OTEC
Vol.26 (2021), 75 ～ 84

The Effect of Low Temperature on Biochemical Properties and Bioactivity of Selected Malaysian Macroalgae Species

Ong Kuan Hung[*1], Kishneth Palaniveloo[*1] and Phang Siew Moi[*1,2]

[*1] Institute of Ocean and Earth Sciences, University of Malaya, Wilayah Persekutuan Kuala Lumpur 50603, Malaysia.
[*2] Department of Biotechnology, UCSI University, UCSI Heights, 1, Jalan Puncak Menara Gading, Taman Connaught, 56000 Cheras, Wilayah Persekutuan Kuala Lumpur.

Abstract

Seaweeds are commercial valuable products with high biochemical properties and bioactive compounds. The effects of low temperature (15℃, 20℃, 24℃ and 28℃) on the biochemical properties and bioactivity of *Caulerpa lentillifera, Ulva* sp and *Gracilaria* sp will be evaluated. The total protein, amino acid, lipid, fatty acid, moisture, ash and carbohydrate content of seaweed cultured under different low temperatures will be compared. The seaweeds' metabolite profile changes will be determined using spectrometry and chromatographic techniques. The antibacterial and antioxidative properties of all seaweed extracts will be evaluated.

Keywords: Temperature, Biochemical, Metabolite, Seaweed, *Caulerpa, Ulva, Gracilaria*

1. Introduction

Seaweeds are multicellular autotrophic marine organisms, which consist of seagrass and macroalgae. For decades, coastal communities have widely consumed a variety of seaweed species. Recently, they have increased the commercial value of seaweed in the global market, both as an edible source for commercial applications and as a reservoir for pharmaceutical research[1,2]. Approximately 40% of seaweeds were farmed for food, and over 23 million tonnes of aggregated production is from China and Indonesia[1]. Many western countries have started to farm seaweed to compensate for the increased market demand[2]. Seaweeds are also gaining popularity among vegetarians and vegans dueto greater nutritional properties as an alternative to terrestrial plant plan. Therefore, many organizations are interested in the exploratory studies of seaweed's nutritional value to promote and protect public health [3].

Seaweeds have varying biochemical profiles, leading to different tastes and consumption benefits[4]. *Caulerparacemosa,* a Chlorophyte is a top-tier delicacy that is commonly eaten raw as salad in South-East Asia, while *Saccharinajaponica*, a Phaeophyte is used to make broth in both China and Japan[4,5,6]. Seaweeds are also rich in carbohydrate content,especially dietary fibre. *Ulva lactuca* was found to have 54% dietary fibre (dry weight) which is higher than broccoli leaves (32% of dry weight)[7,8]. On the other hand, their protein content varies by seaweed groups. For instance, low proteincontents have been found in brown macroalgae (5-24% dry weight) while higher protein content was found in green and red macroalgae (10-47% dry weight)[9]. As food, seaweeds are packed with flavour enhancers, glutamic acid which are responsible for the umami flavour when consuming [4,10,11]. Most edible seaweed had been found to contain levels of beneficial fatty acids,such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[10,12,13]. Both EPA and DHA are omega-3 fatty acidswhich are linked to proper foetal development, improving cardiovascular function and preventing mild Alzheimer's disease[14]. Furthermore, seaweeds gained popularity in commercial product and pharmaceutical research industries. Alginate have been extracted from Phaeophyta since 1881 for wound dressing which promotes wound healing from surgery[15]. Fucoxanthins have been found to exhibit antiproliferative effects on multiple cancer cells on animal model[16]. They also exhibited positive results in reducing body weight during clinical trials[16]. Moreover, Caulerpin, unique to the genus *Caulerpa* displayed antinociceptive, anti-tuberculosis, antiviral and anti-inflammatory activities in pre-clinical trial[17,18,19].

Received date: 2021.11.30
E-mail of corresponding author: s2043080@siswa.um.edu.my

Like other agriculture products, the iochemical content of seaweed is heavily affected by geolocation and seasonal factors [20]. These environmental factors could affect the yield of seaweed, its metabolites and even its morphology [21]. A study that observed seaweeds from temperate regions showed to have accumulated more total lipid content than seaweeds from tropical regions and have higher omega 3 fatty acids[13]. Furthermore, the biochemical composition of tropical seaweeds are influenced by variation in light, salinity and water temperature [22]. Secondary metabolites are a biological response toward environmental changes and stresses. Extract from *Sargassum* collected during monsoon season demonstrated increased anti-cancer property and Caulerpa during the rainy season also showed a higher concentration of caulerpin than dry season [23,24]. Few studies have shown seaweed could accumulate heavy metal content in the environment and even suggested them as a bioindicator for pollution [25,26]. These variations caused by the environment was always a challenge faced by seaweed farming and this created a demand for the research on the quantitative effect of environment on the seaweed growth and metabolites level [1,27]. Thus, it is importanceto understand the impact of growing factor such as temperature, salinity, light and water nutrient on seaweed growth and metabolite level. This research will attempt to investigate the nutritional and metabolites profile as well as metabolites bioactivity of selected Malaysian seaweed species under a series of controlled low-temperature conditions (15℃, 20℃, 24℃ and 28℃).

