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# Screening of Mexican tropical seaweeds as sources of α-amylase and α-glucosidase inhibitors

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ABSTRACT

A key therapeutic strategy to prevent metabolic syndrome is the inhibition of α-amylase and α-glucosidase. Derivatives isolated from naturally-sourced seaweeds may act as inhibitors of these enzymes. The aims of this study are to evaluate *in vitro* the α-amylase and α-glucosidase inhibition of 45 crude extracts from 31 species of Ochrophyta, Rhodophyta, and Chlorophyta present in Mexican seashores, describe their acute toxicity, and putatively identify some of the potential bioactive compounds by using untargeted metabolomics. Also, active extracts were evaluated in the brine shrimp lethality test. Samples were collected during rainy and dry seasons in the rocky shores of Paraíso, Villa Rica and Muñecos, in Veracruz, Mexico. Crude extracts were obtained by maceration and then tested on both enzymes. Chemical profiling was done by accurate mass spectrometry and data was analyzed using statistical tools. The results showed that seaweeds from Veracruz are sources of α-amylase and α-glucosidase inhibitors. The highest α-amylase and α-glucosidase inhibition ($IC_{50}$ values) were observed in *Cladophora dalmatica* (116.99 ± 11.59, 27.86 ± 2.95 μg $mL^{-1}$), *Ectocarpus siliculosus* (679 ± 68.17, 276.86 ± 11.20 μg $mL^{-1}$), *Padina boergesenii* (567.01 ± 65.20, 43.89 ± 5.46 μg $mL^{-1}$) and *P. gymnospora* (> 1000, 59.92 ± 7.45 μg $mL^{-1}$) species, respectively. Active extracts were more effective inhibitors of α-glucosidase compared to acarbose (> 1000 μg $mL^{-1}$), used as drug reference. *C. dalmatica* showed high toxicity ($LC_{50}$ = 37.55 ± 1.04 μg $mL^{-1}$), whilst the rest of the active extracts did not. Fatty acids and terpenoids were tentatively identified in the active extracts as potential inhibitors of tested enzymes. In conclusion, Mexican seaweeds constitute sources of metabolites that could reduce hyperglycemia postprandial by the inhibition of α-amylase and α-glucosidase. Ochrophyta species are the best sources to look for these inhibitors because their extracts are not toxic and displayed lower α-amylase inhibitory activities.

## 1. Introduction

The amylopectin form of starch is the most consumed carbohydrate in the human diet, [1,2]. α-Amylase and α-glucosidase enzymes carry out the hydrolysis of starch, releasing glucose into the bloodstream after food intake [1–4]. Diets rich in carbohydrates, unhealthy life habits, and genetic predisposition all lead to high blood glucose levels after meals, known as postprandial hyperglycemia [2]. This has been considering one of the major risk factors to develop oxidative stress in cells, obesity and metabolic syndrome [2,5,6]. The consequences of these conditions are the development of cardiovascular diseases, diabetes mellitus type 2, cancer, among others [2,6].

These diseases are the main causes of worldwide deaths, and generate high costs to public health systems, especially in developing countries [5–7]. Several efforts have been made in order to avoid these serious chronic diseases [6]. One alternative is by preventing risk factors of health such as postprandial hyperglycemia [5]. The current therapies include the administration of acarbose, which reduces glucose

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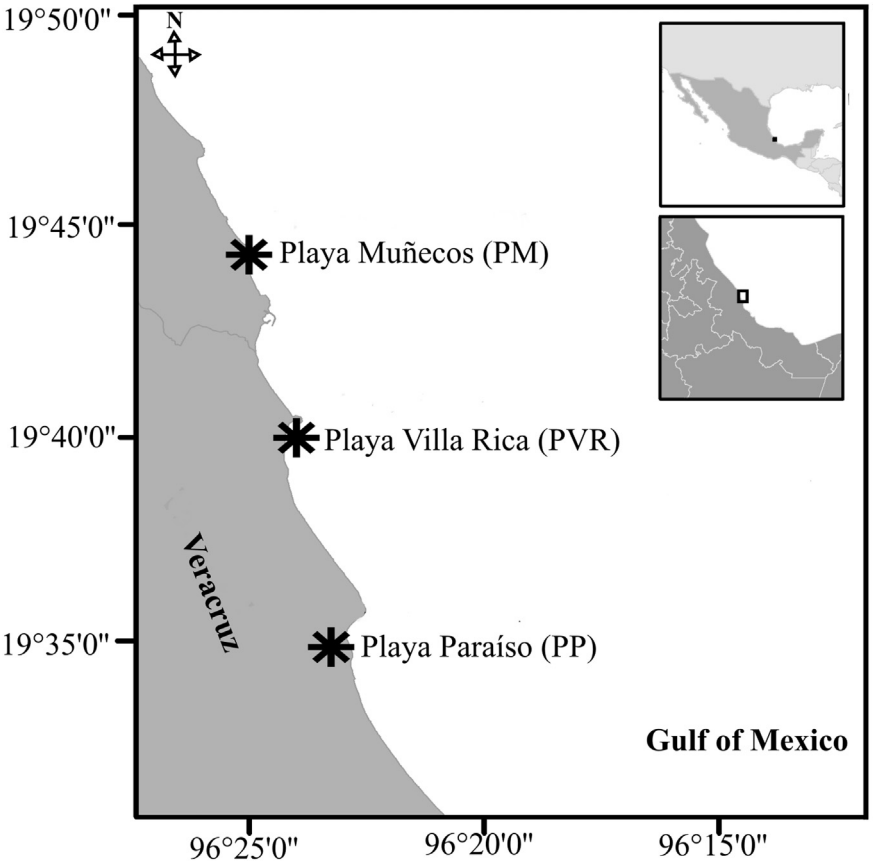


**Fig. 1.** Sites of seaweed collection. Actopan municipality, Veracruz, Mexico.

absorption and decreases the postprandial hyperglycemia by inhibiting the activity of α-amylase and α-glucosidase enzymes [2,4,6]. However, acarbose also causes adverse gastrointestinal side effects due to the excessive inhibition of α-amylase [2,6,7]. These effects lead patients to neglect or reject the therapy [2,6]. Consequently, the search for new inhibitors of these enzymes has focused on good efficacy, safety and a reduction of side effects in comparison to current drugs.

Marine taxa are a rich source of biologically active metabolites and could potentially furnish molecules which are useful in pathologies associated with hyperglycemia [8]. Seaweeds are cosmopolitan photosynthetic organisms that have been used since ancient times in traditional Asian medicine to treat obesity, cardiovascular diseases and diabetes [9,10]. Currently, only a select number of species are consumed and used due to safety in humans [11]. These organisms inhabit harsh environments and are exposed to many biotic and abiotic stress factors such as herbivory, competition, desiccation, fast-changing salinity, temperature, and irradiation [9,12]. Consequently, their chemical composition varies according to species and season collection. In most of the cases, this will affect their biological activity, their metabolic content and including toxic compounds [9]. For that reason, even that > 3200 compounds have been described in seaweeds, the chemistry of many species remains undescribed [9], although some species could share several metabolites due to their phylogenetic arrangement [13]. In this sense, screening guided by biological assays enabled us to identify the natural sources of bioactive compounds [14–16] as well to discriminate between those which are non-toxic, in order to contribute with the first steps in drug discovery [14,17]. In that regard, metabolomics can be useful to reveal patterns and correlations between seaweeds taxonomy, chemical compositions, and displayed bioactivity rankings [18]. Hence, untargeted analysis along with chemical profiling by accurate mass spectrometry identifies chemotaxonomic correlation of species, and allows putative identification of bioactive compounds as many as possible in one or more species in a particular biological group [9,13,18].

Seaweeds have previously been reported as sources of natural products capable to inhibit α-amylase and α-glucosidase enzymes [10,19,20]. Most of the studies have evaluated the bioactivity of natural products from temperate taxa belonging to genus *Ascophyllum*, *Ecklonia*, *Fucus*, and *Laminaria*, revealing that enzymes inhibitors could be phlorotannins, terpenes, halogenated compounds and polysaccharides [21–23]. Other authors have described the potential of species which reach tropical latitudes, and some genus evaluated by them were *Padina, Sargassum*, *Acanthophora*, *Laurencia*, *Gracilaria*, *Grateloupia*, *Caulerpa* and *Ulva*, among others [16,24]. Nonetheless, these studies have focused on describing the activity of crude extracts against enzymes, and very little information is known about active compounds and their safety.

