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# شناسایی و معرفی دو هورمون تنظیم‌کننده گیاهی استخراج شده از جلبک‌های سارگاسوم موتیکوم و گراسیلاریا کورتیکاتا در جنوب ایران

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**چکیده.** در مطالعه حاضر، دو هورمون گیاهی تنظیم کننده، آبسزیک اسید و اکسین، از دو جلبک ماکروسکوپی، یعنی سارگاسوم موتیکوم و گراسیلاریا کورتیکاتا، برای اولین بار استخراج شدند. نمونه برداری هر ماه با سه تکرار از سواحل استان بوشهر واقع در ناحیه شمالی خلیج فارس برای شش ماه متوالی انجام شد. تغییر بیوماس جلبکها و تأثیرات احتمالی تعدادی عوامل محیطی اندازه گیری شدند. فیتوهورمون های استخراج شده با استفاده از روش HPLC جداسازی شدند و از طریق تزریق استانداردها شناسایی شدند. بیشترین مقدار فیتوهورمون ABA در هر دو گونه در آبان رخ داد. همچنین، بیشترین مقدار اکسین در سارگاسوم موتیکوم در اردیبهشت گزارش شد، در حالی که بیشترین مقدار این هورمون در گراسیلاریا کورتیکاتا در دی ماه مشاهده شد. در ماه دی بیشترین میانگین بیوماس در سارگاسوم موتیکوم ۶۷۹ گرم بر مترمربع بود، در حالیکه کمترین میانگین ۲۰٫۶۶ گرم بر مترمربع در ماه شهریور مشاهده شد. علاوه بر این، بیشترین بیوماس متوسط در گراسیلاریا کورتیکاتا ۴۲۳٫۳۳ گرم بر مترمربع در اسفند و کمترین میانگین ۱۵۸ گرم بر متر مربع در آبان گزارش شد. نتایج آزمون آنوا و چای اسکوِر تفاوت قابل ملاحظه ای را در همه نمونه ها نشان داد. این فیتوهورمونها می توانند در تهیهٔ کود مایع جلبکی در مطالعات آتی به کار برده شوند.

**واژه‌های کلیدی**. بیوماس جلبکی، آبسزیک اسید، اکسین، استخراج هورمون، جلبکهای ماکروسکوپی

# Determination and quantification of two regulatory phytohormones extracted from *Sargassum muticum* and *Gracilaria corticata* seaweeds in the south of Iran

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**Abstract.** In the present study, two regulatory phytohormones, abscisic acid and auxin, were extracted from two different macroalgae, *Sargassum muticum* and *Gracilaria corticata*, for the first time. Sampling was performed each month with three replicates, from Bushehr Province shores located in the north region of Persian Gulf for six alternative months. The alteration of seaweeds biomass and the probable impacts of some environmental factors were measured. The extracted phytohormones were isolated employing HPLC method and identified by injection of standards. The highest amount of ABA phytohormone in the two species, occurred in November. Also, the highest amount of Auxin in *Sargassum muticum* was reported in May, while the highest amount of this hormone in *Gracilaria corticata* was observed in January. In January, the maximum mean biomass in *Sargassum muticum* was 679gr/m$^2$, while the minimum mean of 20.66gr/m$^2$ was observed in September. Moreover, the highest average biomass in *Gracilaria corticata* was 423/33gr/m$^2$ in March and the lowest mean of 158g/m$^2$ was reported in November.

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ANOVA and Chi-squared test results showed a significant difference in all samples. These phytohormones could be applied in preparing algae liquid fertilizer in future studies.

**Key words.** algal biomass, abscisic acid, auxin, hormone extraction, macroalgae

## Introduction

Seaweed extracts are organic and natural plant growth stimulants that could be considerably applied in constant and organic crop production (du Jardin, 2015; Mahmoud et al., 2019). They are widely recognised as compounds that alleviate abiotic stress and increase plant productivity (Prieto et al., 2011; Stirk et al., 2013, 2014). Therefore, phytohormones are regarded as signaling molecules for regulating the physiological functions of plants. The small number of hormones control developmental processes and stress responses (Dobrev et al., 2005; Mori et al., 2017). The seaweed extracts are now available in liquid and powder forms, mainly brown algae as herbal stimulants, and are used as a mixture with soil or spray. These growth regulators can produce physiological responses in small amounts (Stirk et al., 2003). Regarding the existence of higher natural phytohormones and nutrient contents in Phaeophyta extracts in contrast with other types of marine algae, they are considered natural plant growth stimulants or bio-fertilizers that could play an important role in organic and modern agriculture (Hong et al., 2007; Craigie, 2011; Hernández-Herrera et al., 2014; Mahmoud et al., 2019). The genus *Sargassum* C. Agardh (Sargassaceae) represents the most complex and species-rich brown marine macroalgae morphologically that is distributed in tropical and inter-tropical regions worldwide (Mattio & Payri, 2011). Some species of *Sargassum* are regarded as the most economically significant algae, especially in Asia that could be used for various purposes including nutritional, cosmetic, liquid fertilizer preparation and medicine (Hong et al., 2007). *Gracilaria* Grev. (Gracilariaceae), belonging to Rhodophyta, could be found in tropical to cool temperate coasts. In addition to its undeniable importance for nutritional and pharmaceutical purposes, it is considered a great source of agar (Winchester, 1969; Barsanti & Gualtieri, 2006; Iyer et al., 2004). These two mentioned above genera are among the most abundant seaweeds distributed in the northern shores of the Persian Gulf (S Iran).

