ANTIMICROBIAL ASSAY OF MICROALGAE *OSCILLATORIA* SPP. FROM GUNUNG STONG, JELI, KELANTAN ON *STREPTOCOCCUS AGALACTIAE* AND *AEROMONAS HYDROPHILA*.

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CERTIFICATION

This is to certify that we have read this research paper entitled Antimicrobial Assay of Microalgae *Oscillatoria* spp. from Gunung Stong, Jeli, Kelantan on *Streptococcus agalactiae* and *Aeromonas hydrophila* by Adib Dzafirah binti Mohd Khairul Fidzal, and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course DVT 55204 – Research Project.

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Thank You

DEDICATIONS

I dedicate my dissertation work to my family and friends. My eternal gratitude to my parents, Aiza and Mohd Khairul Fidzal, for their undying love, support and encouragement. To my siblings, Dzaheen and Dzayra, for giving me strength.

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Table of Contents

List of Tables

List of Figures

Figure 12. The results of AST of *Oscillatoria* spp. cultured in soil water on *S. agalactiae*.

List of Appendices

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement on the course DVT 55204 – Research Project.

Oscillatoria spp. is a cyanophyceae commonly found in both fresh and marine waters of Malaysia. It contains bioactive compounds such as flavonoids, phenolics, and terpenoids that are said to have antimicrobial properties. In this study, *Oscillatoria* spp. collected from Gunung Stong, Jeli, Kelantan, was cultured using four different water mediums to observe its growth. Extraction using ethanol and dimethyl sulfoxide (DMSO) was done to perform an antimicrobial assay against *Aeromonas hydrophila* and *Streptococcus agalactiae*. *Oscillatoria* spp. was present in all 15 samples collected from the river. Also, it was observed that the algae was able to grow better in fish tank water and soil water as compared to tap water and distilled water. Next, the antimicrobial assay of the DMSO and ethanolic extract of *Oscillatoria* spp. against *S. agalactiae* and *A. hydrophila* showed bacterial resistance. Multiple factors of the unsuccessful antimicrobial assay includes the usage of raw extract of *Oscillatoria* spp., agar well diffusion error and the unsuitability of procedure respective to its extracts.

Keywords: *Oscillatoria* spp., Aeromonas *hydrophila, Streptococcus agalactiae,* Antimicrobial assay.

ABSTRAK

Abstrak daripada kertas penyelidikan dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan untuk memenuhi sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Oscillatoria spp. ialah cyanophyceae yang dijumpai di air tawar dan air laut di Malaysia. Ia mempunyai bahan antimikrob seperti flavonoids, phenolics, dan terpenoids. Kajian ini termasuklah pengutipan *Oscillatoria* spp. dari Gunung Stong, Jeli, Kelantan. Seterusnya, adalah pengkulturan *Oscillatoria* spp. tersebut menggunakan empat jenis air untuk memerhati ketumbuhan alga. *Oscillatoria* spp. juga telah melalui ekstrasi menggunakan etanol dan dimetil sulfoksida (DMSO), justeru ujian antimikrob telah dijalankan terhadap *Aeromonas hydrophila* dan *Streptococcus agalactiae*. *Oscillatoria* spp. telah dijumpai di dalam setiap sampel yang diperolehi dalam air sungai Gunung Stong. Alga tersebut juga mampu tumbuh dengan lebih baik dalam air tangki ikan dan air tanah, manakala tiada pertumbuhan alga di dalam air paip dan air suling. Seterusnya, ekstraksi alga *Oscillatoria* spp. menggunakkan etanol dan DMSO terhadap *S. agalactiae* dan *A. hydrophila* telah menunjukkan rintangan antimikrob. Faktor-faktor yang menyebabkan kegagalan ujian antimikrob termasuklah penggunaan ekstraksi mentah alga tersebut, penyalahgunaan cara "agar well diffusion," dan ketidaksesuaian prosedur untuk ekstraksi tertentu.

Kata kunci: *Oscillatoria* spp., Aeromonas *hydrophila, Streptococcus agalactiae,* Ujian Sensitiviti Antimikrob.

1.0 INTRODUCTION

Microalgae have been abundant in Malaysian waters, where up to eight significant classes (Chlorophyceae, Rhodophyceae, Charophyceae, Xanthophyceae, Euglenophyceae, Bacillariophyceae, Cryptophyceae and Dinophyceae) were discovered (Phang et al., 2015). Microalgae are hardy microorganisms that can thrive in extreme temperatures and pH levels alongside being photosynthetic. Microalgae have been part of the food chain of fishes and planktons. In Gunung Stong, Jeli, Kelantan, there are three main classes of microalgae discovered, including Cyanophycyeae, Chlorophyceae, and Bacillariophyceae (Merican et al., 2006). Of the class cyanophyceae, *Oscillatoria* spp. was found in the river of Gunung Stong. *Oscillatoria* spp. is characterized by its simple filamentous, myxophyceae structure with non-mucoid, single trichome (Barsanti & Gualtieri, 2014). Its antimicrobial properties include flavonoids, phenolics, and terpenoids (Mansor et al., 2013).

