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ORIGINAL RESEARCH PAPER

Antioxidant and antibacterial activity of red seaweed; *Kappaphycus alvarezii* against pathogenic bacteria

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ABSTRACT

Seaweeds can produce variety of bioactive components for the benefits of humans. Malaysia is one of the countries to produce red seaweeds, which are popular secondary metabolites. *Kappaphycus* species, largest tropical red algae exhibit the high growth rate. It was reported that its biomass can grow double in just 15 to 30 days. Therefore, this investigation emphasized on two extraction methods such as hot water and ethanolic Soxhlet extraction to extract the bioactive compounds from *Kappaphycus alvarezii*. Both of these extractions were screened to produce antimicrobial and antioxidants compounds. Total phenolic content and ferric reducing assays were employed to quantify antioxidant properties. Whereas, the disc diffusion assays were used to study antibacterial activity. The results reported the highest phenolic content for ethanolic extract (20.25 ± 0.03 mg gallic acid equivalents per gram of extract). On the other hand, the value of phenolic content was slightly decreased 19.1 ± 0.81 mg gallic acid equivalents per gram of extract for the hot water extract. It was also found that both the extracts were potentially capable of balancing reactive oxygen species. Disc diffusion assay results indicated that the extract of red alga *K. alvarezi* were more efficient against *B. cereus*. Among the fatty acids determined levoglucosenone and 4-Pyridinemethanol were present in high percentages in hot water extract whereas Hexamethyl- cyclotrisiloxane followed by 1, 2, 5-Thiadiazole-3-carboxamide, 4-[(2-chloroethyl) amino]-N-(2-hydroxyethyl) were present in ethanolic extract of *K. alvarezi.* The present study concluded that, hot water extracts of *K. alvarezii* can be used for large scale production of bioactive compounds utilizing an easily available potential seaweed. Future research of red seaweed will be highly important for pharmaceutical and medicinal field as well as a homogenizer in milk products, toothpaste and jellies in other industrial applications.

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INTRODUCTION

Algae refers to a large group of different organisms from different phylogenetic groups that consist of various taxonomic divisions (Mabberley, 2017). Generally, algae can be considered as plant-like organisms that are usually photosynthetic and aquatic but lacking of true roots, stems, leaves, vascular tissue and possess simple reproductive structures. (Asmida *et al.,* 2017) have reported that the marine algae in Malaysia consists of 381 taxa where 105, 186, 73, and 17 of that amounts are coming from taxa Chlorophyta, Rhodophyta, Phaeophyta and Cyanophyta respectively. Algae are significantly classified into two major groups which are microalgae and macro algae. Seaweeds live generally attached to rock or other hard substrata in coastal areas (Munir *et al.,* 2013). They are distinguished based on thallus color, placing them into three broad groups namely brown, red and green seaweed. Each of them belongs to phylum Phaeophyta, Rhodophyta and Chlorophyta respectively. However, differentiation of these phyla is not only depending on their color but also based on other substantial differences such as pigmentation, ultra-structural and biochemical features such as photosynthetic pigments, storage compounds, and composition of cell wall. As we know, living conditions of seaweed are exposed to a wide range of environmental stress such as light, rapid fluctuations in temperature, osmotic stress and desiccation. However, they rarely suffer any serious photodynamic damage regardless being subjected to such conditions that could enhance the formation of free radicals and other strong oxidizing agent (Ahmed *et al.,* 2016). This clearly indicates that seaweeds possess special characteristics that are able to protect themselves from the hazard caused by free radicals as well as reactive oxygen species. As mentioned by most researchers, seaweeds contain a wide range of bioactive compounds which could exhibit antioxidant, antibacterial, antiviral, and antifungal activities (Quirós *et al.,* 2010; Vallinayagam *et al.,* 2009). This statement is agreed by Hwang *et al.,* (2010) who have claimed that marine seaweed is a rich source of antioxidants. While the presence of free radicals and reactive oxygen species (ROS) threaten human well-beings, pathogenic bacteria are harmful as they release poisonous substances called endotoxins and exotoxins which are responsible for the bacterial infections. *E. coli*, *Salmonella, Bacillus cereus* are an example of common pathogenic bacteria for the main cause of food poisoning. Thus, demands for therapeutic drugs are increasing prior to treatment of pathogenic infections and diseases caused by bacteria. As natural sources are more preferable due to its novel bioactive properties Cornish and Garbary (2010), especially, seaweeds can be considered as the potential target as it is highly available in marine environment. Physically red seaweed belongs to key of genera Phaeophyta, the bushy thallus is made up of dichotomous and irregular branching. Chemical composition of *Kappaphycus alvarezii* includes moisture 0.89%, carbohydrate 65.20%, protein 3.40%, fat 1.10% and ash 11.57% (Khalil *et al.,* 2018). Moreover, findings from the previous studies have proven that seaweeds possess bioactive constituents (Souza *et al.,* 2011) that can benefit mankind in terms of inhibiting bacterial growth (Kanatt *et al.,* 2015). The examples of compound responsible for antimicrobial and antibacterial activities in seaweeds include phenols, terpenes, acetogenins, indoles, fatty acids and volatile halogenated hydrocarbon (Shafay *et al.,* 2016; Alencar *et al.,* 2014). In fact, the biological potentials of seaweed resources of marine environment in Malaysia has not been adequately explored. So, the present study was undertaken to identify the species of marine edible seaweed to evaluate bioactive substances using two extraction methods, such as hot water extraction and ethanolic extraction by using Soxhlet apparatus. Here, we report the two extraction methods utilized for the extraction of bioactive compounds from Kappaphycus alvarezii. Both extractions were screened for the production of antimicrobial compounds and antioxidant properties. Total phenolic content and ferric reducing assay were used to quantify antioxidant properties while disc diffusion assay was used to study antibacterial activity. This study has been carried out in University of Malaysia Pahang, Malaysia in 2018.

