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Original Research Article

Anti-UV, SPF, and Antibacterial Activities of Gorgonian *Mopsella* cf. *aurantia*

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ABSTRACT

Gorgonia is one of the marine organisms with bioactive potential that has yet to be widely exploited. This research aimed to determine the anti-UV, the Sun Protection Factor (SPF), and antibacterial properties of the extract and fractions of gorgonian *Mopsella* cf. *aurantia*. The extraction was carried out using ethyl acetate as a solvent. The anti-UV and SPF determinations were carried out by ultraviolet spectrophotometry, while the antibacterial property was carried out using the modified Kirby-Bauer method. The result shows that the extract has anti-UVB activity and obtained an SPF value of 7.71 at a concentration of 250 ppm, which is categorized as a minimal level of protection capability. Further bioactivity testing of this gorgonian shows robust activity against Gram-negative *Escherichia coli* DSM498 and Gram-positive *Bacillus megaterium* DSM32T strains. The antimicrobial and anti-UV effects in the related species were located in the ethanol fraction.

***Keywords*:** Gorgonian, *Mopsella* cf. *aurantia*, antibacterial, anti-UV, SPF, *Escherichia coli* DSM498, *Bacillus megaterium* DSM32T

Introduction

Coral reefs are typical tropical ecosystems with distribution centers in the Indo-Pacific region. Nearly 800 species of coral have been identified from the Scleractinia group. Of these, 600 species are found in Southeast Asia, especially Indonesia and the Philippines, so biogeographically, this area is declared the center of coral distribution globally.[1]

Due to the very high biodiversity, there is competition between species for survival.[2] Such conditions make various types of marine biota synthesize toxic secondary metabolites (bioactive compounds) for self-defense against bacterial, fungal, and viral infections,[3-5] against predation and competition between themselves to maintain their growth area.[6, 7]

Several marine resources, such as gorgonians (sea fans), are significant to research. Gorgonia is a biota component of the coral-reef ecosystem with untapped bioactive potential. Some gorgonians with antibacterial, antifouling, and other properties are often composed of lipids, steroids, and terpenes.[8] Defensive compounds have been identified origin of gorgonian species from around the world, including in the Caribbean,[9, 10] the Pacific,[11] the North Atlantic,[12] and the South Atlantic.[13]

The demand for innovative drugs to assist humans in fighting various ailments is never-ending. Bacterial resistance, viral infections (e.g., Dengue Hemorrhagic Fever and SAR-COV-2),[5] a sharply increasing incidence of fungal infections, various types of tumors,[7] parasitic infections (e.g., malaria), and various problems in patients with transplanted organs in today's world population as a result of our inability to address not only health problems, [14-17] but also the agricultural sector.[18]

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Biodiversity in a biosphere describes the diversity of chemical content in it. Tropical waters, like Indonesia, are excellent examples of this environment. In coral-reef ecosystems, for example, the resistance of sessile organisms against competitors, pathogens, and predation is quite significant, resources are abundant, but natural pressures are very high. Sessile organisms such as sponges and corals may be the source of new structural compounds with engaging biological activities.

Gorgonians have a settled adult life phase and are firmly attached to the rigid substrate. The body is a polyp with eight pinned tentacles followed by eight mesenteries, namely soft tissue in the form of septa that hang and divide the cavity in the body into eight parts. Its allelopathic properties make this organism produce compounds to fight competitors in the space around its habitat. The presence of bioactive metabolites in sessile organisms such as gorgonians also reflects the ecological adaptations formed during a long evolutionary process as a defense mechanism.

