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Seaweeds' carbohydrate polymers as plant growth promoters

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ABSTRACT

Currently, the high food demand led to a mass agriculture practices, whereas crop productivity is one of the major concerns for the producers. However, the intensification of the use of synthetic fertilizers has led to serious harmful impacts for human health and for the environment. The quest for novel and natural agricultural products is a current trend, hence seaweed polysaccharide bioactivity can contribute for this pursuit. The seaweed principal carbohydrates (agar, carrageenan and alginate) were extracted and chemically characterized from five red seaweeds, namely *Gracilaria gracilis* (Slender wart weed), *Asparagospis armata* (Harpoon weed), *Calliblepharis jubata* (False eyelash weed), *Chondracanthus teedei* var. *lusitanicus* and *Grateloupia turuturu* (Devil's tongue weed); and three brown seaweeds *Colpomenia peregrina* (Oyster thief), *Sargassum muticum* (Wireweed) and *Undaria pinnatifida* (Wakame). Afterwards, it was evaluated the impact of these sulphated polysaccharides on kale (*Brassica oleracea*) growth. Among the polymers tested, iota-carrageenan (from *Calliblepharis jubata*), kappa/ iota-carrageenan (from the female gametophyte of *Chondracanthus teedei* var. *lusitanicus*) and agar (from *Gracilaria gracilis*) showed a positive effect on kale growth. Through the quantification of polysaccharides and uronic acids, it appears that the polymer chemical structure is the decisive factor that affects the plant biostimulant activity.

1. Introduction

Brassica oleracea L. (kale) is one of the most important vegetable crops from Brassicaceae family, being a human food source cultivated worldwide (Rakow, 2004). Annually, it is produced near 105 million tons of crop vegetables belonging to the genus *Brassica* (Cartea, Lema & Francisco, 2011; Sanlier & Guler, 2018). Due to the exponential human population growth, food demand is a current challenge that undergo through sustainable vegetable crop production. An efficient and environmentally friendly management of the agricultural resources and practices is in fact aligned with the Sustainable Development Goal #12, presented in the 2030 Global Agenda (UN, n.d.). Thus, the replacement of synthetic fertilizers by natural products is a current need.

Enhancing seed germination and consequently plant growth and productivity for food security is a challenging task (Vijay Anand, G., Eswaran & Ghosh, 2018). Seeds and plants can be highly influenced by abiotic and biotic stresses, which limits their germination efficiency, growth, and productivity. However, seed germination and plant growth can be induced when treated with various abiotic and biotic elicitors (Shukla, Borza, Critchley & Prithiviraj, 2016). In this scenario, seaweed-based plant biostimulants are potential tools to develop novel products that improve seed germination and crop productivity in a sustainable way and alleviate climate change effects (Vijay Anand et al., 2018).

In this context, seaweeds are marine resources that produce multiple primary and secondary metabolites can have high impact in agriculture crops (Elgubbi, Zrmoh, Alzarride, Adam & El-Zaidy, 2019; Mzibra et al., 2018). Several studies demonstrated that various seaweed extracts can enhance seed germination (Demir, Dural & Yildirim, 2006; Hernández-Herrera, Santacruz-Ruvalcaba, Zañudo-Hernández &

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Hernández-Carmona, 2016; Silva, Bahcevandziev & Pereira, 2019; Sousa, Cotas, Bahcevandziev & Pereira, 2020), plant growth and development (Di Filippo-Herrera, Muñoz-Ochoa, Hernández-Herrera & Hernández-Carmona, 2019; Khan et al., 2009; Mireya Hernández-Herrera et al., 2018). Moreover, the application of seaweed compounds can have a positive effect on crop chemical characterization, such as chlorophyll and carotenoid content increment (Bharath, Nirmalraj, Mahendrakumar & Perinbam, 2018; Khan et al., 2009; Michalak, Dmytryk, Schroeder & Chojnacka, 2017).

Currently, the marine environment is being endangered by the overgrowth of non-indigenous seaweed species, threatening coastal fauna, flora and the ecosystem services they provide (European Union, 1979; van Kleunen, Weber & Fischer, 2010). The Iberian Peninsula has been targeted by several non-indigenous seaweeds, in which the red seaweeds *Asparagopsis armata, Grateloupia turuturu* and the brown seaweeds *Sargassum muticum, Undaria pinnatifida* and *Colpomenia peregrina* have been considered serious threats to the environment, due to their overgrowth (Pacheco et al., 2020). Thus, the assessment of the biotechnological applications and economical value of algal resources should be conducted, not only on native seaweed species, but also on non-indigenous seaweed species (Milledge, Nielsen & Bailey, 2016; Salvaterra, Green, Crowe & O'Gorman, 2013).

As the major constitution of seaweed biomass is composed by polysaccharides, this led to a growing interest on their commercial exploitation, particularly in agar, carrageenan, and alginate. Meanwhile, seaweeds polysaccharides structural and molecular diversity is not yet fully elucidated (Ermakova, Kusaykin, Trincone & Tatiana, 2015), being different according to the species and their life cycle, abiotic and biotic conditions. Henceforth, polysaccharides bioactivity is strongly related with its chemical composition and structure, so there is a need to characterize algal polysaccharides to understand their bioactivity (Mzibra et al., 2018).

For instance, seaweeds belonging to phylum Rhodophyta, class Florideophyceae, have a triphasic life cycle, exhibiting a gametophyte (female and male, this generation is mostly macroscopic), carposporophyte generation (first sporous generation, which is attached in female gametophyte until the release of the carpospores, which will lead to the development of the tetrasporophyte generation) and tetrasporophyte (which give gametophyte generation, through the production of tetraspores) (Garci-a-Jiménez & Robaina, 2015). Red seaweeds are commonly carrageenan producers, however in some cases the same species does not synthesize the same type of carrageenan along the life cycle (Pereira, 2004). Previous research shows that the seaweeds *Grateloupia turuturu* and *Chondrachantus teedei* var. *lusitanicus*, produce different polysaccharides according to the generation of the life cycle (Cardoso et al., 2019; Pereira, 2004; Soares, Fernandes, Silva, Pereira & Gonçalves, 2016). This could be caused by the differential gene expression in the different phases of seaweeds life cycle (Bi & Zhou, 2014; Lipinska, Collén, Krueger-Hadfield, Mora & Ficko-Blean, 2020).

The different generations of the life cycle of *G. turuturu* can be distinguished through morphological differences. Thus, the blade of the tetrasporophyte *G. turuturu* exhibits a smooth texture. While the non-fructified gametophyte presents spherical cystocarps at the blade level, the fructified gametophyte contains prominent cystocarps and cruciate tetrasporangia (Cardoso et al., 2019; Katsanevakis et al., 2014). In the species *C. teedei* var. *lusitanicus* the tetrasporophyte exhibits dark tetrasporangial sori on lateral branches, while the female gametophyte exhibits along the thallus, spherical and multiaxial cystocarps (Pereira & Silva, 2020). Hence, it is highlighted the presence of florid starch inside the tetrasporocysts and cystocarps (Pereira, 2004).

Considering that macroalgal polysaccharides can have a positive effect on plant growth (Abir Mzibra et al., 2020), the goal of this study was to evaluate how different algal polysaccharides extracted from eight seaweeds (native and non-indigenous) of the Portuguese coast can affect kale (*B. oleracea*) seed germination and plant growth.

2. Material and methods

2.1. Seaweed harvesting and preparation

During January, May and October 2020, five red seaweeds, namely *Gracilaria gracilis* (Slender wart weed), *Asparagospis armata* (Harpoon weed), *Calliblepharis jubata* (False eyelash weed), *Chondracanthus teedei* var. *lusitanicus* and *Grateloupia turuturu* (Devil's tongue weed); and three brown seaweeds *Colpomenia peregrina* (Oyster thief), *Sargassum muticum* (Wireweed) and *Undaria pinnatifida* (Wakame) were collected in two Portuguese seashores, in Buarcos Bay (Figueira da Foz) and Praia do Quebrado (Peniche) (Table 1). Afterwards seaweeds were transported in plastic blags in a coolbox to the laboratory and were frozen at −20 °C for further utilization, whereas samples that were used for biochemical analysis were stored at −80 °C.

Afterwards, the seaweeds were washed with filtered seawater to remove sand, epiphytes, and other detritus from the seaweed biomass. Due to carrageenan type variation, the red seaweed *G. turuturu* and *C. teedei* var. *lusitanicus* were separated according to their life cycle generations, using a binocular magnifying glass (Kern & Sohn GmbH, Germany). Then, the biomass was washed with distilled water to remove the salt content of seawater, placed in plastic trays, and dried in an air-forced oven (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain) during 48 h at 60 °C. After this procedure, the biological samples were milled (< 1 cm) with a commercial grinder (Taurus aromatic, Oliana, Spain) and stored in sterile flasks in a dark and dry place at room temperature (23 °C).