Due to the high density of the brown and red seaweeds in the coastline of the Persian Gulf, there is a potential to extract plant growth regulators to increase the growth of manufacturing products from macroalgae. Therefore, the main goal of the present study was to compare the amounts of extracted auxin and abscisic acid as two opposite regulatory phytohormones from two different macroalgae, *Gracilaria cordicata* and *Sargassum muticum*, in six alternative months of the year in order to achieve the highest level of hormones.

## Materials and Methods

### Seaweeds collection and preparation

*Sargassum muticum* and *Gracilaria corticata* were collected from the northern shores of Persian Gulf (zone 39 of UTM in Bushehr coastal area) (a longitude of 50° 48′ 53″ and latitude of 28° 54 ′ 41″ ). Samples were collected from January 2015 to November 2016 between the full tide of water in the area and the intertidal zone of Bushehr seashore. A hypothetical line perpendicular to the shore was considered for sampling. The seaweeds were recognized by their morphological properties applying botanical and the online algal electronic database (https://algaebase.org) (Sohrabipour & Rabii, 1999; Jha et al., 2009). The biomass of macroalgae was evaluated using 50 × 50 $cm^2$ random quadrats. Three replications were prepared for each sample. Some environmental factors including water temperature, salinity, oxygen levels, and water pH were measured and recorded at the sampling site employing thermometers, portable salinity gauges, oximeters, and pH determinants (APHA, 2005). After washing the samples with distilled water and separating epiphones and epiphytes in sterile and acid-washed containers, the two macroalgae were transferred to Razi Laboratory Complex (Tehran) for extraction of phytohormones. The weight of the macroalgae at the sampling site was measured and recorded using a digital scale with an accuracy of three decimal digits.

### Extraction protocol

For extraction, 10 gr of lyophilized seaweed samples were mixed with 60 mL of the solution including Methanol-Chloroform-Ammonium hydroxide (2N). The homogenization was performed to dissolve the phytohormones well in zero centigrade temperature, overnight. The homogenized mixture was then filtered using Whatman No.1 mesh, and the resulting extract was transferred into a decanter. Water was added twice and then, distilled and stirred vigorously. The chloroform as a supernatant was discarded, and the Water-Methanol phase was kept. The lower phase was collected and evaporated using a rotary until the evaporation of the remaining chloroform and methanol, and their volume reached to 35 mL. The

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pH of the aqueous phase was increased to 2.5 employing HCl (1N) and then transferred into a decanter with appropriate volume. 15 mL of ethyl acetate was added to the decanter. The supernatant was separated and collected in another container. The stage of adding ethyl acetate was repeated twice more, and all three supernatant phases were collected in one container. This solution was including indole-3-acetic acid (IAA) and free Abscisic Acid (ABA).

**Phytohormone analysis**

The samples passed through a 45% polytetrafluoroethylene (PTFE) filter, and then injected into the HPLC column. The solution compounds obtained by HPLC were separated using a $C_{18}$ column, UV detector with 0.7 mL/min flow rate, 0.2% Acetic acid solvent, and 95% of Methanol at 40˚ C.

The statistical analysis of variance (one-way ANOVA) and Chi-square test were used as a first-order autoregression. The correlation structure of different months (six months) in the mentioned model and the correlation of the desired variables with the response variable were evaluated and reported. The SPSS (ver. 21.0) software was used for the statistical analyses.

## Results

**Environmental factors**

The mean results of various environmental factors including temperature, salinity, dissolved oxygen and pH levels are represented in Table 1, Fig. 1. The results demonstrated that, there is a direct correlation between the mean water temperature and salinity amount as well as salinity quantity and pH level from January 2015 to November 2016 (Fig. 1). However, this proportion was not significant between dissolved oxygen and pH levels.

The results of the mean temperature in coastal regions of Bushehr province are shown in Fig. 1. In July 2016, the mean temperature was at its highest level (38.0 ± 2.11). Meanwhile, in two months i.e., January and March, it experienced its lowest average. However, the temperature was remained almost steady during the spring and fall. Chi-square test and ANOVA results showed a significant difference between the studied months ($P < 0.05$; n = 6). The mean salinity quantity reached a peak of 48.33 ppt in July, while in September hit its lowest point (40.33 ppt) (Fig. 1, Table 1). The average of six months salinity level was 44.44 ± 0/77 ppt. One-way ANOVA, and Chi-square test showed significant differences within months ($P < 0.05$; n = 6). Also, the pH average reached a maximum level of 7.91 ± 1.43 in July and bottom out to 7.12 ± 0.24 in January. The pH level saw a minimal fluctuation (Fig. 1, Table 1).

The highest amount of water-soluble oxygen was observed in September (2.80 ± 0.35). Meanwhile, the lowest level occurred in January (2.16 ± 0.09). There was a minimal fluctuation in dissolved oxygen amounts during the six alternative months.

**Biomass amount**

The biomass amount for two different macroalgae i.e., *Sargassum muticum* (brown algae) and *Gracilaria corticata* (red algae) at the Bushehr Province station is shown in Fig. 2. The highest mean biomass amount for *Sargassum muticum* (679 $gr/m^2$) was observed in January 2015. However, the lowest mean (20.66 $gr/m^2$) was obtained in September 2016 during one year of sampling. The biomass level was dramatically decreased between January and September. However, in November, it experienced a considerable rise. There was a significant difference between *Sargassum muticum* biomass amounts during the six alternative months ($P < 0.05$). Also, the biomass's highest and lowest mean levels in *Gracilaria corticata* occurred in March (423.33 $g/m^2$) and November (158 $g/m^2$) respectively. The difference was also significant ($P < 0.05$). The minimum and maximum biomass levels during six alternative months were allocated to *Sargassum muticum* (Fig. 2). In general, during winter, the amount of algal biomass in two species was increased significantly.

