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ISOLATION AND BIOLOGICAL ACTIVITY OF THE GORGONIAN EXTRACT FROM MALUKU

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ABSTRACT

Isolation and biological activity of gorgonians from the Maluku Sea have been carried out. Isolation using n-hexane: ethanol (1:1) solvent resulted in 3 crude extracts from all parts (HEL), axial (HEA), and skin (HEK) of gorgonians. Analyzed crude extracts using LC-MS/MS and tested biological activity with an evaluation of the antioxidant and antibacterial activity. The compounds obtained from the gorgonian extract were derived from sesquiterpenes, phenol lipids, terpenoids, aldehydes, and steroid groups. An antioxidant test assessed DPPH radical-scavenging activity with results between 31,688 - 40,916 %. In addition, the antibacterial activity was tested by disc diffusion against two human pathogenic bacteria *Escherichia coli* IFO 3301*, Salmonella typhimurium* IFO 12529*, Bacillus subtilis* IFO 13719*,* and *Staphylococcus aureus* IFO 13276. All extracts showed positive results in most of the tests.

Keywords: Antibacterial, Antioxidant, Crude Extracts, Gorgonian, LC-MS/MS.

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INTRODUCTION

Natural products from land have been widely processed as essential ingredients in the pharmaceutical, cosmetic, and drug industries. This is due to the large number of synthesized drugs used to cure a disease which has adverse effects on human health. Natural compounds (secondary metabolites) are the best sources of prospective therapeutic leads.[1] However, the continuous use of natural materials from land causes the depletion of these resources. Pharmaceutical companies started to understand that the ocean, which makes up 70% of the planet's surface, would have a distinctive biodiversity and be a source for prospective drug candidates.[2] Therefore, a breakthrough is needed to find new sources of abundant marine biota to provide maximum benefits for the community. Researchers began to divert research looking for new natural products from land to the sea. Various studies have shown that bioactive compounds from marine biota act as medicinal raw materials. Some marine animals and plants contain active compounds in their tissues that can be isolated and extracted as new drug-candidate bioactive compounds. Gorgonian corals are distinguished by their fan-like form, which offers other marine life food and protection.[3,4] Marine invertebrate species known as gorgonians contain various bioactive compounds, including antibacterial, antifungal, cytotoxic, and immunostimulant compounds.[5] Secondary metabolites produced by gorgonian corals are needed to survive and maintain their stability. This is because, unlike hard corals, gorgonians do not have a $CaCO_3$ framework for self-protection.[3] Secondary metabolites that are used as bioactive drugs have problems in the form of supply problems.[6] Therefore, it is crucial to conduct scientific research in the laboratory to obtain secondary metabolites found in gorgonians and to characterize these compounds in their development as medicinal ingredients in the pharmaceutical field that are beneficial to humans. The solvent utilized significantly impacts how successfully marine organisms are extracted. Five zoanthoxanthin alkaloid compounds were isolated from the gorgonian *Echinogorgia pseudossapo* using ethanol and chloromethane as solvents.[7] The study by Sun *et al.*[8] stated that six new tetraprenylated alkaloid compounds of the same gorgonian species were found with the same solvent. The secondary flavonoid

metabolites detected were thought to come from the color of the colonies on the gorgonian. Colony color is influenced by the pigment content of zooxanthella unicellular algae that live in symbiosis in their coenzyme tissue.[9] Secondary metabolites of phenol hydroquinone were detected in the gorgonian extract using n-hexane and methanol as solvents; this shows that phenol hydroquinone is soluble in polar and non-polar solvents when compared to semi-polar solvents such as ethyl acetate. The research of Cheng *et al.*[10] shows that soft coral *Sinularia capillosa* contains capilloquinol with extraction using acetone. Steroid secondary metabolites were detected in all species of gorgonian extracts with various solvents. The results of other studies reported that secondary steroid metabolites were also produced using acetone solvent from various soft corals, including *Sarcophyton* sp. and *Nephthea chabrolii.*[11] Triterpenoids were detected in the ethyl acetate, n-hexane, and methanol extracts of the skin and the axial gorgonian extract. The research of Chung *et al.*[12,13] stated that *Rumphella antipathies* from a mixture of methanol and dichloromethane solvents also found triterpenoid compounds from the sesquiterpenoid group, namely rumphellclovanes C-E of and 4,5-seco-caryophyllane namely rumphellaones B and C. Three new triterpene glycosides from the sponge *Ectyoplasia ferox* using methanol and dichloromethane as solvents.[14] In this study, we investigated the bioactive compounds of gorgonians found in the waters of Maluku, Indonesia. Extracts of organic compounds obtained were then analyzed for their biological potential for antioxidants and antibacterial against pathogens.