In Mexico, the seaweed richness includes > 800 species along with its territory [25]. Only five temperate species distributed in the northwest Mexican coast have been screened as sources of inhibitors of these enzymes, pointing out that extracts of *Ecklonia arborea* and *Silvetia compressa* were active [26]. This revealed that Mexican seaweeds are an undervalued source as α-amylase and α-glucosidase inhibitors since < 1% of the seaweed's richness have been studied for this purpose. Veracruz state located in the central east of Mexican territory along the Gulf of Mexico, has 447 recorded species of seaweeds [27]. Rocky shores from this state are unique and environmentally influenced by the foothill of Neovolcanic Axis having two distinguishable climatic seasons named rainy and dry season, which occurs in July to October and November to June, respectively [28]. Seaweeds from this region are characterized as tropical flora [27]. Some of the species distributed in this region belong to genus previously reported as natural sources of α-amylase and α-glucosidase inhibitors such as *Padina*, *Sargassum*, *Acanthophora*, *Laurencia*, *Gracilaria*, *Codium*, and *Ulva*, among others

[16,24,29–31]. Based on this information, we hypothesize that the natural products of seaweeds from Veracruz, Mexico, have the potential to inhibit α-amylase and α-glucosidase enzymes. Furthermore, these metabolites are potentially non-toxic and structurally novel. Overall, the main goals of this investigation are to describe α-amylase and α-glucosidase inhibitory activity of crude extracts from 31 species of Ochrophyta, Rhodophyta and Chlorophyta collected in different seasons and present in selected Mexican rocky shores. In addition, we aim to evaluate the acute toxicity in the active crude extracts using brine shrimp lethality model. We aim to accomplish this using an untargeted metabolomics approach.

## 2. Materials and methods

### 2.1. Collection, taxonomic determination, and cleaning samples

Seaweed samples were collected in three different beaches named "Playa Muñecos" (19° 44′ 38″ N y 96° 24′ 27″ O), "Playa Paraíso" (19° 35′ 14″ N y 96° 23′ 05″ O) and "Playa Villa Rica" (19° 40′ 14″ N y 96° 23′ 37″ O) (Fig. 1), located in Actopan Municipality, Veracruz, Mexico. Collections were carried out on the rainy season (September 2016) and dry season (May 2017). A total of 45 samples from 31 species of Ochrophyta, Rhodophyta, and Chlorophyta (Supplementary Table 1) were randomly taken and detached manually from the substrate with a spatula between rocky intertidal to 2 m in depth. The species *Acanthophora spicifera*, *Alsidium triquetrum*, *Digenea simplex*, *Cymopolia barbata*, *Ulva lactuca*, and *Padina boergesenii* were collected in both seasons in "Playa Paraíso". Also, *Laurencia obtusa* was collected in both seasons in "Playa Muñecos". The remaining species were collected only in the rainy or dry season, respectively (Supplementary Table 1). All samples were rinsed with running water to eliminate sand and epiphytes and then were frozen at −20 °C for storage until further use. Algal duplicate samples were preserved in a 4% formalin/seawater solution. Then, phycological material was determined using taxonomic keys of Taylor [32], Littler and Littler [33], Gurgel et al. [34] and De Souza-Gestinari et al. [35]. Reference specimens are deposited at herbarium ENCB of Instituto Politécnico Nacional, Mexico City, Mexico.

### 2.2. Crude extracts preparation

The frozen samples were freeze-dried with a lyophilizer (Freezone 1, LABCONCO®, USA). Then, dried samples were milled with a food processor (NBR-0801, Nutribullet®, USA). Each sample was macerated with methanol (MeOH) (1:10, *w/v*) for 24 h with a magnetic stirrer (EW-03407-10, Cole-Parmer®, USA) at 350 rpm. The supernatant was separated from non-soluble materials by vacuum filtration and concentrated in a rotary evaporator (RII, Büchi, Switzerland) under reduced pressure, at 40 °C. The residual materials were re-extracted at least twice more using the same conditions. Finally, the total yield of each crude extract was quantified based on the dry weight of the seaweeds.

### 2.3. Inhibition assay of α-amylase enzyme

Assays were carried out using the method reported by Gella et al. [36], in a 96 well microplate. Briefly, a 40 mM phosphate buffer (17,202, Sigma Aldrich, USA) solution (PB) was prepared, and the pH was adjusted with HCl to 6.8. This solution was used to dissolve all reagents of the experiment. Then 60 μL of an extract solution (1 mg $mL^{-1}$) was mixed with 120 μL of 2-Chloro-4-nitrophenyl-α-D-maltotrioside (0.35 mM) (93,834, Sigma Aldrich, USA) and incubated for 5 min at 35 °C. After that, 30 μL of the enzyme α-amylase of porcine (A3176, Sigma Aldrich, USA), diluted to a working concentration of 4 U $mL^{-1}$ was added to the reaction and incubated for 10 min at 35 °C in a microplate photometer reader (Multiskan™ FC, Thermo Scientific®, USA). Absorbance was measured at 405 nm at the beginning and end of the assay. Acarbose (A8980, Sigma Aldrich, USA) was used as a positive control (3 mM in PB). The calibration curve of *p*-nitrophenyl (241,326, Sigma Aldrich, USA) was done for each microplate. The half-maximal inhibitory concentration ($IC_{50}$) was calculated by testing a dilution series of active extracts from 1 to 1000 μg $mL^{-1}$. Assays were carried out in triplicate and percentages of inhibition were calculated with the following formula:

$$\%of\ inhibition = \frac{[Ac - (A_i - A_b)]}{Ac \times 100}$$

where: Ac is the absorbance of the negative control, $A_i$ absorbance of the inhibitor (extracts or acarbose) at 30 min, and $A_b$ is the absorbance of blanks of inhibitors tested.

### 2.4. Inhibition assay of α-glucosidase enzyme

Assays were carried out in a 96-wells microplate system, according to Kellogg et al. [37]. Briefly, 0.005 mg $mL^{-1}$ of an α-glucosidase enzyme from *Saccharomyces cerevisiae* (G0660, Sigma Aldrich, USA), diluted in 100 mM of phosphate buffer solution (PB) (17202, Sigma Aldrich, USA) pH 7.2. Then, 20 μL of extracts (1 mg $mL^{-1}$ in PB) was mixed with 100 μL of 4-nitrophenyl-α-D-glucopyranoside (N1377, Sigma Aldrich, USA) at 1 mM in PB. The mixture was incubated for 5 min at 30 °C. After the incubation time, the enzyme was added to each well, and the microplate was incubated for 30 min at 30 °C. Absorbance was measured at 405 nm in the microplate reader. The positive control was 30 mM acarbose in PB (A8980, Sigma Aldrich, USA). Assays were carried out in triplicate. The calibration curve, percentage of inhibition and $IC_{50}$ were all calculated in the same way as the assay mentioned above, considering the concentration of serial dilutions.

### 2.5. Acute toxicity of seaweed extracts on brine shrimp Artemia franciscana Kellog

Toxicity assay was carried out only with the most active extracts ($n = 8$), according to Colegate and Molyneux [17]. In a 96 well microplate system, by quintuplicate. Briefly, crude extracts were diluted in seawater (28 ppm, pH = 8.8) in concentration ranges of 1, 10, 100, 250, 500, 1000 and 2000 μg $mL^{-1}$. Then, 400 μL of each dilution was added to each well, and 10 individuals of *A. franciscana* were inoculated. Microplates were incubated for 24 h, at 24 °C. After, surviving nauplios were counted. The positive control was colchicine (C2580000, Sigma Aldrich, USA) at the same concentrations as the extracts. The $LC_{50}$ was calculated with the concentration of serial dilutions. The toxicity interval was based according to Meenakshi et al. (2018): non-toxic ($LC_{50}$ > 1000 μg $mL^{-1}$), low toxicity ($LC_{50}$ = 500–1000 μg $mL^{-1}$), moderate toxicity ($LC_{50}$ > 100–500 μg $mL^{-1}$) and high toxicity ($LC_{50}$ < 100 μg $mL^{-1}$).

