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Unique Marine Insects (Hemiptera) From North Sulawesi and Self-Defense Mechanism Against Sun Exposure

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ABSTRACT

Marine insects are constantly exposed to ultraviolet radiation. How the marine insect Hemiptera: Gerridae responds to UV stress and the molecular mechanisms underlying this adaptation are still unknown. This study analyzed the adaptability of marine insects that are constantly exposed to UV radiation by extracting anti-UV compounds produced by these marine insects. These marine insects have the ability to produce anti-UV B compounds and anti UV A compound with a high point of absorbance, because it is able to absorb UV-B (280 nm- 320 nm) with the highest absorbance value at point 4 with a wavelength of 280 nm - 300 nm, and is also capable to absorb UV-A (320 nm – 400 nm) with the highest absorbance value at point 3.5 with a wavelength of 340 nm.

Keywords: Anti-UV A, Anti-UV B, Insect, Marine.

Introduction

Insects can be found in anywhere on land but are rarely discovered in the sea. A lot of research has been done to know the bioecology of insects that live on land, rivers and lakes, but research on insects that live in the sea and marine ecosystem is still rare. Insects can inhabit areas with varying levels of salinity such as brackish water and sea water. Most of the insects that live in marine waters belong to the order Coleoptera, Hemiptera, and Diptera. However, insects that live on the sea surface are members of the Gerridae family of the Hemiptera order. These insects are always exposed to ultraviolet radiation because their entire life cycle is between the surface of the sea and the air. In some marine organisms that live in shallow waters and areas, tides, organic compounds that have anti-UV properties were also found, namely: Mycosporine-like amino acids (MAAs).[1] MAAs are compound that has low molecular weight and can be discovered in diverse aquatic organisms. In recent study, MAAs has been found in 501 species of maroalgae.[2] Forming mechanism mycosporine is one of the marine organisms defense systems against UV radiation. Mycosporine was first discovered in funguses that lives on the water surfaces. During exposure to sunlight, MAAs produced secondary metabolites that are capable to provide protection against UV radiation. Various MAAs compounds have been found in marine organisms, including MAAs-glycine, MAAs-shinorine, MAAs-palythine, MAAs-porphira, MAAs-palythinol and MAAs-asterine. MAAs-Glycine is the main component of MAAs, which functions as a natural anti-UV substance (λmax = 310 nm).[3]
Halobates sp., a genus of the order Gerridae that lives on the open sea surface, are exposed to Ultra Violet (UV) radiation during daylight, where there is no place to hide. Therefore they have some means of protection from damage due to UV radiation. Examination of the cuticle portion of *Halobates sericeus* revealed the presence of a UV-absorbing dark layer at wavelengths between 260 nm and 320 nm.[4] Through this study, we discovered marine insects that live on the sea surface at Tasik Ria Mokupa, North Sulawesi, Indonesia. These insects are always exposed to UV radiation, hence this research was geared towards revealing the adaptability of this species against UV radiation exposure.

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Materials and Methods

Type, Time and Place of research
This research was an experimental study, conducted between May and December 2022 in Faculty of Fisheries and Marine Science and Faculty of Mathematics and Natural Science's laboratory of Sam Ratulangi University with samples extracted from Mokupa beach, North Sulawesi, Indonesia. The research comprised the following stages: confirmation of marine insect identification, insect extraction using maceration and evaporation method, anti-UV activity testing of marine insects extract towards UV-FIS spectrophotometer after separation with Thin Layer Chromatography and Column Chromatography and determination chemical structure of anti-UV substance from marine insect using HPLC Tosso 802.

Confirmation of Marine Insects Identification
Samples in this study were collected using Neuston nets and a modified collecting method by Dias and Lopes.[5] Collecting process were carried out randomly in seashores and mangrove areas. Salinity and water temperature were measured in every collecting site. Samples collected were then placed into sample bottles that were labeled with place and time of sampling. Samples obtained from Neuston net were preserved in 95% alcohol before being separated from various components and non-insect organisms. These samples were then separated based on size and pattern on the sample's head and thorax. Subsequently, samples with the best proportion and complete body parts were chosen and then placed on microscope slides containing 95% alcohol. Afterwards, samples are placed under dissecting microscope with a camera where various segments of the samples' body are measured. Measurements were taken based on the body structured used as benchmark for confirmation identification purposes.

