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Comparative Analysis of Green and Brown Morphotypes of *Kappaphycus alvarezii* Doty (Doty): Morphology, Total Phenol Content, Antioxidant Activity and Antimicrobial Activity

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ABSTRACT

Kappaphycus alvarezii Doty (Doty), commonly known as elkhorn sea moss, is a popular seaweed cultivated globally. It is a well-known source of carrageenan, which is a thickening agent used in food and other industries. Even though different morphotypes of *K. alvarezii* are reported globally, it has been cultivated and marketed as a single genotype. However, such information is important, especially to produce high-value products such as nutraceuticals. Therefore, this study aimed to evaluate differences in microscopic structure, antimicrobial and antioxidant properties, and total phenolic contents of green and brown morphotypes of *K. alvarezii* cultivated in Jaffna, Sri Lanka. Except for the colour, there was no significant difference in macroscopic parameters. There was no significant difference observed in cross-sections under the light microscope. Antimicrobial properties of ethanol, methanol and water extracts were evaluated by disc diffusion assay against, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Antioxidant properties and total phenolic contents were determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and Folin–Ciocalteau method, respectively. Interestingly, the antibacterial activity, antioxidant properties and total phenolic content of *K. alvarezii* depend on the morphotype. The green morphotype was rich in total phenolic compounds along with high antibacterial and antioxidant properties. *Staphylococcus aureus* was the most susceptible bacteria to antimicrobial agents of *K. alvarezii* and both ethanol and methanol extractions were efficient without significant differences between them in all the assays. The current data suggests better performance of the green morphotype of *K. alvarezii* as a natural source of antibacterial and antioxidant compounds.

INTRODUCTION

Kappaphycus alvarezii Doty (Doty), a member of the family Solieriaceae, holds a significant position in the global seaweed industry due to its multifaceted applications, ranging from food and pharmaceuticals to agrochemicals and biofuels (Hurtado & Critchley, 2018). It is commonly known as Elkhorn sea moss (Zhang et al., 2022) and a well-known source of carrageenan a cell wall polysaccharide, a thickening agent used in many food applications (Seth & Shanmugam, 2016). Further, carrageenan is used in bioactive packaging films and its residues are utilized in glucose production for use in the bio-refinery industry (Kanatt et al., 2015). *Kappaphycus alvarezii* is also a source of proteins, carbohydrates, dietary fibres, fatty acids, vitamins and minerals such as iron, calcium and zinc (Fayaz et al., 2005). It is rich in secondary metabolites, which possess antioxidants, antibacterial, anti-diabetic and anticancer properties as well as anti-hyperlipidemia effects (Araújo et al., 2020).

Initially, *Kappaphycus* was collected from the wild and later it was domesticated in the Philippines, followed by Indonesia and Malaysia (Araújo et al., 2020). Thereafter, these *Kappaphycus* seaweeds were introduced to other countries, mainly to be used as raw materials in aquaculture (Campbell & Hotchkiss, 2017). Sri Lanka also has imported *Kappaphycus alvarezii* from Malaysia, the seed materials have been cultivated in Valaipadu and Vidathalaitivu areas, and the pilot-scale trials have been conducted in different locations of Jaffna, Mannar, and Kilinochchi in Northern province (Shanmugam et al., 2017). In the past, vegetative cuttings were the only means to propagate *Kappaphycus*. Hence, during those transplantations from its original grown area to other areas, *Kappaphycus* has adopted new morphological changes that occurred due to different conditions of new culture sites (Campbell & Hotchkiss, 2017).

The presence of colour morphotypes of *K. alvarezii* with varied pigmentation as green, brown and red has been reported (Muñoz et al., 2004). Different morphotypes show differences in pigment, protein content, carrageenan yield, and physiological responses (Hayashi et al., 2007; Araújo et al., 2014; Masarin et al., 2016). Differences in growth performance and photosynthesis of different morphotypes have also been studied (Ohno et al., 1994; Hurtado et al., 2001; Muñoz et al., 2004). However, despite these differences, most studies on *K. alvarezii* have been conducted with mixed morphotypes. Especially, studies on the biological properties and chemical composition of *K. alvarezii* have been reported with mixed morphotypes or without any specificity on morphotypes (Ling et al., 2015). Araújo et al. (2020) and Araújo et al. (2022) reported a specific comparison between green, red, brown and G11 strains in antioxidant properties, and green morphotypes consisting of high antioxidant properties.

Notably, different colour morphotypes of *K. alvarezii,* commonly observed as brown and green are present in Sri Lanka. Despite their coexistence in various marine habitats, little is known about the key differences and biochemical significance between these colour morphotypes of *K. alvarezii*. Due to a lack of knowledge on the significance of morphotypes, mixed morphotype farming of *K. alvarezii* is common in Sri Lankan waters. It is vital to have detailed studies about these different morphotypes to improve the quality and quantity of products and for specific product development. Therefore, the present study was conducted to comparatively analyze the brown and green colour morphotypes of *K. alvarezii*, exploring their morphological differences, antibacterial properties, antioxidants, and total phenolic contents that were grown in shallow seawater of Jaffna peninsula in Sri Lanka.