### 2.6. Chemical profiling by untargeted metabolomics approach (UHPLC-MS-QTOF-ESI)

Chemical profiling of active extracts was done with an ultrahigh-performance liquid chromatographic (UHPLC) system (Class I, Waters™, UK) coupled to a quadrupole-time of flight mass spectrometer (QTOF) (Synapt G2-Si HDMi, Waters™, UK). The chromatography was carried out on an Acquity BEH column (1.7 μm, 2.1 × 50 mm) with a column and sample temperatures of 40 °C and 15 °C, respectively. The mobile phase consisted of (A) water (MS grade, Sigma Aldrich, USA) and (B) acetonitrile (MS grade, Sigma-Aldrich, USA), both with 0.1% of formic acid. The gradient conditions of the mobile phases were 0–13 min linear gradient 1–80% B, 13–14 min 80% B isocratic, 14–15 min linear gradient 80–1% B (total run time 20 min). The flow rate was 0.3 mL $min^{-1}$ and 1 μL of the extract was injected. The mass spectrometric analysis was performed with an electrospray ionization source in negative and positive mode with a capillary, sampling cone

and source offset voltages of 3000, 40 and 80 V, respectively. The source temperature was 100 °C and the desolvation temperature was 20 °C. The desolvation gas flow was 600 L $h^{-1}$ and the nebulizer pressure was 6.5 Bar. Leucine-enkephalin was used as the lock mass (556.2771, $[M + H]^+$; 554.2615, $[M - H]^-$). The conditions used for MS analyses were: mass range 50–1200 Da, Function 1 CE, 6 V, function 2 CER 10–30 V, scan time 0.5 s. The data was acquired and processed with MassLynx (version 4.1, Waters™, UK) and MarkerLynx (version 4.1, Waters™, UK). Spectrometric data was compared with those previously reported in the literature and public databases such as Chemspider, PubChem and Seaweed Metabolite Database (SWMD). Global analysis of the 45 extracts (from dry and rainy seasons) was carried out for contrasting chemical profiles between species, localities and season collected.

### 2.7. Statistical analyses

A general linear model (GLM) with a quassipoisson link function [38] analyzed the inhibition percentages by each extract on both enzymes. The model took as factors the species and seasons. Then, an ANOVA was applied to the model and the Tukey test was carried out in order to determine the significance of inhibition, using *vegan* package. For calculating the half-inhibitory concentration of enzyme ($IC_{50}$) and the half-lethal concentration ($LC_{50}$) for acute toxicity, logistic regression models were done according to Ritz et al. [39]. All statistical analyses were carried out in R Studio software (3.4.3 version). Intensities of the mass/charge ratio ($m/z$) data was used to perform a principal component analysis (PCA) of the 45 extracts in MassLynx software. Then, in R Studio software a hierarchical cluster analysis based in Euclidian distance was done with *vegan* and *gclus* packages [40] to identify variation in chemical profiling between species and season collects. Only results from $ESI^+$ are presented since data of this experiment exhibited tendency to form groups. All figures were edited in GNU Image Manipulation Program (GIMP, 2.10).

## 3. Results

### 3.1. Inhibition of α-amylase and α-glucosidase enzymes

The intervals of inhibition observed in crude extracts of Rhodophyta (Fig. 2) and Chlorophyta (Fig. 3) species on α-glucosidase were between 8–15 and 10.1–18.7%, respectively; while on α-amylase were between 10–36.1% and 8–22.8%, respectively. In both cases, the inhibition observed in Rhodophyta and Chlorophyta species were significantly lower ($p < .05$) than inhibition observed by acarbose on α-glucosidase (88.5 ± 0.3%) and α-amylase (99.8 ± 0.1%) (Figs. 2, 3). Only the extract obtained from the Chlorophyta *Cladophora dalmatica* exhibited percentages of inhibition higher than 90% on both enzymes. On the other hand, crude extracts from Ochrophyta species were the most active because some of them showed an inhibition higher than 90% on both enzymes. Indeed, the intervals of inhibition on α-glucosidase were between 8.2 and 99%, while on α-amylase were between 7 and 90.9% (Fig. 4).

Concerning species collected in rainy and dry seasons, the extracts of *P. boergesenii* (Fig. 4) and *Laurencia obtusa* (Fig. 2) showed a significantly ($p < .05$) variation in bioactivity on both enzymes. Extracts of *P. boergesenii* increased their α-amylase inhibition and decreased their α-glucosidase inhibition when samples were collected in the rainy season. An inverse pattern was observed in bioactivity results exhibited by extract of *L. obtusa*. Extracts of *Alsidium triquetrum* and *Digenea simplex* showed an increase only on α-amylase inhibition, when species were collected on the rainy season; while non-significant differences were found on α-glucosidase inhibition in both seasons collection. Finally, extracts of *Acanthophora spicifera*, *Cymopolia barbata*, and *Ulva lactuca* did not exhibit differences ($p > .05$) in bioactivity independently of the season collection (Supplementary Table 1).

The most active extracts were obtained from the Chlorophyta species *Cladophora dalmatica*, and extracts of Ochrophyta species *Asteronema breviarticulatum*, *Ectocarpus siliculosus*, *Padina boergesenii*, and *P. gymnospora*, exhibited a higher inhibition of α-glucosidase than acarbose ($p < .05$), and lower α-amylase inhibition than acarbose ($p < .05$). According to $IC_{50}$, the extract from *C. dalmatica* was the most active to inhibit α-amylase ($IC_{50}$ = 116.9 ± 11.6 μg $mL^{-1}$) and α-glucosidase ($IC_{50}$ = 27.8 μg $mL^{-1}$ ± 3.1) enzymes (Table 1).

### 3.2. Acute toxicity of seaweed extracts on brine shrimp Artemia franciscana

A total of five active extracts were evaluated in this test. The crude extract of *C. dalmatica* showed high toxicity ($LD_{50}$ = 37.55 ± 1.04 μg $mL^{-1}$) (Table 2). The toxicity of this extract was 12 times lower than observed on colchicine ($LD_{50}$ = 3.1 ± 0.5 ± 1.04 μg $mL^{-1}$). In contrast extracts of species *A. breviarticulatum*, *E. siliculosus*, *P. boergesenii*, and *P. gymnospora* exhibited $LD_{50}$ values higher than 1000 μg $mL^{-1}$, which means that these extracts were not toxic (Table 2).

Data is the half maximal lethal doses ($LD_{50}$) calculated on a serial dilution ($n = 5$) of each extract and their three replicates. Superscripts denote the significance ($p < .05$) of the sample according to the logistic regression model.

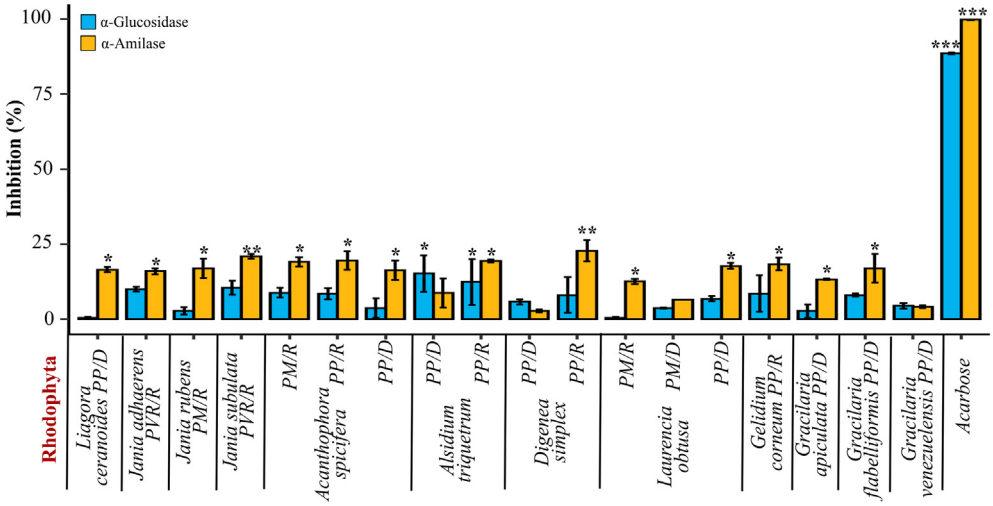


**Fig. 2.** Percentages of α-amylase and α-glucosidase inhibition of Rhodophyta crude extracts. Data is the mean ($n = 3$) ± standard deviation (SD) of crude extracts evaluated at the concentration of 1 mg $mL^{-1}$. Significant differences are based on generalized linear model (GLM) analysis (quassipoisson family, logit link) ($p < .05$). Significance codes for percentages inhibition are: > 10% of inhibition (*), 20–50% (**) and > 60% (***). Symbology: Playa Paraíso (PP), Playa Muñecos (PM) Playa Villa Rica (PVR); Rainy season (R), Dry season (D).

