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Algal Biomass as a Source of Bioactive Compounds for Biotechnological Applications

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Algae are a source of biomass for obtaining bioactive compounds such as antioxidants and sulfated polysaccharides, which have beneficial properties for different biotechnological applications, such as nanomaterials synthesis. By means of a 2^3 factorial design, the present study determined the pH, temperature and concentration of the biomass (%) conditions for obtaining *Sargassum sp.* extracts for further application in the synthesis of nanomaterials; the response variables were the antioxidant capacity, concentration of carbohydrates, organic and inorganic sulfates. The results of the experimental design showed that the biomass concentration factor was the most important to explain the response variables except for the antioxidant capacity response, where the factor that explains the highest percentage of the response was the temperature. The condition that allowed obtaining the maximum concentration of antioxidant power and esterified sulfates was found to be using 90°C, pH of 4.5 and 11% of the biomass concentration, resulting in a concentration of 6.1 ± 0.2 mM/L of FRAP and 0.4 ± 0.09 mg/mL organic sulfates.

1. Introduction

Seaweeds emerged as an alternative sustainable source of biomass with potential for application in several areas of biotechnology (Álvarez-Gómez et al., 2016). This is due to their quick growth even in the absence of chemical additives, high biomass productivity, potential to be harvested several times per year (Fouda et al., 2022), as well as their ability to produce diverse natural bioactive compounds as antimicrobial and antioxidant agents, such as pigments, polyphenols, flavonoids, lipids and carbohydrates of pharmaceutical, nutraceutical, cosmetic and nanobiotechnological interest (Harb et al., 2021).
Aquatic seaweeds such as sargassum, besides being a rich source of antioxidants, is also an environmental problem, as they alter the photosynthetic processes of different plants and seaweeds species because they block the sunlight. In addition, sargassum has caused economic losses in the tourism sector in the coastal areas of the Mexican Caribbean since 2016, as they accumulate in large quantities on the beaches, and cause soil contamination due to organic decomposition processes (Lopez-Miranda et al., 2023).
Therefore, some species of sargassum, since they are a rich source of antioxidants, are used in nanobiotechnology for the synthesis of different nanomaterials, by using bioactive compounds (reducing agents) for the nanoparticles synthesis (Flieger et al., 2021). This is achieved through a process known as bioreduction, where the metal ions used for the synthesis of nanomaterials are reduced to atoms by redox reactions, by reductive agents present in algae. The reducing agents not only serve for the synthesis of nanomaterials, but also provide certain properties to the nanoparticles such as stability, biocompatibility, specificity and even antimicrobial capacity, depending on the bioactive compounds produced by the algae (Antunes et al., 2023; Kidgell et al., 2019).
Among the compounds that can be produced by seaweeds for the nanomaterials synthesis are polyphenols, flavonoids, pigments, alkaloids, fucoidan, alginic acid, carrageenans, xylans, galactans, laminaria and different sulfated polysaccharides (Kidgell et al., 2019; Kothai et al., 2022). All these compounds are potent antioxidants with anticoagulant, antinflammatory, and antitumor properties and even employed for the treatment of bacterial,

