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# Seasonal variations in growth and phytochemical compounds of cultivated red alga, *Hypnea flagelliformis*, in southern coastlines of Iran

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**Abstract**

Global utilization of seaweeds for food, chemicals, pharmaceuticals, and production of polysaccharide is increasing and seaweeds are becoming the most important cultivated marine organisms. This study assessed the cultivation potential of the red alga, *Hypnea flagelliformis*, along the southern coastlines of Iran using monoline plastic rope method, with regard to several environmental parameters of seawater over a year (November 2017 to October 2018). Correlations between relative growth rate (RGR) and environmental parameters were investigated using Pearson correlation analysis. Biochemical composition contents (moisture, ash, protein, and lipid) of the cultivated samples were measured during the experiment. Yield and structural properties of the extracted carrageenan using aqueous and alkali-treated extraction of the samples were investigated using Fourier transform infrared (FT-IR) spectroscopy. This study showed that this species can grow only in 6 months of the year (November to April) in outdoor conditions. The highest relative growth rate ($9 \pm 0.4\%$ day$^{-1}$) was obtained in December. Salinity and temperature had significant impacts on the growth of *H. flagelliformis*. The biochemical composition content range for moisture (86.76-91.76% fw), ash (30-39% dw), total protein (1.40-3.03% dw), and lipid (1.08-3.15% dw) varied during the experiment. The yield of alkali-treated carrageenan (mean $34.5 \pm 2.5\%$ dw) was higher than aqueous method (mean $20.7 \pm 1.3\%$ dw). FT-IR analysis indicated that the extracted hydrocolloids are mainly from κ-carrageenan type. The findings demonstrate that *H. flagelliformis* has good potential for cultivation and as a carrageenan source.

**Keywords** Rhodophyta · Biochemical composition · Carrageenan · Ecological parameters · FT-IR · RGR

## Introduction

Dramatic increasing trend of the world population shows the necessity to look for new resources for food supply. Furthermore, shortage in freshwater resources and massive degradation of agriculture soils due to the long-term overuse are the main factors stimulating research on marine plants and seaweeds as sustainable and commercially renewable photosynthetic organisms to produce food (Kim 2011). Severe issues such as the energy crisis, environmental pollution, climate change, and greenhouse gas phenomenon make it important to pay more attention to cultivation and exploitation of seaweed resources (Holdt and Kraan 2011; Athithan 2014). Global production of seaweed has reached 24 million tonnes with around 10 billion US$ of commercial value with 90% of the production in Asian countries (FAO and UNICEF 2018). Several species of algae are cultivated, especially various species of Gelidiaceae and Gracilariaceae grown for agar and agarose, *Kappaphycus* spp., *Chondrus* spp., and *Hypnea* spp. for carrageenan and *Macrocystis* spp., *Laminaria*/*Saccharina* spp., *Sargassum* spp., and *Turbinaria* spp. for alginate production (Titlyanov and Titlyanova 2010; Edwards and Dring 2011).

Carrageenans as a group of phycocolloids are commercially valuable hydrophilic polysaccharides which have functional roles in the matrix material of cell walls in numerous species of red marine macroalgae. Based on their solubility and gelling properties, carrageenans have classified in different

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groups such as kappa ($k$), iota ($\iota$), and lambda ($\lambda$) carrageenan, and have widespread commercial uses in food, cosmetic, and other industries as thickening, suspending, and gelling agents. The average annual production of carrageenan is about 28,000 t year$^{-1}$, which is worth US$ 240 million. Global carrageenan production has exceeded 56,000 t since 2013 and is valued at US$ 762 million (Anonymous 2020). This biopolymer can be extracted mainly from algae of the order Gigartinales and species belonging of *Kappaphycus*, *Eucheuma*, *Chondrus*, *Palmaria*, *Gigartinia*, *Mazzella*, and *Hypnea*. Carrageenans are large and very flexible molecules with helical structure that allow them to form different types of gels at normal temperatures (FAO 2003).

*Eucheuma* and *Kappaphycus* as the most popular carrageenophyte species account for over 80% of world's carrageenan production. Based on FAO (2003), *Kappaphycus alvarezii* and *Eucheuma denticulatum* are the most used species for carrageenan production. Commercial cultivation of these two species has successfully spread to several countries over the world. Well-established cultivation techniques and high productivity of *K. alvarezii* make it a great candidate to introducing it to many tropical and subtropical countries (Bixler and Porse 2010; Porse and Rudolph 2017). Several researchers have studied the invasive effects of *Kappaphycus*/*Eucheuma* in tropical regions (Castelar et al. 2015). For example, Williams and Smith (2007) reported that although all alien species are invasive, some of them showed an invasive potential more than 10 years after introduction and were associated with coral reefs. Invasions of the two species *E. denticulatum* and *K. alvarezii* have been reported from Hawaii, Venezuela, and India (Chandrasekaran et al. 2008; Conklin and Smith 2005).

Predictions about invasions of any introduced species require study to analyze the interactions among several factors, like the similarity of the environmental condition of the original environment and the target introduced environment or competition by consumers and the susceptibility of the introduced environment to invasion (Schaffelke et al. 2006; Pickering et al. 2007). Castelar et al. (2016) in their comparative study on the growth performances of the native carrageenophyte, *Hypnea musciformis* and introduced *K. alvarezii* in both indoor and outdoor condition found that *H. musciformis* has higher daily growth rate (DGR) (10.8, 0.6% day$^{-1}$) compared to *K. alvarezii* (5.0-1.2% day$^{-1}$) indoors, but in sea conditions *H. musciformis* did not survive while *K. alvarezii* grew better and its carrageenan yield was high (33.7-50.8%). However, cultivation indoors showed that *H. musciformis* can be used as a complementary source of carrageenan.