MATERIAL AND METHODS

Algal preparation

Red algal materials were purchased from a local market in Semporna, Sabah and it was stored in plastic bags and transported to the laboratory under iced conditions. The sample was washed thoroughly with tap water and then washed with fresh water to remove sand and adhered substances. The algal species were identified based on comparing its

morphological structures and features to seaweed manual guide described by (Dennis, 2003, Gegg and Wells, 2019). Sample was shade dried for three days and further placed in a drying oven to ensure complete removal of its water content. Once dried, the sample was cut into small pieces and grounded to a coarse powder form using grinder machine with 0.5 mesh. The powdered sample was stored in a sterile container for further use for different extraction methods.

Crude extracts preparation of algal species

Extraction of powdered algal sample was done using ethanol and hot water according to Hwang and Thi (2014); Godlewska *et al.,* (2016) with some modifications. *For ethanol extraction* 10 g of the powdered seaweed sample was soaked in 150 mL of 70 % ethanol for 30 minutes. Then, the soaked samples were homogenized at room temperature, centrifuged for 20 min. at 5000 rpm and the supernatant was filtered using filter funnel and Whatman No. 1 filter paper and concentrated at 70°C using a rotary evaporator. For hot water extraction, 10 g of powdered seaweed was soaked in 150 mL of distilled water and heated up to 70 ˚C on a hot plate for 3 hours. The mixture was occasionally stirred throughout the heating period. Then, the mixture was allowed to cool down before filtering it through muslin cloth. The filtrate was stored in -80°C.

Determination of antioxidant activity

A stock solution of 2 mg/ml of extracts were prepared. In order to determine the antioxidant property of the seaweed, total phenolic content and ferric reducing power were determined by using Folin-Ciocalteu's method described by Quirós *et al.* (2010); Hwang *et al.* (2010), respectively.

Determination of total phenolic content

0.1 ml of extracts from the stock (2 mg/mL) solutions were pipetted into respective test tubes. 2 mL of 2 % Na_2CO_3 were added into the sample and incubated for two minutes at room temperature. 0.1 mL of Folin-Ciocalteu was added and mixed by shaking and then it was incubated for 30 minutes in dark. Absorbance were measured by using micro plate reader at 700 nm. All measurements were done in triplicate with control. Different concentration of gallic acid ranging from 0.2-1.0 mg/mL was used to plot standard curve. Results were expressed as mg GAE/g of extract.

Determination of ferric reducing power

Different concentration of sample (0.5 mg/mL to 2.0 mg/mL) was prepared and 0.75 mL of pH 6.6 phosphate buffer was added into sample solution. Then, 0.75 mL of 1 % potassium hexacyanoferrate was added into each tube. The mixture was incubated in water bath for 20 minutes at 50°C followed by the addiction of 0.75 mL of 10 % trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 min. Supernatant was collected and mixed with 1.5 mL of distilled water and 1.5 mL phosphate buffer of pH 6.6. 0.1 mL. Finally, 0.1% ferric chloride was pipetted into the solutions. Blank was prepared consisting of the same chemicals except sample while ascorbic acid was used as positive control. Solutions from all test tubes were pipetted into 96 well plate to measure absorbance at 700 nm using micro plate reader (Ganesan *et al.,* 2008).

Determination of antibacterial activity

Antimicrobial activities of the selected red algal species *K. alvarezii* were tested using pathogenic bacteria included *Escherichia coli* and *Bacillus cereus* isolated in the Microbiology laboratory. The bacterial strains were grown in Nutrient agar medium at 37°C. Stock cultures were maintained at 4°C and sub-cultured at regular intervals. The nutrient agar medium was prepared using g/L: 5 g peptone, 3 g beef extract, 5 g NaCl and 20 g agar in distilled water. Antibacterial activity was determined using disc diffusion assay in petri dishes. Briefly, sterile paper discs 9mm in diameter, were loaded with 25 µl of extracts and air dried. Disc containing standard concentration of ampicillin was used as positive control. Plates were incubated at 37°C for 24 h and the inhibition zone formed around the impregnated discs were measured in millimetre. Each set was prepared in triplicates.

Identification of crude extract compounds

GC-MS analysis of ethanolic extract

The analysis of ethanolic extract using GC-MS was done following the method described by Arulkumar *et al.,* (2018). The column used was capillary column (30 mm × 0.25 mm 1D × µM). An electron ionizing system with ionizing energy of 70eV was used while

helium gas (99.999 %) was utilized as carrier gas at constant flow rate 1 mL/min. Injection volume of 2 µL was employed with split ratio of 10:1. The injector temperature was 250°C and ion source temperature was 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, finished with 9 minutes isothermal at 280°C. Mass spectra were taken at 70eV with a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total running time was 36 minutes.

GC-MS analysis of hot water extract

DB-5 column (30 mm × 250 µm × 0.25 µm) was utilized in hot water analysis. Helium gas (99.999 %) acted as the carrier gas at constant flow rate 1 mL/min with split ratio of 1:50. The inlet temperature was 250°C. The initial oven temperature was set at 60°C, hold for 1-minute ramp, with an increase of 10°C/min to 301°C and hold for 5 minutes. The detector temperature was 230°C while transfer line temperature was 310°C. Total running time was 32 minutes.

