

## *Gracilaria gracilis* – A Review of Ecological Knowledge, Chemical Composition, Cultivation, and Applications

Rajae Mouedden<sup>1\*</sup>, Souad Abdellaoui<sup>1,2</sup>, Faid El Madani<sup>3</sup>, Najib El Ouamari<sup>3</sup>,  
Douaa Slimani<sup>1</sup>, Khaoula Kasmi<sup>1</sup>, Mohamed Taibi<sup>1</sup>, Ilham Zahir<sup>4</sup>, Khalid Chaabane<sup>1</sup>

<sup>1</sup> Laboratory for the Improvement of Agricultural Production, Biotechnology and the Environment, FSO, Mohammed First University, Oujda, Morocco

<sup>2</sup> Regional Center of the National Institute of Fisheries Research, Fishing Laboratory, Casablanca, Morocco

<sup>3</sup> Regional Center of the National Institute of Fisheries Research, Fishing Laboratory, Nador, Morocco

<sup>4</sup> Polyvalent Team in Research and Development, Department of Biology, Faculté Polydisciplinaire Beni Mellal, Morocco

\* Corresponding author's e-mail: [mouedden.rajae@gmail.com](mailto:mouedden.rajae@gmail.com)

### ABSTRACT

*Gracilaria gracilis* is a benthic, intertidal red macroalga (Rhodophyta) of the commercially important *Gracilaria* genus. It is highly valued in scientific research for its ability to produce high-quality agar, a valuable polysaccharide widely utilised in various industries. This review serves as a guide to *G. gracilis*, encompassing various aspects of its biology, chemical composition, cultivation methods, and the main environmental factors influencing its growth as well as its different uses. The method used involved a descriptive analysis of the articles sourced from Google Scholar, ScienceDirect, and Springer. The results of the review showed that *G. gracilis* is one of the best candidates for cultivation, giving an excellent resilience to salinity and temperature fluctuations as well as offering ease of vegetative reproduction, among others traits. It can grow at temperatures ranging from 20 to 30°C, salinities between 25–35‰ and up to 42‰, depths ranging from 0.5 to 2.5 m, as well as light intensity of around 70 and 210  $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ . On the basis of its chemical composition, *G. gracilis* has many potentials as a source of high-value compounds and extracts for various uses in cosmetic, food, pharmaceutical, and biomedical industries. It is also used as a raw material or nutritious dietary item in the human diet, and can be suggested as a potential novel commercial source of phycobiliproteins.

**Keywords:** *Gracilaria gracilis*; biology; cultivation techniques; ecological knowledge; chemical composition.

### INTRODUCTION

The marine environment, with its great richness and quantity of creatures, promises a limitless reservoir of compounds that may be exploited and utilised to benefit human well-being. Macroalgae are important oceanic natural resources, rising from 10.6 million tonnes in 2000 to 32.4 million tonnes in 2018. Seaweeds represented 97.1 percent of 32.4 million tonnes of wild-collected and produced aquatic algae combined [FAO, 2020]. As an agarophyte, the genus *Gracilaria* is of significant commercial value and it is the most prevalent and

promising source of agar. It comprises over 150 species in temperate and subtropical zones [Vuai, 2022]. *Gracilaria* species are exploited and employed in a variety of sectors, including direct consumption as human food [Jensen, 2004; Dillehay et al., 2008; Gordon, 2017], in medicine to treat intestinal constipation, dysentery, enteritis, thyroid diseases, urinary disorders, respiratory disease, and diarrhea [Khare, 2007; Costa et al., 2016; Fu et al., 2016; Leódidido et al., 2017]. These species contain many bioactive chemicals with various biological characteristics, such as anti-cancer [Zandi et al., 2010; Sakthivel et al., 2016; Yi et al., 2022],

anti-inflammatory [Chaves et al., 2013; Chen et al., 2013; da Costa et al., 2017], anti-diabetic [Makkar and Chakraborty, 2017], anti-oxidant and anti-bacterial [Widowati et al., 2014; Afonso et al., 2021]. The extracts of *Gracilaria* are also used in agriculture as biostimulants for lettuce [Torres et al., 2018] and against pathogens like the root-rot fungus, *Phytophthora cinnamomi* [Jiménez et al., 2011], in aquaculture as an immunity booster for shrimp [Lin et al., 2011; Chen et al., 2016], as a feed [Valente et al., 2006; Al-Asgah et al., 2016] or as a supplement in feed [Lozano et al., 2016; Magnoni et al., 2017]. Furthermore, *Gracilaria* species were evaluated as insecticides [Leite et al., 2005; Madhiyazhagan et al., 2016], nematicides [Rizvi and Shameel, 2006; Khan, Abid and Hussain, 2015], and acaricides [Lima et al., 2005; Ruangsomboon and Pumnuan, 2016].

*Gracilaria gracilis* is a benthic, intertidal red macroalga (Rhodophyta) that belongs to the commercially valuable *Gracilaria* genus, to the order Gracilariales and Family Gracilariaceae. It is highly valued in scientific research for its ability to produce high-quality agar and a variety of essential organic components, such as proteins, lipids, fatty acids, phenols, sterols, and carbohydrates. The demand in the market coupled with insufficient crop management practices has resulted in the overharvesting of natural *G. gracilis* stands in several locations. This has led to *Gracilaria* scarcity, price rises, and a demand for stable supply and quality. As a result, farming this species has sparked a lot of interest, and numerous cultivating techniques have been created to make the

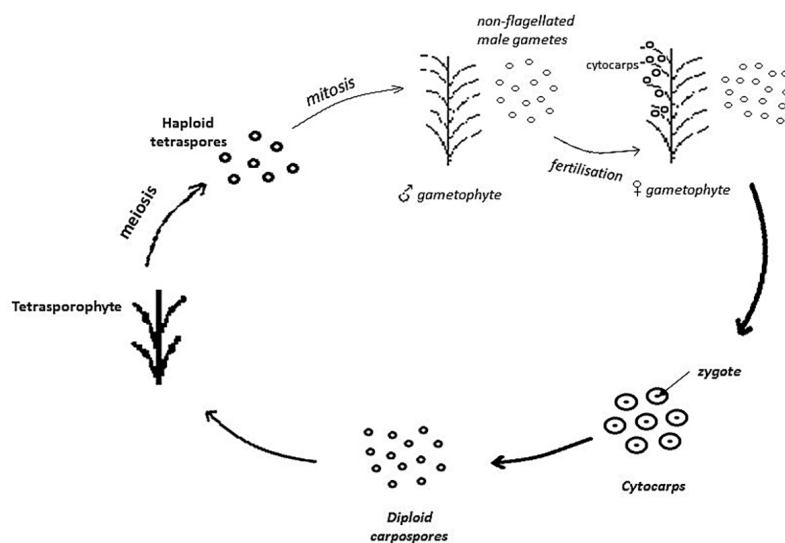
farmed *Gracilaria* crops more significant than wild *Gracilaria* crops. Therefore, it is crucial to comprehend the biology of *G. gracilis*, its habitat, and the environmental factors influencing its growth. This understanding is essential for cultivating *G. gracilis* on a large scale, contributing to successful and sustainable production of this alga species. In the shed of this data, this review aimed to offer a comprehensive guide to the agarophyte *Gracilaria gracilis*, aiming to identify and explore new research opportunities. To reach this goal, the current state of knowledge about this red macroalga was summarized by providing the information about its biology, chemical composition, cultivation methods, environmental factors affecting its growth, and the uses of this Rhodophyta.