The genus *Hypnea* J.V. Lamouroux, with 61 accepted species belonging to the family of Cystocloniaceae (Rhodophyta, Gigartinales), is one of the economic red algal genera in intertidal and subtidal zones of tropical and warm temperate marine biogeographical regions (Geraldino et al. 2009; Yokoya et al. 2020). *Hypnea* is an economically important genus of seaweeds for the production of kappa carrageenan in food industry. Properties of carrageenan extracted from several species have been studied (Prajapati et al. 2014; Perez Recalde et al. 2016) and **c**onsistent occurrence of kappa carrageenan in the members of genus *Hypnea* has been confirmed by IR spectral analysis (Rajasulochana and Gunasekaran 2009).

Some studies also have been focused on growth and reproduction performance of some *Hypnea* species (Ganesan et al. 2006). Nauer et al. (2019) demonstrated the life cycle of *Hypnea* for *Hypnea pseudomusciformis*, which consists of two diploid and one haploid phase in the form of Polysiphonia type.

*Hypnea* has been considered an alternative for *Kappaphycus* in Brazil and India (Yokoya et al. 2020). Moreover, although *K. alvarezii* is the main source of kappa carrageen in the world, *Hypnea* spp. are exploited and used as a source of carrageenan (FAO 2018). Greer et al. (1984) demonstrated that *Hypnea* has high-quality hydrocolloids compared to those obtained from *Kappaphycus*. This alga with its mild marine taste and texture has characteristics of functional foods and can be used in the food industry (Pereira et al. 2009). Accordingly, the development and introduction of *Hypnea* to culture as a carrageenophyte seaweed are very convenient and may elevate the socio-economic conditions of local coastal communities.

There are several native commercial seaweeds with potential of commercial cultivation in tropical waters of the Persian Gulf in the southern coastlines of Iran (Sohrabipour and Rabiei 1999, 2007, 2008; Sohrabipour et al. 2004; Bellorin et al. 2008; Zarei Jeliani et al. 2017a) and one of the commercially important groups of the red algae in this area is the genus *Hypnea* with ten species of this genus having been reported (Kokabi and Yousefzadi 2015). *Hypnea musciformis*, as a common species in the area, has been experimentally cultivated in Chabahar port in southeastern Iran and its production has increased from 500 g of initial weight to 4600 g in 60 days, which has shown a 9-fold increase in biomass (Farahpour et al. 2009).

*Hypnea flagelliformis* is one of the native species on the seashores of Bandar Abbas in the south of Iran. Biochemical composition (Saeidnia et al. 2009; Jassbi et al. 2013), antioxidant and antibacterial properties (Moein et al. 2015), sterol composition (Nasir et al. 2011), and carrageenan content (Rajasulochana and Gunasekaran 2009) of the species have already been investigated, demonstrating the commercial potential of the species.

Therefore, in this study, considering the destructive effects of invasive non-native species on the coral communities of the Persian Gulf, we evaluated the possibility of cultivating the native species *H. flagelliformis* which is the common species in the sandy beds of the intertidal pools on the seashores of Bandar Abbas City in the south of Iran. Using monoline plastic rope method that is the usual method for filamentous marine seaweed cultivation, the effects of some environmental conditions such as season and physicochemical factor effects on biochemical compositions and carrageenan yield of *H. flagelliformis* were investigated in the cultured samples.

## Material and methods

### Site selection and cultivation method

The aim of this study is to evaluate the possible cultivation of native seaweed to establish a socio-economic activity for local rural communities in the southern coast of Iran. Most of the intertidal regions of the southern coastlines of Iran are covered with sands and muds that mix with the skeletal debris of the local sea life (Evans 2020). Therefore, we tried to work on algal species that are compatible with the sandy substrates in these areas. The cultivation site of the current study was located at Suroo Beach (27° 98′ N; 56° 13′ S) in west of Bandar Abbas in Hormozgan Province (south of Iran), and the experiment was carried out from November 2017 to October 2018.

The wild form of *Hypnea flagelliformis* grows creeping on sandy bottom of pools in the lower intertidal zones of the selected site. For the first seeding, specimens of *H. flagelliformis* were collected from the natural beds around the cultivation site and then, in each cycle of harvest, a part of the harvested biomass of the cultivated seaweed was used as seed for the next cycle. *Hypnea flagelliformis* appears in this site from the late autumn in November and continues to the middle of spring season in April. After that, the temperature increases in the area and environmental conditions are not suitable for *Hypnea* growing. Therefore, the cultivation was possible for only 6 months and therefore six cultivation cycles (30 days each) were tested in six experimental periods between November 2017 and October 2018.

There are several methods for cultivating algae and the most common method is cultivation using single-stranded ropes that are attached to wooden poles fixed in the sea bed. In this study, cultivation was done with slight changes according to the monoline plastic rope method described by Zarei Jeliani et al. (2017b) (Fig. 1). After the thalli were cleaned of any visible fouling organisms, around 400 g of seedlings (10 cm length) was inserted in each 2-m-long nylon rope string at intervals of 20 cm. Four ropes with seedling were attached at intervals of 1 m to the two parallel ropes which were tightened to four wooden poles (Fig. 1a) and empty drink bottles were used to float the ropes. In spite of the proposed methods that suggested a depth of 50 cm as a proper depth, for cultivation rope establishment in shallow tidal pools for *H. flagelliformis* cultivation, we had to fasten the ropes in 20-30 cm above the sea bottom, because its thallus is a little brittle and is easily pulled out from the ropes by wave activity.

### Biomass evaluation

*Hypnea flagelliformis* growth is maximized between 25 and 30 days and if the produced biomass of the ropes is not harvested, it will be completely lost. Therefore, each month, all ropes of the ten sets were weighed and the produced biomass of the two sets was harvested monthly for phytochemical analysis. Thus, for a new cycle of cultivation, all 40 ropes of these ten sets were carefully cleaned and again transplanted using some of the harvested algal biomass. Six cultivation cycles were tested in six experimental periods from November 2017 to October 2018. Because of harsh environmental condition, especially high temperature of the area, no growth was observed in the other months of the year. The biomass of each rope was measured as growth estimation (Fig. 1b). Relative growth rate (RGR) was estimated using the following equation (Bezerra and Marinho-Soriano 2010):

$$\mathrm{RGR}\ \left(\%\mathrm{day}^{-1}\right) = \left[\ln\left(W_{\mathrm{f}}/W_{\mathrm{i}}\right)/T\right] \times 100$$

where $W_{\mathrm{i}}$ is the initial weight (approx. 400 g rope$^{-1}$), $W_{\mathrm{f}}$ is the final weight (g), and $T$ is the cultivation time (between 20 and 25 days).

