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Antioxidant Activities and Bioactive Compound in The Extract of *Lobophytum* sp.

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Abstract

This study is a descriptive exploratory research that aims to know the activity of soft coral Lobophytum sp. extract as an antioxidant. Sample extraction was done by maceration using methanol. Viscous extract obtained was then partitioned using n-hexane until gained methanol and n-hexane fraction. The chemical compositions of the methanol extract of Lobophytum sp were investigated using Gas chromatography-Mass spectrometry. The yield result of soft coral Lobophytum sp. extract is 2,59%. The weight of extract partition result were methanol fraction 8,76 g and n-hexane fraction 1,02 g. Antioxidant activity test using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Ascorbic acid was also used as positive antioxidant control. The percentage of inhibition and IC50 were measured. Antioxidant activity test which gains the best antioxidant activity is methanol fraction then followed by n-hexane fraction, both showed DPPH free radical 50% can be inhibited the activity with IC50 rate in a sequence 16,93 ppm and 30,93 ppm. GC-MS analysis, 50 kinds of chemical composition are identified in the Lobophytum sp. The results showed that the soft coral extracts of Lobophytum sp. has antioxidant activity.

Keywords: soft coral *(Lobophytum sp)*, GC-MS, fractions metahol and hexane.

INTRODUCTION

Indonesia is known as one of the countries possessing extensive marine resources. Being located in the equator, with more than 78% areas comprised of shallow water seas, Indonesia has a diverse amount of aquaculture species. Invertebrate species, such as shrimps, sea cucumbers and molluscs, can be harvested from seagrass meadows throughout Indonesia (Hutomo & Moosa, 2005)

Soft coral is a type of macroinvertebrate animal in marine waters. Soft coral is known as a type of coral reef that is found in all marine waters in Indonesia with different diversity. The role of soft corals as part of coral reefs is very diverse, including as a place to live, take refuge, breed various marine life, and also as a biological source of high economic value biological resources. Coral reef is very diverse and, complex marine ecosystem which commonly occur in shallow tropical water (U.S. Coral Reef Task Force Working Group on Ecosystem Science and Conservation, 2000). It has important function and role for marine environment and the life of biota well as for human life. Coral reefs are often characterized as an underwater tropical rain forest with high biodiversity and primary productivity (Hubbell, 1997); (Jaap, 2000) Marine soft corals are known to produce a wide array of secondary metabolites, particularly diterpenoids and steroids, and often characterized by uncommon structural features and potent bioactivities (Putra & Tutik M, 2016). There are more than 20 publications on bioactive compounds of Indonesian soft corals (*Cladiella* sp, *Lobophytum* sp,

Simularia sp) which were reported during 1997–2004. These bioactive compounds showed various pharmacological activities such as antimicrobial, anti-inflammatory and cytotoxicity (Putra and Tutik, 2016). Previously, our study on soft corals of *Sarciphyton trocheliphorum* and *Lobophytum sphave* found numerous secondary metabolites possessing antibacterial and antitumor activities (Al-Footy *et al*., 2016; Zubair *et al*., 2018). In continuing our research on marine organism, particularly from Indonesian marine, our study now starting on soft corals collected off Bulukumba water, South Sulawesi.

RESEARCH METHODS

Sample preparation

In this study, soft coral samples were collected from the water of Bulukumba, Bulukumba Regency, using tools (masks, fins, and snorkels). The sample that has been obtained is put into a heat-resistant plastic bag, then placed in a cool box. The sample was taken to the Biology Laboratory at the Biology Department, Faculty of Mathematics and Natural Sciences, Makassar State University. All samples were cleaned of impurities using running water. Samples that had been cleaned were then cut into small pieces and been weighed. Each sample was labeled and numbered.