2.2. Polysaccharide extraction

2.2.1. Agar

Agar extraction was based in the method described by Li, Yu, Jin, Zhang and Liu (2008) with modifications. The extraction was done using dried seaweed (20 g) and it was added to 600 ml distilled water. Agar extraction was performed in an electric pressure cooker (Aigostar 300008IAU, Aigostar, Madrid, Spain) at a temperature of 115 °C with an air pressure of 80 Kpa, for 2 h. The solution was hot filtered, under vacuum, through a cloth filter supported in a Buchner funnel. After that, the extract was filtered under vacuum with a Goosh 2 silica funnel. The filtrated solution was allowed to gel at room temperature (23 °C), frozen overnight and thawed. Then, the thawed gel was dried in an air-forced oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

Table 1
Seaweed harvesting sites and sampling date.

Seaweed species	Location	GPS	Date of collection
Rhodophyta (red seaweed)			
*Asparagopsis armata**	Praia do Quebrado	39.368258, −9.372303	20/10/2020
Calliblepharis jubata	Buarcos Bay	40.165867, −8.885556	19/10/2020
Chondracanthus teedei var. *lusitanicus*	Buarcos Bay	40.165867, −8.885556	27/05/2020
Gracilaria gracilis	Buarcos Bay	40.165867, −8.885556	19/10/2020
*Grateloupia turuturu**	Buarcos Bay	40.165867, −8.885556	13/01/2020
Ochrophyta (brown seaweed)			
*Colpomenia peregrina**	Praia do Quebrado	39.368258, −9.372303	20/10/2020
*Sargassum muticum**	Buarcos Bay	40.165867, −8.885556	19/10/2020
*Undaria pinnatifida**	Buarcos Bay	40.165867, −8.885556	13/01/2020

* non-indigenous seaweed species.

2.2.2. Carrageenan

Carrageenan extraction was performed according to the method described by Pereira and van de Velde (2011). Before extraction, the milled seaweed (1 g) was pre-treated with an acetone (Fisher Chemicals, Portugal): methanol (VWR Prolabo Chemical, Portugal) (1:1) solution in a final concentration of 1% (m/v) (final volume: 100 ml; 50 mL acetone: 50 mL methanol) for 16 h, at 4 °C, to eliminate the organic-soluble fraction. The liquid solution was decanted, and the seaweed residues obtained were dried in an air-forced oven (Raypa DAF-135, R. Espinar S. L., Barcelona, Spain) at 60 °C before the extraction.

The dried samples were placed in 150 mL of NaOH (Applichem Panreac, USA) (1 M) (1 g of initial seaweed: 150 mL of NaOH solution) in a hot water bath system (GFL 1003, GFL, Burgwedel, Germany), at 85–90 °C, for 3 h. The solutions were hot filtered, under vacuum (Laborport N820, Lisbon, Portugal) through a cloth filter supported in a Buchner funnel. After that, the extract was again filtered under vacuum with a Goosh 2 silica funnel. The extract was evaporated (rotary evaporator model: 2,600,000, Witeg, Germany) under vacuum to one-third of the initial volume (50 mL). The carrageenan was precipitated by adding twice its volume of 96% ethanol (José Manuel Gomes dos Santos, Portugal) (100 mL). The carrageenan precipitated was washed with ethanol 96%, 48 h at 4 °C before dry in an air force oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

2.2.3. Alginate

The alginic acid was extracted using the modified method of Sivagnanavelmurugan, Radhakrishnan, Palavesam, Arul and Immanuel (2018). The milled seaweed were added to a solution of HCl (Fisher Chemicals, Portugal) at 1.23% (1:30 v:v) (3 mL of HCl: 87 mL of distilled water per 3 gs of dried seaweed) was added and kept at room temperature (23 °C) for 48 h. After 48 h, the solution was removed by filtration, under vacuum with a Goosh 2 silica funnel. The residue was washed with distilled water for 2 to 3 times. Then, the residue was alkali extracted in a 2% sodium carbonate (Fisher Chemicals, Portugal) (90 mL for the initial weight of the dried biomass; 1:30 m:v) for 48 h and the extract was filtered through a cloth filter supported in a Goosh 2 silica funnel, under vacuum to remove the residues from the alginate solution. Then 37% HCl (Fisher Chemicals, Portugal) was added to the filtrate for precipitation of alginic acid (1 ml of 37% of HCl: 30 ml of the final solution). The precipitate was separated by centrifugation (Christ Universal Junior II, Christ, Osterode/ Harz, Germany) (4000 rpm, for 15 min) and then the alginate was dried in an air force oven (60 °C, 48 h) (Raypa DAF-135, R. Espinar S.L., Barcelona, Spain).

2.3. Carbohydrate characterization

2.3.1. Carbohydrate and uronic acids analysis

Carbohydrate analysis from the dried algal biomass comprised the quantification of polysaccharides (analysed in the form of monosaccharides) and uronic acids. Samples were subjected to a simultaneous initial hydrolysis for both sugars' determinations, and then, to different procedures, depending on the type of sugar.

For uronic acids analysis, hydrolysis was stopped after 1 h and an aliquot of 0.5 ml was retrieved from each sample to a new tube. Samples were then subjected to the procedures described in Selvendran, March and Ring (1979) and Coimbra, Delgadillo, Waldron and Selvendran (1996), consisting on the reduction of the samples to neutral sugars and proceeding to total uronic acids quantification using a Biochrom EZ Read 2000 Microplate reader, reading at an absorbance of 520 nm wavelength. Galacturonic acid (Merck KGaA, Darmstadt, Germany) was used to create the calibration curve for the measurements and the colorimetric reagent used was 3-phenylphenol (Merck KGaA, Darmstadt, Germany).

Polysaccharide samples were run through a Thermo Scientific Trace 1310 chromatography equipment equipped with a flame ionization detector (GC-FID). A TG-WAXMS A (30 m length, 0.32 mm i.d., 0.25 μm film thickness) GC column was used, and the oven was programmed to an initial temperature of 180 °C, following a linear temperature increase of 5 °C min^{-1} until the final temperature of 230 °C, maintaining this temperature for 12 min. The carrier gas was Helium at a flow rate of 2.5 mL min^{-1}. The monosaccharides were identified by retention time comparison with standards. Quantification of sugars was performed by comparison of the sugar chromatographic peaks to the peaks obtained for the internal standard used (2-desoxyglucose). So, the standard was 2-desoxyglucose (Merck KGaA, Darmstadt, Germany).

2.3.2. Carbohydrate FTIR-ATR analysis

The Fourier-Transform Infrared Spectroscopy - Attenuated Total Reflection (FTIR-ATR) analysis is a method of infrared spectroscopy technique, which is widely used to study and characterize carbohydrates present in seaweeds (among other compounds) and it was based on the protocol described by Pereira, Gheda and Ribeiro-Claro (2013).

For FTIR-ATR analysis, the dried polysaccharides samples from the previous polysaccharide extraction stages, were powdered using a commercial mill, and subjected to direct analysis without any further preparation. This technique only needs dried milled (<1 mm) sample (without humidity) to be analysed.

FTIR-ATR spectra were recorded on an Perkin Elmer Spectrum 400 spectrometer (Waltham, MA, USA), with no need for sample preparation, since these assays only required dried samples (Pereira et al., 2013). All spectra are the average of two independent measurements from 650 to 1500 cm^{-1} with 128 scans, each at a resolution of 2 cm^{-1}.

2.4. Seed germination assay

Polysaccharide solution preparation was done by milling the dried polysaccharide and adding distilled water (1 mg/mL), under constant agitation (Labinco Model L34, Breda, Netherlands) until the complete dissolution of the polysaccharide. Afterwards, the polysaccharide solution was immediately used in the germination assay.

A pH meter (pH meter 3310 Jenway, Staffordshire, UK) and a portable electric conductivity meter (Portable conductivity meter ProfiLine Cond 3310 WTW, Oberbayern, Germany) were used to determine the pH and electric conductivity of the polysaccharide solution.

Kale seeds were disinfected through emersion for 1 min in a solution of sodium hypochlorite (José Manuel Gomes dos Santos, Portugal) (NaClO) 2% and rinsed for 3 min in a volume of 250 mL of distilled water (Rayorath et al., 2008). Previously, sterilized Petri dishes were prepared with cotton and filter paper. Subsequently, 70 mL of each polysaccharide solution, was added. The control was done with addition of distilled water in the same volume. Then, 25 disinfected kale seeds were sown in each Petri dish, sealed with parafilm, and incubated (Heraeus B5090E Incubator, Thermo Scientific, Osterode, Germany) at 25 ± 1 °C in darkness, for 9 days.

