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RESEARCH

Effect of Growing Environment on the Quality and Quantity of *Kappaphycus alvarezii* (Doty) Doty Yield

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ABSTRACT

Kappaphycus alvarezii Doty (Doty) is a commercially important red algae known for carrageenan, a cell wall polysaccharide and thickening agent. It is also a source of major and minor nutrients and secondary metabolites with antioxidant, antibacterial, anti-diabetic, and anticancer properties. However, comprehensive studies are limited to answer the scientific questions in relation to morphotypes and their influence on quantity and quality of carrageenan. This study aimed at analyzing the growth rate, carrageenan yield, water holding capacity of carrageenan, antimicrobial properties, antioxidant properties, and total phenols of green and brown morphotypes of *K. alvarezii* cultivated in Pesalai and Valaippadu of Sri Lanka. The seaweed was harvested after 45 days of cultivation (n=12) and analyzed using standard methods. The antibacterial properties of ethanol extracts were evaluated by disc diffusion assay against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antioxidant properties and total phenolic contents (TPC) were determined using DPPH radical scavenging assay and Folin-Ciocalteu method, respectively. There were no significant ($P>0.05$) location effect and morphotype effect in terms of growth rate, carrageenan yield, and water holding capacity. Irrespective of the cultivation location, the green morphotype showed significantly high ($P<0.05$) antibacterial and antioxidant properties and significantly high ($P<0.05$) TPC. This study suggests that the green morphotype of *K. alvarezii* performs better as a natural source of antibacterial and antioxidant compounds, while both morphotypes of *K. alvarezii* are suitable for carrageenan production. The Mannar sea area of Sri Lanka has conditions for commercial farming of *K. alvarezii* with an average 4.21±0.03 % growth rate.

INTRODUCTION

Kappaphycus alvarezii Doty (Doty), a well-known seaweed as a source of carrageenan (a cell wall polysaccharide) is used in many food applications (Seth and Shanmugam, 2016). This seaweed is rich in secondary metabolites, which possess antibacterial, antioxidants, anti-diabetic, anticancer properties and antihyperlipidemic effects (Araújo et al., 2020). Furthermore, *K. alvarezii* is also a source of carbohydrates, proteins, dietary fibres, fatty acids, minerals and vitamins (Fayaz et al., 2005). Seaweed farming is also a supplementary income generating activity to existing fishing communities (Shanmugam et al., 2017) and it provides employment opportunities for women (Bindu, 2011). Commercial farming of *K. alvarezii* was developed in the Philippines in the late 1960s, and subsequently introduced to other countries. Sri Lanka has imported *K. alvarezii* from Malaysia and acclimatized it in Valaippadu and Vidathalaitivu areas of Sri Lanka (Shanmugam et al., 2017). Since *K. alvarezii* is very sensitive to small changes in environmental conditions (Ateweberhan et al., 2015), comprehensive studies are needed to assess the quality and quantity of the yield. However, to the best of our knowledge, no study has been conducted to investigate aforementioned aspects.

Different colour morphotypes of *K. alvarezii* with varied pigmentation as green, brown and red morphotypes are reported (Muñoz et al., 2004). In Sri Lanka and most other countries, *K. avarezii* is cultivated in mixed cultivations (Araújo et al., 2020). Elsewhere, limited data are available on the effect of morphotypes on the growth, carrageenan content and biochemical properties of *K. alvarezii* (Araújo et al., 2020; Araújo et al., 2022). In Sri Lanka, even though Rajapaksha et al., (2024) reported significantly higher total phenols, antioxidant properties and antimicrobial properties in the green colour morphotype than brown colour morphotype of *K. alvarezii* grown in Jaffna, Sri Lanka, no data on the growth and carrageenan yield of different morphotypes.

Most of the studies on the growth and biochemical properties of *K. alvarezii* have been reported with mixed morphotypes of *K. alvarezii* or without specifying morphotypes (Ling et al., 2015). Therefore, the present study aimed determine the effect of the environment on growth, carrageenan yield, total phenolic content, antioxidant properties and antimicrobial properties of the green and brown morphotypes of *K. alvarezii*.

METHODOLOGY

Location of the study

The field study was conducted in Valaippadu (9°20'40.5"N 80°03'07.7"E) and Pesalai (9°05'15.8"N 79°49'37.1"E) coastal areas of Sri Lanka, during January to March, 2024. These two distant locations were selected to represent the Mannar coastal area of Sri Lanka (Figure 1).

The seabed of Pesalai location was sandy without seagrass patches whereas in Valaippadu location was characterized with sandy seabed with seagrass patches. The average water depth at both locations during low tides and high tides were 1 m and 3 m, respectively. The wave speed of Pesalai location was relatively high compared to the Valaippadu location.

Experimental design

The experiment was a two-factor factorial experiment, laid out in a completely randomized design (CRD). The two factors considered were Location (A) and morphotype of *K. alvarezii* (B). Factor A consisted of two levels, namely, Pesalai location and Valaippadu location. Factor B consisted of two morphotypes of *K. alvarezii*, namely, green and brown (Figure 2 A and B).

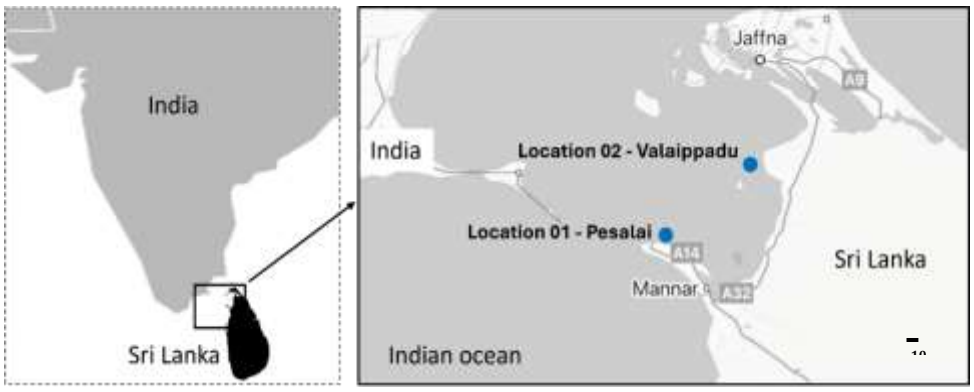

Figure 1. Map showing the locations of the two experimental sites on the Mannar coast, Sri Lanka