1.1 Research problem

Previous study by Merican et al. (2006) found *Oscillatoria* spp. in Gunung Stong, Jeli, Kelantan. However, the location of where the algae was found was not mentioned. As for the algal culture, there are studies and recommendations on using soil water as a media (Barsanti & Gualtieri, 2014). In addition, there had been reports of algal growth in fish tank due to the abundance of macronutrients present (Cassidy, 2009). Yet, there are lack of study on the usage of tap water and distilled water for algal culture. Mansor et al. (2013) had studied the antimicrobial activity of the *Oscillatoria* spp. against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* by identifying natural bioactive compounds with potential applications as broad-spectrum antibiotics in which a zone of inhibition of *Oscillatoria* spp. was evident against the four bacteria. According to Correia et al. (2011), bioactive compounds have therapeutic capabilities in regulating processes of metabolism alongside having properties of inhibiting receptor activities, inhibiting and inducing enzymes and expression of genes. Cazarin et al. (2022) stated that bioactive compounds are now studied as alternatives for disease prophylaxis and treatment. Nevertheless, there was no study done on the antimicrobial activity of *Oscillatoria* spp. against *Aeromonas hydrophila* and *Streptococcus agalactiae*. Therefore, in this study, it was aimed to determine the availability of *Oscillatoria* spp. throughout the perimeters of the river of Gunung Stong. Secondly, it was to determine the best water media for algal culture, where a comparison of algal culture between soil water, fish tank water, distilled water and tap water was observed in this study. Thirdly, it was to study the antimicrobial properties of *Oscillatoria* spp. against *A. hydrophila* and *S. agalactiae.*

1.2 Research questions

1.2.1 Is *Oscillatoria* spp. widely present in Gunung Stong, Jeli, Kelantan?

1.2.2 Which water media is best to be used to culture algae?

1.2.3 Does *Oscillatoria* spp. have antimicrobial activities against *Aeromonas hydrophila* and *Streptococcus agalactiae*.

1.3 Research hypothesis

1.3.1 *Oscillatoria* spp. is widely present and can be isolated from the water, sediments and algae adhered to rocks off the river in Gunung Stong, Jeli.

1.3.2 The soil water and fish tank water media would be the best for the algal culture.

1.3.2 *Oscillatoria* spp. have antimicrobial activities against *Aeromonas hydrophila* and *Streptococcus agalactiae*.

1.4 Objectives

1.4.1 To isolate *Oscillatoria* spp. from Gunung Stong, Jeli, Kelantan.

1.4.2 To determine the best algal culture media.

1.4.3 To observe the antimicrobial activity of *Oscillatoria* spp. against *Aeromonas hydrophila* and *Streptococcus agalactiae*.

2.0 LITERATURE REVIEW

2.1 Freshwater microalgae in Gunung Stong, Jeli, Kelantan

Microalgae are aquatic, photosynthetic microorganisms composed of simple vegetative structures (Sheath & Wehr, 2015). Microalgae possess chlorophyll pigments allowing photosynthesis to occur. Microalgae can be classified as unicellular, in colonies, pseudo filamentous, filamentous, pseudoparenchymatous structures, parenchymatous forms, or coenocytic, each having their unique characteristics. In Gunung Stong, Jeli up to 25 species of microalgae have been recorded, in which the most common groups were Cyanophyceae, Chlorophyceae, and Bacillariophyceae (Merican et al., 2006). According to Sheath and Wehr (2015), cyanophyceae, also known as blue-green algae, are prokaryotic cells that have no membrane-bound organelles, alongside having unstacked thylakoids, phycobiliprotein pigments, cyanophycean starch and peptidoglycan matrices and walls. Chlorophyceae, or green algae, have chloroplasts with no endoplasmic reticulum, stacked thylakoids, photosynthetic pigments, true starch and cellulose walls. Lastly, the Bacillariophyceae or diatom microalgae have membranous chloroplasts, stacked thylakoids, fucoxanthin, chrysolaminarin and siliceous frustule. Bacillariophyceae can be found in both standing and flowing waters with planktonic and benthic habitats.

2.2 Cyanophyceae *Oscillatoria* spp. and its antimicrobial properties

Oscillatoria spp. is a simple, unbranched filamentous cyanophyceae commonly found in both marine and freshwater as well as on moist soil and rocks. According to Sahoo and Seckbach (2015), it is categorized as a myxophyceae, whose characteristics include the absence of a true cell wall, nucleus, true starch, flagella and phycoerythrin. However, it is halophytic as it can grow in a high salinity environment, up to 80 parts per thousand (ppt). It is also a psychrophile and acidophile, where it can grow in cold and very acidic environments (pH 2.0). Based on Barsanti and Gualtieri (2014), *Oscillatoria* spp. can initiate nitrogen fixation with absence of light, alongside being capable to thrive in environments with low

concentrations of nitrogen and high nitrogen-to-phosphorus ratio (N:P). Its morphology is presented with a single or unbranched trichome with the absence of a mucus sheath (Vuuren et al., 2006).

Sosa-Hernandez et al. (2018) mentioned that due to the evolving issue of antimicrobial resistance (AMR), multiple studies on algal-based bioactive compounds have been conducted to observe their antimicrobial potential. As for *Oscillatoria* spp., Mansor et al. (2013) studied the bioactive compound and antibacterial properties against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa. Oscillatoria* spp. A phytochemical analysis done on *Oscillatoria* spp. by Mansor et al. (2013) showed that it consists of flavonoids, phenolics, and terpenoids which act as an antimicrobial. The role of flavonoids against bacteria includes inhibition of the synthesis of the cell envelope and nucleic acid, inhibition of bacterial toxins and virulence factors, and inhibition of efflux pump (Biharee, 2020). Phenolics play a role in alternating the permeability of cell membrane and the rigidity of cell wall alongside being highly lipophilic (Bouarab-Chibane, 2019). Next, according to Mahizan et al. (2019), the function of terpenoids includes the inhibition of oxidative phosphorylation. Thus, with the presence of antimicrobial properties in the algae, the bacteria mentioned above are susceptible against the ethanolic extract of *Oscillatoria* spp..