2. Method

a) Seaweed stock establishment:

Fresh *Caulerpa lentillifera*, *Ulva* sp. and *Gracilaria* sp will be obtained from various sources to be cultured under laboratory conditions. Collected seaweed will be washed clean with artificial seawater before quarantine and acclimatization in an aerated water tank at 28℃, 40 μmol photons m^{-2} s^{-1} with 12 h photoperiod. Salinity will be maintained at 30 ppt. Seaweed growth curve will be determined based on changes in biomass and maximum photosynthetic efficiency ($F_v/F_{m)}$ will determined by using chlorophyll fluorometer (>0.5).

b) Experiment design:

To study the effect of temperature, each respective seaweed will be cultured in four different temperatures; 15℃, 20℃, 24℃ and 28℃ in a decreasing pattern. The temperature reduction will be controlled with a marine tank chiller. One kilogram of seaweed will be cultured in the highest temperature for 30 days before being reduced gradually for a week at a rate of 1℃ a day to prevent sudden stress in temperature changes. This process will repeat for every temperature reduction. After 30 days at each temperature, 1.2 kg wet weight of cleaned seaweed will be harvested for biochemical analysis (1 kg crude extracted in industrial grade ethanol, 200 g oven dried at 60℃ to prevent loss of valuable minerals). Dried seaweed will be milled by using powder blender and stored airtight under -20℃ for analysis.

