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Screening of Antibacterial and Antioxidant Activity of Soft Corals Sinularia sp. and Sarcophyton sp. from Palu Bay Central Sulawesi, Indonesia

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Abstract

This study aimed to evaluate the potential antibacterial and antioxidant activities of *Sinularia* sp. and *Sarcophyton* sp. from the Palu Bay, Central Sulawesi, Indonesia. Soft corals were identified as *Sinularia* sp. (SC1), *Sinularia* sp. (SC2), and *Sarcophyton* sp. (SC3). Antibacterial activity was examined using agar diffusion well method. Antioxidant activity was measured by the DPPH radical scavenging method. The samples were macerated in MeOH: DCM. The crude extracts were partitioned with DCM, EtOAc, and BuOH. The crude extract of *Sinularia* sp. (SC2) showed a very strong antibacterial activity as it was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* up to 10 mg/mL. *Sinularia* sp. (SC3) crude extract showed strong activity against *S. aureus*, whereas it showed weak against *E. coli*. The partition fractions of the three soft coral extracts had the potential to be a potent antioxidant agent.

Keywords: DPPH, Escherichia coli, Sarcophyton, Sinularia, Staphylococcus aureus

1. Introduction

Soft corals are marine invertebrates that produce various kinds of terpenoid compounds. The terpenoids function as the main chemical defense mechanism against natural predators. Soft corals have been the subject of bioactivity studies since the 19th century (Elkhateeb et al., 2014). Soft corals of the genus *Sinularia* and *Sarcophyton* have known as sources of terpenoids and steroids (Wang, Hsieh, & Duh, 2012; Tseng, Wang, & Duh, 2012). Soft corals have also been shown to have varied biological activities, such as anti fungal (Yang et al., 2011), anti inflammatory (Hsiao et al., 2015), cytotoxic (Januar, Putri, Soedharma, & Chasanah, 2017), anticancer (Rajaram et al., 2013; Nair, Kumar, Byju, Anuradha, & Vasundhara, 2014), antibacterial (Zubair et al., 2016) and antioxidant (Byju et al., 2015). The antibacterial bioactive substance of soft corals has also been widely reported. The results of research between 2009-2016 reported antibacterial pharmacological potency of soft coral extracts of the genus *Sinularia* (Sun et al., 2012; Liang et al., 2013b; Rozirwan, Bengen, Zamani, Effendi, & Chaidir, 2014; Rajaram et al., 2014; Afifi, Abdel-Nabi, & El-Shaikh, 2016; Putra, Wibowo, Murniasih, & Rasyid, 2016) and *Sarcophyton* (Liu et al., 2014; Gomaa et al., 2015; Zubair et al., 2016; Marican, Edros, Mohammad, & Salleh, 2016).

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Palu bay is a semi-closed waters bordering the Makassar Strait. Palu bay has a high biodiversity of coral reef biota, where soft corals are also alive (Moore & Ndobe, 2009). Soft coral is one of the original organism inhabitants of the coral reef ecosystem, in addition to the sponge (Zubair et al., 2018). Soft corals live to share one another with other coral reef organisms in need of the substrate as a place of life (Hoeksema, 2011). The Palu Bay was grouped into the transition zone grouping by Wallace, which is known to have high biodiversity (Chasanah, 2008). However, from the perspective of the bioactive substance, the vast potential of marine life is theoretically not followed by an increase in the discovery of bioactive from marine organisms.

The results of previous studies reported the potential of soft corals bioactive from Sulawesi between 2002 -2018. Soft coral Xenia sp. reported potential as cytotoxic (Anta, González, Santafé, Rodríguez, & Jiménez, 2002; Fattorusso et al., 2008) and antibacterial agents (Kantor, Wewengkang, & Wullur, 2015). Lobophytum sp. showed as cytotoxic (Fattorusso et al., 2009) and antibacterial agent (Kowal et al., 2018). Soft coral Sarcophyton sp. reported reducing TNF- α (Kapojos et al., 2010). Sinularia sp. showed potential as an antibacteria agents (Tanod, Aristawati, Putra, & Muliadin, 2018), antifeedant (Tanod, Aristawati, Nurhani, & Mappiratu, 2017), antimitotic (Tanod, Mangindaan, & Kapojos, 2015), and as an inhibitor of NO (Fattorusso et al., 2011; Putra et al., 2012). Soft coral Nephthea sp. reported as an antibacteria agents (Rumengan, 2013). However, only few studies have revealed the antibacterial and antioxidant potency of soft coral from Central Sulawesi, especially Palu Bay.