2.3 Media for Algal Culture

An artificial environment that was established imitating its natural environment to promote algal growth can define algal culture (Devi & Sahoo, 2015). Soil extract is a recommended medium for algal culture as it naturally provides the algae nutrients, vitamins and trace metal chelators (Barsanti & Gualtieri, 2014) as well as being a natural pH buffer (Belcher & Swale, 1982). Next, fish tank water consists of abundant nutrients which promotes algal growth, such as ammonia, nitrites and phosphate (Cassidy, 2009). However, there is no study or evidence as to the usage of tap water and distilled water for algal culture. Lighting also plays a vital role for algal growth as algae relies on light for photosynthesis. Although daylight is sufficient to promote algal growth, artificial lighting can also be used for indoor algal culture. Imitating long day countries such as Malaysia, the lighting supplementation

can be provided with 14 hours: 10 hours (Light to Dark Ratio, L:D) to 16 hours: 18 hours (L:D) (Andersen, 2005). However, Kuack (n.d.) mentioned that 24 hours of light supplementation allows an increase in production rate of algae.

2.4 *Aeromonas hydrophila*

Commonly found in brackish and fresh water, *Aeromonas hydrophila* is a gram-negative, rod-shaped, facultative bacteria that causes food- and water-borne illnesses in animals and humans. *A. hydrophila* is one of the main organisms found in fishes and shellfishes (Lampila & McMillin, 2012). Based on Quinn et al. (2011), virulence factors produced by *A. hydrophila* include adhesins, exoenzymes, haemolysin and enterotoxins. Fishes infected with *A. hydrophila* would show clinical signs such as ulcerations on the skin and fin rot. Moreover, in fishes with severe stress and physical trauma, this bacteria would cause secondary bacterial infection, leading to motile aeromonad septicemia (Plumb & Hanson, 2011). *A. hydrophila* infection can result in up to 80% of mortality rate in young fishes, while it causes up to 35% mortality rate in older fishes (Lio-Po et al., 2001). Furthermore, it is considered as a zoonotic disease transmitted through ingestion of infected fishes, causing diarrhoea and progresses into septicemia, meningitis and endocarditis in humans.

Bacterial culture of *A. hydrophila* on a nutrient agar or Tryptic Soy Agar (TSA) would show cream-colored, rounded and shiny colonies. According to Odeyemi and Ahmad (2017), *A. hydrophila* is completely resistant to Ampicillin, Sulfamethoxazole and Trimethoprim, yet it is susceptible to Tetracycline, Gentamicin and Oxytetracycline.

2.5 *Streptococcus agalactiae*

S. agalactiae is a gram-positive, obligate, beta-hemolytic bacteria that is epidemic in fishes cultured in brackish water and net cages (Plumb & Hanson, 2011). Commonly known as group B streptococcus, it can produce virulence factors such as beta-hemolysin, cytolysin and CAMP factor (Zastempowska et al., 2022). Infection of *S. agalactiae* in fishes would cause unilateral or bilateral exophthalmia alongside

petechial haemorrhage and congestion of the fin base and operculum. Based on Lio-Po et al. (2001), infected fishes would also show erratic swimming, corneal opacity and presence of skin ulcers. Consequently, it can progress into meningoencephalitis and Streptococcosis septicemia. Thus, it causes up to 75% mortality rate in fishes. Humans infected with *S. agalactiae* would show symptoms such as sepsis, pneumonia, meningitis and osteomyelitis (Geng et al., 2011). The diagnostic workup of *S. agalactiae* through bacterial culture on TSA mixed with 0.5% glucose would show small, yellowish, translucent, rounded and slightly raised colonies. According to Geng et al. (2011), *S. agalactiae* has a sensitivity against antibiotics including amoxicillin, chloramphenicol and vancomycin, while it is resistant against gentamicin, penicillin and streptomycin.

2.6 Ethanol and DMSO for algal bioactive compound extraction

Bioactive compounds are nutrients, categorized either as terpenes and terpenoids, alkaloids or phenolic compounds. *Oscillatoria* spp., known as the algal biome of the freshwater region, contain phenolic compounds (Sosa-Hernandez et al., 2018). Extraction was done to extract the bioactive compounds of the algae. One factor that should be considered was the solvent used for bioactive compound extraction, where the extraction plays a role in transferring bioactive compounds of the algae from river water into the solvent (Chen & Wang, 2017). Therefore, the solvent should have good solubility and be volatile to allow efficient extraction. Previously, Mansor et al. (2013) used methanol as the solvent for *Oscillatoria* spp. extraction. However, no study was done on the ethanolic and DMSO solvent used in *Oscillatoria* spp. extraction. Based on Farraj et al. (2019), it is best to use ethanol as an extraction solvent considering that it is able to extract the active compounds from plants, hence it maximizes the antimicrobial activities of the algae. Borges et al. (2020) also encouraged the usage of ethanol extraction for the study of an antimicrobial activity of a compound as it can efficiently extract phenolic compounds. As for DMSO, it is highly polar and water miscible, which allows solubility of polar and nonpolar molecules alongside it easily crosses cell membranes of animal (Capriotti & Capriotti, 2012).

3.0 MATERIALS AND METHODS

3.1 Sample collection

The algae was collected from the river of Gunung Stong located in Jeli, Kelantan with three methods. The first method, the algae adhered to rocks was scraped and collected into a sterile container. The second method, using a pipette, the algae was collected midstream of the river and stored in the sterile container. For the third method, the sedimentations and sands of the river were collected directly using the sterile container. In total, there were 15 sterile containers filled with algae.

3.2 Sample preparation

Algal identification

The algae was placed onto a clean glass slide and viewed under a light microscope using magnifications of 10x, 40x and 100x. The morphology of the algae was then identified by referring to Agriculture and Agri-Food Canada's Algae Identification Field Guide (2006).

Algal culture

All samples of the algae collected from Gunung Stong were accumulated and combined for algal culture. The algal culture was done using four different types of water media, including tap water, distilled water, soil water and fish tank water. The tap water and distilled water was obtained from the Aquatic Laboratory of Faculty of Veterinary Medicine (FPV), University Malaysia Kelantan (UMK). The fish tank water was also collected from a fish tank in the Aquatic Laboratory of FPV UMK. As for the soil water, unfertilized soil was collected in FPV UMK and added into distilled water. The solution was then boiled at 120°C and left to be cooled down. All of the water mediums were then added into a plastic tank with dimensions 18.0 cm (length), 11.0 cm (width) and 9.5 cm (height). Duplicates of each media were made

where each tank consists 650 ml of media. Then, the algae were distributed accordingly into each tank containing the water medium. Four of the tanks with different water media were then placed for two weeks under sunlight, while four other tanks were placed under artificial light for 24 hours daily respectively.