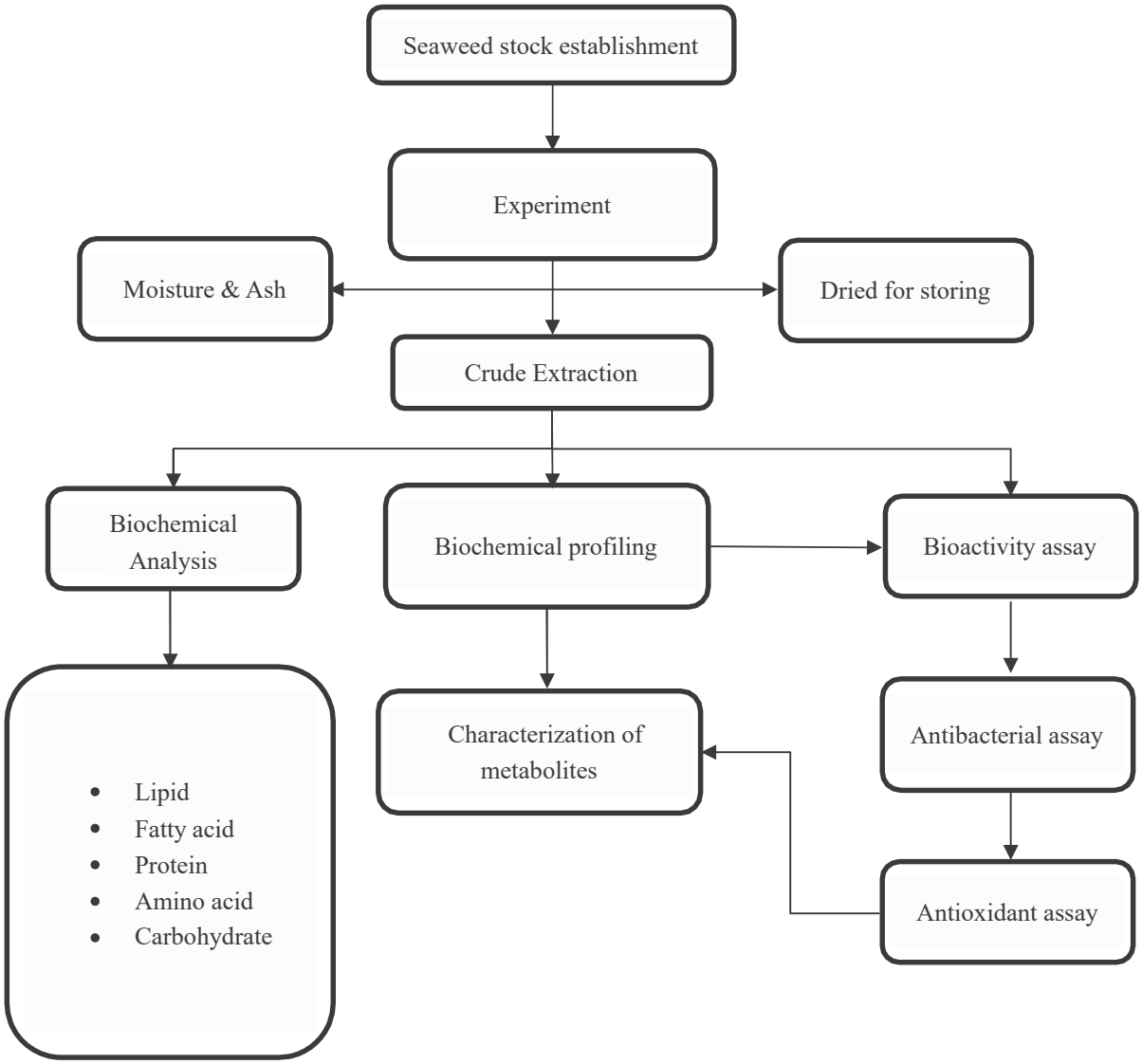

Fig. 1 Experiment methodology flow chart.

c) Biochemical Analysis:

Seaweed chemical composition (moisture, ash, lipid, fatty acid, protein, amino acid and carbohydrate) will be determined according to official method of analysis by AOAC [28].

1) Moisture and ash content:

Moisture content will be estimated by measuring the weight loss of 100g fresh seaweed after drying in hot air oven at 105°C until constant weight was obtained [29]. The moisture content will be expressed as a percent of moisture in the fresh seaweed mass (% moisture). The ash content was calculated using weight loss of 5g oven-dried seaweed powder from moisture experiment after incineration at 550°C for 18h using electric muffle furnace. The ash content will be expressed as a percentage of ash in the seaweed mass (%ash) [28].

2) Lipid content:

Crude lipid content will be determined with modified Folch extraction method described [31]. Two-hundred milligram of dried seaweed powder will be weighed and homogenized with 5mL chloroform:methanol (2:1, v/v). The mixture will be light agitated using vortex mixer at room temperature for 15 minute. The lipid containing chloroform:methanol extract will be concentrated. The total lipid content will be determined gravimetrically with the weight between the residue and dried seaweed powder. The lipid content will also be expressed as percentage of lipid content of dried seaweed powder (%lipid).

3) Fatty acid content:

Hundred milligram of lipid extract eluted over hexane: ethyl acetate (9:1) using a silica gel column will be subjectedto fatty acid methyl ester (FAME) conversion. The concentrated eluent will be trans-methylated using 2.7ml of hexane and 0.3ml of 2M sodium methoxide to obtain FAME. The sample will be subjected to continuous stirring with magneticstirrer for 3h at room temperature. Transmethylation reaction in the sample will be stopped using 1ml chloroform- methanol (1:1) mixture. The sample will be dried using rotary evaporator until yellowish oil is obtained. Fatty acid composition will be quantified using gas chromatography mass spectrometry (GC-MS) through a Silica BPX70 capillarycolumn (60 m, with a film thickness of 0.25 µm) according to method described in articles[29]. Input for GC-MS will be adjusted as followed: temperature injector-225°C; carrier gas-helium; flow rate-0.75mL/min; capillary column (Rt-2560,100 m, 0.25 mm ID, 0.2 um df) according to method describe in articles [29]. Run method will be temperature gradient between 150 to 240 °C for 60 min. Identification and quantification of FAMEs will be accomplished by comparing theretention times of the peaks to internal library, National Institute of Standards and Technology (NIST) and the Flavors and Fragrances of Natural and Synthetic Compounds library (FFNSC). Concentration of FAME will be calculated based onGC area of a peak compared to standards used and expressed as a percentage of individual FAs in the lipid fraction.

4) Protein content:

Crude protein content will be estimated based on nitrogen composition in the sample analyzed using a CHNS analyzer multiplied by a conversion factor of five [34]. Hundred mg of dried seaweed powder will be packed in tin capsulewith an oxidizer which will be combusted in the analyzer. The analyzer will quantify the % nitrogen content (% N) in thesample which will convert to % crude protein content (% crude = %N *5). The non-protein nitrogen content (%NPN) will be obtained from precipitated 250mg seaweed powder with 10% trichloracetic acid. Total protein content will be determined from difference between %crude and %NPN and expressed as percentage of protein of dried seaweed powder(%protein) [35].