METHODOLOGY

Sample collection

Kappaphycus alvarezii seaweeds were cultivated in Allaipiddy area of Jaffna, Sri Lanka (9°36'59.9"N 79°57'33.4"E). The seabed was sandy and characterized by spreading seagrass patches. The depth during low tides was 1 m and high tides were 4 m. Seaweed cultivated period was November and

December of 2022. The average rainfall and average maximum temperature were 7.6 mm and 26.5 °C, respectively (measured at regional meteorological office in Jaffna, Sri Lanka). Seaweed was harvested after 45 days of cultivation that were cultivated using Monoline method of cultivation. After harvesting, a part of the samples was fixed freshly in a solution of FAA (formaldehyde, acetic acid, and 50% ethyl alcohol) and stored at 4 °C until microscopic analysis. Another part of the samples was sun-dried for two days, and then oven-dried at 45 °C until reaching constant weight. The dried samples were stored at room temperature (25 °C) until the preparation of crude extract.

Comparison of thallus diameter

The diameter of the tertiary thallus at the point of 2 cm after the emerging place from the secondary thallus was measured using a vernier caliper. Analysis was performed using three technical replicates and three biological replicates for each morphotype.

Comparison of microscopic structure

Thallus specimens were taken from the tertiary thallus at the point of 2 cm after the emerging place from the secondary thallus. After hand sectioning, sections were mounted on slides and staining was accomplished with toluidine blue. Slides were examined under a light microscope and number of cells (greater than 150 µm) were counted in four random sections obtained after subdivision of specimen into eight pieces. Photographs of light microscopic observations were captured through the Cell Sens Standard 1.16 software. Analysis was performed using three technical replicates and three biological replicates for each morphotype.

Preparation of seaweed crude extract

Crude extracts preparation of seaweed samples were done using methanol (≥99.8%), ethanol (≥99.8%) and hot water according to Bhuyar et al. (2020) and Deepa et al. (2018) with some modifications. Brown and green colour morphotypes of *K. alvarezii* seaweed was used for the crude extract preparation. The dry seaweed was ground into a fine powder using Knife mill sample grinder (RETSCH GM 200). For methanol and ethanol extraction, 30 g of seaweed powder were subjected to extraction with 200 mL of methanol or ethanol for 24 hrs at room temperature (25 °C). The hot water extraction was performed at 70 °C on a hot plate for 3 hrs with periodical stirring. The extracts were filtered and centrifuged at 2147×g for 20 minutes (Thermo Fisher Scientific-Sorvall ST 8R). Resulted supernatants were evaporated to dryness using vacuum concentrator at 45 °C. The final powdered extracts obtained were stored at -20 °C until used for further analysis.

Determination of antimicrobial properties

The crude extract of *K. alvarezii* was tested against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) bacteria species. Disc diffusion assay was performed according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards in a 4 mm thick Muller-Hinton Agar (MHA) medium (Bandusekara et al., 2023b). Three species of bacteria were cultured in nutrient agar to obtain single colonies and then they were sub-cultured in LB broth at 37 °C for 24 hrs. Immediately after the turbidity adjustment, Muller-Hinton Agar plates were inoculated with 1 mL of bacteria inoculum over the entire sterile media surface. This step was repeated twice, rotating the plate approximately 60° to ensure an even spread of inoculum. After inoculation, the partially opened Petri dishes were left for 5 minutes to absorb any excess surface moisture. A dried-seaweed crude extract of 5 mg 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 seaweed extracts and air dried. Sterile solutions of 25 µL of DMSO and 10 µL of 0.3% w/v gentamicin were used as negative and positive controls, respectively. Thereafter, the impregnated disks were placed on the surface of the inoculated plates using sterile forceps. The Petri dishes were placed in an inverted position and incubated at 37 °C for 20 hrs. The antimicrobial activity was evaluated by measuring the inhibition zones diameter expressed in millimeters of inhibition against

the tested organisms. All treatment combinations were tested in triplicates within the same culture plate for separate bacteria species (Technical replicates) and the same was repeated in three separate culture plates for each bacteria species (Biological replicates).

1, 1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

Scavenging ability of stable 1,1- diphenyl-2-picrylhydrazyl (DPPH) radicals by different extractions of *K. alvarezii* were measured using the method described by Kumar et al. (2008) with some modifications. A 5 mg sample of the dried crude extract was dissolved in 1 mL of 10% DMSO (Araújo et al., 2020). Then, 1 mL of 1 mM of DPPH was mixed with 1 mL of crude extract. The mixture was vortexed and kept for 30 min at room temperature (25 °C) in the dark. Then absorbance was measured at 517 nm (BIO Spectrometer, Kinetic-Eppendorf). The percentage of radical scavenging activity was calculated using Equation 1;

$$Radical\ scavenging\ activity\ (\%) = [A_{Control} - A_{Sample}/A_{Control}] \times 100 \quad \text{Equation 1}$$

