

Carotenoid and Chlorophyll Profiles in Five Species of Malaysian Seaweed as Potential Halal Active Pharmaceutical Ingredient (API)

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Abstract— Seaweed, also known as macroalgae, consist of various species that have been gaining the interest of researchers due to the presence of functional bioactive compounds that can be extracted from their biomass. This research aimed to study the chlorophyll and carotenoid content from five species of Malaysian seaweed as potential Active Pharmaceutical Ingredient (API). Three species of red macroalgae, (*Euclima denticulatum*, *Gracilaria tikvahiae*, and *Kappaphycus striatum*), green macroalgae (*Caulerpa lentillifera*) and one brown macroalga (*Padina pavonica*) were selected and their chlorophyll and carotenoid contents were determined by using UV-Vis spectrophotometer and HPLC. Their antioxidant and antimicrobial activities were investigated using DPPH Radical Scavenging activity and Disc Diffusion methods, respectively. In terms of carotenoid content, *P. pavonica* contained the highest amount of carotenoid (100.9 ± 14.7 ug/g DW) and chlorophyll content (7.5 ± 1.5 ug/g DW). Meanwhile, based on individual carotenoid content, *K. striatum* had the highest lutein content (38.6 ug/g DW). Zeaxanthin, beta-carotene, and violaxanthin were significantly higher in *C. lentillifera* at 21.3 ug/g DW, 10.7 ug/g DW, and 8.9 ug/g DW respectively. Besides that, the antioxidant test showed that *P. pavonica* presented the strongest DPPH activity with the percentage of inhibition (I %) of ($61.0 \% \pm 0.9$). Finally, for the antimicrobial test, strong antimicrobial activities were shown by all the seaweed samples toward *E. coli* and *P. aeruginosa*. Overall results of the antifungal test demonstrated moderate antifungal action by all seaweed samples towards *M. gypseum* and *Fusarium sp.*

Keywords— antimicrobial; antioxidant; carotenoid; chlorophyll; seaweed; halal.

I. INTRODUCTION

This study compared the carotenoid and chlorophyll contents of brown (Phaeophyta), green (Chlorophyta) and red (Rhodophyta) seaweeds from coastal area of Sabah, Malaysia. The classification of these seaweeds are based on their nutritional values and chemical constituents [1][2]. Classification and biodiversity studies are very important to determine their value as additional ingredient in food and pharmaceutical as well as other valuable products [3]. The bioactive compounds found in seaweed are responsible for their antioxidant, antimicrobial and antifungal properties.

The study of seaweed bioactive properties is essential due to the high demand by consumers for fresh and natural products. In line with enhanced awareness of food safety and quality, industry players are turning to and exploiting the natural products as alternatives to synthetic additives and ingredient [4]. Algae possesses high content of carotenoid, a potential Active Pharmaceutical Ingredient (API) of

secondary metabolite which acts as antioxidant agent [5][6][7].

API is used in finished pharmaceutical products that renders pharmacological activity to disease treatment [8] and restores and improves human's physiological functions. Previous study of food carotenoid had determined carotenoid composition and identified provitamin A and non-provitamin A carotenoids [9].

This present research aims to determine the chlorophyll and carotenoid content from five species of Malaysian seaweed as potential Active Pharmaceutical Ingredient (API).

II. MATERIALS AND METHOD

A. Sample Collection and Process

Five species of Malaysian seaweed namely *Euclima denticulatum* (Rhodophyta), *Gracilaria tikvahiae* (Rhodophyta), *Kappaphycus striatum* (Rhodophyta), *Caulerpa lentillifera* (Chlorophyta), and *Padina pavonica*

(Phaeophyta) were collected from a private seaweed plantation company in Semporna, Sabah (Table I). The samples were washed using distilled water to remove dirt. They were cut into a smaller size before being dried using freeze dryer (Martin Crist, Germany; Alpha 1-4 LD Plus with an RZ2.5 vacuum pump) for four d. The freeze-dried samples were ground using a heavy-duty blender and stored in -20 °C freezer until further analysis.