RESULTS AND DISCUSSION

Seaweed identification

The identification of seaweed was done by comparing its morphological structures to the published keys and journals. Identified seaweed belongs to key of genera Phaeophyta seaweed, the bushy thallus is made up of dichotomous and irregular branching. These long and cylindrical main branches appear almost unnoticeable by the presence of lateral branches with pointed tips. Reef flat and reef edge, 1 to 17 m deep. Loosely attached to broken coral, or unattached fragments floating in shallow and deep waters. Can form large, moving mats of unattached thalli. This seaweed possesses the taxonomic rank of *Kappaphycus alvarezii* and which was purchased from Semporna, Sabah.

Extraction yield

The extraction yield of *Kappaphycus alvarezii* crude extracts obtained from ethanolic and hot water extraction are shown in Table 1 The highest value obtained in hot water extraction and its yield was 32.534 % followed by ethanolic extraction which differs by 17.065 % from hot water extraction. These values were calculated by using the formula obtained from Mansuya *et al.,* (2010) such as Eq. 1.

Table 1: Percentage of extraction yield

Type of extraction	Extraction yield (%)
Ethanolic	15.469
Hot water	32.534

$$\frac{Dry\ weight\ of\ crude\ extract}{Initial\ weight\ of\ sample} \times 100 \quad (1)$$

Ethanolic extraction was done by using 70 % (v/v) ethanol while distilled water was utilized in hot water extraction. The higher value in hot water extraction indicates that compounds in *K. alvarezii* are mostly high in polarity and soluble in water. According to Naveena and Prakash, (2013) solubility of compounds in solvents of different polarities were affected by its structure of phenolic compound. Both solvents used in this study were polar solvent. However, since water has higher polarity index and shorter chain compared to ethanol, it is more effective in extracting solutes due to the mentioned features (Kolanjinathan *et al.,* 2009). Besides polarity, other factors that affect the percentage yield include extraction temperature, extraction time and solvent to solid ratio. These factors are in line with Hwang and Thi (2014) who claimed that extraction condition affected the antioxidant potentials in red seaweed based on their study on effects of extraction and processing methods towards Laver (Porphyra tenera). In present study, extraction temperature as well as extraction time were different in each method. Total extraction time of seaweed in ethanol was approximately less than two hours while hot water extraction was going on for more than three hours. Extraction temperature also varied as ethanolic extraction took place in room temperature while hot water extraction was carried out at a very high temperature (70 ºC). Thus, this might explain why extraction yield of hot water is higher than ethanolic extraction (Ibrahim *et al.,* 2012).

Determination of antioxidant activity

Determination of total phenolic content

In order to find the values for gallic acid equivalent, concentration of gallic acid in both samples were calculated by using Eq. 2.

$$y = mx + c \quad (2)$$

From the calibration curve, the equation established was y = 2.0561x - 0.0794 with correlation coefficient of 0.9889. This indicates that the model is 98.89 % fit to the data obtained from this experiment. Next, calculation of total phenolic content was made according to Eq. 3.

$$T = \frac{C \times V}{M} \quad (3)$$

In Eq. 3 where T represents total phenolic content of extract expressed as mg GAE/g of extract while C is concentration of gallic acid (mg/mL). V and M denote volume of extract solution in mL and weight of extract (g) respectively. Total phenolic content of *K. alvarezii* from both extraction methods were expressed as mg GAE/g in the table below. Values shown are in terms of mean ± standard deviation.

As seen in Table 2, ethanolic extract has higher phenolic content compared to hot water extract. The value obtained from ethanolic extract is 20.25 ± 0.03 mg GAE/g while hot water extract is 19.1 ± 0.8 mg GAE/g. The phenolic content of the K. alvarezii was compared with L. digitata and P. palmata by Cox et al, 2010 and reported that, phenolic content was higher in methanolic extract than ethanolic extract. Therefore, the present study could also perform the extraction with methanol for better yield for further study. Also, the content of phenolic compound in the present study contradicts from Ling *et al.,* (2015) who reported that extraction of phenolic compounds is more effective in water compared to ethanol. Generally, recovery of phenolic compound is believed to depend on type of solvent used, its polarity index and the solubility of phenolic compounds in the extraction solvents. A previous study by Ahmad *et al.,* (2016), showed that the highest phenolic content was recorded in green seaweed followed by brown and red seaweed. Both green and brown seaweed showed significantly higher phenolic content compared to those in red seaweeds tested. Previous studies agreed than green seaweed has the higher capacity of scavenging free-radical (Ganesan *et al.,* 2008).Phenolic content of seaweed varies depending on the location it was grown. *K. alvarezii* obtained from Langkawi showed lower phenolic content compared to the one obtained from Semporna with total phenolic content of 54.35 mg GAE/100 g and 73.25 mg GAE/100 g respectively (Abirami and Kowsalya, 2011).

Determination of ferric reducing power

Ferric-reducing antioxidant power (FRAP) was determined with the method described by Ganesan

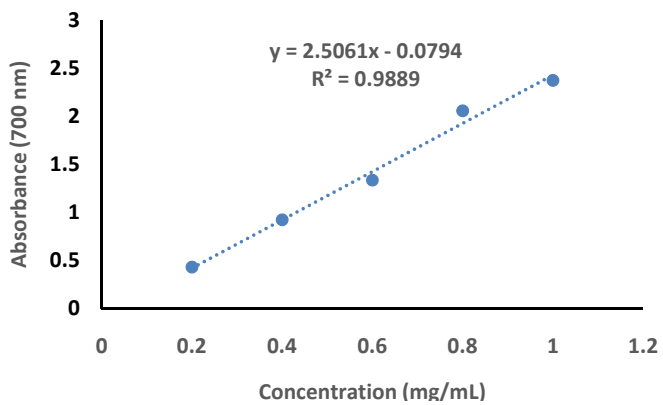

Fig. 1: Gallic acid standard curve (R^2=09889)

Table 2: Total phenolic content of *Kappaphycus alvarezii* (mg GAE/g of extract)

Seaweeds	Extraction method	Total phenolic content (mg GAE/g)	Reference
Laminaria digitata	Methanolic extract	37.66±0.00	Cox *et al.,* 2010
Palmaria palmata	Methanolic extract	42.83±3.2	Cox *et al.,* 2010
Kappaphycus alvarezii	Hot water extract	19.1 ± 0.8	Present study
Kappaphycus alvarezii	Ethanolic extract	20.25 ± 0.03	Present study

et al. (2008). The absorbance was read at 700 nm using a microplate reader (Biochrom Asys UVM 340). BHT, BHA and quecertin were used as positive controls. Greater absorbance indicated greater FRAP. Hot water extract was showing extensively increasing results compare to ethanolic extract and ascorbic acid as shown in Fig. 2. The maximum absorbance was observed at 2 mg/mL extract.