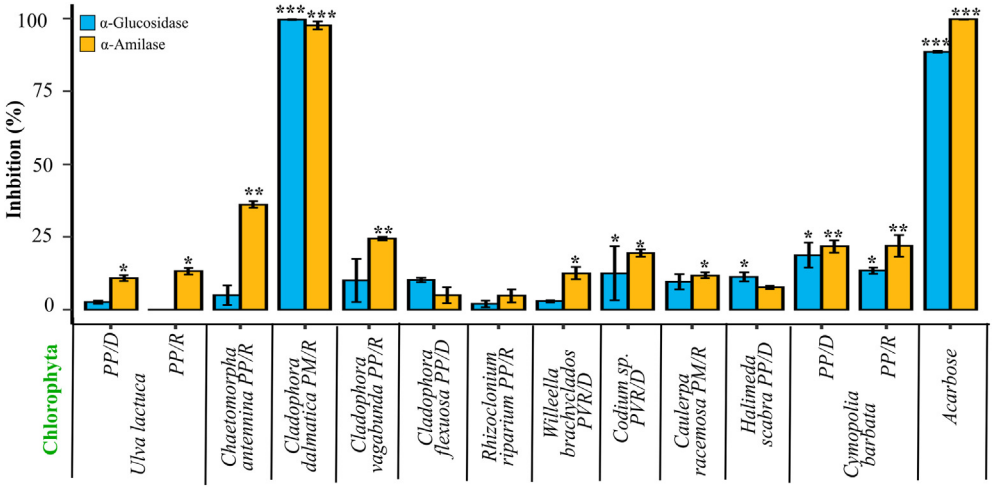


**Fig. 3.** Percentages of α-amylase and α-glucosidase inhibition of Chlorophyta crude extracts. Data is the mean ($n$ = 3) ± standard deviation (SD) of crude extracts evaluated at the concentration of 1 mg $mL^{-1}$. Significant differences are based on generalized linear model (GLM) analysis (quassipoisson family, logit link) ($p$ < .05). Significance codes for percentages inhibition are: > 10% of inhibition (*), 20–50% (**) and > 60% (***). Symbology: Playa Paraíso (PP), Playa Muñecos (PM) Playa Villa Rica (PVR); Rainy season (R), Dry season (D).

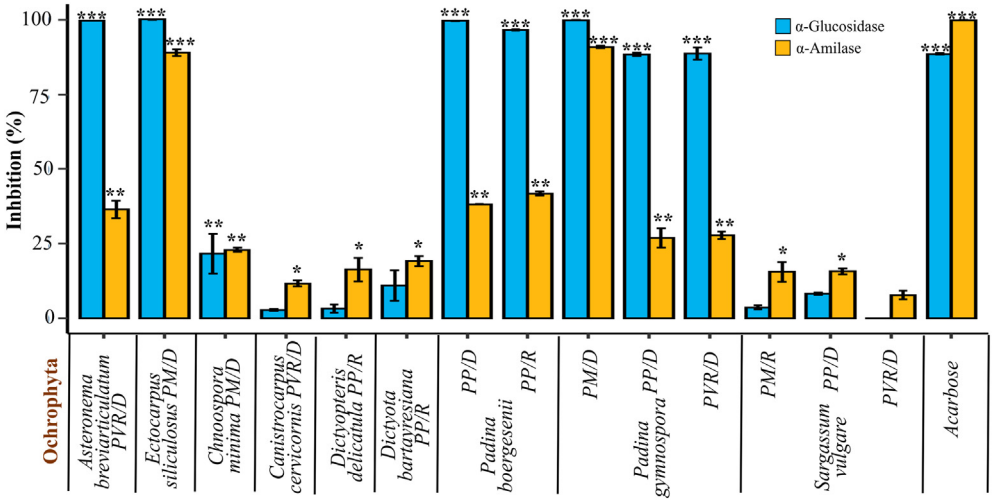


**Fig. 4.** Percentages of α-amylase and α-glucosidase inhibition of Ochrophyta crude extracts. Data is the mean ($n$ = 3) ± standard deviation (SD) of crude extracts evaluated at the concentration of 1 mg $mL^{-1}$. Significant differences are based on generalized linear model (GLM) analysis (quassipoisson family, logit link) ($p$ < .05). Significance codes for percentages inhibition are: > 10% of inhibition (*), 20–50% (**) and > 60% (***). Symbology: Playa Paraíso (PP), Playa Muñecos (PM) Playa Villa Rica (PVR); Rainy season (R), Dry season (D).

**Table 1**
Half maximal inhibitory concentration ($IC_{50}$ = μg $mL^{-1}$) of Mexican seaweed extracts on α-amylase and α-glucosidase enzymes.

| Sample | α-glucosidase | α-amylase |
|---|---|---|
| *Cladophora dalmatica* | 27.8 ± 3.1[a] | 116.9 ± 11.6[a] |
| *Asteronema breviarticulatum* | 168.3 ± 8.1[b] | NA |
| *Ectocarpus siliculosus* | 276.8 ± 11.2[c] | 679.6 ± 68.2[b] |
| *Padina boergesenii* | 43.8 ± 5.5[d] | 567 ± 65.2[b] |
| *Padina gymnospora* | 59.9 ± 7.5[e] | 1507.8 ± 448.5[c] |
| Acarbose | 1659.1 ± 152.4[f] | 40.6 ± 3.6[d] |

Data is expressed in μg $mL^{-1}$. The $IC_{50}$ was calculated on a serial dilutions ($n$ = 5) of each extract and their three replicates. Superscripts denote the significance ($p$ < .05) of the sample according to the multicomparative logistic model. NA = No activity.

**Table 2**
Toxicity of methanolic extracts of seaweeds on *Artemia franciscana* assay.

| Muestra | $LD_{50}$ (μg $mL^{-1}$) |
|---|---|
| *Cladophora dalmatica* | 37.5 ± 1.04[b] |
| *Padina boergesenii* | 1570 ± 1.2[d] |
| *Padina gymnospora* | 22,569 ± 3.2[f] |
| *Ectocarpus siliculosus* | 1007 ± 1.07[c] |
| *Asteronema breviarticulatum* | 3578 ± 1.07[e] |
| Colchicine | 3.1 ± 0.5[a] |

### 3.3. Chemical profiling by untargeted metabolomics approach (MS-UHPLC-QTOF-ESI)

#### 3.3.1. Chemical profiling by principal component analyses (PCA)

Chemical profiles of crude extracts obtained by accurate MS clustered the species in three distinguishing groups according to PCA (Fig. 5). The clusters are a) Dictyotaceae and Sargassaceae species (Ochrophyta), b) all Rhodophyta species, and c) Codiaceae, Caulerpaceae, Dasycladaceae, Halimedaceae (Chlorophyta), Asteronemataceae, Ectocarpaceae and Scytosiphonaceae (Ochrophyta) species (Fig. 3). Crude extracts of Cladophoraceae and Ulvaceae were not clustering in any group. The most active extracts were clustered in separated groups: *Padina* spp. are in "a" group, *E. siliculosus* and *A. breviarticulatum* are in "c" and *C. dalmatica* did not cluster with any group.