Gorgonia that live in tropical waters such as Indonesia has promising potential as a source of new bioactive compounds to be developed into commodities of high economic value.[6] Gorgonians are rich in bioactive metabolites with anti-UV, cytotoxic, antitumor, and antimicrobial activities that have attracted the interest of researchers.[19] More than 28 marine natural products are currently in the clinical trial phase, and more are in the preclinical trial phase. For example, Eupalmerin Acetate is a new anticancer compound from gorgonians that grows in Caribbean Waters. This compound induces cell death (apoptosis) in malignant glioma cancer cells through the c-Jun NH2-terminal kinase pathway.[20]

Materials and Methods

Sampling

Gorgonian sample *Mopsella* cf. *aurantia* was taken in Bunaken Island, Manado-Indonesia's coastal waters at N 136'24.4" and E 12446'58.0" (Figure 1) in April 2022. The sample was collected using SCUBA diving at 4-5 m depth. The specimen is presented in Figure 2.

The sample was sliced using a knife directly from the substrate following, photographed, and rinsed using fresh water to remove the remaining salt within the body. The identification of the specimen was referred to the World Register of Marine Species: WoRMS (http://www.marinespecies.org/).

Extraction and fractionation

Gorgonian *Mopsella* cf. *aurantia* was extracted with a maceration method using ethyl acetate as solvent.[3, 21] Samples were sliced and soaked in a ratio of 1:3 for 24 hours at room temperature. The extraction process is homogenized using an *automatic shaker*. The extract was then filtered and evaporated using *a rotary vacuum evaporator*. The extract was then liquid fractionated using n-octane:ethanol: water with a ratio of 1:1:1 to obtain three fractions, e.g., n-octane (non-polar), ethanol (semipolar), and water fraction (polar). The fractions were then retested for activities to determine the active fraction.

Anti-UV Activity Testing and Determination of SPF Value

The anti-UV activity testing uses a UV-Visible Spectrophotometer. The sample extract obtained previously was tested at a wavelength of 280-400 nm to see if the sample had anti-UV by observing the spectrophotometric absorbance. The UV-A light emits from 320 to 400 nm, while UV-B has wavelengths ranging from 280 to 320 nm.

Determination of the value of SPF (*Sun Protection Factor*) is an advanced step in testing anti-UV compounds. Determination of SPF aims to determine the effectiveness of sunscreens carried out *in-vitro* by determining the absorption characteristics of sunscreens using spectrophotometric analysis of the dilution results of the tested sunscreens.[22] This test was conducted by extracting the sample dissolved in ethyl acetate and made in 50, 125, and 250 ppm concentrations. The absorption of each extract was measured on a spectrophotometer in the wavelength range of 290-320 nm with 5 nm intervals three times. Mansur *et al.*,[23] developed a straightforward mathematical equation that substitutes the *in vitro* method, utilizing UV spectrophotometry and the following equation: [23, 24]

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I\ (\lambda) \times Abs(\lambda)$$

Where EE: Erythemal Effect Spectrum, I: Solar Intensity Spectrum, Abs: Absorbance of the measured extract, and CF: Correction Factor (=10). EE x I value is presented in Table 1.

The results of the analysis determining the level of sunscreen ability of the tested extracts and grouped based on the ultraviolet index value are presented in Table 2.

Bacterial growth media preparation

Nutrient Agar (NA) was prepared by dissolving 23 grams of media in 1 L of demineralized water. Sterilized by autoclaving at 121ºC for 15 minutes. The media was poured into sterile petri dishes to solidify. For slant agar, 10 ml of media was transferred into a test tube and covered with sterile cotton, and allowed to solidify at a slope of 15°.

Brain Heart Infusion (BHI) was prepared by dissolving 37 grams of media in 1 L of demineralized water and sterilizing by autoclaving at 121ºC for 15 minutes.

Müller Hinton Agar (MHA) was prepared by dissolving 38 grams of media in 1 L of demineralized water. Sterilized by autoclaving at 121ºC for 15 minutes. The media was kept at 50 C to prevent solidification. A bacteria culture was added to it to reach a final density of $1x10^5$ cells/ml, followed by pouring it into Petri dishes to solidify.

