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SOFT CORAL *Sinularia gibberosa* EXTRACTS ORIGIN PALU BAY, CENTRAL SULAWESI WITH ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY

Muliadin[1], Didit Kustantio Dewanto[1], Roni Hermawan[1], Putut Har Riyadi[2] and Wendy Alexander Tanod[3*]
[1]Institute of Fisheries and Marine (Sekolah Tinggi Perikanan dan Kelautan – STPL Palu), Palu,
Jalan Soekarno Hatta KM. 6 Kampus Madani, Mantikulore, Palu, 94118 Central Sulawesi, Indonesia
[2]Department of Fisheries Post Harvest Technology, Faculty of Fisheries and Marine Science, Universitas Diponegoro,
Jalan Prof. Soedarto, SH Tembalang, Semarang, 50275 Central Java, Indonesia
[3]Department of Fisheries and Marine, Politeknik Negeri Nusa Utara,
Jalan Kesehatan No.1, Sawang Bendar, Tahuna, 95812 Sangihe Archipelago, North Sulawesi, Indonesia
E-mail: wendytanod@gmail.com

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ABSTRACT

Sinularia gibberosa is one of the soft coral species reported to produce bioactive compounds for pharmaceutical's potential. Soft corals produce compounds with antibacterial and antioxidant properties. This study aimed to obtain a bioactive profile of *S. gibberosa*, which could scavenge DPPH radicals and inhibit the growth of *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. The research included a sampling of soft corals, extraction by maceration, identification of chemical profiles using LC-HRMS, the assay for antibacterial activity (well-diffusion method), antioxidant assay (DPPH method), and total phenolic content (Folin–Ciocalteu method). Sampling was conducted in Palu Bay, Central Sulawesi, Indonesia. Based on monomorphic colonies and sclerites, soft coral samples were identified as *Sinularia gibberosa*. The chemical profiles of *S. gibberosa* extracts was dominated by trimethylethanolamine (22.03%), arachidonic acid (14.13%), (3R)-3-hydroxy-4-(trimethylazaniumyl)-butanoate (10.90%), (5Z,8Z,11Z,14Z,16R)-16-Hydroxy-5,8,11,14-icosatetraenoic acid (5.64%), Octyl decyl phthalate (4.18%), and N-Methylnicotinic acid (3.63%). The antibacterial assaying at 300 mg/mL of *S. gibberosa* extracts showed moderate to strong antibacterial activity, with the inhibition zone diameter of *L. monocytogenes* (15.11 ± 0.70 mm), *P. aeruginosa* (13.44 ± 0.84 mm), and *S. typhimurium* (19.78 ± 0.39 mm). *S. gibberosa* extracts showed strong antioxidant activity, with an IC_{50} of 54.69 ± 0.34 µg/mL and total phenolic content was 5423.76 ± 14.00 mg GAE/25 mg dry extract. Based on the results, *S. gibberosa* shows the potential for antibacterial and antioxidant activity. This study indicates that soft corals origin Palu Bay, Central Sulawesi, Indonesia, are potential organisms in the discovery and development of antibacterial and antioxidant agents.

Keywords: Bioactive compounds; *Listeria monocytogenes*, soft coral, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*

INTRODUCTION

Indonesia's marine biodiversity is very high and has significant potential in the economy (Supriharyono, 2000). Biodiversity is directly proportional to the diversity of natural products isolated from organisms. The bioprospection of natural compounds from the marine environment has produced thousands of active compounds with pharmacological functions. Thousands of active compounds produced by marine organisms confirm that marine organisms are an important source for the research and development of new drugs (Tanod *et al*., 2015).

Eastern Indonesian waters are waters with high biodiversity of marine organisms (Tapilatu, 2015). Based on this, eastern Indonesia's waters can become the center for discovering potential bioactive from marine organisms (Leal *et al*., 2012). Marine organisms have physiologically unique metabolites to survive in extreme marine environments. The unique metabolites of marine organisms are chemically and biologically beneficial and not produced by terrestrial organisms (Blunt *et al*., 2013). Soft corals contain various bioactive substances and have antibacterial and antioxidant biological activities (Bishara *et al*., 2007; Ishii *et al*., 2009). There have been many explorations of the search for pharmacological agents from Indonesian waters. However, the investigation of bioactive substances from soft corals from Sulawesi waters has not been widely carried out, especially uncovering the antibacterial and antioxidant potential.

Soft coral genus *Sinularia* is one of the genera reported to produce many bioactive compounds and has enormous potential in pharmacology (Byju *et al*., 2015). *Sinularia* was reported to produce flavonoids, saponins, alkaloids, steroids, phenolics and triterpenoids (Tanod et al., 2019a; Salanggon *et al*., 2020). Romansyah (2011) reported that soft corals could produce compounds that can be used as antibacterial and antioxidant agents. Antibacterial are substances that can kill or inhibit bacterial growth. The need for active antibacterial agents continues to increase (Wright and Sutherland, 2007). The World Health Organization reports the increasing resistance of pathogenic bacteria to available antibacterial active agents (Rice, 2008; Radić and Strukelj, 2012). Antioxidants are substances that function to protect cells from damage caused by free radicals. Antioxidant substances can scavenge reactive oxygen species (ROS) to prevent oxidative stress in cells (Liu *et al*., 2015).