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fungal, viral, ulcer and cancer diseases (Kothai et al., 2022). For example, some brown seaweeds such as *Sargassum vulgare*, *Sargassum muticum*, and *Sargassum wightii* were reported to contain phlorotannins, flavonoid fucoxanthins, heteropolymers, and sulfated polysaccharides such as fucoidans (Afreen et al., 2023; Kumar et al., 2018; Milledge et al., 2016). However, there are only few reports related with nanomaterial synthesis that analyzed the content and concentration of bioactive compounds involved in the synthesis and just limited to mention the possible components that may be present in the algae (Ahmouda et al., 2022; Goodarzi et al., 2014; Martínez-Cabanas et al., 2021). Thus, the present work attempts to evaluate the potential of components of the sargassum extracts for the synthesis of nanomaterials, by assessing the antioxidant capacity, reducing sugars and organic and inorganic sulfates as indicators of the presence and content of sulfated polysaccharides well known for their nutraceutical, therapeutic, adjuvant and biomedical properties.
The extraction of antioxidant compounds as sulfated polysaccharides from seaweed biomass for nanomaterials biosynthesis is performed by exposing the organic material to different polar solvents such as water, ethanol and methanol (Antunes et al., 2023). However, the extraction of these compounds also depends on their composition and chemical structure, thus the extraction parameters are different for each algal species (Kidgell et al., 2019). Kidgell et al. (2019) reported that the extraction of sulfated polysaccharides with antioxidant power depends mainly on three factors: pH, temperature and time of extraction. Data was confirmed by Harb et al. (2021), where they found that aqueous extracts of 13 seaweeds; eight red, four brown, and one green species, contain mainly sulfated polysaccharides when the biomass was heated to 80° for 3 h. However, they observed that the extraction of such compounds is not selective, since it also depends on the algae species, geographic location where it was collected, stage of its development, environmental conditions, among other factors (Kidgell et al., 2019). Therefore, the aim of this research was to determine the pH, temperature and concentration of the biomass (%) conditions for obtaining aqueous extracts of *Sargassum sp.* for future application in the synthesis of nanomaterials.

2. Materials and methods

2.1 Selection of the optimal conditions for sargassum extract obtention

The seaweed of the *Sargassum* genus obtained from the Mexican coast, was rinsed with running water to eliminate salt residues and dried at room temperature. The seaweed was then dehydrated at 60°C for 24 h and pulverized with the aid of an electric grinder. A factorial design 2^3 was performed using Desing-Expert v11 software (Table 1), which evaluated three factors at two levels temperature (70 and 90°C), pH (2.5 and 4.5) and concentration of the dry weight of sargassum dissolved in water (5 and 11%), as well as four responses: ferric antioxidant reducing power (FRAP), reducing carbohydrates, inorganic sulfates and organic or esterified sulfates (Kidgell et al., 2019). For this, seaweed biomass and distilled water were mixed at the concentration of 5 and 11% (w/v) at a final volume of 30 mL. Subsequently, the pH was adjusted to 2.5 or 4.5 according to the design and heated to the indicated conditions (70 and 90°C). The mixture was kept in agitation for 1 h under the established conditions and subsequently the supernatant was filtered on Whatman™ grade 608 paper. The extracts were kept in freezing at -20°C until further use (Kidgell et al., 2019).

*Table 1: Factorial design 2^3 for obtaining aqueous extracts of Sargassum sp.**

Run	Temp. (°C)	pH	Conc. (%)	Run	Temp. (°C)	pH	Conc. (%)
1	90	4.5	5	13	90	4.5	11
2	90	2.5	11	14	90	2.5	11
3	70	4.5	11	15	70	4.5	5
4	90	4.5	5	16	70	2.5	5
5	70	4.5	11	17	90	2.5	11
6	90	2.5	5	18	90	4.5	11
7	70	2.5	5	19	70	2.5	11
8	70	4.5	5	20	90	4.5	11
9	90	2.5	5	21	70	4.5	5
10	90	2.5	5	22	70	2.5	11
11	70	4.5	11	23	90	4.5	5
12	70	2.5	11	24	70	2.5	5

*****The factorial design 2^3 was applied in order to study the combined effect of several factors (pH, temperature and concentration) on a single or more response variables, individually or combined. The design allows the identification of the conditions that improve the response variables.

2.2 Determination of ferric antioxidant reducing power (FRAP)

The reducing power of the extracts was measured based on the technique reported by Vijayalakshmi and Ruckmani (2016), using a calibration curve from 0.001 to 1 mM/L ascorbic acid and the value was reported in mM/L FRAP by multiplying the value of the calibration curve by two. For this, 1 mL of the sample, 2.5 mL of phosphate buffer and 2.5 mL of 1% potassium ferricyanide were mixed, and incubated for 20 min in a water bath at 50°C. Then, 2.5 mL of 10% trichloroacetic acid was added and centrifuged for 10 min at 3000 rpm. Subsequently, 2.5 mL of the supernatant were added to 0.5 mL of 0.1% ferric chloride and then kept for 10 min. Finally, the sample was analyzed by UV-VIS at 700 nm, using distilled water as blank. The composition of the phosphate buffer was prepared at 0.2 M, and pH 6.6 with the following reagents (g/L): 8; NaCl, 0.2; KCl, 1.44; Na_2HPO_4, 0.24; KH_2PO_4 (Vijayalakshmi and Ruckmani, 2016).

