Biota
Biologi dan Pendidikan Biologi

http://biota.ac.id/index.php/jb
DOI: http://dx.doi.org/10.20414/jb.v17i1.489

Research Article

Bioactivity Diversity (Antibacterial and Antioxidant) of Macroalgae in the Ekas Beach Area, Lombok

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Abstract

Macroalgae in the southern coastal area of Lombok has not been explored much in terms of bioactivity. The aim of this research is to explore the usefulness of 9 types of macroalgae on Ekas Lombok beach by searching for their bioactivity capabilities, whether each macroalgae has no bioactivity at all, has antibacterial and antioxidant activity, only has antibacterial activity but no antioxidant activity or vice versa and whether there are macroalgae that even have anticancer activity. The method used in this research consists of several steps: Macroalgae Sampling, Morphological Analysis of Macroalgae Samples, Isolation of active compounds from macroalgae and Cytotoxic Test on Breast Cancer Cells in Vitro which consists of 3 tests: Culture and sub culture of MCF-7 Breast Cancer Cells, Cell Viability and Cytotoxicity Test with MTT and DNA Isolation of MCF-7 Breast Cancer Cells. The research results show that the macroalgae with the best antibacterial activity is the red macroalgae *Acanthophora spicifera* and the macroalgae with the best antioxidant activity is the green macroalgae *Ulva reticulata.*

Keywords: *Bioactivity diversity, antiocsidant, antibacterial, macroalgae, Ekas beach*.

1. Introduction

Indonesia is a maritime countries that has a very diverse range of macroalgae (seaweed). This diversity is not accompanied by the use of seaweed in Indonesia, which is still far behind when compared to other seaweed producing countries such as Japan, Korea, Taiwan and China. The use of seaweed production in Indonesia is still limited to the food sector and commodity raw materials for export. Meanwhile, in other countries, seaweed has been widely used as an ingredient in industries other than food, such as the pharmaceutical, cosmetic, medical and agricultural industries (Liu et al, 2019).

One of the islands in Indonesia that has abundant macroalgae diversity is the island of Lombok, both the east, west, north, central and even the south coast of Lombok have a variety of macroalgae. 9 types of macroalgae have been successfully identified morphologically and genetically, 4 types of red macroalgae (*Acanthophora spicifera, Eucheuma sp., Gracilaria foliifera* and *Wurdemannia miniata*), 3 types of brown macroalgae (*Hormophysa cuneiformis, Padina australis* and *Turbinaria ornata*) and 2 types of green macroalgae (*Ulva intestinalis* and *Ulva reticulata*) (Furqan et al, 2023).

Macroalgae identified from other areas such as beaches in West Java (most data has been successfully explored at Sayang Heulang Beach) have been studied for their

bioactive compound content and have been successfully tested for their bioactivity as antioxidants, antibacterials, anti-inflammatory and even anticancer. Therefore, researchers are interested in conducting research related to the diversity of bioactivity of 9 types of macroalgae on Ekas beach, Jerowaru (South Coast of Lombok).

The aim of this research is to explore the usefulness of 9 types of macroalgae on Ekas Lombok beach by searching for their bioactivity capabilities, whether each macroalgae has no bioactivity at all, has antibacterial and antioxidant activity, only has antibacterial activity but no antioxidant activity or vice versa and whether there are macroalgae that even have anticancer activity.

2. Materials and Methods

2.1 Materials

The tools used in the study were clear plastic, cooler box, micro pipettes measuring 1–10 L, 10–100 L, and 100–1000 L (Biologix), mortar and pestle, tweezers, 100 mL measuring cup (Iwaki), Erlenmeyer 100 mL (Iwaki). The instruments used consisted of a DSC-H300 digital camera (Sony), MAC-601 autoclave (EYELA), micro centrifuge (Prism TM R), TM water bath (MyBath), vortex mixer (Labnet), refrigerator (Toshiba), analytical balance (Ohaus Pioneer), PCR machine (BioRad), agarose gel electrophoresis apparatus (Biorad), GPS (Global Positioning System) (Garmin), thermometer, multiparameter HI 9828 (Hanna Instruments), light microscope (Nikon) and UV wave transilluminator 254 nm (Vilber Lourmat).