EXPERIMENTAL

Material and Equipment

A sampling of gorgonians was carried out at 3°30'10.2"S 128°19'25.5"E, Liang waters, Maluku, Indonesia, by diving in May 2022. The samples were put in a cool box, taken to the laboratory, and stored at -20 °C until the test was carried out. For column chromatography, the materials employed in isolation were n-hexane P. A Merck, ethanol P.A Merck, and Silica gel 60 Merck (0.063-0.200 mm). Structure elucidation was carried out using LC-MS/MS (The Waters Xevo TQD).

Extraction of Secondary Metabolites

The extracted gorgonian consisted of whole tissue (HEL), axial (HEA), and outer skin (HEK) sections. Gorgonian powder (about 100 g dry weight) and axial (at least 50 g dry weight) were macerated with n-hexane: ethanol (1:1) for 72 hours. The macerate is filtered and the filtrate is concentrated with a rotary vacuum evaporator at 55 ºC with pressure. The crude extract of gorgonian was weighed and the yield was calculated.

Identification of the Isolated Compound

Analysis with LCMS/MS with the condition: On a UPLC BEH C18 column (50 mm x 2.1 mm x 1.7 m), HPLC separation was carried out. Two solvents made up the mobile phase: solvent (A) was water with 0.1% formic acid and solvent (B) was CH_3CN with 0.1% formic acid. Conditions for mass spectrometry: The mass analyzer scanned the m/z range between 50 and 1200 amu. The ionization parameters were as follows: positive ion mode, capillary voltage 3000 V, cone voltage 30 V. By comparing experimental spectra to spectrum libraries, suspected compounds dereplication was performed using MS/MS data.

Assessment of Antioxidant Activity

The gorgonian extracts HEL, HEA, and HEK were tested for antioxidant activity in vitro by using the DPPH radical (2,2-diphenyl1-picrylhydrazyl). The gorgonian extract was dissolved in methanol to obtain a 500 µg/mL concentration. Afterward, 500 µL of the extract was mixed with DPPH (500 µL, final concentration: 0.01 mM) and incubated for 30 min in the dark. Absorbance measurements were carried out at 550 nm with methanol as a blank. As the test standard, ascorbic acid was dissolved in methanol at a concentration of 500 µg/mL, while DPPH and methanol solution were used as negative controls. The formula calculates the calculation of the percentage of DPPH inhibition percentage:

$$\%\ inhibition = 100\ x\ (1 - \frac{AS}{AC})$$

Where AC represents the control absorbance value and AS is the sample absorbance.

Assessment of Antibacterial Activity

Four pathogens (*Escherichia coli* IFO 3301*, Salmonella typhimurium* IFO 12529*, Bacillus subtilis* IFO 13719*,* and *Staphylococcus aureus* IFO 13276) were evaluated for the antibacterial potential of HEL, HEA, and HEK extract using the disc diffusion method. Pathogenic bacteria were grown in a nutrient broth (NB) medium at 35 °C for 24 hours. Then 100 µl (10^8 CFU/ml - McFarland 0.5) of the test bacteria were spread on Mueller Hinton Agar medium on a Petri dish. Discs measuring 6 mm (Whatman) were immersed in 300 mg gorgonian extracts which had been dissolved in methanol and evaporated to dryness. After that, the disc was placed on the MHA medium, which had been planted with the test bacteria. Petri dishes were incubated at 35 °C, and the zone of inhibition was measured after 24 hours. In this test, gentamicin 50 mg was used as a positive control. This test was repeated three times for each sample.