The plant growth parameters evaluated were:

- Germination percentage (GP): calculated according to Hernández-Herrera, Santacruz-Ruvalcaba, Ruiz-López, Norrie and Hernández-Carmona (2014)
- GP = (number of germinated seeds/total number of seeds) × 100
- Seedling shoot (measured from the cotyledon base to the apical bud) and radicular length, using a ruler.
- Fresh weight of the cotyledon's aerial and radicular parts, using an analytical scale (Kern, Germany).

2.5. Statistical analysis

All the polysaccharide extraction and characterization methods were done in triplicated, and the seed germination was done with four replicas.

Macronutrient profiles of the algae species studied were statistically analyzed and compared through non-metric multidimensional scaling

(nMDS), associated to Analysis of Similarities (ANOSIM) and Similarity Percentage Analysis (SIMPER), as well as Analysis of Variance (ANOVA), to assess differences in the studied components between species.

The seed germination assay statistical analysis was performed with the software Sigma Plot v.14. It was employed an ANOVA analysis to assess statistically differences between the germination percentage. While, Bonferroni multiple comparison t-test was used after the rejection of the ANOVA null hypothesis, to discriminate the differences between radicular and aerial part length and weight. The statistical analysis was performed comparing the different treatments with the control, being considered statistically different when p-value <0.05.

3. Results

3.1. Carbohydrate characterization

Carbohydrate characterization, essential to analyze chemical and structural differences between the polysaccharides analysed, allowing the comprehension of its overall impact on plant growth.

3.1.1. Polysaccharide yield and solution characterization

In Table 2, it is demonstrated the carbohydrate extraction yields for each sample and the corresponding main polysaccharide present (alginate/ agar/ carrageenan), based on the literature (Imeson, 2009; Pereira et al., 2013). The extraction procedure was based in the previous literature and from small scale extractions before this work, when we optimized method for each species, mainly the hybrid polysaccharide seaweeds. In this case we choose the polysaccharide extraction technique, according to the polysaccharide in higher concentration.

It is clear that the highest yield of polysaccharide was from the female and male gametophyte of *C. teedei* var. *lusitanicus*, (40.9% and 42.1%, respectively), when compared with all the samples analyzed.

Moreover, the pH varied between the polysaccharides solutions, in which alginophytes have an acidic pH, like the agarophyte (*G. gracilis*) and the agar/carrageenan hybrid (*A. armata*). On the alkaline pH, we had the carrageenophytes fraction of both, female and male gametophyte of *C. teedei* var. *lusitanicus*. Regarding the conductivity, alginophytes presented a higher conductivity; while, the carrageenophytes had low conductivity demonstrating variations between the polysaccharides analyzed.

3.1.2. Polysaccharide and uronic acids profile

Polysaccharide analysis identified six different residues (Table 3), namely ribose, arabinose, xylose, mannose, galactose and glucose, although the content of galactose and glucose was provided jointly. Galactose and glucose (Gal + Glc) content was, in general, the most abundant residue, apart from *S. muticum* and *U. pinnatifida*, where ribose was the most abundant residue. Significant differences regarding the content of each residue were assessed between the species studied. The polysaccharide profile of *S. muticum* stands out from the remaining species due to the species' ribose and mannose content, particularly higher than those of the remaining species, and because this species does not present arabinose in its profile. *Undaria pinnatifida, G. gracilis* and *C. peregrina* also stand out due to their relatively high mannose content, while there is a clear dominance of the residue xylose in the species *C. jubata, A. armata* and the three phases of the life cycle of *C. teedei* var. *lusitanicus* and *G. turuturu*.

Uronic acid analysis (Table 3) allowed the observation of significant differences among the groups, with *G. gracilis* standing out from the remaining species, exhibiting a considerable higher content of uronic acids. The female gametophyte of *C. teedei* var. *lusitanicus*, on the other hand, presented the lowest content in uronic acids.

3.1.3. Polysaccharide profile

The extracted polysaccharides were analyzed by FTIR-ATR. This spectroscopic technique allowed the polysaccharide characterization in a rapid, nondestructive manner, demanding low amounts of sample (Pereira & Mesquita, 2003). The obtained spectra were reviewed with bibliographic support (Chandía, Matsuhiro, Mejías & Moenne, 2004; Pereira et al., 2013; Pereira, Amado, Critchley, van de Velde & Ribeiro-Claro, 2009; Rupérez, Ahrazem & Leal, 2002). Due to the three main types of polysaccharides (which have different FTIR-ATR spectra), we divided the spectra into different divisions, based in the polysaccharide profile (agar/ hybrids; carrageenan; alginate) (Fig. 1: agar and hybrid agar/carrageenan, Fig. 2: carrageenan and Fig. 3: alginate). Moreover, the idealized structure of the chemical unites of agar and the different main types of carrageenan is presented in the Fig. 2. Due to the similarity of FTIR-ATR peaks in the red seaweeds, Table 4 presents the FTIR-ATR bands identification and characterization peaks of red seaweeds, while Table 5 is for brown seaweeds.

Hence, it is emphasized that *A. armata* (Fig. 1a) and *G. gracilis* (Fig. 1b) are more similar than *G. turuturu* (tetrasporophyte, non-fructified and fructified gametophyte) samples (Fig. 1c, d, e), which have higher sulfate esters content (1240 cm^{-1}) and two different peaks (830 and 845 cm^{-1}). However, the region between 690 and 800 cm^{-1} is similar with all the samples.

In Fig. 3 there are two identical spectra, corresponding to both gametophytes of *C. teedei* var. *lusitanicus* (Fig. 3c, d). However, between them and other carrageenan there is a high dissimilar spectrum,

Table 2
Extraction yield and polysaccharides solution (1 mg/mL) characterization. The extraction yield results are expressed in mean ± standard deviation ($n = 3$). NA – Not applicable.

Seaweed species	Polysaccharide described in the literature (Imeson, 2009; Pereira et al., 2013)	Extraction technique	Extraction yield (%)	pH	CE (μS cm^{-1}; 25 °C)
Gracilaria gracilis	Agar	agar	27.0 ± 2.2	3.1	349
Asparagopsis armata	agar/ carrageenan	agar	10.6 ± 3.3	2.6	906
Grateloupia turuturu **(non-fructified gametophyte)**	carrageenan/ agar	carrageenan	15.0 ± 3.4	7.5	270
Grateloupia turuturu **(fructified gametophyte)**	carrageenan/ agar	carrageenan	7.6 ± 0.3	5.8	283
Grateloupia turuturu **(tetrasporophyte)**	carrageenan/ agar	carrageenan	23.0 ± 3.9	6.7	269
Calliblepharis jubata	carrageenan	carrageenan	10.4 ± 0.3	4.4	272
Chondracanthus teedei **var.** ***lusitanicus*** **(male gametophyte)**	carrageenan	carrageenan	42.1 ± 4.5	9.0	244
Chondracanthus teedei **var.** ***lusitanicus*** **(female gametophyte)**	carrageenan	carrageenan	40.9 ± 1.5	8.7	256
Chondracanthus teedei **var.** ***lusitanicus*** **(tetrasporophyte)**	carrageenan	carrageenan	28.1 ± 8.1	7.3	210
Colpomenia peregrina	alginate	alginate	13.0 ± 0.6	3.1	975
Sargassum muticum	alginate	alginate	15.1 ± 0.2	2.9	758
Undaria pinnatifida	alginate	alginate	8.7 ± 1.3	3.1	667
NA	distilled water	NA	NA	7.0	1.9

Table 3
Uronic acids and polysaccharide residues profile and content of each residue of the algae species studied. Rib – ribose, Ara – arabinose, Xyl – xylose, Man – mannose, Gal + Glc – joint content of galactose and glucose. The results are expressed in mean ± standard deviation. Statistically significant differences found in the content of a residue among the studied species are expressed by letters.