## RESEARCH METHODOLOGY

The methodology comprised a literature review utilizing key search terms in such databases as Google Scholar, ScienceDirect, and Springer. A total of 83 articles were identified, focusing on biology, chemical composition, cultivation techniques, growth, and uses. The collected articles underwent systematic review and analysis to contribute to the literature review.

### General biology

*G. gracilis* follows the typical pattern of most Rhodophyta, the triphasic Polysiphonia-type life history [Engel et al., 2001; Leitao, 2005; Haddy,



**Figure 1.** Haploid–diploid life cycle in *G. gracilis*; italic characters and thin lines show haploid phase stages; bold characters and thick lines indicate diploid phase stages; italic characters and thick show carposporophyte phase stages

2011; Freitas et al., 2021]. As in all sexual life cycles, the three stages are related via meiosis and syngamy [Engel et al., 2001] (Fig. 1). The first phase is a diploid phase where tetraspores are produced from the tetrasporophytes via meiosis and develop into haploid isomorphic gametophytes (second phase), whereby the gametes are formed by mitosis. Male gametes, which lack flagella, are released into the water column and adhere to the trichogyne (female carpogonium extensions that extend slightly from the surface of the female thallus) [Engel et al., 2002; Engel, Destombe, and Valero, 2004] and fertilize the female gamete. The fertilized female gamete develops into cystocarps (a hemispherical fruiting body that is macroscopic and made of both maternal as well as zygotic tissues), forming the additional diploid (carposporophyte) phase [Engel et al., 2002; Engel, Destombe and Valero, 2004; Polifrone, De Masi and Gargiulo, 2006]. The zygote within the cystocarp multiplies by mitosis, releasing thousands of identical carpospores. These carpospores disperse grow into diploid tetrasporophytes [Freitas et al., 2021].

*G. gracilis* generally appears in the lower intertidal and upper subtidal, where it is found attached to stones, boulders, bedrocks, and sandy shores by a perennial holdfast [Haddy, 2011]. At low tide, it is commonly observed growing through up to 20 cm of sand under running water. It is found in temperate waters at 0 and 20 m [Gioele et al., 2017].

### Chemical composition of *Gracilaria gracilis*

Seaweeds may produce a variety of chemical compounds in varying concentrations, so the research field devoted to the discovery of bioactive components in algae is practically unlimited [Rodrigues et al., 2015]. Seaweeds are considered low in calories and high in polysaccharides, minerals, steroids, vitamins, proteins, and dietary fibers, making them increasingly popular commercially [Lordan, Ross and Stanton, 2011]. Table 1 shows the biochemical composition of *Gracilaria gracilis*.

### Proximate biochemical composition

#### Protein content

Red seaweed protein contents are comparable to meat protein contents (18–25%) and several legume proteins, such as peas or beans (19–22%) [Rodrigues et al., 2015]. Therefore, these algae might be utilized to make protein-balanced,

low-cost meals that could replace vegetable protein sources like legumes and cereals.

In a study by [Özen et al., 2018] to determine the effects of salinity stress on *G. gracilis*, the highest total protein content (88.47 mg/g wet weight) was found at a salinity level of 48 ‰ on day 2 of the experiment. Day 7 had the lowest value, 2.35 mg/g wet weight at the same salinity. Greenlight (500–550 nm) stimulates protein accumulation, reaching 29 mg·g<sup>-1</sup> DW. There is a 56% increase, as compared to the control. Nevertheless, the other light qualities had no apparent impact. The protein values obtained from wild populations of LOBS (14.20%) were slightly higher than those from FFBC populations (11.80%), but lower than cultured *G. gracilis* (21.58%) [Freitas et al., 2021]. The protein values obtained for cultured *G. gracilis* were similar to those reported by [Rodrigues et al., 2015] for wild Portuguese populations (20.2%). However, [Rasyid, Ardiansyah, and Pangestuti, 2019] reported significantly lower values (10.86%) for wild *G. gracilis* collected in Indonesia.

The crude protein content (the total nitrogen content multiplied by 6.25) varied significantly from 2.96% at a depth of 2.5 m with an initial fragment weight (IFW) of 40 g to 5.83% at a depth of 0.5 m with an IFW of 5 g [Ben Said et al., 2018]. This indicates that the IFW and depth influence the crude protein content.

#### R-phycoerythrin content

*G. gracilis* is a valuable source of the red pigment R-phycoerythrin [Nguyen et al., 2020]. After extraction and purification, it is used as a natural colorant and fluorescent probe with diverse uses in the cosmetic, food, pharmaceutical, and biomedical sectors. The R-phycoerythrin content reported by [Francavilla et al., 2015] for the Italian *G. gracilis* was substantially higher (7 mg·g<sup>-1</sup>) than that obtained by [Özen et al., 2018], who obtained the highest value (2.048 mg·g<sup>-1</sup>) for Turkish *G. gracilis* populations (Izmir Bay) at a salinity level of 37‰ on day 2 of the experiment. The lowest value was obtained on Day 7 at 0.356 mg·g<sup>-1</sup> at 25‰ salinity, and by, *G. gracilis* – 0.907 mg/g. Moreover, the R phycoerythrin content of *G. gracilis* from the Bizerte lagoon ranged from 0.011 mg·g<sup>-1</sup> at a depth of 2.5 m and an IFW of 40 g to 0.050 mg·g<sup>-1</sup> at a depth of 0.5 m and a starting weight of 5 g [Ben Said et al., 2018].

### Carbohydrate, 3,6-anhydrogalactose, and sulphate content

Carbohydrates stand out as a predominant storage compound within plants, serving as a source of food and fiber for humans and animal feed [Chibbar and Båga, 2003]. They also play a crucial role in metabolism, serving as the primary source of energy needed for respiration and other metabolic processes [Khairy and El-Shafay, 2013]. The carbohydrate content was the most abundant component of the proximate composition in *G. gracilis* studied by [Rasyid, Ardiansyah and Pangestuti, 2019], accounting for 63.13%. This finding was higher than that published by [Freitas et al., 2021] for cultured *G. gracilis* (38.35%) and wild FFBC and LOBS populations (40.72% and 44.12%, respectively); [Ben Said et al., 2018] reported 9.52% for Tunisian *G. gracilis* harvested at 2.5 m with an IFW of 40 g.

The 3,6-anhydrogalactose content varied from 20.12% to 47.17%. The minimum and the maximum percentages were noted in the algae with an IFW of 5 g and 40 g at 0.5 m, respectively [Ben Said et al., 2018]. The sulphate content of Tunisian *G. gracilis* was observed to vary from 3.98 to 5.51%. The highest and the lowest values were obtained from the agar samples cultivated at 0.5 m and 2.5 m and with an IFW of 5 g and 40 g, respectively [Ben Said et al., 2018].