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Confirmation identification was carried out with the help of various literatures, including studies by Moreira, *et al.*[6]

Insect Extraction

The extraction method used a modified method from Suh *et al*.[7] Extraction started by homogenizing the collected insects with 80% methanol in a ratio of 1:10 using an electric blender. Then the sample was incubated for 5 hours at 4^{o}C. Then filtered to remove the cells that were not needed. Supernatant filter results with Whatman Glass GF/C filter paper was then evaporated to remove methanol with the help of a rotary evaporator (rotovap) at a temperature of 30^{o}C. Samples were stored at -20^{o}C.[7]

Anti-UV Activity Testing

To find out whether the insect has anti-UV substances quantitatively, the results of insect extracts were tested using a UV-VIS spectrophotometer such as method from Libkind *et al*.[8] The extract was dissolved in a 20% methanol solution and then inserted in to suprasil cuvette (1 cm) and tested on Hewlett Packard UV-VIS spectrophotometer at 310 nm.[8] The amount of light absorbed at length a given wave is proportional to the number of molecules that absorb radiation. anti-substance Mycosporine-derived UV can absorb radiation at 310 – 362 (nm).[9] Insects that have absorbance values that qualify as anti-UV were extracted. A total of 5 grams of insects were extracted using 200 ml methanol 80 %, mixed to homogenize, then placed in a orbital shaker in the dark for 5 hours at 4^{o}C, then filtered with Whatman paper GF/C filters. Methanol in the filtrates was removed by evaporation at 30^{o}C.

These filtrates were then freeze-dried and stored at -20^{o}C. Afterwards, 4 grams of filtrates were mixed with 2 ml of methanol then further isolated on the Preparative Thin Layer Chromatography (PTLC).

PTLC were used for initial separation of active compounds based on the molecular weight. Separation was done using silica gel plate with hexane and ethyl acetate solvents with 1:1 ratio and C18 gel plate with water:methanol solvents with 1:10 ratio to determined whether the resulting compounds were polar or non-polar. Based on these results obtained, the processed were then followed by using column chromatography to obtained sufficient extract to purified.

These extracts were then purified using column chromatography using 50 ml titration pipette filled with silica gel (Wakogel 100 C18) for stationary phase. Whereas methanol:water were used for mobile phase. Column chromatography started with pre-column by flowing methanol:water with a starting ratio of 100:0, and then continued with ratio of 75:25 to 0:100. Samples were inserted after pre-column with the addition of methanol:water in a ratio of 0: 100 to 100: 0, every 3 ml of samples from column were collected in a test tube. All of the collected test tubes were tested on a spectrophotometer at a wavelength of 320 nm. The results obtained at this stage were then tested again on a UV-FIS spectrophotometer with a length at 220-400 nm.

Anti-UV Substance Chemical Structure Determination

The purification was done using method by Carreto *et al*.[10] Extracts were dissolved in 500 l in 100% methanol (v/v), then 20 l of the extract solution was added to the phenosphere 5 m C18 column with solvent or mobile phase 100% water and 0.2% TFA at a flow rate of 2.0 ml/min and detected at a wavelength of 334 nm using HPLC Toso-8022. Sample collection was carried out when a peak began to form on the monitor until almost at the end of the peak. Each peak was collected in a different tube, then evaporated. The collected peaks were then analyzed for absorbance capability on a UV-VIS spectrophotometer.[10]

Results and Discussion

Samples of marine insects were taken in the Mokupa area on the coast of Tasik Ria, Manado, North Sulawesi, Indonesia at location coordinate 1.411382.124.706696 (1^{o} 24'41.0"N124^{o} 42'24.1"E); 1.411407.124.706 519 (1^{o} 24' 41.1"N 124^{o} 42'23.5"E); 1.410926.124.706246 (1^{o} 24'39.3 "N124^{o} 42'22.5"E) and 1.411169.124.706180 (1^{o} 24'40.2"N 124^{o} 42'22.2"E). This beach area is a special beach area because it is a combination of mangrove areas with a muddy substrate, dead coral and white sand. Various types of mangrove trees can be found at the location where the marine insects were collected, such as *Rhizophora apiculata*; *Sonneratia alba* and *Avicennia marina*. Insects were found in the bottom of the mangrove tree, between the roots and location without wave currents. These insects can also be found on the surface of the water in coral reef areas, even at open seas, but can not be found in areas that are dry.