TABLE I
SEAWEED SAMPLE CLASSIFICATION

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Kingdom	Plantae	Plantae	Plantae	Plantae	Chromista
Sub-kingdom	Biliphyta	Biliphyta	Biliphyta	Viridiplantae	-
Phylum	Rhodophyta	Rhodophyta	Rhodophyta	Chlorophyta	Ochrophyta
Sub-phylum	Eurodhophytina	Eurodhophytina	Eurodhophytina	Chlorophytina	-
Class	Floridophyceae	Floridophyceae	Floridophyceae	Ulvoophyceae	Phaeophyceae
Order	Gigartinales	Graciliales	Gigartinales	Bryopsidales	Dictyotales
Family	Solieriaceae	Gracilariaceae	Solieriaceae	Caulerpaceae	Dictyotaceae
Genus	<i>Eucheuma</i>	<i>Gracilaria</i>	<i>Kappaphycus</i>	<i>Caulerpa</i>	<i>Padina</i>
Species	<i>denticulatum</i>	<i>tikvahiae</i>	<i>striatum</i>	<i>lentillifera</i>	<i>paronica</i>

B. Extraction and Saponification Process

An amount of 1 g of powdered sample was rehydrated with distilled water for 2-3 h. A volume of 10 ml of acetone: methanol (7:3) in the presence of calcium carbonate (CaCO₃) was mixed into the sample solution. The mixture was centrifuged and the supernatant was collected. Then, an equal amount of hexane and distilled water was added to the supernatant. The upper layer of the mixture was collected. This step was repeated until the supernatant became colourless. The supernatant was dried under a gentle stream of oxygen-free nitrogen (NO₂), capped and sealed with parafilm and stored at -20 °C until further analysis.

For saponification method, the freeze-dried sample was resuspended with 1000 µl ethyl acetate. A volume of 50 µl of sample mixture was pipetted into 2000 µl Eppendorf tube. The sample was mixed with 350 µl of acetonitrile (ACN): water (9:1) to make a total volume of 400 µl. Then, 400 µl of 10% w/v methanolic potassium hydroxide (MeKOH) was added to the sample. The mixture was identified as mixture A, vortexed and allowed to stand overnight in a dark prior second stage of saponification.

A volume of 800 µl of the mixture A was added with 400 µl of hexane and 400 µl of 10% NaCl; and centrifuged for 2 min at 13500 rpm. The upper layer was collected and transferred to Eppendorf tube. The process was repeated until the supernatant became colourless. The supernatant was collected, washed thrice with distilled water and dried under oxygen-free nitrogen (NO₂) stream [10].

C. Determination of Chlorophyll and Carotenoid Content

Chlorophyll and carotenoid contents were determined by using UV-Vis Spectroscopy (Shimadzu UV-160 spectrophotometer) method [11][12]. Dried crude extract and dried saponified extract were used to determine

chlorophyll and carotenoid contents, respectively. Both samples were mixed with 300 µl ethyl acetate. A volume of 50 µl of the mixture was mixed with volume 950 µl chloroform. The total chlorophyll and carotenoid contents were measured at, 480 nm, 648 nm, and 666 nm, respectively.

The result for total chlorophyll obtained was then substituted in the following formula [11]:

$$Ch_a (\mu\text{g/ml}) = 10.91 (A666) - 1.2 (A648) \quad (1)$$

$$Ch_b (\mu\text{g/ml}) = 16.36 (A648) - 4.57 (A666) \quad (2)$$

$$\text{Total chlorophyll } (\mu\text{g/ml}) = Ch_a + Ch_b \quad (3)$$

The result for total carotenoid content obtained was substituted into the following equation [13]:

$$Ca_a (\mu\text{g/ml}) = 10.91 (A666) - 1.2 (A648) \quad (1)$$

$$Ca_b (\mu\text{g/ml}) = 16.36 (A648) - 4.57 (A666) \quad (2)$$

$$Ca_{x+c} (\mu\text{g/ml}) = \frac{(1000 A648 - 1.42 Ca_a - 46.09 Ca_b)}{202} \quad (4)$$

Where Ca_a = carotenoid concentration at 666 nm; Ca_b = carotenoid concentration at 648 nm; and Ca_{x+c} = total carotenoid concentration at 480 nm.