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

Total phenolic content (TPC)

Total phenolic content (TPC) of the seaweed extract was estimated by Folin–Ciocalteau as described by Bhuyar et al. (2020) and Araújo et al. (2020) with some modifications. A 5 mg sample of the dried crude extract was dissolved in 1 mL of 10% DMSO. Then, 0.1 mL of extract was mixed with 2 mL of 2% Na_2CO_3. Mixture was incubated for two minutes at room temperature (25 °C) followed by adding 0.1 mL of Folin-Ciocalteu and mixing. Then, the mixture was kept in dark for 30 minutes for incubation. Absorbance was measured at 760 nm (BIO Spectrometer, Kinetic-Eppendorf). All measurements were done in triplicate with a control. Gallic acid standard curve was plotted using concentrations of gallic acids ranged from 0.2 to 1.0 mg/mL. Results were expressed as mg of Gallic Acid Equivalent per gram (GAE/g) dry weight (DW) of extract.

Data analysis

Data are presented as mean ± standard deviation. The data were tested for normality and homogeneity of variance and then one-way ANOVA was performed to analyze for significant differences among different extraction methods of two morphotypes of *K. alvarezii* separately for antimicrobial assay, antioxidant assay and total phenolic content determination. When differences were observed, Tukey post hoc test at 95% significance level (P=0.05) was used to identify significant differences. For seaweed thallus diameters and microscopic structure comparison between two morphotypes, Student's t-test and Wilcoxon rank sum test were used, respectively. All the analysis was performed using SAS OnDemand academic edition (2023).

RESULTS AND DISCUSSION

Morphological comparison of two colour morphotypes of *K. alvarezii*

The tertiary thallus diameter was not significantly different (P<0.05) between the two morphotypes. The mean diameters of green and brown morphotypes were 7.0±0.6 mm and 7.5±1.0 mm, respectively (Figure 1).

The microscopic structure of the thallus was compared to see whether there are any identifiable differences between them. Both morphotypes consisted of scattered cellular structure without any morphotype specific pattern (Figure 2 A). Furthermore, cortex, medulla area and the axial hyphae are identified in both morphotypes (Figure 2 B) (Phang et al., 2010). In a cross section of *K. alvarezii* cortex, we could identify small cortical cells, which tend to become elongated towards the outer layers, and medulla of *K. alvrezii* has made up of large globular shape cells with a central axial hyphae area [Figures 2 C, 2 D and 2 E]. The number of cells that are greater than 150 µm in diameter were counted in four random pieces after dividing the cross section into eight pieces. The number of cells were not significantly different between

brown and green colour morphotypes of *K. alvarezii* ($P > |Z|$ 0.7532)

Figure 1. Brown and green colour morphotypes of *Kappaphycus alvarezii*
Note: Brown morphotype (A), Green morphotype (B).