Some insects were also found in coastal areas with sandy substrate, probably because they were carried away by waves or wind. Insects with a high population density live in groups on the surface of the water under the shade of mangrove trees. This is in line with several research results which states that these insects can be found in various water areas fresh, brackish and seawater, especially in coastal areas.[11-14] Insects that live on the surface of the water from the family Gerridae have a wide distribution area because these insects are known to have the ability to adapt to high temperature. Several studies that have been carried out discusses the distribution of insects that live on the surface of the water, some even research several regions in Indonesia.[15-18] However, no one has reported the types of insects found in the current study. The sample in this study is a member of the order Hemiptera because it has a piercing mouth type sucking (stylet) in the shape of a beak that segmented and arising from the front of the head (Figure 1.)[18-20]

The results of the separation of crude extracts from marine insects by column chromatography were then analyzed with a spectrophotometer at a wavelength of 320 nm, producing two peaks. The first peak is the peak with a high absorbance value which is 3, while the second peak only has a value of 2.6. (Figure 3.A). Each of these peaks were then further analyzed on a UV-FIS spectrophotometer with a wavelength of 220-400 nm as shown in Figure 3.B. and Figure 3.C. The first peak (S.4.1) can absorbs a wavelength of 230 nm – 300 nm with an absorbance value at point 4 then begins to decrease to 3 at 310 nm and then rises again to 3.5 at a wavelength of 340 nm and begins to decrease again to a point 0.5 at a wavelength of 380 nm. These results indicate that the peak of S.4.1 is able to absorb UV-B (280 nm- 320 nm) with the highest absorbance value at point 4 with a wavelength of 280 nm – 300 nm, then decreases until point 3 at a wavelength of 319 nm. Furthermore, this peak is also able to absorb UV-A (320 nm – 400 nm) with highest absorbance is at point 3.5 with a wavelength of 340 nm. On the second peak (S.4.2)) capable of absorbing a wavelength of 230 nm – 270 nm with an absorbance value at point 4 then began to decline at 295 with point 1, and rose again to point 1.3 at wavelength 320 and starts to decline again to 0.5 at wavelength 350 nm.

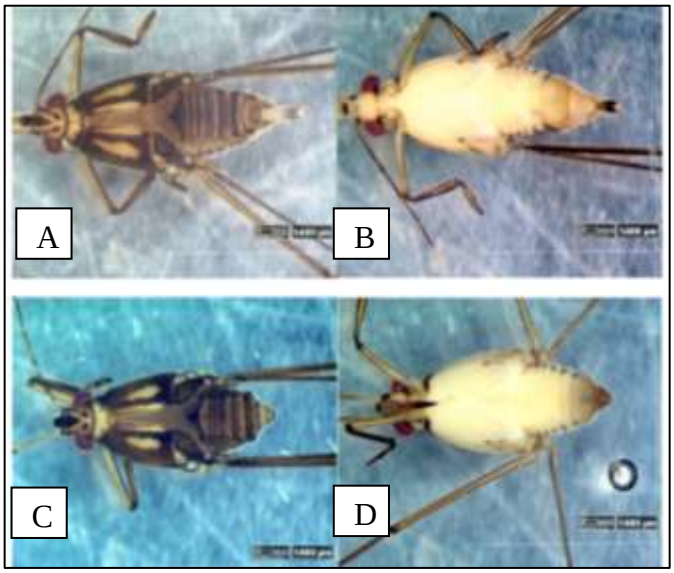

Figure 1: Marine insects Hemiptera found in coastal areas Mokupa Beach Manado A. Dorsal view of male insect; B. Ventral view of male insect; C. Dorsal view of female insect; D. Ventral view of female insect