**Auxin and Abscisic acid phytohormones in *Sargassum muticum***

The highest rate of abscisic acid (ABA) hormone in *Sargassum muticum* species was recorded in November (20.667%) (equivalent to 2.667% of 1gr of *Sargassum*). However, there was no trace of this hormone in May and July (Fig. 3). On the other hand, the auxin (IAA) amount extracted from *Sargassum muticum* in May was at its highest level (14.113%). Though, in January and March there was no trace of this hormone. Overall, the maximum level of extracted abscisic acid was much higher than that recorded for auxin during six months of sampling. Chi-squared parent test and one-way ANOVA results showed a significant difference ($P < 0.05$; n = 6).

**Auxin and abscisic acid phytohormones in *Gracilaria corticata***

The highest percentage of abscisic acid extracted from 10 grams of *Gracilaria corticata* was observed in November 2016 (60.667%). Its amount was gradually increased from July to November. Meanwhile, there was no trace of this hormone in the other three months (Fig. 4). The highest level of auxin was reported in January 2015 (18.513%). Nevertheless, the presense of this hormone was only confirmed in two months of sampling. Chi-squared parent test and one-way ANOVA showed a significant difference between the six- month sampling period over two years ($P < 0.05$, n = 6).

## Discussion

In the present study, the main objective was to isolate two regulatory phytohormones as organic compounds from two different macroalgae (*Gracilaria corticata* and *Sargassum muticum*) for the first time, to determine the phytohormones quantity in the two mentioned above macroalgae during six alternative months. The growth of macroalgae is completely reliant on some environmental factors including nutrients bioavailability, photosynthetic quantum yield, and irradiance (Hwang et al., 2007), temperature, salinity (Hanisak & Samuel, 1987), and wave exposures (Andrew & Viejo, 1998; Engelen et al., 2005). Since, the macroalgal biomass could be applied as a raw material for the production of value-added products, pharmaceutical and cosmetic purposes, fertilizers, plant growth biostimulants, and toxic metal ions sorbents from wastewater (Michalak & Messyasz, 2021), its alteration was measured during six months of sampling in both mentioned red and brown algae. The results demonstrated that, the amount of biomass products is closely related to the environmental conditions and seasonal changes (Van den Hoek et al., 1995; Higgins et al., 2005; Pikosz et al., 2017; Michalak & Messyasz, 2021). Some other factors, such as the type of macroalgae species, type of cultivation, and time of the year could affect biomass amounts. Pedersen et al. (2005) was previously reported that, *Sargassum muticum* has highly variable biomass amounts among seasons in contrast with its taxonomically close species, i.e., *Halidrys siliquosa.* This biomass variability is related to its more 'ephemeral' characteristics. According to Nejatkhah Manavi et al. (2010) studies, the maximum growth level of *sargassum* occurs in cold temperature. Due to the brown algal resistance to undesirable conditions, their biomass amounts are more than other types of algae. In the present study, the biomass amount was at its highest level during (January and March, 28 C°) for both mentioned algae whereas, there was a significant decline in biomass level during summer (July and September), that could be related to the temperature as well as low level of nutrients (Orduna-Rojas et al., 2002; Michalak & Messyasz, 2021). Also, the biomass level in *Sargassum muticum* was higher than *Gracillaria corticata* from November to March, which is conform to the previous study (Nejatkhah Manavi et al., 2010). The results of the recent investigations in Australia demonstrated that, *Sargassum* biomass increases during winter and early spring, and stabilizes during late spring and early summer, before decreasing during late summer and early autumn, which is completely in line with the results of the present study (Hoang et al., 2016). Moreover, seasonal changes in the physicochemical parameters of seawater, undoubtedly effects on alterations in this algal canopy structure (Ang & De Wreede, 1992; Arenas & Fernández, 2000; Rivera & Scrosati, 2006; Ateweberhan et al., 2009). Although minimal fluctuation was determined for dissolved oxygen amounts, the subsequent rise and occurance of the peak in September were unclear. The results also showed a significant correlation between some environmental factors such as pH and water temperature, as well as pH and salinity. There was a considerable alteration in pH amounts during six months of the study. The pH was in the range from 7.12 ± 0.24 in January to 7.91± 1.43 in July, completely conforming to the temperature. Therefore, the temperature is considered the major driver of seasonality in pH (Takahashi et al., 2014; Hagens & Middelburg, 2016).

**Table 1.** The mean results of the environmental factors during six alternative months from January 2015 to November 2016 (± SE; n=6).

| Month | Temperature (C°) | Salinity (ppt) | Dissolved Oxygen (DO) | pH |
|---|---|---|---|---|
| January 2015 | 28.0±0.52 | 44.33±1.01 | 2.16±0.09 | 7.12±0.24 |
| March 2015 | 28.0±1.11 | 47.00±1.22 | 2.36±0.17 | 7.50±1.31 |
| May 2016 | 32.0±1.16 | 44.66±1.35 | 2.22±0.12 | 7.51±0.10 |
| July 2016 | 38.0±2.11 | 48.33±1.22 | 2.27±0.09 | 7.91±1.43 |
| September 2016 | 35.0±1.84 | 40.33±0.89 | 2.80±0.35 | 7.16±0.36 |
| November 2016 | 33.0±1.77 | 42.00±0.97 | 2.42±0.22 | 7.48±0.73 |
| Average in 6 months | 32.3±1.21 | 44.44±0/77 | 2.37±0.19 | 7.45±0.29 |
| Max | 38.0±2.11 | 48.33±1.22 | 2.80±0.35 | 7.91±1.43 |
| Min | 28.0±0.52 | 40.33±0.89 | 2.16±0.09 | 7.12±0.24 |