### Environmental parameters

We evaluated several environmental parameters monthly during a year to determine optimal conditions for planting. Seawater temperature, salinity, and pH were recorded weekly using a portable 340i WTW multimeter (WTW, Germany). Water samples (in three replicate) were filtered through Whatman GF-F filters and stored in propylene vials at −4°C for further nutrient analysis. Dissolved nutrients ($NH_4^+$, $NO_3^-$, $NO_2^-$, and $PO_4^-$) were measured using a UV-visible spectrophotometer (Hatch DR2800, Germany), following the Nessler, cadmium reduction, diazotization, and amino acid methods, respectively (Zarei Jeliani et al. 2017b).

### Evaluation of biochemical compositions

The moisture and ash content were determined according to the Association of Official Analytical Chemists (AOAC 2000). The total protein was determined by the Bradford (1976) method. using bovine serum albumin (BSA) as the standard and expressed in percentage of dry weight (% dw). Total lipid content was estimated gravimetrically by the Bligh and Dyer (1959) method and expressed as a percentage of dry weight.

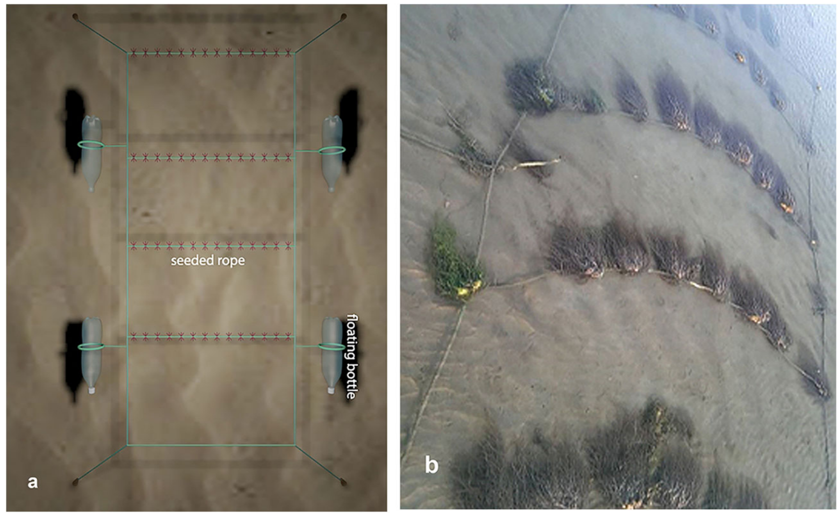



**Fig. 1** **a** Schematic representation of monoline plastic rope method for the cultivation experiment. **b** Cultivation of *Hypnea flagelliformis* with initial seedling and grown plants

## Carrageenan extraction

During the extraction process, 10 g of the 2-day sun-dried *H. flagelliformis* was hydrated overnight in 200 mL deionized water before direct extraction at room temperature (25 °C) for 12h, followed by depigmentation using 100 mL of methanol-acetone (1:1) mixture for 1h to eliminate the organic soluble fraction of lipids. Alkali-treatment was carried out using a slightly modified procedure of the method described by Rhein-Knudsen et al. (2017). Briefly, 500 mL NaOH (6%, w/v) was added to 10 g of the soaked and depigmented seaweed samples and reaction was carried out at 60 °C for 2h. NaOH was removed by washing the seaweed samples with tap water. Carrageenan aqueous and alkali-treated extraction was then performed in 250 mL deionized water at 95 °C for 1.5h. The extracts were filtered using a cotton cloth followed by a glass microfiber filter (Whatman GF/D). Then, the extracts were precipitated in 95% ethanol (1:3, v/v), centrifuged, and freeze-dried. Finally, yield percentage was determined by weighing.

## Fourier transform infrared spectroscopy

To investigate the carrageenan functional groups, a mixture of hydrocolloids corresponding to each aqueous-treated and alkali-treated sample was selected for Fourier transform infrared spectroscopy (FT-IR) analysis using a Fourier transform infrared spectrophotometer (FT-IR Bruker, Germany) equipped with the OPUS 3.1 software. The extracted carrageenan was ground with KBr powder and then pressed into pellets for FT-IR measurement in the frequency range of 400–4000 cm$^{-1}$. The spectra of the samples were obtained and compared with spectrum of commercial carrageenan (Sigma-Aldrich).

**Table 1** Environmental parameters during the cultivation period

| Parameters | *Hypnea flagelliformis* absent[a] | | | *Hypnea flagelliformis* present [b] | | |
|---|---|---|---|---|---|---|
| | Mean ± SD | Max | Min | Mean ± SD | Max | Min |
| Temperature ** (°C) | 32.1 ± 2.62 | 35.6 | 27.1 | 23.3 ± 0.77 | 29.7 | 17.45 |
| Salinity ** (ppt) | 38.1 ± 0.17 | 38.5 | 37.6 | 37.1 ± 0.20 | 37.4 | 36.7 |
| pH ** | 8.4 ± 0.17 | 8.6 | 8.3 | 8.4 ± 0.07 | 8.4 | 8.2 |
| Nitrate* (mmol L$^{-1}$) | 2.14 ± 0.11 | 2.3 | 2.0 | 1.95 ± 0.11 | 2.4 | 1.5 |
| Nitrite* (mmol L$^{-1}$) | 0.001 ± 0.00 | 0.008 | 0.005 | 0.01 ± 0.001 | 0.015 | 0.005 |
| Ammonium* (mmol L$^{-1}$) | 5.6 ± 0.28 | 7.4 | 4.1 | 5.8 ± 0.35 | 6.2 | 5.1 |
| Orthophosphate* (mmol L$^{-1}$) | 1.7 ± 0.09 | 2.0 | 0.8 | 2.0 ± 0.44 | 2.8 | 0.8 |