2.3 Determination of reducing carbohydrates

The reducing carbohydrate quantification was carried out by Miller's method using a calibration curve from 0.2 to 1 mg/mL of glucose (Núñez Ávila et al., 2012). In a 15 mL tube, 0.5 mL of the sample, 0.5 mL of the reagent 3,5-dinitrosalisylic acid (DNS) were added and incubated for 5 min in boiling water bath. Subsequently, the reaction was stopped in cold water bath, 5 mL of distilled water was added and the reaction was mixed and kept for 15 min. The samples were analyzed by UV-VIS at 540 nm, using distilled water as blank. The DNS reagent was obtained by dissolving in order 0.8 g of sodium hydroxide, 15 g of sodium potassium tartrate tetrahydrate and 0.5 g of DNS in 50 mL of distilled water. The DNS reagent can be kept in amber bottles refrigerated at 4°C (Núñez Ávila et al., 2012).

2.4 Determination of inorganic, organic and total sulfates

Sulfate quantification was performed using some modifications of the barium chloride-gelatin turbidimetric method reported by Torres et al. (2018) from the original research of Dodgson and Price (1962). For this purpose, a sulfate ion (SO_4^{2-}) calibration curve from 0 to 2 mg/mL was prepared from a concentrated solution of 10 mg/mL SO_4^{2-} from 14.79 mg/mL sodium sulfate (Na_2SO_4) dissolved in 0.5 M HCl. Subsequently, hydrolysis of the extract was performed in a tube for the determination of total sulfates, therefore, 5 mL of sample, 5 mL of 0.5 M HCl were added, mixed and incubated at 105°C for 2 h. Afterwards, the sample was centrifuged at 12,000g for 10 min to obtain the supernatant. The non-hydrolyzed extract for the determination of inorganic sulfates was performed as described above, but without the heat treatment (Torres et al., 2018). Then, 0.5 mL of the sample supernatant, 1 mL of turbidimetric agent, 2.5 mL of ultrapure water were mixed and maintained in agitation for 5 min to perform UV-VIS analysis at 405 nm. The turbidimetric agent was obtained by dissolving 250 mg of animal gelatin in 50 mL of boiling ultrapure water. Then 2.5 g of barium chloride ($BaCl_2$) and 2.5 g of sodium chloride (NaCl) were added. As a negative control, 0.5 M HCl was used. The esterified or organic sulfate was calculated with the following formula:

$$ES = H - \mathrm{NH} \quad (1)$$

Where ES is the organic or esterified sulfate concentration expressed as mg/mL, H the value of the hydrolyzed sample and NH the value of the non-hydrolyzed sample, both in mg/mL (Torres et al., 2018).

3. Results and discussion

3.1 Factorial design 2^3 analysis of the response variables

The results of the experimental design showed that the most important factor contributing to the statistical model for obtaining reducing sugars, inorganic and organic sulfates during the obtention of aqueous extracts of *Sargassum sp.* was the concentration of the biomass (%), reaching 48.7, 56.6 and 53.8%, respectively (Table 2). Temperature was another factor that contributed a large percentage (28.7%) to the statistical model for obtaining inorganic sulfates, but not for reducing sugars (3.2%) and organic sulfates (7.6%). In contrast, pH contributed 17.6 and 8.9% for reducing sugars and organic sulfates, but was not significant for FRAP. On the other hand, the factor with the highest contribution in the statistical model in terms of obtaining FRAP was temperature with 47.7%, followed by biomass concentration with 29.8% (Table 2). Interactions between factors contributed less than 8% in the statistical models.