#### 3.3.2. Hierarchical cluster analyses (HCA)

The results of hierarchical clustering (Fig. 6) showed five groups. Although clear patterns were not observed in all extracts, it can be distinguished that Rhodophyta species tend to cluster with species from the same division. Ochrophyta and Chlorophyta have a major tendency to cluster together as was observed in PCA. The active extracts from *A. breviarticulatum*, *E. siliculosus*, and *C. dalmatica* tend to cluster in the same group whilst *Padina* spp. are in a separate cluster. For species recollected in both seasons (*L. obtusa*, *A. triquetrum*, *C. barbata*, and *P. boergesenii*), their extracts tend to separate in a different cluster, while extracts of *A. spicifera*, *D. simplex*, and *U. lactuca* clustered in the same groups (Fig. 4).

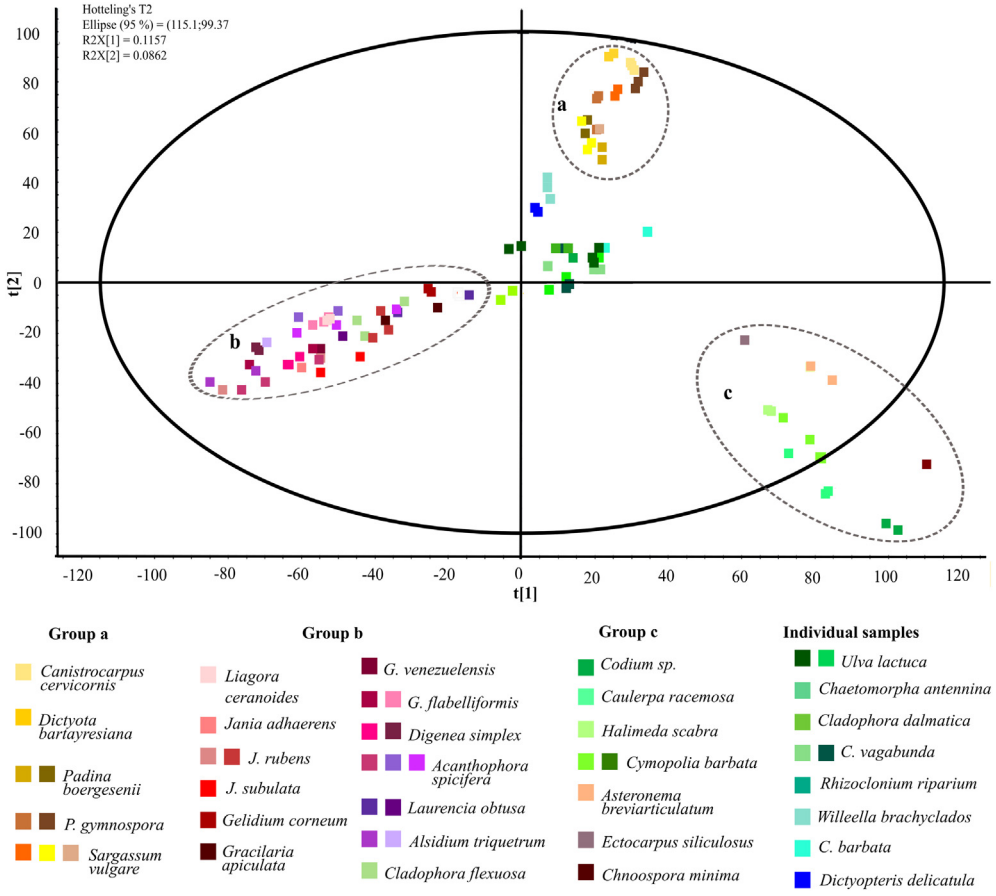


**Fig. 5.** Ordination (PCA plot) of 45 methanolic crude extracts from 31 Mexican seaweeds based on *m/z* and retention time values (variance 38%). The three major seaweeds clades (indicated by circles and letters) tended to group together mainly chemotaxonomically.

### *3.3.3. Tentative chemical identification of crude extracts*

Tentative identifications (mass error < 5 ppm) were conducted with *Padina boergesenii*, *P. gymnospora*, *A. breviarticulatum* and *E. siliculosus* (Table 3). Most of the species are characterized by the presence of fatty acids. Mannitol is reported by the first time in *P. gymnospora* as well as stypoldione in *A. breviarticulatum*. In addition, cystoseirol, sargachromenol, and stypoldione are reported for the first time in *E. siliculosus*. For *C. dalmatica*, none of the major *m/z* peaks matched with previous compounds reported in the literature and databases.

## 4. Discussion

The present study has evaluated crude extracts from 31 seaweeds species of three phyla: Ochrophyta, Rhodophyta and Chlorophyta. This is the first report that has analyzed an important number of tropical species from Mexican coast around the world as α-amylase and α-glucosidase inhibitors. The results have shown that extracts of Rhodophyta (Fig. 2) and Chlorophyta (Fig. 3) species had significant lower bioactivity ($p < .05$) compared with acarbose, which was used as a drug reference. It is important to mention, that these crude extracts are a complex mixture of compounds that can act with either synergic or antagonist effects [9,14]. In accordance to previous reports, the α-glucosidase inhibition reported for extracts of Rhodophyta species *Grateloupia elliptica* (42%) [24] and *Polyopes lancifolius* (52.4%) [41] increased significantly when the fractions and bromophenols obtained from both extracts were tested. The same pattern was observed in the extracts of Ochrophyta *Sargassum serratifolium* ($IC_{50}$ = 24.1 μg $mL^{-1}$) [30] and *Ecklonia stolonifera* ($IC_{50}$ = 6.3 μg $mL^{-1}$) [42], after chromatographic separations. Although Chlorophyta and Rhodophyta crude extracts showed an interval inhibition on enzymes between 10 and 25% (Supplementary Table 1), it is expected that their bioactivity will increase if the antagonist compounds are removed, as well if the active compounds are isolated and purified [14]. For that reason, it is advisable continuing the study of the extracts from Mexican seaweeds taxa even if they showed low bioactivity. The chromatographic partitioning approaches may be suggested for this purpose.

Some of the species evaluated in this screening belong to genera previously reported as sources of inhibitors of these enzymes. In the specific case of α-amylase inhibition, extracts of *Gracilaria apiculata* (13.3%), *G. flabelliformis* (16.9%), *G. venezuelenzis* (4.1%), *Laurencia obtusa* (6.5–17.7%), and *Ulva lactuca* (10.9–13.8%), showed lower inhibition than extracts reported of *G. edulis* (57%), *G. gracilis* (68%) [43], *L. dendroidea* (27%) [16], and *U. lactuca* (65.8%) [44]. On the other hand, the inhibition on α-glucosidase observed by extract of *Halimeda scabra* (11.2%), was also lower than reported for *H. macroloba* (70%) [45]; while extracts of *U. lactuca* did not exhibit inhibition on the enzyme. This contrasts the results previously reported for *U. lactuca* (22–87%) [38,45] and *U. pertusa* (8.3%) [37].