D. Quantification of Carotenoid Content

Quantification of carotenoid content was determined using HPLC instrument according to Othman (2009) [10]. The dried saponified extract was mixed with 300 µl ethyl acetate, filtered and subjected for HPLC analysis equipped with diode array detector (Agilent, USA). A volume of 10 µl sample was injected into the HPLC system and eluted by acetonitrile: water (9:1) as solvent A and ethyl acetate as solvent B. The gradient of the solvent used was developed as followed: 0-40 % solvent B (0-20 min), 40-60 % solvent B (20-25 min), 60-100 % solvent B (25-25.1 min), 100 % solvent B (25.1-35 min) and 100-0 % solvent B (35-35.1 min) at a flow rate of 1.0 ml/min. The carotenoid peaks were separated by Zorbax Eclipse SD-C18, end-capped 5 µm, 4.6 x 250 mm reverse phase column at 20 °C and detected at 452 nm, 454 nm, 447 nm and 441 nm for zeaxanthin, beta-carotene, lutein and violaxanthin, respectively. These identified peaks were confirmed by individual comparison to standards and quantified based on calibration curve using 0.02 µg/µl, 0.04 µg/µl, 0.06 µg/µl, 0.08 µg/µl and 0.10 µg/µl standards.

E. Antioxidant Activity

1) *Scavenging DPPH Radical*: The stock sample was prepared to the initial concentration of 1 mg/ml extract solution. First, using a multichannel pipette (12 tips), 100 µl of methanol was filled into 96-well microplate from wells B1-B12 until H1-H12. Then, 200 µl of sample 1 (1 mg/ml) was pipetted into wells A1-A6 while sample 2 was pipetted into wells A7-A12. All samples were transferred from A1-A12 into the wells in row B and mixed thoroughly. The samples were diluted continuously until row H. Then, 100 µl of the mixture from the last row was transferred into waste plate. Methanol in 100 µl of the volume was added in A to H (only 4th-6th wells and 10th-12th wells). Next, 100 µl of 1,1-diphenyl-2-picrylhydrazyl (DPPH) methanolic solution was added into A to H wells (only 1st-3rd wells and 7th-19th wells). The microplate was covered and stored in the dark

cabinet for 40 min at 27°C prior absorbance reading using Microplate reader spectrophotometer at 517 nm.

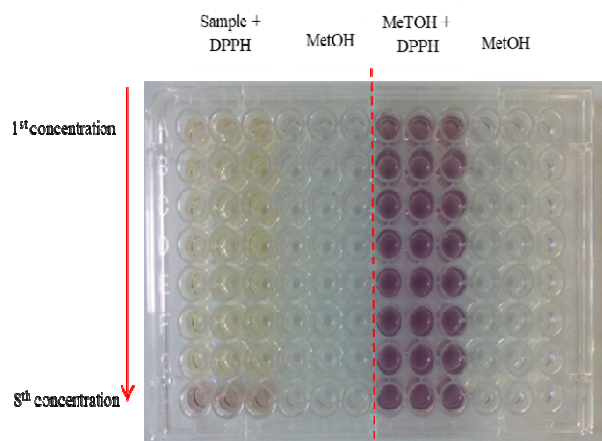


Fig.1 DPPH radical scavenging activity of sample and blank in the a 96-well plate with different concentration from the highest concentration 1µg/µl of selected species of Malaysian seaweed

F. Antimicrobial and Antifungal Activity Assay

1) *Disc Diffusion Method*: Disc Diffusion Method (Kirby-Bauer Method) was conducted to ascertain antibacterial and antifungal activities of the extracts. [14]. A volume of 100 µl of selected bacteria and fungi suspensions was spread onto 20 ml of sterile agar plates using sterile cotton swab and allowed to dry for about 1 min. Sterile 6 mm dia. Of filter paper discs was arranged on the spread-plated agar and impregnated with 5 µl of 2.5 mg/ml, 5 mg/ml and 10 mg/ml extracts. Tetracycline and clotrimazole were used as antibacterial and antifungal positive controls, respectively while ethanol was used as negative control. The plates were incubated at 37°C for 24 h (bacteria) and 27 °C for 72 h (fungi) to allow growth. The diameter of inhibition zone (mm) was measured after subtracting the diameter of filter paper disc. Each extract was analyzed in triplicates.