* ($n$ = 10)

** ($n$ = 4)

[a] May, Jun, Jul, Aug, Sep, and Oct (2018)

[b] Nov, Dec (2017), Jan, Feb, Mar, and Apr (2018)

**Table 2** Pearson correlation analysis between related growth rate (RGR% $day^{-1}$) and environmental parameters

| *Hypnea flagelliformis* | Environmental parameters | | | | | | |
|---|---|---|---|---|---|---|---|
| | Temperature | Salinity | pH | Nitrite | Nitrate | Ammonium | Orthophosphate |
| Correlation coefficient | −0.793** | −0.798** | −0.112 | 0.469** | −0.399* | 0.124 | −0.165 |
| Sig. (2-tailed) | 0.01 | 0.01 | 0.514 | 0.01 | 0.05 | 0.471 | 0.336 |
| *n* | 36 | 36 | 36 | 36 | 36 | 36 | 36 |

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

## Statistical analysis

All data of the various analyses are expressed in terms of mean ($n = 4$) ± standard deviation (SD). One-way analysis of variance (ANOVA, $p \leq 0.05$) was used to determine any significant differences in relative growth rate (RGR% $day^{-1}$) and biochemical composition data. Pearson's correlation analysis was used to compute the correlation between RGR and environmental parameters. The statistical analyses were carried out with SPSS (version 21) software.

## Results

### Environmental parameters and growth rate

Results of the environmental factor measurements are shown in Table 1. Water temperature significantly varied between 17.45 °C in December to 35.60 °C in July. Salinity showed minimum values in February (36.7 ppt) and maximum levels in July (38.5 ppt). There were little changes in pH and ammonium in the period of study. The highest concentrations of $PO_4^{3-}$ (2.8 mmol $L^1$) and $NO_2^-$ (0.015 mmol $L^{-1}$) were recorded in March and February, respectively. The maximum value of $NO_3^-$ (2.4 mmol $L^{-1}$) was detected in April (Table 1, Fig. 3).

The growth rates from consecutive harvests are shown in Fig. 2. Figure 3d shows the monthly biomass of *H. flagelliformis* (kg $m^{-2}$) and the cumulative biomass curve which estimates the annual biomass product of the cultivated species. The maximum growth rate was obtained in December (9 ± 0.57% $day^{-1}$) followed by January (8.16 ± 0.57% $day^{-1}$), with a decreasing trend by April (5.1 ± 0.78% $day^{-1}$). ANOVA showed significant differences in growth rate of the cultivated macroalgae ($p \leq 0.05$) (Fig. 2). Pearson analysis depicted a negative correlation between RGR value and salinity ($r = -0.798$, $p = 0.01$), water temperature ($r = -0.793$, $p = 0.01$), and $NO_3^-$ ($r = -0.399$, $p = 0.01$), while a positive correlation was observed between RGR and nitrite ($r = 0.469$, $p = 0.01$) (Table 2, Fig. 3a-c). The other parameters showed no significant correlation with RGR ($p > 0.05$) (Table 2).

### Biochemical composition

The lowest moisture, ash, protein, and lipid contents (86.76, 30, 1.40, and 1.08%, respectively) were detected in April and the highest were obtained in winter months (Table 3). The ash

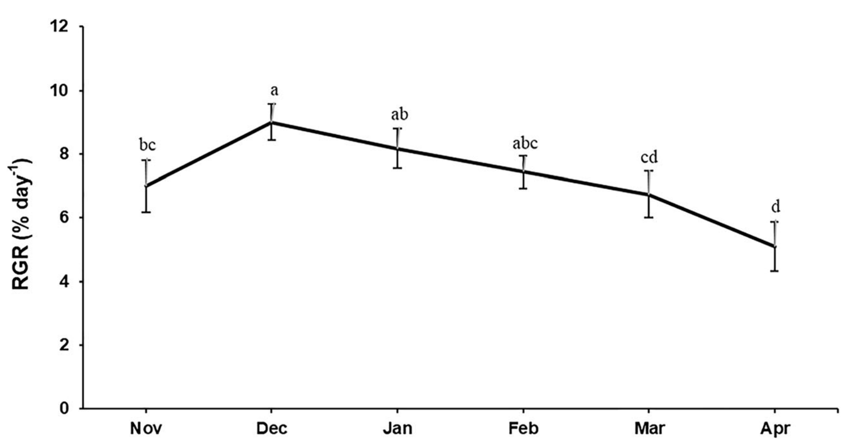
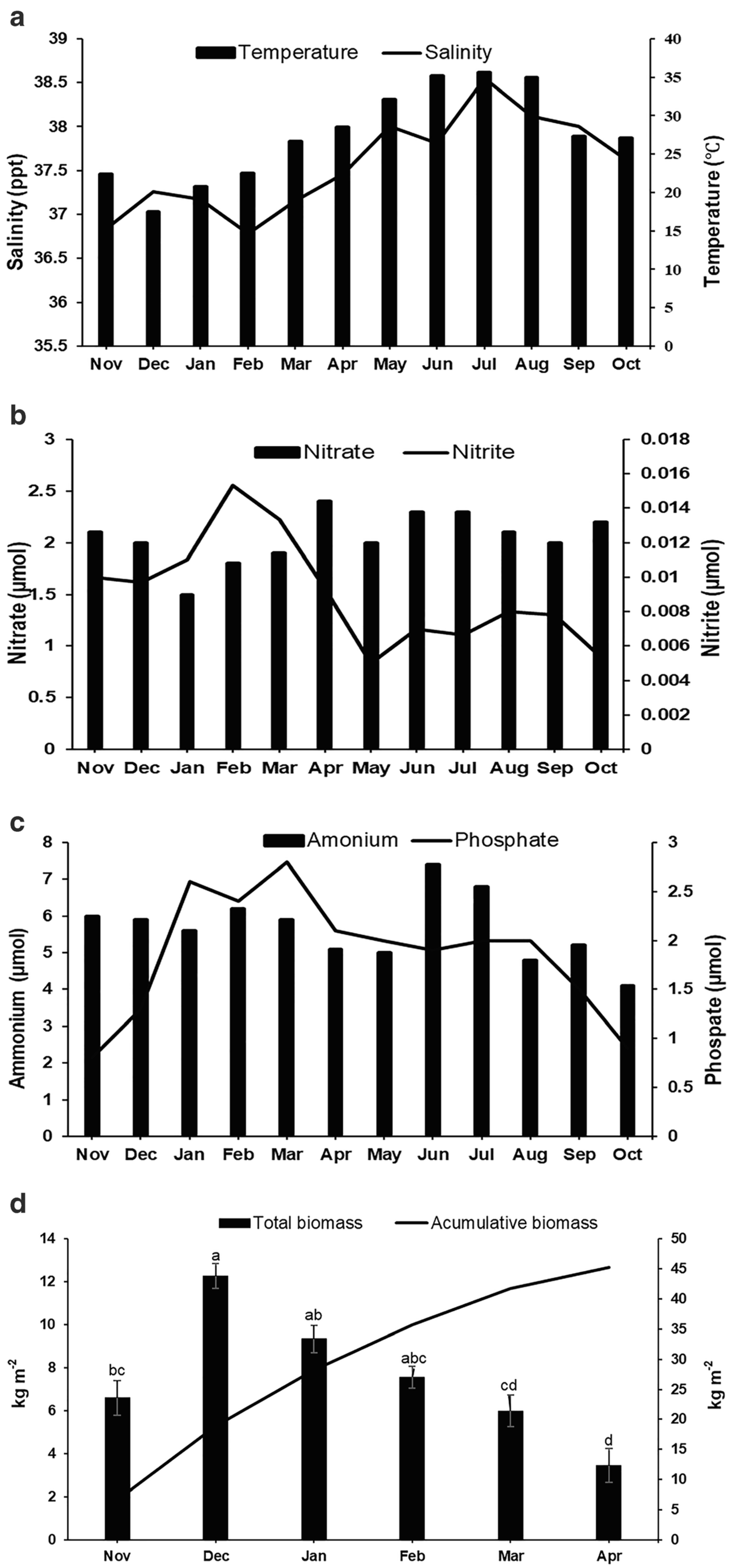