Previous research has reported the pharmacological potential of soft corals from Palu Bay, namely as an antibacterial against the growth of *Escherichia coli*, *Staphylococcus aureus* (Tanod *et al*., 2018a; Tanod *et al*., 2019b), and *Pseudomonas aeruginosa* (Salanggon *et al*.,

2020); antioxidants by DPPH method (Dewanto *et al*., 2019; Tanod *et al*., 2019c); antifeedant to inhibit feeding activity of *Plutella xylostella* larvae (Tanod *et al*., 2018b); and nitric oxide and nuclear factor kappa beta inhibitors (Riyadi *et al*., 2019; Tanod *et al*., 2019a). However, the chemical profiles of the soft coral extract has not been identified. In addition, research on the antibacterial activity of *Sinularia* only observed the inhibitory growth of *E. coli* and *S. aureus*. Even though many pathogenic bacteria threaten human health, this study aimed to obtain a chemical profile of *Sinularia gibberosa*, which can scavenge DPPH radicals and inhibit the growth of *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*.

RESEARCH METHODS

Study Area

The Palu Bay is a semi-closed water area located in Central Sulawesi, Indonesia, and borders the Makassar Strait. A sampling of *S. gibberosa* from Palu Bay's coastal area in Lero, Donggala, Central Sulawesi, Indonesia at coordinates 0°37'44.76" South Latitude and 119°48'40.60" East Longitude (Figure 1).

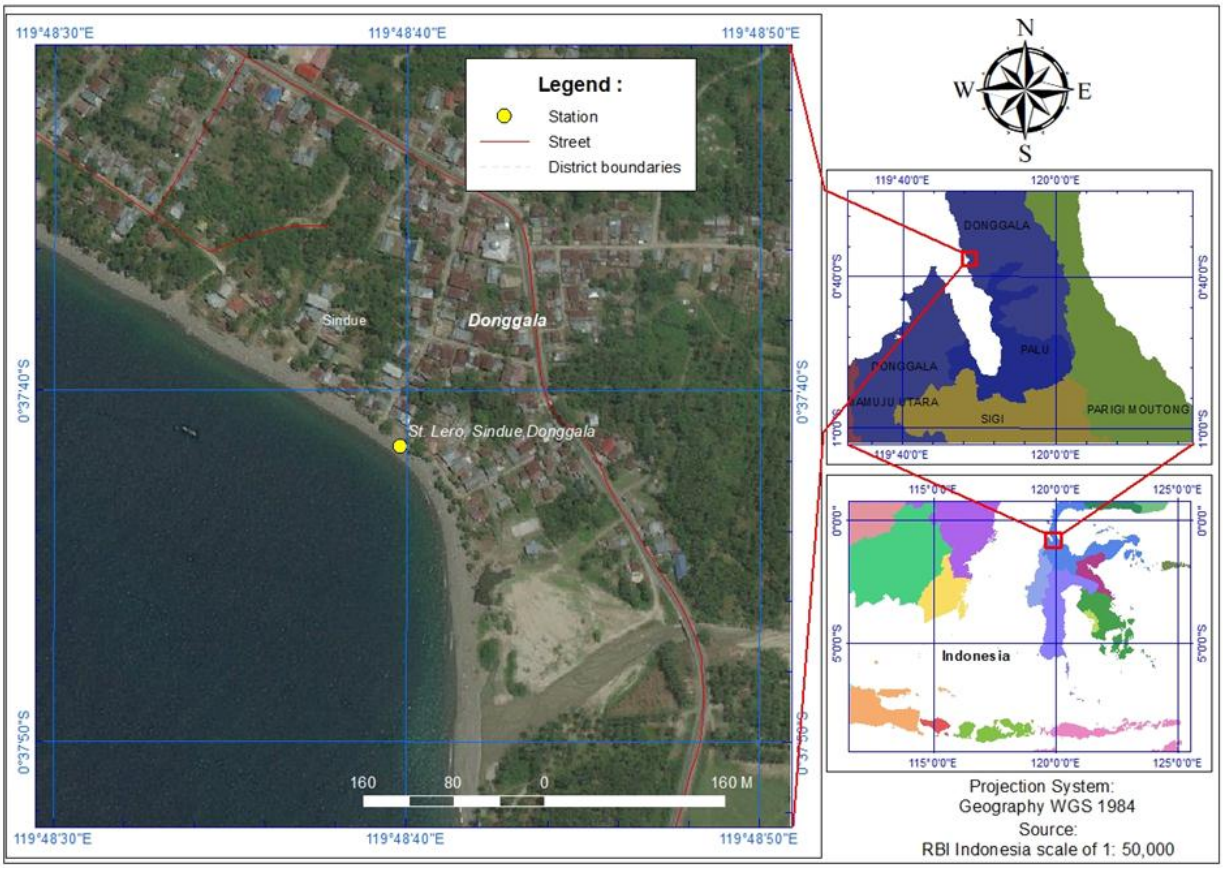

Figure 1. Sampling Location of *Sinularia gibberosa* at Lero, Central Sulawesi, Indonesia

Chemical and Reagents

Nutrient agar (Merck), nutrient broth (Merck), bacteriological agar (Hi-media), dichloromethane (Merck), methanol (Merck), 1,1-Diphenyl-2-picrylhydrazyl, free radical (DPPH, Merck), Folin–Ciocalteu reagent, gallic acid, Na_2CO_3, and aquadest were purchased form CV. Amani Media Malang and CV. Intraco Makassar, Indonesia.

