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Isolation and Identification of Marine Algae (*Oscillatoria* Sp.) From Kuantan Coastal Region Malaysia and Studies on Their Bioactive and Antibacterial Properties

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Abstract: Marine algae is an incredible group of prokaryotic organisms that produce a variety of industrially and clinically important secondary metabolites. In this study explore the marine cyanobacteria (*Oscillatoria* Sp.) was isolated from the Kuantan coastal area and on their anti-microbial activity and bioactive compounds. The ethanolic *Oscillatoria* Sp. extract was subjected to GC-MS analysis to determine the bioactive compounds. Also the ethanolic extract used for antibacterial activity by disk diffusion assay using four different types of pathogenic bacteria such as *P. aurogenosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli.* The zone of inhibition was observed and measured in the experimental petri plates. The antibacterial activity showed that the ethanol extract of *Oscillatoria* Sp. was effective against *P. aeruginosa* the 16mmzone of inhibition was observed. The microalgae crude extract was highly significant in against tested gram-positive bacteria. Moreover, GC-MS analysis data shows the present of several compounds such as phenol derivatives and fatty acid derivatives in the sample. The compounds are mainly responsible for controlling the gram positive bacteria.

Key words: Marine algae · Antibacterial · GC-MS · Phytochemicals · *Oscillatoria* Sp

INTRODUCTION

Natural product has supplied significant source of therapies in order to cure acute and chronic diseases of mankind. The plant and their derivatives are acting as functional food that contains one or more active ingredients that provides health benefits to living things [1, 2]. Phytochemicals in herbal plants and algae are preventing the oxidative stress, mutation and other communicable diseases. Microalgae are a vital source of food and medicinal compounds that they supply a variety of bioactive compounds such as alkaloids, phenolics, flavonoids, vitamins such as A, B1, B2, B6, niacin and C and are rich in iodine, potassium, iron, magnesium and calcium [3]. Hence the commercially cultivated microalgae including cyanobacteria are marketed as nutritional supplements. The microalgae *Chlorella* Sp. and *spirulina* Sp. rich in vitamin C, beta carotene, phenolics and proteins [4]. Some of the algae species have a rich source of oils in which high levels of unsaturated fatty acids, arachidonic acid. It mainly uses for biofuel and bioethanol production [5]. Among them algae contains essential omega-3 fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoicacid (EPA) biologically active compounds are highly involved in the therapeutic function. The micro algae has emerged in recent years as a popular source of secondary metabolites, it is used for treating various endemic diseases [6]. *Oscillatory* Sp. is an aerobic photosynthetic cyanobacteriam. It mainly involved in the nitrogen fixation mechanisms. Oscillatoria Sp. produces anatoxin and homoanatoxin, it is harmful toxin product. The compound is adverse effect to the human system [7].

In this present study was carried out novel marine microalgae identified from the Kuantan coastal region. The identified marine microalgae was isolated and mass

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ultured in lab scale. Further cultured *Oscillatoria* Sp. was analyzed their phytochemical constituents and antimicrobial efficacy.

MATERIALS AND METHODS

Sample Collection and Identification: The marine algae sample *Oscillatoria* Sp. was collected from Kuantan, East Coastal region of Peninsular Malaysia. The sample was collected by using 0.50µm mesh size of plankton net which made of bolting silk cloth. The collected sample was cultured using BG-11 nutrient medium under fluorescent light at 25±2°C. The cultured micro algae was identified by microscope [8].

Extraction and Preliminary Phytochemical Analysis: The mass cultured algae was separated from the culture medium by centrifugation at 5000rpm for 15min and their pellets were dried at 55°C for 24 hours. The dried *Oscillatoria* Sp. sample was ground into a fine powder by using mortar and pestle. Powdered sample was immersed in methanol solvent (10 ml) and placed in a shaking incubator for 24 hours at 25°C. The solvent extracts were concentrated under reduce pressure at 40ºC by vacuum evaporator. The concentrated sample used for phytochemical assays [8]. The dried extracts was resuspended in 3 ml of solvent to make into different concentration (50, 75 and 100mg/ml) and preserved at 4°C till further use of antimicrobial assays [9].