**Fig. 2** Relative growth rate (RGR% $day^{-1}$) of *Hypnea flagelliformis* from November to April. The data are expressed as mean ± SD ($n = 10$)


a
Temperature
Salinity
Salinity (ppt)
Temperature (°C)
39
38.5
38
37.5
37
36.5
36
35.5
40
35
30
25
20
15
10
5
0
Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct
b
Nitrate
Nitrite
Nitrate (µmol)
Nitrite (µmol)
3
2.5
2
1.5
1
0.5
0
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct
c
Amonium
Phosphate
Ammonium (µmol)
Phospate (µmol)
8
7
6
5
4
3
2
1
0
3
2.5
2
1.5
1
0.5
0
Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct
d
Total biomass
Acumulative biomass
kg m-2
kg m-2
14
12
10
8
6
4
2
0
50
45
40
35
30
25
20
15
10
5
0
bc
a
ab
abc
cd
d
Nov Dec Jan Feb Mar Apr

◀ **Fig. 3** Average values of **a** seawater temperature and salinity, **b** nitrate and nitrite, **c** ammonium and phosphate, and **d** annual and monthly biomass yield

content significantly varied between 39 and 30% dw during the cultivation. The maximum ash level was recorded in November and the minimum was in April. Notably, protein and lipid contents in *H. flagelliformis* were relatively low (Table 3). The maximum contents of protein were obtained in January and February (3.03 and 2.95% dw, respectively), and did not vary significantly in November, December, March, and April ($p > 0.05$). The lipid content in February was significantly higher than that of the other months. Likewise, no significant differences were observed in lipid content among December, January, and March ($p > 0.05$).

## Carrageenan yield

As shown in Fig. 4, slight significant differences were observed in hydrocolloid yield at different months of cultivation period ($p \leq 0.05$). The highest and lowest carrageenan yields (aqueous-treated and alkali-treated) were detected in January and April, respectively. Furthermore, no significant differences were observed between January and February ($p > 0.05$). The carrageenan yields varied between 30 and 37% dw in alkali-treated, and 18 and 22% dw in aqueous-treated extraction methods.

## FT-IR analysis

The FT-IR spectral profile of aqueous and alkali-treated carrageenan revealed typical absorption bands depend on sulfated polysaccharide (Fig. 5). The broad signals at 3300–3500 $cm^{-1}$ are associated with O–H stretching vibrations and signals at 2929–2989 $cm^{-1}$ are related to C–H stretching vibrations which indicates polysaccharide signals (Wu et al. 2012). The strong signal around 1645 $cm^{-1}$ can be attributed to the asymmetric stretching C=O group (Zarei Jeliani et al. 2017b). Furthermore, signals around 1230–1275 $cm^{-1}$ correspond to the asymmetric stretching vibrations of sulfate ester S=O bond (Alves et al. 2016). The occurrence of strong bands at approximately 930-940 and 1070 $cm^{-1}$ in the FT-IR spectra indicated the presence of 3,6-anhydro-D-galactose and is assigned as C–O bonds. These two peaks are typical for phycocolloids (Souza et al. 2018). As shown in the FT-IR spectra, the moderately strong bands at around 840–850 $cm^{-1}$ are attributed to the D-galactose-4-sulfate (Pereira et al. 2009).

## Discussion

As a possible candidate species for commercial cultivation along the southern coast of Iran, *H. flagelliformis* was considered in the current study. In order to evaluate the affective environmental factors on yield and growth of *H. flagelliformis*, experimental cultivation of the species was carried out in the intertidal pools of Bandar Abbas.