### Total lipid content

Lipids form an extensive category of naturally occurring compounds that includes sterols, waxes, fat, fat-soluble vitamins (such as vitamins A, B, D, and K), phospholipids, monoglycerides, diglycerides, carotenoids, and others [Bernal et al., 2011]. They contribute to various biological purposes, including serving as energy storage molecules, important signaling molecules, and structural components of cell membranes [Bernal et al., 2011]. The total lipid content ranged between 1.37% and 3.58%. The maximum lipid content was found in algae cultivated at 2.5 m from initial weights of 5 g, whereas the lowest was found at 0.5 m from initial fragment weights of 5 g (Ben Said et al., 2018). Moreover, [Francavilla et al., 2013] reported that the lipid content of *G. gracilis* gathered from Lesina Lagoon (Italy) assorted from 1.19% to 1.98% dw. Likewise, [Freitas et al., 2021] found that the values from cultivated biomass varied from 1.21% to 1.40 % from LOBS wild populations.

### Total phenolic and total flavonoid content

The total phenol content of the methanolic extract of *G. gracilis* was 29.39 mg gallic acid, equivalent·g<sup>-1</sup> of extract. In contrast, the ethyl acetate extract was calculated to be 35.53 mg·g<sup>-1</sup> [Ebrahimzadeh, Khalili, and Dehpour, 2018]. The total phenolic content of the Portuguese *G. gracilis* was 228 mg catechol equiv·g<sup>-1</sup> dry seaweed [Rodrigues et al., 2015]. On the basis of a study, the light quality considerably impacts phenolic compounds. The highest accumulation, 2.92 mg·g<sup>-1</sup> DW, was found under blue light, 68% more than the control. Nevertheless, under UV and red-light conditions, the concentration of phenolic compounds decreased by 17% and 25%, respectively (Table 1). Regarding total flavonoid content, the ethyl acetate extract of *G. gracilis* species was rich in flavonoid compounds (66.48±1.87 mg quercetin equivalent·g<sup>-1</sup> of extract). However, the methanolic extract contained fewer flavonoid compounds (26.47±1.203 mg·g<sup>-1</sup>) [Ebrahimzadeh, Khalili and Dehpour, 2018].

### Cultivation methods

The natural deposits of *Gracilaria* are dwindling worldwide due to overexploitation. This situation has prompted the development of various cultivation techniques aimed at augmenting biomass. Therefore, cultivation has the best potential to conserve natural resources and meet the high demand for agar. All planting methods depend on the ability of *Gracilaria* to create an underground thallus system that ties the algae to the soft bottom.

#### Open water systems

The cultivation of seaweeds in the sea is usually done in protected bays and estuaries. *Gracilaria* crops are planted using one of two methods: bottom culture and suspended culture (Table 2). In these two approaches, the spores settled on lines and ropes, vegetative thalli or cuttings tied to or inserted into line, or rope can be used as planting material.

#### Land-based systems

The cultivation of *Gracilaria* in land-based systems can be divided into intensive (tank farming) and non-intensive (pond farming) cultivation systems (Table 3). Tanks are typically constructed

**Table 1.** Biochemical composition of *Gracilaria gracilis*

Reference	Moisture (% dw)	Organic matter (%)	Ash (%)	Carbohydrate (%)	3,6 anhydrogalactose (%)	Sulphate (% ww)	Protein	R-phycoerythrin (mg/g dw)	Total lipid (% dw)
Freitas et al. (2021)	72.02-82.04	72.85-81.04	18.96-27.15	38.35-44.12	nd	nd	11.80-21.58%	nd	1.21-1.40
Rasyid et al. (2019)	19.04	nd	6.78	63.13	nd	nd	10.86%	nd	nd
Rodrigues et al. (2015)	7.99 ± 0.02	67.21 ± 0.01	24.8 ± 0.03	nd	nd	nd	20.2 ± 0.6%	nd	nd
Özen et al. (2018)	nd	nd	nd	nd	nd	nd	2.35-88.47 mg/g ww	0.356-2.048	nd
Ben Ghedifa et al. (2021)	Nd	nd	nd	nd	nd	nd	29±4.32 mg/g dw	0.907±0.34	nd
Ben Said et al. (2018)	Nd	nd	19.04-35.25	5.38-9.52	20.12- 47.17	3.98-5.51	2.96-5.83%	0.011-0.050	1.37-3.58
Francavilla et al. (2015)	1.32-9.13	nd	19.98-20.88	24.8-34.1	nd	nd	31–45%	7	1.19-1.98
Mollet et al. (1998)	Nd	nd	nd	nd	36.6-50.4	2.1-6.6	nd	nd	nd

**Note:** Nd – not defined; dw – dry weight; ww – wet weight.

**Table 2.** Open water cultivation methods

Type	Methods	Tools	Requirements
Bottom culture	1- Transplanting rocks or securing plants to substrates 2- Insert thalli into the sandy bottom (direct method) 3- Fixing thalli into sandy bottom (Plastic tube method) 4- Inoculating spores (either tetra- or carpospores) onto ropes (Spore method)	1- Raffia, rubber bands... 2- Weights or forks 3- Polyethylene tubes filled with sand 4- Ropes	The spore method requires: - Nursery unit (indoor tanks and inoculation chamber) - Prepare out-planting site
Suspended culture	1- Suspended between stakes 2- Supported by buoys or a raft 3- Anchored to seafloor 4- Inoculating tetra or carpospores into lines or nets (spore method)	Weaving plant materiel through rope fibers or tying with Raffia or nylon	The spore method requires: - Nursery unit (indoor tanks and inoculation chamber) - Prepare out-planting site

of concrete or plastic and have a water agitation system. They are regarded as the most expensive method of cultivating seaweeds, while ponds are always composed of open earthen structures and lack an artificial water agitation system.

### Environmental factors affecting the growth of *gracilaria gracilis*

Seaweeds live in a dynamic and complex ecological environment that can be classified as an extreme environment because abiotic (e.g., temperature, light intensity, salinity, and nutrients) and biotic (e.g., epiphytism) factors can fluctuate widely and rapidly, requiring seaweeds to adapt quickly [Cotas *et al.*, 2020]. Therefore, understanding how these factors affect growth and production is important in cultivating and managing this marine macroalgae [Njobeni, 2006].

### Temperature

Temperature is the primary physical factor determining the seasonal and latitudinal distribution of seaweed [Gebrekios, 2003]. Understanding its effects on growth can help predict seasonal productivity fluctuations [Morgan, 2000]. In a study conducted at Klein Oesterwal, Langebaan Lagoon, South Africa, it was found that the growth of *G. gracilis* was positively affected by higher temperatures, with growth measurements increasing between 22°C and 30°C, but decreasing at 18°C [Beltrand *et al.*, 2022]. Likewise, [Mensi *et al.*, 2020] reported that the optimum growth had been observed in restricted temperature ranges, between 20°C and 28°C.

### Light

Light, just like temperature, significantly impacts the growth of *Gracilaria*. It provides the

**Table 3.** Cultivation methods for land-based systems

Type	Description	Requirements	Strengths	Limitations
Pond farming	<ul style="list-style-type: none"> <li>- Open earthen structure (including natural lagoons or artificial excavations);</li> <li>- Large (0.5 to 2 ha) and shallow (&lt;1m)</li> <li>- Optimal location: Sheltered areas with minimal wind strategically positioned near the sea for effective tidal water exchange.</li> <li>- Alga are often free-floating and properly spaced throughout the bottom surface of ponds.</li> </ul>	<ul style="list-style-type: none"> <li>- Water pumping</li> <li>- Nutrient addition (fertilizers) if needed</li> </ul>	<ul style="list-style-type: none"> <li>- Requires less control</li> <li>- The simplest and cheapest method</li> </ul>	<ul style="list-style-type: none"> <li>- Certain factors need to be considered (temperature, salinity, and pH)</li> </ul>
Tank farming	<ul style="list-style-type: none"> <li>- Small concrete or industrial tanks (from liters to cubic meters)</li> </ul>	<ul style="list-style-type: none"> <li>- Water agitation system</li> <li>- Sufficient supply of nutrients (phosphorous and nitrogen);</li> <li>- Compressed air or paddle wheels to maintain movement;</li> <li>- Additional factors (light, temperature, CO<sub>2</sub> supply, pH).</li> </ul>	<ul style="list-style-type: none"> <li>- Production control</li> <li>- The most significant productivity per unit area</li> <li>- Sustainable and high production</li> </ul>	<ul style="list-style-type: none"> <li>- Requires a significant amount of energy and capital investment.</li> </ul>

initial energy for photosynthesis [Gebrekios, 2003], and it is used as a signal for life processes, such as reproduction and growth during the life cycle of seaweeds [Gebrekios, 2003].