5) Carbohydrate content:

Total carbohydrate content will be estimated by weight difference using the following equation [20, 36],

$$\text{Carbohydrates} = [100\% - (\%\ \text{protein} + \%\ \text{lipid} + \%\ \text{ash} + \%\ \text{moisture})].$$

6) Amino Acid content:

The amino acid content will be determined with automatic online OPA/FMOC derivatization by RP-HPLC using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump, DGU-20AS degasser and photodiode array SPD-M20A (PAD), and fluorescence RF-10AXL (FLD) detectors online and the procedures will be according to [20,29]. Two-gram dried seaweed powder will be hydrolysed with 2ml, 6M hydrochloric acid in screwed-capped test tube for 24h at 110°C. After hydrolysis, the mixture will be evaporated to dryness under vacuum then it will be reconstituted in 2 ml of 0.1 M hydrochloric acid. Twenty microlitre sample will be derivatized with o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), prepared according to protocol[30]. Derivatized sample will be filtered through 0.22µm microfiltration membrane to remove microbe contamination. Filtered sample will be injected onto the RP-HPLC system equipped with an HPLC column (Zorbax Eclipse XDB-C18 (4.6 × 250 mm) conditioned at 27 ± 0.1 ◦C. The OPA-3-MPA derivatives will be detected by a programmable fluorimeter with excitation (λex) and emission (λem) wavelengths set at 338 and 262 nm, respectively, while the FMOC derivatives will be identified at λex 260nm and λem 315 nm; wavelength change occurred at 18.5 min. The mobile phase used will be a combination of 10 mM disodium hydrogen phosphate buffer: 10mM disodium tetraborate and 5 mM sodium azide adjusted to pH 8.2 eluent

(A), and a mixture of acetonitrile, methanol, and water with a ratio of 45:45:10 (v/v/v) as eluent B, at a flow rate of 1.0 mL min^{-1}. The amino acid standards mix (AAS18, Sigma Aldrich, MO, USA) will be prepared by dissolving in 0.1 M hydrochloric acid then be diluted appropriately to obtain a working solution with norvalin (Merck, MO, USA) used as the internal standard [20]. Concentration of amino acids will be calculated based on peak area with reference to standard and expressed as a percentage.

d) Biochemical Profiling

To establish secondary metabolite profile, a series of chromatographic tools, mass spectrometer (MS) will be used.

1) HPLC Profiling:

Moreover, high performance liquid chromatography (HPLC, Shimadzu) will be used to profile extracts from each experiment to observe for changes in production of secondary metabolites across the respective seaweeds cultured in varying temperature conditions. One milligram of dried seaweed extract will be dissolved in 1ml, HPLC grade methanol to prepare a stock solution of 1mg/ml. The 0.22µm PTFE membrane filtered stock solution will be subjected to HPLC analysis equipped with a Shimadzu C18 (100 mm × 4.6 µm) column and a Photodiode Array Detector. The solvent used for mobile phase will be water (A) and acetonitrile (B), both will be acidulated with 0.1% formic acid analysed on a gradient profile. Any compound of interest will be subjected to isolation.

2) Mass Spectrometry (LC-MS/MS)

The crude extracts will be diluted in deionized water and filtered through a 0.45µm nylon syringe filter. A 50ppm of the filtrate will be prepared in triplicates and used for LCMS analysis. The LCMS analysis will be performed using liquid chromatography G6550A system (Agilent Technologies, Inc., USA), which consisted of a degasser, a G4220A binary pump with a pressure gradient unit, a dual AJS ESI MS QTOF detector and a G4226A HiP AutoSampler. A 4.6 x 100 mm Shimadzu C18 column will be utilized for separation (30 eV at 45°C and 20µL at 0.8 mL/min). A mobile phase of 24% of acetonitrile and 76% of water containing 0.2% formic acid for a separation time of 30 min will be applied following the previous methods with minor modifications [37]. Identification of compounds by the acquisition of mass spectra will be conducted in both positive and negative modes, respectively, between m/z 100 and 1500.

e) Characterization of Metabolites

Based on the initial HPLC profile, extracts will be selected for targeted isolation. The dried seaweed extracts will be subjected to column chromatography, a series of thin layer chromatography; derivatized with phosphomolybdic acid solution 5 wt. % in ethanol were used as a derivatization reagent for observation of phenolics, hydrocarbon waxes, alkaloids and steroids, and repeated preparative thin layer chromatography (PTLC) for compound isolation. Purification will involve the use of HPLC. Pure isolates will be subjected to nuclear magnetic resonance spectroscopy (NMR) for 1D (^{1}H, ^{13}C, DEPT-135) and 2D-NMR (HSQC, ^{1}H -^{1}H COSY, HMBC, NOESY) experiments for characterization.

f) Bioactivity

1) Antibacterial assay:

All extracts and compounds will be selected for antibacterial potential evaluation by determining the Minimum Inhibitory Concentration (MIC) of extracts or compound according to Nagappan's study [33]. Tested microbe are *Escherichia coli* and *Staphylococcus aureus* and will be preculture in respective growth medium and used for assay. Colony forming unit (CFU/mL) of test bacteria will be obtained using serially diluted with ten-fold dilution factor in test tube. Fractions will be diluted by two-fold in growth medium. About 10µl of diluted fraction will be serially added into each of the sterile 96 well of microdilution tray loaded with 90µl of bacteria solution. Hundred microlitre of bacteria (negative control) and bacteria solution added with imipenem (positive control) will be used for comparison. Mixture and control will be incubated at 37°C for 24h and checked for the presence of whitish sedimentation that indicates dead bacterial cells. Maximum turbidity in positive control will indicate minimum bacterial growth. Minimum inhibition concentration (MIC) will be determined when a single skipped well occur. Assay will be carried out in triplicate for fractions tested.