Animal Materials

A sampling of *S. gibberosa* soft corals was carried out in July 2020 with sunny weather conditions and hot sunlight. Sampling using SCUBA at a depth of 5-6 m. Soft corals identified using instructions Fabricus and Alderslade (2001) by observing the form of monomorphic colonies and interior sclerites. Soft coral was cut into smaller sizes and stored in the freezer before extraction. Soft coral samples were kept at the Institute of Fisheries and Marine (Sekolah Tinggi Perikanan dan Kelautan-STPL Palu).

Extraction

A total of 500 g of *S. gibberosa* samples were dried using an oven (Finco OV50) at 60^{o}C during 25-30 hours. After drying, the sample mashed until it becomes flour. Then 100 g of *S. gibberosa* flour macerated in methanol:dichloromethane (1:1) for 48 hours (Hsiao *et al*., 2015; Putra *et al*., 2016a). After that, the extracts filtered and dried with a rotary vacuum evaporator (EYELA N-1100). The maceration process was carried out three times. Then, the dried extracts that have been weighed and stored in the refrigerator.

Chemical Profiles Screening

Chemical profiles screening of *S. gibberosa* extracts using Liquid Chromatography and High-Resolution Mass Spectrometry (LC-HRMS) with an injection volume of 1 µL. Bioactive profile analysis was performed using the LC-HRMS Shimadzu-8040, Japan, equipped with an autosampler, binary pump, column compartment, and a diode array detector for

scanning spectroscopy. Chromatographic separation was performed using a C-18 column, Shim Pack FC-ODS (2 mm ø x 150 mm, 3 µm). Two solvents prepared included solvent A (H_2O:MeOH, 8:2) with 0.1% formic acid) and solvent B (0.1% formic acid in acetonitrile). The two solvents were adjusted to 95:5 ratios, respectively, with an elution gradient of 0/0 at 0 minutes, 15/85 at 5 minutes, 20/80 at 20 minutes, 90/10 at 24 minutes. Mass spectroscopy (MS) analysis was performed by Electrospray Ionization (ESI) with positive ions as the source. MS data were obtained through collision energy traps starting at 5.0 V. The ESI source parameters were regulated, including a capillary voltage of 3.0 kV, source temperature of 100^{o}C, desolvation temperature of 350^{o}C, sampling cone 23 V, and desolvation gas flow of 6 L / hour. The chromatogram data obtained were compared with the data profile from the mzCloud Library system.

Antibacterial Assay

The antibacterial assay used the well-diffusion method on a petri dish, as shown by Balouiri *et al*. (2016); Alhaddad *et al*., (2019), with modifications. The modification was carried out by adding a 2 g nutrient agar composition with 2 g of bacteriological agar in 100 mL of aquadest as the base medium layer. The well-diffusion method used two media layers: the base media and the seed media layers. The base media was made by dissolving nutrient agar and bacteriological agar, then sterilizing it and pouring it into a petri dish. The seed medium layer was made from 70% nutrient agar in 100 mL of aquadest, then put into a glass tube containing 9 mL and sterilized.

Furthermore, 1 mL of warm seed medium was added to the test bacteria with a density of 1×10^7 colony/mL (bacterial solution compared to the standard McFarland-Hi-media solution). Isolates *Listeria monocytogenes* ATCC 49594, *Salmonella typhimurium* ATCC 13311, and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the Laboratory of Microbiology, Faculty of Medicine, University of Brawijaya Malang, Indonesia. The seed medium that has been added with the test bacteria was vortexed. Then it is poured over the base media layer. After the media hardens slightly, a well-hole was made at a certain distance. Each well was filled with 50 µL of *S. gibberosa* extracts at concentrations of 50, 100, 200, 300, 400 mg/mL, and incubated at 37°C for 24 hours. Ciprofloxacin 10 mg/mL and Pine Oil 1% were used as comparison controls. After that, the zone of inhibition was observed and measured. All experimental measurement data were carried out in three replications and expressed as Mean ± Standard Deviation (n = 3). The results of the inhibition zone measurement evaluated by the minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) using the Bloomfield method (Bloomfield, 1991). The MIC value was determined by plotting the (ln) extract concentration on the x-axis, while the y-axis is the inhibition zone's squared value. The linear regression y = a + bx with the x-axis as the value for Mt. The MIC value is 0.25×Mt and the MBC value is 4×MIC.

Antioxidant Assay

Antioxidant activity was determined using the DPPH free radical scavenging method (Molyneux, 2004; Dewanto *et al*., 2019). A total of 25 mg of *S. gibberosa* extracts was placed in a glass tube. Then, 125 mL of ethanol was added to obtain a 200 µg/mL extract. After that, serial dilutions of 20, 40, 60, 80, 100 µg/mL were made. A 2 mL aliquot of the extract solution of each concentration was added to 2 mL of the 50 µM DPPH solution. The mixture was homogenized and left for 30 minutes in a dark room at room temperature before measuring the free radical absorption at a wavelength of 517 nm with a Spectrophotometer (UV-VIS spectrophotometer T90 + PG Instruments Ltd). The absorbance value of the DPPH solution was also measured and determined by IC_{50} (The half maximal inhibitory concentration). Vitamin E was used as a control. Then, the percentage of inhibition was plotted on the y-axis and the x-axis as a series of dilutions of the extract concentrations to obtain a linear regression equation ($y = a + bx$). IC_{50} was determined as the concentration of the extract solution required to scavenge 50% DPPH free radicals. The assay was carried out in three repetitions, and the measurement results were expressed with a standard deviation. The percentage inhibition of the sample was calculated using the equation:

$$DPPH\ Scavenging\ Effect\ (\%) = \frac{Blank\ Absorbance\ -\ Sample\ Absorbance}{Blank\ Absorbance} \times 100 \quad (1)$$