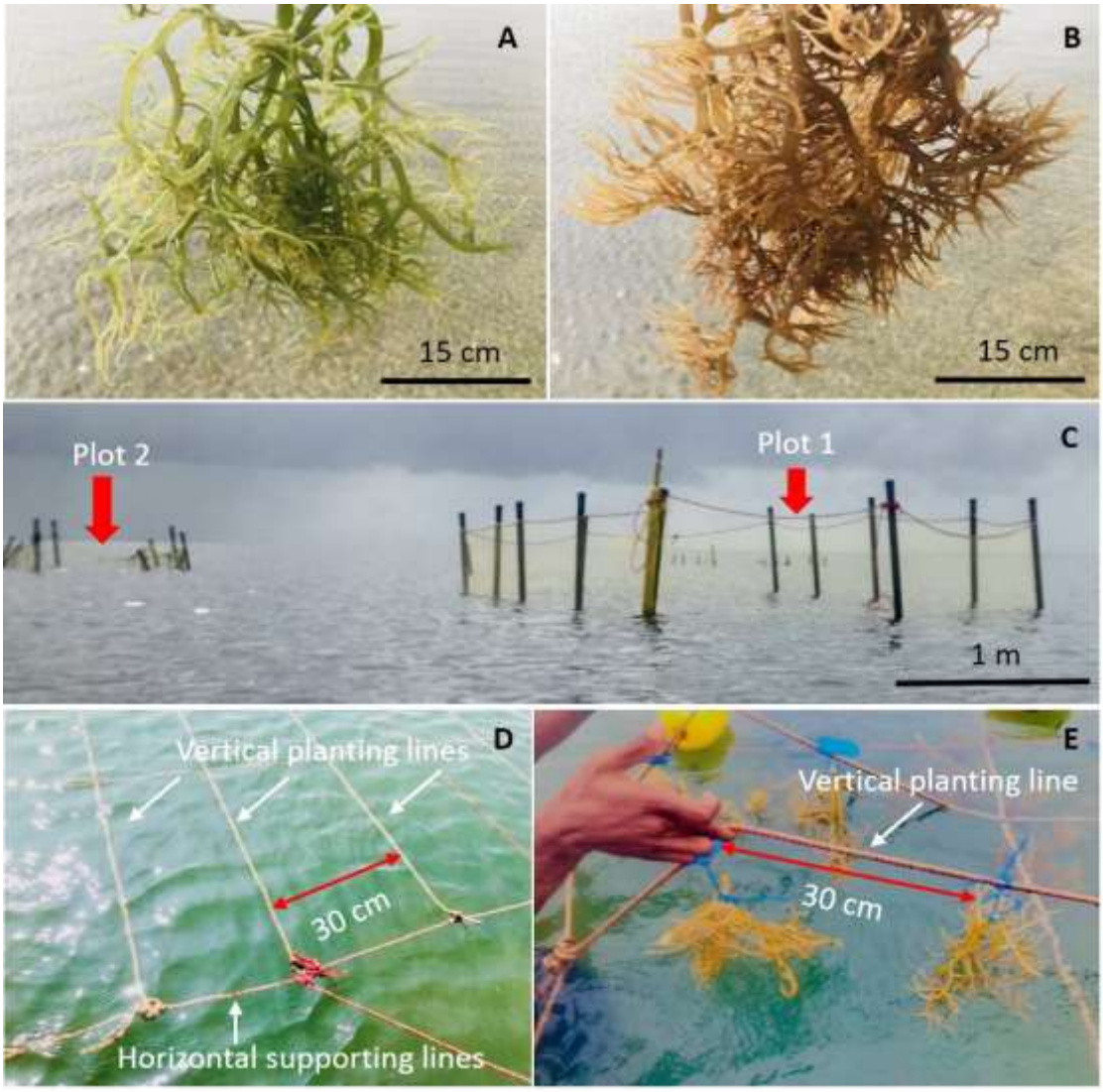

Figure 2. Establishment of *K. alvarezii* plots in the seawater: (A) Green colour morphotype and (B) Brown colour morphotypes of *K. alvarezii*, (C) Monoline plot and protected by fishing net, (D) Arrangement of plots with vertical and horizontal supporting lines, (E) Initial seedlings on a vertical planting line

Preparation of *K. alvarezii* plots

The monoline method of *K. alvarezii* cultivation was employed in the present study. In each location, three replicate monoline plots were established (Figure 2C). The size of a monoline plot was 4.0 m × 2.0 m and seven polypropylene planting lines of 3 m in length (5 mm diameter) were established perpendicular to horizontal 5 mm diameter ropes, tied to wooden posts. The plot area was covered with a 1.5 cm mesh size fishing net (Figure 2D).

Establishment of *K. alvarezii* cultivation, farming operation and sample collection

Kappaphycus alvarezii cultivations were initiated in January 2024, simultaneously at the two locations. Initial seedling stock was collected from an existing farm at Mannar, Sri Lanka. The *K. alvarezii* seedlings of around 100 g and composed of primary, secondary, tertiary and growing tips were selected. All seedlings were mixed properly before being allocated to different treatments to minimize possible errors due to the heterogeneity of seedlings. The distance between seedlings in a row and a column was 30 cm (Figure 2E). Planting lines of *K. alvarezii* were set at a depth of 30 cm from the surface of the seawater body allowing them to grow at that 30 cm depth throughout their growth period. Daily farming operations included fixing up loose knots and removing debris which were carried out throughout the cultivation process. Harvesting and samples were collected 45 days post-cultivation. Twelve seaweed plants were harvested from each morphotype of *K. alvarezii* in each location, i.e., four seaweed plants from each plot and total plots were three for each morphotype in each location. The seaweed plants fresh weight of each harvested seaweed was measured separately, kept for three days of sun drying followed by oven drying at 45 ˚C. Dried samples were stored at room temperature (25 ˚C) until further analyses.

Determination of water quality parameters

Water quality parameters were measured at the two farming sites during the experimental period at the same depth as seaweed, i.e., 30 cm depth and data were recorded on 15, 30 and 45 days post-cultivation. Each water quality parameter was measured near to each cultivation plot separately in triplicates. Salinity (ppt), Temperature (˚C), pH, and Total dissolved solids (ppt) were measured on-site using a HI-98194 Multiparameter waterproof meter (HANNA instruments, Romania). The water motion of the sea was measured using a floating drogue with a 5 m length line. A stopwatch was used to measure the time from release until the line became taut and the water motion (cm min^{-1}) was calculated using equation 1.

$$Water\ motion\ = (5\ m)/t \ldots Equation\ 1$$

Where, 't' is the time taken for the drogue line to become taut.

Determination of the growth rate of *K. alvarezii*

The biomass growth rate was evaluated after 45 days of cultivation. The percentage growth rate was calculated using the formula previously used by Kumar et al., (2016) (Equation 2).

$$Growth\ rate\ (\%) = l\ \mathrm{n}\ (W_f/W_0)/t \times 100 \ldots Equation\ 2$$

Where, W_f is the final dry weight (g) on day t, W_o is the initial dry weight (g), and 't' is the number of cultivation days.