The seaweeds used in this study were 4 types of red macroalgae (*Acanthophora spicifera, Eucheuma sp., Gracilaria foliifera* and *Wurdemannia miniata*), 3 types of brown macroalgae (*Hormophysa cuneiformis, Padina australis* and *Turbinaria ornata*) and 2 types of green macroalgae (*Ulva intestinalis* and *Ulva reticulata*) taken from Ekas beach, Lombok. Breast cancer cells (MCF-7) were obtained from the cell culture laboratory, Faculty of Medicine, Mataram University Teaching Hospital. Solvents used for extraction and column fractionation were n-Hexane (p.a, Merck), methanol (p.a, Merck), acetone (p.a, Merck), chloroform (p.a, Merck), 0.05 M phosphate buffer solution (pH 7, 6) which is made from KH2PO4 and K2HPO4 solutions.

The materials used to test antibacterial activity were Escherichia coli, Staphylococcus aureus and P. acne (bacteria obtained from the Mataram Health Laboratory), Tryptone, yeast extract, NaCl, Agar and Tetracycline and kanamycin as positive controls. The ingredients used to test antioxidant activity were DPPH (1,1-diphenyl-2-picryhydrazyl) (Sigma) and Gallic Acid (Sigma) as positive controls.

The materials used for cell culture and cell harvest were RPMI (Gibco), FBS 10% (Gibco), Penicillin-Streptomycin (Gibco), PBS (Invitrogen), DMSO (Merck) and EDTA (Merck). For the cytotoxicity test, MTT (3-(4-5-dimethylthiazol-2-yl-)-2,5-diphenyltetrazolium bromide) (Sigma) and Doxorubicin (Dankos) were used as positive controls.

Cancer cell protein isolation using the Nuclear Extraction Kit (Abcam ®), DNA Methyltransferase Activity Test using the DNMT1 Inhibitor Screening Assay Kit (Abcam ®).

2.2 Methods

Macroalgae Sampling

Sampling of macroalgae was carried out in tidal beach conditions with sunny weather. Seawater conditions at the time of sampling were analyzed with several parameters, including: pH, temperature (°C), conductivity (µS/cm), total dissolved solids (ppt) and salinity (PSU). Seawater analysis was carried out on each square that was submerged in seawater during sampling.

Morphological Analysis of Macroalgae Samples

Morphological analysis was carried out based on visual observations and documented using digital camera photos. Parameters observed included color, shape and branching of the thallus. The morphology of macroalgae samples was evaluated and compared with the morphology of macroalgae found in the literature (Guiry and Guiry, 2018).

Isolation of active compounds from macroalgae

Samples of 9 types of macroalgae, each of which had been dried and finely ground, were then extracted using the method from Kumari et al., (2018) with several modifications. The dried samples were macerated for 24 hours at room temperature using methanol (p.a), chloroform (p.a) and 50 mM phosphate buffer pH 7.6 with a ratio of 2 : 1 : 0.8 (v/v). Then filtered to get the first filtrate. The remaining residue was re-extracted 3 times using the same solvent in a ratio of 1 : 1 : 0.8 (v/v) for 30 minutes, filtered and a second filtrate was obtained. The extraction was repeated until the fourth filtrate was obtained. The collected filtrate was then added to Milli-Q in a ratio of 9 : 2 and centrifuged for 15 minutes at 2100 xg. The results of centrifugation will separate the mixture into 2 phases, namely the chloroform phase and the methanol-water phase. The two phases are separated and each phase is concentrated using an evaporator. The chloroform phase obtained was then weighed, while the methanol-water phase obtained was dried using a freeze dryer to obtain dry powder from the crude methanol-water extract and weighed.