In previous studies, it was concluded that the three soft coral crude extracts used in this study had nitric oxide (NO) inhibitory release activity (Tanod, Yanuhar, Maftuch, Putra, & Risjani, 2019). Besides that, the three soft coral crude extracts showed DPPH scavenging properties at concentrations 10, 30, 50, 70, 90 μg/mL (Tanod, Yanuhar, Maftuch, Wahyudi, & Risjani, 2019). Therefore, this research will explore the antibacterial and antioxidant activity of *Sinularia* sp. and *Sarcophyton* sp. from Palu Bay, Central Sulawesi. The aims of this research were to evaluate the potential antibacterial and antioxidant activity of *Sinularia* sp. and *Sarcophyton* sp. from Palu Bay, Central Sulawesi, Indonesia.

2. Material and Methods

2.1. Study Area

The Palu Bay is a semi-enclosed waters area, located on Central Sulawesi, Indonesia. Soft coral samples were collected from the coastal area of Palu Bay, Central Sulawesi, at the coordinates of 43.32 South Latitude and 119.47 East Longitude (Figure 1).

2.2. Chemical and Reagents

Nutrient agar (Merck), nutrient broth (Merck), bacteriological agar (Hi-media), aquades, dichloromethane (Merck), methanol (Merck), dimethyl sulfoxide (Merck), n-butanol (Merck), Ethyl Acetate (Merck) and 1,1-Diphenyl-2-picrylhydrazyl, free radical (DPPH, Merck) were purchased form CV. Amani Media Malang and CV. Intraco Makassar, Indonesia.

2.3. Animal Materials

Soft corals sampling was conducted in December 2016. Soft corals were collected using SCUBA at a

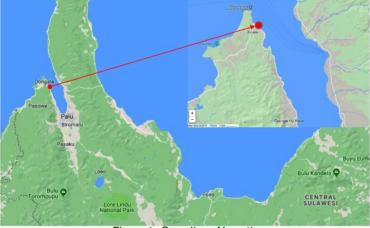


Figure 1. Sampling of Location

depth of 4-5 m. Soft corals were identified using Fabricus & Alderslade (2001) instructions by observing the shape of monomorphic colonies and interiors sclerit. Based on the identification keys from the Australian Institute of Marine Science, the samples were identified as *Sinularia* sp. (SC1), *Sinularia* sp. (SC2), and *Sarcophyton* sp. (SC3). Soft corals were cut into smaller sizes and stored in a freezer before extracted. Samples were stored at the Institute of Fisheries and Marine, Palu, Indonesia.

2.4. Extraction

A total of 500 g (wet weight) sample were macerated in methanol: dichloromethane (1:1) for 48 hours (Hsiao et al., 2015; Putra, Murniasih, et al., 2016a). After that, it was filtered, evaporated (Rotary Vacuum Evaporator EYELA N-1100), dried, weighed and coded as samples SC1, SC2, and SC3. The maceration was performed three times for each of the soft coral samples. The crude extracts of soft coral were partitioned with Dichloromethane (DCM), Ethyl Acetate (EtOAc), and n-butanol (BuOH) for 24 hours. Then, it was evaporated and weighed.

2.5. Antibacterial Assay

The antibacterial activity assay used the well diffusion plate method as described by Balouiri, Sadiki, and Ibnsouda (2016) with modification. Modifications was made by adding nutrient agar composition from 2 g/100 mL to 4 g/100 mL. This method used two media layers, namely the base medium layer and the medium seeding layer. The base medium was prepared by dissolving nutrient agar and bacteriological agar in 100 mL aquades, then sterilized. The seeding medium layer was made with from 70% nutrient agar in 100 mL of aquades, then inserted to tube of 9 mL, and then sterilized. Furthermore, in warm seeding medium, one mL of test bacteria was added with a density of 1×107 CFU/mL (Bacterial solution were compared using standard McFarland-Hi-media). Isolate of S. aureus ATCC 33591 and E. coli ATCC 25922 were obtained from Microbiology Laboratory, Faculty of Medicine, Brawijaya University Malang. Media that had been added with the bacterial solution were vortexed, poured over the base medium layer, and dropped into the wells. In each of the well, it was filled with a 50 μ L of crude extracts with 1.000, 100 and 10 mg/mL concentrations, and incubated at 37 °C for 24 hours. Chloramphenicol and ampicilin were used as a comparative control with dose of 100, 10 and 1 mg/ mL. After that, it was observed and measured for the inhibition zone. All experimental measurement data were performed in three replicates and expressed as Mean \pm SD (n = 3).