Algal extraction

The algae were collected from the respective cultures and placed onto a petri dish to be dried at 55°C for 24 hours. Then, the dried algae were scraped into a mortar and pestle and ground into fine powder. The weight of the powder was measured, then placed into 10.0 ml of ethanol solvent and shaken in the shaking incubator under 250 rpm for 24 hours. After that, the solvents were then placed into a petri dish and dried at 40°C for 24 hours. Lastly, the dried extracts were placed into a 3.0 ml of ethanol and dimethyl sulfoxide (DMSO) respectfully and chilled for antimicrobial assay. The concentration of the algal extract for both ethanol and DMSO were 300 mg/ml.

3.3 Antimicrobial assay

Firstly, inoculum of *A. hydrophila* and *S. agalactiae* obtained from archived samples of Faculty of Veterinary Medicine UMK were produced and compared with 0.5 McFarland Standard. Next, the inoculum of each bacteria were streaked onto 2 Mueller Hinton agar (MHA) plates respectively. The antimicrobial assay is performed using the agar well diffusion method. Using a cork borer of 0.9 mm in diameter, four holes were punched aseptically through the MHA. 150 µl of ethanol extract, DMSO extract, ethanol and DMSO were added into the wells, followed by the placement of oxytetracycline antibiotic disc onto the surface of the agar. Oxytetracycline antibiotic discs were placed as a positive control. It was then labeled respectively, where A was the ethanolic extract, B was ethanol, C was DMSO extract, D was DMSO and E was Oxytetracycline. The agars were then incubated at 34° Celsius for 24 hours. Lastly, the agar plates with zones of inhibition were observed and measured. The four MHA agar plates were presented respectively in Figure 1, Figure 2, Figure 3 and Figure 4.

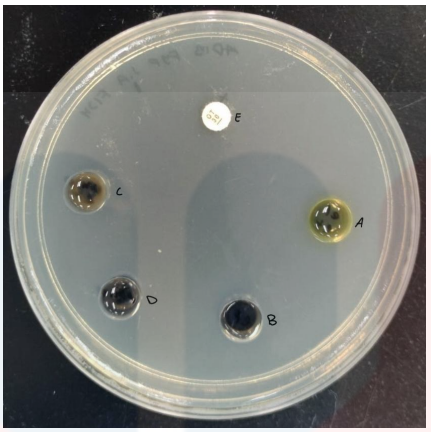

Figure 1. Antimicrobial Sensitivity Test (AST) of *Oscillatoria spp.* cultured in fish tank water done on *Streptococcus agalactiae.*

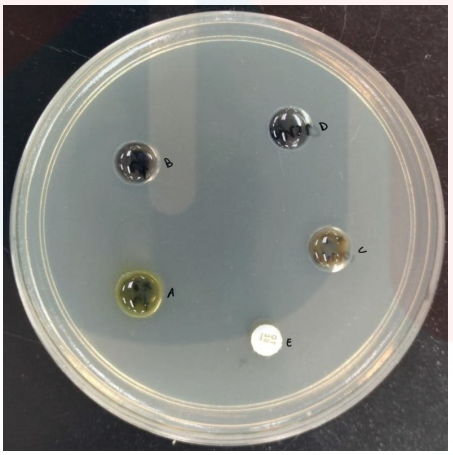

Figure 2. Antimicrobial Sensitivity Test (AST) of *Oscillatoria spp.* cultured in fish tank water done on *Aeromonas hydrophila.*

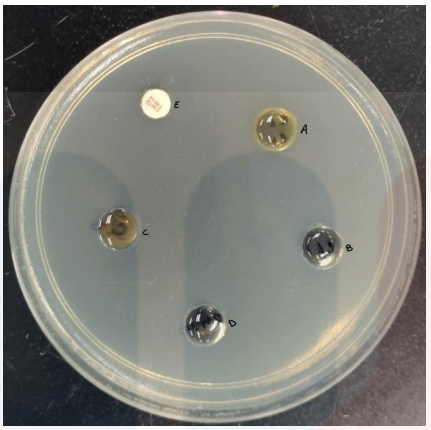

Figure 3. Antimicrobial Sensitivity Test (AST) of *Oscillatoria spp.* cultured in soil water done on *Aeromonas hydrophila.*

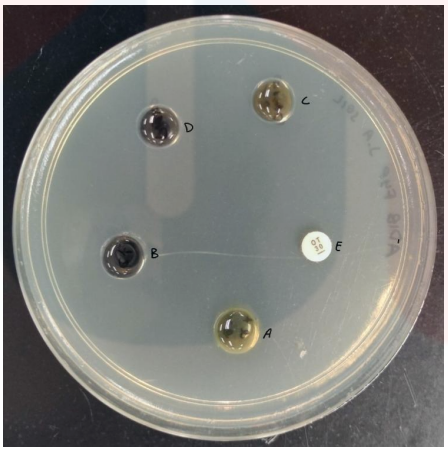

Figure 4. Antimicrobial Sensitivity Test (AST) of *Oscillatoria spp.* cultured in soil water done on *Streptococcus agalactiae.*

4.0 RESULTS

4.1 Algal identification

Under the microscope with magnification of 40x, 100x, and 400x, the morphology of the algae observed was a straight and unbranched algae. In addition, there was no mucus sheath present.