Antibacterial assay

Gram-negative bacteria *E. coli* DSM498 and Gram-positive bacteria *B. megaterium* DSM32T strains obtained from The Leibniz Institute-German Collection of Microorganisms and Cell Cultures GmbH (Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)), Braunschweig, Germany. Both strains were overnight culture-refreshed on nutrient slope agar. The cultures were harvested and grown overnight with BHI broth medium, followed by a measure for their density. The initial bacterial density was checked using a UV-Vis spectrophotometer (λ = 600 nm). The value of OD_{600} of 1 is equivalent to a bacterial density of $8x10^8$ cells/ml. The cultures were diluted until $1x10^5$ cells/ml prior to use, and 1 ml of the bacterial culture was subjected and seeded onto the MHA agar. After solidification, 20 µl of the extract of the sample or fractions were spotted onto the plates. Amoxicillin (1 mg/ml) and 96% ethanol served as positive and negative controls, respectively. Plates were incubated at 37°C for 24 hours and checked for inhibition zones. All were repeated in triple.

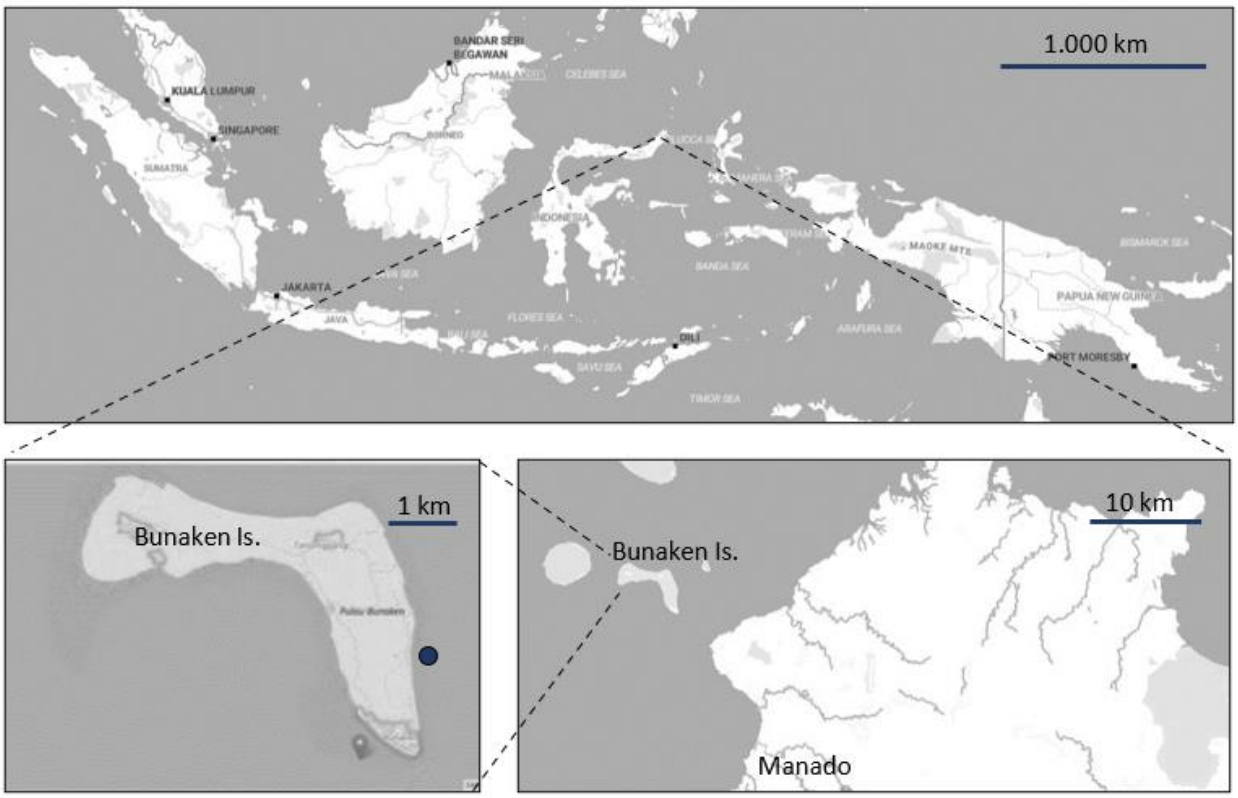

Figure 1: Sampling area of *Gorgonia Mopsella cf. aurantia*, Bunaken Island, Indonesia
Blue dot = sampling point.