Carrageenan extraction from *K. alvarezii*

Carrageenan was extracted from *K. alvarezii* using a modified method by Hayashi et al., (2007a). Dry seaweed samples of 20 g weight were soaked in 1 L of 6 % KOH for 24 hours at 25 ˚C, washed with distilled water, followed by incubation of seaweed in 400 mL of 6 % KOH at 80 °C for 2 hours period. The incubated samples were washed with distilled water and carrageenan was extracted in 400 mL of distilled water at 80 °C for 2 hours on a hot plate. The extraction was filtered using no. 80 mesh and the filtrate was jellified in 0.2 % KCl solution. The jellified samples were frozen and thawed in two cycles to remove excess water followed by freeze drying (Telstar, LYOQUEST

-85 PLUS, Spain) until constant weight. The carrageenan yield was determined according to the formula used by Ilias et al., (2017) (Equation 3).

$$Carrageenan\ yield = (W_c/W_d) \times 100 \ldots Equation\ 3$$

Where, W_c is the weight of carrageenan and W_d is seaweed dry weight (g).

Determination of water holding capacity of carrageenan

The water holding capacity of carrageenan gel was measured according to the Chan et al., (2013) with minor modifications. Briefly, a 4 % (w/v) carrageenan solution was set in a 50 mL centrifuge tube (n=9) to form carrageenan gel with a height of 5 cm. The initial weight of the gel was measured (W_i) and stored at 4 ˚C temperature for 24 hours followed by centrifugation at 4000 rpm for 30 minutes at 25 ˚C. After centrifugation, the supernatants were decanted, and the weight of the gel were measured immediately (W_t). Water holding capacity was calculated using Equation 4.

$$Water\ \ holding\ capacity\ (\%) = (W_t/W_i) \times 100 \ldots Equation\ 4$$

Where, W_i is the initial weight of the carrageenan gel and W_t is the weight of the carrageenan gel after the centrifugation.

Preparation of *K. alvarezii* crude extract

Crude extracts of *K. alvarezii* were prepared using ethanol (≥99.8%) according to Rajapaksha et al., (2024). Pooled samples of dried *K. alvarezii* for each morphotype in each plot of two locations were prepared by mixing 10 g of dried seaweed plant sample. Then *K. alvarezii* was ground into a fine powder using a knife mill sample grinder (RETSCH GM 200). Then, 30 g of *K. alvarezii* powder was subjected to extraction with 200 mL of ethanol for 24 hours at 25 °C. The extracts were filtered and vacuum dried at 45 °C. Crude extracts were prepared from *K. alvarezii* of harvested from all treatment plots.

Determination of antimicrobial properties

The antimicrobial properties of *K. alvarezii* were tested against standard *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) bacteria species. According to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards, a Disc Diffusion Assay was performed in a 4 mm thick Muller-Hinton Agar (MHA) medium (Rajapaksha et al., 2024). Briefly, bacteria were cultured in nutrient agar to obtain single colonies. Then they were sub-cultured in Lysogeny Broth (LB broth) at 37 °C for 24 hours. Thereafter, Muller-Hinton Agar plates were inoculated with 1 mL of bacteria inoculum immediately after the turbidity adjustment. This step was repeated twice, rotating the plate 60° to ensure an even spread of inoculum on the media surface. After inoculation, the partially opened Petri dishes were kept for 5 minutes to absorb excess moisture of the surface. A 5 mg of *K. alvarezii* crude powder sample was dissolved in 1 mL of 10 % dimethyl sulfoxide (DMSO). Sterile filter paper disks (Whatman's No. 1, 6 mm in diameter) were impregnated with 25 µL of *K. alvarezii* crude that dissolved in DMSO and air dried. Furthermore, 25 µL of DMSO and 10 µL of 0.3 % w/v gentamicin were used as negative and positive controls, respectively. Impregnated disks were placed on the surface of the inoculated plates. The Petri dishes were placed in an inverted position followed by incubation at 37 °C for 24 hours. The antimicrobial activity was measured based on the inhibition zone diameter expressed in millimeters against the tested bacteria species. *K. alvarezii* crude from all treatment plots were tested in triplicates within the same culture plate for each bacteria species.

1, 1- Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay

The radical scavenging ability of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) by crude extractions of *K. alvarezii* samples from each treatment were measured using the method described by Rajapaksha et al., (2024). A 5 mg of *K. alvarezii* crude powder sample was dissolved in 1 mL of 10 % dimethyl sulfoxide (DMSO). Then, 1 mL of 1 mM of DPPH was

added to 1 mL of that crude solution followed by vortex. Then the mixture was subjected to incubation in the dark for 30 minutes at 25 °C. The absorbance of the incubated samples was measured at 517 nm using a spectrophotometer (BIO Spectrometer, Kinetic-Eppendorf). The radical scavenging activity was calculated using Equation 5.

$$Radical\ scavenging\ activity\ (\%) = [A_{Control} - A_{Sample}/A_{Control}] \times 100 \quad \text{... Equation 5}$$

Where, $A_{Control}$ is the absorbance of the control (DPPH) and A_{Sample} is the absorbance of the sample.

Total phenolic content (TPC)

Total phenolic content (TPC) of *K. alvarezii* was measured using Folin–Ciocalteau method as described by Rajapaksha et al., (2024). A 5 mg of *K. alvarezii* crude powder sample was dissolved in 1 mL of 10 % Dimethyl Sulfoxide (DMSO). Then, 0.1 mL of crude solution was mixed with 2 mL of 2 % Na_2CO_3 and then the mixture was incubated for two minutes at 25 °C. After the incubation, 0.1 mL of Folin-Ciocalteu was added and kept in dark for 30 minutes for incubation. Absorbance of the resulted dark incubated solution was measured at 760 nm using a spectrophotometer (BIO Spectrometer, Kinetic-Eppendorf). Gallic acid standard curve was plotted using concentrations of gallic acids ranging from 0.2 to 1.0 mg/mL and results were reported as mg of Gallic Acid Equivalent per gram (GAE/g) dry weight (DW) of crude extract.

Statistical analysis

The data were tested for normality and homogeneity of variance and then ANOVA was performed. A two-way ANOVA was performed to identify the factor effects. The Tukey post hoc test at 95 % significance level (α=0.05) was used to identify significant differences between the means. Furthermore, a comparison was made between the water quality parameters of the Pesalai and Valaippadu locations using t-test and correlations among water quality parameters of each location were analyzed using the Pearson correlation test. All statistical analyses were performed using the SAS Academic; Cary, NC: SAS Institute Inc.