The above suggests that the antioxidant activity comes mainly from other compounds that may be present in the extract and not from the sulfated polysaccharides, such as polyphenols, flavonoids, or pigments, all of them were reported for the genus *Sargassum* (Afreen et al., 2023; Kumar et al., 2018; Milledge et al., 2016). This because the temperature factor explained more than 40% of the FRAP response but was not significant for reducing sugars, and only explained 7.6% for organic sulfates (Table 2).

Table 2: Factors contribution to the statistical model of the response variables assessed in obtaining Sargassum sp. extracts

Factors	Contribution to the statistical model (%)			
	FRAP	Reducing sugars	Inorganic sulfates	Organic sulfates
Temperature (A)	47.7**	3.2	28.7**	7.6*
pH (B)	0.1	17.6**	2.0**	8.9*
Concentration (C)	29.8**	48.7**	56.6**	53.8**
AB	0.3	0.6	0.6	0.02
AC	6.2**	4.6	5.2**	0.4
BC	4.5*	7.1*	1.5*	0.5
ABC	0.6	0.006	1.7**	4.9
Error	10.5	17.8	3.3	23.6

AB; AC, BC, ABC: interactions between the factors Temperature (A), pH (B) and Concentration (C). * Significant with 95% confidence interval. ** Significant with 99% confidence interval

Furthermore, the FRAP concentration response analyzed in the design of experiments for obtaining *Sargassum sp.* extracts showed that the maximum concentration of antioxidant power was obtained when using 90°C, pH 4.5 and 11% biomass concentration (Figure 1a), whereas the maximum concentration of reducing sugars, inorganic and organic sulfates was obtained using 90°C, pH 2.5 and 11% biomass concentration (Figure 1b – 1d).

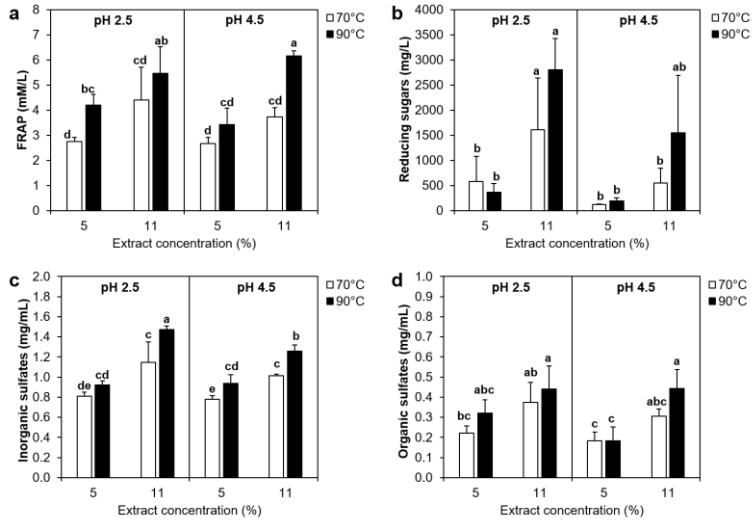

Figure 1: Concentration analysis of a) FRAP, b) reducing sugars, c) inorganic and d) organic sulfates in aqueous extracts of Sargassum sp. Significant differences (P < 0.05) are shown with lowercase letters above the bars.

The last indicates that the content of organic sulfates and reducing sugars are related to each other. In addition, both responses were influenced by pH (Table 2), suggesting that the extracted reducing sugars present sulfate esters and, therefore, may be forming part of the structure of the sulfated polysaccharides. This is because the chemical structure of such polymers consists mainly of sulfated rhamnose and xylose, which are reducing sugars, as well as glucuronic acid and iduronic acid in disaccharide repeats with α- and β-bonds (1 - 4) (Kidgell et al., 2019).

The design results also showed that the organic sulfates are sensitive to temperature, but not the reducing sugars (Table 2), which suggests that the sulfated polysaccharides present in the seaweed could be degraded by temperature. This is because the composition and chemical structure of the sulfated polysaccharides are different for each type of algae, resulting in modifications of the physicochemical properties of these polymers (Kidgell et al., 2019).