Determination of antibacterial activity

Antibacterial activities of *Kappaphycus alvarezii* was assessed against two pathogenic bacteria by using disc-diffusion assay. *Escherichia coli* was used for gram negative microorganism and for gram-positive bacterium, *Bacillus cereus* was used to check antibacterial activity of the extracts. The efficacy of the seaweed's antibacterial activities was evaluated based on the inhibition zone formed around the impregnated discs. Different concentration of extracts was compared with commercial 10 µg ampicillin paper disc and distilled water as positive and negative control respectively. The results were represented in Tables 3 and 4. The (-) symbol indicates the absence of inhibition zone while (+) indicates presence of inhibition zone with less than 10 mm of diameter. (++) indicates that the inhibition zone is present with diameter of more than 10 mm but less than 25 mm.

From the result, it can be said that *E. coli* showed resistant towards various concentrations of ethanolic and hot water extracts since no inhibition zone on LB agar plates. As for *B. cereus*, very thin line of clearer zone around the discs were spotted in both ethanolic and hot water extracts at varying concentrations. The common concentration showing inhibition zone in both extracts is 2 mg/mL Therefore, it can be said that this concentration is probably the most effective to test antibacterial activity. The type of bacteria has been highly associated with its antibacterial performance. Gram negative bacteria appears to be less susceptible towards antibacterial agents compared to gram negative bacteria due to its morphological structure

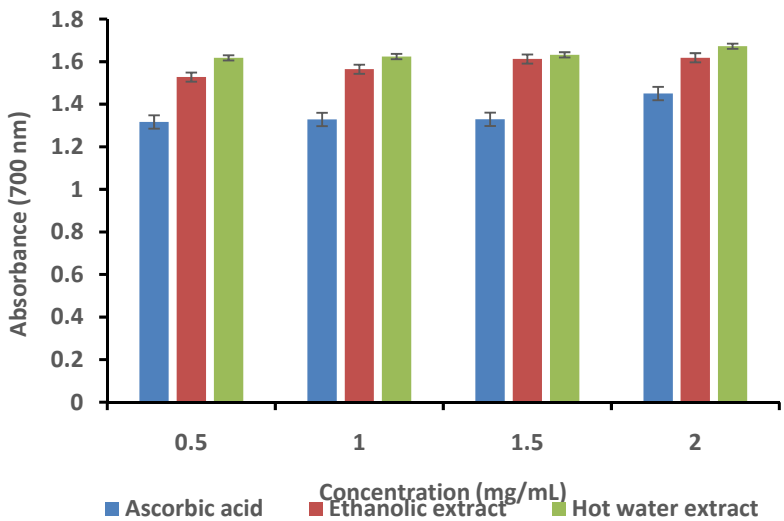

Fig. 2: Ferric reducing power of *Kappaphycus alvarezii* ethanolic and hot water extract

Table 3: Inhibition zone of *E. coli*

	Formation of inhibition zone									
E. coli	Control		Concentration of ethanolic extract (mg/mL)				Concentration of hot water extract (mg/mL)			
	10 µg Ampicillin	Distilled water	0.5	1	1.5	2	0.5	1	1.5	2
Replicate 1	++	-	-	-	-	-	-	-	-	-
Replicate 2	++	-	-	-	-	-	-	-	-	-
Replicate 3	++	-	-	-	-	-	-	-	-	-

Table 4: Inhibition zone of *B. cereus*

Bacillus cereus	Formation of inhibition zone									
	Control		Concentration of ethanolic extract (mg/mL)				Concentration of hot water extract (mg/mL)			
	10 µg Ampicillin	Distilled water	0.5	1	1.5	2	0.5	1	1.5	2
Replicate 1	++	-	-	+	+	+	+	+	+	+
Replicate 2	++	-	-	+	-	+	-	-	-	+
Replicate 3	++	-	+	+	+	+	+	-	-	+