III. RESULTS AND DISCUSSION

A. Determination of Chlorophyll and Carotenoid Content

Fig. 2 shows that the total chlorophyll content for *C. lentillifera* and *P. pavonica* were slightly similar with *C. lentillifera* at 7.3 µg/g DW and *P. pavonica* at 7.5 µg/g DW. Meanwhile, the result for *K. striatum*, *G. tikvahiae* and *E. denticulatum* showed lower amounts of total chlorophyll content with *K. striatum* at 4.5 µg/g DW, *G. tikvahiae* at 2.8 µg/g DW and *E. denticulatum* at 2.9 µg/g DW. Thus, the total chlorophyll content for all types of seaweed used can be summarized as Phaeophyta > Chlorophyta > Rhodophyta. In red seaweed, the presence of phycoerythrin and phycocyanin pigment contribute to their red colour. However, the results were not in line with the study conducted by Kumar et al. (2009) [15]. The research reported that Chlorophyta had the most amount of the total chlorophyll content, followed by Phaeophyta and Rhodophyta, respectively. However, the results reported by Vimala & Poonghuzhali (2013) supported the findings of this research whereby total amount of chlorophyll content is the highest in green seaweed species sample by using different types of solvent in the extraction method. [3]. Regarding the chlorophyll A and B content, a significant difference ($p < 0.05$) was found only in

the green seaweed species. Meanwhile, in terms of total chlorophyll content, *K. striatum* (red seaweed) was found to be significantly different ($p < 0.05$) in comparison to other species. Chlorophyll content is contributed by many factors such as the solvent used in pigment extraction. Dere et al. (1998) indicated in their research that the most suitable solvent to extract chlorophyll was methanol. Acetone performed better than diethyl ether as a solvent but no significant differences were reported between these two solvents [16]. Therefore, a combination of acetone and methanol solvent (7:3) was utilized in the current study to ensure the extraction of the semi-polar compound that are present in the seaweed samples. This was further supported by Sumanta et al. (2014) they indicated an intense peaks of chlorophyll absorption when the acetone was used and therefore it can be concluded that the acetone provided a good benefit as a solvent for the chlorophyll assay purpose [17].

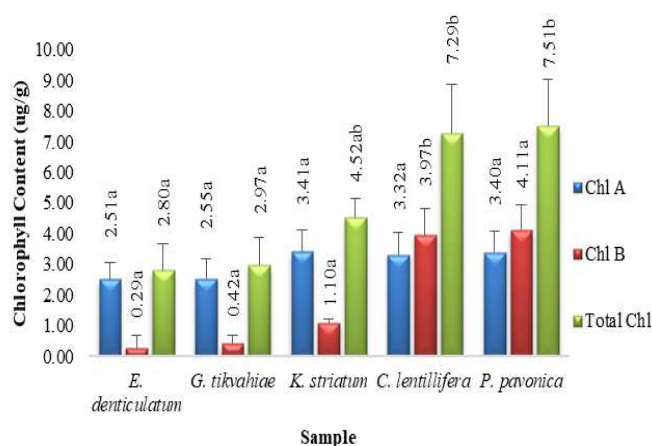


Fig. 2 Amount of total chlorophyll of selected species of Malaysian seaweed.

Fig. 3 shows the total carotenoid for the five Malaysian seaweed species investigated in this study. *P. pavonica* recorded the highest total carotenoid content, which was 100.9 ± 14.7 µg/g DW and *G. tikvahiae* have the lowest carotenoid content (25.1 ± 9.4 µg/g DW). The other species of seaweed which were *C. lentillifera*, *K. striatum*, and *E. denticulatum* recorded 63.5 ± 13.0 µg/g DW, 57.0 ± 15.8 µg/g DW and 33.0 ± 16.9 µg/g DW of total carotenoid content, respectively. The summary of the order based on total carotenoid content in the five species of Malaysian seaweed was Phaeophyta (brown seaweed) > Chlorophyta (green seaweed) > Rhodophyta (red seaweed). *P. pavonica* and *G. tikvahiae* species were significantly different on the amount of total carotenoid content. The analysis of variance that observed the total carotenoid content (with the unit of µg/g DW) in five species of Malaysian seaweed showed that there was a significant difference ($p < 0.0005$). Chakraborty & Santra (2008) also recorded that among all seaweed types, brown seaweed was found to have the biggest carotenoid content followed by red seaweed and finally green seaweed. However, the results of this present research showed that the total amount of carotenoid content was higher in green seaweed compared with red seaweed [18].