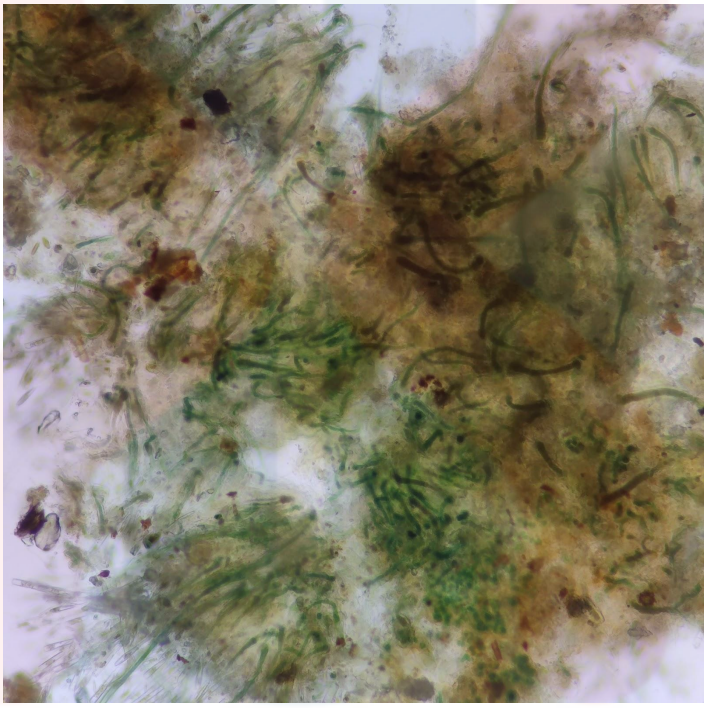

Figure 5. *Oscillatoria* spp. present in a drop of river water from Gunung Stong, Jeli, Kelantan under the

microscope with 40x magnification.

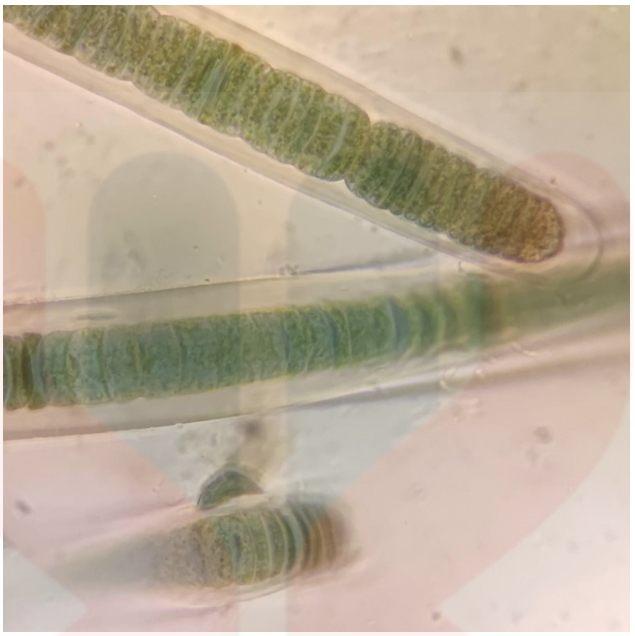

Figure 6. The observation of *Oscillatoria* spp. under the microscope with 400x magnification.

4.2 Algal culture

The growth of algae was determined grossly, based on the changes of the aquarium tank as well as the gross changes of the algae present. Firstly, the algae exposed to sunlight with 12 hours: 8 hours (Light to Dark Ratio, L:D) grew better compared to the algae that was exposed to 24 hours of artificial light. Furthermore, amongst the algae exposed to sunlight, the algae cultured in fish tank water media had a better growth rate, followed by the algae cultured in soil water. The walls and the water of the aquarium tank containing the algae in the fish tank water media turned green which indicated algal growth as shown in Figure 7. As for the soil water, there was presence of algae adhered to the surface of the soil as presented in Figure 8. Unfortunately, there was no algal growth in tap water and distilled water media due to no changes to the water and algae.

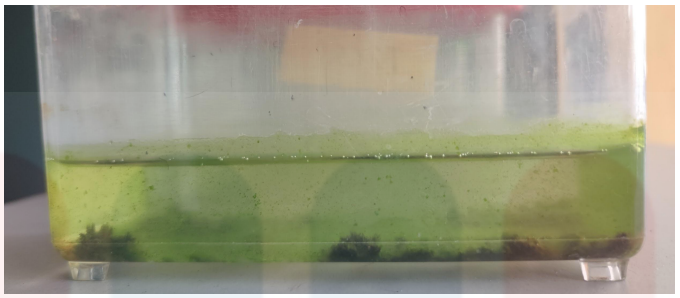

Figure 7. The algal culture using fish tank water.

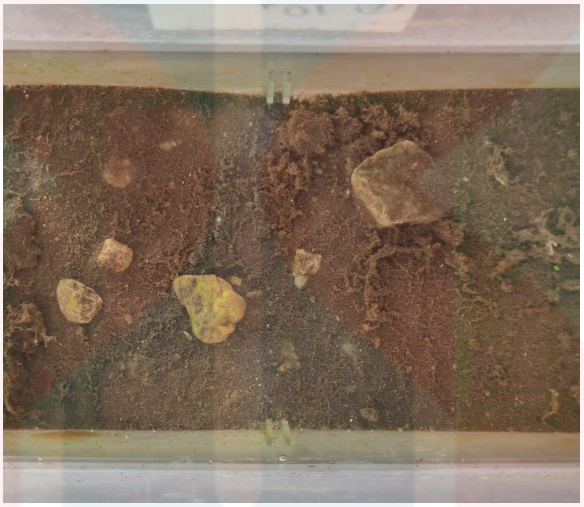

Figure 8. The algal culture using soil water.