2) Antioxidant assay:

Total antioxidant activity of the samples will be determined according to Prieto's study [38]. Selected fractions (100µl) will be added with 900µl solution comprised of 28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM ammonium molybdate. The mixture will be incubated at 95 ºC for 90 min in a water bath. The absorbance will be measured at 695nm using UV-Vis 2450 spectrophotometer (Shimadzu, Japan) and a standard curve (between 50 µg/ml and 250µg/ml) will be determined using ascorbic acid. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid in milligram per millilitre of extract (mg ascorbic acid/ ml).

The radical scavenging effect of the sample will be determined by 2,2-Diphenyl-1-picrylhydrasyl (DPPH) assay described by Manoj's study [39,40]. DPPH radical solution (0.5mM) will be prepared in methanol and series of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) standard will be prepared using DPPH solution, 0, 100, 200 and 400 µg/ml. One millilitre of DPPH solution and 3ml of sample will be mixed. Sample and standards will be incubated for 30min in the dark, then absorbance at 517nm will be measured. Decreasing the absorbance of DPPH solution indicates an increase in DPPH radical scavenging activity. The radical scavenging effect will be calculated as %DPPH, using formula [39,40].

% DPPH radical scavenging = [(control absorbance - sample absorbance)/(control absorbance)] × 100

h) Statistical analysis:

Results will be obtained from experiments performed on separate days. All data will be expressed in term of mean ± standard deviation. The means of the carbohydrate, lipid, protein, ash and moisture content will be examined for statistical significance ($p<0.05$) between temperature treatments via one-way analysis of variance (ANOVA). The means of data from antibacterial and antioxidant experiment will be for statistical significance ($p<0.05$) between temperature treatments via one-way analysis of variance (ANOVA).

3. Experiment Progress

3.1 Stock establishment

Caulerpa was received from local latok seller who was supplied by a local farmer in Kuala Lumpur, Malaysia. The green seaweed came in as 3.1kg but after washing and selection, only 2.5 kg of *Caulerpa* was able to use. *Caulerpa* was washed according to method mentioned and seaweed with intact stolon, rhizoids and fronds was selected. Soft and squishy *Caulerpa* was removed or discarded. The green seaweed was washed three times before put into stock tank. Stock tank condition is 28℃, 40 µmol photons m^{-2} s^{-1} with 12 h photoperiod and 30 ppt salinity. Stock tank is a three combinedtank with aerated and filtered system. Therefore, approximately 1.25 kg of each *Caulerpa* was added in a tank and total of two tank was used. Weekly photo image was taken to monitor the growth visually, Fig 2 showed the seaweed growth in one tank fromtime points (1 Sept, 28 Sept, 6 Oct, 21 Oct, 8 Nov and 26 Nov). From Fig 2, seaweed in tank 3 had obvious visual growthafter a month in stock tank. The *Caulerpa* was observed to gradually fill up the tank and new stolon, rhizoids and frondscan be observed along the acclimatization period. Another tank showed the same pattern so it is not showed. On 26 Nov, seaweed in tank 3 was weighted and 2.215kg was recorded. Using weekly specific growth rate (SGR) equation, %SGR

$= [(weight_t – weight_0)/(weight_0 \text{ x weeks})]\text{x } 100\%$, weekly specific growth rate of 6.43% was achieved. This showed an average of 6% increment in fresh seaweed biomass every week for 2 months. A growth curve will be needed to determine the life cycle stage of the *Caulerpa* and Fv/Fm will also be used as indication for seaweed stress to standardize the seaweed growth condition as stock. Currently, the frequency for replenishing artificial seawater will be increased for theseaweed to establish growing seaweed stock. After successful establish growing seaweed stock, the temperature experiment will be initiated.