Algal growth is significantly influenced by light intensity. [Ben Ghedifa et al., 2021] stated that the highest daily growth rate (DGR) ( $7.24\% \text{ day}^{-1} \pm 1.06$ ) was observed under red light (620–670 nm) compared to the control ( $95 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ ), which was  $2.17\% \pm 0.2$ . Following this, the samples exposed to blue light (400–450 nm) exhibited a growth rate of  $5.73\% \text{ day}^{-1} \pm 0.17$ . The supplemented green light (500–550 nm) increased the growth rate compared to the control. However, compared to the other samples, it is still the lowest ( $3.77\% \text{ day}^{-1} \pm 0.42$ ). [Mensi and Ben Ghedifa, 2019] found that the optimum DGR ( $5\% \text{ day}^{-1}$ ) was attained at an irradiance of  $198 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ , while the impact of light reached a maximum of roughly  $195 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ , decreasing on both sides of this value. A reduction in growth rate exceeding this light limit could indicate the beginning of photoinhibition. A light intensity increase induces photoinhibition and diminishes light use efficiency [Mensi and Ben Ghedifa, 2019]. These authors also reported that under the following light conditions ( $70\text{--}210 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ ), *G. gracilis* could be cultivated with an acceptable DGR exceeding 3% per day. However, *G. gracilis* growth did not show enhancement at lower light intensities ( $70 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$ ).

### Depth

Due to its negative association with light intensity, depth is a significant and complex factor influencing algal production [Yang *et al.*, 2015]. A study by [Ben Said et al., 2018] showed that *G. gracilis* could be cultivated at 0.5 and 2.5 m depths, with the maximum DGR found at 0.5 m. The cultivation experiment of *G. gracilis* in the Bizerte lagoon (BL), Tunisia, showed that the shallowest depth (1 m) yielded the highest DGR values, while the deepest (>3 m) yielded the lowest. In comparison, the DGR of *G. gracilis* in Bizerte Bay (BB) remained unaffected at a depth of 4 m, suggesting that sufficient light levels were available in the studied depths [Mensi *et al.*, 2020]. In Izmir Bay, Aegean Sea-Turkey, the best results were obtained with the plants suspended at 0.6–0.7 m depth [Dural, Demir and Sunlu, 2006], where the plants were suspended near the bottom. Near the water, surfaces were subjected to epiphytism or fish consumption, affected by waves or currents, or died due to sunlight [Dural, Demir and Sunlu, 2006].

### Salinity

A study conducted by [Cirik *et al.*, 2010] showed that *G. gracilis* had a wide salinity tolerance range, and the optimum production was determined at 42‰ salinity. Moreover, [Özen et al., 2018] found that *G. gracilis* grows well in the salinity ranges between 25‰ and 35‰. However,

these authors only focused on the impact of different salt concentrations on the biochemical composition of *G. gracilis*. They did not investigate other factors that could affect the growth and survival of the alga.

### Nutrients

Several studies showed that nutrient levels, particularly nitrogen levels, play a crucial role in influencing agar yield, quality, and the growth of *Gracilaria* [Yang *et al.*, 2015; Ben Said *et al.*, 2018]. It has been shown that the DGR of *G. gracilis* augmented with increasing dissolved inorganic nitrogen ( $\text{NH}_4^+ + \text{NO}_3^-$ ) concentration, reaching 300  $\mu\text{mol}$  [Mensi and Ben Ghedifa, 2019]. Among nutrients, ammonium is important in controlling the growth of *G. gracilis*. For example, [Mensi and Ben Ghedifa, 2019] demonstrated that under the following ammonium conditions (10–80  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{L}^{-1}$ ), *G. gracilis* could be cultivated with an acceptable DGR exceeding 3% per day. Nevertheless, the maximum DGR (5%  $\text{day}^{-1}$ ) was reported at a nitrogen concentration of 80  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{L}^{-1}$  for ammonium. Also, it has been highlighted that the growth rate of *G. gracilis* was the highest (DGR: 5%  $\text{day}^{-1}$ ) when nitrate concentrations reached 210  $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{L}^{-1}$  [Mensi and Ben Ghedifa, 2019]. Photosynthetic parameters, thalli nitrogen content, and nitrogen uptake rates encourage higher productivity. In a comparison between the BL and BB, [Mensi *et al.*, 2020] found that the nitrogen concentration in the two sites was insufficient (<50  $\mu\text{mol}$ ) to maintain the high seaweed DGR needed for biomass production as indoor culture (>1000  $\mu\text{mol}$ ) because of the highly nitrophilic character of *G. gracilis*. Hence, the growth rate of BL exceeded that of BB, possibly due to the enrichment of nitrogen from surface runoff into the lagoon. This nitrogen influx aids algae in fulfilling their nitrogen requirements.

### Epiphytism

Seaweed biodiversity and abundance are affected by herbivores and other organisms [Yang *et al.*, 2015]. Primary consumption by herbivores can diminish thallus mass as well as influence the growth and reproductive processes of seaweeds [Dethier, Williams and Freeman, 2005; Williams, Bracken and Jones, 2013]. Moreover, epiphytes can impact seaweed production either directly, by shading the seaweed and constraining its growth, or indirectly, by causing the sinking of culture

lines [Asaeda *et al.*, 2004]. At Baha Bustamante, Argentina, the *G. gracilis* population serves as a substrate for various epiphytes with varying degrees of attachment and/or infection. Twenty-nine algal species were reported as *G. gracilis* epiphytes. They included 17 Rhodophyta, 9 Heterokontophyta (class Phaeophyceae), 2 Chlorophyta, and 1 Cyanophyta species. Among the numerous species observed, *Calothrix confervicola* stood out as one of the most abundant. This epiphyte, which exhibited weak attachment to the host surface (*G. gracilis* surface), did not induce any damage to the host tissue. In contrast, species like *C. rubrum*, *Polysiphonia abscissa*, and other Ceramiales were more detrimental, causing harm by penetrating the cortical portion of the host thallus, with their rhizoids occasionally reaching the medullary tissue [Martín *et al.*, 2013]. According to [Nabivailo, Skriptsova and Titlyanov, 2005], the associated algal species *Enteromorpha prolifera f. prolifera* (*Ulva prolifera*), *Chaetomorpha linum*, and *Polysiphonia sp.* inhibited the photosynthetic activity of *G. gracilis* both in nature, during their blooms, and in laboratory culture. These authors proposed that the inhibition of *G. gracilis* photosynthesis is related to the influence of extracellular metabolites secreted by *Enteromorpha prolifera f. prolifera*, *Chaetomorpha linum*, and *Polysiphonia sp.*