4.3 Antimicrobial assay

For antimicrobial assay of *A. hydrophila*, firstly, Figure 9 shows the results for the *Oscillatoria* spp. cultured in fish tank water, the zone of inhibition of the ethanolic extract was 15.0 mm, while for ethanol, the zone of inhibition was 13.0 mm. There was no zone of inhibition present for DMSO extract and DMSO. Moreover, the zone of inhibition for the Oxytetracycline disc was 35.0 mm.

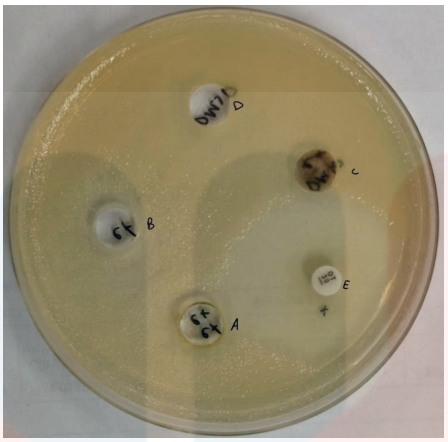

Figure 9. The results of AST of *Oscillatoria* spp. cultured in fish tank water on *A. hydrophila*.

Next, it was observed, as shown in Figure 10, that the zone of inhibition of the ethanolic extract of *Oscillatoria* spp. cultured in soil water against *A. hydrophila* was 14.0 mm. For ethanol, the zone of inhibition was 13.0 mm. There was also no zone of inhibition present for the DMSO extract and DMSO. Lastly, the zone of inhibition for the Oxytetracycline disc was 33.0 mm. The zones of inhibitions against *A. hydrophila* comparing *Oscillatoria* spp. cultured in fish tank water and soil water were stated in Table 1.

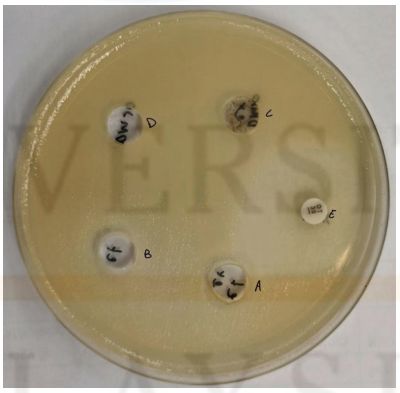

Figure 10. The results of AST of *Oscillatoria* spp. cultured in soil water on *A. hydrophila*.

	Zone of Inhibition (mm)	
	Fish Tank Water	Soil Water
Ethanolic extract	15.0	14.0
Ethanol	13.0	13.0
DMSO extract	0	0
DMSO	0	0
Oxytetracycline	35.0	33.0

Table 1. Zone of inhibitions recorded on AST of *A. hydrophila.*

For the antimicrobial assay of *S. agalactiae,* represented by Figure 11, the zone of inhibition of the ethanolic extract the *Oscillatoria* spp. cultured in fish tank water and ethanol was 13.0 mm. No zone of inhibition was observed for the DMSO extract and DMSO. The zone of inhibition for the Oxytetracycline disc was 33.0 mm.

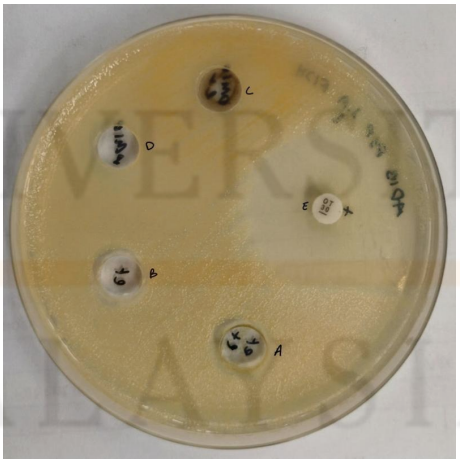

Figure 11. The results of AST of *Oscillatoria* spp. cultured in fish tank water on *S. agalactiae*.

Furthermore, for the *Oscillatoria* spp. cultured in soil water against *S. agalactiae*, it was shown in Figure 12. The ethanolic extract and ethanol showed that the zone of

inhibition was 13.0 mm. Also, no zone of inhibition was present for DMSO extract and DMSO. Lastly, the zone of inhibition for the Oxytetracycline disc was 33.0 mm. The results of the antimicrobial assay comparing *Oscillatoria* spp. cultured in fish tank water and soil water against *S. agalactiae* were noted in Table 2.

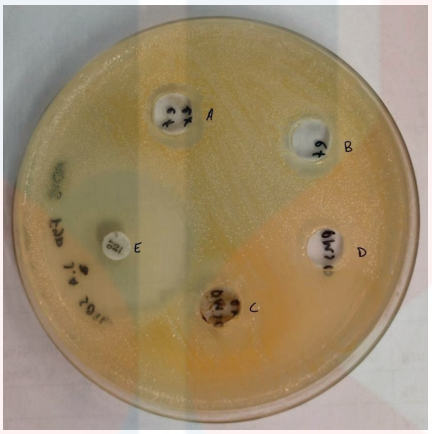

Figure 12. The results of AST of *Oscillatoria* spp. cultured in soil water on *S. agalactiae*.

	Zone of Inhibition (mm)	
	Fish Tank Water	Soil Water
Ethanolic extract	12.0	13.0
Ethanol	12.0	13.0
DMSO extract	0	0
DMSO	0	0
Oxytetracycline	33.0	33.0

Table 2. Zone of inhibitions recorded on AST of *S. agalactiae.*

5.0 DISCUSSION

For this study, there was the presence of *Oscillatoria* spp. in each sterile container that was filled with samples of the river of Gunung Stong, Jeli. This indicated the availability of the algae throughout the perimeters of the river. Based on Okogwu and Ugwumba (2009), increased levels of phosphate due to increased eutrophication and anthropogenic activities promotes cyanophyceae growth. Canonical correspondence analysis (CCA) also showed that cyanophyceae growth relies on the parameters of pH, Biochemical Oxygen Demand (BOD), dissolved oxygen, water velocity and width. Unfortunately, a water analysis of the river of Gunung Stong was not done to identify its condition which promotes *Oscillatoria* spp. growth.