Fractionation of the crude extract of each methanol-water was carried out using the column chromatography method according to the method of Behbahani, et al (2013) with several modifications, namely by using column chromatography, G60 7733 silica with a height of 10 cm and a diameter of 1.2 cm. Elution was carried out in stages using a mixture of hexane and acetone with a ratio of 8:2, 5:5, 2:8 (v/v) respectively and ended with methanol (p.a). The total volume of each eluent is 24 mL for the mixture of hexane and acetone, and 50 mL for the methanol eluent. The silica gel to be used is activated by

heating at 100°C and equilibrated with hexane: acetone in a ratio of 8: 2 (eluent 1) overnight to prevent the silica from breaking. The crude extract (0.1 – 0.5 grams) was added with 5 drops of methanol then put into the column. The elution flow rate was 6 – 8 drops per minute and fractions were collected every 2 mL in microtubes. Fractionation of the chloroform crude extract was carried out in the same way as the methanol-water crude extract fractionation method with different ratios of the stepwise elution gradient used. The eluent ratio (Hexane: Acetone) used sequentially is 7:3; 3:7; 1:1 and 1:9 (v/v) (Panjaitan, 2014).

Cytotoxic Test on Breast Cancer Cells in Vitro

Culture and sub culture of MCF-7 Breast Cancer Cells

MCF-7 cancer cells will be obtained from the cell culture laboratory of the Faculty of Medicine, Teaching Hospital, Mataram University. Cancer cell culture is carried out using cancer cells that have been frozen at -80°C. Thawing cancer cells is done by warming the cells to a temperature of 37°C then adding new medium. Centrifuge at 1500 xg for 5 minutes. Resuspend again using 1 mL of media. The cells were then transferred into a flask filled with 4 mL of complete media (RPMI 1640 Media, Pen-strep 1% (v/v) and FBS 10% (v/v)). Cells were incubated at 37°C, 5% CO2 for 24 hours or until cells were obtained with 80% confluence. Next, the cells can be directly harvested and tested or sub-cultured. Cell harvesting was carried out by changing the growth medium and washing the cells using 2 mL of PBS (Phosphate Buffer Saline). A total of 2 mL of Trypsin-EDTA 0.05% in PBS and incubated in an incubator for 5 minutes. The cells were observed with a microscope and the cells that had separated from the bottom of the flask were transferred into a 15 mL centrifuge tube containing 2 mL of complete media. Next, centrifugation was carried out at a speed of 1500 xg, 4°C for 5 minutes. Cancer cell sub-culture is carried out by transferring the suspension into two to three flasks containing complete media and growing in an incubator at a temperature of 37°C, 5% CO2. The number of cells was counted using a hemacytometer and counter. Cells were stained first using trypan blue in a ratio of 1:1 until a blue color appeared. The staining results are viewed using a microscope. Dead cells will absorb trypan blue so they will appear blue, while living cells will remain transparent. Next, determine how many cells will be used for the cytotoxic test using seaweed extract using the MTT method.

Cell Viability and Cytotoxicity Test with MTT

Cells that are ready to be harvested and diluted using culture media as needed are transferred to a test plate with 96 wells with 100 µL in each well. Next, the cells were re-incubated in a 5% CO2 incubator at 37°C for 24 hours to recover the cells that had been harvested. Meanwhile, extract sample preparation was carried out by making a stock solution by dissolving a certain amount of dry extract with 5% (v/v) DMSO (in sterile ddH2O) to obtain an extract concentration of 2000 µg/mL. Next, graded dilutions were made from the mother liquor using culture media with varying concentrations between

1000 µg/mL – 15.625 µg/mL. Furthermore, after 24 hours, the culture medium was discarded and replaced with media that had been added with extracts and fractions with various concentrations of 200 µL for each well. The same treatment was also carried out on control cells, media control and positive control Doxorubicin 1 µg/mL. Next, the cells were incubated again in a 5% CO2 incubator at 37°C for 48 hours. After 48 hours of incubation, the medium was replaced with 100 µL of new medium to which MTT had been added and incubated for 4 hours. After 4 hours the condition of the cells was checked to see the formation of formazan crystals using a microscope. If formazan crystals have formed, then 100 µL of DMSO is added to each well to stop the reaction and the absorbance is measured on an ELISA reader with a wavelength of 550 nm.