2.6. Antioxidant Assay

The antioxidant activity was measured using the DPPH radical scavenging method (Molyneux, 2004). The crude extracts of soft coral were prepared in 200 μ g/mL, while the IC₅₀ was determined using different concentrations of 20, 40, 60, 80, and 100 µg/mL. Vitamin E was used as a comparative control. The extract was added with 50 µM DPPH. Then, this mixture was homogenized and incubated in a dark room for 30 minutes. Then, it was analyzed with a spectrophotometer at 517 nm wavelength (UV-VIS spectrophotometer T90+ PG Instruments Ltd). The measurement process was carried out three times and expressed in Mean ± SD. Data processing was done using Microsoft Excel 2013. The DPPH scavenging effect was measured using the following equation:

DPPH Scavenging Effect (%) = (Blank absorbance-Sample absorbance) x 100% (Blank absorbance)

3. Results and Discussion

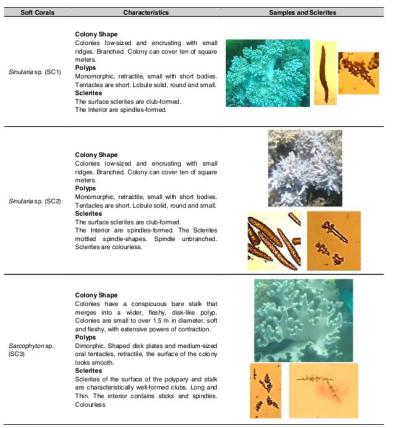
Soft coral samples were identified based on colony shape, interior, and surface sclerit using identification keys from the Australian Institute of Marine Science. The results of identification were morphologically identified as *Sinularia* sp. (SC1), *Sinularia* sp. (SC2), and *Sarcophyton* sp. (SC3). The morphological characteristics of soft corals used in this research can be seen in Table 1.

The evaluation of antibacterial activity were conducted by observing the inhibition of the growth of *S. aureus* ATCC 33591 (gram-positive) and *E. coli* ATCC 25922 (gram-negative). The results of the antibacterial activity assay of all soft coral's crude extracts can be seen in Table 2 and Figure 2.

The results showed that the crude extracts of all soft corals have shown different antibacterial activities against *S. aureus* and *E. coli*. According to the category of inhibition zone by Paudel et al. (2014), there are four categories of antibacterial activity: very strong (inhibition zone \emptyset 20 mm), strong (inhibition zone, \emptyset 15 - 20 mm), moderate (inhibition zone \emptyset 10 - 15 mm), and weak (inhibition zone \emptyset 10 mm). The crude extracts of all soft corals showed various antibacterial potentials from very strong to weak against *S. aureus* and *E. coli* bacteria.

The crude extract of *Sinularia* sp. (SC2) showed very strong antibacterial activity because it was able to inhibit the growth of *S. aureus* and *E. coli* up to 10 mg/mL. *Sinularia* sp. (SC1) crude extract showed strong activity against *S. aureus*, whereas it showed moderate against *E. coli. Sarcophyton* sp. (SC3) crude

Table 1. Characteristics of soft coral Sinularia sp. and Sarcophyton sp.



Keys Identification by Fabricus & Alderslade (2001)

extract showed moderate activity against *S. aureus*, whereas it showed weak activity against *E. coli*.