	Uronic Acids (μg.g $^{-1}$ of dried weight seaweed)	Polysaccharide (mg.g $^{-1}$ of dried weight seaweed)					
Seaweed Species		Rib	Ara	Xyl	Man	Gal + Glc	N
Asparagopsis armata	204.00 ± 8.37^{a}	0.70±0.14^{c}	0.36±0.04	2.69±0.58^{a}	0.73±0.08^{d}	32.86±1.35	5
Calliblepharis jubata	313.97±45.99^{c}	0.16±0.09^{c}	0.35±0.01	4.80±0.30^{b}	0.00±0.00	44.74±4.58	4
***Chondracanthus teedei* var. *lusitanicus* (male gametophyte)**	295.13±12.94^{c}	0.46±0.16^{c}	0.22±0.16	4.79±1.47^{b}	0.00±0.00	74.99±9.06	4
***Chondracanthus teedei* var. *lusitanicus* (female gametophyte)**	57.54 ± 11.63^{d}	0.72±0.28^{c}	0.22±0.16	4.27±1.07^{b}	0.00±0.00	74.99±9.06	4
***Chondracanthus teedei* var. *lusitanicus* (tetrasporophyte)**	295.34±11.58^{c}	0.36±0.08^{c}	0.13±0.02	5.38±0.20^{b}	0.08±0.11^{c}	45.10±15.21	5
Colpomenia peregrina	214.14±13.47^{a}	6.26±0.31^{d}	0.29±0.02	1.52±0.42^{a}	2.34±0.64^{b}	9.56±3.21	5
***Grateloupia turuturu* (non-frutidied gametophyte)**	147.85±50.96^{a}	0.55±0.08^{c}	0.44±0.05	20.99±0.11^{b}	0.29±0.01^{c}	24.48±3.27	5
***Grateloupia turuturu* (frutified gametophyte)**	143.24±50.29^{a}	0.45±0.03^{c}	0.24±0.03	6.01±0.27^{b}	0.27±0.01^{c}	19.74±3.03	5
***Grateloupia turuturu* (tetrasporophyte)**	156.64±50.44^{a}	0.49±0.09^{c}	0.30±0.05	4.23±2.29^{d}	0.23±0.11^{c}	24.80±11.72	5
Gracilaria gracilis	612.02±90.28^{b}	0.38±0.06^{c}	0.66±0.28	3.13±1.85^{d}	3.25±0.72^{b}	74.40±18.92	5
Sargassum muticum	147.23±50.04^{a}	15.05±1.88^{a}	0.00±0.00	1.63±0.16^{a}	20.71±4.59^{a}	4.37±0.38	4
Undaria pinnatifida	143.34±89.76^{a}	4.04±0.25^{b}	0.41±0.03	0.42±0.02^{c}	4.02±0.20^{b}	3.53±0.26	5

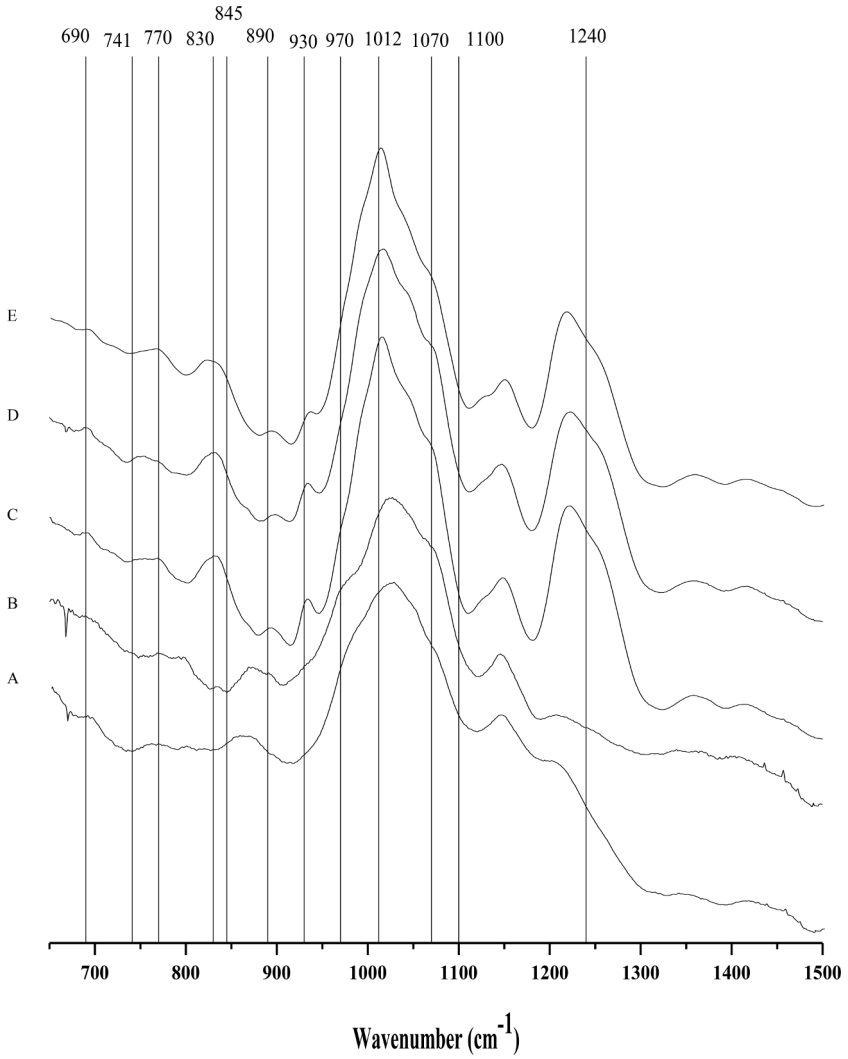

Fig. 1. FTIR-ATR spectra of the agarophyte and hybrids agar/carrageenan producers: (A) *Asparagopsis armata*, (B) *Gracilaria gracilis*, (C) *Grateloupia turuturu* tetrasporophyte, (D) *G. turuturu* non-fructified and (E) *G. turuturu* fructified gametophyte.

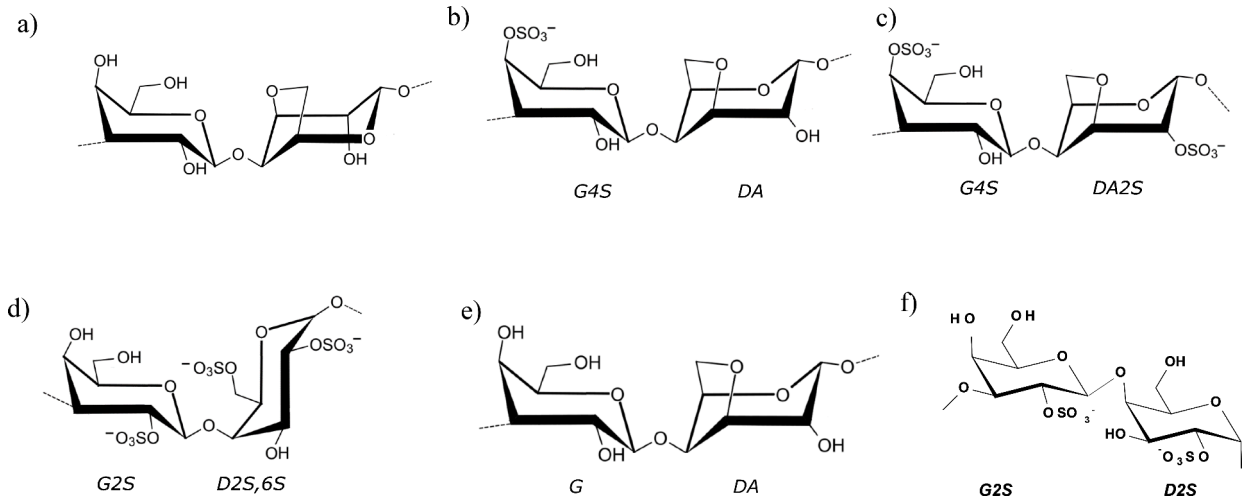

Fig. 2. Idealized structure of the chemical units of a) agar and the different main types of carrageenan b) kappa-carrageenan; c) iota-carrageenan; d) lambda carrageenan, e) beta-carrageenan and f) xi-carrageenan.