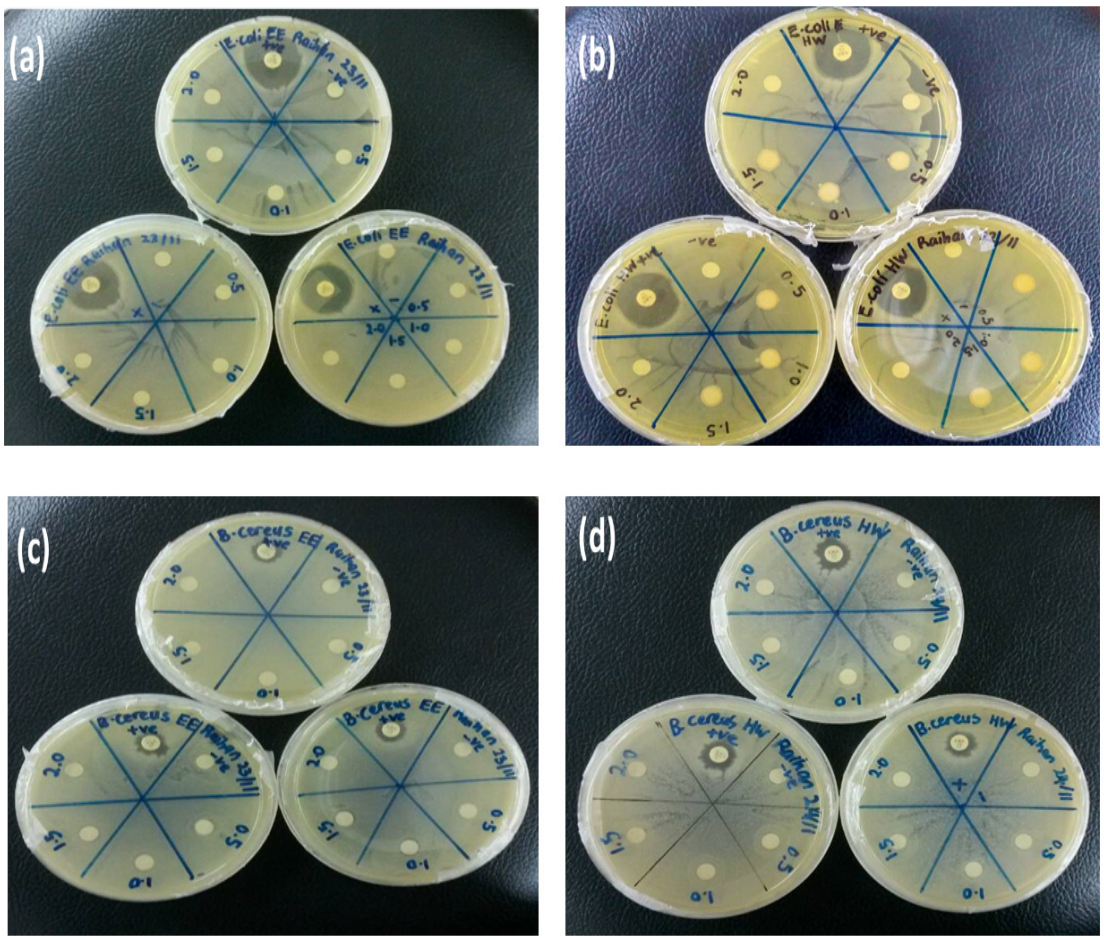

Fig. 3: Antimicrobial activity assay (a); Inhibition zone of *E. coli* (b); Inhibition zone of *B. cereus* (c)

and composition (Rebecca *et al.,* 2012). This is because outer membrane of the gram-negative bacteria consists of lipopolysaccharide which acts as a barrier and constrain the penetration of antibacterial as well as antimicrobial agents. The passage through the outer membrane of gram-negative bacteria is regulated by the presence of hydrophilic channels which usually inhibit the entry of hydrophobic compounds. However, this type of bacteria is effective in hydrophobic diffusion as it lacks phosphoglycerides in the outer membrane. As stated by Monte *et al.,* (2014), the values for minimum inhibitory concentration in *E. coli* was higher than those in *S. aureus* which is a gram-positive bacteria.

Table 5: GC-MS analysis of *K. alvarezii* ethanolic extract

Peak	Retention time (RT)	Area (%)	Name of the compounds	Similarity
1	1.947	66.18	Hexamethyl- cyclotrisiloxane	49
2	2.439	10.52	1,2,5- Thiadiazole-3-carboxamide, 4- [(2-chloroethyl) amino]-N-(2-hydroxyethyl)	36
3	3.543	9.76	4-Aminopyrimidine	47
4	3.870	4.23	1,3-Butadiene-1-carboxylic acid	43
5	4.259	4.59	5-Deoxypyridoxal	40
6	6.370	0.25	5-Hexyl-2-furaldehyde	9
7	14.615	1.24	Propanamide	4
8	19.222	0.27	Neopentyl isothiocyanate	4
9	21.299	0.28	Phthalic acid, 5-methylhex-2-yl, heptadecyl ester	4
10	25.138	0.22	1H-1,2,4-Triazole	2
11	25.893	1.10	1,2-bis(1-buten-3-yl)- benzene	43
12	26.174	1.20	Diazoprogesterone	22
13	29.453	0.16	1-(4-Hydroxyphenethyl) isoquinoline	3

Table 6: GC-MS analysis of *K. alvarezii* hot water extract

Peak	Retention time (RT)	Area (%)	Name of compounds	Similarity
1	1.203	0.07	2-fluoro-acetamide	2
2	1.730	6.54	4,5-dimethyl-1H-Imidazole	78
3	2.256	0.18	4-Methyl-1,4-heptadiene	5
4	2.422	2.10	1-(3-thienyl)- ethanone	5
5	3.206	3.17	1-Hydroxymethyl-2-methyl-1-cyclohexene	9
6	3.578	48.90	Levoglucosenone	90
7	2.824	2.34	1,3-Butadiene-1-carboxylic acid	47
8	4.144	28.21	4-Pyridinemethanol	64
9	4.487	0.15	Azetidine, 2,2,3,3-tetramethyl-	3
10	4.671	0.20	Cyclopentanone, 3-methyl-	2
11	5.140	1.14	Cyclopentanone, 3-methyl-	9
12	5.277	0.13	2-Furanmethanamine	5
13	5.586	0.03	2-Azabicyclo [2.2.1] heptane	3
14	6.330	1.70	2-Oxopropionic acid, dimethylhydrazone, methyl ester	9
15	6.484	0.11	1-(2-buten-2-yl)-aziridine	3
16	6.559	0.10	N-2-propynyl- acetamide	3
17	6.748	1.54	Furan, 2,3-dihydro-	4
18	8.298	0.25	Cyanamide, (trimethylphosphoranylidene)	2
19	10.261	0.06	5H-Tetrazol-5-amine	4
20	19.302	0.21	2,3-Pyridinedicarbonitrile	2
21	25.172	0.30	2-Propynenitrile, 3-fluoro-	2