The antibacterial assay also was compared with chloramphenicol and ampicillin as comparative controls. Chloramphenicol inhibited test bacteria with a larger inhibition zone than ampicillin and soft coral extracts. The assay results can be seen in Table 2 and Figure 2. This is because chloramphenicol is an antibacterial compound that is very stable and diffuses well in agar media. Jawetz, Melnick, and Adelberg (1998) mentioned that antimicrobial activity influenced by factors such as environmental pH, media composition, the stability of antimicrobial compounds, the amount of inoculum, duration of incubation, and metabolic activity of microorganisms.

The resistance of gram-negative and gram-positive bacteria to antibacterial compounds showed different respon. Gram-negative bacteria are generally sensitive to polar antimicrobial compounds, while gram-positive bacteria are sensitive to non-polar antibacterial compounds (Branen & Davidson, 1993). The difference of antibacterial response to gram-positive and gramnegative bacteria related to the structure in their cell walls, such as the amount of peptidoglycan (the presence of receptors, pores, and lipids), cross-linking properties, and autolytic enzyme activity. These components are factors that determine penetration, binding, and the activity of antimicrobial compounds (Jawetz, 1998).

Furthermore, crude extracts of *Sinularia* sp. (SC1), *Sinularia* sp. (SC2), and *Sarcophyton* sp. (SC3) were partitioned by DCM, EtOAc, and BuOH. Then, it was evaluated by observing the inhibition of the growth of *S. aureus* and *E. coli* at a dose of 250 mg/mL. The assay results are shown in Table 3. All fractions showed weak antibacterial activity at 250 mg/mL. However, fractions of *Sarcophyton* sp. (SC3) showed the potential to inhibit the growth of *S. aureus* and *E.*

Table 2. Antibacterial activity of crude extracts, chloramphenicol, and ampicilin (comparative control) at concentration of 1000, 100, 10 and 1 mg/mL

	Soft Corals Extract	Mean Diameter of Inhibition Zone (mm)				
	Concentrations	Sinularia sp. (SC1)	Sinularia sp. (SC2)	Sarcophyton sp. (SC3)	Chloramphenicol	Ampicilin
	1000 mg/mL	16.33 ± 1.53	22.63 ± 1.74	13.87 ± 1.96		
	100 mg/mL	12.33 ± 0.58	14.07 ± 1.42	6.73 ± 0.51	34.50 ± 1.15	25.50 ± 2.48
	10 mg/mL	6.73 ± 1.69	10.40 ± 1.59	NZ	27.63 ± 2.52	21.00 ± 2.65
S. aureus	1 mg/mL				10.67 ± 1.42	13.63 ± 1.91
	1000 mg/mL	12.63 ± 0.35	21.40 ± 1.31	10.10 ± 1.15		
E. Coli	100 mg/mL	8.60 ± 1.00	15.50 ± 1.01	7.63 ± 0.58	25.83 ± 2.56	19.73 ± 1.38
	10 mg/mL	NZ	6.33 ± 0.58	NZ	21.53 ± 1.47	16.73 ± 2.14
	1 mg/mL				1.83 ± 1.40	8.07 ± 1.36

NZ: No inhibition zone detected

Table 3. Antibacterial activity assay of fractions of Sinularia sp. and Sarcophyton sp. at 250 mg/mL

Soft Corals	Fractions	Mean Diameter of Inhibition Zone (mm)		
con contra	Tractions	S. aureus	E. coli	
	DCM	NZ	2.00 ± 0.10	
Sinularia sp. (SC1)	EtOAc	NZ	4.97 ± 0.35	
	BuOH	3.80 ± 0.20	5.87 ± 0.51	
	DCM	NZ	NZ	
Sinularia sp. (SC2)	EtOAc	NZ	NZ	
	BuOH	7.63 ± 0.35	5.93 ± 1.53	
	DCM	4.47 ± 1.04	NZ	
Sarcophyton sp. (SC3)	EtOAc	6.37 ± 0.93	5.40 ± 0.17	
	BuOH	5.53 ± 0.50	3.60 ± 0.61	

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NZ: No inhibition zone detected

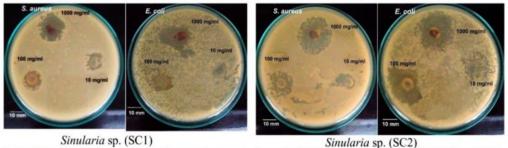
coli, while fractions of *Sinularia* sp. (SC1 and SC2) did not show good potential activity in inhibiting both tested bacteria. It does not mean that soft coral extract is not potential as an antibacterial. It is suspected that if higher concentration is used in assay, the extracts will show a better ability to inhibit bacteria.