GC-MS Study: The gas chromatography coupled with mass spectrometry is used for the detection of secondary metabolites. They were analysed in Agilent 7890A Gas Chromatography (GC) system equipped with a HP-5 column (30m×250µm ID, 0.25µm filmthickness, Agilent 122-0132) and agilent 5975C inert Mass Spectrometer Detector (MSD)with triple-axis detection using helium as carrier gas. The flow rate of 1.50 ml/min; injector and column oven temperature 280°C and 80°C; injection mode is split the ratio 20:1.The chemical constituents were identified by a computer-based library search with retention indices and comparing their visual interpretation of the mass spectra with the NIST (National Institute of Standards and Technology) and fiehn mass spectral libraries.

Antibacterial Studies: The *Oscillatoria* Sp. extract was tested with different bacterial cultures using disc diffusion method by Kirby - Bauer technique [10]. Petri plates were prepared with 20 ml of nutrient agar. The tests were conducted using three different concentrations of the methanol extract of *Oscillaria Sp.*. (50 mg, 75mg and 100mg/ml). Antibacterial activities from green algae were compared with commercial antibiotics streptomycin which used as control. Sterilized discs were made from Whatman No. 1 filter paper and extract different concentration were loaded into the disc and dried completely under sterile conditions. The *Oscillatoria Sp.* extracts were screened for antibacterial activities against the Gram positive pathogenic bacteria such as *S. aureus* and *B. Subtilis* and for Gram negative such as *E. coli* and *P. aeruginosa*. The bacterial culture was uniformly inoculated by using sterile L- glass rod and the extract loaded discs were placed on the seeded plates by using a sterile forceps. After that, inoculated plates were incubated at 35°C for 24 hours. Then the plates were observed andmeasuredthe zone of inhibition by using a scale. Clear inhibition zones around the discs indicate the presence of antimicrobial activity.

Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) was performed by the serial dilution technique using 96-well microplates. A series of different concentrations of *Oscillatoria Sp.* ethanolic extract which were 10, 20, 30 and 40µg/ml were prepared. Themicroplates were incubated for 24 h at 37°C. The tests were compared with the ethanol as negative control. The optical density of each well wasmeasured at a wavelength of 530 nm using micro plate reader [11].

RESULTS

Fig. 1 shows the identified marine micro algae species in the Kuantan coast region and micro algae flask scale production. Phytochemical analysis of marine algae *Oscillatoria* Sp. extract was identified various chemical constituents such as flavonoids, phenolics, carbohydrates, proteins and terpenoids (Table 1). The presence of these phytochemical compounds is effectively acting as antibiotic agents against harmful human pathogens. It was found that the methanolic extract of *Oscillatoria* Sp. has some potential bioactive compounds are inhibit the growth of pathogenic bacteria. Also the GC-MS of methanolic extract of the micro algae sample has been studied two important clusters of phytochemicals especially phenolics and fatty acids. Similarly other medicinal important bioactive compounds are detected from *Oscillatoria* Sp. solvent extract such as flavonoids and esters. The major chemical constituents of are summarized in Table 2 (Fig. 2).