The second objective was to study the best method for algal culture. Firstly, the algal culture using artificial light was unsuccessful as compared to algal culture under sunlight. For this study, based on the value obtained by the manufacturer, the artificial light supplies white light with a light temperature of 6500K which should imitate daylight. However, there was no light intensity recorded. Although artificial lighting allows a decrease in irradiance level fluctuations, due to light to dark ratio (L:D) from sunlight, the type of artificial lighting is also important (Blanken et al., 2013). Therefore, light-emitting diodes which emit red and blue light should be used as artificial lighting for light energy supplementation of algae growth (Brzychczyk et al., 2020). Andersen (2005) also suggested coloured fluorescent tubes or photodiodes which provide illumination of red-light.

Secondly, the algal culture of *Oscillatoria* spp. in tap water and distilled water was unsuccessful. Based on Andersen (2005), algae requires macronutrients such as nitrogen and phosphorus to thrive and survive, usually in a ratio of 16 Nitrogen: 1 Phosphorus (Nitrogen to Phosphorus ratio, N:P). In soil water, it is considered as a natural source of nitrogen and phosphorus, while in fish tank water, it commonly has accumulated nitrogen and phosphorus that comes from waste product and food waste of the fishes. In comparison, there is absence of the required nutrients in distilled water and tap water, thus causing the death of the algae.

As for the antimicrobial assay results for *S. agalactiae* and *A. hydrophila*, the DMSO extract and ethanolic extract of *Oscillatoria* spp. in both soil water and fish tank water had no zone of inhibitions. The ethanolic extract of *Oscillatoria* spp. in fish

tank water had the same width of zone of inhibition of ethanol, thus it was invalid. There are several factors that may affect the study. Firstly, there may be human error and mishandling during the extraction of the algae. During the administration of ethanolic extract of *Oscillatoria* spp. and ethanol into the respective wells, it was observed that the liquids disperse around its well. It may cause an error to the study where the dispersed liquid affected the growth of bacteria around the well. A repeated study should be conducted using a disc diffusion method, which involves saturating the disc with the solvents, which are ethanolic extract, DMSO extract, ethanol and DMSO, then administered onto MHA similarly to antibiotic disc placements. Further and repeated study of the antimicrobial assay of *Oscillatoria* spp. should be done properly to minimize error and to further confirm the resistance of *S. agalactiae* and *A. hydrophila.*

Furthermore, a phytochemical analysis conducted by Mansor et al. (2013) found an antimicrobial property known as phenolics in *Oscillatoria* spp. Based on Bouarab-Chibane (2019), phenolics are highly lipophilic and its mechanism of action includes alternating the permeability of cell membrane and the rigidity of cell wall. According to Koivikko (2008), the concentration of phenol in algae depends on the habitat, time of harvest, as well as the light and nutrient supplementation availability. Based on Machu et al. (2015), the type of solvent used for extraction alongside the different extraction methods respective to its solvent may also determine the total phenolic content extracted. According to El-Aty et al. (2014), phenolics were highly soluble in highly polar solvents such as methanol. Methanol can dissolve phenolics better than ethanol as it has smaller and flexible aliphatic fragments as compared to ethanol, thus it can surround phenols with substituted carbons inside their aromatic ring (Galanakis et al., 2012). On the other hand, ethanol has a longer aliphatic fragment, thus affecting the efficacy of phenolic solubility of *Oscillatoria* spp. Other than that, DMSO claims to be polar aprotic solvent and polyfunctional which is able to solubilize both polar and apolar substances (Sricharoen et al., 2015). Unfortunately for this study, the extraction procedure referred from a study by Mansor et al. (2013) which extracts *Oscillatoria* spp. using methanol was unsuitable for ethanolic extraction although both ethanol and methanol are from the same alcohol group. For phenolic extraction of *Oscillatoria* spp., Sricharoen (2015) also studied and concluded that the most efficient phenolic extraction using DMSO was

through the Microwave Assisted Extraction (MAE) method as it produced the highest extraction yield by being an antioxidant and scavenge hydroxyl free radicals.

However, this study only included the ethanolic extraction of *Oscillatoria* spp. without phenolic extraction. Al-Katib and Amin (2020) had conducted a study comparing the antibacterial activity of raw ethanolic extract and phenolic extract against *Staphylococcus aureus, Bacillus subtillus, Pseudomonas aeroginosa* and *Escherichia coli*. As a result, there was no zone of inhibition observed for both gram-positive and gram-negative bacteria using raw ethanolic extract, while there was a zone of inhibition observed using the phenolic extract. Therefore, raw ethanolic extract is insufficient to produce antimicrobial activities as compared to a phenolic extract. To obtain the phenolic extract from the raw ethanolic extract, based on Al-Katib and Amin (2020), the raw ethanolic extract was evaporated and underwent acid decomposition. Then, ethyl acetate was added and the phenol was concentrated through the rotary evaporation to obtain its phenolic extract. Another study by Alara et al. (2020) extracted phenol from raw extract by adding 0.2 ml Folin-Ciocalteu phenol reagent into 100 µl of extract, After, it was incubated for 5 minutes in a dark room, followed by addition of 0.6 ml of 0.2 mM sodium carbonate solution and incubation for 2 hours. The phenolic extraction is then confirmed by using a spectrophotometer. The Folin-Ciocalteu reagent confirms the phenolic is present when there is molybdenum-tungsten blue produced at 760 nm when observed spectrophotometrically (Malta et al*.,* 2014). Limitations of this study include the unavailability of reagents, such as ethyl acetate and Folin-Ciocalteu reagent, that does not allow us to proceed with phenolic extraction. Without these reagents, the phenolic extraction and results may not be accurate and significant for the antimicrobial assay of *S. agalactiae* and *A. hydrophila*.