3.2 Selection of the conditions for obtaining *Sargassum sp.* extract

The experimental design results showed that the maximum concentration of organic sulfates, which refer to sulfate esters present in the sulfated polysaccharides, was obtained using a temperature of 90°C and 11% of biomass concentration at pH 2.5 and 4.5 (Table 3). These conditions allowed obtaining the maximum FRAP concentration in the *Sargassum sp.* extract, which resulted in 5.4 ± 1.0 and 6.1 ± 0.2 mM/L, respectively.

However, in terms of FRAP concentration, no statistically significant differences were found between the two conditions (Table 3).

Table 3: Concentration of FRAP, reducing sugars, inorganic and organic sulfates in Sargassum sp. extracts.

Sargassum sp. extracts			FRAP (mM/L)	Reducing sugars (mg/L)	Inorganic sulfates (mg/mL)	Organic sulfates (mg/mL)
70°C	pH 2.5	[5%]	2.7 ± 0.1^{d}	578.7 ± 499.9^{b}	0.8 ± 0.04^{de}	0.2 ± 0.04^{bc}
90°C	pH 2.5	[5%]	4.2 ± 0.4^{bc}	364 ± 175.3^{b}	0.9 ± 0.04^{cd}	0.3 ± 0.07^{abc}
70°C	pH 2.5	[11%]	4.4 ± 1.3^{cd}	1608.7 ± 1034.7^{a}	1.1 ± 0.2^{c}	0.3 ± 0.1^{ab}
90°C	pH 2.5	[11%]	5.4 ± 1.0^{ab}	2798.9 ± 620.5^{a}	1.4 ± 0.03^{a}	0.4 ± 0.1^{a}
70°C	pH 4.5	[5%]	2.6 ± 0.2^{d}	118.4 ± 12.1^{b}	0.7 ± 0.04^{e}	0.1 ± 0.04^{c}
90°C	pH 4.5	[5%]	3.4 ± 0.6^{cd}	192.7 ± 61.4^{b}	0.9 ± 0.09^{cd}	0.1 ± 0.07^{c}
70°C	pH 4.5	[11%]	3.7 ± 0.3^{cd}	547.7 ± 294.1^{b}	1.0 ± 0.01^{c}	0.3 ± 0.03^{abc}
90°C	pH 4.5	[11%]	6.1 ± 0.2^{a}	1549.4 ± 1142^{ab}	1.2 ± 0.06^{b}	0.4 ± 0.09^{a}

a, b, c, d, e: Tukey's statistical analysis with a 95% confidence interval. Means that do not share a letter are statistically significant.

Therefore, the condition that allowed maximizing the concentration of organic sulfates and FRAP in the *Sargassum sp.* extract was to use 90°C, pH 4.5 and 11% biomass concentration, giving values of 6.1 ± 0.2 mM/L of FRAP, 1549.4 ± 1142 mg/L of reducing sugars, 1.2 ± 0.06 and 0.4 ± 0.09 mg/mL of inorganic and organic sulfates. These results are in agreement with data reported by Ahmouda et al. (2022), in which a FRAP concentration from 1.6 to 6.9 mM was obtained from *Rosemarinus officinalis*, *Juniperus phoenicia*, *Matricaria pubescen*t, and *Artemisia herba-alba* plant extracts for the synthesis of iron oxide nanoparticles. On the other hand, in the synthesis of silver and cadmium sulfide nanoparticles, values of 0.034 and 3.2 mM FRAP respectively, have been reported (Loa et al., 2023; Phongtongpasuk et al., 2016). Regarding the content of reducing sugars and organic and inorganic sulfates, the data suggest the presence of sulfated polysaccharides since the analysis of organic sulfates identifies the concentration of sulfate esters, which are predominant in these polymers, as well as the relationship between reducing sugars and organic sulfates. However, additional purification steps are required to obtain these polymers, due to the fact that during the extraction process several bioactive compounds are also obtained (Kidgell et al., 2019).