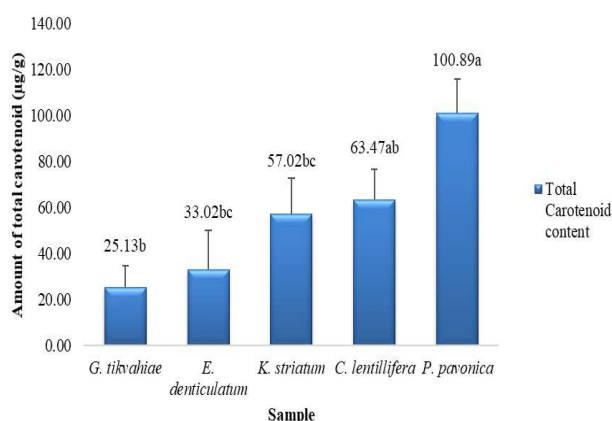


Fig. 3 Amount of total carotenoid content ($\mu\text{g/g}$ DW) for 5 species of Malaysian seaweed included the analysis of the differences with a confidence interval of 95% (a,b and c)

B. Determination of Individual Carotenoid Content

Fig. 4 shows four types of carotenoids found in the seaweed samples, which are zeaxanthin, lutein, beta-carotene, and violaxanthin. *K. striatum* (red seaweed) showed the highest lutein content, which was $38.6 \mu\text{g/g}$ DW, while *P. pavonica* (brown seaweed) showed the lowest lutein content, which was only $7.2 \mu\text{g/g}$ DW. Meanwhile, the highest content of zeaxanthin was found in *C. lentillifera* ($21.3 \mu\text{g/g}$ DW) while *E. denticulatum* showed the lowest amount ($3.6 \mu\text{g/g}$ DW). As for beta-carotene content, *C. lentillifera* ($10.7 \mu\text{g/g}$ DW) exhibited the highest content while *E. denticulatum* ($2.4 \mu\text{g/g}$ DW) was reported to have the lowest amount. The last type of carotenoid found in the seaweed samples was violaxanthin, which was found only in the green seaweed species, *C. lentillifera* ($8.9 \mu\text{g/g}$ DW). Overall, the result showed green and brown seaweed were significantly different in terms of zeaxanthin content ($p < 0.05$) whereas the significant difference was not found between all the red seaweed species. Seaweed species were not significantly different for lutein and beta-carotene content. The findings of the current study were supported by Burtin (2003) [19]. According to the study, brown seaweeds are rich in carotenoids especially fucoxanthin, beta-carotene, and violaxanthin. In red seaweed, the main carotenoids are beta-carotene and alpha-carotene, as well as their dihydroxylated derivatives consisting of zeaxanthin and lutein. For green seaweed species, the main carotenoids accumulated were similar to the higher plant, which are beta-carotene, lutein, violaxanthin, antheraxanthin, zeaxanthin, and neoxanthin. In comparison with the current results, violaxanthin was only detected in green seaweed. Green seaweed was also found to have an average level of each carotenoid, which was similar to the higher plant samples. Mcdermid & Stuercke (2003) reported the amount of beta-carotene content was higher in *C. lentillifera* (green seaweed) compared to the carotene content of *E. denticulatum* (red seaweed). This result was in accordance with the current study where *C. lentillifera* recorded higher amount of beta-carotene content compared to *E. denticulatum* [20].