DNA Isolation of MCF-7 Breast Cancer Cells

DNA isolation of MCF-7 breast cancer cells was carried out using a kit from Geneaid according to the instructions provided. Cells that had been treated with the active fraction which had activity to inhibit DNMT1 were incubated for 48 hours. Cells that had been trypsinized and washed with PBS were centrifuged and the cell pellet was taken, added with 200 µL of GB buffer and transferred into a 1.5 µL microtube and incubated at 60°C for 10 minutes. Add 200µL absolute ethanol and vortex. Place the GD column in the collection tube. Transfer the solution to a GD column and centrifuge at 12,000 xg for 2 minutes and discard the supernatant. Add buffer W1 and centrifuge at 12,000 xg for 60 seconds and discard the supernatant. Add 600 µL wash buffer and centrifuge at 12,000 xg for 60 seconds and discard the supernatant. Transfer the column into a new microtube. Add 100 µL of elution buffer and centrifuge at 12,000 xg for 60 seconds. The DNA obtained was observed using 1% (w/v) agarose electrophoresis.

3. Results and Discussion

3.1 Results

Figure 1. Crude extract, the result of maceration and evaporation of one of the green macroalgae.

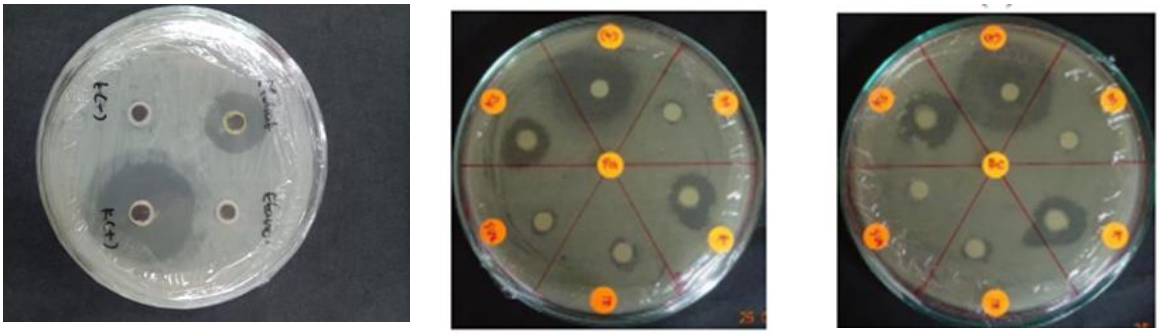

Figure 2. Image of test results for the anti-bacterial activity of several macroalgae.

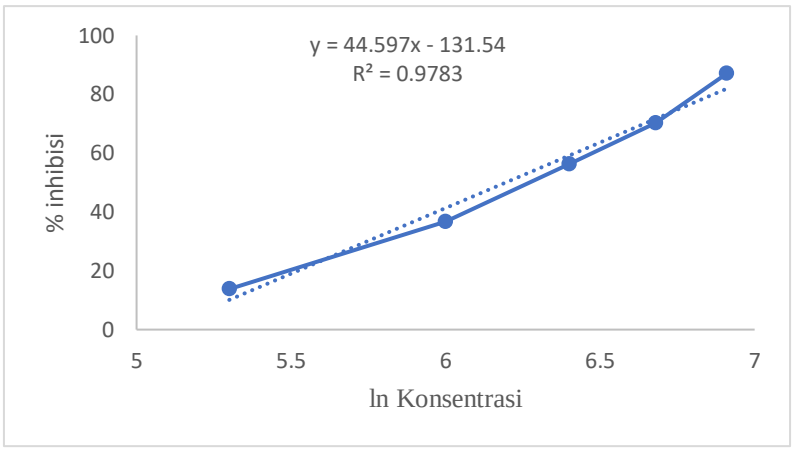

Figure 3. Graph of macroalgae inhibitory test against free radicals.