### Uses of *Gracilaria gracilis*

Globally, seaweeds have been used for thousands of years as feed, food supplements, sources of medicine, and fertilizers in agriculture [Ben Said *et al.*, 2018] and to control the postharvest diseases of fruits (Bahammou N, 2017). On the basis of the nutraceutical and nutritional value of *Gracilaria gracilis*, shown by the research published worldwide [Paiva *et al.*, 2014; Rodrigues *et al.*, 2015; Freitas *et al.*, 2021], it is suggested that this seaweed species can potentially be used as a raw material or as a food or supplement for humans and various species of fish and shellfish. Additionally, the generally high protein content gives this species relevance as a health promoter, nutraceutical agent, and healthy and nutritious gastronomic ingredient [Mouritsen, Rhatigan and Pérez-Lloréns, 2019]. Moreover, algae are a known source of vitamins and are valued for their metabolic processes, antioxidant activity, and other health advantages. In particular, the water-soluble vitamin C in *G. gracilis* decreases

blood pressure and reduces the risk of cancer [Škrovánková, 2011; Freitas *et al.*, 2021]. In addition, *G. gracilis* is considered an economically valuable resource for its agar content [Marinho-Soriano and Bourret, 2003; Rodríguez *et al.*, 2009]. *G. gracilis* can be suggested as a potential novel commercial source of phycobiliproteins, proteins that function as photosynthetic accessory pigments in red algae, cyanobacteria, cryptophytes, and glaucophytes. Phycoerythrin (R-phycoerythrin) [Cotas *et al.*, 2020], which, after extraction and purification, is applied as a natural colorant in food [Pereira *et al.*, 2020] and cosmetics and as fluorescent probes in diagnostic assays and diverse studies, can be taken as an example [Dumay and Morançais, 2016]. *G. gracilis* is also a valuable source of organic compounds such as proteins, lipids, fatty acids, phenols, sterols, and carbohydrates [Francavilla *et al.*, 2013]. Additionally, the macroalgae has a high phenol and flavonoid content, which allow it to possess excellent antioxidant properties [Freitas *et al.*, 2021] and great radical scavenging activity [Francavilla *et al.*, 2013; Barbosa *et al.*, 2018]. Furthermore, the ethanol and methanol extracts from *G. gracilis* could help identify compounds that serve as antibacterial agents against several infectious agents, e.g., species from the genus *Vibrio* and *Bacillus subtilis* [Capillo *et al.*, 2018; Freitas *et al.*, 2021], and also could be used for the treatment and prevention of fish diseases due to *Vibrio* species [Cavallo *et al.*, 2013]. Also, *G. gracilis* has the potential to serve as a promising source for the development of antiseptic and cleansing products [Capillo *et al.*, 2018]. On the basis of the presence of antimicrobial and antioxidant compounds, According to [Barbosa *et al.*, 2018], seaweed extracts have been proposed as a potential source of preservative compounds for use in chilled fish storage. The inclusion of *G. gracilis* extracts in the icing system demonstrated a positive impact on the quality retention of chilled hake. This was evidenced by a reduction in microbial activity (trimethylamine formation) as well as an inhibitory effect on the development of lipid oxidation (tertiary oxidation compounds) [Barbosa *et al.*, 2018]. In aquaculture, *G. gracilis* has proven successful in feed applications, primarily for abalone, and has been employed as a biofilter in integrated multi-trophic aquaculture (IMTA) systems. In these systems, *G. gracilis* is utilized alongside fishes, shrimps, and abalones [Njobeni, 2006; Smit *et al.*, 2007] since they have

excellent bioremediation ability in removing inorganic nutrients. For example, [Henriques *et al.*, 2015] found that *G. gracilis* showed massive bioaccumulation capabilities, accumulating as much as 209 mg of mercury (Hg) per gram of macroalgae (d.w.), this corresponds to the 99% removal of Hg from the contaminated seawater. Furthermore, according to [Jacinto *et al.*, 2018], living *G. gracilis* has been recognized for its potential in removing and recovering rare critical elements from wastewater, achieving removal efficiencies of up to 70% within 48 h and recovering nearly 100% of all yttrium (Y), cerium (Ce), neodymium (Nd), europium (Eu), and lanthanum (La) in seaweed biomass in a 300-fold more concentrated solution.

## CONCLUSIONS

*Gracilaria gracilis* is a popular seaweed because it produces high-quality agar and a variety of other organic compounds, such as proteins, lipids, fatty acids, phenols, sterols, and carbohydrates. Decreased natural beds have encouraged agar producers and dealers to investigate other production methods. As a result, cultivation of this seaweed has increased in recent years, with promising results. A well-established “protocol” appears critical for allowing large-scale production of this alga species. Recent research has revealed that this alga might be used in innovative ways. Further research is warranted to investigate the influence of temperature and salinity on the growth of this species. Encouraging additional studies will contribute to developing novel cultivation techniques and agar extraction procedures to enhance yields and quality as well as discover new uses for this species and new applications based on agar exploitation. Undoubtedly, there is a vast field of research to be studied in quest of active chemicals and new sources.

## REFERENCES

1. Afonso, C., Correia, A.P., Freitas, M.V., Mouga, T., Baptista, T. 2021. *in vitro* evaluation of the antibacterial and antioxidant activities of extracts of *Gracilaria gracilis* with a view into its potential use as an additive in fish Feed. Applied Sciences, 11(14), 6642.
2. Al-Asgah, N.A., Younis, E.-S.M., Abdel-Warith, A.-W.A., Shamlol, F.S. 2016. Evaluation of red



