Potential of Autotroph Microalgae (*Spirulina plantentis*) as Antimicrobial agent

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Abstract. Microalgae is a phytoplankton that has antimicrobial activity. From 20 blue-green microalgae species, 78% have antibacterial activity and 42% antifungal activity, but there are still many other microalgae species that have unknown their activities. One of the microalgae whose unknown antimicrobial potential is *Spirulina plantentis*. The purpose of this study was to determine the antimicrobial potential of autotrophic microalgae (*Spirulina plantentis*) which can be used as antimicrobial compounds from natural materials. This study used the agar diffusion method to see the antimicrobial activity of autotrophic microalgae. Microalgae suspension was tested their antimicrobial activities by using *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The result shows that the MICs of *Spirulina plantentis* suspension against *C. albicans*, *E. coli* and *S. aureus* were 62.5 ppm, 125 ppm and 500 ppm. The MBCs against *C. albicans*, *E. coli* and *S. aureus* were 125 ppm, 250 ppm and 500 ppm. Antimicrobial activity of *Spirulina plantentis* suspension exhibited week against *C. albicans* and *E. coli*, but no antimicrobial activity against *S. aureus*. *Spirulina plantentis* produced inhibition zone against *C. albicans* by 3.9 mm at 125 ppm and 15.6 mm at 250 ppm, against *S. aureus* and *E. coli* by 1.4 mm at 500 ppm and 1.6 mm at 1000 ppm. Against *E. coli* by 8.55 mm at 500 ppm and 12 mm at 1000 ppm.

1. Introduction

Antibiotics are antimicrobial compounds widely used. The Weaknesses of antibiotic are the resistance effects and components which can also provide toxic effects. Thus using of antibiotic began to alternatively use natural antimicrobial agent [9].

Microalgae or phytoplankton are microorganisms which have its own distinctive characteristics. Each of these microorganisms can be distinguished on cell size, color, and place of habitat in the sea other than the cell which is unicellular and can be either photoautotrophic or heterotrophic creatures [10].

Microalgae has long been used as food and organic products, besides it is currently widely used as energy products, waste processing materials, and extracts can also be molluscidal, anti-inflammatory, antifungal, and antibacterial [8].

Microalgae have secondary metabolites which can act as antimicrobials, including alkaloids, phenols, saponins, and quinones [12]. Falch in 1995 stated that 20 blue-green microalgae strains extracted using
hydrophilic solvents produced 54 extracts of which 78% had antibacterial activity and 45% had antifungal activity [8]. Microalgae in cyanobacteria class also contain microcystin compounds which have antimicrobial activity [11].

Many microalgae species haven’t been cultivated caused limited research on antimicrobials in microalgae. Therefore in this study antimicrobial activity will be tested by using heterotrophic microalgae *Spirulina plantentis* which has unknown antimicrobial potential using the agar diffusion method.

**2. Materials and Methods**

Materials and tools used are petri dish, inoculating, bunsen burner, Erlenmeyer, beaker, measuring cup, media bottle, parchment paper, fatty cotton, plastic wrap, heat-resistant plastic, aluminum foil, label paper, pH meter, water bath, incubator, autoclave, centrifuge tube, test tube, test tube rack, stirring rod, spatula, vial, micropipette with tip, freezer, measuring pipette, filler, drop pipette, oven, balance analytic, refrigerators, autotrophic microalgae (*Spirulina plantentis*), 0.9% NaCl sterile solution, 0.36 N H2SO4, 1% BaCl2, distilled water, dimethyl sulfoxide (DMSO), MHA media (Mueller Hinton Agar), SDA media (Sabouraud Dextrose Agar), NA media (Nutrient Agar), NB media (Nutrient broth) ampicillin, ketoconazole, chloramphenicol, *Staphylococcus aureus* ATCC 29737, *Escherichia coli* ATCC 10536, and *Candida albicans* ATCC 10231.

Microalgae suspension will be used for testing the minimum inhibitory concentration (MIC), minimum bacteriocidal concentration (MBC) and anti-microbial activity tested against gram-positive bacteria, gram-negative bacteria, and fungi.

**2.1. Minimum Inhibitory Concentration (MIC) Test**

Autotrophic microalgae (*Spirulina plantentis*) and antibiotics as positive controls made of concentration dilutions 0.975 ppm; 1.9 ppm; 3.9 ppm; 7.8 ppm; 15.6 ppm; 31.25 ppm; 62.5 ppm; 125 ppm; 500 ppm; 1000 ppm; 2000 ppm. NB media that has been made is mixed with microbial tests, by piping microbes into 1000 µL sterile Petri dishes and ± 20 mL of NB media. The mixture of bacteria and media was taken as much as 150 µL into micropole. Furthermore, each microalga, antibiotic (positive control) and DMSO (negative control) were put into series with a concentration of 50 µL into each well. Micropole was closed tightly, then incubated for 24 at 37°C for bacteria and 48 hours at 20°C for fungi. The KHM test results were observed visually, which was indicated by the absence of turbidity. The concentration of the test solution which produces clear color is continued for the KBM test.

**2.2. Minimum Bactericidal Concentration (MBC) Test**

Sterilized sterile Petri dishes poured into NA media and let it solidify. The MIC test results were tested by taking concentrations that did not occur bacterial growth. Then streak using a sterile inoculating into the Petri dishes then incubated for 24 hours at 37°C. MBC concentration showed no microbes colony was formed after incubated.