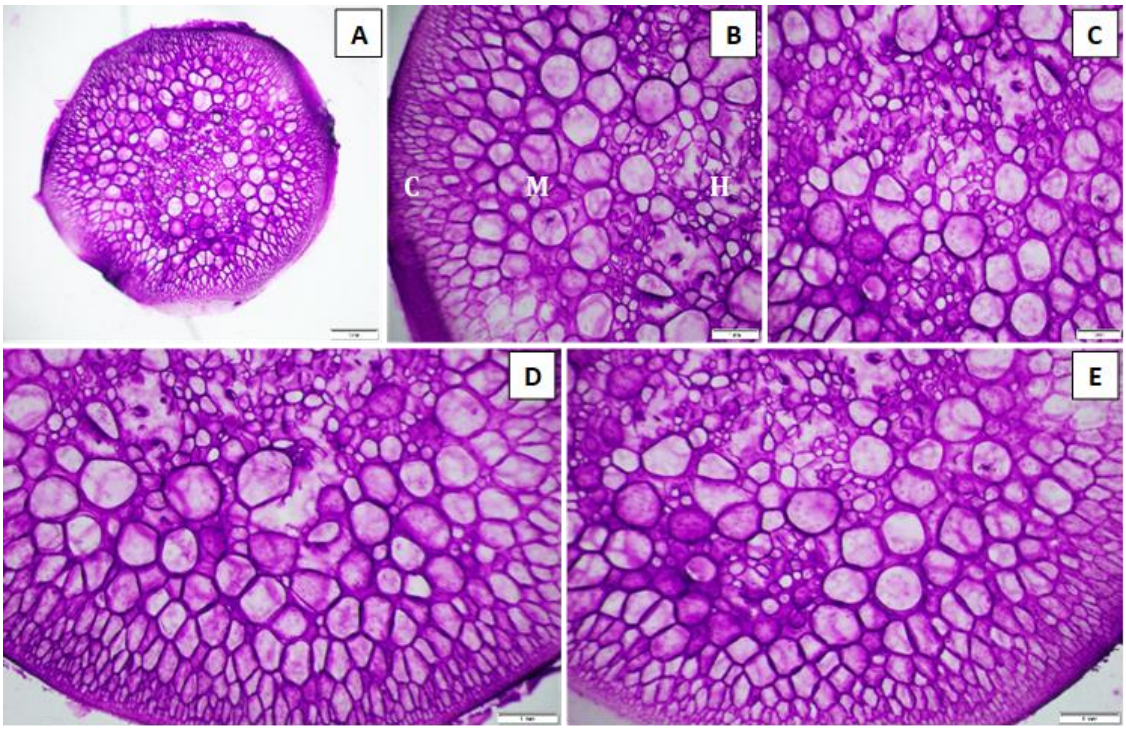

Figure 2. Microscopic structure of brown and green morphotypes of *K. alvarezii*
Note: Cellular arrangement of green and brown morphotypes of *K. alvarezii* (A), Different cell layers in both morphotypes of *K. alvarezii;* C= Cortex M=Medulla H=Axial hyphae (B), Hyphae region of green and brown morphotypes of *K. alvarezii* (C). Elongated, globular and irregular shapes of cells in cortex, medulla and hyphae areas respectively in brown colour morphotype of *K. alvarezii* (D), Elongated, globular and irregular shapes of cells in cortex, medulla and hyphae areas respectively in green colour morphotype of *K. alvarezii* (E) (1 bar = 1 mm).

Several authors have reported the presence of different colour morphotypes or colour strains of *K. alvarezii* (Risjani & Abidin, 2020; Araújo et al., 2020). Risjani & Abidin (2020) reported the presence of green and brown morphotypes in Indonesia, which are also commonly available in Sri Lanka. There are no previous records on comparative analysis of microscopic structures of green and brown morphotypes of *K. alvarezii.* Even though there were no differences at the low magnification light microcopy, there may be

differences at the ultrastructure, and therefore, further high-magnification microscopic analysis is recommended.

Antimicrobial assay

As the positive control, gentamicin showed the highest inhibition zones for all species of bacteria. Hot water, ethanol, and methanol extracts of the green colour morphotype showed significantly higher inhibition zones for all bacteria species over that of the brown colour morphotype (Table 1).

Pseudomonas aeruginosa and *E. coli* showed complete resistance to all types of extracts from brown colour morphotype. However, ethanol and methanol extracts resulted in inhibition zones, against *S. aureus*. Accordingly, *S. aureus* was identified as the most susceptible species of three tested ones, for all type of extracts from both morphotypes. As such the inhibition effect of morphotype depended on the bacterial species. Gram-negative bacteria seem to be less susceptible to antibacterial agents compared to gram-positive bacteria. This is attributed to their morphological structure and composition (Rebecca et al., 2012). The outer membrane of the Gram-negative bacteria consists of lipopolysaccharide which controls the penetration of antimicrobial agents. As reported by Monte et al. (2014) and Bhuyar et al. (2020), required minimum inhibition concentration for *E. coli* (Gram-negative) was higher than in *S. aureus* (Gram-positive), which are in line with the present study. Through a similar study, Prabha et al. (2013) reported the absence of inhibition zones of methanolic extract of *K. alvarezii* against *P. aeruginosa*. Our data also showed the absence of inhibition zones for same species for brown colour morphotype. However, we observed the inhibition zones in green colour extracts. As Prabha et al. (2013) has not mentioned the phenotype, a direct comparison with our results is difficult. There were no studies reported comparing the antibacterial properties of different colour morphotypes of *K. alvarezii*. Therefore, we suggest expanding our work to more bacterial species.

Ethanol and methanol extracts showed higher inhibition effects for both morphotypes without significant difference between two extraction methods. Hot water extracts had the lowest inhibition effect. Pushparaj et al. (2014) studied the antibacterial activity of *K. alvarezii* with different types of solvents, including acetone, chloroform, ethanol, ethyl acetate and methanol. They identified the ethanol extract as the best solvent because it has shown inhibition zones against all the pathogens tested, such as *Bacillus subtilis, S. aureus, Lactobacillus acidophilus, E. coli, P. aeruginosa and Proteus mirabilis.* Rebecca et al. (2012) also reported that ethanol extracts show better results compared to methanol and chloroform extracts against *E. coli* bacteria. Therefore, bioactive metabolite extraction using a polar extractant such as ethanol seems to be a healthy, efficient, and a low-cost process with low content of non-polar substances from *K. alvarezii* (Figure 3).