Our findings demonstrate that the growth of *H. flagelliformis* was related to the changes in environmental factors, especially salinity and temperature of seawater. As shown in Figs. 2 and 3, the relative growth rate (RGR) and monthly biomass of *H. flagelliformis* (kg $m^{-2}$) were maximum in December and after that decreased. Cumulative biomass data curve estimates the annual production of around 45 kg $m^{-2}$ of the cultivated species which is equivalent to 45 t $ha^{-1}$ that is a considerable crop production. These data indicate that despite the sensitivity to growth conditions, *H. flagelliformis* can be a good option for cultivation in tidal pools.

*Hypnea flagelliformis* morphological characteristics differ from *H. musciformis* with its branches having a stolon-like behavior which attach to the sea bed at some points along their length. This characteristic makes its cultivation different from *H. musciformis*. In the case of *H. flagelliformis*, the cultivation ropes must be close to the seabed, because the thallus is slightly brittle and factors such as wave and severe water current can

**Table 3** Percentage, range, and mean of chemical composition of cultivated *Hypnea flagelliformis* during the experiment

| | % Moisture | % Ash | Total protein (% dw) | Total lipid (% dw) |
|---|---|---|---|---|
| Nov (2017) | $88.42 \pm 0.85^{bc}$ | $39 \pm 0.017^{a}$ | $1.46 \pm 0.04^{b}$ | $1.08 \pm 0.56^{c}$ |
| Dec (2017) | $91.23 \pm 1.01^{a}$ | $37 \pm 0.012^{b}$ | $1.91 \pm 0.38^{b}$ | $1.64 \pm 0.95^{bc}$ |
| Jan (2018) | $91.76 \pm 0.97^{a}$ | $38 \pm 0.005^{ab}$ | $3.03 \pm 0.61^{a}$ | $1.63 \pm 0.29^{b}$ |
| Feb (2018) | $89.36 \pm 0.78^{b}$ | $37 \pm 0.004^{b}$ | $2.95 \pm 0.59^{a}$ | $3.15 \pm 0.32^{a}$ |
| Mar (2018) | $87.20 \pm 0.81^{c}$ | $31 \pm 0.004^{c}$ | $1.91 \pm 0.38^{b}$ | $1.80 \pm 0.65^{b}$ |
| Apr (2018) | $86.76 \pm 0.73^{c}$ | $30 \pm 0.001^{c}$ | $1.40 \pm 0.30^{b}$ | $1.08 \pm 0.38^{c}$ |
| Range | 86.76-91.76 | 30-39 | 1.40-3.03 | 1.08-3.15 |
| Mean | $89.12 \pm 2.1$ | $35.3 \pm 3.8$ | $2.11 \pm 0.7$ | $1.8 \pm 0.7$ |

All the values are mean ± SD ($n = 4$)

[a,b] Values with different letters are significantly different in the same row ($p \leq 0.05$)

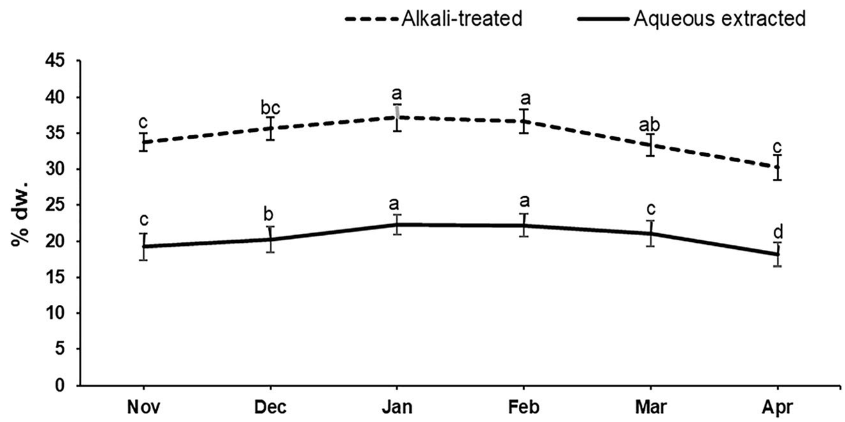



**Fig. 4** Carrageenan yield (%) of *Hypnea flagelliformis* using different methods (aqueous-treated and alkali-treated) during the cultivation experiment. The data are expressed as mean ± SD ($n = 4$). Different letter superscripts indicate significant difference ($p \leq 0.05$)

detach the thallus from the ropes. The decreasing trend of RGR may partly be attributed to this particular characteristic of *H. flagelliformis*, because the tips of thallus attached to the seabed and after around 1 month, a considerable part of branches is attached to the sea bed and will detach from the ropes whenever they were harvested. Another reason for decreasing trend of RGR and measured biomass is related to the increasing water temperature. Biomass yield of the cultivated seaweeds also follows the same rhythm as the wild specimens. The issue reveals the marked effect of temperature on the physiology of *H. flagelliformis*. With respect to environmental factors, seawater temperature increase leads to increase of evaporation and this leads to increased salinity and osmotic stress on the algae. Accordingly, this tends to lead to water and moisture loss in the alga which affects the cell physiology and disturbs the cell walls and finally leading to the detachment of the alga thallus from the ropes. In a study on *H. musciformis* which investigated the combined effects of seawater from three urbanized areas (natural runoff, runoff and sewage with treatment, and untreated sewage) and three different temperatures (15, 25, and 30 °C) on growth and photosynthetic characteristics (De Faveri et al. 2015), they found that after 4 days of cultivation, the biomass of *H. musciformis* differed due to the area of water source and temperature treatments. This study showed the specific effect of temperature (specifically, in 35 °C) in reducing the photosynthetic parameters $ETR_{max}$, $\alpha$(ETR), $\beta$(ETR), and $F_v/F_m$ and also changed cell morphology, with reduction in photosynthetic pigments and drastic reduction in growth rates.

