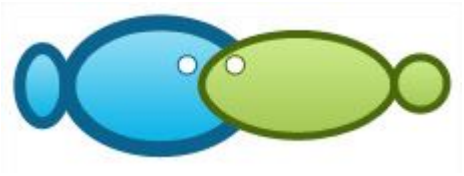

The potential of marine ascidians as sources of natural antioxidant and antibacterial agents from Manado, North Sulawesi

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Abstract. The ascidians are an excellent source of bioactive compounds as natural antioxidants, and they have particular interest due to their beneficial effects on health. Sample preparations for antioxidant and antibacterial activity evaluation of the ascidians *Lissoclinum patella, Didemnum molle* and *Herdmania momus* used methanol as extraction media. In the present study, the antioxidant activity was determined through superoxide dismutase (SOD) assay and the antibacterial activity was tested against pathogenic bacteria *Enterococcus faecalis*. Results showed that methanol extracts of *L. patella, D. molle* and *H. momus* had an antioxidant activity against SOD with IC_{50} of 300, 82 and 52 ppm, respectively. On the other hand for antibacterial activity, two ascidian extracts (*D. molle* and *H. momus*) showed inhibition zones values of 9.2 and 6.9 mm at 10% together with chlorhexidine as positive control, while the ascidian *L. patella* was inactive. These data indicated that ascidians had potential bioactive compounds as source of natural antioxidants.

Key Words: ascidians, antioxidant activity, antibacterial activity, SOD.

Introduction. Marine invertebrates are an important resource for the discovery of bioactive natural products. Chemical investigations on marine ascidians have been prosperous, leading to the isolation of various metabolites possessing unique structural and potent biological properties (Blunt et al 2018). Ascidians (tunicates) are marine invertebrate chordates and prolific producers of a wide variety of biologically active compounds and several of them have properties which make them be candidates for potential new drugs to treat diseases, such as tumor/cancer (Tatsuta 2017; Sumilat 2018; Watters 2018), bacteria (Liu et al 2004), and as inhibitor of PTP1B enzyme (Sumilat et al 2017).

In the present study, we found the potential of ascidians as a source of antioxidant (SOD) and antibacterial agent from *Enterococcus faecalis*. Superoxide dismutase (SOD) is a detoxification enzyme that converts superoxide to hydrogen peroxide, which can subsequently be converted to water. Superoxide dismutase (SOD) activities in various diseases appear to be of clinical interest. SOD has powerful antiinflammatory activity. For example, treatment with SOD decreases reactive oxygen species generation and oxidative stress and, thus, inhibits endothelial activation. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease (Seguí et al 2004). Superoxide radicals are one of the most toxic reactive oxygen species and its damaging effects lead to a variety of detrimental health conditions including cardiovascular diseases, neurodegenerative disorders and other types of age-related diseases (Iranzo 2011). Following Nature example, chemists have designed manganese complexes that mimic the protecting action of the superoxide dismutases (SOD), metalloenzymes that catalyze the conversion of superoxide radical to the less toxic oxygen and hydrogen peroxide (Iranzo 2011).

On the other hand, the biological activity of SOD in ascidians has not been reported, and we found for the first time the biological activity of ascidians as a source of antioxidant (SOD) to be an important target for therapeutic research.

Material and Method

General experimental procedures. Chemicals including solvents were used without further purification in the preparation and Superoxide Dismutase (SOD) assay and Antimicrobial assay have been purchased from Sigma-Aldrich.

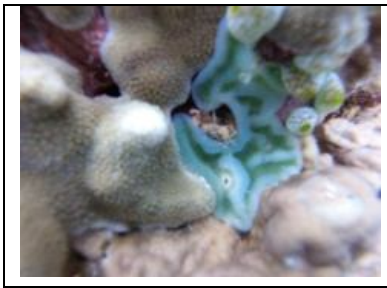
Collection and extraction of ascidians. Samples were collected using SCUBA in the coral reef of Malalayang, Manado, Indonesia, in March 2018. They were cut into small pieces right after collection and extracted for 24 hours three times in 500 mL of ethanol. The voucher specimens are deposited at the Faculty of Fisheries and Marine Science, Sam Ratulangi University.