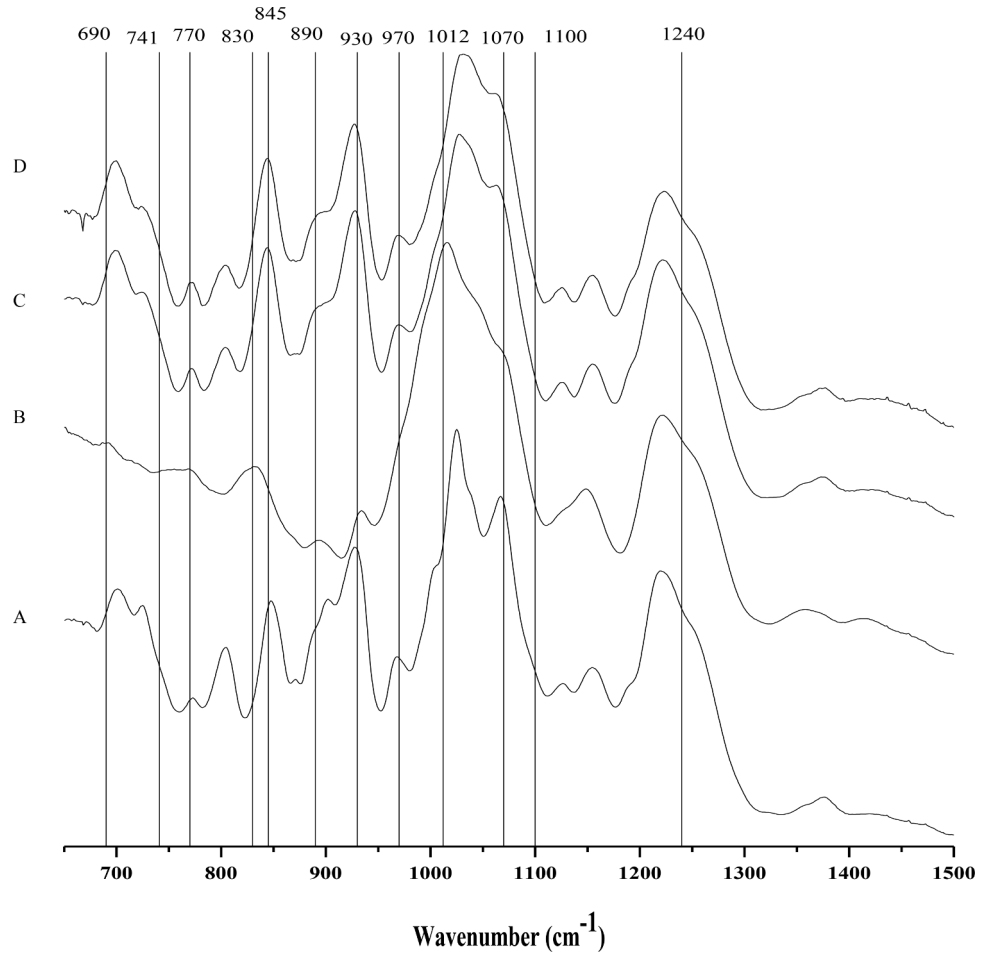

Fig. 3. FTIR-ATR spectra of the carrageenophytes: (A) *Calliblepharis jubata*, (B) *Chondracanthus teedei* var. *lusitanicus* tetrasporophyte, (C) *C. teedei* var. *lusitanicus* male and (D) *C. teedei* var. *lusitanicus* female gametophytes.

demonstrating different types of carrageenan. The similar peaks are 1012 cm^{-1} region peak, 930 cm^{-1} and 1240 cm^{-1}.

Gracilaria gracilis FTIR-ATR (Fig. 1b) demonstrates peaks indicating agar linkages with low sulfate esters as *A. armata* sample (Fig 1a) (agar peaks: 690, 741 and 790 cm^{-1}), unlike *G. turururu* samples which showed higher sulfate ester content (Fig. 1c, d, e). Thus, the FTIR-ATR analysis of *A. armata* (Fig. 1a) and *G. turururu* (in all the life cycle) (Fig. 1c, d, e) demonstrates a hybrid polysaccharide consisting in agar/ carrageenan or carrageenan/ agar form, respectively. The red seaweed *G. turururu* (Fig. 1c, d, e) demonstrated that the three phases had the same hybrid polysaccharide. The peaks presented in the spectra support the presence of a hybrid kappa/ iota/ theta carrageenan with some

Table 4
FTIR-ATR bands identification and characterization of the red seaweed polysaccharides (agar and carrageenan), based on literature (Pereira et al., 2009; 2013). AA- *Asparagopsis armata*, GG- *Gracilaria gracilis*, GTT- *Grateloupia turuturu* (tetrasporophyte), GTGNF- *G. turuturu* (non-fructified gametophyte), GTGF- *G. turuturu* (fructified gametophyte), CJ- *Calliblepharis jubata*, CTGF- *Chondracanthus teedei* var. *lusitanicus* (female gametophyte), CTTNF- *C. teedei* var. *lusitanicus* (male gametophyte), CTT- *C. teedei* var. *lusitanicus* (tetrasporophyte).

Wave number (cm^{-1})	Bound	Compound	AA	GG	GTT	GTGNF	GTGF	CJ	CTGF	CTTNF	CTT
690	3,6- anhydro-L-galactose (agar)	Agar	+	+	+	+	+		-	-	-
741	C-S/C-O-C bending mode in glycosidic linkages of agars	Agar	+	+	+	+	+	-	-	-	-
790	Characteristic of agar-type in second derivative spectra	Agar	+	+	+	+	+	-	-	-	-
805	C–O–SO3 on C2 of 3,6-anhydrogalactose	DA2S	+	+	-	-	-	+	+	+	-
815–820	C–O–SO3 on C6 of galactose	G/D6S	+	-	-	-	-	-	-	-	-
825–830	C–O–SO3 on C2 of galactose	G/D2S	-	-	+	+	-	-	-	-	+
845	D-galactose-4-sulfate	G4S	-	+	+	-	+	+	+	+	-
867	C–O–SO3 on C6 of galactose	G/D6S	+	-	-	-	-	-	+	+	-
890–900	Unsulphated b-D-galactose	G/D	-	+	-	+	+	-	+	sh	sh
905	C–O–SO3 on C2 of 3,6-anhydrogalactose	DA2S	+	-	-	-	-	+	Sh	sh	sh
930	C–O of 3,6-anhydrogalactose (agar/carrageenan)	(DA)	-	Sh	+	+	+	+	+	+	sh
970–975	Galactose	G/D	-	-	-	-	-	+	+	+	-
1012	Sulphated esters	S = O	+	+	+	+	+	+	+	+	+
1070	C–O of 3,6-anhydrogalactose	DA	-	Sh	sh	sh	sh	+	+	+	sh
1100	Sulphated esters	S = O	+	+	+	+	+	+	+	+	+
1240–1260	Sulphated esters	S = O	+	+	+	+	+	+	+	+	+

Sh- shoulder (where peak demonstrate intensity, but not enough to be considered a peak due to the surrounding peak intensities).

Table 5
FTIR-ATR bands identification and characterization of the brown seaweed polysaccharides (alginate), based on literature (Pereira et al., 2013). CP- *Colpomenia peregrina*, SM - *Sargassum muticum*, UP- *Undaria pinnatifida*.

Wave number (cm^{-1})	Bound	CP	SM	UP
788	Mannuronic acids residues	+	+	+
806	Guluronic acids residues	+	+	+
1020	Alginic acid	+	+	+
1232	Fucoidan	+	+	+
930–950	C-O stretching vibration of uronic acids	+	+	+

vestigial presence of agar (agar: 690, 741 and 790 cm^{-1}; kappa: 930 and 845 cm^{-1}; iota: low peak at 805 cm^{-1}; theta: low shoulders at 905, 930, and 1070 cm^{-1}). These results demonstrate that *G. turuturu* has a high percentage of kappa carrageenan, with low content of agar, theta carrageenan and iota carrageenan. On the other hand, the *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 3b) has a hybrid xi/ theta carrageenan, due to the presence of three shoulder peaks at 905 cm^{-1}, 930 cm^{-1}, and 1070 cm−1 (DA) in the FTIR spectrum, which is related to the presence of theta-carrageenan (Pereira et al., 2009; Soares et al., 2016) (similar to *G. turuturu* spectra). On the other hand, the *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 3b) does not have peaks in the agar typical bonds, which demonstrate that this species does not have the presence of agar as the *G. turuturu*. In other hand, the *C. teedei* var. *lusitanicus* tetrasporophyte was wide peak in 830 cm^{-1} which is typical of two main peaks near, which are typical from the xi-carrageenan. In this case, the wide and standout peak demonstrates that the *C. teedei* var. *lusitanicus* tetrasporophyte (Fig. 3b) has a xi/ theta carrageenan (Soares et al., 2016).

The male and female *C. teedei* var. *lusitanicus* gametophytes (Fig. 2c, d) presented similar FTIR-ATR spectra, which corresponds to a hybrid kappa/iota carrageenan (presence of the peaks: kappa: 930 and 845 cm^{-1}; iota: 805 cm^{-1}). The FTIR-ATR analysis of *C. jubata* (Fig. 3a) has predominance of bounds that indicates the presence of iota-carrageenan with a low content in kappa-carrageenan (presence of the peaks: iota: 805 cm^{-1}, and kappa: low intense peaks at 930 and 845 cm^{-1}). Also the FTIR-ATR analysis demonstrate the inexistence of glucose typical bonds between 1106 and 1150 cm^{-1}, which demonstrate that there is a low hypothesis of glucose presence in the polysaccharides, although as demonstrated by the FTIR-ATR analysis there is a high content in galactose units (Bartošová, Soldán, Sirotiak, Blinová & Michaliková, 2013; Z. Li et al., 2017; Pereira et al., 2009).