- seaweed *Gracilaria arcuata* as dietary ingredient in African catfish, *Clarias gariepinus*. *Saudi Journal of Biological Sciences*, 23(2), 205–210.
3. Asaeda, T., Sultana, M., Manatunge, J., Fujino, T. 2004. The effect of epiphytic algae on the growth and production of *Potamogeton perfoliatus* L. in two light conditions. *Environmental and Experimental Botany*, 52(3), 225–238.
  4. Bahammou, N., O. Cherifi, H. Bouamama, K. Cherifi, T. Moubchir and M. Bertrand, 2017. Postharvest control of gray mold of tomato using seaweed extracts. *J. Mater. Environ. Sci.*, 8: 831-836.
  5. Barbosa, R.G., Trigo, M., Dovale, G., Rodríguez, A., Aubourg, S.P. 2018. Antioxidant and antimicrobial behaviour of alga *Gracilaria gracilis* extracts during hake (*Merluccius merluccius*) chilled storage. *Bulgarian Chemical Communications*, 50, 118–124.
  6. Beltrand, M., Dineen, A., Hitzeroth, C., Baum, B., Cerff, C.D., Vos, C.D., Lewis, J., Zaroufis, S., Pillay, D. 2022. Warming effects on two autogenic engineers (*Zostera capensis* and *Gracilaria gracilis*): consequences for macrofaunal assemblages and benthic heterogeneity in intertidal sandflat ecosystems. *Estuaries and Coasts*, 45, 247–259.
  7. Ben Ghedifa, A., Vega, J., Korbee, N., Mensi, F., Figueroa, F.L., Sadok, S., 2021. Effects of light quality on the photosynthetic activity and biochemical composition of *Gracilaria gracilis* (Rhodophyta). *Journal of Applied Phycology*, 33, 3413–3425.
  8. Ben Said, R., Mensi, F., Majdoub, H., Ben Said, A., Ben Said, B., Bouraoui, A. 2018. Effects of depth and initial fragment weights of *Gracilaria gracilis* on the growth, agar yield, quality, and biochemical composition. *Journal of Applied Phycology*, 30, 2499–2512.
  9. Bernal, J., Mendiola, J.A., Ibáñez, E., Cifuentes, A. 2011. Advanced analysis of nutraceuticals. *Journal of Pharmaceutical Biomedical Analysis*, 55(4), 758–774.
  10. Capillo, G., Savoca, S., Costa, R., Sanfilippo, M., Rizzo, C., Lo Giudice, A., Albergamo, A., Rando, R., Bartolomeo, G., Spanò, N., Faggio, C. 2018. New insights into the culture method and antibacterial potential of *Gracilaria gracilis*. *Marine Drugs* 16(12), 492.
  11. Cavallo, R. A., Acquaviva, M. I., Stabili, L., Cecere, E., Petrocelli, A., Narracci, M. 2013. Antibacterial activity of marine macroalgae against fish pathogenic *Vibrio* species. *Central European Journal of Biology* 8(7), 646–653.
  12. Chaves, L. D. S., Nicolau, L.A.D., Silva, R.O., Barros, F.C.N., Freitas, A.L.P., Aragão, K.S., Ribeiro, R. de A., Souza, M.H.L.P., Barbosa, A.L. dos R., Medeiros, J.V.R. 2013. Antiinflammatory and antinociceptive effects in mice of a sulfated polysaccharide fraction extracted from the marine red algae *Gracilaria caudata*. *Immunopharmacology and Immunotoxicology* 35(1), 93–100.
  13. Chen, K.J., Tseng, C.K., Chang, F.R., Yang, J.I., Yeh, C.C., Chen, W.C., Wu, S.F., Chang, H.W., Lee, J.C. 2013. Aqueous extract of the edible *Gracilaria tenuistipitata* inhibits hepatitis C viral replication via cyclooxygenase-2 suppression and reduces virus-induced inflammation. *PLoS One* 8(2), e57704.
  14. Chen, X. Q., Liu, Q., Jiang, X.Y., Zeng, F. 2005. Microwave-assisted extraction of polysaccharides from *Solanum nigrum*. *Journal of Central South University of Technology*, 12, 556–560.
  15. Chen, Y.Y., Chen, J.C., Kuo, Y.H., Lin, Y.C., Chang, Y.H., Gong, H.Y., Huang, C.L. 2016. Lipopolysaccharide and  $\beta$ -1,3-glucan-binding protein (LGBP) bind to seaweed polysaccharides and activate the prophenoloxidase system in white shrimp *Litopenaeus vannamei*. *Developmental & Comparative Immunology*, 55, 144–151.
  16. Chibbar, R.N., Bâga, M., 2003. Genetic modification of primary metabolism. *Carbohydrates*.
  17. Cirik, Ş., Çetin, Z., Ak, İ., Cirik, S., Göksan, T. 2010. Greenhouse cultivation of *Gracilaria verrucosa* (Hudson) Papenfuss and Determination of Chemical Composition. *Turkish Journal of Fisheries and Aquatic Sciences*, 10(4), 559–564.
  18. Costa, D.S., Araújo, T.S., Sousa, N.A., Souza, L.K., Pacifico, D.M., Sousa, F.B.M., Nicolau, L.A.D., Chaves, L.S., Barros, F.C.N., Freitas, A.L.P., Medeiros, J.V.R. 2016. Sulphated polysaccharide isolated from the seaweed *Gracilaria caudata* Exerts an antidiarrhoeal effect in rodents. *Basic & clinical pharmacology & toxicology*, 118(6), 440–448.
  19. Cotas, J., Leandro, A., Pacheco, D., Gonçalves, A.M., Pereira, L., 2020. A comprehensive review of the nutraceutical and therapeutic applications of red seaweeds (Rhodophyta). *Life*, 10(3), 19.
  20. Da Costa, E., Melo, T., Moreira, A. S., Bernardo, C., Helguero, L., Ferreira, I., Cruz, M., Rego, A., Domingues, P., Calado, R., Abreu, M., Domingues, M. R. 2017. Valorization of lipids from *Gracilaria* sp. through lipidomics and decoding of antiproliferative and anti-inflammatory activity. *Marine Drugs*, 15(3), 62.
  21. Dethier, M.N., Williams, S.L., Freeman, A. 2005. Seaweeds under stress: manipulated stress and herbivory affect critical life-history functions. *Ecological Monographs* 75(3), 403–418.
  22. Dillehay, T.D., Ramírez, C., Pino, M., Collins, M.B., Rossen, J., Pino-Navarro, J.D. 2008. Monte Verde: seaweed, food, medicine, and the peopling of South America. *Science*, 320(5877), 784–786.
  23. Dumay, J., Moranchais, M. 2016. Proteins and Pigments. *Seaweed in Health and Disease Prevention*. Nantes, France.
  24. Dural, B., Demir, N., Sunlu, U. 2006. A Pilot-scale Unit for suspended cultivation of *Gracilaria gracilis* in Izmir Bay, Aegean Sea-Turkey. *Pakistan Journal of Biological Sciences*, 9(6), 1043–1046.