RESULTS AND DISCUSSION

Water quality parameters

Mean values of water temperature, pH, salinity, total dissolved solids (TDS) and water motion at the experimental locations and their pair-wise correlatins are presented in Table 1. Except for the water motion, there were no significant differences (P>0.05) in the tested water quality parameters between Pesalai and Valaippadu. Pesalai location had significantly high (P<0.05) water motion. It indicates the prevalence of frequent wave actions on Pesalai coast than on the Valaippapdu coast. Furthermore, in both Pesalai and Valaippadu cultivation locations, no significant strong correlations (P>0.05) were recorded among salinity, temperature, pH, total dissolved solids (TDS) and water motion.

According to Reddy et al., (2018), 24-31 °C, 7.9-8.5 pH, and 24-35 ppt are the suitable water temperature, pH, and salinity conditions, respectively, for seaweed farming. Reported water quality parameters of both Pesalai and Valaippadu locations of the present study are in accordance with those ranges. It reflects the availability of appropriate seaweed farming seawater conditions in Mannar coast of Sri Lanka.

Growth rate and final yield of *K. alvarezii*

Within or between locations, there was no significant difference (P>0.05) in growth rate (Figure 3A), fresh weight and dry weight (Table 2) of *K. alvarezii* morphotypes. The internationally recommended commercial growth rate of *K. alvarezii* is 3.5 % and the mean growth rate of *K. alvarezii* (4.21±0.03 %) of the present study is complying that requirement (Periyasamy et al., 2019).

Table 1. Seawater quality parameters of locations of *Kappaphycus alvarezii* cultivation

Mean values of water quality parameters			**Pearson correlation coefficients of water quality parameters**					
Parameter	Pesalai (Mean±SD)	Valaippadu (Mean±SD)	Parameter	Salinity	Tem	pH	TDS	Water motion
Salinity (ppt)	29.22±0.67[a]	29.45±0.53[a]	Salinity	1	-0.49*	0.54*	0.03*	-0.14*
Tem (˚C)	28.71±0.55[a]	28.96±0.79[a]	Tem	-0.30*	1	-0.39*	-0.11*	0.30*
pH	7.91±0.32[a]	7.92±0.24[a]	pH	0.35*	-0.43*	1	0.27*	0.05*
TDS (ppt)	31.34±1.01[a]	31.68±1.13[a]	TDS	-0.30*	-0.12*	0.21*	1	-0.04*
Water motion (cm min^{-1})	52.52±0.91[a]	40.92±0.95[b]	Water motion	-0.23*	0.01*	-0.12*	-0.35*	1

TDS= Total Dissolved Solids, Tem= Temperature. Means followed by the same letter are not significantly different at p=0.05. For each treatment combination, means of nine replicates were used. In the correlation coefficient matrix; lower triangle is Pesalai location ( ) and upper triangle is Valaippadu location ( ), Pearson correlation coefficients which are followed by the superscripted asterisk mark (*) are not significant correlations.

Therefore, the data suggest the potential for successful commercial farming of *K. alvarezii* in Mannar sea of Sri Lanka. The fresh and dry seaweed conversion ratio was around 10:1, i.e., 1000 g of fresh seaweed yielded 100 g oven-dried seaweed from both morphotypes of *K. alvarezii* with no effect on the location (Table 2).

Shanmugam et al., (2017) also recommended the Valaippadu location of Sri Lanka as a suitable location for commercial farming of *K. alvarezii* who have reported a maximum growth rate of 4.22±0.37 % for *K. alvarezii*, which is comparable to 4.26±0.22 % growth rate of the present study. However, previous studies did not mention the morphotype and there are no studies on comparative studies on the commercial viability of green and brown colour morphotypes of *K. alvarezii* in Sri Lanka. Considering the commercial farming recommendations, *K. alvarezii* was harvested at 45 days of cultivation in the present study (Hayashi et al., 2007b; Periyasamy et al., 2019). The growth rates obtained in this study for the 45-day growth period of *K. alvarezii* were higher than that was reported by Periyasamy et al., (2019) for the same species that was cultivated in southeast coast of India which recorded a growth rate of 3.46±0.11 %. However, Periyasamy et al., (2019) have not specified the morphotype of *K. alvarezii.* Muñoz et al., (2004), Hayashi et al., (2007a), and Hayashi et al., (2011) reported no significant differences in growth rate among the morphotypes of *K. alvarezii* which is in line with the present study. Nevertheless, Hung et al., (2009) reported higher growth rates of the brown morphotype, whereas Hurtado, (1995) reported higher growth rates of the green morphotype of *K. alvarezii*.

Carrageenan yield and carrageenan gel water holding capacity

No significant difference (P>0.05) was recorded between the two morphotypes of *K. alvarezii* in terms of yield and water-holding capacity of the carrageenan gel. Furthermore, in terms of the yield and the water holding capacity of carrageenan gel, a significant interaction effect was not observed among the locations of cultivation and morphotypes of *K. alvarezii* (Figure 3B and Figure 3C). Muñoz et al., (2004), Hayashi et al., (2007a), and Hayashi et al., (2011) also reported no significant differences in carrageenan yield among *K. alvarezii* morphotypes. The carrageenan yield recorded here is above the minimum requirement of 25 % carrageenan for commercial farming of *K. alvarezii* (Firdaus et al., 2021). The carrageenan yield ranged from 31.21±2.14 % to 32.56±3.21 % without significant differences among morphotypes or locations of cultivation. Visually also there was no difference of carrageenan extracted from green and brown morphotypes of *K. alvarezii* (Figure 4). The water-holding capacity of carrageenan was excellent (>95 %) in both morphotypes indicating good functional properties of locally produced carrageenan (Chan et al., 2013).

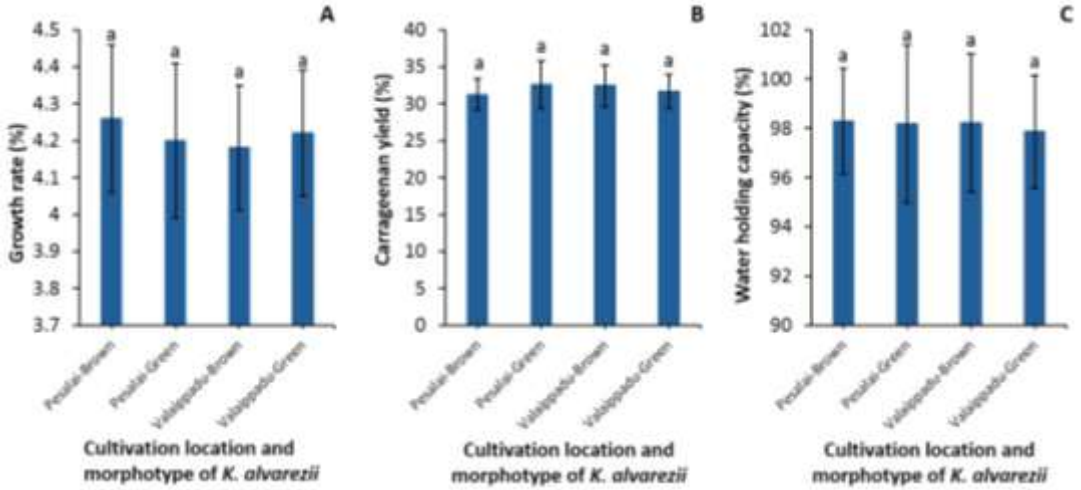

Figure 3. Comparison of growth rate, carrageenan yield and water holding capacity of *Kappaphycus alvarezii*: (A) Daily growth rate (%), (B) Carrageenan yield (%), (C) Water holding capacity (%). Note: The same letters over the error bars denotes the absence of significant differences at p=0.05. For each treatment combination, means of twelve replicates were used.