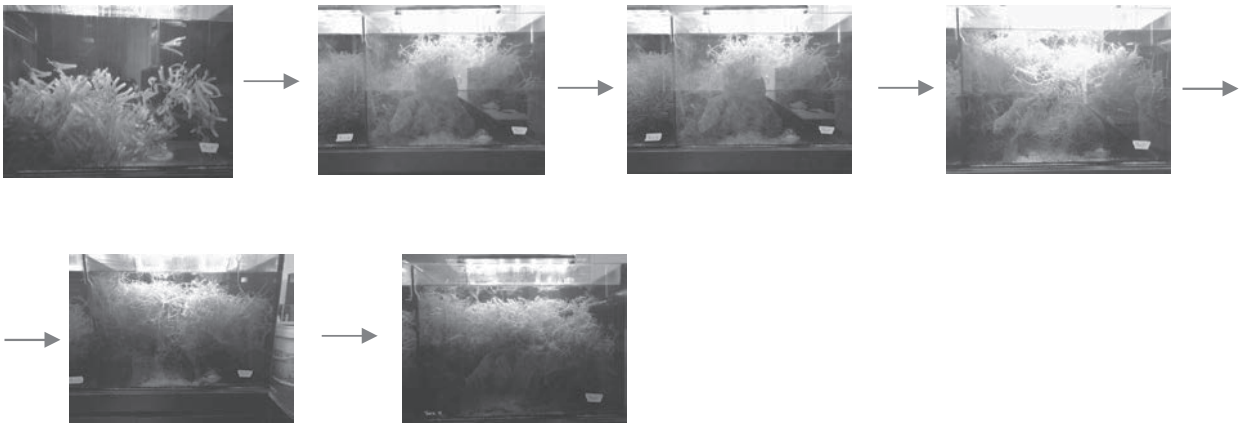

Fig. 2 Photo image of stock *Caulerpa* growth, chronologically (1 Sept, 28 Sept, 6 Oct, 21 Oct, 8 Nov and 26 Nov).

Fig. 3 Photo images of new growth of a) stolon, b) rhizoid and c) fronds.

4. Expected Output

As mentioned, seaweeds growth and biochemical level are easily affected by environmental factors (light intensity, temperature, water nutrient and salinity) [13,20,21]. Therefore, it is expected to find changes in seaweed biochemical level (carbohydrate, protein, amino acid, lipid, fatty acid, ash and moisture) due to seaweed growing in low temperature. A different biochemical levels can lead to changes in seaweed metabolite profile in different temperatures. Therefore, it is expected to discover new bioactive compound that is produced by the changes in seaweed metabolite profile. This research will also isolate and characterize the new compound for its bioactivity and aim to develop the commercial potential of the compound. Studies that found the increased bioactivity of seaweed extract in the rainy season also suggested that environmental changes can increase seaweed bioactive compound level [23,24]. Therefore, this experimentis expected to find an increased in bioactive compound level from seaweed cultured in low temperature. Increased bioactive compound level and production of new bioactive compound also would suggest an increased in the bioactivity level of seaweed extract. It is to be expected to increase the antibacterial and antioxidant level of the seaweed extract.

5. Acknowledgement

This is supported by the Science and Technology Research Partnership for Sustainable Development (SATREPS) Program entitled ‘Development of Advanced Hybrid Ocean Thermal Energy Conversion (OTEC) Technology for Low Carbon Society and Sustainable Energy System: First Experimental OTEC Plant of Malaysia’ funded by Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA), and Ministry of Higher Education Malaysia (MoHE) and led by the Institute of Ocean Energy Saga University (IOES) of Japan, and UTM Ocean Thermal Energy Centre (UTM OTEC), Universiti Teknologi Malaysia (UTM). Registered Program Cost Centre: R.K130000.7809.4L887, Project [Cost Centre: Project No IF045-2019].