**2.3. Antimicrobial Activity Test**

Antimicrobial activity test was using the agar diffusion method. The media used for testing were MHA (Mueller Hinton Agar) media for testing *S. aureus* and *E. coli*, SDA media (Sabouraud Dextrose Agar) was used for testing *C. albicans*

Microbial suspension as much as 200 µL was put into sterile Petri dishes using micropipettes. Then in sterile Petri dishes, MHA agar media for bacteria and SDA media for mushrooms as much as 20 mL were added. A media mixture and suspension of bacteria are shaken to mix bacteria with the media. After that, the mixed media was allowed to solidify. On top of the media placed four sterile disc paper. Microalgae suspensions were dissolved using DMSO solvents and tested. In each disc, microalgae suspension was inserted, standard antibiotics as positive controls, and DMSO solvents as negative controls were added 20 µL. The standard antibiotics used for gram-positive bacteria are 10 µg / ampicillin disk. for gram-negative is chloramphenicol 30 µg / disk. and 50 mg/disk of ketoconazole
for fungi [5]. Then incubated 24 hours at 37 °C for bacteria and 24-48 hours for fungi at 20 °C. After incubation, the diameter of the inhibition is measured using a caliper.

3. Result and Discussion
The minimum inhibitory concentration (MIC) is the lowest concentration that causes a growth inhibition of microorganisms. MIC testing was used by dilution method which can be seen visually. Microalgae showed MIC at 62.5 ppm in *C. albicans*, 125 ppm in *E. coli* and 500 ppm in *S. aureus*. Microbial inhibition is shown by a clear color of the test solution, which is caused by microbial growth inhibition.

| Types of microbe       | MIC (ppm) | MBC (ppm) |
|------------------------|-----------|-----------|
| *Staphylococcus aureus*| 500       | 500       |
| *Escherichia coli*     | 125       | 250       |
| *Candida albicans*     | 62.5      | 125       |

The test is continued by taking solution in the micropole which was indicated by the absence of turbidity. The liquid was taken using a sterile inoculating then streak on the NA media. After being incubated for 24 hours at 37°C, it was found at concentrations of 125 ppm, 250 ppm, and 500 ppm respectively *C. albicans*, *E. coli* and *S. aureus* did not form colonies. This concentration is an MBC that can be used for microbial activity testing.

Antimicrobial activity test was used agar diffusion method to determine antimicrobial activity from autotrophic microalgae (*Spirulina plantentis*). The results of antimicrobial activity testing can be seen in Table 2.

| Types of microbe       | Inhibitory Zone Diameter (mm) |
|------------------------|-----------------------------|
|                        | 125 ppm | 250 ppm | 500 ppm | 1000 ppm |
| *Staphylococcus aureus*| 0       | 0       | 1,4     | 1,6      |
| *Escherichia coli*     | 0       | 0       | 8,55    | 12       |
| *Candida albicans*     | 3,9     | 15,6    | -       | -        |

According to Greenwood (1995), antimicrobial activity can be classified into 4 based on the diameter of the inhibitory zone formed. The antimicrobial activity was strong if it produces an inhibitory zone diameter > 20 mm, medium activity 16-20 mm, weak activity 11-15 mm, and no activity 10 mm. So that antibacterial activity of *Spirulina plantentis* showed weak activity against *C. albicans* and *E. coli*, and have no antimicrobial activity against *S. aureus*. *Spirulina plantentis* showed 3.9 mm inhibitory zone against *C. albicans* at 125 ppm and 15.6 mm at 250 ppm, inhibiting *S. aureus* and *E. coli* by 1.4 mm at 500 ppm and by 1.6 mm at 1000 ppm. inhibits *E. coli* by 8.55 mm at 500 ppm and by 12 mm at 1000 ppm.
Negative control (DMSO) did not show antimicrobial activity, while positive controls for gram-positive (ampicillin), gram-negative (chloramphenicol) and fungi (ketoconazole) each provided activity with the inhibitory power of 19.1; 18.9 and 24.7 mm in tested microbes (S. aureus, E. coli, and C. albicans). Ampicillin and chloramphenicol is beta-lactam group which is part of a broad spectrum antibiotic, which can inhibit both grams negative and positive bacteria. In gram-negative bacteria, beta-lactam enters the cell through the porin duct on the outer membrane. Whereas, in gram-positive bacteria, beta-lactam diffuses across the cell wall. Furthermore, beta-lactam binds to penicillin-binding proteins (PBPs) which are enzymes needed to synthesize cell walls (autolysis enzymes). Binding of beta-lactam molecules to PBPs occurs on the surface of the cytoplasmic membrane, thus inhibiting its function and causing cell walls to become damaged and lysis and death occur [4]. Ketoconazole inhibits ergosterol biosynthesis in C. albicans which is the main sterol in mycelium formation [13].

Microalgae produce allelopathic secondary metabolites, which can inhibit the growth of competitors, both microorganisms and other predators [9]. Spirulina plantensis belongs to the class of cyanobacteria. In all cyanobacteria, there is microcystin which is a cyclic heptapeptide [3]. Microcystin extracted from Microcystis aeruginosa is known to have antimicrobial activity against Mycobacterium tuberculosis and nontuberculosis bacteria [11].

Ethanol extract of Spirulina plantensis is contained antimicrobial compounds including alkaloids, saponins, phenols, and quinones. Alkaloid compounds can interfere with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is damaged causing cell death. Saponin compounds work like detergents, which can cause a decrease in voltage between the bacterial cell wall and damage to membrane permeability. Phenol compounds can denaturate cell proteins. The bond of hydrogen formed between phenol and protein causes the protein structure to break down. Cell walls and cell membranes which are composed of proteins also become disrupted by permeability, causing lysis due to an imbalance of macromolecules and ions in the cell. Quinone compounds will bind to proteins cell so that protein loses its function which will cause disruption of cell metabolism [12].

4. References
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