Some soft coral extracts that do not show inhibition zone, however, it does not mean that they do not have antibacterial compounds (Setyaningsih, Nurhayati, Nugraha, & Gunawan, 2012). The compounds in the extract may works synergistically.

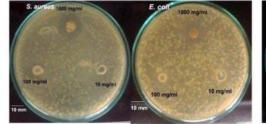
According to the antibacterial assay result, we assumed that the partitioning process was not optimal. However, there is a possibility that it will show

a stronger antibacterial if the appropriate minimum sample concentration for antibacterial assay was used. The bioactives substances from soft coral is thought to interact with each other to provide antibacterial and antioxidant properties on soft coral extracts. Bioactive compounds from natural products can work in a synergy between the compounds (Merzenich, Panek, Zeitler, Vetter, & Wagner, 2010). Natural products can work through multi-compound and multi-target synergistic modes (Long, Yang, Xu, Hao, & Li, 2015).

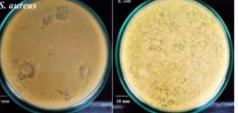
The development of pathogenic bacterial resistance against existing antibacterial drugs resulted in uncontrolled bacterial infections (Zaidan et al., 2005). In addition, the unwanted side effects of certain



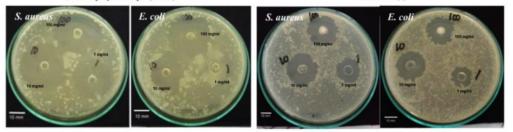
Sinularia sp. (SC1)



Sarcophyton sp. (SC3)



Control (-)



Chloramphenicol

Ampicilin

Figure 2. Antibacterial activity assay of Sinularia sp. and Sarcophyton sp. crude extracts, chloramphenicol, and ampicilin

antibiotics have led to the search for new antibacterial agents especially from marine organisms (Marican et al., 2016). Soft coral is a very important marine organism in chemical ecology and has various biological activities such as antibacterial (Afifi et al., 2016).

The previous research have shown that bioactive compounds and their potential biological activity are not the same among soft coral species. Soft corals was also reported that the antibacterial potency of S. trocheliophorum from Yalong Bay, China, showed antibacterial activity against methicillin-sensitive S. aureus Newman strains (Liang, Lan, Taglialatela-Scafati, & Guo, 2013). Sarcophyton trocheliophorum Weizhou Island origin, China, was reported that it could inhibit the growth of Xanthomonas vesicatoria, Agrobacterium tumefaciens, Pseudomonas lachrymans, B. subtilis, and S. aureus (Liu et al., 2014). Sarcophyton trocheliophorum from the Red Sea

was also reported to exhibit antibacterial activity of inhibiting the growth of S. aureus, Bacillus cereus, Salmonella typhi, E. coli and P. aeruginosa (Gomaa et al., 2015). The soft coral Sarcophyton sp. of Kedah origin, Malaysia was able to inhibit the growth of S. aureus (Marican et al., 2016). Sarcophyton trocheliophorum that was collected from the Red Sea also showed a wide range of inhibitory potential for the growth of Acinetobacter baumannii, E. coli, K. pneumoniae, P. aeruginosa, S. aureus, S. epidermidis, and S. pneumoniae (M. Zubair et al., 2016). The soft coral of Selayar Island origin, Indonesia S. trocheliophorum showed moderate inhibitory activity against S. aureus, B. subtilis, and Vibrio cholera (Putra, Saparhadi, Karim, Murniasih, & Swasono, 2016).