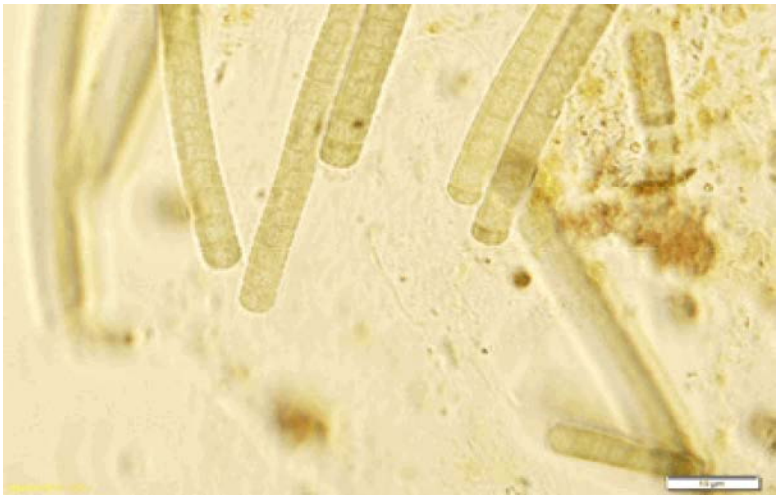

Fig. 1: The microscopically identified marine algae *Oscillatoria* Sp. from the Kuantan coastal region, Malaysia

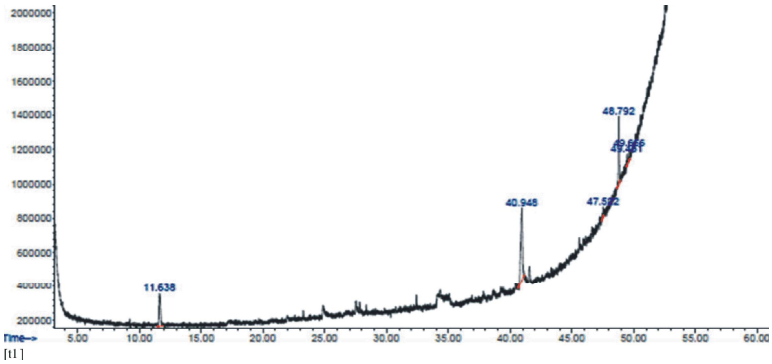

Fig. 2: GC-MS results of marine algae ethanolic extract

Table 1: Preliminary confirmation of secondary metabolites from methanol extract of *Oscillatoria sp*

No	Chemical Constituents	Results obtained
1	Alkaloids	+
2	Flavanoids	+
3	Saponins	-
4	Tannins	-
5	Essential oils	+
6	Phenols	+
7	Sterols	-
8	Carbohydrates	+
9	Proteins	+

Table 2: GC-MS identification of biological active compounds in ethanolic extract of *Oscillatoria sp*

Compounds name	Compound nature	Molecular formula	Retention Time (min)
4,6-bis (diethylamino)-1,3,5-triazine	Triazine derivative	$C_3H_3N_3$	26.544
5-Nitro-3-cyano-2(1H)-pyridone	Pyridone derivative	$C_5H_4NH(O)$	32.401
Triphenylphosphine oxide	Phenyl derivative	$C_{18}H_{15}OP$	40.946
1,4 benzenedicarboxamide	Phathalic acid derivative	$C_6H_4(COOH)_2$	47.525
Benzenemethanol	Benzyl alcohol derivative	C_7H_8O	49.460
trimethylsilyl ether	Ether derivative	$C_5H_{12}OSi$	49.927
2-Methyl-7-phenyl indole	Indole derivative	C_8H_7N	57.984
Acetic acid	Fatty acid	$C_2H_4O_2$	58.026
Silicic acid	Carboxylic acid	$Si(OH)_4$	58.292
9-Oxo-9,10-dihydro acridine-4-yl	Acridine derivative	$C_{13}H_9N$	59.823
2-[p-Fluorophenyl]-6-methylcinchoninic acid	Cinchoninic acid	$C_{10}H_7NO_2$	60.003