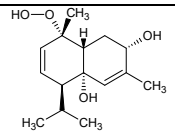
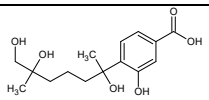
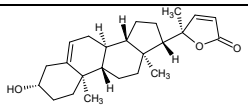
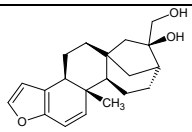
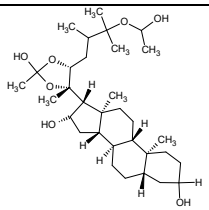
RESULTS AND DISCUSSION

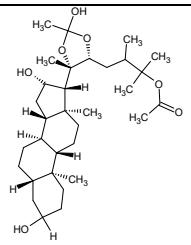
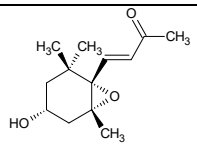
Isolation and identification of bioactive compounds in coral are important to determine the potential of secondary metabolites. In previous studies, various compounds in gorgonians have been reported, including the group of alkaloids, flavonoids, saponins, terpenoids, polyphenols, steroids, and tannins. It has been found that the gorgonian extracts in methanol, ethanol, ethyl acetate, and n-hexane all contain these secondary metabolites.[14,15]

Isolation and Identification Compounds as Crude Extract Gorgonian

The results of the extraction of gorgonian (HEL) using n-hexane: ethanol (1:1) as solvent was tested using LC-MS/MS can be seen in Table-1. The results showed that seven bioactive compounds were found in the extract with mass 224.3711, 268.3487, 298.5436, 370.5261, 314.7307, 551.0091, and 552.9449, which came from the class of compounds sesquiterpene, phenol lipids, steroid, and terpenoid.

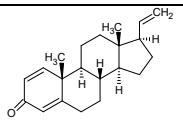
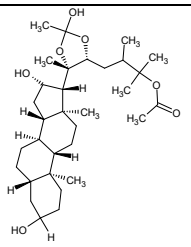
Table-1: Compound Identified by the LC-MS/MS in HEL Extract

	Compound name	Structure	Class	Exact Mass
1	(2*S*,4a*S*,5*S*,8*S*,8a*R*)-8-hydroperoxy-3,8-dimethyl-5-(1-methylethyl)-1,5,8,8a-tetrahydronaphthalene-2,4a(2*H*)-diol (heterofusceterpene)		Sesquiterpene	268.3487
2	3-hydroxy-4-(1,5,6-trihydroxy-1,5-dimethylhexyl)benzoic acid		Phenol lipids	298.5436
3	3β,20R-dihydroxycholest-5,22-dien-24-oic acid γ-lactone		Steroids	370.5261
4	(3bS,5aS,7R,8R,10aR,10bS)-3b,4,5,6,7,8,9,10,10a,10b-Decahydro-7-hydroxy-10b-methyl-5a,8-Methano-5aH-cyclohepta(5,6)naphtho(2,1-b)furan-7-methanol		Terpenoids	314.7307
5	Hippurinsterol		Steroids	552.9449

6	ortho-hippurinsterol		Steroids	551.0091
7	3β-hydroxy-5α,6α-epoxy-7-megastigmen-9-one		Hydroperoxy steroids	224.3711

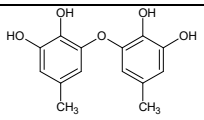
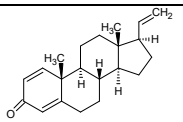
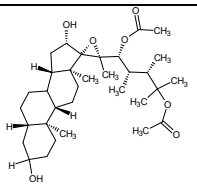
Axial extraction from gorgonian (HEA) using n-hexane: ethanol (1:1) as the solvent can be seen in Table-2. In the HEA extract, only two compounds were found from the steroid group, namely pregna-1,4,20-trien-3-one and ortho-hippurinsterol, which were also found in the HEK extract.

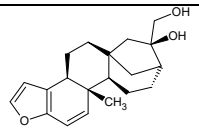
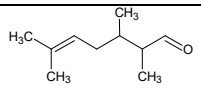
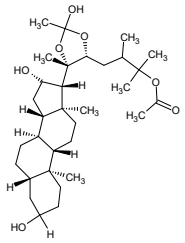
Table-2: Compound Identified by the LC-MS/MS in HEA Extract

	Compound name	Structure	Class	Exact Mass
1	pregna-1,4,20-trien-3-one		Steroids	296.4490
2	ortho-hippurinsterol		Steroids	551.0091

The identification of compounds in the HEK extract showed the presence of compounds from steroids, terpenoids, aldehydes, and violaceol groups (Table-3).