Table 2. Weight of the final harvest of *K. alvarezii* cultivated in different locations

Location	Morphotype	Fresh weight (g) (Mean ± SD)	Dry weight (g) (Mean ± SD)	Fresh: Dry weight
Pesalai	Green	789.85±3.70[a]	76.69±3.53[a]	10.10: 1
	Brown	791.61±3.40[a]	79.14±5.37[a]	10.40: 1
Valaippadu	Green	792.00±4.15[a]	79.23±3.00[a]	10.11: 1
	Brown	790.67±3.67[a]	78.22±2.60[a]	10.01: 1

Separately under each parameter, means followed by the same letter are not significantly different at p=0.05. For each treatment combination, means of twelve replicates were used.

Figure 4. Production of carrageenan from *Kappaphycus alvarezii*: (A) Carrageenan from green morphotype of *K. alvarezii*, (B) Carrageenan from brown morphotype of *K. alvarezii*: (x) Extracted carrageenan gel after precipitation in 0.2 % KCl, (y) Dried and powdered carrageenan, (z) Set carrageenan gel from 4 % (W/V) carrageenan solution.

Antimicrobial properties

Rajapaksha et al., (2024) studied the antibacterial properties of green and brown colour morphotypes of *K. alvarezii* which had grown in Allaipiddy, Jaffna, Sri Lanka using the same bacteria species, i.e., *E. coli*, *S. aureus* and *P. aeruginosa*. In that study, the green colour morphotype showed significantly higher antimicrobial properties than the brown colour morphotype. To test whether it was a location-specific property, the same assays were conducted using the harvests of both locations.

Gentamicin showed the highest inhibition zones for all species of bacteria as the positive control. No significant difference was recorded in the inhibition effect of gentamicin against *S. aureus*, *P. aeruginosa* and *E. coli* (Table 3). Extracts of the green colour morphotype of *K. alvarezii* showed significantly higher ($P<0.05$) inhibition zones for all bacteria species compared to the brown colour morphotype irrespective of the location (Figure 5). No significant interaction ($P>0.05$) effect was recorded between the location and morphotype of *K. alvarezii* for antimicrobial properties. *P. aeruginosa* and *E. coli* showed resistance to extract from brown colour morphotype but not for *S. aureus*. Accordingly, *S. aureus* was identified as the most susceptible species of the three tested bacteria species, for both morphotypes of *K. alvarezii*. Gram-positive bacteria seem to be more susceptible to antibacterial agents compared to gram-negative bacteria. This is attributed to the difference of morphological structure and composition between gram-positive and gram-negative bacteria (Rebecca et al., 2012). Mainly, the outer membrane of the gram-negative bacteria consists of lipopolysaccharide which enables the ability to control the antimicrobial agents' penetration into their cell. Monte et al., (2014) and Bhuyar et al., (2020) also have reported that, the required minimum inhibition concentration for *E. coli* (Gram-negative) was higher than in *S. aureus* (Gram-positive). The same results were reported in a previous study by Rajapaksha et al., (2024).

Prabha et al., (2013) reported the absence of inhibition zones from the extract of *K. alvarezii* against *P. aeruginosa*. In the present study, too, brown colour morphotype did not report inhibition zones. However, the green colour morphotype of *K. alvarezii* has given the inhibition zones in the present study. Since Prabha et al., (2013) have not specified the morphotype of *K. alvarezii*, direct comparison with the present study is difficult. Rebecca et al., (2012), Pushparaj et al., (2014) and Rajapaksha et al., (2024) reported that the bioactive metabolite extraction using ethanol is a healthy, efficient, and low-cost process from *K. alvarezii.* Therefore, the present study was continued with ethanol extraction from *K. alvarezii*.

Table 3. Inhibition zones against pathogenic bacteria by two morphotypes of *Kappaphycus alvarezii*

Bacteria species	Inhibition zone (Mean ± SD mm)		Morphotype	Location	
	Gentamicin	DMSO		Pesalai	Valaippadu
Staphylococcus aureus	17.03±0.16[a]	0	Green	10.55±0.18[b]	10.59±0.17[b]
			Brown	7.50±0.10[d]	7.40±0.13[d]
Pseudomonas aeruginosa	17.00±0.25[a]	0	Green	8.28±0.07[c]	8.28±0.10[c]
			Brown	0	0
Escherichia coli	17.13±0.16[a]	0	Green	8.25±0.13[c]	8.21±0.12[c]
			Brown	0	0

DMSO=Dimethyl sulfoxide, i.e., Negative control, Gentamicin=Positive control. Means followed by the same letter are not significantly different at p=0.05. For each treatment combination, means of nine replicates were used.