Sample Extraction

The extraction of the samples was carried out using the maceration method. After all samples were cleaned, cut into small pieces, and weighed as much as 400 g of fresh weight, the samples were then blended until smooth. The solvent used in the maceration process was methanol p.a 80% as much as 800 ml. Samples that have been macerated for 24 hours are filtered and the concentrated suspension is obtained in a centrifuge for 15 minutes at a speed of 3500 rpm. The concentrated extract obtained was stored in a refrigerator for further bioactivity testing using the GC-MS method (Tulika & Agarwal, 2017) and antioxidant activity using the DPPH method (Brand-Williams *et al*., 1995)

The GC-MS analysis of the extracts was performed using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 60m length, 0.25mm dia. and 0.25mm film thickness Injection Mode: Split, Flow Control Mode: Linear Velocity, Pressure: 173.3 kPa, Linear Velocity: 28.9cm/sec, Purge Flow: 3.0 mL/min, Split Ratio: 10.0. For GC-MS detection [GC-2010], an electron ionization system with ionization energy of 70eV was used. Helieum gas (99.99%) was used as a carrier gas at a constant flow rate- total flow: 16.3 mL/min. and column flow: 1.21 mL/min. Injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed (Column Oven Temp.: 100.0°C and Injection Temp.: 270.00°C) from 70 to 220°C at 10°C/min, held isothermal for 1min and finally raised to 300°C AT 10°C/min. 2ml of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass scan of 50-600 amu. Total running time of GC-MS is 48 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass. The relative percentage of each extract constituents was expressed as percentage with peak area normalization.

Antioxidant Activity

Preparation of DPPH solution, DPPH solution with a concentration of 0.05 μM was prepared by weighing 1.97 mg of DPPH powder dissolved with 100 ml pro-analysis ethanol.

Preparation of sample solutions. The 2.5 mg soft coral sample was dissolved in 2.5 ml of p.a ethanol and then homogenized as a stock solution. The stock solution was made with various concentrations of 90, 70, 50, 30, and 10 ppm.

Measurement of antioxidant power Measurement of antioxidant power blank. The test was carried out by pipetting 1.0 ml of DPPH added by 1.0 ml of ethanol p.a. the solution was vortexed and incubated for 30 minutes at 37oC in a dark room. The absorbance of the blank solution was measured using a spectrophotometer (λ 515 nm).

Measurement of antioxidant power of soft coral methanol extract. Tests were carried out by pipetting 1.0 ml of sample solution from various concentrations (90 ppm, 70 ppm, 50 ppm, 30 and 10 ppm). Each sample solution was added 1.0 ml of DPPH. Sample solution was vortexed and incubated for 30 minutes at 37oC in a dark room. The absorbance of the sample solution was measured using a spectrophotometer (λ 515 nm).

RESULTS AND DISCUSSION

The comparison of mass spectra of the contituents with NIST library, nineteen peaks were obtained; all the constituents were characterized and identified (Table 1). The retention time (RT) are in minutes.

Table 1. Components identified in the methanolic extract of *Lobophytum sp* GC-MS

No	R. Time	Compound Name	Molecular Formula	Molecular weight	Peak area (%)
1	17.933	Hexadecanoic acid, methyl ester	C17H34O2	270	0.20
2	18.179	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methyle	C20H32	272	0.17
3	18.374	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methyle)	C20H32	272	0.24
4	18.882	3-Oxatricyclo[20.8.0.0(7,16)]triaconta-1(22),7(16),9,13,23,29-hexaene	C29H42O	406	0.19
5	19.042	Methyl n-hexadecyl ketone	C18H36O	268	0.13
6	19.231	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-meth)	C15H24	204	2.46
7	19.647	Cedren-13-ol, 8-	C15H24O	220	0.18
8	19.881	2,7,11-Cyclotetradecatrien-1-ol, 1,7,11-trimethyl-4-(1-methyleth)	C20H34O	290	0.94
9	20.102	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	C21H34O2	318	0.37
10	20.279	6-(p-Tolyl)-2-methyl-2-heptenol	C15H22O	218	0.86
11	20.410	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	306	0.29
12	20.553	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-			0.53
13	20.809	10-12-Pentacosadiynoic acid			0.70
14	21.225	Cedren-13-ol, 8-	C15H24O	220	1.27
15	21.332	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	306	5.19
16	21.425	Bicyclo[3.1.1]hept-2-ene, 2,2'-(1,2-ethanediyl)bis[6,6-dimethyl-	C20H30	270	0.43
17	21.541	1-Methyl-3-(2,6,6-trimethyl-1-cyclohexen-1-yl) propyl acetate	C15H26O2	238	0.50
18	21.688	3-Carene, 4-isopropenyl-	C13H20	176	0.53
19	21.824	1-Heptatriacotanol	C37H76O	536	0.39
20	21.877	Spiro[4.4]nona-1,3-diene, 1,2-dimethyl-	C11H16	148	0.34
21	21.946	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	306	0.72
22	22.133	5-(7A- isopropenyl-4,5-dimethyl-octahydro-inden-4-yl)-3-methyl-	C20H32O	288	0.41