The chemical composition of seaweeds varies according to prevailing biotic and abiotic factors in the collection site [9,12]. This could explain the marked differences between extracts of species evaluated in this work and extracts of their congeners. Recently, tools such as metabolomics based on accurate mass spectrometry lead us to have a bigger view of the type and quantity of metabolites in a specific biological sample [13,18], which fluctuations respond to environmental cues, defense strategies and to anthropogenic pressures [18]. Evaluation of chemical profiles using metabolomics tools, PCA and HCA, provide a procedural plan capable of distinguishing the individual characteristic of several seaweeds [13,18]. The results of this work provide evidence that metabolic fingerprints show differences in

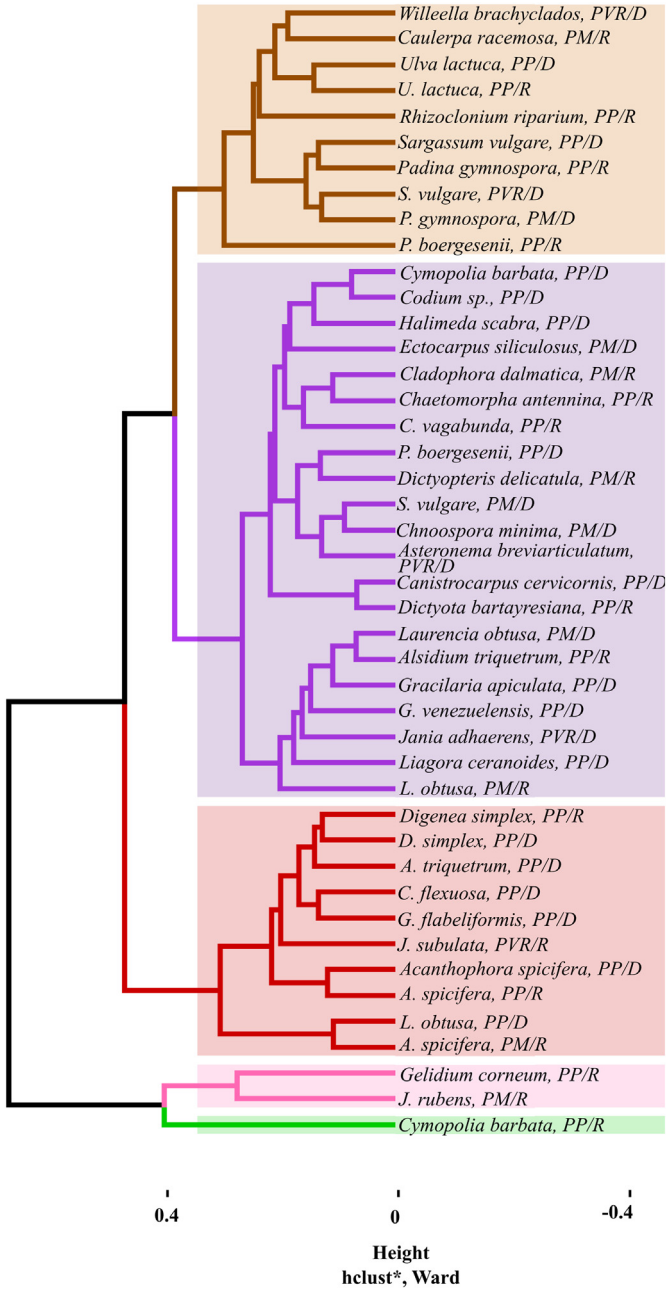


**Fig. 6.** Hierarchical cluster analyses of methanolic crude extracts of Mexican seaweeds. Dendogram is based in Euclidean distance. Symbology: Playa Paraíso (PP), Playa Muñecos (PM) Playa Villa Rica (PVR); Rainy season (R), Dry season (D).

composition among species, and these differences may be strongly related to the phylogenetic order more than any other factor. This affirmation could be supported by the PCA analyses (Fig. 5) where no differences in chemical profiles were observed between species collected in rainy and dry seasons or between the site collecting points (Playa Paraíso, Playa Villa Rica or Playa Muñecos).

In addition, the HCA (Fig. 6) shows some consistent results with the PCA analyses, in particular for Rhodophyta species, which tend to cluster with species from the same division. Both analyses may indicate that Ochrophyta and Chlorophyta species have a major relationship with their metabolic content than with Rhodophyta species, even the distance that prevails in their phylogenetic arrangement [13,46]. The main metabolites reported in Ochrophyta species are terpenes and polyphenols, while in Chlorophyta are terpenes and halogenated compounds [9]. Finally, Rhodophyta have mainly halogenated metabolites [9]. The presence of terpenes in Chlorophyta and Ochrophyta species may be related to the chemotaxonomy cluster between these taxa, while halogenated metabolites could be a strong chemotaxonomic feature which may be related with the clear separation of Rhodophyta species. Nonetheless, this should be further confirmed using integrative approaches that also considerer genomics and transcriptomics evidence [13].

Regarding the species collected in dry and rainy seasons, the HCA evidenced that *L. obtusa*, *A. triquetrum,* and *P. boergesenii* vary their metabolic contents according to the collection season. These results are expected since biotic and abiotic factors that prevail in both site and season collection will affect metabolic seaweeds content and the effect of their derivatives on biological activities [13,47]. In the case of extracts of *L. obtusa* and *P. boergesenii*, these showed a marked variation in their inhibition percentages on both enzymes. This tendency could be due to metabolic variation evidenced by HCA. In a contrary case, the extract of *C. barbata* did not show variation in their biological activity, but the HCA evidenced strongly metabolic differences in relation to the season collection of this species. As a result of this evidence, the metabolic content of seaweed from Neovolcanic rocky shores of Veracruz could vary according to season collection. This may be due to the presence or absence of specific metabolites and not their total metabolic content, as was observed in PCA. This variation could influence their potential for α-amylase and α-glucosidase inhibition.

In the crude extract of green seaweeds, *C. dalmatica* was the only that has induced 90% inhibition on both enzymes (Fig. 3). In correspond with $IC_{50}$ values, this extract inhibited α-amylase (116.9 ± 11.6 μg $mL^{-1}$) three times less than the control acarbose (40.6 ± 3.6 μg $mL^{-1}$) and inhibited α-glucosidase (27.8 ± 3.1 μg $mL^{-1}$) with higher potency than drug reference (1659.1 ± 152.4 μg $mL^{-1}$) (Table 1). So far, extracts of *C. rupestris* have been evaluated for the same bioactivity and did not show inhibitory activity on either enzymes [48]. In general, MeOH extracts of green seaweeds have shown inhibition only on the α-amylase enzyme. In contrast with previous reports, the extract of *C. dalmatica* has lower α-amylase inhibition than MeOH extracts of *Caulerpa racemosa* ($IC_{50}$ = 90 μg $mL^{-1}$) [49] and *Chlorodesmis* sp. ($IC_{50}$ = 47.6 μg $mL^{-1}$) [48]. Also, *C. dalmatica* had a higher α-amylase inhibitory activity than MeOH extracts of *Chaetomorpha aerea* ($IC_{50}$ = 408.9 μg $mL^{-1}$) [48] and *Ulva lactuca* ($IC_{50}$ = 232 μg $mL^{-1}$) [44].

Moreover, for extracts of the Ochrophyta species, the percentage inhibition of *Asteronema breviarticulatum* (99.69 ± 0.01%), *Ectocarpus siliculosus* (100.07 ± 0.1%), *Padina boergesenii* (96.6 ± 0.3–99.6 ± 0.07%) and *P. gymnospora* (88.4 ± 0.6–99.8 ± 0.04%) were significantly higher ($p < .05$) than acarbose on α-glucosidase enzyme (supplementary table 2). The $IC_{50}$ of *A. breviarticulatum* (168.3 ± 8.07 μg $mL^{-1}$) and *E. siliculosus* (276.8 ± 11.2 μg $mL^{-1}$) had a major inhibition than MeOH extracts of *Stoechospemum marginatum* (508 μg $mL^{-1}$) [45] and *Tolypiocladia glomerulata* (608 μg $mL^{-1}$) [50] (Supplementary Table 2). *P. boergesenii* and *P. gymnospora*, exhibited higher percentages inhibition than reported for aqueous extracts of *P. distromatica* (70.4%) [51] and *P. sulcata* (24.1–42.1%) [45,51], as well as MeOH extract of *P. arborescens* (71.9%) [52]. The $IC_{50}$ of methanolic extracts of *P. boergesenii* (43.8 ± 5.5 μg $mL^{-1}$) and *P. gymnospora* (59.9 ± 7.5 μg $mL^{-1}$) were higher than *S. marginatum* [44], *T. glomerulata* [50], and also higher than extracts of *P. arborescens* (260 μg $mL^{-1}$) [52], *Sargassum ringgoldianum* (120 μg $mL^{-1}$) [53], and *Spyridia fusiformis* (60 μg $mL^{-1}$) [44] (Supplementary Table 2).