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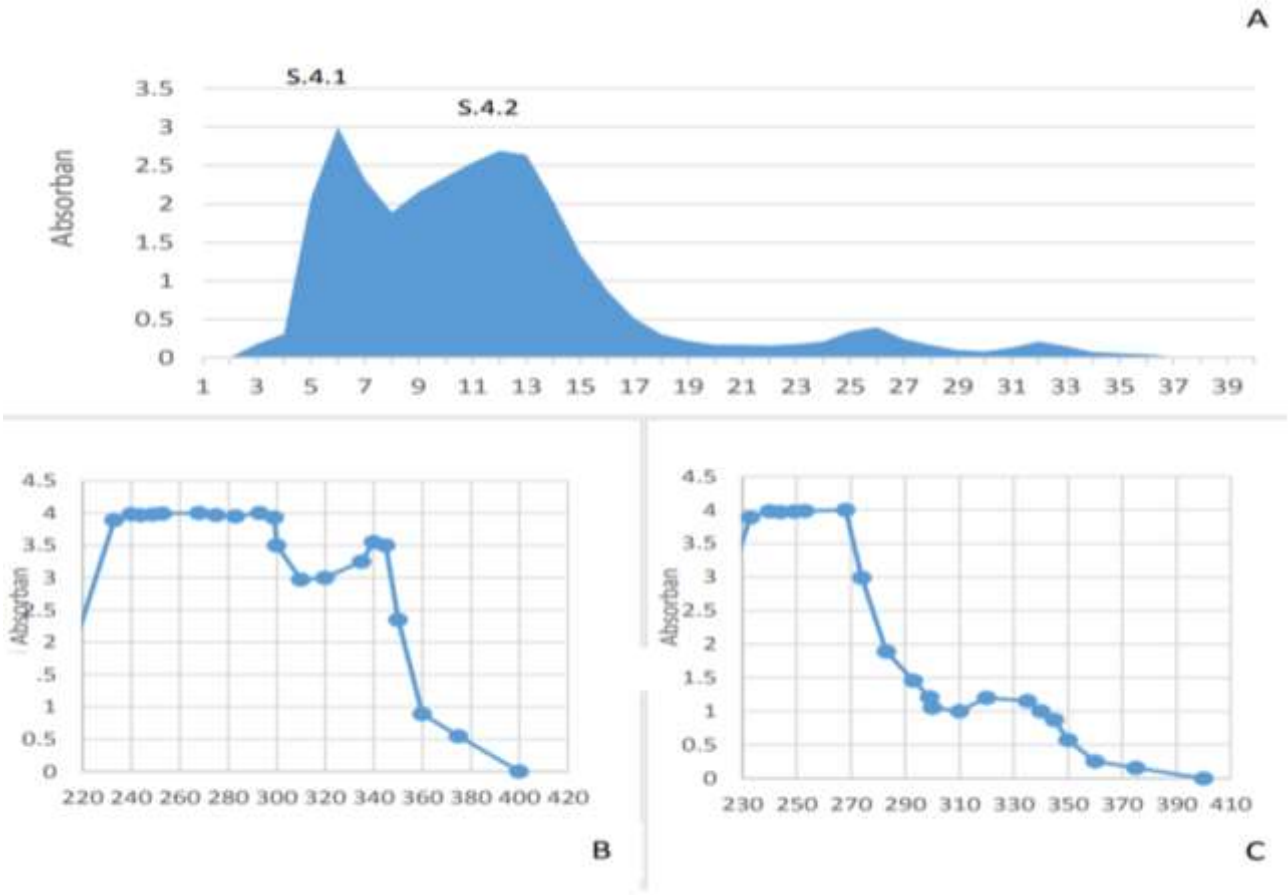

Figure 2: Potential of marine insect samples to produce anti-UV substances
A. The absorbance value of column chromatography results at a wavelength of 320 nm.
B. UV-FIS spectrophotometer results for the first peak (S.4.1).
C. UV-FIS spectrophotometer results for the second peak (S.4.2)