The spectra of alginophytes have differences between the seaweed analyzed, with *U. pinnatifida* (Fig. 5c) being the most different, demonstrating that the alginate structure can be different as observed, particularly, by peaks at 950 and 788 cm^{-1} and by the sulfate esters at 1232 cm^{-1}. Moreover, in the Fig. 4 is presented the idealized chemical structure of the a) alginic acid and b) fucoidan.

The brown seaweeds FTIR-ATR spectra showed typical alginic acid peaks, which indicates more units of mannuronic than guluronic acid (ratio m/g, 788>806). Only *S. muticum* presented an identical concentration of this two uronic acids (Chandía et al., 2004), the *C. peregrina* have more mannuronic acid and *U. pinnatifida* have more guluronic acid (Pereira et al., 2013).

3.2. Seed germination assay

Regarding germination percentage, it was not observed statistically

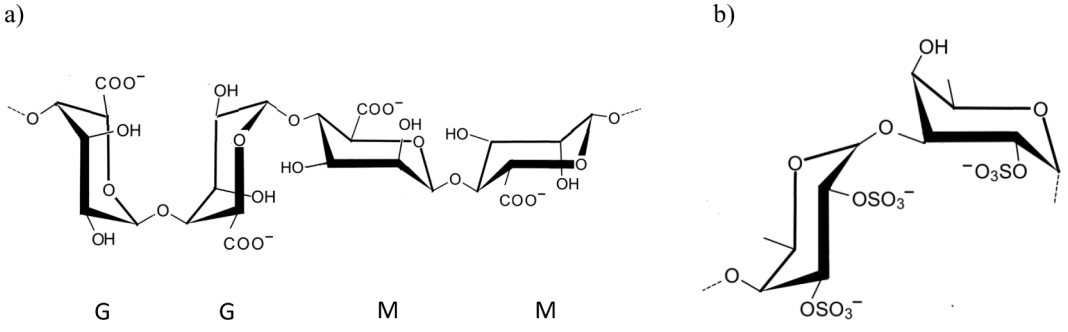

Fig. 4. Idealized structure of the chemical units of a) alginic acid and b) fucoidan.

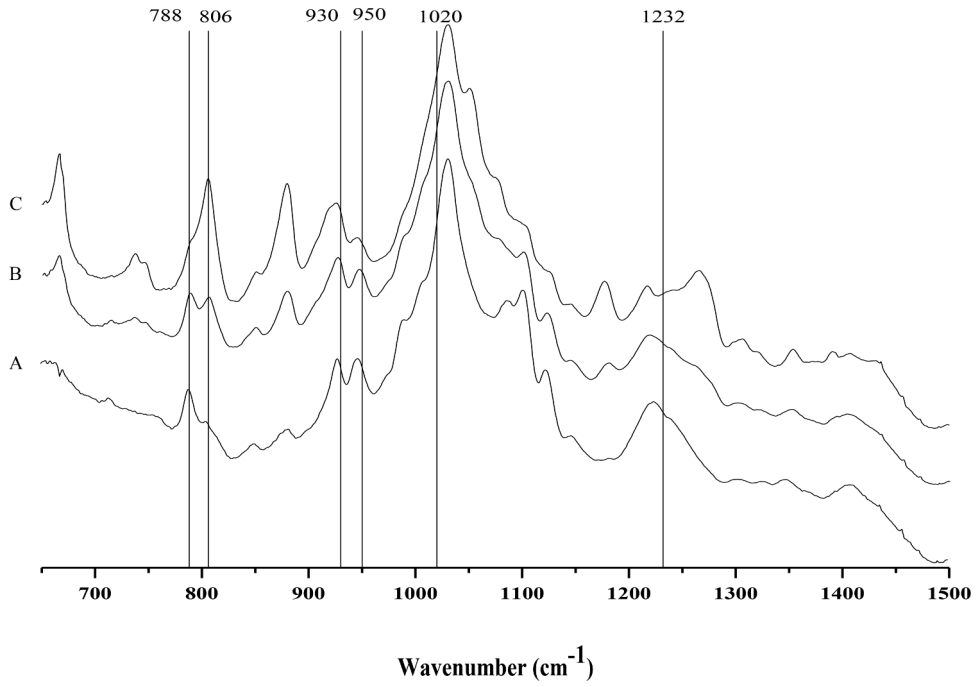

Fig. 5. FTIR-ATR spectra of the alginophytes: (A) *Colpomenia peregrina*, (B) *Sargassum muticum* and (C) *Undaria pinnatifida*.

differences between the treatments (Fig. 6a). Meanwhile, all the polysaccharides' solutions seemed to cause a negative effect on the radicular growth and weight, presenting average values lower than the control (Fig. 6b and 6 c). Only, the female gametophyte of *C. teedei* var. *lusitanicus* showed differences from the control, regarding the radicular length and weight.

The positive effect that *C. jubata, G. gracilis* and the female gametophyte of *C. teedei* var. *lusitanicus* revealed on the aerial part length, as they exhibited higher average values when compared with the control (Fig. 6d). This positive effect is also reflected on the aerial part weight, while the female gametophyte of *C. teedei* var. *lusitanicus* stands out positively from the control (Fig. 6e).

After 9 days of incubation, it was evaluated the growth parameters of the germination assay (Fig. 7). It is possible to see a dark color in one of the four replicates of alginate from *S. muticum*, that could be caused by the hydrolyzation of the seed capsule. Contrarily, the polysaccharide from *A. armata* did not promote seed germination, demonstrating an inhibitory effect.

4. Discussion

Agar extraction from *G. gracilis* demonstrates a slightly lower percentage than found from other authors, such as Marinho-Soriano and Bourret (2003) or Martín et al. (2013). However, it is necessary to consider the different geographical locations of the sampling sites. For instance, *G. gracilis* from the study conducted by Marinho-Soriano and Bourret (2003) was performed with a seaweed collected in the Mediterranean Sea, whereas Martín et al. (2013) harvested the algae in the Patagonian coast of Argentina. Regarding the non-native species, *A. armata* collected from Peniche coast, it was reported by Marcia, Clélia, Teresa and Paulo (2014) a yield of extraction of 16%. While, for *G. turuturu*, there is no bibliographic information regarding their polysaccharide extraction yield with the same methodology employed in this study. The carragenophyte, *C. jubata* demonstrated a lower content of carrageenan when compared to other studies of Araujo et al. (2020) and Zinoun and Cosson (1996), which were conducted in Buarcos Bay (Portugal) and in the Normandy coast (France), respectively. However identical results were obtained in the same season and geographical sampling site by Pereira (2004). The carrageenan extraction yield of *C. teedei* var. *lusitanicus* (female and male gametophyte, tetrasporophyte) is in line with the results of Pereira (2004), whereas the sampling site was the same of this study. Regarding the alginate extracted from *S. muticum*, this study reveals a lower yield when compared with the same species harvested in Morocco (El Atouani et al., 2016). Only *C. peregrina* presented a higher yield when compared to the literature (Beacham et al., 2019; Rostami, Tabarsa, You & Rezaei, 2017).

This variance in the yield of polysaccharide within the life cycle phase could be explained by the negative correlation between the seaweed dry weight and the carrageenan content, as well as the hygroscopic properties of carrageenan (Pereira, 2013). Moreover, there are several abiotic and biotic factors (such as, light intensity, temperature, salinity, pH, herbivory, wave exposure and weather conditions) that can affect the polysaccharide yield on seaweeds. Previous research showed that it is on spring and in the beginning of summer that seaweeds synthesize more polysaccharides quantity (Cotas, Figueirinha, Pereira & Batista, 2019; Pereira & Mesquita, 2004; Zinoun & Cosson, 1996).

The FTIR-ATR analysis of *C. jubata* is in concordance with the analysis of Pereira et al. (2009), which observed an iota-carrageenan with low/residual content of kappa-carrageenan. The FTIR-ATR analysis of *C. teedei* var. *lusitanicus* (female and male gametophyte, tetrasporophyte), were also similar to the results obtained by Pereira (2004) and Soares et al. (2016).

All the brown seaweeds spectra presented alginate peaks, but it was also detected sulfate esters which can be derived from sulphated polysaccharides, such as fucoidan and laminarin present in the wave number 1220 cm^{-1}. At 790 and 800 cm^{-1}, there is a peak demonstrating sulfate groups of the uronic acids (mainly, from guluronic) (Pereira et al., 2013).