23	22.193	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	306	0.59
24	22.365	Cedren-13-ol, 8-	C15H24O	220	9.18
25	22.578	Cedren-13-ol, 8-	C15H24O	220	2.39
26	22.657	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethlidene)-,	C15H22O	218	1.67
27	22.762	Corymbolone	C15H24O2	236	0.70
28	22.856	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3.beta.,4.alpha.,5.alpha.)-	C32H52O2	468	0.49
29	22.997	2(3H)-Furanone, 3-(2-(decahydro-6-hydroxy-5-(hydroxymethyl)-5	C20H30O5	350	0.69
30	23.189	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	C15H24	204	1.15
31	23.369	2-heptanone, 6-methyl-6-[3-methyl-3-(1-methylethenyl)-1-cyclo	C15H240	220	0.29
32	23.492	7-(5-Hexynyl)tricyclo[4.2.2.0~2,5~]dec-7-ene	C16H22	214	0.19
33	23.671	Cedren-13-ol, 8-	C15H24O	220	1.19
34	23.819	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one	C15H22O	218	4.43
35	23.914	Longiverbenone	C15H22O	218	1.99
36	24.035	2,6,6,11-Tetramethyltricyclo[5.4.0.0~2,8~]undec-10-en-9-one	C15H22O	218	1.44
37	24.452	2-Propenal, 3-(2,4,5,6,7,7A-hexahydro-3,7-dimethyl-1h-inden-4-yl)-2	C15H22O	218	1.08
38	24.613	d-Norandrostane (5.alpha.,14.alpha.)	C18H30	246	1.54
39	24.684	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-di	C15H25I	332	2.03
40	24.962	d-Norandrostane (5.alpha.,14.alpha.)	C18H30	246	35.61
41	25.190	d-Norandrostane (5.alpha.,14.alpha.)	C18H30	246	3.27
42	25.304	6-Isopropenyl-4,8A-dimethyl-1,2,3,5,6,7,8,8A-octahydro-2,3-naphth	C15H24O2	236	4.99
43	25.429	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-di	C15H25I	332	0.67
44	25.550	Longifolenaldehyde	C15H24O	220	0.19
45	25.644	6-Isopropenyl-4,8A-dimethyl-1,2,3,5,6,7,8,8A-octahydro-2,3-naphth	C15H24O2	236	0.84
46	26.350	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	C20H34O2	306	0.25
47	26.819	Pentacyclo[9.1.0.0(2,4).0(5,7).0(8,10)]dodecane, 3,3,6,6,9,9,12,12-octamethyl-, anti,ant	C20H32	272	0.20
48	37.782	Tetradecanoic acid, hexadecyl ester	C30H60O2	452	2.94
49	39.225	26,26-Dimethyl-5,24(28)-ergostadien-3.beta.-ol	C30H50O	426	0.72
50	39.415	Ergost-5-en-3-ol, (3.beta.,24R)-	C28H48O	400	2.21

Table 2. Activity of Bioactive compound identified in the extracts of *Lobophytum sp.*

Sr. No	Name of compound	Activity	Reference
1	d-Norandrostane (5.alpha.,14.alpha.)	Anabolic-androgenic steroids	Zöllner *et al.*, 2009
2	Cedren-13-ol, 8-	sex pheromones and antimicrobial	Zhao *et al.*, 2018; Amri *et al.*, 2017
3	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one	Antimicrobial, anti-inflammatory, antihyperlipidemic, antioxidant	Pressy P *et al.*, 2015
4	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylethlidene)-,	Antimicrobial, antioxidant, deterrent effects against herbivores, insecticidal activity against mosquitoes,	Pressy P *et al.*, 2015

		antibacterial	
5	Hexadecanoic acid, methyl ester	Antioxidant, Pesticide, Flavor, 5-Alpha reductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide and Antialopecic	Ponnamma & Manjunath , 2012
6	Corymbolone	Antioxidant, antimicrobial activity	Azzaz *et al*., 2014

Antioxidant activity of soft coral extracts of *Lobophytum* sp The methanol fraction and n-hexane fraction were tested quantitatively using the DPPH (2,2-diphenyl-1-picrylhidrazyl) method. The results of testing for antioxidant activity can be seen in Table 2.