Table 1. Inhibition zones against pathogenic bacteria by water, ethanol, and methanol extracts of green and brown morphotypes of *K. alvarezii* seaweed

	Inhibition zone (Mean ± SD mm)			Inhibition zone (Mean ± SD mm)		
	Gentamicin	**DMSO**	**Morphotype**	**Water**	**Ethanol**	**Methanol**
S. aureus	17.1±0.1	0	Green	7.6±0.1^{c}	10.5±0.1^{a}	10.6±0.2^{a}
			Brown	0	7.4±0.1^{d}	7.4±0.1^{d}
P. aeruginosa	18.1±0.2	0	Green	7.4±0.1^{cd}	8.2±0.1^{b}	8.2±0.1^{b}
			Brown	0	0	0
E. coli	17.2±0.2	0	Green	7.4±0.1^{cd}	8.2±0.1^{b}	8.2±0.1^{b}
			Brown	0	0	0

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

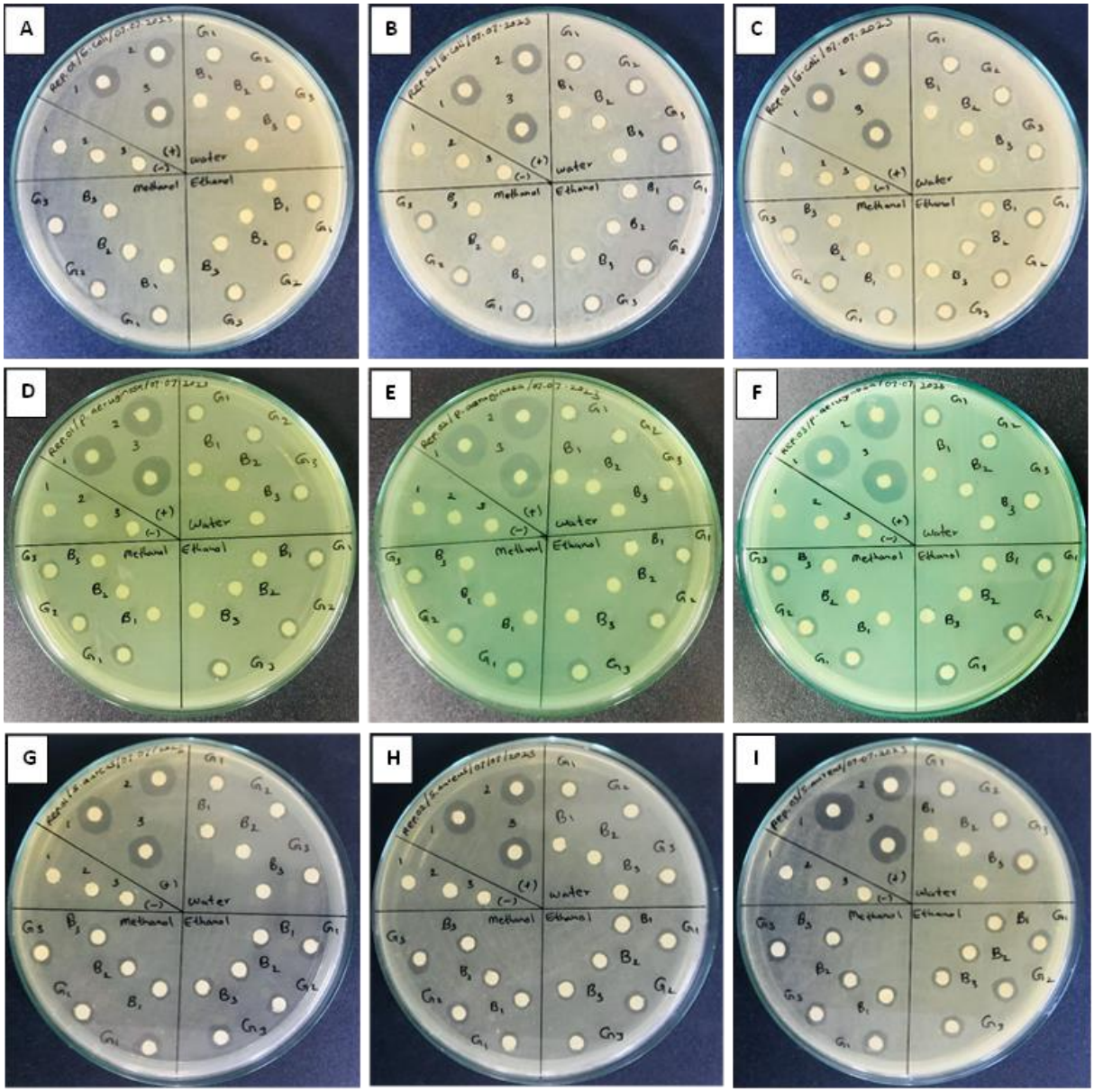

Figure 3. Antibacterial activity assay

Note: *E. coli* (A B C), *P. aeruginosa* (D E F), *S. aureus* (G H I); G= Green morphotype, B= Brown morphotype, (+) = Gentamicin, (-) = DMSO.

In the present study, these three species of bacteria were selected as testing organisms due to their high level of pathogenicity against humans and allowing them to represent both Gram negative and Gram-positive bacteria. *Staphylococcus aureus* can cause a wide variety of diseases, ranging from moderately severe skin infections to fatal pneumonia and sepsis. Furthermore, treatment of *S. aureus* (Gram positive) infections is complicated by antibiotic resistance. The absence of a working vaccine also a critical problem in *S. aureus* infections (Cheung et al., 2021). *Pseudomonas aeruginosa* and *E. coli* (Gram negative) have been identified as opportunistic human pathogens that cause high morbidity and mortality (Lee et al., 2015). Kanatt et al. (2015) have reported that water, ethanol, and methanol are the best solvents for seaweeds extraction during bioprospecting. Furthermore, these polar solvents are able to extract phenolic compounds, sulphated polysaccharides, soluble proteins, glycosides, and organic acids like bioactive metabolites efficiently (Diyana et al., 2015). Based on those records, hot water, ethanol, and methanol extraction methods were tested for antimicrobial properties.