25. Ebrahimzadeh, M.A., Khalili, M., Dehpour, A.A. 2018. Antioxidant activity of ethyl acetate and methanolic extracts of two marine algae, *Nannochloropsis oculata* and *Gracilaria gracilis* - an in vitro assay. *Brazilian Journal of Pharmaceutical Sciences*, 54, 1–6.
26. Engel, C., Åberg, P., Gaggiotti, O.E., Destombe, C., Valero, M. 2001. Population dynamics and stage structure in a haploid-diploid red seaweed, *Gracilaria gracilis*. *Journal of Ecology*, 89, 436–450.
27. Engel, C., Valero, M., Lagadeuc, Y., Destombe, C. 2002. Non-random mating in controlled multiple-donor crosses in *Gracilaria gracilis* (Gracilariaceae, Rhodophyta). *European Journal of Phycology*, 37(2), 179–190.
28. Engel, C.R., Destombe, C., Valero, M. 2004. Mating system and gene flow in the red seaweed *Gracilaria gracilis*: effect of haploid-diploid life history and intertidal rocky shore landscape on fine-scale genetic structure. *Heredity*, 92(4), 289–298.
29. FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome.
30. Francavilla, M., Franchi, M., Monteleone, M., Caroppo, C. 2013. The red seaweed *Gracilaria gracilis* as a multi products source. *Marine Drugs*, 11(10), 3754–3776.
31. Francavilla, M., Manara, P., Kamaterou, P., Monteleone, M., Zabaniotou, A. 2015. Cascade approach of red macroalgae *Gracilaria gracilis* sustainable valorization by extraction of phycobiliproteins and pyrolysis of residue. *Bioresource Technology*, 184, 305–313.
32. Freitas, M.V., Mougá, T., Correia, A.P., Afonso, C., Baptista, T. 2021. New insights on the sporulation, germination, and nutritional profile of *Gracilaria gracilis* (Rhodophyta) grown under controlled conditions. *Journal of Marine Science and Engineering*, 9(6), 562.
33. Fu, X.M., Zhang, M.Q., Shao, C.L., Li, G.Q., Bai, H., Dai, G.L., Chen, Q.W., Kong, W., Fu, X.J., Wang, C.Y. 2016. Chinese marine materia medica resources: status and potential. *Marine Drugs*, 14(3), 46.
34. Gebrekiros, S.T. 2003. Characterisation of nitrogen stress response genes of the marine alga *Gracilaria gracilis*. Ph.D. Thesis, University of Cape Town, South Africa.
35. Gioele, C., Marilena, S., Valbona, A., Nunziacarla, S., Andrea, S., Antonio, M. 2017. *Gracilaria gracilis*, source of agar: A short review. *Current Organic Chemistry*, 21(5), 380–386.
36. Gordon, A. 2017. Food Safety and Quality in Developing Countries. Case study: Improving the quality and viability of a traditional beverage – Irish Moss, Kingston, Jamaica.
37. Haddy, S.M. 2011. Development of a transformation protocol and cell culture system for the commercially important species of red macroalga, *Gracilaria gracilis* Ph.D. Thesis, University of Cape Town, South Africa.
38. Henriques, B., Rocha, L.S., Lopes, C.B., Figueira, P., Monteiro, R.J., Duarte, A.C., Pardal, M.A., Pereira, E. 2015. Study on bioaccumulation and bio-sorption of mercury by living marine macroalgae: Prospecting for a new remediation biotechnology applied to saline waters. *Chemical Engineering Journal*, 281, 759–770.
39. Jacinto, J., Henriques, B., Duarte, A.C., Vale, C., Pereira, E. 2018. Removal and recovery of Critical Rare Elements from contaminated waters by living *Gracilaria gracilis*. *Journal of hazardous materials*, 344, 531–538.
40. Jensen, M.N. 2004. Cultivating Edible Seaweed in Hawaii: New technique helps local farmers. *Arizona Land and People*, 49(1) 12–13.
41. Jiménez, E., Dorta, F., Medina, C., Ramírez, A., Ramírez, I., Peña-Cortés, H. 2011. Anti-phytopathogenic activities of macro-algae extracts. *Marine Drugs*, 9(5), 739–756.
42. Khairy, H.M., El-Shafay, S.M. 2013. Seasonal variations in the biochemical composition of some common seaweed species from the coast of Abu Qir Bay, Alexandria, Egypt. *Oceanologia* 55(2), 435–452.
43. Khan, S.A., Abid, M., Hussain, F. 2015. Nematicidal activity of seaweeds against *Meloidogyne javanica*. *Pakistan Journal of Nematology*, 33(2), 195–203.
44. Leitao, D. 2005. Effects of Commercial Kelp Extract and plant growth regulators on growth of *Gracilaria gracilis* in culture. Ph.D thesis, University of Cape Town, South Africa.
45. Leite, Y.F.M.M., Silva, L.M.C.M., Amorim, R.C. das N., Freire, E.A., de Melo Jorge, D.M., Grangeiro, T.B., Benevides, N.M.B. 2005. Purification of a lectin from the marine red alga *Gracilaria ornata* and its effect on the development of the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Biochimica Biophysica Acta (BBA) - General Subjects*, 1724(1-2), 137–145.
46. Leódidio, A.C.M., Costa, L.E., Araújo, T.S., Costa, D.S., Sousa, N.A., Souza, L.K.M., Sousa, F.B.M., Filho, M.D.S., Vasconcelos, D.F.P., Silva, F.R.P., Nogueira, K.M., Araújo, A.R., Barros, F.C.N., Freitas, A.L.P., Medeiros, J.V.R. 2017. Anti-diarrhoeal therapeutic potential and safety assessment of sulphated polysaccharide fraction from *Gracilaria* intermedia seaweed in mice. *International Journal of Biological Macromolecules*, 97, 34–45.
47. Lima, M.E.P., Carneiro, M.E., Nascimento, A.E., Grangeiro, T.B., Holanda, M.L., Amorim, R.C.N., Benevides, N.M.B. 2005. Purification of a Lectin from the Marine Red Alga *Gracilaria cornea* and Its Effects on the Cattle Tick *Boophilus microplus* (Acari: Ixodidae). *Journal of Agricultural and Food Chemistry*, 53(16), 6414–6419.
48. Lin, Y.C., Yeh, S.T., Li, C.C., Chen, L.L., Cheng, A.C., Chen, J.C. 2011. An immersion of *Gracilaria*