Regarding polysaccharide and uronic acids composition, information in literature is scarce or null about the seaweeds used in this study. However, it was found that *G. gracilis* collected in the South Africa exhibited 7.03% ribose, 6.89% arabinose, 0.06% xylose, 3.33% galactose and 6.16% glucose (Olasehinde, Mabinya, Olaniran & Okoh, 2019). The results in this study are in accordance with the ones reported by the previous cited work, excluding the xylose concentration which hereby presented lower values.

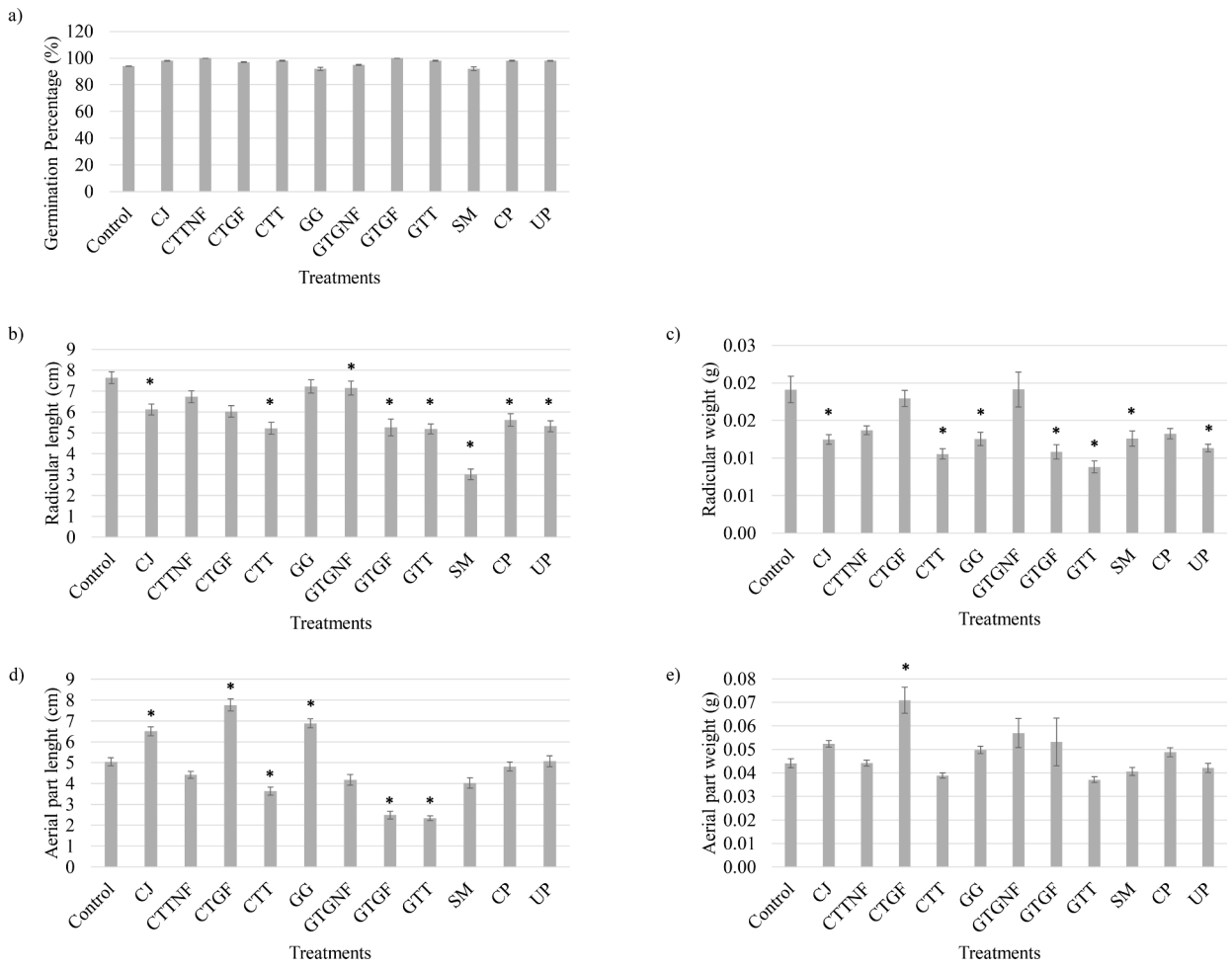

Fig. 6. a) Germination percentage; b) radicular length and c) weight; d) aerial part length and e) weight. * p-value < 0.05, comparing with the control. The graphs present average values and standard error ($n = 4$). CJ- *Calliblepharis jubata*, CTTNF- *Chondracanthus teedei* var. *lusitanicus* (male gametophyte), CTGF- *C. teedei* var. *lusitanicus* (female gametophyte), CTT- *C. teedei* (tetrasporophyte), GG- *Gracilaria gracilis*, GTGNF- *Grateloupia turuturu* (non-fructified gametophyte), GTGF- *G. turuturu* (fructified gametophyte), GTT- *G. turuturu* (tetrasporophyte), SM- *Sargassum muticum*, CP- *Colpomenia peregrina*, UP- *Undaria pinnatifida*.

Regarding the polysaccharide solutions applied in the seed germination assay, all of them presented a conductivity lower than 1 000 μS cm^{-1}, which is essential to seed germination (Li, Shi & Fukuda, 2010). The electrical conductivity (EC) is directly related to salinity, thus the increase of the EC values will have a negative impact on plant cell homeostasis, causing a lower water absorbency, compromising metabolic pathways (Kaya, Okçu, Atak, Çıkılı & Kolsarıcı, 2006; Uçarlı, 2020; Wong & Wong, 1989). The pH also affects seed germination and development (Shoemaker & Carlson, 1990). For instance, neutral pH is optimal for seed germination, conversely acid or basic pH can inhibit seed germination (Laghmouchi, Belmehdi, Bouyahya, Skali Senhaji & Abrini, 2017). The pH and EC were different within the samples, demonstrating that, even at the same concentration, but from different seaweed sources, the polysaccharides had affected differently the EC and pH. There are several physico-chemical parameters that affect the polysaccharides pH and their rheological properties, such as the polysaccharide concentration and temperature. For instance, low agar concentrations result in a lower pH (Yu et al., 2020). The different types of carrageenan also presents different rheological properties and pH sensitivity, being very stable under pH above 6, while between 3.5 and 6 pH values, some of their bioactivities can be affected (CP Kelco, 2002). As alginate contains carboxylate groups in its backbone that are protonated, forming hydrogen bonds, alginate solutions can reach a pH between 3–3.5 (Lee & Mooney, 2012). Uronic acids are an integral component of polysaccharides, such as pectin and alginate, commonly presenting an acidic pH (Mehtiö et al., 2016; Ohtani, Okai, Yamashita, Yuasa & Misaki, 1995).

The presence and location of sulfate groups makes seaweeds polymers, such as agar, alginate, and carrageenan biologically active. However, these bioactivities are affected by the sulphation degree, their concentration and oxidation (Zhong et al., 2020). Typically, alginophytes present the lower content of sulfate groups, while carrageenophytes present the higher content (Cunha & Grenha, 2016; Ma et al., 2017; Zhong et al., 2020). Nevertheless, carrageenan chemical structure is very heterogenous, and depending on the seaweeds species and the extraction method employed, there are three main types of carrageenan that can be obtained: kappa, iota, and lambda. However, there are other types of carrageenan reported, such as xi, mu or theta (Cunha & Grenha, 2016). These different types of carrageenan mainly differ on the sulphation degree and the position of the sulfate groups on the molecule. In this context, according to the literature, kappa-carrageenan exhibits 25–30% sulfate content, while iota-carrageenan presents 28–30% and lambda-carrageenan contains the highest sulfate concentration (32–39%) (Cunha & Grenha, 2016; dos Santos & Grenha, 2015).Despite the overall lower radicular length when compared with the control, *G. gracilis* and both male and female gametophyte of *C. teedei* var. *lusitanicus* achieved the highest results. In these treatments, EC (349, 244 and 256 μS cm^{-1}, respectively) were relatively similar, but the pH (3.1, 9.0 and 8.7, respectively) was different, suggesting that pH may influence the radicular growth.