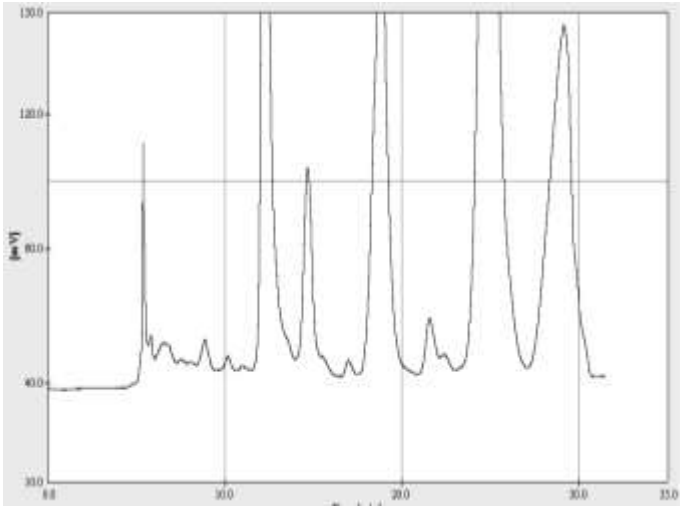

Figure 3: HPLC Profile of MI with 100 % H2O + 0.05 % TFA

These results indicate that the peak of S.4.2 is capable of absorbing UV-B (280 nm-320 nm) with the highest absorbance value at point 1.5 with a wavelength of 280 nm then it decreases until point 1.2 at a wavelength of 319 nm. This peak is also able to absorb UV-A (320 nm – 400 nm) with the highest absorbance value at point 1.2 with a wavelength of 320 nm.

The purification results were then further analyzed on HPLC especially at the peak that shows the ability to absorb UV A with a high absorbance value of 3.5. Comparing the HPLC results of the samples obtained (Figure 3.) with the results obtained by Carreto *et al.*, it can be seen on peak number 1 sample in the analysis results at HPLC has the same retention time position as peak number 1 in the results research from Carreto *et al.*, namely Mycosporine palithyne serine sulfate.[10] The marine insect sample were shown to have strong ability to absorb the Mycosporine Palythine Serine Sulfate compound, because it reaches a value of 3 to absorb ltraviolet light at a long 321 nm wave, this value is far above the ability of MAAs Shinorine in *Actino-mycetales* sp., which only reaches a value of 1.8 at wavelength 333 nm.[21] The results of the study are different from the results of the study by Andersen and Cheng, where they discovered 4 types of mycosporine in Halobates, namely: mycosporine-glycine, shinorine, orphyra 334, and palythine.[4] In another study, they obtained skin parts from several species of the Family Gerridae, which contain layers that are believed to be layers of UV absorbers.[22]

Results obtained from several studies shows that various organism produce bioactive substance that function as anti-UV or have photoabsorbance ability, for example *Oryza sativa* var. that contain glutinose which has potential as a suncsreen;[23] *Caesalpinia sappan L* that can be used as antioxidant and sunscreen;[24] *Elephantopus mollis* which have been tested in vivo and in vitro as photoprotective.[25]

Sunscreen compound's mechanism of action in protecting cell consists of transforming absorbed sunlight into thermal energy. However some of the active compounds used in commercial sunscreen products have a negative effect. This is because in the chemical process, changes that turned sunlight into thermal energy, will produce intermediate

products, generally in the form of free radicals, which will actually cause damage to the DNA and become potentially carcinogenic.
In contrast, natural active compounds such as MAAs doesn't produce intermediate compounds during the process of protecting cells from UV rays, as reported by Conde *et al.*[26] This is due to the presence of antioxidants in MAAs extracts, considering that some antioxidants, such as 4-deoxygadusol, which is known as MAAs precursors. The protective functions of MAAs compound is based on the effectiveness of these chemical groups in absorbing UV-A and UV-B radiation, to determine whether these compounds can function as stable sunscreens to protect against exposure to UV radiation over a long period of time.[1]

Conclusion

Marine insects Hemiptera: Gerridae found at Mokupa beach, North Sulawesi, Indonesia are constantly exposed to UV radiation produced anti-UV compounds as an adaptation to exposure to ultraviolet radiation. This insect has the ability to produce anti-UV B compounds with the ability to absorb UV light at a wavelength of of 270 nm – 300 nm with an absorbance value at point 4 and ability to produce anti-UV A compound with the ability to absorb UV light at a wavelength 340 nm with an absorbance value at point 3.5.

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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