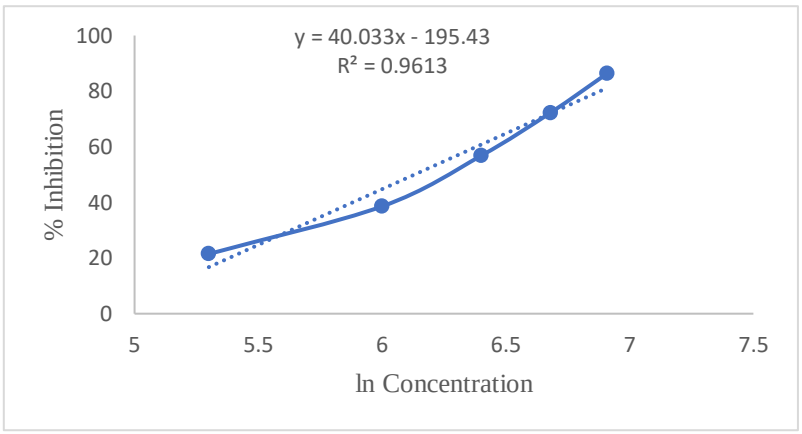

Figure 4. Graph of Vitamin C inhibitory test against free radicals

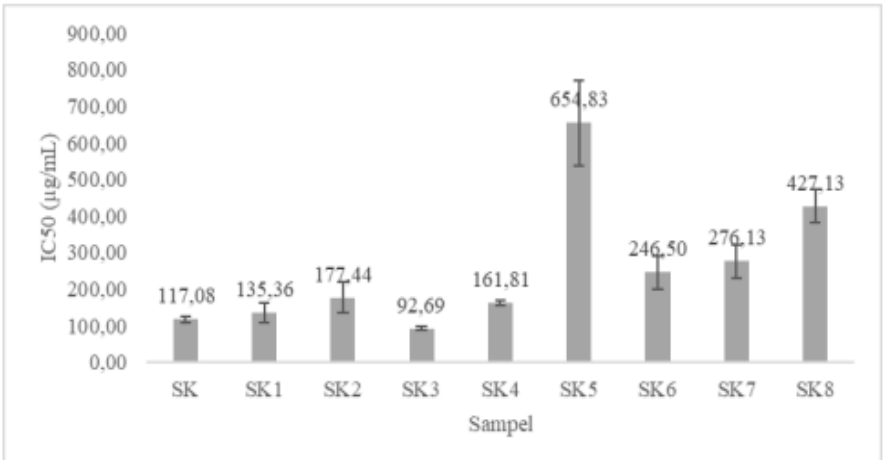

Figure 5. Graph of cytotoxic test results for 9 macroalgae samples

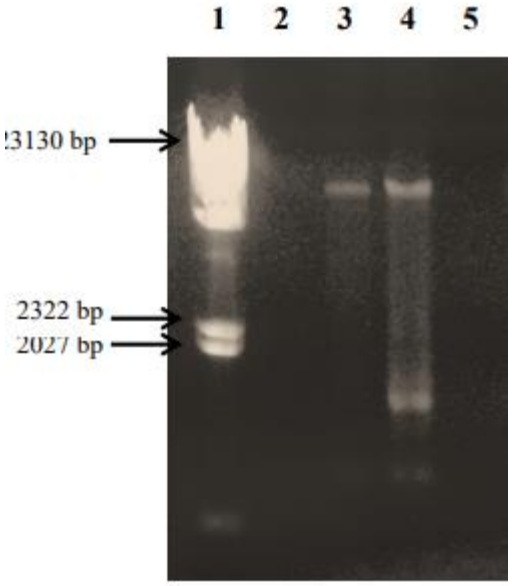

Figure 6. Image of DNA fragmentation results, where number 1 is marker, number 2 is negative control, number 3 is MCF-7 cancer cell DNA, number 4 is positive control, and number 5 is macroalgae sample.

3.2 Discussion

Samples and determinations have been successfully obtained with confirmation of the following brown macroalgae (*Hormophysa cuneiformis, Padina australis, Turbinaria ornata*), green macroalgae (*Ulva intestinalis, Ulva reticulata*) and red macroalgae (*Acanthophora spicifera, Eucheuma sp, Gracilaria foliifera, Wurdemannia miniata*).

The 9 macroalgae were dried first, then the simplicia were made. After that, the compound was isolated using the maceration method using ethanol solvent and evaporation to remove the solvent to obtain a macrolagae extract.