When *H. flagelliformis* was abundant in the natural habitat at the selected site, the cultivated specimens also showed high biomass yield. Similar observations were reported for *H. musciformis* (Ganesan et al. 2006). The present study illustrated that environmental factors such as temperature, salinity, and nitrite had significant ($p < 0.01$) impacts on the growth of *H. flagelliformis* (Table 2). Ganesan et al. (2006) also found that water temperature and salinity had negative correlation with the biomass of *H. musciformis*. In the current study, biomass of the harvested sets was increased more than 100% in a period of 20-25 days. The maximum relative growth rate

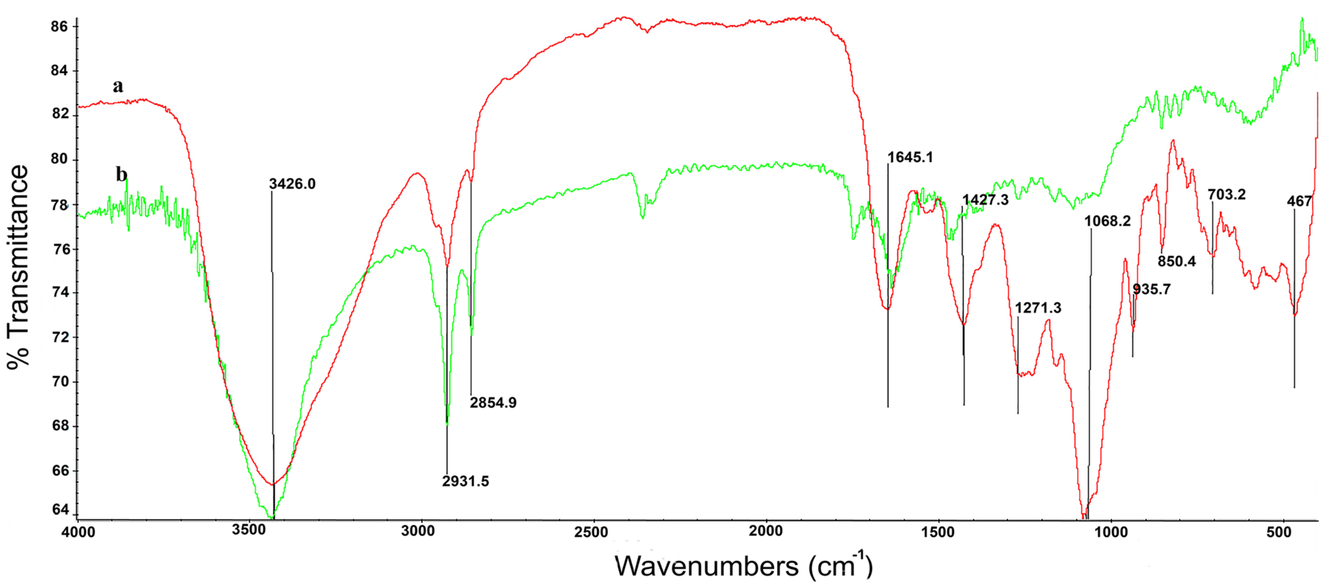



**Fig. 5** FT-IR spectra of carrageenan isolated from *Hypnea flagelliformis* using different methods. **a** Aqueous-treated. **b** Alkali-treated

of *H. flagelliformis* (9% day$^{-1}$ in Dec) in the present study was higher than other published data for the same genus (Faccini and Berchez 2000; Ganesan et al. 2006). Whitehouse and Lapointe (2015) concluded that the fastest growth rate of *H. musciformis* (0.35 doublings day$^{-1}$) was in November.

Due to the biogeographical location of the Persian Gulf in subtropical regions, the temperature of water in surface layers almost is not less than 17 °C and this work showed that the favorable temperature for the maximum growth of *H. flagelliformis* ranged between 17.45 and 22.5 °C in December, January, and February. Similar results have been reported by Guist Jr et al. (1982) who stated that the highest growth rate of *H. musciformis* was obtained between 18 and 24 °C water temperature. Thus, according to the results of Pearson correlation (Table 2), seawater temperature and salinity are the most influencing factors that control and limit the growth of *H. flagelliformis*.

Increase of temperature leads to elevation of salinity and both led to lower growth, of *H. flagelliformis*. The reduction in biomass and growth rate under increasing salinity can be due to an excess energy utilization for osmoregulation rather than cell growth and photosynthesis (Lawton et al. 2015). Changes in salinity have a serious effect on the cell membrane (Ding et al. 2013) and could change water balance and ion balance causing oxidative stress which leads to various signaling which affects cell membrane lipid composition and protein activity and all together can change membrane permeability and fluidity to induce ion homeostasis, water potential regulation, and detoxification (Guo et al. 2019). Durako and Dawes (1980) investigated the diminishing impact of high salinity on biomass of *H. musciformis* in the summer in Florida.

The findings from the physicochemical parameters illustrated that there is no noticeable annual variation in the pH, $NH_4^+$, and $PO_4^{3-}$ (Table 1). Further, Pearson correlation showed that there is a positive correlation between *H. flagelliformis* growth and $NO_2^-$, and a negative correlation with $NO_3^-$. These findings of the current study are not consistent with those of Zarei Jeliani et al. (2017b), who found ammonium nitrogen had a significantly positive correlation with biomass production of *Gracilariopsis persica*. Some researchers have argued that high nutrient concentrations stimulate macroalgal growth rates (Whitehouse and Lapointe 2015; Zarei Jeliani et al. 2017b).

Silkin et al. (2012) modeled nitrate and nitrite absorption by *Gelidium latifolium* and revealed the dependence of maximum content of nitrites in biomass on the initial nitrate concentration in the medium and depicted the positive correlation with the initial medium concentration of nitrite that reach to maximum (7 μmol g$^{-1}$) in 80 μmol of nitrite concentration, while about nitrate, the maximum content of nitrate in biomass reaches to maximum amount (15 μmol g$^{-1}$) in 20 μmol of nitrate concentration and then goes to steady state and shows no increase at higher concentration of nitrate. They stated if algae are transported from depleted nitrogen medium to sea water with high nitrate concentration, gene expression takes place, which are responsible for nitrate reductase synthesis, and 2 h later, the content of this enzyme increases. Decrease in time of nitrate concentration in the medium results in lowering of nitrate reductase synthesis. Thus, nitrate concentration is a factor, which controls the nitrate reductase content in the cell. Martin et al. (2011) found the brown color strain of *H. musciformis* absorbed more nitrate than the light green strain of the species. They also found that light green strains store the nitrate mainly in protein form while the brown strain storage is pigment and protein. Compared with the current study results with the results of the Martin et al. (2011), we concluded that during the winter when the nitrite level is higher, the color of *H. flagelliformis* is almost brown and when the nitrate concentration in the water depleted, thallus color changed to light green and gradually bleached and died in high water temperature.