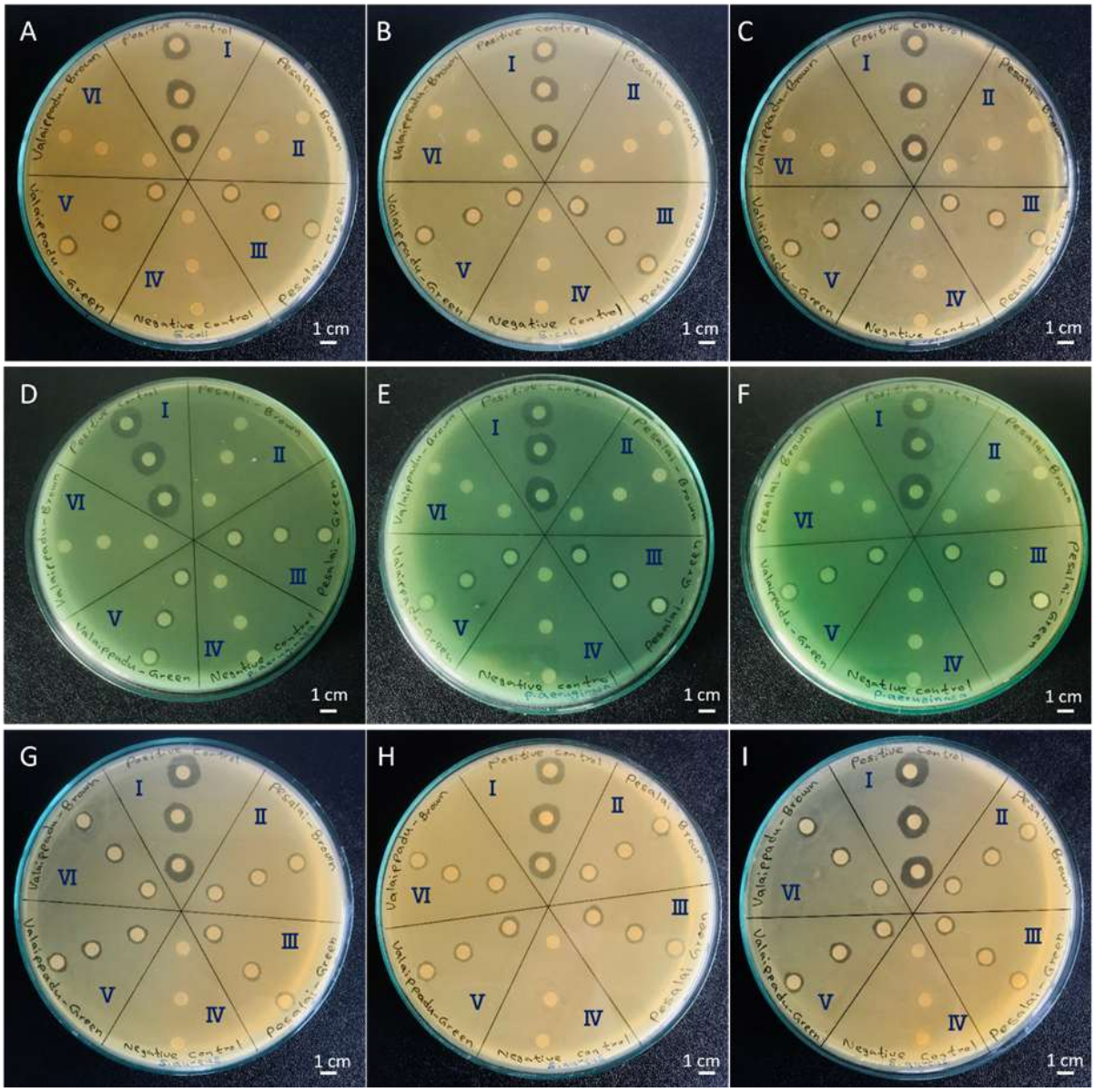

Figure 5. Antibacterial activity assay of *Kappaphycus alvarezii*: (A B C) *Escherichia coli*, (D E F) *Pseudomonas aeruginosa*, (G H I) *Staphylococcus aureus*. Note: Details of a plate; (I)Gentamicin positive control, (II)Brown morphotype that cultivated in Pesalai location, (III) Green morphotype that cultivated in Pesalai location, (IV) Dimethyl sulfoxide (DMSO) negative control, (V) Green morphotype that cultivated in Valaippadu location, (VI) Brown morphotype that cultivated in Valaippadu location.

Antioxidant properties

The DPPH free radical scavenging assay is the most commonly used antioxidant assay method owing to rapid results, simplicity, and low cost, and previously used successfully for *K. alvarezii* (Rajapaksha et al., 2024). As previously reported, significant difference ($P<0.05$) of antioxidant properties was recorded between the green and brown colour morphotypes of *K. alvarezii*. However, there was no significant effect ($P>0.05$) of cultivation location on the antioxidant properties of both morphotypes of *K. alvarezii*. Green colour morphotypes had significantly high ($P<0.05$) antioxidant properties compared to the brown colour morphotype (Figure 6A). Araújo et al., (2020) compared antioxidant properties of green, red, brown and G11 strains of *K. alvarezii* that were grown

in Brazilian water. Araújo et al., (2020) have revealed that high antioxidant properties of the green colour morphotype using different test methods including DPPH assay, Folin–Ciocalteu reducing potential, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and ferric reducing antioxidant power (FRAP) assay methods. In the DPPH assay, antioxidant activity has ranged from 7.15 % to 31.21 %. However, high values for antioxidant properties of *K. alvarezii* have been recorded in FRAP method which ranged from 27.24 % to 95.95 %. Similar to the present study, Rajapaksha et al., (2024) have comparatively studied the antioxidant properties of green and brown colour morphotypes of *K. alvarezii* which had grown in Allaipiddy, Jaffna, Sri Lanka. Their findings revealed that the green colour morphotype had significantly high antioxidant properties (46.1 %) when compared to the brown colour morphotype (36.7%). Furthermore, antioxidant activity values reported in the current study are higher than that was reported for *K. alvarezii* by Kumar et al., (2008) (20 %) and Farah Diyana et al., (2015) (19.35 %). However, direct morphotype vice comparison of antioxidant properties with the present study is not possible due to absence of colour morphotype comparison in Kumar et al., (2008) and Diyana et al., (2015). Furthermore, there were no reported studies on the effect of growing environment on the antioxidant properties of *K. alvarezii.*

Total phenolic content

Considering the identified differences of antimicrobial and antioxidant properties between green and brown morphotypes of *K. alvarezii*, a comparison was made between them again based on the total phenolic content. Because the phenolic compounds in living organisms are potent antioxidants and antibacterial agents (Rajapaksha et al., 2024).

Total phenolic content of *K. alvarezii* was ranging from 33.39±2.21 mg of GAE/g dry weight to 48.63±1.12 mg of GAE/g dry weight in the present study (Figure 6B). When comparing two colour morphotypes, a significantly high ($P<0.05$) total phenolic content was recorded in green colour morphotype of *K. alvarezii* and no interaction effect were recorded between morphotype and cultivated location. The high level of total phenolic content of green colour morphotype can be introduced as a factor for the higher antimicrobial and antioxidant properties of green colour morphotype of *K. alvrezii*. This could be due to the positive correlations among phenolic compounds, antioxidant properties and antibacterial properties (Rajapaksha et al., 2024). The values resulted in the present study on total phenolic content were similar to Rajapaksha et al., (2024) that ranged from 32.4±1.46 mg of GAE/g dry