The results of their study also revealed that gram positive bacteria were more susceptible to crude extract of seaweed than gram negative bacteria. None of the samples showed antibacterial activities against *E. coli* even after 24 hours of incubation. Similar finding was reported in an antibacterial study of seaweed ethanol extracts against *E.coli* (Kolanjinathan *et al.,* 2014). It is believed that the type of extraction solvent used contributes to the properties of antibacterial in seaweeds. According to Chuah *et al.* (2017) inhibition zone was present in methanolic extract of *Kappaphycus alvarezii* against *Proteus mirabilis* but absent in acetone extract of the same sample and bacteria. Yet, when acetone extract was used, maximum inhibition zone of 10 mm was observed against *Bacillus subtilis*. Rebecca *et al.* (2012) claimed that chloroform and isoamyl alcohol were the best organic solvent to extract antibacterial materials based on his study regarding antibacterial activities of *Kappaphycus* against

Table 7: Various seaweeds and its therapeutic properties

Seeweeds	Therapeutic compounds and properties	References
A. orientalis, S. marginatum	Antibacterial activity against Pseudomonas aeruginosa	Shanmughapriya *et al.,* 2008
Sargassum muticum	methanol extract (SMME) showed antiangiogenic activity; antioxidant effects	Namvar *et al.,* 2013
Fucus vesiculosus	Fucoidan (sulfated polysaccharide derived from brown algae) anticancerous activity	Xue *et al.,* 2012; Kim *et al.,* 2010
Porphyra dentata	Sterol fraction (containing cholesterol, β-sitosterol, and campesterol) decreased the reactive oxygen species (ROS) and arginase activity.	Kaslowska *et al.,* 2013
Lophocladia sp.	Lophocladines A and B are 2,7-naphthyridine alkaloids; Cytotoxic to lung tumour cells and breast cancer cells	Gross *et al.,* 2006
Laurencia sp.	Dactylone	Federov *et al.,* 2007
Ishiae okamurae	Ethanol extracts	Kim *et al.,* 2009
Gracilaria termistipitata	Methanol extracts	Ji *et al.,* 2012
Phoma herbarum	Ethanol extracts	Kim *et al.,* 2009
Kappaphycus alvarezii	Ethanol and Hot water extract	**Present study**

infectious pathogens. In similar studies of green seaweed against *Staphylococcus aureus, E. coli, Proteus mirabilis, Klebseilla pneumoniae,* and *Salmonella typhimurium*, the methanolic extract showed significant results and strong antibacterial activities. In fact, *Bacillus cereus and Proteus mirabilis* were the most susceptible towards the extract as they formed the largest inhibition zone.

GC-MS analysis

The identified compounds from ethanolic and hot water extracts were tabulated in Tables 5 and 6 respectively. However, the detected compounds showed very low similarity to the library. Theoretically, the data should be more than 90 % similar to library in order to say that the particular compound is present. In this case, it can be said that the compounds are likely to be unknown since it shows less than 50 % similarity. Another possible reason for this occurrence might be due to the outdated version of database. This is because the data was obtained from National Institute of Standards and Technology database version 2011. Therefore, some compounds are probably not registered. Nonetheless a compound from hot water extract was detected having 90 % similarity to the library, which is levoglucosenone, a type of preservative.

Mala *et al.,* 2017 claimed that levoglucosenone exhibit anti-tumour activity which has comparatively increased the cytotoxic effects. Table 7 showed the comparative extraction method and properties of the compound extracted from different seaweeds.The qualitative phytochemical screening of *Kappaphycus alvarezii* ethanol extract showed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids and trepenoids which can potentially act as antibacterial agents (Farah *et al.,* 2015; Hayashi *et al.,* 2012).

CONCLUSION

In conclusion the results of the present study on the selected red alga *Kappaphycus alvarezii* indicated scope for deriving bioactive compounds which are potential inhibitors of pathogenic bacteria. The presence of fatty acids in both extracts were Levoglucosenone, which has highly functionalized chiral structure that can be used as key intermediates of biologically active compounds; Pyridinemethanol , a functional pyridine which is an intermediate in the pharmaceutical industries; hexamethylcyclotrisiloxane used widely in medicine, military aviation and other petrochemical industries; 1,2,5- Thiadiazole-3-carboxamide, 4- [(2-chloroethyl) amino]-N-(2-hydroxyethyl)], can be used for antimicrobials. In addition, significant level of levoglucosenone (48.9%) and 4-Pyridinemethanol (28.21%) were detected in hot water extract through GC-MS analysis could possibly exhibit the anti-tumour activity. Further, the main cultivated species of seaweed for superfood in East Malaysia, Semporna has the potential to develop drugs from useful seaweed *K. alvarezii* to cure chronic human diseases. Further investigations towards the development of drugs from red alga have great scope.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

ABBREVIATIONS

%	Percent
^0C	Degree Celsius
B. cereus	Bacillus cereus
BHA	Beta hydroxy acid
BHT	Butylated hydroxytoluene
CFU	Colony Forming Unit
Da	Dalton
DRS	Doctoral Research Scholarship
E. coli	Escherichia coli
Eq.	Equation
eV	Electron Volt
FRAP	Ferric-Reducing Antioxidant Power
g	Gram
h	Hour
GAE	Gallic Acid Equivalent
GC-MS	Gas chromatography–mass spectrometry
mg	Milligram
min	Minute
mL	Milliliter
Mm	millimeter
Na_2CO_3	Sodium Carbonate
No.	Number
nm	Nanometer
RDU	Research Development Unit
ROS	Reactive Oxygen Species
rpm	Revolution Per Minute
TCA	Trichloroacetic acid
v/v	Volume per volume
µl	Micro Litre
µM	Micro Meter

