteins from the sponge have antimicrobial effects. The highest bioactivity on a 1x24-hour incubation period of sponge protein fractions was obtained by ammonium sulfate saturation level of 50%, was 20.17 mm. The lowest activity was shown by ammonium sulfate saturation of 90%, which was 10.05. Bioactivity against *E. coli* after incubation for 1x24 hours had the highest activity in fractions of proteins with ammonium sulfate saturation level of 30%, which is 14.21 mm.

While the lowest activity is shown in the sponge protein of ammonium sulfate-fraction with a saturation level of 70% equal to 9.05 mm, as comparative, chloramphenicol has inhibitory to *B. subtilis* and *E. coli* by 31.56 mm and 29.55 mm respectively, which can be seen in Table 2. Observations were continued after incubation for 48 hours showed turbidity in the clear zone on the medium of *B. subtilis* tested before, but not so with the medium in which test bacteria *E. coli* clear zone. This indicates that the sponge proteins act as bacteriostatic against *B. subtilis* that only disable its growth.

Table 2. Antimicrobial bioactivity of the protein fraction of *Niphates* sp. against *Bacillus subtilis* and *Escherichia coli*

Extracts	The average diameter of the inhibition zone (mm)	
	Bacillus subtilis	***Escherichia coli***
Crude protein extract	10.01 ±2.21	11.17 ±2.23
Protein fraction 0 – 30%	10.05 ±1.33	14.21 ±1.10
Protein fraction 31 – 50%	12.05 ±2.02	10.59 ±2.46
Protein fraction 51 – 70%	11.08 ±3.41	9.05 ±1.11
Protein fraction 71 – 90%	9.27 ±2.22	13.56 ±3.11
Control (+) chloramphenicol	31.56 ±4.52	29.55 ±7.24

Antioxidant activity assay

Free radicals are unstable, highly reactive, and can take electrons from other molecules to get a pair of electrons. Free radicals formed quickly will attract electrons of biological macromolecules in the surrounding areas such as lipids, proteins, and nucleic acids (DNA) (Fadly et al., 2020). The formation of free radicals will be neutralized by antioxidants produced by the body in several impartial. But the effects of free radicals occur when the amount exceeds the detoxification of the body's antioxidant defense system, causing oxidative stress conditions (Schmidl and Labuza, 2000).

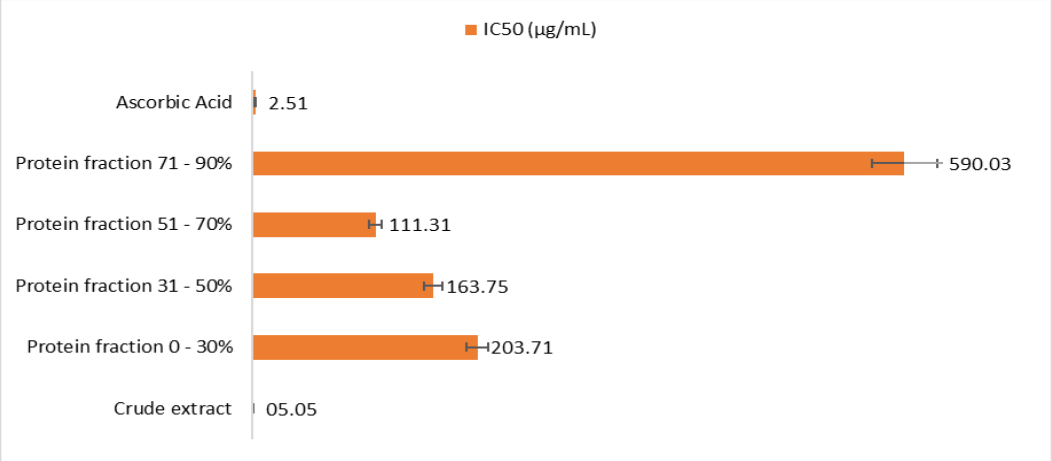

Fig 1. Scavenging effect of *Niphates* sp. protein against free radicals DPPH

Our observation toward free radical DPPH scavenging activities of each protein fraction by saturation level of 0-30%, 30-50%, 50-70%, and 70-90% was displayed in fig 1. The antioxidant activity was expressed as the number of antioxidants to reduce free radical DPPH 50% (IC_{50}) (Minsas et al., 2020).

Based on the assay results, indicate the antioxidant activity of isolated protein against free radicals DPPH with IC_{50} values for crude protein extract results of 5.05 µg/mL, protein fraction 0 – 30% of 203.71 µg/mL, protein fraction 31 – 50% of 163.75 µg/mL, protein fraction 51 – 70% of 111.31 µg/mL, and protein fraction 71 – 90% amounted to 590.03 µg/mL. The results showed that the most significant antioxidant activity present in crude protein extracts, but still less than the antioxidant activity of ascorbic acid (2.51 µg/mL) as a basic reference. The big difference in the crude extract's antioxidant activity and each fraction is likely due to differences between the amino acid composition. Antioxidants' mechanism of action against DPPH depends on the conformation of antioxidant compounds (Sofiana et al., 2020). Generally, the amount of hydroxyl (OH) or a group that can donate hydrogen-like -NH and -SH in the molecular structure would increase the antioxidant activity (Dewi et al., 2020b; Masriani et al., 2020; Rahman et al., 2020; Son and Lewis, 2002). The antioxidant properties of the extract protein fraction of sponge can be indicated that their constituent amino acid of glutathione, which is a natural antioxidant compound produced in the body.

CONCLUSION

In vitro analysis of isolated protein of *Niphates* sp. sponge demonstrates the more significant potential of antimicrobial activity against *E. coli* in comparison with the *B. Subtilis.* The inhibition zone of growth of pathogenic bacteria *E. Coli* is 14.21 mm while for *B. subtilis* is 12.05 mm. Furthermore, the most substantial antioxidant potential as a free radical scavenger shown in crude protein extracts *Niphates* sp. sponge with IC_{50} of 5.05 µg/mL.

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