The soft corals of genus Sinularia sp. also showed antibacterial potential against both gram-negative and gram-positive bacteria. Sinularia humilis Of wegen from

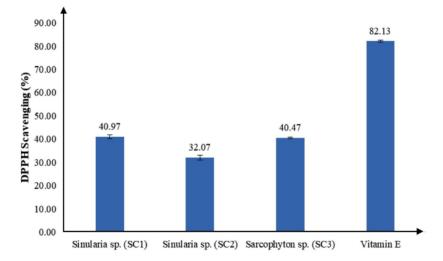
the South China Sea showed antibacterial activity against Bacillus megaterium (Sun et al., 2012). The Steroid compounds isolated from S. depressa Tixier-Durivault of Lingshui Bay origin, China showed an inhibitory activity of S. aureus strain Newman (Liang, Wang et al., 2013). Sinularia kavarattiensis collected from the coast of Mandapam, showed antibacterial activity against S. aureus and S. epidermidis (Rajaram et al., 2014). Two species of Sinularia sp. which collected from the Bandar Al-Khayran area, Oman showed antibacterial activity against Micrococcus luteus, S. aureus, B. subtilis, E. coli, and Salmonella sp. (Dobretsov et al., 2015). Sinularia sp. collected from Lampung, Indonesia showed antibacterial activity against B. subtilis, S. aureus, E. coli, and V. eltor (Putra, Wibowo et al., 2016). Crude extract S. polydactyla of Yanbu origin, Red Sea showed antibacterial activity against S. aureus (Afifi et al., 2016).

This study also evaluated antioxidant activity by calculating the level of purple light intensity of DPPH, proportional to the reduction in DPPH concentration. This reduction is caused by the reaction of the diphenyl-2-picryl hydrazyl molecule with hydrogen atoms released by the molecular components of the sample, thus forming a diphenyl picryl hydrazine compound and causing DPPH to change color from purple to yellow (Huliselan, Runtuwene, & Wewengkang, 2015). Antioxidant activity showed the ability of a compound bioactive to inhibit an oxidation reaction, which was expressed as a percentage of

inhibition (Dewanto, Tanod, Finarti, & Renol, 2018). In this study, the DPPH scavenging percentage was measured for soft coral crude extracts and Vitamin E at a concentration of 200 μ g/mL, as can be seen in Figure 3.

The crude extracts of Sinularia sp. (SC1), Sinularia sp. (SC2), and Sarcophyton sp. (SC3) were partitioned by DCM, EtOAc, and BuOH. The DPPH scavenging effect was evaluated for the determination of IC_{50} (Table 4). The fractions of soft corals indicated antioxidant activities because they were able to donate hydrogen/ electron atoms to react with DPPH radicals. The partition fractions of all soft coral extracts have the potential as antioxidant. According to the Blois (1958), there were four categories of antioxidant activity: very strong (IC₅₀<50 µg/mL), strong (IC₅₀ between 50-100 μ g/mL), moderate (IC₅₀ ranges from 100-150 μ g/mL) and weak (IC₅₀ ranges from 150-200 µg/mL). The assay results showed that the increase in fractions concentration gave an increase in DPPH radical inhibition percentage (Figure 4).

Vitamin E as a comparative control has one hydroxyl group. Vitamin E is a natural antioxidant compound that is often used as a comparative compound in antioxidant activity assay. The assay showed that vitamin E was a very powerful antioxidant compound. From the literature study, IC_{50} values of vitamin E are ranged from 8-23 µg/mL (Rohman, Riyanto, & Hidayat, 2007; Yassa, Masoomi, Rankouhi, & Hadjiakhoondi, 2009; Da'i & Triharman, 2010; Melannisa, Da'i, & Rahmi, 2011; Cheng, Wu, Lin, & Liu, 2013).



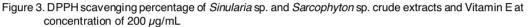


Table 4. IC₅₀ value from antioxidant assay using DPPH scavenging of *Sinularia* sp. and *Sarcophyton* sp. fractions and vitamin E

Soft Corals	Fractions	Mean of IC ₅₀ (μg/mL)
Sinularia sp. (SC1)	DCM	78.95 ± 0.45
	EtOAc	73.58 ± 0.96
	BuOH	50.47 ± 0.21
<i>Sinularia</i> sp. (SC2)	DCM	70.52 ± 0.43
	EtOAc	74.47 ± 0.71
	BuOH	72.09 ± 0.07
<i>Sarcophyton</i> sp. (SC3)	DCM	79.36 ± 0.50
(303)	EtOAc	87.16 ± 0.65
	BuOH	83.13 ± 0.88
Vitamin E		20.83 ± 1.93