Table-3: Compound Identified by the LC-MS/MS in HEK Extract

	Compound name	Structure	Class	Exact Mass
1	3,3'-oxybis(5-methylbenzene-1,2-diol)		Violaceol-I	262.2886
2	pregna-1,4,20-trien-3-one		Steroids	296.4490
3	Hippuristerol		Steroids	485.7853

4	Kahweol		Terpenoids	314.6617
5	2,3,6-trimethylhept-5-enal		Aldehydes	154.4352
6	ortho-hippurinsterol		Steroids	551.0980

Based on the study's results, the compounds obtained from the gorgonian extract were derived from sesquiterpenes, phenol lipids, terpenoids, aldehydes, and steroids. Compounds from the steroid group dominated and ortho-hippurinsterol was found in all analyzed extracts. Previous studies have reported the presence of compounds from the sesquiterpene group[16] and aldehydes[17], while Matulja *et al.*[3] reported the presence of phenol lipid compounds and terpenoids found in gorgonians. Compounds from the steroid group were also found in gorgonian extracts.[16]

Evaluation of Antioxidant Activity

The study results shown in Fig.-1 show that the HEL, HEL, and HEK extracts have antioxidant activity tested through DPPH inhibition, with the highest antioxidant activity in the HEL extract.

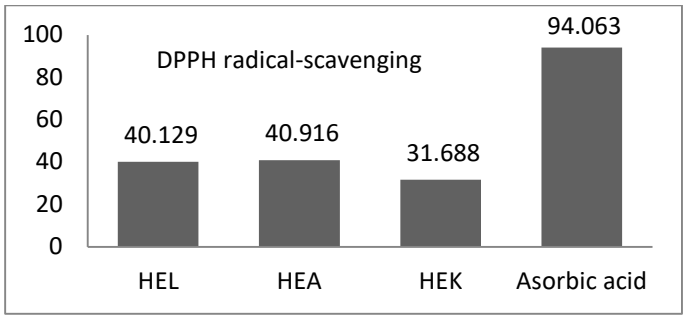


Fig.-1: Antioxidant Activity from HEL, HEA, and HEK Extracts

Previous studies have shown that gorgonian *Eunicella singularis* has a high phenolic content, so the more polar fraction correlates with high DPPH radical scavenging.[18,19] In addition, the carotene content can also be a determinant of the antioxidant activity of high carotenoids (i.e., astaxanthin).[20,21] Research conducted by Shahbudin *et al.*[22] showed that when organisms carry out an inflammatory response, they release non-enzymatic free radical scavengers to counter their side effects, including some gorgonian and other soft coral species. Additionally, during temperature variations linked to environmental changes, corals show increased antioxidant potential.[23] Although there have been many studies on the biological potential of secondary metabolites of gorgonian and other soft corals, their antioxidant potential has not been widely reported.[22,24]

Evaluation of Antibacterial Activity

The diffusion disc assay was used to test the antibacterial activity against the following four bacteria: *Escherichia coli* IFO 3301*, Salmonella typhimurium* IFO 12529*, Bacillus subtilis* IFO 13719*,* and

Staphylococcus aureus IFO 13276. Table-4 summarizes the findings, confirming the inhibition of all tested bacterial species, with *E. coli* showing high inhibition.

Table-4: Antibacterial Activity from HEL, HEA, and HEK Extracts

Samples	Inhibition activity (mm)			
	E. coli	*S. typhimurium*	*B. subtilis*	*S. aureus*
HEL	3	1	0	1
HEA	2.5	1	0	0
HEK	3	3	1	1
Gentamicin	17	17	17	16

Gorgonians have already been shown to be a rich source of secondary metabolites with various biological functions. Terpenoids and steroids had anticancer, anti-inflammatory, antibacterial, antimalarial, and antifoulant activities, making up most of the identified bioactive chemicals.[25] This study has added information about the potential of Indonesian gorgonians as a source of antimicrobials and antioxidants. The search for bioactive compounds continues to be needed to answer the need for new, more promising medicinal raw materials. These organic compounds, which were extracted from marine gorgonian corals, are a valuable source of biologically active substances with important medical applications.

CONCLUSION

This study presents the results of gorgonians' isolation, identification, and biological potential from Maluku, Indonesia. Isolation of gorgonian resulted in 3 crude extracts HEL, HEA, and HEK, which were identified using LC-MS/MS. An antioxidant test on crude extract was carried out by assessing the radical scavenging activity of DPPH with results between 31.688 - 40.916%. Antibacterial activity was tested by disc diffusion against four bacteria: *Escherichia coli* IFO 3301*, Salmonella typhimurium* IFO 12529*, Bacillus subtilis* IFO 13719*,* and *Staphylococcus aureus* IFO 13276. All extracts showed positive results in most of the tests. In order to increase the biological potential, further purification, isolation, and characterization of pure compounds from the most active fractions are indeed being researched.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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