Table 3. Test results fot the percentage of antioxidant activity and the IC50 value on the soft coral fraction (*Lobophytum* sp)

Sample	Concentration (ppm)	% antioxidant activity	Regression Equation	IC50 (ppm)
Methanol fraction soft coral extract	10	17.249	Y= 2.018x + 15.829 R2= 0.9148	16,933
	30	21.139		
	50	21.759		
	70	22.717		
	90	26.550		
N-hexane fraction of soft coral exctract	10	7.553	Y= 1.4431x + 5.366 R2= 0.9151	30.929
	30	7.779		
	50	9.188		
	70	10.598		
	90	13.360		

Gas chromatography and mass spectrometry GC-Mass is an important method to identify chemical compositions of organism which is applied by most recent researches for identification of important compounds. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula (Ghaidaa et al., 2016). The fatty acid constituents were identified by GC/MS analysis; and the results revealed the presence of hexadecanoic acid methyl ester, d-Norandrostane (5.alpha.,14.alpha.) as major unsaturated fatty acid.

Soft corals of the genus Lobophytum, a marine invertebrate of the subclass Alcyonaria, is rich source of diterpenes, lipids, sesquiterpenes and hydroxylated steroids. Cembrane diterpenes previously isolated from Lobophytum species; have shown diverse biological activities as ichthyotoxic, cytotoxic, antiarthritic, antiinflammatory, antibacterial and Ca-antagonist. A sample of *Lobophytum pauciflorum* was collected for chemical investigation and for discovering bioactive substances from the Red Sea marine organisms. Bioassay guided fractionation of the bio-active fractions resulted in isolation and characterization of two bio-active metabolites (nephthenol and gorgost-5-ene-3β-ol) together with other four compounds (heptadecan-1-ol, palmitic acid, stearic acid and batilol) from the dichloromethane and ethyl acetate soluble successive fractions (Hassan *et al*., 2016).

The test results showed that the percentage of antioxidant activity tended to increase with increasing sample concentration. Based on the percentage value (%) of antioxidant activity of soft coral extract, a regression equation has a good correlation coefficient, that is close to 1, meaning that the equation obtained is linear. From this equation the IC50 value is also o btained.

Based on the results obtained, the highest antioxidant activity was found in the methanol fraction followed by the n-hexane fraction, both of which showed that 50% DPPH free radical activity could be inhibited with IC50 values of 16.933 ppm and 30.929 ppm, respectively.

The results of antioxidant activity indicated that the methanol fraction and n-hexane fraction had a greater percentage (%) value of antioxidant activity or tended to increase with increasing solution concentration. This is in accordance with the research of (Mu'nisa *et al*., 2017), the percentage value of antioxidant activity increases with increasing sample concentration due to more antioxidant compounds in the sample that inhibit free radicals. The percentage of inhibition against free radical activity increases with increasing extract concentration (Molyneux, 2004)

The percentage value of antioxidant activity obtained was used to calculate the IC50 value. The IC50 value is obtained from the linear regression equation which states the relationship between the concentration of the test extract as the x-axis and the percentage value of antioxidants as the y-axis (Mu'nisa *et al*., 2017). The IC50 value is the value of the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. The smaller the IC50, the more active the test extract is as a DPPH radical scavenger or antioxidant compound Test of antioxidant activity on soft corals *Lobophytum sp*. Show that the active compounds contained in the soft coral fraction have potential as an antioxidant.

CONCLUSION

Based on the results of the study, it can be concluded that the *Lobophytum* sp extract from the GC-MS test results contains a number of chemical compounds including d-Norandrostane (5.alpha., 14.alpha., Cedren-13-ol, 8-, Tetradecanoic acid, hexadecyl ester, 6-Isopropenyl-4,8 A-dimethyl-1,2,3,5,6,7,8,8A-octahydro-2,3-naphth, 8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo [5.1.0]oct-5-en-2-one and 4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)- *Lobophytum* sp. extract has high antioxidant activity with IC50 values of 16.933 and 30.929, and methanol extract has higher antioxidant activity than n-hexan.

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