Other than that, considering that the content of phenols in algae are only 20% maximum of the dry weight of algae (Balboa et al*., 2013)*, the concentration of *Oscillatoria* spp. in both ethanolic and DMSO extract of this study (300 mg/ml) may be insufficient to proceed with antimicrobial assay as the total phenolic content in this study was only 60 mg/ml. Lastly, there may also be a possibility that the bacteria may be resistant to the antibacterial properties of *Oscillatoria* spp. According to Ajaz et al. (2004), bacterial resistance towards phenol may be due to intrinsic characteristics of the bacteria, mutation or plasmid acquired by the bacteria.

6.0 CONCLUSION AND RECOMMENDATION

In conclusion, *Oscillatoria* spp. is widely available within the perimeter of the river of Gunung Stong, Jeli, Kelantan. Next, fish tank water and soil water is recommended for algal culture. Thirdly, AST of the zone of inhibition using DMSO extract and ethanolic extract of *Oscillatoria* spp. showed bacterial resistance with the absence of a zone of inhibition. The first and second hypothesis could be accepted, while the third hypothesis is rejected.

As a recommendation, algal culture should be done using red or blue light to provide proper light supplementation to the algae. Next, further study using phenolic extract of *Oscillatoria* spp. would produce a better antimicrobial assay againsts *S. agalactiae* and *A. hydrophila.*

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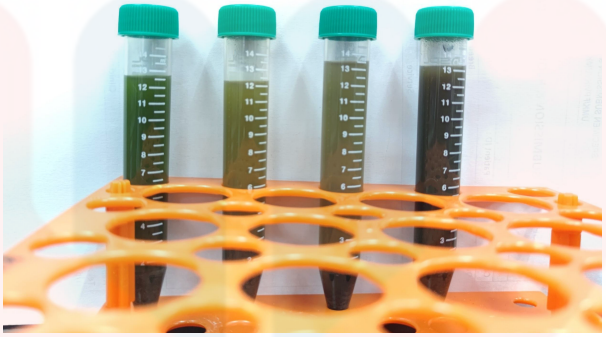
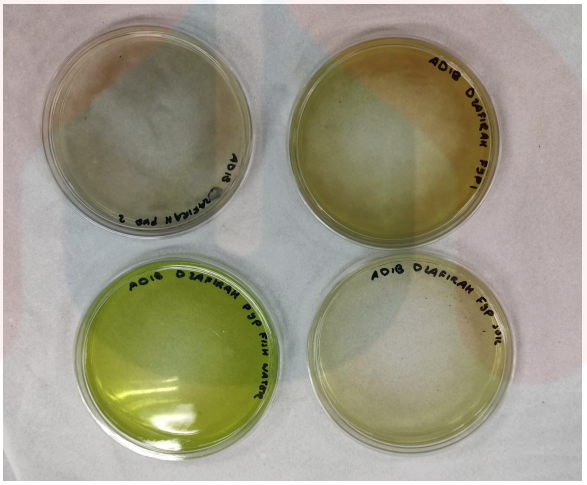
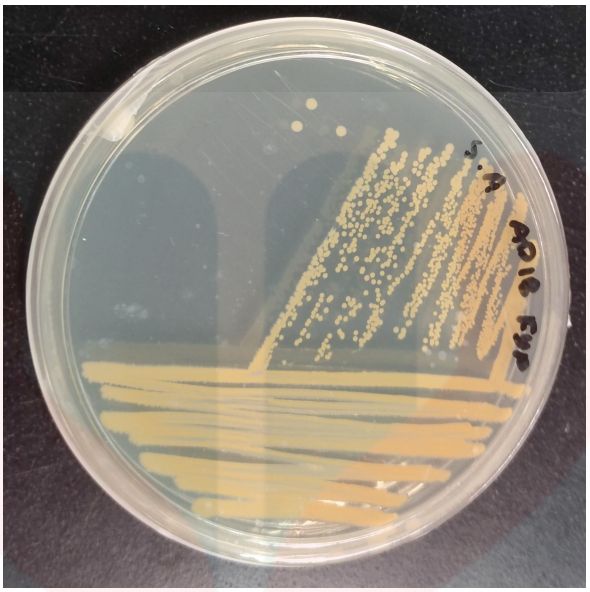
Appendix A



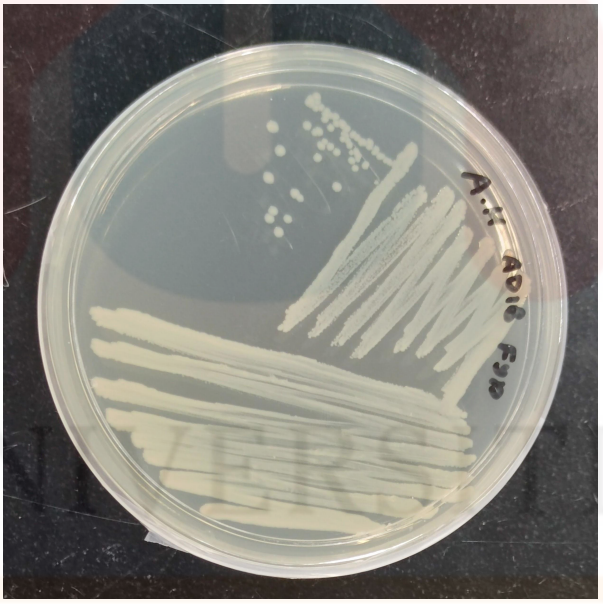
Appendix A.1: Algal extract using ethanol.



Appendix A.2: Algal extract using DMSO.



Appendix A.3: **Bacterial culture of *S. agalactiae.***



Appendix A.4: **Bacterial culture of *A. hydrophila.***