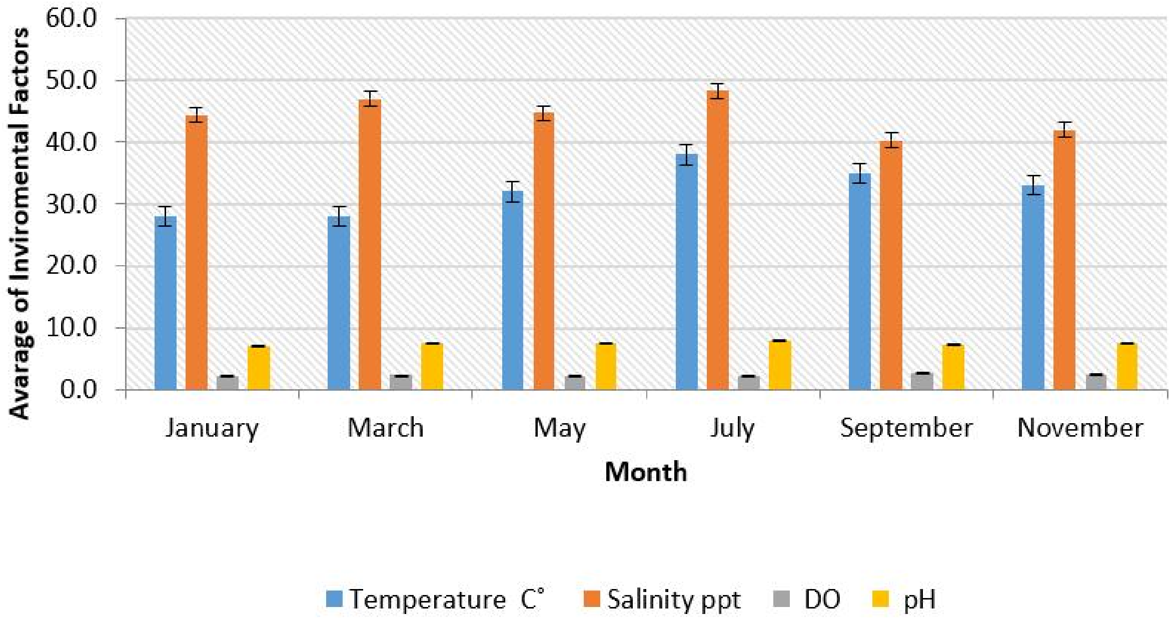


**Figure 1.** Comparison of some environmental factors during the six alternative months from January 2015 to November 2016.

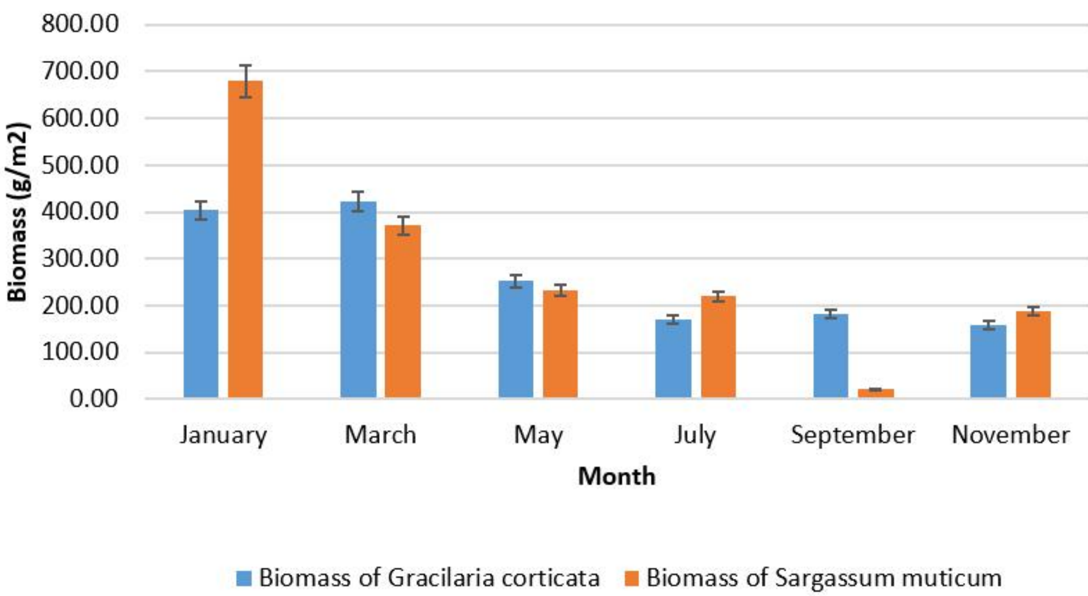


**Figure 2.** The comparison between the biomass average in *Gracilaria corticata* and *Sargassum muticum* (gr/$m^2$) during six alternative months from January 2015 to November 2016.