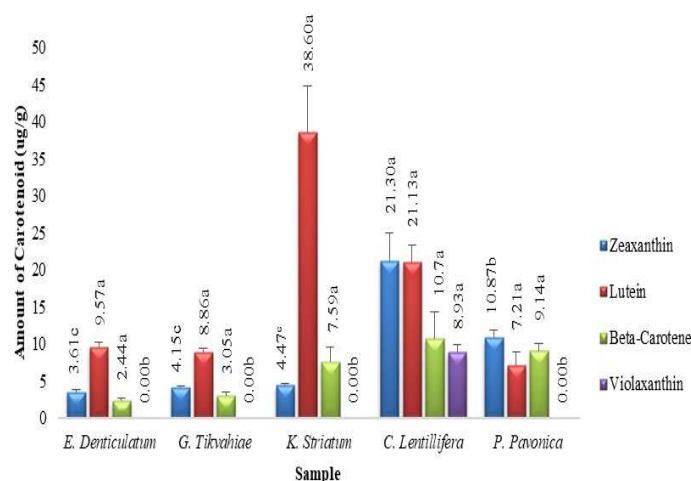


Fig. 4 Total amount of individual carotenoid in selected species of Malaysian seaweed; content 1 g DW .

C. DPPH Scavenging Assay for Antioxidant Properties

The DPPH radical scavenging capacity of five different seaweed species are illustrated in Fig. 5. Generally, the effect of antioxidant on DPPH radical scavenging is the result of its hydrogen donating ability. In this study *P. pavonica* (brown seaweed) presented the strongest DPPH activity with percentage of inhibition (i %) of $61.0 \% \pm 0.9$ followed by *E. denticulatum* (red seaweed) with $57.3 \% \pm 0.2$, *C. lentillifera* (green seaweed) with $47.0 \% \pm 4.3$, *G. tikvahiae* (red seaweed) with $37.8 \% \pm 1.4$ and *K. striatum* (red seaweed) with $35.6 \% \pm 1.7$ at the highest concentration of $1.00 \mu\text{g}/\mu\text{L}$. Overall, only *E. denticulatum* is significantly different compared to the other seaweed tested ($p < 0.05$). Besides that, *P. pavonica* (brown seaweed) recorded the highest antioxidant value. A research conducted by Andrade et al. (2013) indicated that the strongest radical scavenging activity was produced by brown seaweed [21]. The findings of the mentioned study were in accordance with a research conducted by Monsuang et al. (2009) as it discovered that brown seaweed *Sargassum* sp, displayed a lot more significant antioxidant activities compared to the antioxidant activities of both the red and green seaweeds [22]. The differences occurred in radical scavenging activity between the types of seaweed were possibly caused by their chemical composition differences that might result in the major changes in antioxidant activity [23]. In addition, the type of solvent utilized during the extraction was the reason for the antioxidant properties obtained in the extracted sample. In the current study, such a potent antioxidant activity was found during the methanol seaweed extracts. The researchers believe that their free radical-scavenging ability (RSA) were potentially caused by the antioxidant mechanisms of seaweed extracts as the results showed that the aqueous methanol extracts of *S. wightii* and *U. lactuca* were quite similar to the ascorbic acid, one of the most well-known antioxidant. The results displayed that the RSA % of brown seaweed (*S. wightii*) reached 108.06% , considered much higher than the RSA of green seaweed (*U. lactuca*) (at only 14.20%). Apart from that, location and salinity are the parameters that may strongly influence the level of antioxidant present in the algae. The exposure of the sun and its effect, as well as the emersion on *Porphyra umbilicalis*

was investigated in the research conducted by Sampath-Wiley et al. (2008). The study also discovered that during the summer period, the higher antioxidant level was found in the seaweed that is located in upper intertidal regions compared to the antioxidant level possessed by submerged seaweed. All the samples collected in this study were harvested from a seaweed plantation in Sabah [24].

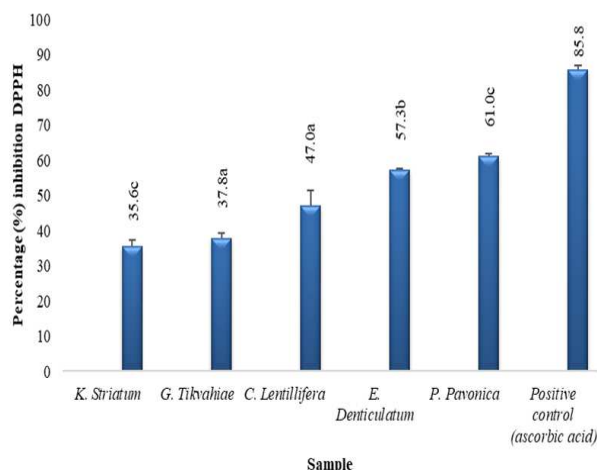


Fig. 5 Percentage inhibition (%) of DPPH for antioxidant activity of selected species of Malaysian seaweed at concentration 1µg/ul.