6. References

1. Buschmann, A. H.; Camus, C.; Infante, J.; Neori, A.; Israel, Á.; Hernández-González, M. C.; Pereda, S. V.; Gomez-Pinchetti, J. L.; Golberg, A.; Tadmor-Shalev, N.; Critchley, A. T., Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology* **2017,** *52*, (4), 391-406.
2. Peñalver, R.; Lorenzo, J. M.; Ros, G.; Amarowicz, R.; Pateiro, M.; Nieto, G., Seaweeds as a Functional Ingredient for a Healthy Diet. *Mar Drugs* **2020,** *18*, (6).
3. Ferdouse, F.; Holdt, S. L.; Smith, R.; Murúa, P.; Yang, Z., The global status of seaweed production, trade and utilization. *Globefish Research Programme* **2018,** *124*, I.
4. Ikeda, K., New Seasonings. *Chemical Senses* **2002,** *27*, (9), 847-849.
5. de Gaillande, C.; Payri, C.; Remoissenet, G.; Zubia, M., Caulerpa consumption, nutritional value and farming in the Indo-Pacific region. *Journal of Applied Phycology* **2017,** *29*, (5), 2249-2266.
6. Patra, J. K.; Das, G.; Baek, K. H., Chemical Composition and Antioxidant and Antibacterial Activities of an Essential Oil Extracted from an Edible Seaweed, Laminaria japonica L. *Molecules* **2015,** *20*, (7), 12093-113.
7. Yaich, H.; Garna, H.; Besbes, S.; Paquot, M.; Blecker, C.; Attia, H., Chemical composition and functional properties of Ulva lactuca seaweed collected in Tunisia. *Food Chemistry* **2011,** *128*, (4), 895-901.
8. Berndtsson, E.; Andersson, R.; Johansson, E.; Olsson, M. E., Side Streams of Broccoli Leaves: A Climate Smart and Healthy Food Ingredient. *International journal of environmental research and public health* **2020,** *17*, (7), 2406.
9. Mohamed, S.; Hashim, S. N.; Rahman, H. A., Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology* **2012,** *23*, (2), 83-96.
10. Magdugo, R. P.; Terme, N.; Lang, M.; Pliego-Cortés, H.; Marty, C.; Hurtado, A. Q.; Bedoux, G.; Bourgougnon, N., An Analysis of the Nutritional and Health Values of Caulerpa racemosa (Forsskål) and Ulva fasciata (Delile)—Two Chlorophyta Collected from the Philippines. *Molecules* **2020,** *25*, (12).
11. Qudus, B. A. A.; Abdul Razak, S.; Palaniveloo, K.; Nagappan, T.; Suraiza Nabila Rahmah, N.; Wee Jin, G.; Chellappan, D. K.; Chellian, J.; Kunnath, A. P., Bioprospecting Cultivated Tropical Green Algae, Caulerpa racemosa (Forsskal) J. Agardh: A Perspective on Nutritional Properties, Antioxidative Capacity and Anti-Diabetic Potential. *Foods* **2020,** *9*, (9).
12. Dawczynski, C.; Schubert, R.; Jahreis, G., Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry* **2007,** *103*, 891-899.
13. Susanto, E.; Fahmi, A. S.; Hosokawa, M.; Miyashita, K., Variation in Lipid Components from 15 Species of Tropical and Temperate Seaweeds. *Mar Drugs* **2019,** *17*, (11).
14. Swanson, D.; Block, R.; Mousa, S. A., Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in nutrition (Bethesda, Md.)* **2012,** *3*, (1), 1-7.
15. Morgan, D., Alginate Dressings: Part 1: Historical Aspects. Journal of Tissue Viability 1997, 7, (1), 4-9.
16. Méresse, S.; Fodil, M.; Fleury, F.; Chénais, B., Fucoxanthin, a Marine-Derived Carotenoid from Brown Seaweeds and Microalgae: A Promising Bioactive Compound for Cancer Therapy. *International Journal of Molecular Sciences* **2020,** *21*, (23), 9273.
17. Ahmed, S. A.; Abdelrheem, D. A.; El-Mageed, H. R. A.; Mohamed, H. S.; Rahman, A. A.; Elsayed, K. N. M.; Ahmed, S. A., Destabilizing the structural integrity of COVID-19 by caulerpin and its derivatives along with some antiviral drugs: An in silico approaches for a combination therapy. *Struct Chem*, 1-22.
18. de Souza É, T.; de Lira, D. P.; de Queiroz, A. C.; da Silva, D. J. C.; de Aquino, A. B.; Campessato Mella, E. A.; Lorenzo, V. P.; de Miranda, G. E. C.; de Araújo-Júnior, J. X.; de Oliveira Chaves, M. C.; Barbosa-Filho, J. M.; de Athayde-Filho, P. F.; de Oliveira Santos, B. V.; Alexandre-Moreira, M. S., The Antinociceptive and Anti-Inflammatory Activities of Caulerpin, a Bisindole Alkaloid Isolated from Seaweeds of the Genus Caulerpa. *Mar Drugs* **2009,** *7*, (4), 689-704.
19. Canché Chay, C. I.; Gómez Cansino, R.; Espitia Pinzón, C. I.; Torres-Ochoa, R. O.; Martínez, R., Synthesis and Anti-Tuberculosis Activity of the Marine Natural Product Caulerpin and Its Analogues. *Mar Drugs* **2014,** *12*, (4), 1757-72.
20. Aroyehun, A. Q.; Palaniveloo, K.; Ghazali, F.; Rizman-Idid, M.; Abdul Razak, S., Effects of Seasonal Variability

on the Physicochemical, Biochemical, and Nutritional Composition of Western Peninsular Malaysia Gracilaria manilaensis. *Molecules* **2019,** *24*, (18).