REFERENCES

Abirami, R.G.; Kowsalya, S., (2011). Nutrient and nutraceutical potentials of seaweed biomass Ulva lactuca and Kappaphycus alvarezii. Nong Ye Ke Xue Yu Ji Shu. 1: 5(1) **(7 pages).**

Ahmad, F.; Sulaiman, M.R.; Saimon, W.; Yee, C.F.; Matanjun, P., (2016). Proximate compositions and total phenolic contents of selected edible seaweed from Semporna, Sabah, Malaysia. Borneo Sci., 6: 31. **(10 pages).**

Alencar, D.B.; Silva, S.R.; Pires-Cavalcante, K.; Lima, R.L.; Pereira, Junior, F.N.; Sousa, M.B.; Viana, F.A.; Nagano, C.S.; Nascimento, K.S.; Cavada, B.S.; Sampaio, A.H., (2014). Antioxidant potential and cytotoxic activity of two red seaweed species, Amansia multifida and Meristiella echinocarpa, from the coast of Northeastern Brazil. Anais da Academia Brasileira de Ciências. 86(1): 251-63 **(13 pages).**

Arulkumar, A.; Rosemary, T.; Paramasivam, S.; Rajendran, R.B., (2018). Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (Gracilaria corticata and Gracilaria edulis) from Palk Bay, India. Biocatal. Agric. Biotechnol., 15: 63-71 **(9 pages).**

Asmida, I.; Akmal, A.N.; Ahmad, I.; Diyana, M.S., (2017). Biodiversity of macroalgae in Blue Lagoon, the Straits of Malacca, Malaysia and some aspects of changes in species composition. Sains Malaysiana. 46(1): 1-7 **(7 pages).**

Cornish, M.L.; Garbary, D.J., (2010). Antioxidants from macroalgae: potential applications in human health and nutrition. Algae. 25(4): 155-171 **(17 pages).**

Cox, S.; Abu, Ghannam, N.; Gupta, S., (2010). An Assessment of the Antioxidant and Antimicrobial Activity of Six Species of Edible Irish Seaweeds. Int. Food Res. J., 17: 205-220 **(15 pages).**

Chuah, X.Q.; Mun, W.; Teo, S.S., (2017). Comparison study of anti-microbial activity between crude extract of Kappaphycus alvarezii and Andrographis paniculata. Asian Pac. J. Trop. Biomed., 7(8): 729-731 **(3 pages).**

Chojnacka, K.; Saeid, A.; Witkowska, Z.; Tuhy, L., (2012). Biologically active compounds in seaweed extracts-the prospects for the application. In The open Conf. Proceed J., 3(1) **(9 pages).**

Dennis, J.; McHugh., (2003). A guide to the seaweed industry., 1-118 **(118 pages).**

El, Shafay, S.M.; Ali, S.S.; El-Sheekh, M.M., (2016). Antimicrobial activity of some seaweed's species from Red sea, against multidrug resistant bacteria. Egypt J. Aqua. Res., 42(1): 65-74

(10 pages).

Farah, Diyana, A.; Abdullah, A.; Shahrul, Hisham, Z.A.; Chan, K.M., (2015). Antioxidant activity of red algae Kappaphycus alvarezii and Kappaphycus striatum. Int. Food Res. J., 22(5) **(8 pages).**

Fedorov, S.N.; Shubina, L.K.; Bode, A.M.; Stonik, V.A.; Dong, Z., (2007). Dactylone inhibits Epidermal Growth Factor–induced transformation and phenotype expression of human cancer cells and induces G1-S arrest and apoptosis. Cancer Res., 67(12): 5914-5920 **(7 pages).**

Ganesan, P.; Kumar, C.S.; Bhaskar, N., (2008). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresour. Technol., 1; 99(8): 2717-2723 **(7 pages).**

Gegg, P.; Wells, V., (2019). The development of seaweed-derived fuels in the UK: An analysis of stakeholder issues and public perceptions. Energy Policy. 133: 110924 **(9 pages).**

Godlewska, K.; Michalak, I.; Tuhy, Ł.; Chojnacka, K., (2016). Plant growth biostimulants based on different methods of seaweed extraction with water. BioMed Res. Int., **(11 pages).**

Hayashi, L.; Reis, R.P., (2012). Cultivation of the red algae Kappaphycus alvarezii in Brazil and its pharmacological potential. Revista Brasileira de Farmacognosia. (4): 748-752 **(5 pages).**

Hwang, P.A.; Wu, C.H.; Gau, S.Y.; Chien, S.Y.; Hwang, D.F., (2010). Antioxidant and immune-stimulating activities of hot-water extract from seaweed Sargassum hemiphyllum. J. Mar. Sci. Technol., 18(1): 41-46 **(6 pages).**

Hwang, E.S.; Do, Thi, N., (2014). Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera). Preventive Nutr. Food Sci., 19(1): 40 **(9 pages).**

Ibrahim, M.F.; Abd-Aziz, S.; Razak, M.N.; Phang, L.Y.; Hassan, M.A., (2012). Oil palm empty fruit bunch as alternative substrate for acetone–butanol–ethanol production by Clostridium butyricum EB6. Appl. Biochem. Biotechnol., 166(7): 1615-1625 **(11 pages).**

Kanatt, S.R.; Lahare, P.; Chawla, S.P.; Sharma, A., (2015). Kappaphycus alvarezii: Its antioxidant potential and use in bioactive packaging films. J. Microbiol. Biotechnol. Food Sci. 5(1): 1 **(6 pages).**