Total Phenolic Assay

The *S. gibberosa* extracts were evaluated for the total phenol content according to the Folin–Ciocalteu method (Blainski *et al*., 2013; Lamuela-Raventós, 2017; Dewanto *et al*., 2021). The first stage was making a standard curve of gallic acid, which is as much as 25 mg of gallic acid was weighed, then dissolved in EtOH:H_2O (1:1) to a volume of 25 mL. Gallic acid solutions were made in a series of dilution concentrations of 5, 20, 40, 60, 80, and 100 mg/L. From each dilution concentration, 1 mL of gallic acid was taken, and 10 mL of aquadest was added. Then 1 mL of Folin–Ciocalteu reagent (homogenization) was added. After that, let stand for 8 minutes, then add 3 mL of 20% Na_2CO_3 solution (homogenization). Then let stand for 2 hours at room temperature. The absorbance value was measured at a wavelength of 750 nm. The standard curve for gallic acid was prepared with gallic acid (mg/L) with an absorbance value.

In the second stage, 25 mg of *S. gibberosa* extracts dissolved in 25 mL of EtOH:H_2O (1:1) solution. Then from the extract solution, 1 mL was taken, and 10 mL of aquadest + 1 mL of Folin–Ciocalteu reagent (homogenization) was added. After that, let stand for 8 minutes and add 3 mL of 20% Na_2CO_3 (let stand for 2 hours at room temperature). The absorption was then measured with a UV-Vis spectrophotometer at a wavelength of 750 nm, which gives a blue color. The total phenolic was determined using the standard curve regression equation for gallic acid.

RESULT AND DISCUSSION

Based on monomorphic colonies, the soft coral sample used in this study was identified as *Sinularia gibberosa* (presented in Figure 2). Soft coral identification procedures follow directions Fabricus and Alderslade (2001). *S. gibberosa* has low-sized colonies with small protruding protrusions, branching, and colonies covering tens of square meters. *S. gibberosa* has monomorphic retraction and small polyps. *S. gibberosa* has short tentacles, hard lobules, is round and small in size. The surface sclerites of *S. gibberosa* are club-shaped, and the interior sclerites are spindles. The sclerite is a spindle, unbranched, and colorless.

The results of screening the bioactive profile of *S. gibberosa* extracts using LC-HRMS detected 154 peaks (113 compounds), with a retention time range of 0.947 - 26,421 min. However, only 36 peaks (29 compounds) were above 85% quality. The chemical profiles screening of *S. gibberosa* extracts was dominated by fatty acid derivatives, such as arachidonic acid, docosahexaenoic acid ethyl ester, and eicosapentaenoic acid. Soft corals produce more than 5800 secondary metabolites, including fatty acid derivatives with rare and unusual structures (Ermolenko *et al*., 2020). Imbs *et al*. (2007) reported the presence of fatty acid compounds such as arachidonic acid and phthalates from *Sinularia* sp. Characteristic of fatty acid strucrure have a carboxyl and hydroxl group. Previous studies have reported several fatty acid derivatives of *S. gibberosa*, such as sinularolides (Li *et al*., 2005); gibberoketosterols (Ahmed *et al*., 2006); Sinugibberosides (Chen *et al*., 2006); and gibberosenes (Ahmed *et al*., 2008).

Kabara *et al*. (1972); Do Nascimento *et al*. (2014) reported that fatty acid derivatives showed antibacterial and antioxidant activity. Fatty acid derivatives from marine organisms have also been reported to exhibit antibacterial and antioxidant properties (Karthikeyan *et al*., 2014; Liu *et al*., 2019; Alves *et al*., 2020). The screening results for the chemical profiles of *S. gibberosa* extracts with LC-HRMS could be seen in Table 1.

Table 1. Screening of the Chemical Profiles of *Sinularia gibberosa* Extracts with LC-HRMS