- tenuistipitata* extract improves the immunity and survival of white shrimp *Litopenaeus vannamei* challenged with white spot syndrome virus. *Fish & Shellfish Immunology*, 31(6), 1239–1246.
49. Lordan, S., Ross, R.P., Stanton, C. 2011. Marine bioactives as functional food ingredients: potential to reduce the incidence of chronic diseases. *Marine Drugs* 9(6), 1056–1100.
  50. Lozano, I., Wacyk, J.M., Carrasco, J., Cortez-San Martín, M.A. 2016. Red macroalgae *Pyropia columbina* and *Gracilaria chilensis*: sustainable feed additive in the *Salmo salar* diet and the evaluation of potential antiviral activity against infectious salmon anemia virus. *Journal of Applied Phycology*, 28, 1343–1351.
  51. Madhiyazhagan, P., Murugan, K., Kumar, A.N., Nataraj, T., Subramaniam, J., Chandramohan, B., Panneerselvam, C., Dinesh, D., Suresh, U., Nicoletti, M., Alsalhi, M.S., Devanesan, S., Benelli, G. 2016. One pot synthesis of silver nanocrystals using the seaweed *Gracilaria edulis*: biophysical characterization and potential against the filariasis vector *Culex quinquefasciatus* and the midge *Chironomus circumdatus*. *Journal of Applied Phycology*, 29, 649–659.
  52. Magnoni, L.J., Martos-Sitcha, J.A., Queiroz, A., Caldach-Giner, J.A., Gonçalves, J.F.M., Rocha, C.M.R., Abreu, H.T., Schrama, J.W., Ozorio, R.O.A., Pérez-Sánchez, J. 2017. Dietary supplementation of heat-treated *Gracilaria* and *Ulva* seaweeds enhanced acute hypoxia tolerance in gilthead seabream (*Sparus aurata*). *Biology Open*, 6(6), 897–908.
  53. Makkar, F., Chakraborty, K. 2017. Antidiabetic and anti-inflammatory potential of sulphated polygalactans from red seaweeds *Kappaphycus alvarezii* and *Gracilaria opuntia*. *International Journal of Food Properties*, 20(6), 1326–1337.
  54. Marinho-Soriano, E., Bourret, E. 2003. Effects of season on the yield and quality of agar from *Gracilaria* species (*Gracilariaceae*, *Rhodophyta*). *Bioresource Technology*, 90(3), 329–333.
  55. Martín, L.A., Miravalles, A.B., Rodríguez, M.C., Leonardi, P.I. 2013. Epiphytism in a subtidal natural bed of *Gracilaria gracilis* of southwestern Atlantic coast (Chubut, Argentina). *Journal of Applied Phycology*, 25, 1319–1329.
  56. Mensi, F., Ben Ghedifa, A. 2019. Optimum ranges of combined abiotic factor for *Gracilaria gracilis* aquaculture. *Journal of Applied Phycology*, 31, 3025–3040.
  57. Mensi, F., Nasraoui, S., Bouguerra, S., Ben Ghedifa, A., Chalhaf, M. 2020. Effect of lagoon and sea water depth on *Gracilaria gracilis* growth and biochemical composition in the Northeast of Tunisia. *Scientific Reports*, 10(1), 10–14.
  58. Morgan, D. 2000. The potential for *Gracilaria* polyculture at Jacobs Bay Sea Products Pty. Bachelor's thesis, University of Cape Town, South Africa.
  59. Mouritsen, O.G., Rhatigan, P., Pérez-Lloréns, J.L. 2019. The rise of seaweed gastronomy: phycogastromony. *Botanica Marina*, 62(3), 195–209.
  60. Nabivailo, Y.V., Skriptsova, A.V., Titlyanov, E.A. 2005. Interactions of Algae within the Community of *Gracilaria gracilis* (*Rhodophyta*). *Russian Journal of Marine Biology*, 31, 288–293.
  61. Nguyen, H.P.T., Morançaïs, M., Délérís, P., Fleurence, J., Nguyen-Le, C.T., Vo, K.H., Dumay, J., 2020. Purification of R-phycoerythrin from a marine macroalga *Gracilaria gracilis* by anion-exchange chromatography. *Journal of Applied Phycology*, 32, 553–561.
  62. Njobeni, A. 2006. Experimental cultivation of the red seaweed, *Gracilaria gracilis* (*Rhodophyta*) in land-based tank culture systems on abalone farms in the Western Cape, South Africa. Ph.D thesis, University of Cape Town, South Africa.
  63. Özen, M., Kozak, A., Dere, Ş., Tüney Kızılkaya, İ.T. 2018. The effects of different salt concentrations on the biochemical contents of *Gracilaria gracilis* Greville (*Rhodophyta*). *Celal Bayar University Journal of Science*, 14(3), 303–307.
  64. Paiva, L., Lima, E., Patarra, R.F., Neto, A.I., Baptista, J. 2014. Edible Azorean macroalgae as source of rich nutrients with impact on human health. *Food Chemistry*, 164, 128–135.
  65. Pereira, T., Barroso, S., Mendes, S., Amaral, R.A., Dias, J.R., Baptista, T., Saraiva, J.A., Alves, N.M., Gil, M.M. 2020. Optimization of phycobiliprotein pigments extraction from red algae *Gracilaria gracilis* for substitution of synthetic food colorants. *Food Chemistry*, 321, 1–9.
  66. Polifrone, M., De Masi, F., Gargiulo, G.M. 2006. Alternative pathways in the life history of *Gracilaria gracilis* (*Gracilariales*, *Rhodophyta*) from north-eastern Sicily (Italy). *Aquaculture*, 261(3), 1003–1013.
  67. Rasyid, A., Ardiansyah, A., Pangestuti, R. 2019. Nutrient composition of dried seaweed *Gracilaria gracilis*. *Indonesian Journal of Marine Sciences*, 24(1), 1–6.
  68. Rizvi, M.A., Shameel, M. 2006. In vitro nematocidal activities of seaweed extracts from karachi coast. *Pakistan Journal of Botany*, 38(4), 1245–1248.
  69. Rodrigues, D., Freitas, A.C., Pereira, L., Rocha-Santos, T.A.P., Vasconcelos, M.W., Roriz, M., Rodríguez-Alcalá, L.M., Gomes, A.M.P., Duarte, A.C. 2015. Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. *Food Chemistry*, 183, 197–207.
  70. Rodríguez, M.C., Matulewicz, M.C., Nosedá, M.D., Ducatti, D.R.B., Leonardi, P.I. 2009. Agar from *Gracilaria gracilis* (*Gracilariales*, *Rhodophyta*) of the Patagonic coast of Argentina – Content, structure and physical properties. *Bioresource Technology*, 100(3), 1435–1441.

71. Ruangsomboon, S., Pumnuan, J. 2016. Acaricidal activities of algal extracts against the house dust mite, *Dermatophagoides pteronyssinus* (Trouesart). *Journal of the Acarological Society of Japan*, 25(Supplement1), S169-S178.
72. Sakthivel, R., Muniasamy, S., Archunan, G., Devi, K.P. 2016. *Gracilaria edulis* exhibit antiproliferative activity against human lung adenocarcinoma cell line A549 without causing adverse toxic effect in vitro and in vivo. *Food & Function*, 7(2), 1155–1165.
73. Škrovánková, S. 2011. Seaweed Vitamins as Nutraceuticals, in: *Advances in Food and Nutrition Research*, 64, 357–369.
74. Smit, A.J., Robertson-Andersson, D.V., Peall, S., Bolton, J.J. 2007. Dimethylsulfoniopropionate (DMSP) accumulation in abalone *Haliotis midae* (Mollusca: Prosobranchia) after consumption of various diets, and consequences for aquaculture. *Aquaculture*, 269(1-4), 377–389.
75. Umamaheswari, M., Chatterjee, T.K., 2008. In vitro antioxidant activities of the fractions of coccinia grandis l. leaf extract. *Afr J Tradit. Complement. Altern. Med*, 5(1), 61–73.
76. Yi, L., Wang, Q., Luo, H., Lei, D., Tang, Z., Lei, S., Xiao, H. 2022. Bioactive components from *Gracilaria rubra* with growth inhibition on HCT116 colon cancer cells and anti-inflammatory capacity in RAW 264.7 Macrophages. *Front. Nutr*, 9:856282.
77. Torres, P., Novaes, P., Ferreira, L.G., Santos, J.P., Mazepa, E., Duarte, M.E.R., Nosedá, M.D., Chow, F., dos Santos, D.Y.A.C. 2018. Effects of extracts and isolated molecules of two species of *Gracilaria* (Gracilariales, Rhodophyta) on early growth of lettuce. *Algal Research*, 32, 142–149.
78. Valente, L.M.P., Gouveia, A., Rema, P., Matos, J., Gomes, E.F., Pinto, I.S. 2006. Evaluation of three seaweeds *Gracilaria bursa-pastoris*, *Ulva rigida* and *Gracilaria cornea* as dietary ingredients in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture*, 252(1), 85–91.
79. Vuai, S.A.H. 2022. Characterization of agar extracted from *Gracilaria* species collected along Tanzanian coast. *Heliyon*, 8(2), 1–6.
80. Widowati, I., Lubac, D., Puspita, M., Bourgougnon, N. 2014. Antibacterial and antioxidant properties of the red alga *Gracilaria verrucosa* from the north coast of java, Semarang, Indonesia. *International Journal of Latest Research in Science and Technology*, 3, 179–185.
81. Williams, S.L., Bracken, M.E.S., Jones, E. 2013. Additive effects of physical stress and herbivores on intertidal seaweed biodiversity. *Ecology*, 94(5), 1089–1101.
82. Yang, Y., Chai, Z., Wang, Q., Chen, W., He, Z., Jiang, S. 2015. Cultivation of seaweed *Gracilaria* in Chinese coastal waters and its contribution to environmental improvements. *Algal Research*, 9, 236–244.
83. Zandi, K., Tajbakhsh, S., Nabipour, I., Rastian, Z., Yousefi, F., Sharafian, S., Sartavi, K. 2010. In vitro antitumor activity of *Gracilaria corticata* (a red alga) against Jurkat and Molt-4 human cancer cell lines. *African Journal of Biotechnology*, 9(40), 6787–6790.