The use of sulfated polysaccharides and their homologous glycosaminoglycans at a concentration of 0.2% (w/v) and 0.5 mM respectively was reported for the coating of silver and gold nanoparticles (Jeon et al., 2021; Kemp et al., 2009). However, these results can not be compared with the data obtained in the present research since most reports mention the total sulfated polysaccharides concentration, while the data reported in this study show only the concentration of esterified sulfates (0.4 ± 0.09 mg/mL) associated with the antioxidant and beneficial properties of these polymers. Therefore, the seaweed used in this research shows potential application in different areas based on esterified sulfates content closely related to sulfated polysaccharides which have been reported with wide benefits.

4. Conclusions

The experimental design showed that pH 4.5, temperature of 90°C and 11% of *Sargassum sp.* biomass were the conditions that enhanced the concentration of reducing power, reducing sugars and organic and inorganic sulfates, obtaining 6.1 ± 0.2 mM/L, 1549.4 ± 1142 mg/L, 1.2 ± 0.06 mg/mL and 0.4 ± 0.09 mg/mL, respectively (Table 3), suggesting the presence of antioxidant agents and sulfated polysaccharides which could be used for the synthesis and coating of nanomaterials. Additional purification steps are required if some of these compounds are employed for specific biotechnological applications.

References

Afreen, A.B., Rasool, F., Fatima, M., 2023. Bioactive properties of brown seaweed, Sargassum wightii and its nutritional, therapeutic potential and health benefits: A review. J. Environ. Biol. 44, 146–158. https://doi.org/http://doi.org/10.22438/jeb/44/2/MRN-5081

Ahmouda, K., Benhaoua, B., Laouini, S.E., Labbi, A., 2022. Plant extract FRAP effect on cation vacancies formation in greenly synthesized wüstite (FexO) nanoparticles: A new contribution. Sustain. Chem. Pharm. 25, 100563. https://doi.org/10.1016/j.scp.2021.100563

Álvarez-Gómez, F., Korbee, N., Figueroa, F.L., 2016. Analysis of antioxidant capacity and bioactive compounds in marine macroalgal and lichenic extracts using different solvents and evaluation methods. Ciencias Mar. 42, 271–288. https://doi.org/http://dx.doi.org/10.7773/cm.v42i4.2677

Antunes, S.F., Dos Santos, M.S., dos Santos, O.A.L., Backx, B.P., Soran, M.L., Opris, O., Lung, I., Stegarescu, A., Bououdina, M., 2023. Biosynthesis of nanoparticles using plant extracts and essential oils. Molecules 28, 101–117. https://doi.org/https://doi.org/10.3390/molecules28073060

Dodgson, K.S., Price, R.G., 1962. A note on the determination of the ester sulphate content of sulphated polysaccharides. Biochem. J. 84, 106–110. https://doi.org/10.1042/bj0840106

Flieger, J., Flieger, W., Baj, J., Maciejewski, R., 2021. Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. Materials (Basel). 14. https://doi.org/10.3390/ma14154135

Fouda, A., Eid, A.M., Abdelkareem, A., Said, H.A., El-Belely, E.F., Alkhalifah, D.H.M., Alshallash, K.S., Hassan, S.E.D., 2022. Phyco-Synthesized Zinc Oxide Nanoparticles Using Marine Macroalgae, Ulva fasciata Delile, Characterization, Antibacterial Activity, Photocatalysis, and Tanning Wastewater Treatment. Catalysts 12. https://doi.org/10.3390/catal12070756

Goodarzi, V., Zamani, H., Bajuli, L., Moradshahi, A., 2014. Evaluation of antioxidant potential and reduction capacity of some plant extracts in silver nanoparticles' synthesis. Mol. Biol. Res. Commun. 3, 165–174.