No	Chemical Profile	Molecular Formula	RT (min)	Area (%)	Quality	PubChem CID
1	(+/-)-8-Hydroxy-5Z,9E,11Z,14Z,17Z-eicosapentaenoic acid	$C_{20}H_{30}O_3$	16.810	0.30	90.9	16061128
2	4-Methylene-2-octyl-5-oxotetrahydro-3-furancarboxylic acid	$C_{14}H_{22}O_4$	8.905	0.09	90.4	4248455
3	(5Z,8Z,11Z,14Z,16R)-16-Hydroxy-5,8,11,14-icosatetraenoic acid	$C_{20}H_{32}O_3$	17.689	5.64	93.5	9548884
4	19-Nortestosterone	$C_{18}H_{26}O_2$	15.746	0.26	87.2	9904
5	2,3-Dinor-8-epi-prostaglandin F2α	$C_{18}H_{30}O_5$	16.005	0.08	86.7	9548881
6	(10E,12Z)-9-Oxo-10,12-octadecadienoic acid	$C_{18}H_{30}O_3$	18.196	0.15	89.5	9839084
7	7H-Purin-6-amine	$C_5H_5N_5$	1.227	0.58	88.8	190
8	Arachidonic acid	$C_{20}H_{32}O_2$	21.475 22.010	14.13	89.7 88.4	444899
9	Trimethylaminoacetic acid	$C_5H_{12}NO_2$	26.386	0.20	86.7	247
10	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	23.310	0.12	90.8	8343
11	Bis(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	23.310	0.24	85.4	7641
12	Trimethylethanolamine	$C_5H_{13}NO$	1.006 1.221 11.615	22.03	94.1 94.1 87.9	305
13	Dibutyl phthalate	$C_{16}H_{22}O_4$	18.313	0.47	88.8	3026
14	Docosahexaenoic acid ethyl ester	$C_{24}H_{36}O_2$	19.651	1.19	88.6	9831416
15	Eicosapentaenoic acid	$C_{20}H_{30}O_2$	17.486	1.40	93.2	446284
16	Eicosapentaenoic acid methyl ester	$C_{21}H_{32}O_2$	19.871 20.100	0.26	91.3 91.3	6421261
17	Erucamide	$C_{22}H_{43}NO$	22.504	0.13	95.0	5365371
18	Hexadecanamide	$C_{16}H_{33}NO$	22.658	0.41	94.0	69421
19	(3R)-3-hydroxy-4-(trimethylazaniumyl)butanoate	$C_7H_{15}NO_3$	0.967 1.205	10.90	90.6 90.6	10917
20	3-Methylcyclopentadecanone	$C_{16}H_{30}O$	20.877	0.12	85.5	10947
21	Nicotinic acid	$C_6H_5NO_2$	1.445	0.04	85.5	938
22	Octyl decyl phthalate	$C_{26}H_{42}O_4$	23.831 24.085 24.552	4.18	86.8 87.3 88.5	8380
23	Oleamide	$C_{18}H_{35}NO$	22.090	1.43	96.4	5283387
24	Oleoyl ethanolamide	$C_{20}H_{39}NO_2$	22.385	0.14	85.6	5283454
25	Palmitoyl ethanolamide	$C_{18}H_{37}NO_2$	20.995	0.16	87.2	4671
26	Pinolenic acid	$C_{18}H_{30}O_2$	17.473	0.16	92.9	5312495
27	Stearamide	$C_{18}H_{37}NO$	24.644	0.21	87.6	31292
28	Thymine	$C_5H_6N_2O_2$	1.556	0.53	92.0	1135
29	N-Methylnicotinic acid	$C_7H_7NO_2$	0.947	3.63	95.7	5571

Figure 2. *Sinularia gibberosa* Collected from Palu Bay, Central Sulawesi, Indonesia

Evaluation of antibacterial activity was carried out by observing the growth inhibition of *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa*. The inhibition zone diameter of *S. gibberosa* extracts effect can be seen in Figure 3. In comparison, the measurement results of the control inhibition zone diameter are presented in Figure 4.

The *S. gibberosa* extracts showed varying inhibition zone diameters against the growth of *L. monocytogenes*, *S. typhimurium*, and *P. aeruginosa*. According to the inhibition zone category by Paudel *et al*. (2014), there are four categories of antibacterial activity: very strong (inhibition zone ø > 20 mm), strong (inhibition zone, ø 15 - 20 mm), moderate (inhibition zone ø 10 - 15 mm), and weak (inhibition zone ø < 10 mm). Extracts of *S. gibberosa* showed antibacterial potential from very strong to weak against *L. monocytogenes*, *S. typhimurium*, and *P. aeruginosa*, depending on the extract concentration.

Figure 3 showed that the 100 and 200 mg/mL extracts of *S. gibberosa* had moderate antibacterial activity against *S. typhimurium*, whereas against *L. monocytogenes* and *P. aeruginosa* showed weak to moderate antibacterial activity. *S. gibberosa* extracts 300 mg/mL showed moderate to strong antibacterial activity. While the 400 mg/mL *S. gibberosa* extracts showed very strong antibacterial activity against *S. typhimurium* and *P. aeruginosa*, because it was able to form an inhibition zone diameter > 20 mm. While *S. gibberosa* extracts, 400 mg/mL showed strong antibacterial activity against *L. monocytogenes*.

Based on the MIC and MBC, *S. gibberosa* extracts are more effective at inhibiting Gram-negative than Gram-positive bacteria. Compounds with carboxyl groups that are thought to act as antibacterials. The carboxyl group was reported to affect antibacterial activity (Maggi *et al*., 1968; Zi *et al*., 2018). The MIC and MBC evaluation of *S. gibberosa* extracts against three tested bacteria were presented in Table 2.

Previous research reports on the antibacterial potential of the genus *Sinularia*, namely *S. polydactyla* collected from the Red Sea, showed the potential to inhibit *S. aureus* (Afifi *et al*., 2016). *Sinularia* sp. collected from Lampung, Indonesia has the potential to inhibit *Bacillus subtilis*, *E. coli*, *S. aureus*, and *Vibrio eltor* (Putra *et al*., 2016b). *Sinularia* sp. from Bandar Al-Khayran, Oman, can inhibit *B. subtilis*, *E. coli*, *Micrococcus luteus*, *S. aureus*, and *Salmonella* sp. (Dobretsov *et al*., 2015). *S. kavarattiensis* collected by the coast of Mandapam, India, can inhibit *S. aureus* and *Staphylococcus epidermidis* (Rajaram *et al*., 2014). *S. depressa* from Lingshui Bay, China, was reported to inhibit *S. aureus* (Liang *et al*., 2013). *S. gibberosa* collected from Pingtung Beach, Southern Taiwan was reported to exhibit the better inhibitory activity of *B. subtilis* than ampicillin (Lin *et al*., 2013). *S. humilis*, collected from the South China Sea, showed activity against *Bacillus megaterium* (Sun *et al*., 2012).