Inhibition of the α-amylase and α-glucosidase enzymes delays the digestion of starch preventing the excessive postprandial rise in the blood glucose levels [2,4,6]. This is a key factor in the prevention of obesity and metabolic disorder [5,6]. Acarbose is a pseudotetrasaccharide isolated from bacteria *Actinoplanes* sp., which acts as a competitive and reversible inhibitor of both enzymes in order to reduce

**Table 3**
Compounds tentatively identified in crude extracts of Mexican seaweeds.

| | RT (min) | Mass detected (m/z) | Tentative formula | Tentative identification | Mass calculated (*m/z*) | Ion type |
|---|---|---|---|---|---|---|
| *Padina boergesenii* | 9.72 | 351.2511 | $C_{19}H_{36}O_4$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| PP, rainy season | 14.54 | 681.4131 | $C_{42}H_{58}O_6$ | Fucoxantine | 704.4028 | $[M + Na]^+$ |
| *P. boergesenii* | 14.54 | 681.4131 | $C_{42}H_{58}O_6$ | Fucoxantine | 704.4028 | $[M + Na]^+$ |
| PP, dry season | 11.21 | 279.2300 | $C_{16}H_{32}O_2$ | Plamitic acid | 302.2197 | $[M + Na]^+$ |
| | 10.56 | 351.2511 | $C_{19}H_{36}O_4$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| | 10.62 | 351.2511 | $C_{19}H_{36}O_5$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| | 11.66 | 303.2300 | $C_{18}H_{32}O_2$ | Linoleic acid | 326.2197 | $[M + Na]^+$ |
| | 11.21 | 279.2300 | $C_{16}H_{32}O_2$ | Palmitic acid | 228.0585 | $[M + Na]^+$ |
| | 11.66 | 303.230 | $C_{18}H_{32}O_2$ | Linoleic acid | 326.2197 | $[M + Na]^+$ |
| *P. gymnospora* | 0.40 | 205.0688 | $C_6H_{14}O_6$ | Mannitol | 228.0585 | $[M + Na]^+$ |
| PM, dry season | | | | | | |
| *P. gymnospora* | 0.42 | 205.0688 | $C_6H_{14}O_6$ | Mannitol | 228.0585 | $[M + Na]^+$ |
| PP, dry season | | | | | | |
| *Ectocarpus siliculosus* | 11.44 | 447.2511 | $C_{27}H_{36}O_4$ | Cystoseirol A | 470.2408 | $[M + Na]^+$ |
| PM, dry season | 11.44 | 447.2511 | $C_{27}H_{36}O_4$ | Sargachromenol | 470.2408 | $[M + Na]^+$ |
| | 11.82 | 449.2668 | $C_{27}H_{38}O_4$ | Stypoldione | 472.2565 | $[M + Na]^+$ |
| | 13.90 | 279.2300 | $C_{16}H_{32}O_2$ | Palmitic acid | 302.2197 | $[M + Na]^+$ |
| | 9.72 | 351.2511 | $C_{19}H_{36}O_4$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| | 9.72 | 279.2300 | $C_{16}H_{32}O_2$ | Palmitic acid | 302.2197 | $[M + Na]^+$ |
| | 13.77 | 303.2300 | $C_{18}H_{32}O_2$ | Linoleic acid | 326.2197 | $[M + Na]^+$ |
| *Asteronema breviarticulatum* | 11.84 | 449.2668 | $C_{27}H_{38}O_4$ | Stypoldione | 472.2565 | $[M + Na]^+$ |
| PVR, dry season | 9.72 | 351.2511 | $C_{19}H_{36}O_4$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| | 10.33 | 351.2511 | $C_{19}H_{36}O_5$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |
| | 10.56 | 351.2511 | $C_{19}H_{36}O_6$ | Nonadecanoic acid | 374.2408 | $[M + Na]^+$ |

Data were identified based on their high-resolution mass spectra (error < 5 ppm) and by comparison with public databases. Symbology: Mass/charge ratio (*m/z*), Playa Paraíso (PP), Playa Muñecos (PM) Playa Villa Rica (PVR) Retention time (RT).

postprandial hyperglycemia [3,4]. This attaches to the carbohydrate-binding site of the enzymes with an affinity exceeding that of the normal substrate due to the presence of the intramolecular nitrogen, stopping the enzymatic reaction because the C-N bonds in the acarviosine unit of acarbose cannot be cleaved [3,54,55]. Nonetheless, only mild α-amylase inhibition is recommended, due to the abnormal fermentation of indigestible carbohydrates, which lead to gastrointestinal side effects [3,4]. For that reason, non-toxic and more selective inhibitors from natural products are sought in order to prevent postprandial hyperglycemia and the risks that this health condition could present [15,21,56]. Interestingly, in this research, active extracts of *A. breviarticulatum*, *E. siliculosus*, *P. boergesenii*, and *P. gymnospora* showed a selective stronger inhibition on α-glucosidase rather than α-amylase, this selectivity is not observed in acarbose. These results suggest that MeOH extracts of Mexican tropical seaweeds may delay carbohydrate absorption with higher efficacy than acarbose, avoiding the excessive inhibition of α-amylase.

Polyphenolic extracts of brown seaweed have been reported as inhibitors which act on the α-glucosidase enzyme rather than α-amylase. Furthermore, they also have a negative correlation between phenolic content and the $IC_{50}$ values on the α-glucosidase inhibition [15,21,42,57,58]. This pattern has been previously reported for other natural products from food sources such as tea and byproducts of grapes [59,60]. Indeed, the efficacy on the inhibition on α-glucosidase observed in *C. dalmatica*, *P. boergesenii*, *P. gymnospora* and *A. breviarticulatum* is higher than those intervals reported for grapes (200–440 μg $mL^{-1}$), black tea (560–580 μg $mL^{-1}$), Oolong tea (980 μg $mL^{-1}$), and green tea (> 1000 μg $mL^{-1}$). Seaweeds and their polyphenolics have been pointed out as valuables sources of nutraceuticals [26]. Our results suggest that Mexican tropical seaweeds could also be raw materials of nutraceuticals to delay starch digestion and reduce hyperglycemia. It is advisable to further complete comprehensive studies that focus on the nutraceutical potential of these seaweeds.

In addition, fatty acids such as linoleic, oleic and palmitic acids have been reported with a strong inhibition on α-glucosidase than on α-amylase enzyme, which means that they are selective inhibitors of this enzyme [61]. Another class of compounds in seaweed reported as inhibitors of the α-glucosidase enzyme are triterpenes [29,30] and ergosterol derivatives [31]. Indeed, the untargeted metabolomics analysis pointed out the presence of fatty acids such as linoleic, palmitic and nonadecanoic acids in active Ochrophyta extracts (Table 3). Additionally, these analyses tentatively identified the following compounds: mannitol, which is a polyol, stypoldione, which is an ortho-quinone [62], cystoseirol A, and sargachromenol, which are meroditerpenoids [63]. The three last compounds are derived from mixed biosynthetic origins, which suggest the presence of complex compounds in the active extracts, as have been reported in seaweeds.

Linoleic acid is a competitive inhibitor on the α-glucosidase enzyme [61], while the terpene sargachromenol has been reported as a non-competitive inhibitor of this enzyme [30]. Linoleic acid was tentatively identified in *P. boergesenii* and *E. siliculosus*, while sargachromenol was tentatively identified in *E. siliculosus* and *A. breviarticulatum*. The selective inhibition on α-glucosidase observed in the extracts of *P. boergesenii*, *E. siliculosus,* and *A. breviarticulatum* could be related to the presence of compounds such as polyphenols, terpenes, and fatty acids. In addition, the presence of these types of compounds in active crude extracts may influence the mechanisms of inhibition on enzymes acting as competitive or non-competitive inhibitors. However, further studies should be conducted in order to isolate and identify these potentially chemical entities and to understand the mechanisms and types of inhibition of these natural products. Selective targeted of known metabolites with previous reports of α-glucosidase inhibition in all active seaweeds extracts showed few matches, which indicates the lack in the knowledge of the chemistry of seaweeds, which still prevails. In this sense, Mexican seaweeds represent natural sources of novel compounds that remain untapped.