Antioxidant properties

Owing to significant differences of antimicrobial properties between brown and green morphotypes, antioxidant properties also were analyzed along with different extraction methods. In terms of antioxidant properties, a significant difference was recorded between the two morphotypes. The percentage of radical scavenging activity ranged from 29.3% to 46.1% and from 23.6% to 36.7% in green and brown morphotypes, respectively. Green colour morphotype had a higher antioxidant property from all types of extracts compared to the brown morphotype. Within each morphotype, ethanolic and methanolic extraction methods were able to give significantly high antioxidant effect compared to hot water extraction (Figure 4).

The present study reported about 20% higher values for antioxidant properties than those of Kumar et al. (2008), and Farah Diyana et al. (2015) with 19.35%±0.78% for the same species of seaweed with no comparison of colour morphotype. A similar finding was recorded by Araújo et al. (2020) on antioxidant properties of *K. alvarezii*, in which they compared green, red, brown and G11 strains grown in Brazilian water. Other than DPPH method used in present study, Araújo et al. (2020) compared these morphotypes using Folin–Ciocalteu reducing potential, 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging and ferric reducing antioxidant power (FRAP) assay methods. For the DPPH method, the antioxidant activity has ranged from 7.15% to 31.21% and highest values have been recorded in FRAP method, ranging from 27.24% to 95.95%. In all those analysis, aqueous extraction has given lower values compared to methanolic extracts while Araújo et al. (2020) has not included ethanolic extracts in the analysis. Furthermore, green colour morphotype showed higher antioxidant properties in those different assays.

Total phenolic content

Out of three extraction methods, ethanol and methanol extractions were obtained with the highest total phenolic content for both morphotypes, ranging from 32.4±1.46 mg of GAE/g DW to 49.12±1.15 mg of GAE/g DW. The lowest total phenolic content was recorded in hot water extraction, which ranged from 10.93±0.73 mg of GAE/g DW to 16.53±0.53 mg of GAE/g DW and is less than half of the TPC content in ethanol and methanol extracts. Weak antibacterial and antioxidant properties recorded in hot water extracts from both morphotypes could be attributed to lower values of total phenolic content. When comparing two colour morphotypes, a significantly higher ($P<0.05$) TPC was recorded in green morphotype in all types of extractions (Figure 5). This can be suggested as a factor for the higher antimicrobial and antioxidant properties of green colour morphotype under different treatment combinations. It is common to have a positive correlation between phenolic compounds and antioxidant or antibacterial properties. This is because phenolic compounds in living organisms are potent antioxidants and antibacterial agents.

The values resulted in the present study on total phenolic content were higher than those reported by Chew et al. (2008), Diyana et al., (2015) and Nurshahida et al. (2020). Reported total phenolic content values by them were 28.4±1.1 mg of GAE g^{-1} DW, 7.51±0.16 – 17.32±1.2 mg of GAE g^{-1} DW and 19.17±0.04 mg of GAE g^{-1} DW respectively. However, the reported values were for the species without differentiation on colour morphotypes. Araújo et al. (2020) reported the TPC values of brown and green morphotypes through serial extractions as 58.49±9.45 mg of GAE 100 g^{-1} DW for green methanolic extracts, 47.54±6.96 mg of GAE 100 g^{-1} DW for brown methanolic extracts, 12.51±1.08 mg of GAE 100 g^{-1} DW for green aqueous extracts, and 9.90±1.71 mg of GAE 100 g^{-1} DW for brown aqueous extracts. Bhuyar et al. (2020) also have reported values as 19.1±0.8 mg of GAE g^{-1} and 20.25±0.03 mg of GAE g^{-1} for hot water and ethanol extractions, respectively. Higher values recorded in the present study could be due to the longer extraction time compared to the previous studies. During the present study, ethanol and methanol extraction times increased from 30 minutes to 24 hours at room temperature as described by Deepa et al.(2018).