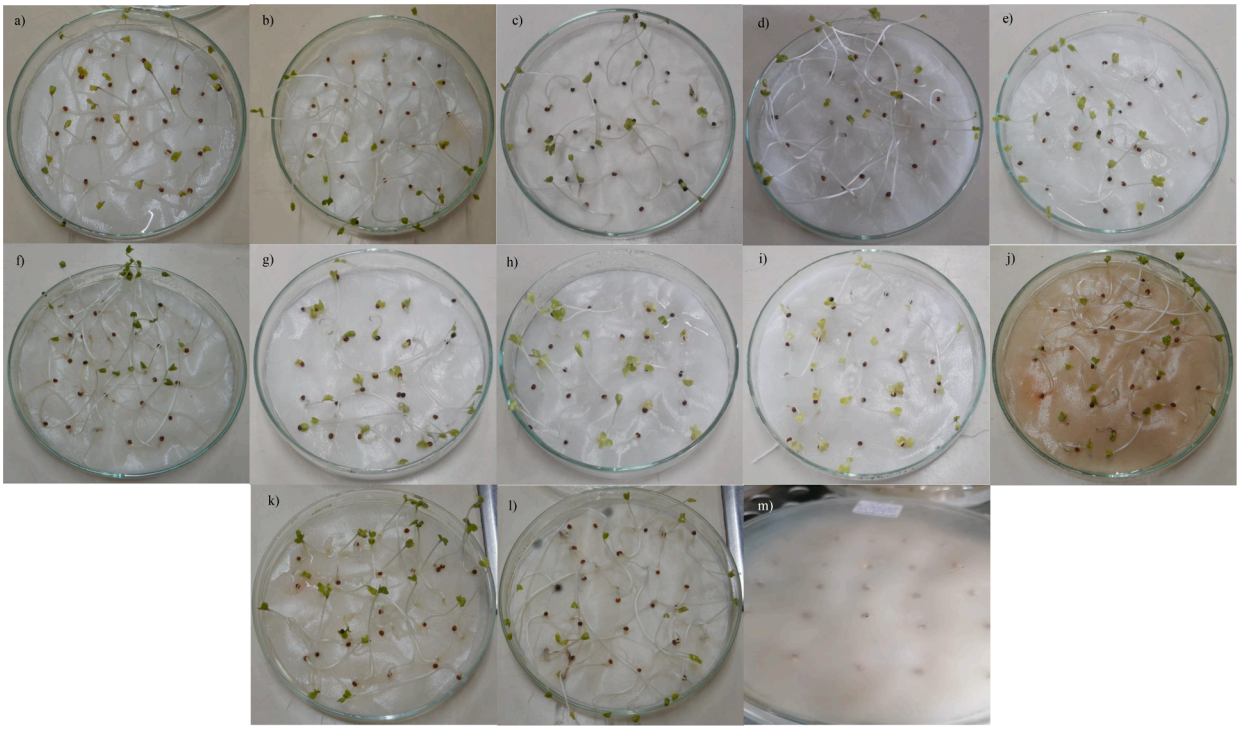

Fig. 7. Photographic record of a) control, b) *Calliblepharis jubata*, c) *Chondracanthus teedei* var. *lusitanicus* (male gametophyte), d) *C. teedei* var. *lusitanicus* (female gametophyte), e) *C. teedei* (tetrasporophyte), f) *Gracilaria gracilis*, g) *Grateloupia turuturu* (non-fructified gametophyte), h) *G. turuturu* (fructified gametophyte), i) *G. turuturu* (tetrasporophyte), j) *Sargassum muticum*, k) *Colpomenia peregrina*, l) *Undaria pinnatifida* and m) *A. armata* after 9 days of incubation.

Moreover, the main difference between the male and female gametophytes of *C. teedei* var. *lusitanicus* and *G. gracilis* is the uronic acids concentration (295.34, 57.54 and 612.02 μg.g $^{-1}$), that can interfere in the plant development, mainly in the cell walls rigidity (Lyczakowski et al., 2017). However, in the mentioned species it was found a similar glucose and galactose concentration, which can be an essential key for the plant development (Hu, Shi, Zhang, Zhang & Li, 2012). Regarding the radicular weight, the control presented the best result, followed by the non-fructified gametophyte of *G. turuturu* and both female and male gametophytes of *C. teedei* var. *lusitanicus,* not showing statistically significant differences from the control.

In the aerial part, only *C. teedei* var. *lusitanicus* (female gametophyte) seemed to be the best treatment, achieving the highest seedling growth and weight. The best treatments (kappa/iota-carrageenan extracted from the female gametophyte of *C. teedei* var. *lusitanicus* (the male gametophyte do not have significant differences from the control) and agar extracted from *G. gracilis*, showed different uronic acids concentration (57.54 and 612.02 μg.g $^{-1}$, respectively), as well as the pH (8.7 and 3.1) and electric conductivity (256 and 349 μS cm^{-1}) of the polysaccharides solutions. The uronic acids show that can acidify and also increase the EC values (Meywes, 2019). Also, the polysaccharide chemical structure profile was different, *G. gracilis* has more anhydrogalactose, and the female gametophyte of *C. teedei* var. *lusitanicus* has galactose and sulphated galactoses, even in monosaccharide content is different, only exhibiting only a similar ribose content (0.46 and 0.38 mg.g $^{-1}$, respectively).

Kappa/iota-carrageenan has a higher potential to promote the radicular and aerial kale growth, when compared with the other polysaccharides assayed. This capacity is possible due to the biochemical profile, pH and conductivity, being the structure the most probable cause of the carrageenan efficiency and the other polysaccharides lack of efficiency in the kale seed germination. According the literature, the sulfate content that carrageenan contains, particularly kappa and iota, in comparison with the other polysaccharides, can also have a positive effect on *B. oleracea* development, due to the sulfur requirements of this plant (Ishida, Hara, Fukino, Kakizaki & Morimitsu, 2014; Koralewska et al., 2007).

In the alginate solution it was observed an acidic pH and higher EC values, which affects directly the plant cell homeostasis and water retention by the plant (Laghmouchi et al., 2017; Uçarlı, 2020). This also can be an explanation to why *A. armata* polysaccharide solution does not present seed germination. Only the agarophyte *G. gracilis* and the carrageenophytes appear to have similar effects, where the conductivity is identical, demonstrating that the cell homeostasis can be the key of seed development.

The darkish color, observed in the four replicates from *S. muticum*, can be due to the lower pH of the solution, that can cause the seed coating phytomelanin hydrolysation and extraction (Glagoleva, Shoeva & Khlestkina, 2020; Keles & Özdemir, 2018).

However, the polysaccharide uronic acid and monosaccharide content does not appear to have direct impact on seed germination, due to the inexistence of a linear correlation between this compound profile and the seed germination in this assay. Concurrently, the polysaccharide chemical and structural characterization can be a key to promote or inhibit the plant development.

In literature, the polysaccharide constitution and uronic acids relationship are not well explored. Hence, the data about this type of assay demonstrates that carrageenan's can enhance plant development by regulating various plants metabolic pathways, such as photosynthesis and ancillary pathways, cell division, purine and pyrimidine synthetic pathways as well as metabolic pathways involved in nitrogen and sulfur assimilation (Shukla et al., 2016). This can, in part, be an explanation of the results obtained from the female gametophyte of *C. teedei* var. *lusitanicus*, associated with low uronic acid and high galactose and glucose concentration. However, the male gametophyte of *C. teedei* var. *lusitanicus* presents a higher concentration of uronic acids which is the only difference between their genotype compositions.

Carrageenan can induce plant cell to produce IAA (Saucedo, Contreras & Moenne, 2015), which is an important hormone, vital to regulate the plant development, the compound effect in the cellular

elongation, differentiation, cellular division, apoptosis, and morphogenesis, however can inhibit the root length growth (Donati, Lee, Leveau & Chang, 2013; Shukla et al., 2016). Moreover, the glucose content also can enhance the production of IAA and consequently the plant development (Mishra, Singh, Aggrawal & Laxmi, 2009). When *Eucalyptus* trees were treated with kappa oligo-carrageenan displayed a concomitant increase in indole acetic acid (IAA) and gibberellic acid (GA3) levels, which is aligned with previous observations showing a reciprocal and positive interaction among auxin and gibberellin in other plants (Abad et al., 2011; González, Contreras, Zúiga & Moenne, 2014).

5. Conclusions

This study highlights those polysaccharides have a significant impact on the stimulation or inhibition of kale seed germination and growth and that the polysaccharide kappa/iota-carrageenan, extracted from the female gametophyte of *C. teedei* var. *lusitanicus* provided the best results. In this analysis, the physico-chemical parameters of the polysaccharide solution (pH and CE) showed to be essential for kale seed germination and development, as well as the polysaccharide chemical structure and uronic acids composition.

Further biochemical and chemical analysis to the polysaccharides that achieved better results are needed to fully understand their ideal properties and to define a good and efficient plant biostimulant. It would be also advantageous to understand how the plant metabolizes the polysaccharides, in order to evaluate its immunomodulatory effect.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Author contributions

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