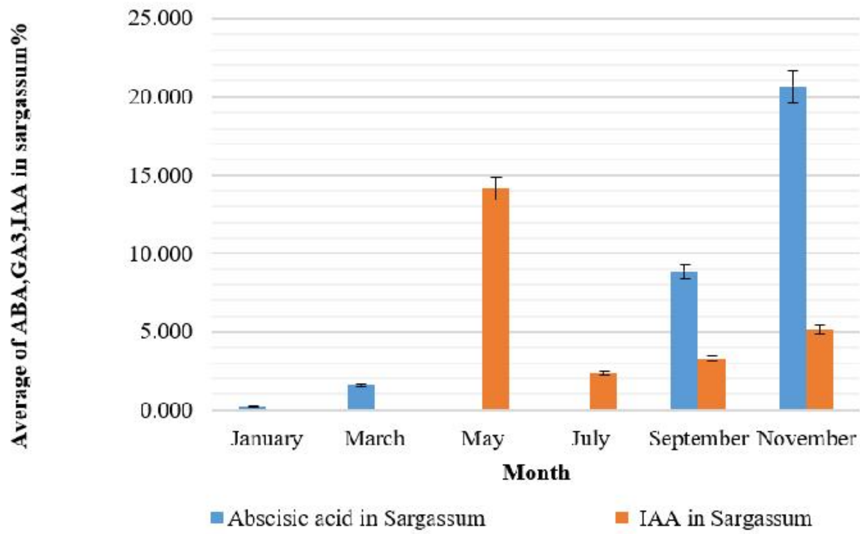


**Figure 3.** The average percentage of auxin (IAA) and abscisic acid (ABA) extracted from *Sargassum muticum* between January 2015 and November 2016.

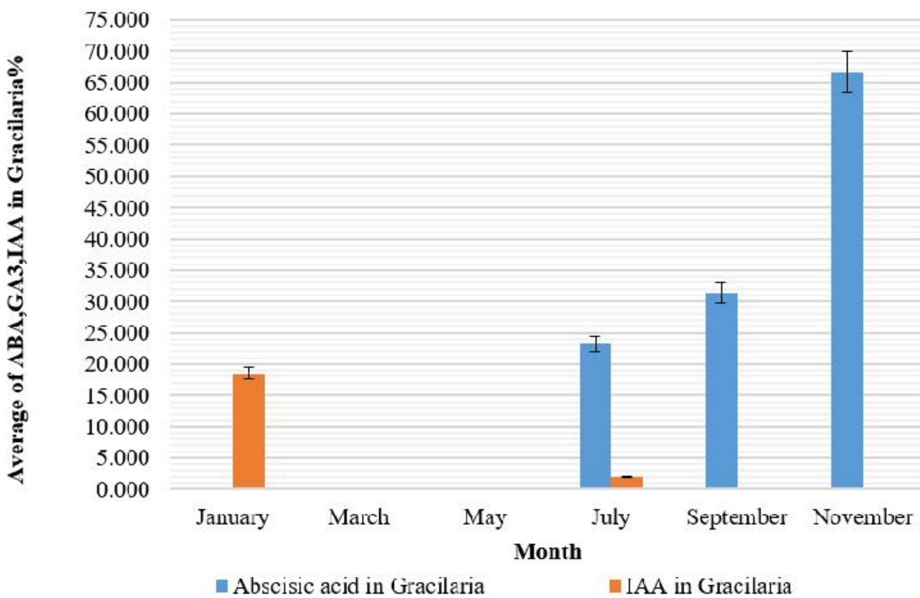


**Figure 4.** The average percentage of auxin (IAA) and abscisic acid (ABA) extracted from *Gracilaria corticata* between January 2015 and November 2016.

The current study was also designed to compare two phytohormones in two different algae during six alternative months. Indole-3-acetic acid (IAA) is commonly regarded as the most active indole component biologically (Woodward & Bartel, 2005). It has been determined in prolific green macroalgae, namely *Caulerpa paspaloides* (Jacobs et al., 1985), and in the extracts from brown algae such as *Ascophyllum, Fucus distichus, F. vesiculosus* and *Dictyota dichotoma* (Stirk & Staden, 1997; Cooke et al., 2002; Sitnik et al., 2003; Bogaert et al., 2019). Also, the recent study on phytohormone concents in liquid extracts of three different *Sargassum* species showed the presence of IAA as a common growth hormone in all mentioned species (Sunarpi et al., 2021). In this study, the highest level of IAA in *Gracilaria corticata* (red algae) was higher than that obtained for *Sargassum muticum* (brown algae). However, the highest level of IAA in *Sargassum muticum* occurred in May, and for *Gracilaria corticata* was observed in January.

Abscisic acid (ABA) was also previously identified in *Ulva lactuca* (Tietz et al., 1989), *Ascophyllum nodosum* (Boyer & Dougherty, 1988) and *Laminaria* species (Schaffelke, 1995). According to Hirsch et al. (1989) and Tietz et al. (1989) two macroalgae i.e., *Dunaliella parva* and *Draparnaldia mutabilis,* grew in the environment with salt stress and increased pH, shows the level of increasing endogenous ABA. The recent studies on red algae have confirmed the presence of IAA, and ABA as two opposite regulatory hormones in *Pyropia yezoensis, Pyropia haitanensis,* and *Bangia fuscopurpurea* thalli (Wang et al., 2014; Mikami et al., 2016; Mori et al., 2017). In the present study, the highest level of abscisic acid in *Gracilaria corticata* was higher than that obtained for *Sargassum muticum* in the same month (November). These differences might be due to the exposure rate of *Gracilaria corticata* to more environmental changes. Since ABA is a growth inhibitor and induces dormancy, IAA acts as a growth stimulant and increases the length of the yarn (Benková 2016). Therefore, it was expected that changing the season from cold to the warm, causes a considerable alteration in the IAA level in contrast with ABA amount. According to Stirk et al. (2009) the cytokinin, auxin, and abscisic acid amounts in *Ulva* and *Dictyota* algae have changed during a year. In March and May, auxin was at the highest level in *Ulva* and *Dictyota,* respectively. Also, the ABA amount was at the highest level in September in both mentioned algae. The findings also showed that, environmental factors such as temperature and salinity have a limiting effect on algal biomass in the presence of growth inhibitors (ABA) and growth stimulants (IAA). Zhu et al. (2022) found that, the IAA level accompanied by some other growth regulatory phytohormones in red algae, *Neoporphyra haitanensis,* decrease significantly upon exposing to cold temperature, representing that algae could adjust to the cold stress by their regulating phytohormones and slowing down growth subsequently. Though, some other phytohormones related to stress resistance, such as ABA, were expressively increase at low temperatures. They investigated that, ABA overexpression can promote cold tolerance. Due to their opposite functions, increasing one of the mentioned hormones give rises to decreasing another one.