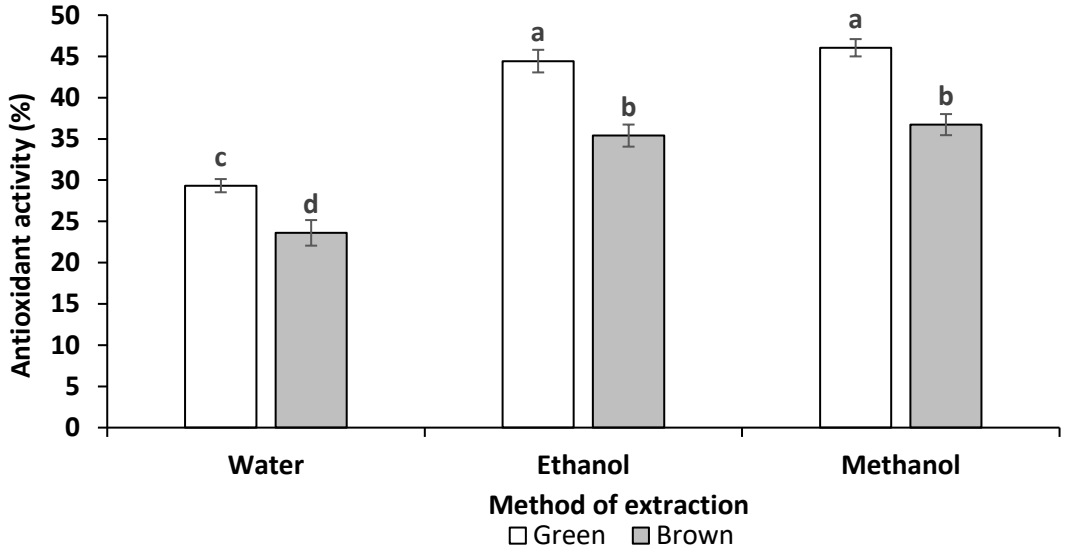

Figure 4. Antioxidant activity (DAPPH) of green and brown morphotypes of *K. alvarezii* extracted from different methods

Note: Bars with the same letter are not significantly different at p=0.05.

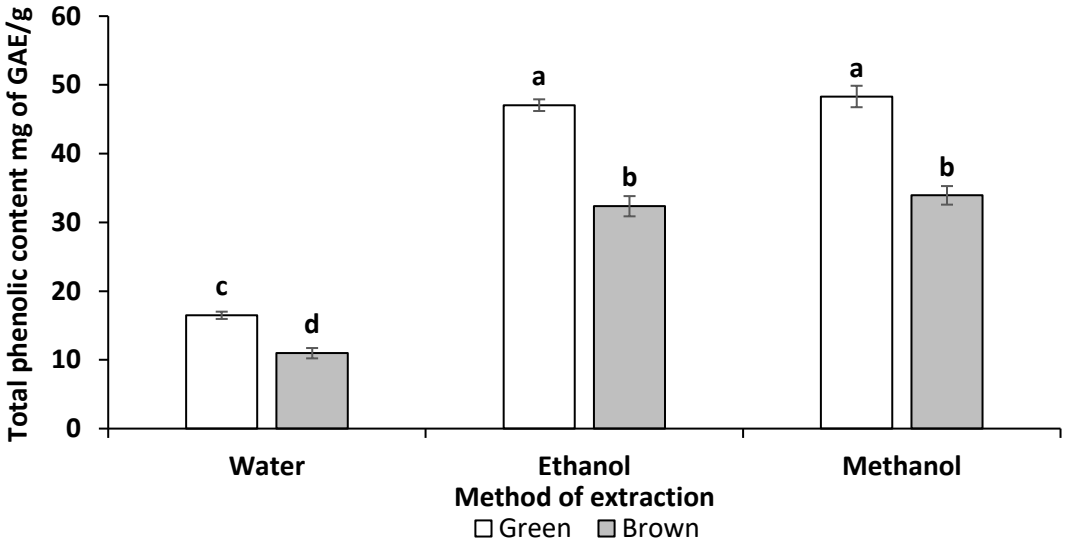

Figure 5. Total phenolic content of green and brown morphotypes of *K. alvarezii* extracted from different methods

Note: Bars with the same letter are not significantly different at p=0.05.

CONCLUSIONS

Our data revealed that, except for the colour, there were no significant differences in other macroscopic parameters and light-microscope cross-sections between green and brown morphotypes of *K. alvarezii*. However, the antibacterial activity, antioxidant properties and total phenolic content of *K. alvarezii* differs in colour morphotypes. The green colour morphotype has a comparatively higher total phenolic compounds along with a higher antibacterial and antioxidant potential than the brown colour morphotype. Hence, the green colour morphotype of *K. alvarezii* may be suitable as a natural source of antibacterial and antioxidant agents. Selection of suitable morphotype is important for large-scale cultivations, especially to produce high-value

products. Among the tested bacteria species *S. aureus* was the most susceptible bacteria to *K. alvarezii* and both ethanol and methanol extractions were effective extraction methods.