Assaying for antibacterial activity in this study used Ciprofloxacin 10 mg/mL and Pine Oil 1% as a control (Figure 4). Ciprofloxacin is an antibacterial agent that is very stable and diffuses well on agar media. Antimicrobial activity is influenced by factors such as pH and media composition, the number of inoculums, the stability of antimicrobial compounds, incubation time, and microorganisms' metabolic activity (Jawetz *et al*., 2005). Ciprofloxacin is an antibacterial agent with a spectrum of activity covering most pathogenic bacteria (Jin *et al*., 2019). Ciprofloxacin is effective against Gram-negative bacteria (such as *E. coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Proteus mirabilis*, and *P. aeruginosa*), but less effective against Gram-positive bacteria (such as *S. aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis*) (Marfuati *et al*., 2017). Meanwhile, Fekih et al. (2014); Tillah et al. (2017) reported that pine oil from pine extracts provides antibacterial effects against *L. monocytogenes*, *Enterococcus faecalis*, *P. aeruginosa*, *Acinetobacter baumanii*, *Citrobacter freundii*, *S. aureus* and *K. pneumoniae*.

The *S. gibberosa* extracts were also evaluated for its antioxidant activity using the DPPH radical scavenging method. The DPPH purple light intensity level is proportional to the decrease in the DPPH concentration. This reduction is caused by the reaction of the hydrazine diphenyl-2-picryl molecule with the hydrogen atoms released by the sample molecule components so that the hydrazine diphenyl picrile compound is formed and causes DPPH to change color from purple to yellow (Huliselan *et al*., 2015). Antioxidant activity shows a bioactive substance's ability to inhibit oxidation reactions, which is expressed as a percentage of inhibition (Dewanto *et al*., 2019). In this study, the measurement of the percentage of DPPH radical scavenging from *S. gibberosa* extracts and vitamin E was used as a control comparison, as could be seen in Figure 5.

The *S. gibberosa* extracts was evaluated for the effect of DPPH radical scavenging for IC_{50} determination (Table 3). The *S. gibberosa* extracts showed antioxidant potential because it can donate a hydrogen atom/electron to react with DPPH radicals. According to Blois (1958), there are four categories of antioxidant activity: very strong (IC_{50}<50 µg/mL), strong (IC_{50} between 50-100 µg/mL), moderate (IC_{50} between 100-150 µg/mL), and weak (IC_{50} between 150-200 µg mL). Based on the IC_{50}, *Sinularia gibberosa* extracts was classified as strong antioxidants. Compounds with carboxyl and hydroxyl groups are thought to play a significant role as antioxidants (Chen *et al*., 2020; Godlewska-żyłkiewicz *et al*., 2020). Compounds with carboxyl and hydroxyl groups can act as proton donors and electron acceptors to scavenge and stabilize free radical molecules (Alavi Rafiee *et al*., 2018). The results showed that increasing the concentration of *S. gibberosa* extracts increased the percentage of DPPH radical scavenging (Figure 5).