Therefore, due to the exorbitant price of phytohormones in Iran and regarding the algal importance as a liquid fertilizer, agriculture and fisheries, and also, the presence of many macroalgae on the shores of the Persian Gulf, this study could be considered a valuable source for isolating these precious hormones from accessible macroalgae in Iran.

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## REFERENCES

**Abo-State, M.A.M., Shanab, S.M.M., Ali, H.E.A. & Abdullah, M.A.** 2015. Screening of antimicrobial activity of selected Egyptian cyanobacterial species. Journal of Ecology of Health & Environment 3: 7-13.

**Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F. & Azwai, S.** 2013. Screening of antibacterial activity in marine green, red and brown macroalgae from the Western Coast of Libya. Natural Science 5: 7-14.

**Andrew, N. & Viejo, R.** 1998. Effects of wave exposure and intraspecific density on the growth and survivorship of *Sargassum muticum* (Sargassaceae: Phaeophyta). European Journal of Phycology 33: 251-258.

**Ang, P.O. & De Wreede, R.E.** 1992. Density-dependence in a population of *Fucus distichus.* Marine Ecology Progress Series 90: 169-181.

**APHA** 2005. Standard Methods for the examination of water and wastewater. 21st edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

**Arenas, F. & Fernández, C.** 2000. Size structure and dynamics in a population of *Sargassum muticum* (Phaeophyceae). Journal of Phycology 36: 1012-1020.

**Ateweberhan, M., Bruggemann, J.H. & Breeman, A.M.** 2009. Seasonal changes in size structure of *Sargassum* and *Turbinaria* populations (Phaeophyceae) on tropical reef flats in the southern Red Sea. Journal of Phycology 45: 69-80.

**Barsanti, L. & Gualtieri, P.** 2006. Algae: anatomy, biochemistry and biotechnology. CRC Press, NewYork, 344 pp.

**Benková, E.** 2016. Plant hormones in interactions with the environment. Plant Molecular Biology 91: 597.

**Bogaert, K.A., Blommaert, L., Ljung, K., Beeckman, T. & De Clerck, O.** 2019. Auxin function in the brown alga *Dictyota dichotoma*. Plant Physiology 179: 280-299.

**Bouhlal, R., Riadi, H., Martínez, J. & Bourgougnon, N.** 2010. The antibacterial potential of the seaweeds (Rhodophyceae) of the strait of gibraltar and the mediterranean coast of Morocco. African Journal of Biotechnology 9: 6365 - 6372.

**Boulho, R., Marty, C., Freile-Pelegrín, Y., Robledo, D., Bourgougnon, N. & Bedoux, G.** 2017. Antiherpetic (HSV-1) activity of carrageenans from the red seaweed *Solieria chordalis* (Rhodophyta, Gigartinales) extracted by microwave-assisted extraction (MAE). Journal of Applied Phycology 29: 2219-2228.

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**Boyer, G.L. & Dougherty, S.S.** 1988. Identification of abscisic acid in the seaweed *Ascophyllum nodosum*. Phytochemistry 27: 1521-1522.

**Cooke, S., Schreer, J.F., Dunmall, K.M. & Philipp, D.P.** 2002. Strategies for quantifying sublethal effects of marine catch and release angling: Insights from novel fresh water applications. American Fisheries Society Symposium 30: 121-134.

**Craigie, J.S.** 2011. Seaweed extract stimuli in plant science and agriculture. Journal of Applied Phycology 23: 371-393.

**Dobrev, P.I., Havl´ıcek, L., Vagner, M.V., Malbeck, J. & Kam´ınek, M.** 2005. Purification and determination of plant hormones auxin and abscisic acid using solid phase extraction and two-dimensional high performance liquid chromatography. Journal of Chromatography A 1075: 159-166.

**du Jardin, P.** 2015. Plant bio-stimulants: definition, concept. main categories and regulation. Scientia Horticulturae 196: 3–14.

**Engelen, A., Breeman, A., Olsen, J., Stam, W. & Åberg, P.** 2005. Life history flexibility allows *Sargassum polyceratium* to persist in different environments subjected to stochastic disturbance events. Coral Reefs 24: 670- 680.

**Hagens, M. & Middelburg, J.J.** 2016. Attributing seasonal pH variability in surface ocean waters to governing factors. Geophysical Research Letters 43: 12528-12537.

**Hamed, I., Özogul, F., Özogul, Y. & Regenstein, J.M.** 2015. Marine bioactive compounds and their health benefits: A review. Comprehensive Reviews in Food Science and Food Safety 14: 446-465.

**Hanisak, M.D. & Samuel, M.** 1987. Growth rates in culture of several species of Sargassum from Florida, USA. Hydrobiologia 152: 399-404.

**Hernández-Herrera, R.M., Santacruz-Ruvalcaba, F., Ruiz-López, M.A., Norrie J. & Hernández-Carmona, G.** 2014. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). Journal of Applied Phycology 26: 619-628.

**Higgins, S.N., Howell, E.T., Hecky, R.E., Guildford, S.J. & Smith, R.E.** 2005. The wall of green: the status of *Cladophora glomerata* on the northern shores of Lake Erie's eastern basin, 1995-2002. Journal of Great Lakes Research 31:547-563.