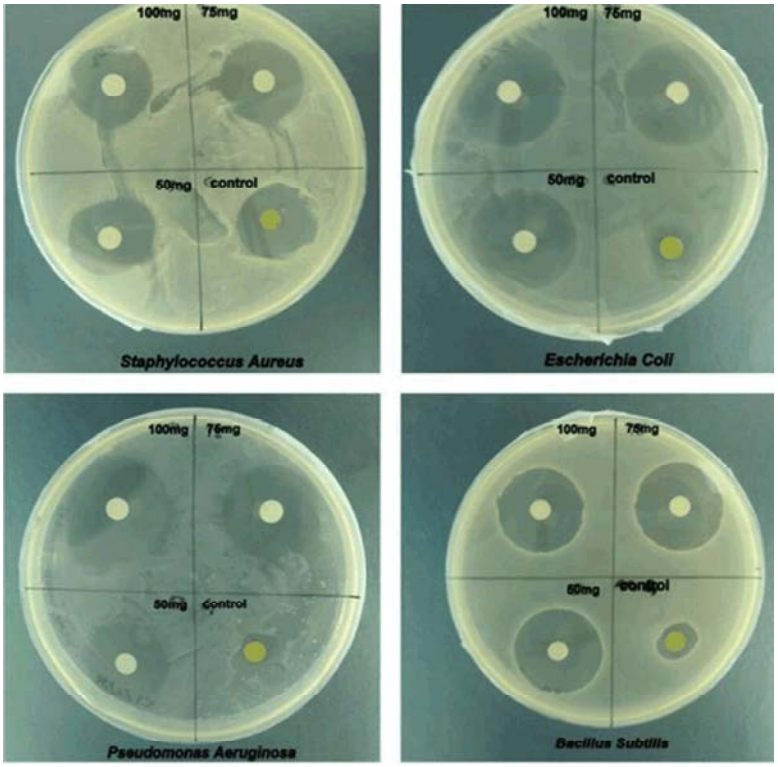

Fig. 3: The antibacterial activity by disc diffusion assay using ethanolic extract of marine algae

The antibacterial results of this study showed that the *Oscillatoria* sp.extract (100mg/ml) showed highest zone of inhibition (22mm) against *P.aeruginosa* followed by *E. coli* (20mm), *B. subtilis* (19mm) and *S. aureus* (15mm) which the gram positive bacteria are more susceptible than gram negative bacteria to methanolic extract of *Oscillatoria* sp. The MIC values of *Oscillatoria* Sp. methanolic extract showed 30µg/ml concentration against *B. subtilis* and *P. aeruginosa* (Fig. 3). This minimal concentration is preferably controlled the bacterial growth. According to that our study explores *Oscillatoria* Sp. ethanolic extract effectivey inhibits the growth of human pathogenic bacteria such as *B. subtilis* and *P. aeruginosa*.

DISCUSSION

The marine algae produced different chemical constituents such as halogenated aliphatic compounds, fatty acids, proteins, phenols and sulfur containing hetero cyclic compounds. It highly reactive with gram negative and gram positive bacteria to control the cell division mechanisms. Naturally the marine organisms are produced various clinical oriented drugs. It is used for the antimicrobial, antifungal and anticancer agents. As well as the natural product have less side effect and stimulate the normal function in cellular system [12]. Hence, the different classes of marine algae contain interesting secondary metabolites that can cure the acute and chronic diseases. Nowadays, leading to the use of natural of medicine is a less adverse effect with an improved nutritional status of human health. The presence of secondary metabolites such as phenolics and flavanids which clearly state that inhibit the growth of microorganisms [13]. The chemical constituents phenolics, carbohydrates, proteins, flavonoids, tannins are in abundantly present in marine algae. They were known to stimulate the immune system against pathogenic microorganisms [14, 15]. Besides the marine derived active ingredients is act as broad spectrum antibiotics against gram positive and gram negative bacteria. Likewise many algal strains extracts were discovered their bioactivity against pathogenic organism including *Salmella typhi, E.coli* and *P.aurogenosa* [16].