21. Verlaque, M.; Durand, C.; Huisman, J. M.; Boudouresque, C.-F.; Le Parco, Y., On the identity and origin of the Mediterranean invasive Caulerpa racemosa (Caulerpales, Chlorophyta). *European Journal of Phycology* **2003,** *38*, (4), 325-339.
22. Marinho-Soriano, E.; Fonseca, P. C.; Carneiro, M. A. A.; Moreira, W. S. C., Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology* **2006,** *97*, (18), 2402-2406.
23. Praiboon, J.; Palakas, S.; Noiraksa, T.; Miyashita, K., Seasonal variation in nutritional composition and anti-proliferative activity of brown seaweed, Sargassum oligocystum. *Journal of Applied Phycology* **2018,** *30*, (1), 101-111.
24. Januar, H. I.; Wikanta, T.; Hastarini, E., Hubungan Antara Musim dengan Kadar Caulerpin dalam Caulerpa racemosa. *Jurnal Penelitian Perikanan Indonesia* **2017,** *10*, (3), 1-6.
25. Bonanno, G.; Veneziano, V.; Orlando-Bonaca, M., Comparative assessment of trace element accumulation and biomonitoring in seaweed Ulva lactuca and seagrass Posidonia oceanica. *Science of The Total Environment* **2020,** *718*, 137413.
26. Farias, D. R.; Hurd, C. L.; Eriksen, R. S.; Macleod, C. K., Macrophytes as bioindicators of heavy metal pollution in estuarine and coastal environments. *Marine Pollution Bulletin* **2018,** *128*, 175-184.
27. Bleakley, S.; Hayes, M., Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods* **2017,** *6*, (5).
28. Cunniff, P.; WASHINGTON, D., Official methods of analysis of AOAC international. *J. AOAC Int* **1997**, *80*, (6), 127A.
29. Aroyehun, Q. B.; Abdul Razak, S.; Palaniveloo, K.; Nagappan, T.; Suraiza Nabila Rahmah, N.; Wee Jin, G.; Chellappan, D. K.; Chellian, J.; Kunnath, A. P., Bioprospecting cultivated tropical green algae, Caulerpa racemosa (Forsskal) J. Agardh: a perspective on nutritional properties, antioxidative capacity and anti-diabetic potential. *Foods* **2020**, *9*, (9), 1313.
30. Heems, D.; Luck, G.; Fraudeau, C.; Vérette, E., Fully automated precolumn derivatization, on-line dialysis and high-performance liquid chromatographic analysis of amino acids in food, beverages and feedstuff. *Journal of Chromatography* **1998**, *798*, (1), 9-17.
31. Gosch, B. J.; Magnusson, M.; Paul, N. A.; de Nys, R., Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. *GCB Bioenergy* **2012**, *4*, (6), 919-930.
32. Carvalho, F. V.; Fonseca Santana, L.; Diogenes A. da Silva, V.; Costa, S. L.; Zambotti-Villelae, L.; Colepicolo, P.; Ferraz, C. G.; Ribeiro, P. R., Combination of a multiplatform metabolite profiling approach and chemometrics as a powerful strategy to identify bioactive metabolites in Lepidium meyenii (Peruvian maca). *Food Chemistry* **2021**, *364*, 130453.
33. Nagappan, T.; Vairappan, C. S., Nutritional and bioactive properties of three edible species of green algae, genus Caulerpa (Caulerpaceae). *Journal of Applied Phycology* **2014**, *26*, (2), 1019-1027.
34. Angell, A. R.; Mata, L.; de Nys, R.; Paul, N. A., The protein content of seaweeds: a universal nitrogen-to-protein conversion factor of five. *Journal of Applied Phycology* **2016,** *28*, (1), 511-524.
35. Dawczynski, C.; Schubert, R.; Jahreis, G., Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry* **2007,** *103*, 891-899.
36. Neto, R.; Marçal, C.; Queirós, A.; Abreu, M.; Silva, A.; Cardoso, S., Screening of Ulva rigida, Gracilaria sp., Fucus vesiculosus and Saccharina latissima as Functional Ingredients. *International Journal of Molecular Sciences* **2018,** *19*, 2987.
37. Tsuge, K.; Seto, Y., Mass spectrometric identification of chemical warfare agent adducts with biological macromolecule for verification of their exposure. *Journal of Health Science* **2009,** *55*, (6), 879-886.
38. Prieto, P.; Pineda, M.; Aguilar, M., Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry* **1999,** *269*, (2), 337-341.
39. Kumar, M.; Kumari, P.; Trivedi, N.; Shukla, M. K.; Gupta, V.; Reddy, C.; Jha, B., Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *Journal of Applied Phycology* **2011,** *23*, (5), 797-810.

40. Kordali, S.; Cakir, A.; Mavi, A.; Kilic, H.; Yildirim, A., Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. *Journal of agricultural and food chemistry* **2005,** *53*, (5), 1408-1416.