Kazłowska, K.; Lin, H.T.V.; Chang, S.H.; Tsai, G.J., (2013). In vitro and in vivo anticancer effects of sterol fraction from red algae Porphyra dentata. Evidence-Based Com. Alte. Med., **(10 pages).**

Khalil, H.A.; Yap, S.W.; Tye, Y.Y.; Tahir, P.M.; Rizal, S.; Fazita, M.N., (2018). Effects of corn starch and Kappaphycus alvarezii seaweed blend concentration on the optical, mechanical, and water vapor barrier properties of composite films. Bio-Resour., 13(1): 1157-1173 **(17 pages).**

Kim, M.M.; Rajapakse, N.; Kim, S.K., (2009). Anti-inflammatory effect of Ishige okamurae ethanolic extract via inhibition of NF-κB transcription factor in RAW 264.7 cells. Phytother Res., 23(5): 628-634 **(7 pages).**

Kim, E.J.; Park, S.Y.; Lee, J.Y.; Park, J.H.Y., (2010). Fucoidan present in brown algae induces apoptosis of human colon cancer cells. BMC gastroenterology., 10(1): 96 **(11 pages).**

Kolanjinathan, K.; Ganesh, P.; Saranraj, P., (2014). Pharmacological importance of seaweeds: A review. World J. Fish Marine Sci., 6(1): 1-15 **(15 pages).**

Kolanjinathan, K.; Ganesh, P.; Govindarajan, M., (2009). Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. Eur. Rev. Med. Pharmacol., Sci. 13(3): 173-177 **(5 pages).**

Ling, A.L.; Yasir, S.; Matanjun, P.; Bakar, M.F., (2015). Effect of different drying techniques on the phytochemical content and antioxidant activity of Kappaphycus alvarezii. J. Appl. Phycol., 27(4): 1717-1723 **(7 pages).**

Mabberley, D.J., (2017). Mabberley's plant-book: a portable dictionary of plants, their classification and uses. Cambridge University Press **(1040 pages).**

Mala, R.; Celsia, A.R.; Devi, S.M.; Geerthika, S., (2017). Comparison on Bactericidal and Cytotoxic Effect of Silver Nanoparticles Synthesized by Different Methods. In IOP Conference Series: Mate. Sci. Eng., 225(1): 012155 **(11 pages).**

Munir, N.; Sharif, N.; Naz, S.; Manzoor, F., (2013). Algae: a potent antioxidant source. Sky J. Microbiol. Res., 1: 22-31 **(10 pages).**

Monte, J.; Abreu, A.; Borges, A.; Simões, L.; Simões, M., (2014). Antimicrobial activity of selected phytochemicals against Escherichia coli and Staphylococcus aureus and their biofilms. Pathogens. 3(2): 473-498 **(26 pages).**

Mansuya, P.; Aruna, P.; Sridhar, S.; Kumar, J.S.; Babu, S., (2010). Antibacterial activity and qualitative phytochemical analysis of selected seaweeds from Gulf of Mannar region. J. Exp. Sci., 1(8): 23-26 **(4 pages).**

Namvar, F.; Mohamad, R.; Baharara, J.; Zafar-Balanejad, S.; Fargahi, F.; Rahman, H.S., (2013). Antioxidant, antiproliferative, and antiangiogenesis effects of polyphenol-rich seaweed (Sargassum muticum). BioMed Res. Int., **(9 pages).**

Naveena, B.E.; Prakash, S., (2013). Biological synthesis of gold nanoparticles using marine algae Gracilaria corticata and its application as a potent antimicrobial and antioxidant agent. Asian J. Pharm. Clin. Res., 6(2): 179-182 **(4 pages).**

Quirós, A.R.; Frecha-Ferreiro, S.; Vidal-Pérez, A.M.; López-Hernández, J., (2010). Antioxidant compounds in edible brown seaweeds. Eur. Food Res. Technol., 231(3): 495-513 **(19 pages).**

Rebecca, L.J.; Dhanalakshmi, V.; Shekhar, C., (2012). Antibacterial activity of Sargassum Ilicifolium and Kappaphycus alvarezii. J. Chem. Pharm. Res., 4(1): 700-705 **(6 pages).**

Souza, B.W.; Cerqueira, M.A.; Martins, J.T.; Quintas, M.A.; Ferreira, A.C.; Teixeira, J.A.; Vicente, A.A., (2011). Antioxidant potential of two red seaweeds from the Brazilian coasts. J. Agric. Food. Chem., 59(10): 5589-5594 **(6 pages).**

Shanmughapriya, S.; Manilal, A.; Sujith, S.; Selvin, J.; Kiran, G.S.; Natarajaseenivasan, K., (2008). Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Ann. Microbiol., 58(3): 535-541 **(7 pages).**

Vallinayagam, K.; Arumugam, R.; Kannan, R.R.; Thirumaran, G.; Anantharaman, P., (2009). Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. Global J. Pharmacol., 3(1): 50-52 **(3 pages).**

Xue, M.; Ge, Y.; Zhang, J.; Wang, Q.; Hou, L.; Liu, Y.; Sun, L.; Li, Q., (2012). Anticancer properties and mechanisms of fucoidan on mouse breast cancer in vitro and in vivo. PLoS one, 7(8): e43483 **(9 pages).**

Yang, J.I.; Yeh, C.C.; Lee, J.C.; Yi, S.C.; Huang, H.W.; Tseng, C.N.; Chang, H.W., (2012). Aqueous extracts of the edible Gracilaria tenuistipitata are protective against H2O2-induced DNA damage, growth inhibition, and cell cycle arrest. Molecules. 17(6): 7241-7254 **(14 pages).**

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