Crude extracts (**Figure 1**) from each (9 types) of macroalgae were also obtained which were then tested for phytochemicals and then tested for antibacterial and antioxidant activity. As for the results, there are those that have antibacterial activity but

no antioxidant activity (weak category antioxidants), conversely there are those that have antioxidant activity but weak category antibacterial activity.

Antibacterial activity test

The antibacterial activity test of macroalgae crude extract was carried out using the Disc Diffusion method and the clear zone formed was observed. Testing was carried out on pathogenic bacteria representing the group of gram-positive bacteria (Staphylococcus aureus and Bacillus cereus) and gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The test was carried out by inserting 100 µL of bacterial liquid culture into solid LB media using the spread method. After the bacterial culture had solidified, sterile paper discs were then inserted and 10 µL of sample was dropped onto each paper disc with a sample concentration of 5 mg/mL which was dissolved in each solvent (ethanol) and solvent as a control solvent. The positive control used was Kanamycin with a concentration of 0.5 mg/mL in ethanol solvent. Observations were made by observing the clear zone that formed around the disc paper and then measuring it using a ruler (Esteller and Herman, 2022).

From the results of the antibacterial activity test (**Figure 2**), only the red macroalga *Acanthophora spicifera* had antibacterial activity higher than the positive control (commercial antibiotic in the form of kanamycin) while the other 4 had antibacterial activity but their inhibition zone values were still below the positive control, the remaining 3 did not even have inhibition zone values.

Antioxidant Activity Test

Antioxidant activity was carried out using the DPPH (2,2-diphenyl-1-picryhydrazyl) method, which is a free radical compound. The measurement carried out using this method is the ability of a compound to capture free radicals. DPPH has a maximum absorption at a wavelength of 517 nm (Chakraborty and Dhara, 2020).

The ability of a compound to inhibit the DPPH free radical compound depends on its ability to form pairs with the free electrons of the DPPH compound (O'Sullivan, 2015). The antioxidant activity test using DPPH is an initial analysis used to determine the potential of an extract as an antioxidant. The DPPH test has been widely used to screen an extract that is thought to have bioactivity as an antioxidant at small concentrations. Antioxidant activity is related to the ability of a bioactive compound to donate hydrogen (H) to form reduced DPPH (DPPH-H) (Chakraborty et al, 2017).

The IC_{50} value was obtained (**Figure 3 and 4**) which was the largest and greater than the positive control in the form of Vitamin C in the green macroalga Ulva reticulata of 56.144 in the very strong inhibition category (the IC_{50} value of vitamin C was 57.378 in the strong category).

Cytotoxic Test

The cytotoxic test results showed that the best inhibitor in terms of the IC_{50} value was the green macroalgae Ulva reticulata of 92.69 (**Figure 5**), so the anticancer activity test using the DNMT1 enzyme inhibition method was continued only for this green macroalgae.

DNMT1 activity and DNA fragmentation assay

The results of tests carried out on the active fraction obtained for DNMT1 activity showed that green macroalgae could inhibit DNMT1 activity by 51.09% at a concentration of 163 µg/mL and could inhibit DNMT1 activity by 67.60% at a concentration of 93 µg/mL. These results indicate that green macroalgae have excellent potential to inhibit epigenetic mechanisms through DNMT1 activity. Observations of DNA fragmentation in MCF-7 cancer cells showed that the two active fractions of macroalgae extract were not able to trigger apoptosis in MCF-7 breast cancer cells. So it is necessary to carry out more research on observing DNA fragmentation in MCF-7 cancer cells to produce better data. Results number 4 & 5 (**Figure 6**) should be the same if you want to get fragmentation results that show green macroalgae are capable of becoming natural compounds as anticancer

Conclusion

The research results show that there are macroalgae that have antibacterial activity but do not have antioxidant activity (weak category antioxidants), conversely there are macroalgae that have antioxidant activity but weak category antibacterial activity, and none have anticancer activity. Where the macroalgae with the best antibacterial activity is the red macroalgae *Acanthophora spicifera* and the macroalgae with the best antioxidant activity is the green macroalgae *Ulva reticulata*.

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