Figure 2: Gorgonia *Mopsella* cf. *auratia*

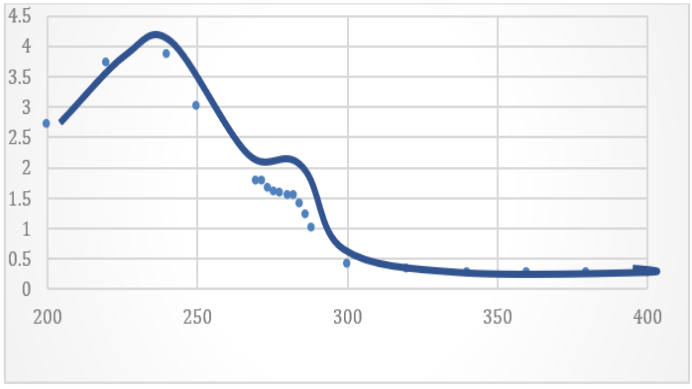

Figure 3: UV Absorption Spectrogram of gorgonian extract *Mopsella* cf. *aurantia.*

Result and Discussion

Anti-UV and SPF

The anti-UV activity of the gorgonian *Mopsella* cf. *aurantia* was measured using a UV-VIS spectrophotometer. The extract of gorgonian *Mopsella* cf. *aurantia* has a UV absorption maximum at a defined wavelength of λ 280 µm, with an absorbance at 2,205 mAU (Figure 3), indicating it has anti-UV-B property. Based on the spectroscopic absorption result, the extract *has* anti-UV activity and continues to determine the SPF value.

This research can be seen from the results of the absorbance value, which has an SPF value that is successively increasing in proportion to the amount of the tested ppm concentration solution. From these data, the ability level of the ultraviolet index can be grouped based on the SPF (*Sun Protection Factor*) according to the US. Food and Drug Administration (FDA; Table 3).[25] Based on the results obtained, it can be seen that the extract of gorgonian extract *Mopsella* cf. *aurantia* with a concentration ranging from 50-250 ppm obtained an SPF value of 3.89 to 7.71, categorized with minimum protection capability The fact that gorgonians reside in the subtidal zone, a layer of water that reduces UV penetration, and the fact that their sclerite is composed of polycrystalline calcite, a type of calcium carbonate, may both contribute to the lower SPF value. Research by Lisnawati *et al*.,[26] tested the ethyl acetate extract of mango leaves with a concentration of 120 ppm, which had an SPF value of 5, including the minimum protection type, a concentration of 240 ppm with an SPF 16 value categorized as the moderate protection. Furthermore, research conducted by Purwaningsih *et al*.,[27] revealed that the use of *Rhizophora mucronata* combined with carrageenan was able to protect the skin. The presence of phenolic substances, tannins, and flavonoids is alleged to act as UV-absorbing agents. According to the findings of this study, the SPF value of the gorgonian extract *Mopsella* cf. *aurantia* has the potential to prevent sunburn with a concentration increase, allowing it to be produced as a sunscreen.

Antibacterial assay

Test of antibacterial activity of crude extract of gorgonian *Mopsella* cf. *aurantia* was carried out with a concentration of 100,000 ppm towards E. *coli* and *B. megaterium* strain. The mean zone of inhibition in this study was classified according to Davis and Stout.[28] The classification of the criteria for the strength of an antibacterial material is as follows, namely the diameter of the inhibition zone <5 mm is categorized as weak, and the zone of inhibition 5-10 is categorized as moderate, while the diameter of the inhibition zone of 10-20 mm is categorized as strong and beyond >20 mm is declared a compound having a robust antibacterial activity.