Harb, T.B., Pereira, M.S., Cavalcanti, M.I.L.G., Fujii, M.T., Chow, F., 2021. Antioxidant activity and related chemical composition of extracts from Brazilian beach-cast marine algae: opportunities of turning a waste into a resource. J. Appl. Phycol. 33, 3383–3395. https://doi.org/10.1007/s10811-021-02446-8

Jeon, M.S., Han, S. Il, Park, Y.H., Kim, H.S., Choi, Y.E., 2021. Rapid green synthesis of silver nanoparticles using sulfated polysaccharides originating from Porphyridium cruentum UTEX 161: evaluation of antibacterial and catalytic activities. J. Appl. Phycol. 33, 3091–3101. https://doi.org/10.1007/s10811-021-02540-x

Kemp, M.M., Kumar, A., Mousa, S., Park, T.J., Ajayan, P., Kubotera, N., Mousa, S.A., Linhardt, R.J., 2009. Synthesis of Gold and Silver Nanoparticles Stabilized with Glycosaminoglycans having Distinctive Biological Activities. Biomacromolecules 10, 589–595. https://doi.org/doi:10.1021/bm801266t.

Kidgell, J.T., Magnusson, M., de Nys, R., Glasson, C.R.K., 2019. Ulvan: A systematic review of extraction, composition and function. Algal Res. 39, 101422. https://doi.org/10.1016/j.algal.2019.101422

Kothai, R., Arul, B., Anbazhagan, V., 2022. Anti - Dengue Activity of ZnO Nanoparticles of Crude Fucoidan from Brown Seaweed S.marginatum. Appl. Biochem. Biotechnol. 195, 3747–3763. https://doi.org/https://doi.org/10.1007/s12010-022-03966-w

Kumar, A., AbdElgawad, H., Castellano, I., Selim, S., Beemster, G.T.S., Asard, H., Buia, M.C., Palumbo, A., 2018. Effects of ocean acidification on the levels of primary and secondary metabolites in the brown macroalga Sargassum vulgare at different time scales. Sci. Total Environ. 643, 946–956. https://doi.org/10.1016/j.scitotenv.2018.06.176

Loa, J.D.A., Cruz-Rodríguez, I.A., Rojas-Avelizapa, N.G., 2023. Colorimetric Detection of Metals Using CdS-NPs Synthesized by an Organic Extract of Aspergillus niger. Appl. Biochem. Biotechnol. 195, 4148–4163. https://doi.org/10.1007/s12010-023-04341-z

Lopez-Miranda, J.L., Molina, G.A., González-Reyna, M.A., España-Sánchez, B.L., Esparza, R., Silva, R., Estévez, M., 2023. Antibacterial and Anti-Inflammatory Properties of ZnO Nanoparticles Synthesized by a Green Method Using Sargassum Extracts. Int. J. Mol. Sci. 24. https://doi.org/10.3390/ijms24021474

Martínez-Cabanas, M., López-García, M., Rodríguez-Barro, P., Vilariño, T., Lodeiro, P., Herrero, R., Barriada, J.L., de Vicente, M.E.S., 2021. Antioxidant capacity assessment of plant extracts for green synthesis of nanoparticles. Nanomaterials 11, 1–14. https://doi.org/10.3390/nano11071679

Milledge, J.J., Nielsen, B. V., Bailey, D., 2016. High-value products from macroalgae: the potential uses of the invasive brown seaweed, Sargassum muticum. Rev. Environ. Sci. Biotechnol. 15, 67–88. https://doi.org/10.1007/s11157-015-9381-7

Núñez Ávila, R., Pérez Rivas, B., Motzezak Hernández, R., Chirlino, M., 2012. Contenido de azúcares totales , reductores y no reductores en Agave cocui Trelease. Multiciencias 12, 129–135.

Phongtongpasuk, S., Poadang, S., Yongvanich, N., 2016. Environmental-friendly Method for Synthesis of Silver Nanoparticles from Dragon Fruit Peel Extract and their Antibacterial Activities. Energy Procedia 89, 239–247. https://doi.org/10.1016/j.egypro.2016.05.031

Torres, P., Novaes, P., Ferreira, L.G., Santos, J.P., Mazepa, E., Duarte, M.E.R., Noseda, 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 Res. 32, 142–149. https://doi.org/10.1016/j.algal.2018.03.016

Vijayalakshmi, M., Ruckmani, K., 2016. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh J. Pharmacol. 11, 570–572. https://doi.org/10.3329/bjp.v11i3.27663