CONCLUSION

The present investigation screening of bioactive compounds and antibacterial from marine algae *Oscillatoria* Sp. The different concentration of

Oscillatoria Sp. extract was carried out the antibacterial activities against human pathogenic bacteria. The important preliminary phytochemicals such as alkaloids, fatty acids were first reported. The GC-MS characterization of the bioactive compounds was identified such as phenolic compound, carboxylic acid and 1,2,4-triazole from *Oscillatoria Sp.* solvent extract. The methanolic *Oscillatoria* Sp. extract has also been described as potential antibacterial agents. The results showed that *P. aeruginosa* has the highest zone of inhibition (22mm) while *S. aureus* has the lowest zone of inhibition (15mm)

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REFERENCES

1. Borowitzka, M.A., 1995. Microalgae as sources of pharmaceuticals and other biologicallyactive compounds. J. Appl. Phycol., 7: 3-15.
2. Plaza, M., S.L. Santoyo, G.G.B. Jaime, M. Reina, F.J. Herrero, S. Se˜noráns and E. Iba˜nez, 2010. Screening for bioactive compounds from algae. Journal of Pharmaceutical and Biomedical Analysis., 51(2): 450-455.
3. Vijayakumar, A., V. Duraipandiyan, B. Jeyaraj, P. Agastian, M.K. Raj and S. Ignacimuthu, 2012. Phytochemical analysis and *in vitro* antimicrobial activity of Illicium griffithii Hook. f. & Thoms extracts. Asian Pacific Journal of Tropical Disease, 2(3): 190-199.
4. Morton, T. and L. Steve, 2008. Modern Uses of Cultivated Alga. Ethnobotanical Leaflets. Southern Illinois University Carbondale, pp: 12-26.
5. Horincar, V.B., G. Parfene and G. Bahrim, 2011. Evaluation of bioactive compound inextracts obtained from three romanian marine algae species. Romanian Biotechnological Letters, 16(6): 72-78.
6. Robles-Centeno, P.O., D.L. Ballantine and W.H. Gerwick, 1996. Dynamics ofantibacterial activity in three species of Caribbean marine algae as a function of habitat and life history. Hydrobiology, 326: 457-462.
7. Kellan, S.J. and J.M. Walker, 1989. Antibacterial activity from marine microalgae. British J. Phycol., 23: 41-44.
8. Khushk, I., M.U. Dahot, S.A. Baloach and M.A. Bhutto, 2010. The Evaluation of Soybean Extracts in Alloxan-Induced Diabetic Rabbits. World Applied Sciences Journal 8 (Special Issue of Biotechnology & Genetic Engineering)., 6: 22-25.
9. Deshmukh, D.V. and P.R. Puranik, 2010. Application of Plackett-Burman Design to Evaluate Media Components Affecting Antibacterial Activity of Alkaliphilic Cyanobacteria Isolated from Lonar Lake. Turkish J. Biochem., 35(2): 114-120.
10. Evans, C.W., 1989. Mexican medicinal plants. J. Ethnopharmacol., Pharmacognosy, 13th Edn. Bailli`ere Tindall, London.
11. Sastry, V.M.V.S. and G.R.K. Rao, 1994. Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. Botonica Marina., 37: 357-360.
12. Smith, V.J., A.P. Desbois and E.A. Dyrynda, 2010. Conventional and Unconventional Antimicrobials from Fish,Marine Invertebrates and Micro-algae. Mar. Drugs., 8: 1213-1262.
13. Santoyo, S., M. Plaza, L. Jaime, G. Garcia-Blairsy Reina, M. Herrero, F.J. Seriorans and E. Ibanez, 2010. Screening for bioactive compounds from algae. J. Pharm. Biomed. Anal., 51(2): 450-5.
14. McGaw, L.J., 2002. Isolation of antibacterial fatty acid from Schotia brachypetala. Fitoterapia., 73: 431-433.
15. Plaza, M., S. Santoyo, L. Jaime, G. Garcia-Blairsy Reina, M. Herroro, F.J. Senorans and E. Ibanez, 2010. Screening for bioactive compounds from algae. J. Pharmaceutical and Biomedical Analysis, 51: 450-455.
16. Battu, G.R., S. Ethadi, P.P. Murthy, V.S. Praneeth and T.M. Rao, 2011. *In-Vitro* Antibacterial Activity And Preliminary Phytochemical Screening Of Three Algae From Visakhapatnam Coast andhra Pradesh, India. International Journal of Pharmacy and Pharmaceutical Sciences., 3(4): 1-5.