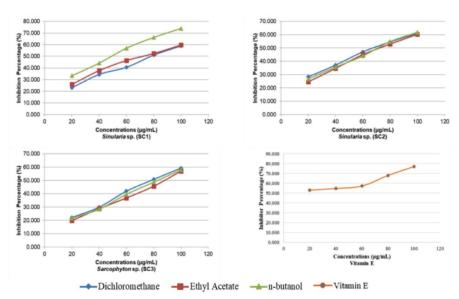


Figure 4. Percentage of DPPH free radical inhibition from *Sinularia* sp. and *Sarcophyton* sp. fractions and vitamin E in different solvents

The previous research showed that soft coral extracts were also potential as antioxidants. Two sesquiterpene compounds were isolated from *Sinularia* sp. collected from Sanya bay Hainan Island of China (Zhang et al., 2006). *Sinularia* sp. and *Lobophytum* sp. collected from Pongok Island, Bangka Belitung, Indonesia were detected flavonoid derivative components that can act as antioxidants (Apri, Zamani, & Effendi, 2013). Twenty-four diterpenoid derivatives extracted from *Sinularia maxima* and

Lobophytum crissum showed peroxyl radical capacityscavenging capacity from moderate to strong (Thao et al., 2015). Ethanolic extract from *Sarcophyton flexuosum* Tixier-Durivault collected from Kavarathi Island, Lakshadweep Islands showed increased of free radical scavenging when the extract concentration increased gradually (Byju et al., 2015).

Some studies revealed the potency of bioactive secondary metabolites from soft corals. This research results showed that soft corals from Palu Bay, Central

Sulawesi produced variations of bioactive compounds, namely antibacterial against *S. aureus* and *E. coli*, and DPPH scavenging. It is suggested that further isolation and purification of their bioactive compounds are needed.

4. Conclusion

This research gives information on the bioactive potency of soft corals *Sinularia* sp. and *Sarcophyton* sp. from Palu Bay, Central Sulawesi as a source of antibacterial and antioxidant compounds. Increased global attention on finding alternative sources of raw materials for antibacterial and antioxidant drugs, soft corals could be one of the options.

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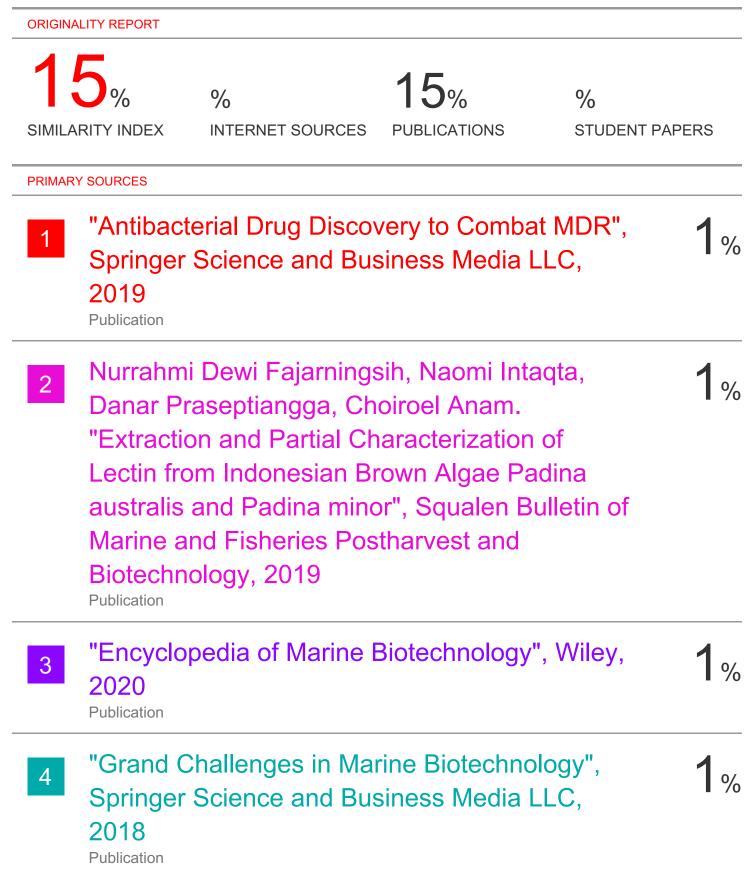
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