D. Antibacterial and Antifungal Activity

The indicator used to measure the antimicrobial activities are based on Rauha et al. (2000) [25] and Sani et al. (2017) [14] where the zone of inhibition excluding the disc diameter was interpreted into antibacterial and antifungal activities respectively. Various concentrations of crude seaweed sample were used (2.5 mg/ml, 5 mg/ml, and 10 mg/ml) to determine the effects of the different seaweed concentration towards the bacteria and fungi tested in this study. Rajasekar and Priyadharshihi (2013) stated that seaweed is a creature that offers a high amount of bioactive metabolites that will be strongly beneficial for the pharmaceutical industry, mainly in the drug development process. They also stated that the bioactive components extracted from the seaweed reduce the growth speed of pathogens produced by Gram-positive and Gram-negative bacteria [26]. In this present study, the strongest antimicrobial effect was observed towards *E. coli* and *P. aeruginosa*. Meanwhile, medium effects were shown by all seaweed samples towards MRSA and *S. epidermidis*. Finally, for the antimicrobial test, *S. pyogenes* and *B. subtilis* were found to be hardly affected by all seaweed samples as reported in Table II. For the antifungal test, all results showed medium antifungal action especially on *M. gypseum* and *Fusarium sp.* as reported in Table III. This study indicated that the used seaweed extracts showcased more potent antibacterial activities compared to their antifungal activities. It is believed that the composition and permeability difference of their cell walls also played an important role regarding the two mentioned activities. Kosanic´ et al. (2012) and Heijenoort (2001) mentioned that both teichoic acids and peptidoglycans are present in the cell walls of Gram-positive bacteria while lipoproteins, lipopolysaccharides, and peptidoglycans were found in the

cell walls of Gram-negative bacteria. It was found that the higher resistance was caused by the poor permeability of the lipid part of the surface membrane of Gram-negative bacteria [27], [28]. Farkas (2003) also stated the low permeability of the fungi cell wall and polysaccharides such as glucan and hitching were also found in the cell wall of fungi [29]. The different levels of sensitivity in fungi, Gram-negative bacteria, and Gram-positive bacteria are affected by the permeability and the structure of the cell wall. Zubia et al. (2008) also found that light, salinity, herbivory, depth, and nutrients were some of the external environmental factors affecting the high variation occurred in the potential antimicrobial components in seaweed. The factors mentioned above could potentially act according to the spatiotemporal regulation on the metabolic expression of the active compounds that eventually leads to distinct quantitative and qualitative variations among identical species at a lower scale than different species. Other factors that influence the antibacterial and antifungal activity include the season when the samples were extracted, the type of solvent utilized during the extraction and the methodology of the sample extraction. Those factors are the reason of the variation occurred in the final outcome [30]. Hediat et al. (2010) claimed that different type of solvents are attributed to the different capacity of the extraction towards the different phytoconstituents and polarity and solubility of the solvents play an important role in that. The present study discusses the selection of the most suitable solvent to extract the active compound from seaweed and it was performed to ensure antibacterial activity [27]. Therefore, it is important that seaweed is extracted using a combination of different solvent systems as it allows optimization in their antibacterial activity. Adersson et al. (1983) and Zubia et al. (2008) displayed that the different anti-pathogenic, anti-inflammatory and antibacterial effects are influenced by the type of solvent used (chloroform, water, dichloromethane, and ethanol) during the seaweed extraction [30].