As seen in Table 3, the ash content of *H. flagelliformis* ranged between 30 and 39%, with mean 35.3 ± 3.8% dw, that was higher than that for *Hypnea pannosa* (18.65%) and *H. musciformis* (21.57%) (Siddique et al. 2013). The higher levels of ash content are correlated with higher values of mineral elements (Rodrigues et al. 2015). The ash contents of seaweeds are generally much higher than some terrestrial vegetables (Rupérez 2002), and can be varied based on species type, environmental factors, and seasons (Yoshie et al. 1994; Kaehler and Kennish 1996). Furthermore, the protein content in *H. flagelliformis* varied between 1.36 and 3.03%, with mean 2.11 ± 0.7% dw, which was higher than the protein content of *H. musciformis* (2.3% dw) reported by Arman and Qader (2012), and (1.21% dw) reported by Balamurugan et al. (2013). However, the findings of the current study support the previous research about protein content of *H. flagelliformis* (1.92% dw) by Moein et al. (2015). The lipid and protein contents of seaweeds can vary according to the species, seasonal changes, and geographic zone (Haroon et al. 2000; Ratana-arporn and Chirapart 2006). The highest and lowest lipid contents for *H. flagelliformis* were recorded 3.15 and 1.08%, respectively, with mean 1.8 ± 0.7% dw. This lipid content was higher than the values reported for *Hypnea japonica* (1.42% dw), *Hypnea charoides* (1.48% dw) (Wong and Cheung 2000), *H. pannosa* (1.56% dw), and *H. musciformis* (1.27% dw) (Siddique et al. 2013).

The current study showed that the moisture content varied between 86.76 and 91.76 with mean of 89.12 ± 2.1% fresh weight. In addition, Pearson correlation showed that there is a negative correlation between the moisture content and seawater temperature ($r$ = −0.879, $p$ = 0.01). With respect to the results, increasing sea water temperature leads to a decrease in the moisture content. As mentioned above, the interpretation for this may be due to the phenomenon that increasing sea water temperature leads to increased salinity and consequently increased osmotic stress on the algae. Accordingly, the algae tend to lose water and moisture. In the study of Ding et al. (2013), the SGR of *Hypnea cervicornis* decreased as salinity and temperature increased. When the salinity level is higher than the biological range of the alga tolerance, the cell wall will change dramatically and gradually led to the decomposition of the alga thallus.

In contrast, based on our findings, the salinity and temperature showed no significant correlation with ash, lipid, and protein contents ($p > 0.05$). Further researches are needed to investigate the effectiveness of the physicochemical factors (e.g., salinity and temperature) on the biochemical composition (e.g., lipid and protein) of *H. flagelliformis*, under controlled conditions in the laboratory.

Our findings showed that carrageenan yield was increased in lower temperature and salinity while carrageenan yield reduced in higher temperature and salinity. Probably winter conditions provided fairly ideal conditions for *H. flagelliformis* to synthesize carrageenan. It has been reported that when under stress conditions, photosynthetic activity may increase the synthesis of other macromolecules and generation of ATP in the plants instead of storage carbohydrates producing (Lobban et al. 1994).

In this work, carrageenans were extracted from *H. flagelliformis* by either the alkaline-treated with 6% w/v NaOH solution or aqueous extraction. The profound difference was observed on the extraction yields with the two different extraction techniques. Extraction yields were highest for the alkaline-treated method 36-37% by weight of dry material while aqueous extraction yielded to 19-20% (Fig. 4), and are in harmony with earlier studies with similar extraction parameters for *H. musciformis* (Aziza et al. 2008; Arman and Qader 2012). Castelar et al. (2016) demonstrated that in controlled conditions, *H. musciformis* had a high carrageenan yield and grew better than *K. alvarezii*. Although there are many reports on carrageenan yields, comparisons are difficult, since yields vary depending on the extraction methodology and alga species. According to the FT-IR spectra of carrageenan hydrocolloids extracted from *H. flagelliformis*, the alkali-treatment carrageenan showed the lower sulfate content (Fig. 5). The spectra of carrageenan from *H. flagelliformis* showed sharp peaks at 850 $cm^{-1}$ for 1,3 linked galactose 4-sulfate and at 935 $cm^{-1}$ for 3,6-anhydrogalactose which was confirmed as kappa carrageenan (Rafiquzzaman et al. 2015). The findings in this work suggest that applying alkali-treated method in hydrocolloid extraction of *H. flagelliformis* can be more beneficial than the aqueous extraction method. As shown in Figs. 2 and 4, carrageenan yield and growth rate had a maximum value in the same time in the winter, which is consistent with those of Qari et al. (2018). In contrast, Rodrigueza and Montaño (2007) found the inverse impact of biomass production on carrageenan yield of *Kappaphycus striatum*.

## Conclusions

This study showed in contrary to the sensitive behavior in the artificial cultivation, *H. flagelliformis*, as a native carrageenophyte species, can be a good candidate for cultivation as carrageenan source. With its relatively high growth rate (4-9% $day^{-1}$) and around annual biomass production of 45 kg $m^{-2}$, the alga can play an important role in aquaculture activities in local communities to increase their socio-economic conditions. Using the native species that are common algal vegetation in the area can create a sustainable seaweed industry and prevent the invasive effect of alien species on the natural fauna and flora of local areas. Because of the commercial value of this species as carrageenan and biochemical composition resource, it needs more study to scale up the cultivation in commercial scales. Finding the optimum culture conditions and the best method for cultivation needs more indoor and outdoor investigation. In conclusion, based on this primary study, the best period to cultivation of *H. flagelliformis* in intertidal areas on southern Iranian coastlines of the Persian Gulf is during the winter to mid spring seasons.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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