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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

Araújo, P. G., Ribeiro, A. L. N. L., Yokoya, N. S., & Fujii, M. T. (2014). Temperature and salinity responses of drifting specimens of *Kappaphycus alvarezii* (Gigartinales, Rhodophyta) farmed on the Brazilian tropical coast. *Journal of Applied Phycology*, *26*(5), 1979–1988. https://doi.org/10.1007/s10811-014-0303-9

Bandusekara, B. S., Pushpakumara, D. K. N. G., & Bandaranayake, P. C. G. (2023b). Comparison of High-Performance Liquid Chromatography (HPLC) Profiles and Antimicrobial Activity of Different *Cinnamomum* Species in Sri Lanka. *Tropical Agricultural Research*. https://doi.org/10.4038/tar.v34i2.8624

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

Campbell, R., & Hotchkiss, S. (2017). Tropical Seaweed Farming Trends, Problems and Opportunities. *Tropical Seaweed Farming Trends, Problems and Opportunities*. https://doi.org/10.1007/978-3-319-63498-2

Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus.* Virulence, *12*(1), 547–569. https://doi.org/10.1080/21505594.2021.1878688

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*, *41*(6), 1067–1072https://doi.org/10.1016/j.lwt.2007.06.013

Deepa, V., 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, F. A., Abdullah, A., Hisham, Z. A. S., & Chan, K. M. (2015). Antioxidant activity of red algae *Kappaphycus alvarezii* and *Kappaphycus striatum*. *Int Food Res J*, *22*.

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

Hayashi, L., Oliveira, E. C., Bleicher-Lhonneur, G., Boulenguer, P., Pereira, R. T. L., Von Seckendorff, R., Shimoda, V. T., Leflamand, A., Vallée, P., & Critchley, A. T. (2007). 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*(5), 505–511. https://doi.org/10.1007/s10811-007-9163-x

Hurtado, A. Q., Agbayani, R. F., Sanares, R., & De Castro-Mallare, M. T. R. (2001). The seasonality and economic feasibility of cultivating *Kappaphycus alvarezii* in Panagatan Cays, Caluya, Antique, Philippines. *Aquaculture*, *199*(3–4), 295–310. https://doi.org/10.1016/S0044-8486(00)00553-6

Hurtado, A. Q., & Critchley, A. T. (2018). A review of multiple biostimulant and bioeffector benefits of AMPEP, an extract of the brown alga Ascophyllum nodosum, as applied to the enhanced cultivation and micropropagation of the commercially important red algal carrageenophyte *Kappaphycus alvarezi. Journal of Applied Phycology*, *30*(5), 2859–2873. https://doi.org/10.1007/s10811-018-1407-4

Kanatt, S. R., Lahare, P., Chawla, S. P., & Sharma, A. (2015). *Kappaphycus alvarezii*: Its antioxidant potential and use in bioactive packaging films. *The Journal of Microbiology, Biotechnology and Food Sciences*, *5*(1), 1.

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

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

Lee, S. A., Gallagher, L. A., Thongdee, M., Staudinger, B. J., Lippman, S., Singhc, P. K., & Manoila, C. (2015). General and condition-specific essential functions of *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(16), 5189–5194. https://doi.org/10.1073/pnas.1422186112

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/10.100 7/s10811-014-0467-3

Masarin, F., Cedeno, F. R. P., Chavez, E. G. S., De Oliveira, L. E., Gelli, V. C., & Monti, R. (2016). Chemical analysis and biorefinery of red algae *Kappaphycus alvarezii* for efficient production of glucose from residue of carrageenan extraction process. *Biotechnology for Biofuels*, *9*(1), 1–12. https://doi.org/10.1186/s13068-016-0535-9

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. S. F., Nazikussabah, Z., Subramaniam, S., Faizal, W. I. W., & Aini, M. A. N. (2020). Physicochemical, physical characteristics and antioxidant activities of three edible red seaweeds (*Kappaphycus alvarezii, Eucheuma spinosum and Eucheuma striatum*) from Sabah, Malaysia. *IOP Conference Series: Materials Science and Engineering*, *991*(1), 12048.

Ohno, M., Largo, D. B., & Ikumoto, T. (1994). Growth rate, carrageenan yield and gel properties of cultured kappa-carrageenan producing red alga *Kappaphycus alvarezzi* (Doty) Doty in the subtropical waters of Shikoku, Japan. *Journal of Applied Phycology*, *6*, 1–5.

Phang, S. W., Yeong, H.-. Y., Lim, P.-. E., Nor, A. R. M., & Gan, K. T. (2010). Commercial varieties of *Kappaphycus* and *Eucheuma* in Malaysia. *Malaysian Journal of Science*, *29*. https://doi.org/10.22452/mjs.vol29no3.4

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.

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

Risjani, Y., & Abidin, G. (2020). Genetic diversity and similarity between green and brown morphotypes of *Kappaphycus alvarezii* using RAPD. *Journal of Applied Phycology*, *32*(4), 2253–2260. https://doi.org/10.1007/s10811-020-02223-z

Seth, A., & Shanmugam, M. (2016). Seaweeds as agricultural crops in India: New vistas. In J. C. Dagar, P. C. Sharma, D. K. Sharma, & A. K. Singh (Eds.), *Innovative Saline Agriculture* (pp. 441–473). Springer India. 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

Zhang, L., Liao, W., Huang, Y., Wen, Y., Chu, Y., & Zhao, C. (2022). Global seaweed farming and processing in the past 20 years. *Food Production, Processing and Nutrition*, *4*(1). https://doi.org/10.1186/s43014-022-00103-2