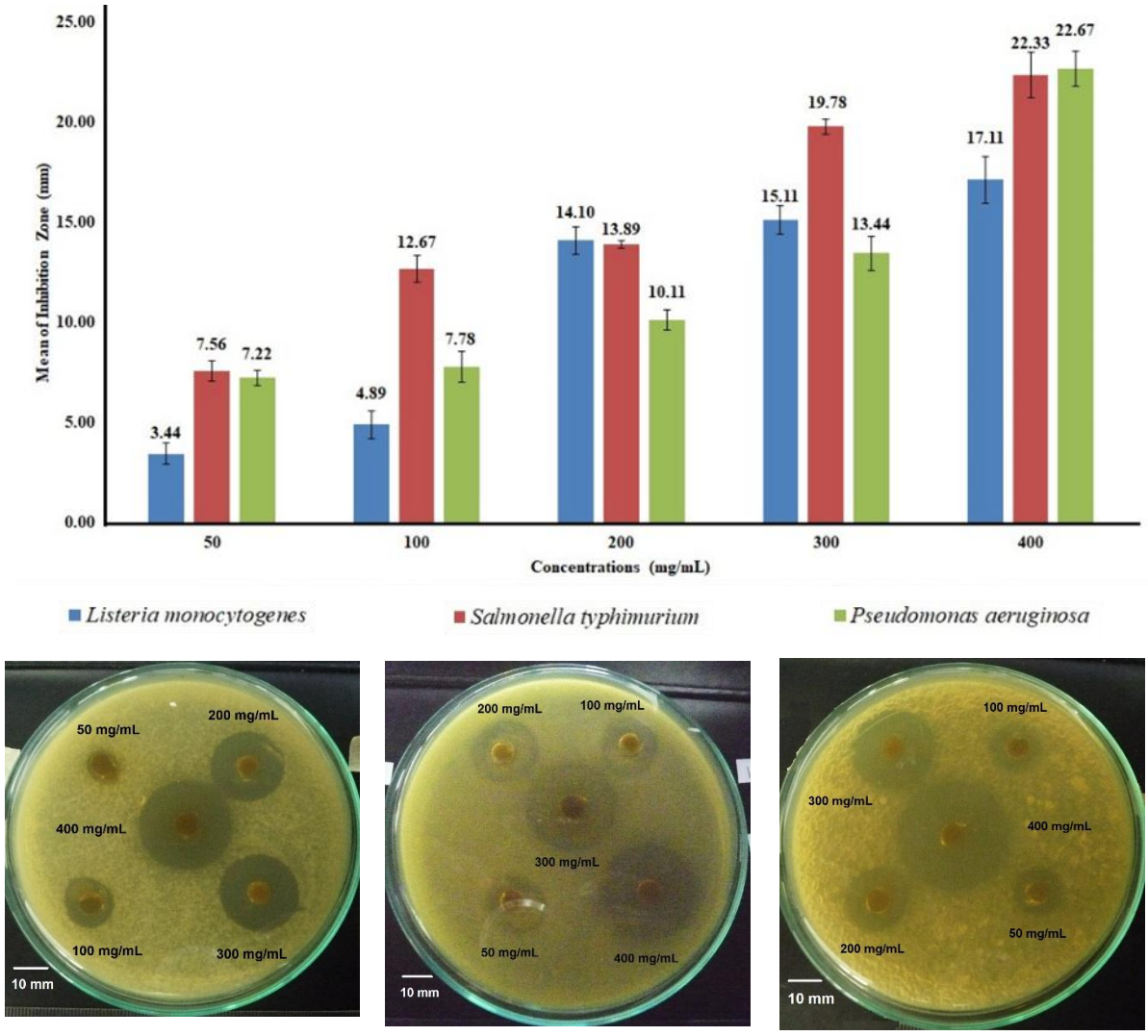

Figure 3. Inhibition Zone of *Sinularia gibberosa* Extracts

Table 2. MIC and MBC of *Sinularia gibberosa* Extracts Against The Tested Bacteria

Bacteria	Regression Equations	R^2	MIC (mg/mL)	MBC (mg/mL)
Listeria monocytogenes	y = 143.87x – 582.91	0.92	1.01	4.05
Salmonella typhimurium	y = 199.33x – 756.93	0.87	0.95	3.80
Pseudomonas aeruginosa	y = 172.89x – 700.22	0.57	1.01	4.05

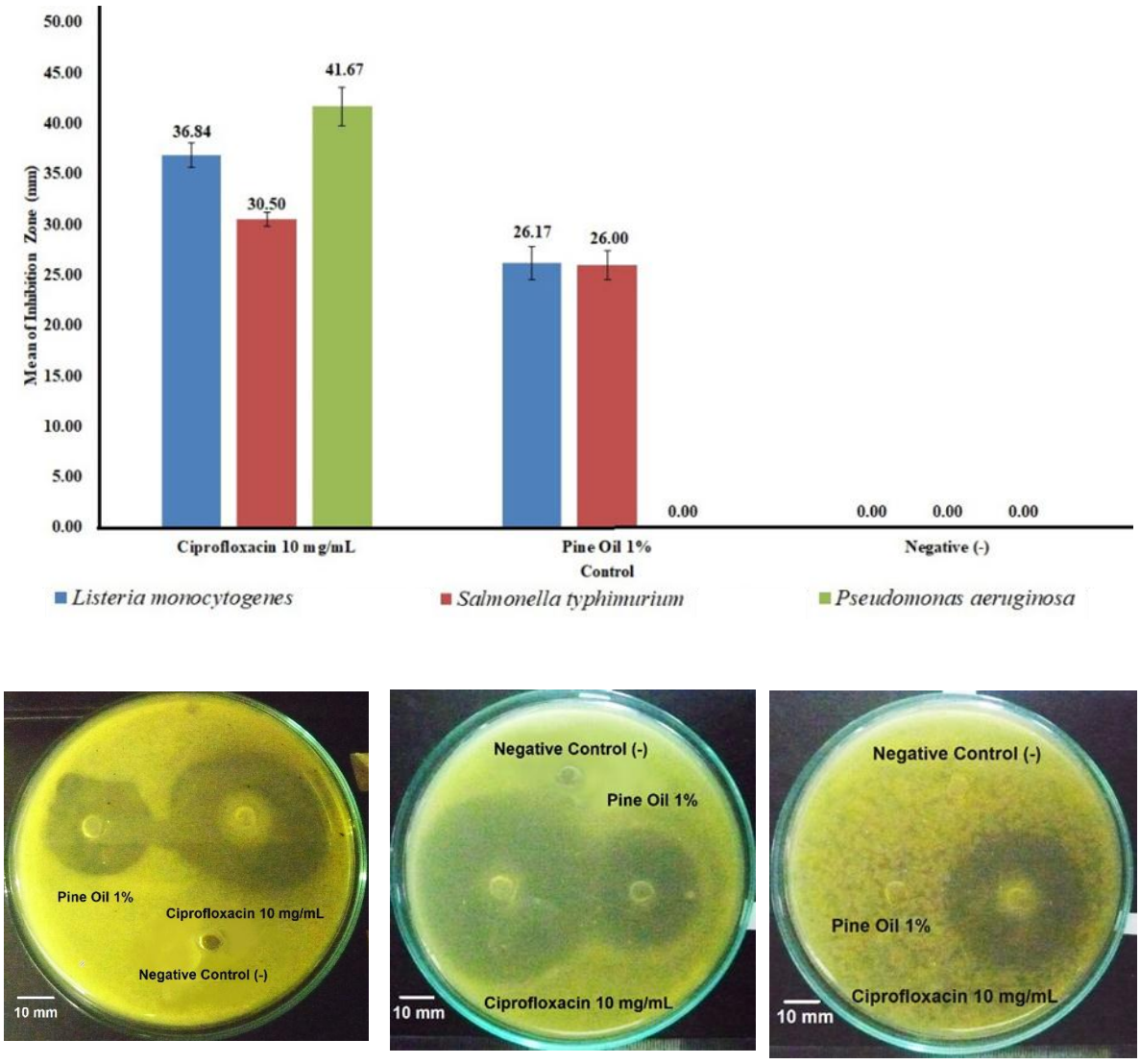

Figure 4. Inhibition zone diameter of positive and negative controls to test bacteria