TABLE II.
ANTIBACTERIAL EFFECT OF SEAWEED; SAMPLE AMOUNT IS 12.5, 25 AND 50µG

Sample	µg/ disc	<i>E. coli</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. pyogenes</i>	MRS-1	<i>P. vulgaris</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>B. subtilis</i>
<i>E. denticulatum</i>	50	--	--	--	-	--	~	--	--	-
	25	--	-	-	~	--	~	--	--	-
	12.5	~	~	~	~	~	~	--	--	-
<i>G. tikvahiae</i>	50	--	~	~	-	--	~	-	-	-
	25	--	~	~	~	-	~	--	~	-
	12.5	-	-	-	~	~	-	~	-	-
<i>K. striatum</i>	50	--	~	~	-	~	-	--	~	-
	25	~	-	-	-	~	~	--	~	-
	12.5	--	-	~	-	-	~	-	~	-
<i>C. lentilifera</i>	50	--	--	--	-	--	--	--	--	--
	25	--	~	~	-	-	-	--	-	--
	12.5	~	~	-	-	-	~	--	-	--
<i>P. pannonica</i>	50	--	-	-	-	--	-	--	--	-
	25	--	~	-	-	-	~	-	~	~
	12.5	--	--	-	-	~	-	~	~	-

a - : No antimicrobial activity, inhibition zone (i.z) of sample < i.z of ethanol +1mm
b ~ : Slight antimicrobial activity, i.z of sample 1-3mm > i.z of ethanol
c + : Moderate antimicrobial activity, i.z of sample 3-4mm > i.z of ethanol
d + + : Clear antimicrobial activity, i.z of sample 4-10mm > i.z of ethanol
e + + + : Strong antimicrobial activity, i.z of sample > i.z of ethanol + 10mm
f () : Effect is only bacteriostatic
g * : Tetracycline as positive control

TABLE III
ANTIFUNGAL EFFECT OF SEAWEED; SAMPLE AMOUNT IS 12.5, 25 AND 50µG

Sample	µg/ disc	<i>C. albicans</i>	<i>M. gypseum</i>	<i>A. niger</i>	<i>Fusarium sp.</i>	<i>Trichoderma sp.</i>	<i>Penicillium sp.</i>
<i>E. denticulatum</i>	50	-	~	~	-	~	-
	25	~	-	-	-	-	-
	12.5	-	-	-	-	-	-
<i>G. tikvahiae</i>	50	~	~	-	-	-	~
	25	~	-	-	~	-	~
	12.5	-	-	~	~	-	-
<i>K. striatum</i>	50	-	--	-	~	-	~
	25	~	-	~	-	-	-
	12.5	-	-	~	-	-	-
<i>C. lentilifera</i>	50	~	~	~	-	-	--
	25	-	-	-	--	-	-
	12.5	-	~	-	-	-	-
<i>P. pannonica</i>	50	-	--	~	~	-	~
	25	~	-	-	-	-	-
	12.5	~	~	-	~	-	-

a - : No antimicrobial activity, inhibition zone (i.z) of sample < i.z of ethanol +1mm
b ~ : Slight antimicrobial activity, i.z of sample 1-3mm > i.z of ethanol
c + : Moderate antimicrobial activity, i.z of sample 3-4mm > i.z of ethanol
d + + : Clear antimicrobial activity, i.z of sample 4-10mm > i.z of ethanol
e + + + : Strong antimicrobial activity, i.z of sample > i.z of ethanol + 10mm
f () : Effect is only bacteriostatic
g * : Tetracycline as positive control

IV. CONCLUSIONS

Seaweed extracts and powder have exhibited bioactive responses as antioxidant, antibacterial and antifungal agents. These positive activities were observed in all seaweed genera used in this study encompassing red, green and brown algae species. The bioactive compounds present in seaweed offer a great opportunity for them to be used as an antioxidant, antimicrobial and Active Pharmaceutical Ingredient (API). The study highlighted the total chlorophyll and carotenoid content in seaweed, which includes the individual carotenoid that is present in the samples. There are four types of carotenoid found in this research including zeaxanthin, lutein, beta-carotene, and violaxanthin. In addition, it was observed that brown seaweed had higher antioxidant activity; $61.0\% \pm 0.9$ than the other seaweed species. Antibacterial activity of seaweed samples showed the highest activity towards Gram-negative bacteria. Overall, the seaweed extracts were more effective as antimicrobial agents as compared to antifungal.

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