**Hirsch, R., Härtung, W. & Gimmler, H.** 1989. Abscisic acid content of algae under stress. Botanica Acta 102: 326-334.

**Hoang, T.C., Cole, A.J., Fotedar, R.K., O'Leary, M.J., Lomas, M.W. & Roy, Sh.** 2016. Seasonal changes in water quality and Sargassum biomass in southwest Australia. Marine Ecology Progress Series 551: 63 -79.

**Hong, D.D., Hien, H.M.M. & Son, P.N.** 2007. Seaweeds from Vietnam used for functional food, medicine and biofertilizer. Journal of Applied Phycology 19: 817- 826.

**Hwang, E.K., Park, C.S. & Baek, J.M.** 2007. Artificial seed production and cultivation of the edible brown alga, *Sargassum fulvellum* (Turner) C. Agardh: Developing a new species for seaweed cultivation in Korea. In: Anderson, R., Brodie, J., Onsøyen, V. & Critchley, A. (eds.), Eighteenth International Seaweed Symposium. vol. 1: 25-31. Springer, Netherlands.

**Iyer, R., De Clerck, O., Bolton, J.J. & Coyne, V.E.** 2004. Morphological and taxonomic studies of *Gracilaria* and *Gracilariopsis* species (Gracilariales, Rhodophyta) from South Africa. South African Journal of Botany 70: 521-539.

**Jacobs, W.P., Falkenstein, K. & Hamilton, R.H.** 1985. Nature and amount of auxin in algae. Plant Physiology 78: 844-848.

**Jeyaseelan, E.C., Kothai, S., Kavitha, R., Tharmila, S. & Thavaranjit, A.C.** 2012. Antibacterial activity of some selected algae present in the costal lines of Jaffna Peninsula. International Journal of Pharmaceutical & Biological Archive 3: 352-356.

**Jha, B., Reddy, C.R.K., Thakur, M.C. & Rao, M.U.** 2009. Seaweeds of India: The diversity and distribution of seaweeds of the Gujarat Coast. Springer, Neatherlands.

**Karabay-Yavasoglu, N.U., Sukatar, A., Ozdemir, G. & Horzum, Z.** 2007. Antimicrobial activity of volatile components and various extracts of the red alga *Janiarubens.* Phytotherapy Research 21: 153 - 156.

**Kausalya, M. & Rao, G.M.N.** 2015. Antimicrobial activity of marine algae. Journal of Algal Biomass Utilization 6: 78- 87.

**Lourenço-Lopes, C., Fraga-Corral, M., Jimenez-Lopez, C., Pereira, A.G., Garcia-Oliveira, P., Carpena, M., Prieto, M.A. & Simal-Gandara, J.** 2020. Metabolites from macroalgae and its applications in the cosmetic industry: A circular economy approach. Resources 9: 101.

**Mahmoud, S.H., Salama, D.M., El-Tanahy, A.M.M., & Abd El-Samad, E.H.** 2019. Utilization of seaweed (*Sargassum vulgare*) extract to enhance growth, yield and nutritional quality of red radish plants. Annals of Agricultural Science 64: 167-175.

**Malingin, D.L., Hongayo, M.C. & Larino, R.C.** 2012. Antibacterial and antioxidant effects of brown alga *Padina australis* Hauck crude extract. IAMURE International Journal of Science and Clinical Laboratory 2: 35 - 70.

Mattio, L. and Payri, C.E. 2011. 190 Years of *Sargassum* taxonomy, facing the advent of DNA phylogenies. The Botanical Review 77: 31-70.

**Michalak, I. & Messyasz, B.** 2021. Concise review of *Cladophora* spp.: macroalgae of commercial interest. Journal of Applied Phycology 33:133-166.

**Mikami, K., Mori, I.C., Matsuura, T., Ikeda, Y., Kojima, M., Sakakibara, H. & Hirayama, T.** 2016. Comprehensive quantification and genome survey reveal the presence of novel phytohormone action modes in red seaweeds. Journal of Applied Phycology 28: 2539-2548.

**Mori, I.C., Ikeda, Y., Matsuura, T., Hirayama, T. & Mikami, K.** 2017. Phytohormones in red seaweeds: a technical review of methods for analysis and a consideration of genomic data. Botanica Marina 60: 153-170.

**Nejatkhah Manavi, P., Rafiee, F., Shariat zadeh, S. & Seifi, S.H.** 2010. Identification and biomass of macroalgae in intertidal zone of Bushehr Province. Marine Science and Technology Research 5: 81- 88. [In Persian]

**Orduña-Rojas, J., Robledoa, D. & Dawesd, C.J.** 2002. Studies on the tropical agarophyte *Gracilaria cornea* J. Agardh (Rhodophyta, Gracilariales) from Yucatán, Mexico. I. Seasonal physiological and biochemical responses. Botanica Marina 45: 453-458.

[ DOR: 20.1001.1.24236330.2022.9.2.3.5 ]

[ DOI: 10.52547/nbr.9.2.115 ]

**Oumaskour, K., Boujaber, N., Etahiri, S. & Assobhel, O.** 2013. Anti-inflammatory and antimicrobial activities of twenty-three marine algae from the coast of SidiBouzid (El Jadida-Morocco). International Journal of Pharmacy and Pharmaceutical Sciences 5: 145- 149.

**Pepper, I.L. & Gentry, T.J.** 2015. Microorganisms found in the environment. In: Pepper, I.L., Gerba, C.P. and Gentry, T.J. (eds.), Environmental microbiology, 2: 9–36, Academic Press, Cambridge, MA, USA.

**Pikosz, M., Messyasz, B. & Gąbka, M.** 2017. Functional structure of algal mat (*Cladophora glomerata*) in a freshwater in western Poland. Ecological Indicators 74:1-9.