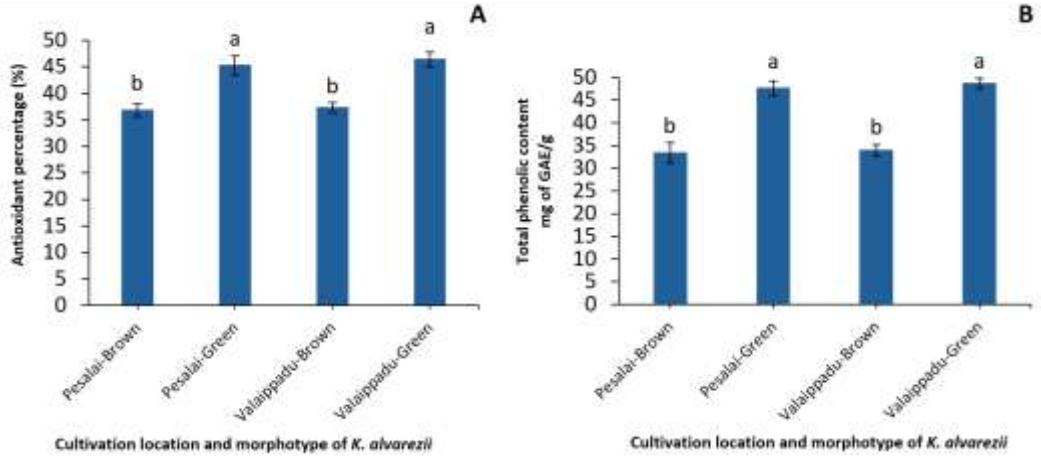

Figure 6. Antioxidant properties and total phenolic content of *Kappaphycus alvarezii*: (A) Antioxidant properties, (B) Total phenolic content. Note: Same letters over the error bars are not significantly different at p=0.05. For each treatment combination, means of nine replicates were used.

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weight to 49.12±1.15 mg of GAE/g dry weight. Rajapaksha et al., (2024) have further revealed that green colour morphotype of the *K. alvarezii* has high total phenolic content than brown colour morphotype, which is in line with the present study. The total phenolic content values resulted in the present study were higher than those reported by Chew et al., (2008), Diyana et al., (2015) and Nurshahida et al., (2020) which were 28.4±1.1 mg of GAE g^{-1} dry weight, 7.51±0.16–17.32±1.2 mg of GAE g^{-1} dry weight and 19.17±0.04 mg of GAE g^{-1} dry weight, respectively. Comparison of morphotype level in the present study was not possible because their reported values were for the *K. alvarezii* species without specification on colour morphotypes of *K. alvarezii*. Higher total phenolic content recorded in the present study could be owing to the extended extraction time over the previous studies. In the current study, room temperature extraction time had been increased from 30 minutes to 24 hours as described by Deepa et al., (2018). However, no previous studies were reported on a comparative study on the effect of cultivation location along with morphotype of *K. alvarezii* on total phenolic content.

CONCLUSION

This study on the mariculture of *K. alvarezii* comparing the Pesalai and Valaippadu cultivation locations of Mannar coast, Sri Lanka has identified that *K. alvarezii* farming in both locations can be employed without significant difference in growth and other physicochemical properties. For carrageenan production, both morphotypes are suitable with no quantitative and qualitative differences. However, the antibacterial activity, antioxidant properties and total phenolic content of *K. alvarezii* depend on the morphotype, where the green colour morphotype has a high level of total phenolic compounds along with high antibacterial and antioxidant properties. Furthermore, this study ascertains the feasibility of *K. alvarezii* farming along the Mannar coast of Sri Lanka. Therefore, promoting commercial farming of *K. alvarezii* is suggested as a viable source of income for the coastal community.

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REFERENCES

Araújo, P. G., Nardelli, A. E., Duran, R., Pereira, M. S., Gelli, V. C., Mandalka, A., Eisner, P., Fujii, M. T., & Chow, F. (2022). Seasonal variation of nutritional and antioxidant properties of different *Kappaphycus alvarezii* strains (Rhodophyta) farmed in Brazil. *Journal of Applied Phycology*, *34*(3), 1677–1691. https://doi.org/10.1007/s10811-022-02739-6

Araújo, P. G., Nardelli, A. E., Fujii, M. T., & Chow, F. (2020). Antioxidant properties of different strains of *Kappaphycus alvarezii* (Rhodophyta) farmed on the Brazilian coast. *Phycologia*, *59*(3), 272–279. https://doi.org/10.1080/00318884.2020.1736878

Ateweberhan, M., Rougier, A. & Rakotomahazo, C. (2015). Influence of environmental factors and farming technique on growth and health of farmed *Kappaphycus alvarezii* (cottonii) in south-west Madagascar. *Journal of Applied Phycology*, 27(2), 923–934. https://doi.org/10.1007/s10811-014-0378-3.

Bhuyar, P., Rahim, M.H., Sundararaju, S., Maniam, G.P. & Govindan, N. (2020). Antioxidant and antibacterial activity of red seaweed; *Kappaphycus alvarezii* against pathogenic bacteria. *Global Journal of Environmental Science and Management*, 6(1), 47–58. https://doi.org/10.22034/gjesm.2020.01.04.

Bindu, M.S. (2011). Empowerment of coastal communities in cultivation and processing of *Kappaphycus alvarezii* - a case study at Vizhinjam village, Kerala, India. *Journal of Applied Phycology*, 23(2),157-163. Available at: https://doi.org/10.1007/s10811-010-9570-2.

Chan, S.W., Mirhosseini, H., Taip, F.S., Ling, T.C. & Tan, C.P. (2013). Comparative study on the physicochemical properties of κ-carrageenan extracted from *Kappaphycus alvarezii* (doty) doty ex Silva in Tawau, Sabah, Malaysia and commercial κ-carrageenans. *Food Hydrocolloids*, *30*(2), 581-588.

Chew, Y.L., Lim, Y.Y., Omar, M. & Khoo, K.S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *Lwt Food Science and Technology*, 41(6),1067–1072. https://doi.org/10.1016/j.lwt.2007.06.013.

Deepa, V.H., Acharya, A., Gupta, P., Kumar, R. & Sarkar, P. (2018). Antimicrobial Potential of *Kappaphycus Alvarezii* Against Plant Pathogens. *International Journal of Current Research in Life Sciences*, 7(04), 1420–1425. http://vipspublisher.com/ijcrls.com/sites/default/files/issues-pdf/IJCRLS-2.pdf.

Diyana, A.F., Abdullah, A., Hisham, Z.S. & Chan, K.M. (2015). Antioxidant activity of red algae *Kappaphycus alvarezii* and *Kappaphycus striatum*. *International Food Research Journal*, 22(5), 1977.