Observation of antibacterial activity on the tested bacterial media after overnight incubation showed that the crude extract inhibited the growth of *E. coli* and *B. megaterium* strains. The inhibition zone of gorgonians *Mopsella* cf. *aurantia* extract towards *B. megaterium* and *E. coli* showed an inhibition zone of 18.0 mm ± 1.00 and 15.6 mm ± 251, respectively (Figure 4), which is characterized as potent inhibition. No inhibition was recorded from the negative control.

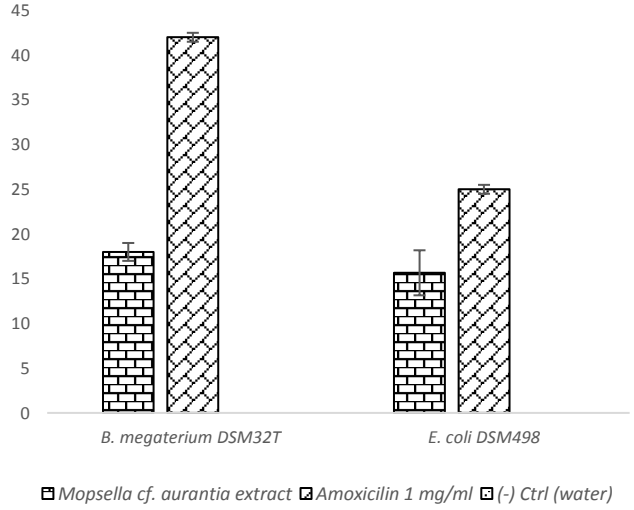

Figure 4: The average inhibition of gorgonian *Mopsella* cf. *aurantia* extract towards *B. megaterium* DSM32T and *E. coli* DSM498.

Table 1: EE x I value at wavelength 290-320 nm

Wavelength (µm)	EE × I
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1846
315	0.0839
320	0.018

Source: [23, 29]

Antibacterial compounds can be classified as broad to narrow spectrum. The term "broad spectrum" refers to the compound's activity against a wide range of bacterial strains, including Gram-positive and Gram-negative bacteria. A narrow spectrum indicates that a substance actively works against only one type of bacteria and solely against Gram-positive or Gram-negative bacteria.[30] The results of this study indicate that the antibacterial compound of the gorgonian *Mopsella* cf. *aurantia* extract could classify as broad-spectrum because of its ability to inhibit both Gram-positive and negative. Several research studies were conducted concerning gorgonian antimicrobial activity, gorgonian *Mopsella* sp., shown to be active against the *Pseudomonas aeruginosa*

strain.[31] Four Pseudopterogorgia species, e.g. *Pseudopterogorgia acerosa, P. americana, P. rigida*, and *P. nanna* from Caribbean Waters, were shown to be potent towards several bacterial strains.[32]

The findings of this study are fascinating since the related gorgonian has a significant activity array against Gram-negative *E. coli*. Gram-negative infection control has become a major issue in modern medicine. The characteristics of the specific group, such as their dense and compact peptidoglycan layer, inhibit many antibiotics' ability to reach bacterial cellular mechanisms. It also has an "efflux-pump mechanism," a compound removal mechanism that does not require in bacterial cellular biotransformation processes via their type-III secretion system.[33, 34]

Further investigation of the gorgonian fractions *Mopsella* cf. *aurantia* showed that the active compounds responsible for the anti-UV effects and antibacterial in the related species were located in the ethanol fraction.

Table 2: SPF value of gorgonian extract *Mopsella* cf. *aurantia*.

Conc. (ppm)	SPF value measurement	UV protection level
50	3.89	Minimum
125	6.48	Minimum
250	7.71	Minimum

Table 3: the SPF values according to the US. Food and Drug Administration.

SPF Range	Category
2-11	Minimum
12-29	Moderate
≥30	High

Source: [25]

Conclusion

The *Mopsella* cf. *aurantia* extract showed vigorous antimicrobial activity towards *Escherichia coli* DSM498 and *Bacillus megaterium* DSM32T strains. The particular extract also has anti-UVB activity. Further investigation shows that the related extract has a minimum SPF value at a concentration of 250 ppm. The antimicrobial and anti-UV effects in the related species were located in the ethanol fraction.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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