Superoxide dismutase (SOD) assay. The SOD-mimic activity of complexes was evaluated using an indirect method of riboflavin photoreduction as described previously (Kostyuk et al 2007; Deawati et al 2017). The method involves the competitive reaction between the complex and reduced NBT (NBT = nitroblue tetrazolium) for $O_2^{\cdot-}$ generated by riboflavin under illumination at room temperature (25°C). The sample mixture (240 µL) contained the complex (11 different concentrations), 6 µM riboflavin (Thermo Scientific), 0.8 µM of N,N,N',N'-tetramethylethylene-diamine (TMEDA) (Biorad) in 0.016 M phosphate buffer (pH 7.4) and 85 µM NBT (Thermo Scientific). The reaction was stopped by switching off the light after 15min (4 fluorescence tubes, Philips TLD/20 W, 20 cm distance) and the absorbance of reduced NBT was measured at λ 560 nm with a Multiskan Go Thermo Fischer Scientific UV/Vis double beam spectrophotometer.

Antimicrobial assay. The antibacterial activity against *E. faecalis* ATCC 29212 was determined with the Kirby-Bauer disk diffusion and Muller Hinton broth and Muller Hinton agar as medium and chlorhexidine as positive control. Paper discs (7 mm) were impregnated with 20 µL of each sample and then the discs was loaded with compounds were placed onto the surface of the agar. Tests were performed in duplicate. The bacterial cells were pre-cultured in Muller Hinton broth at 37°C under aerobic conditions and incubated in the presence of compounds with the concentrations obtained by serial two-fold dilution at 37°C without shaking in the same broth for 24 h. Methanol used for dissolving crude extracted that of methanol have no effect to bacterium.

Results and Discussion

Collection and extraction of ascidians. Marine ascidians were collected by scuba dives in the coral reef at Malalayang Manado, Indonesia, in 2018 and identified as *Lissoclinum patella, Didemnum molle* and *Herdmania momus* (Figure 1). The marine ascidians were thawed, cut into small pieces, and extracted three times in ethanol. The ethanol extract was evaporated to dryness.



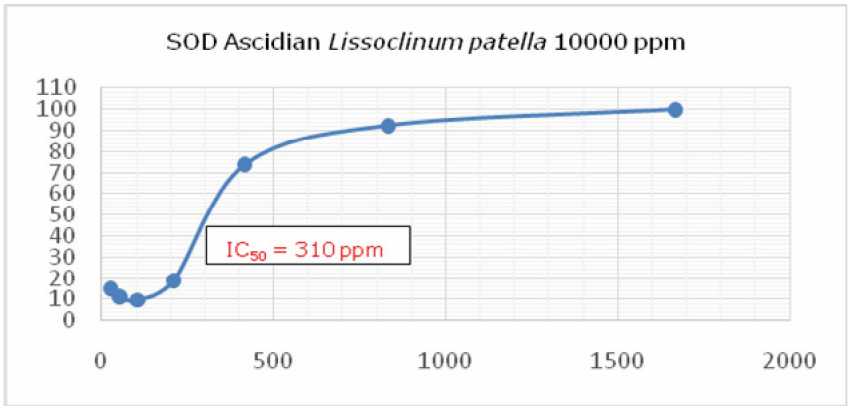
Lissoclinum patella

Didemnum molle

Herdmania momus
(Source: Sumilat (2013))

Figure 1. Marine ascidians.