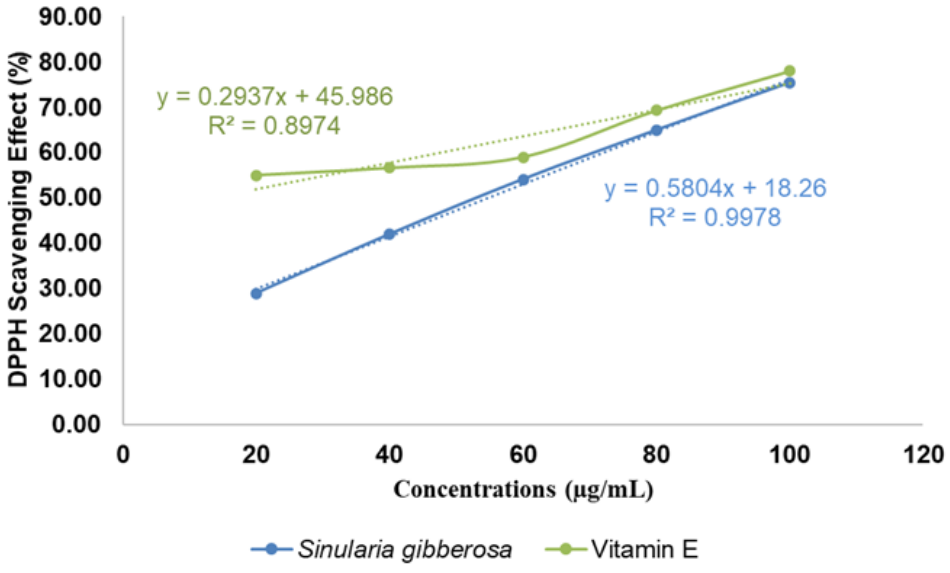

Figure 5. DPPH Scavenging Effect from *Sinularia Gibberosa* Extracts Compared to Vitamin E

Table 3. IC_{50} of *Sinularia gibberosa* extracts compared with Vitamin E.

Samples	IC_{50} (µg/mL)
Sinularia gibberosa	54.69 ± 0.34
Vitamin E	15.87 ± 4.47

Vitamin E, as control, has one hydroxyl group. Vitamin E is a natural antioxidant compound often used as a comparison compound in antioxidant activity assays. This assay showed that vitamin E is a very strong antioxidant compound. From literature studies, the IC_{50} of vitamin E ranges from 8-23 µg/mL, depends on DPPH concentrations (Cheng *et al*., 2013; Melannisa *et al*., 2011; Da'i and Triharman, 2010; Yassa *et al*., 2009; Rohman *et al*., 2007).

The results of total phenol measurement from *S. gibberosa* extracts obtained 5423.76 ± 14.00 mg GAE/25 mg dry extracts. Previous studies have also reported the total phenol content of *S. polydactyla*, *S. variablis*, and *S. compresa* in the range of 47.60 - 63.32 mg/L (Yegdaneh *et al*., 2020). The total phenol content is an indicator to determine the amount of phenolic content in the sample. The phenolic compounds contained in the sample have redox properties, allowing them to act as antioxidants (Johari and Khong, 2019). Phenolic compounds have hydroxyl groups and are responsible for facilitating free radical scavenging (Aryal *et al*., 2019).

Previous research has shown that soft coral extracts from the genus *Sinularia* also showed potential as antioxidants. Twenty-four diterpenoid derivatives isolated from *S. maxima* showed moderate to a strong peroxyl radical scavenging capacity (Thao *et al*., 2015). *Sinularia* sp., which was collected from Pongok Island, Indonesia, detects flavonoid derivative components that can act as antioxidants (Apri *et al*., 2013). Two sesquiterpene compounds were isolated from *Sinularia* sp. collected from the Sanya bay, China (Zhang *et al*., 2006).

Bioactive substances from soft corals are thought to interact with each other to provide antibacterial and antioxidant properties. Bioactive compounds from organisms (including marine organisms) can work synergistically between one compound and another (Merzenich *et al*., 2010). Bioactive compounds can work through multi-compound and multi-target synergistic modes (Long *et al*., 2015). The results of this study proved that *Sinularia gibberosa* soft corals from Palu Bay, Central Sulawesi, Indonesia produced various compounds with antibacterial and antioxidant properties.

CONCLUSION

Based on the results, soft coral *Sinularia gibberosa* from Palu Bay, Central Sulawesi, showed moderate to strong antibacterial activity against the growth of *L. monocytogenes*, *S. typhimurium*, and *P. aeruginosa*. The extracts of *S. gibberosa* are more effective at inhibiting Gram-negative than Gram-positive bacteria. Apart from that, *S. gibberosa* extracts also showed strong antioxidant potential with an IC_{50} of 54.69 ± 0.34 µg/mL The chemical profiles of *S. gibberosa* extracts using LC-HRMS obtained 29 compounds with quality above 85% and was dominated by compounds of fatty acid derivatives. This study indicated that soft corals from Palu Bay, Central Sulawesi, Indonesia, could be potential organisms in discovering and developing antibacterial and antioxidant agents.

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