Fayaz, M., Namitha, K. K., Murthy, K. N. C., Swamy, M. M., Sarada, R., Khanam, S., Subbarao, P. V., & Ravishankar, G. A. (2005). Chemical composition, iron bioavailability, and antioxidant activity of *Kappaphycus alvarezzi* (Doty). *Journal of Agricultural and Food Chemistry*, *53*(3), 792–797. https://doi.org/10.1021/jf0493627

Firdaus, M., Nurdiani, R., Prihanto, A.A., Lestari, E.P. & Amam, F. (2021). Carrageenan characteristics of *Kappaphycus alvarezii* from various harvestages. In I*OP Conference Series: Earth and Environmental Science*, 860(1) 012067. https://doi.org/10.1088/1755-1315/860/1/012067.

Hayashi, L, Paula, E.J.D, & Chow, F. (2007a). Growth rate and carrageenan analyses in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of Sao Paulo state, Brazil. *Journal of Applied Phycology,* 19,393-399.

Hayashi, L., Oliveira, E.C, Lhonneur, B.L,

Boulenguer, P., Pereira, R.T.L., Seckendorff, R.V., Shimoda, V.T., Leflamand, A., Vallée, P., & Critchley, A.T. (2007b). The effects of selected cultivation conditions on the carrageenan characteristics of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in Ubatuba Bay, São Paulo, Brazil. *Journal of Applied Phycology* 19, 505–511.

Hayashi, L., Santos, A.A., Faria, G.S., Nunes, B.G., Souza, M.S., Fonseca, A.L., Barreto, P.L., Oliveira, E.C. & Bouzon, Z.L. (2011). *Kappaphycus alvarezii* (Rhodophyta, Areschougiaceae) cultivated in subtropical waters in Southern Brazil. *Journal of Applied Phycology,* 23, 337-343.

Hung, L.D., Hori, K. & Nang, H.Q. (2009). Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga Kappaphycus alvarezii cultivated in Camranh Bay , Vietnam. *Journal of Applied Phycology*,21,265–272. https://doi.org/10.1007/s10811-008-9360-2.

Hurtado-Ponce, A.Q. 1995. Carrageenan properties and proximate composition of three morphotypes of *Kappaphycus alvarezii* Doty (Gigartinales, Rhodophyta) grown at two depths.. *Botanica Marina,* 38(1978), 215–219.

Ilias, M.A., Ismail, A. & Othman, R. (2017). Analysis of carrageenan yield and gel strength of *kappaphycus* species in semporna sabah. *J. Trop. Plant Physiol*, 9(1985), 14 -23.

Kumar, K.S., Ganesan, K., Subba Rao, P.V. & Thakur, M.C., (2016). Seasonal studies on field cultivation of *Kappaphycus alvarezii* (Doty) Doty on the northwest coast of India. *Journal of Applied Phycology*, 28(2), 1193–1205. https://doi.org/10.1007/s10811-015-0629-y.

Kumar, K.S., Ganesan, K. & Rao, P.V.S. (2008). Antioxidant potential of solvent extract of *K. alvarezii* (Doty) Doty - an edible seaweed. *Food Chemistry*, 107(1), 289-295. https://doi.org/10.1016/j.foodchem.2007.08.016.

Ling, A. L. M., Yasir, S., Matanjun, P., & Abu Bakar, M. F. (2015). Effect of different drying techniques on the phytochemical content and antioxidant activity of *Kappaphycus alvarezii*. *Journal of Applied Phycology*, *27*(4), 1717–1723. https://doi.org/10.1007/s10811-014-0467-3

Monte, J., Abreu, A.C., Borges, A., Simões, L.C. & Simões, M. (2014). Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. *Pathogens*, 3(2), 473–498. https://doi.org/10.3390/pathogens3020473.

Muñoz, J., Freile-Pelegrín, Y. & Robledo, D. (2004). Mariculture of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) color strains in tropical waters of Yucatán, México. *Aquaculture*, 239(1–4), 161–177. https://doi.org/10.1016/j.aquaculture.2004.05.043.

Nurshahida, M.F., Nazikussabah, Z., Subramaniam, S., Faizal, W.W. & Aini, M.N. (2020). Physicochemical, physical characteristics and antioxidant activities of three edible red seaweeds (*Kappaphycus alvarezii*, *Eucheuma spinosum* and *Eucheuma striatum*) from Sabah, Malaysia. *in IOP Conference Series: Materials Science and Engineering*, 12048.

Periyasamy, C., Subba Rao, P. V. & Anantharaman, P. (2019). Harvest optimization to assess sustainable growth and carrageenan yield of cultivated *Kappaphycus alvarezii* (Doty) Doty in Indian waters. *Journal of Applied Phycology*, 31(1), 587–597. https://doi.org/10.1007/s10811-018-1562-7.

Prabha, V., Prakash, D.J. & Sudha, P.N. (2013). Analysis of Bioactive Compounds and Antimicrobial activity of marine algae *Kappaphycus alvarezii* using three solvent extracts. *International Journal of Pharmaceutical Sciences and Research*, 4(1), 306–310.

Pushparaj, a, Raubbin, R.S. & Balasankar, T. (2014). Antibacterial activity of

Kappaphycus alvarezii and *Ulva lactuca* extracts against human pathogenic bacteria. *International Journal of Current Microbiolog and Applied Sciences*, 3(1), 432–436.

Rajapaksha, G.D.S.P., Gunathilake, P.M.P.C., Nirooparaj, B., Vidanarachchi, J.K., Jayawardana, B.C., De Croose, M.D.S.T., Wijesekara, I. & Bandaranayake, P.C.G. (2024). Comparative Analysis of Green and Brown Morphotypes of *Kappaphycus alvarezii* Doty (Doty): Morphology, Total Phenol Content, Antioxidant Activity and Antimicrobial Activity. *Tropical Agricultural Research*, *35*(1), 61-72.

Rebecca, L.J., Dhanalakshmi, V. & Shekhar, C. (2012). Research Article Antibacterial activity of *Sargassum Ilicifolium* and *Kappaphycus alvarezii*. *Journal of Cheical and Pharmaceutical Research*, 4(1), 700–705.

Reddy, C.R.K., Eswaran, K., Ganesan, M., Thiruppathi, S. & Mantri, V.A. (2018). Manual on best practise of seaweed cultivation : *Kappaphycus alvarezii*. *Central Salt and Marin e Chemicals Research Institute, India publication*, p.23.

Seth, A., & Shanmugam, M. (2016). Seaweeds as agricultural crops in India: New vistas. *Innovative Saline Agriculture*, 441–473. https://doi.org/10.1007/978-81-322-2770-0_20

Shanmugam, M., Sivaram, K., Rajeev, E., Pahalawattaarachchi, V., Chandraratne, P. N., Asoka, J. M., & Seth, A. (2017). Successful establishment of commercial farming of carrageenophyte *Kappaphycus alvarezii* Doty (Doty) in Sri Lanka: Economics of farming and quality of dry seaweed. *Journal of Applied Phycology*, *29*(6), 3015–3027. https://doi.org/10.1007/s10811-017-1161-z