**Prieto, R.E., Cordoba, N.M., Montenegro, A.M. & Gonzalez-Mariño, G.E.** 2011. Production of indole-3-acetic acid in the culture medium of microalga *Scenedesmus obliquus* (UTEX 393). Journal of Brazilian Chemical Society 22: 2355-2361.

**Schaffelke, B.** 1995. Abscisic acid in Sporophytes of three *Laminaria* Species (Phaeophyta). Journal of Plant Physiology 146: 453- 458.

**Rivera, M. & Scrosati, R.** 2006. Population dynamics of *Sargassum lapazeanum* (Fucales, Phaeophyta) from the Gulf of California, Mexico. Phycologia 45: 178-189.

**Silva, A., Silva, S.A., Carpena, M., Garcia-Oliveira, P., Gullón, P., Fátima Barroso, M., Prieto, M.A. & Simal-Gandara, J.** 2020. Macroalgae as a source of valuable antimicrobial compounds: extraction and applications. Antibiotics 9: 642.

**Sitnik, K.M., Musatenko, L.I., Vasyuk, V.A., Vedenicheva, N.P., Generalova, V.M., Martin, G.G. & Nesterova, A.N.** 2003. Hormonal complex in plants and fungi. Akademperiodika, Kiev, Ukraine, pp: 28-43.

**Sohrabipour, J. & Rabii R.** 1999. A list of marine algae of seashores of Persian Gulf and Oman sea in the Hormozgan Province. Iranian Journal of Botany 8: 131-162.

**Spalding, H.L., Amado-Filho, G.M., Bahia, R.G., Ballantine, D.L., Fredericq, S., Leichter, J.J., Nelson, W.A., Slattery, M. & Tsuda, R.T.** 2019. Macroalgae. In: Loya Y., Puglise K.A. and Bridge, T.C.L. (eds.), Mesophotic Coral Ecosystems. Springer, Cham, Switzerland.

**Stirk, W.A. & Staden, J.V.** 1997. Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L. Journal of Applied Phycology 9: 327- 330.

**Stirk, W.A., Novák, O., Strnad, M. & van Staden, J.** 2003. Cytokinins in macro-algae. Plant Growth Regulation 41: 13- 24.

**Stirk, W.A., Nov´ak, O., Hradeck´a, V., Pěnčĺk, A., Rolčĺk, J., Strnad, M. & van Staden, J.** 2009. "Endogenous cytokinins, auxins and abscisic acid in *Ulva fasciata* (Chlorophyta) and *Dictyota humifusa* (Phaeophyta): towards understanding their biosynthesis and homoeostasis". European Journal of Phycology 44: 231-240.

**Stirk, W.A., Bálint, P., arkowská, D., Novák, O., Maróti, G., Ljung, K., Turečková, V., Strnad, M., Ördög, V. & van Staden, J.** 2013. Hormone profiles in microalgae: gibberellins and brassinosteroids. Plant Physiology and Biochemistry 70: 348-353.

**Stirk, W.A., Bálint, P., arkowská, D., Novák, O., Maróti, G., Ljung, K., Turečková, V., Strnad, M., Ördög, V. & van Staden, J.** 2014. Effect of light on growth and endogenous hormones in *Chlorella minutissima* (Trebouxiophyceae)". Plant Physiology and Biochemistry 79: 66-76.

**Sunarpi, H., Nikmatullah, A., Ambana, Y., Ilhami, B.T.K., Abidin, A.S., Ardiana, N., Kirana, I.A.P., Kurniawan, N.S. H., Rinaldi, R., Jihadi, A. & Prasedya, E.S.** 2021. Phytohormone content in brown macroalgae *Sargassum* from Lombok coast, Indonesia. IOP Conf. Series: Earth and Environmental Science 712: 1- 5.

**Takahashi. T., Sutherland, S.C., Chipman, D.W., Goddard, J.G., Ho, C., Newberger, T., Sweeney, C. & Munro, D.R.** 2014. Climatological distributions of pH, $pCO_2$, total $CO_2$, alkalinity, and $CaCO_3$ saturation in the global surface ocean, and temporal changes at selected locations. Marine Chemistry 164: 95-125.

**Tietz, A.**, **Ruttkowski, U., Koehler, R. & Kasprik, W.** 1989. Further investigations on the occurrence and the effects of abscisic acid in algae. Biochememie und Physiologie der Pflanzen 184: 259-266.

**Van den Hoek, C., Mann, D.G. & Jahns, H.M.** 1995. Algae: an introduction to phycology. Cambridge University Press, Cambridge, 623 pp.

**Vuong, D., Kaplan, M., Lacey, H.J., Crombie, A., Lacey, E. & Piggott, A.M.** 2018. A study of the chemical diversity of macroalgae from South Eastern Australia. Fitoterapia 126: 53-64.

**Wang, X., Zhao, P., Liu, X., Chen, J., Xu, J., Chen, H. & Yan, X.** 2014. Quantitative profiling method for phytohormones and betaines in algae by liquid chromatography electrospray ionization tandem mass spectrometry. Biomedical Chromatography 28: 275-280.

**Winchester, A.M.** 1969. Biology and its relation to mankind. D.Van Nostrand Reinhold Company. pp. 172-173.

**Woodward, A.W. & Bartel, B.** 2005. Auxin: regulation, action, and interaction. Annals of Botany 95: 707-735.

**Zhu, S., Gu, D., Lu, C., Zhang, C., Chen, J., Yang, R., Luo, Q., Wang, T., Zhang, P. & Chen H.** 2022. Cold stress tolerance of the intertidal red alga *Neoporphyra haitanensis*. BMC Plant Biology 22: 114.

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