SOD-mimic activity. The mSOD activities of the three crude extract of *L. patella, D. molle* and *H. momus* were evaluated quantitatively using the indirect method of riboflavin photoreduction in the presence of TMEDA. Riboflavin was reduced photochemically in air generating superoxide anions, which in turn reduced NBT. The result showed that methanol extract of ascidians *L. patella, D. molle* and *H. momus* were showed antioxidant activity against SOD with IC_{50} of 300, 82 and 53 ppm, respectively (Figure 2). These IC_{50} values are in the wide range of the IC_{50} of mSOD (Iranzo 2011), since the ethanol extract was still crude. Therefore, the results indicated that the effects of superoxide anion radical scavenging from marine ascidians *L. patella, D. molle* and *H. momus* were inhibiting NBT reduction and the riboflavin photoreduction mSOD assay showed crude extract affect SOD activity.



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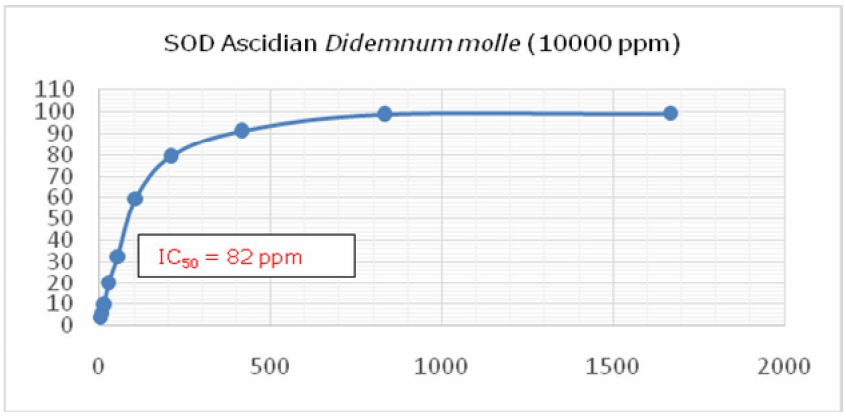

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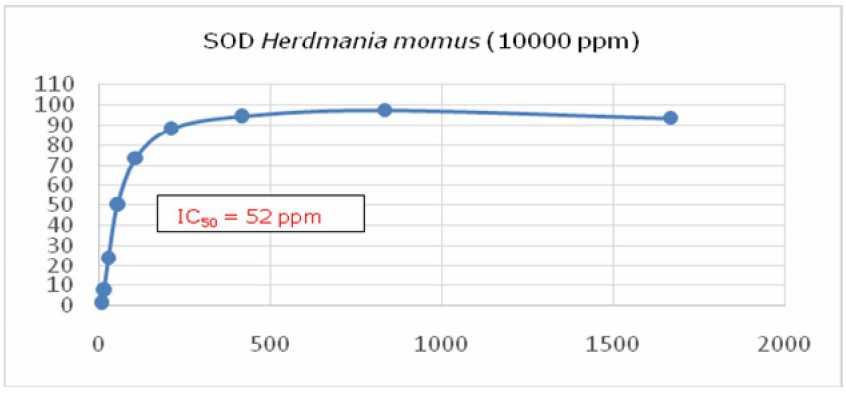

C

Figure 2. The mSOD activity curves of marine ascidians (A) *L. patella*, (B) *D. molle*, (C) *H. momus* and the IC_{50} values.

Antibacterial activity. Antibacterial activity of two ascidians extract *D. molle* and *H. momus* showed inhibition zones values of 9.95 and 6.9 mm at 10% together with chlorhexidine as positive control, while the ascidian *L. patella* was inactive (Table 1).

Table 1

Antibacterial activity of ascidians against *E. faecalis*

No.	*Ascidians*	*Concentration (%)*	*Inhibition zone (mm)*
1.	*Lissoclinum patella*	10	0
2.	*Didemnum molle*	10	9.2
3.	*Herdmania momus*	10	6.9
Control	Chlorhexidine (CHx)	10	23.6

Conclusions. SOD mimic assay was the preliminary study and first time for marine ascidians. Therefore, SOD mimic activity was determined and indicated that structural features affected SOD activity. These research data also reflected that marine ascidians had potential bioactive compounds as source of natural antioxidants.

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