Finally, compounds such as cystoseirol A, and stypoldione have been reported as toxic agents [62,63]. These compounds were tentatively identified in MeOH extracts of *A. breviarticulatum* and *E. siliculosus*. According to the toxicity evaluation (Table 2), the extracts of *A. breviarticulatum*, *E. siliculosus*, *P. boergesenii*, and *P. gymnospora* were not toxic against *A. franciscana* due to their $LD_{50}$ being higher than 1000 μg $mL^{-1}$. Only the $LD_{50}$ of *C. dalmatica* extract (37.55 ± 1.04 μg $mL^{-1}$) showed high toxicity, but this was lower than the activity observed in colchicine (3.15 ± 0.45 μg $mL^{-1}$) (Table 2).

Colchicine is an alkaloid isolated from terrestrial plant *Colchicus* spp. and is characterized by their toxic effects in humans [14,64]. The toxicity assay with brine shrimp *Artemia* spp. has a correlation with cytotoxic activities, and lets us evaluate rapidly and at low cost the toxicity of several extracts [17,65,66]. Also, this toxicity assay could guide the isolation of colchicine from plant extracts [67]. This suggests that the sensibility of *Artemia* sp. is a useful tool to alert us of the presence of compounds as toxic as colchicine in crude extracts. In fact, only a few species of seaweeds have been screened on the *Artemia* toxicity model [30,68,69]. There are reports in the literature that Ochrophyta, Rhodophyta and Chlorophyta are potentially toxic species. As previously mentioned, crude extracts are complex mixes of metabolites that could cause toxicity when scaled in other biological systems [70]. Then, the toxicity of extracts may be related to the chemical composition of each species in the overall mixture. For example, *Cladophora* possesses mainly halogenated and terpenoids metabolites [71] whilst *Padina* spp., *A. breviarticulatum*, and *E. siliculosus* have mainly terpenoids and polyphenols compounds. *Artemia* spp. nauplii have shown to be very sensitive to terpenoid compounds [17,65]. Indeed, the assay may confirm the presence of these compounds in the extracts and could indicate that *C. dalmatica* extract possess a larger content of them than Ochrophyta species. Additionally, the tentative toxic compounds identified in *A. breviarticulatum* and *E. siliculosus* could be in low concentration and therefore their toxic effects could be masked by other metabolites or these chemicals could not be as toxic as colchicine [14]. For *Padina* spp. extracts, the results may suggest that these do not contain toxic metabolites. Either way, if the search for bioactive metabolites derived from tropical Mexican seaweeds continues, it will be necessary to carry out toxicity assays in a finer level of discrimination for all seaweeds taxa.

## 5. Conclusions

Mexican tropical seaweeds represent an important natural source of compounds capable of reducing hyperglycemia postprandial, because their metabolic content targeted the inhibition of α-amylase and α-glucosidase enzymes. Seaweeds from the Neovolcanic rocky shores of Veracruz vary their metabolic content according to season collection, but only the variation of specific metabolites could influence the α-amylase and α-glucosidase inhibition. Ochrophyta species, particularly those belonging to *Padina* genus, are an important natural sources to look for metabolites capable of inhibiting α-amylase and α-glucosidase. While Chlorophyta and Rhodophyta species remain as potential natural sources of inhibitory metabolites. Extracts of *A. breviarticulatum*, *E. siliculosus*, *P. boergesenii*, and *P. gymnospora* are the most attractive since they exhibited selective inhibition on α-glucosidase and displayed no toxicity in preliminary assays against *A. franciscana*. The selective inhibition by these natural products may reduce the adverse effects associated with the excessive inhibition of the α-amylase enzyme. Their biological activity could be related to the fatty acid, terpene and polyphenol content. The low numbers of compounds identified in active extracts indicate that these natural sources have a wide range of potentially active metabolites which remain undiscovered such as we observed in *C. dalmatica*.

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## CRediT authorship contribution statement

**Cristina Landa-Cansigno**:Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing.**Eric E. Hernández-Domínguez**:Methodology, Supervision, Resources, Writing - review & editing.**Juan L. Monribot-Villanueva**:Methodology, Supervision, Formal analysis, Writing - review & editing.**Alexei F. Licea-Navarro**:Methodology, Supervision, Validation, Writing - review & editing.**Luz E. Mateo-Cid**:Supervision, Validation, Writing - review & editing.**Aldo Segura-Cabrera**:Methodology, Supervision, Writing - review & editing.**José A. Guerrero-Analco**:Conceptualization, Methodology, Project administration, Supervision, Resources, Funding acquisition, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2020.101954.

## References

[1] N.G. Asp, Dietary carbohydrates: classification by chemistry and physiology, Food Chem. 57 (1996) 9–14, https://doi.org/10.1016/0308-8146(96)00055-6.
[2] B.E. Goodman, Insights into digestion and absorption of major nutrients in humans, AJP Adv. Physiol. Educ. 34 (2010) 44–53, https://doi.org/10.1152/advan.00094.2009.
[3] H. Bischoff, Pharmacology of alpha-glucosidase inhibition, Eur. J. Clin. Investig. 24 (Suppl. 3) (1994) 3–10, https://doi.org/10.1111/j.1365-2362.1994.tb02249.x.
[4] D.C. Whitcomb, M.E. Lowe, Human pancreatic digestive enzymes, Dig. Dis. Sci. 52 (2007) 1–17, https://doi.org/10.1007/s10620-006-9589-z.
[5] WHO, Global action plan for the prevention and control of noncommunicable diseases 2013-2020, World Heal. Organ. 102 (2013) (doi:978 92 4 1506236).
[6] WHO, Noncommunicable disease, World Heal. Organ. 157 (2018), https://doi.org/10.1002/9781119097136.part5.
[7] M.E. Triana-Mantilla, La hiperglicemia y sus efectos tóxicos. Un concepto patogénico para la micro y macroangiopatía diabética, Rev Cuba. Angiol Y Cir Vasc. 2 (2001) 131–141 http://bvs.sld.cu/revistas/ang/vol2_2_01/ang10201.pdf.
[8] C. Lauritano, A. Ianora, Marine organisms with anti-diabetes properties, Mar. Drugs. 14 (2016) 220, https://doi.org/10.3390/md14120220.
[9] D.B. Stengel, S. Connan, Natural Products from Marine Algae: Methods and Protocols, 1st ed., Human Press, 2015, https://doi.org/10.1007/978-1-4939-2684-8.
[10] K.G. Collins, G.F. Fitzgerald, C. Stanton, R.P. Ross, Looking beyond the terrestrial: the potential of seaweed derived bioactives to treat non-communicable diseases, Mar. Drugs. 14 (2016) 1–31, https://doi.org/10.3390/md14030060.
[11] F. Ferdouse, S.L. Holdt, R. Smith, P. Murúa, Z. Yang, The global status of seaweed production, trade and utilization, FAO Globefish Res. Program. 124 (2018) 120

http://www.fao.org/in-action/globefish/publications/details-publication/en/c/1154074/.
[12] C.D. Amsler, Algal chemical ecology, 1st ed., Springer-Verlag, Berling Heidelberg, 2008, https://doi.org/10.1007/978-3-540-74181-7.
[13] I. Belghit, J.D. Rasinger, S. Heesch, I. Biancarosa, N. Liland, B. Torstensen, R. Waagbø, E.J. Lock, C.G. Bruckner, In-depth metabolic profiling of marine macroalgae confirms strong biochemical differences between brown, red and green algae, Algal Res. 26 (2017) 240–249, https://doi.org/10.1016/j.algal.2017.08.001.
[14] G.E. Trease, W.C. Evans, D. Evans, W.B. Saunders (Ed.), Trease and Evans Pharmacognosy, 15th ed., 2002, https://doi.org/10.1017/CBO9781107415324.004 Edimburg.
[15] S. Lordan, T.J. Smyth, A. Soler-Vila, C. Stanton, R. Paul Ross, The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts, Food Chem. 141 (2013) 2170–2176, https://doi.org/10.1016/j.foodchem.2013.04.123.
[16] K. Pirian, S. Moein, J. Sohrabipour, R. Rabiei, J. Blomster, Antidiabetic and antioxidant activities of brown and red macroalgae from the Persian Gulf, J. Appl. Phycol. 29 (2017) 3151–3159, https://doi.org/10.1007/s10811-017-1152-0.
[17] S.M. Colegate, R.J. Molyneux, Bioactive Natural Products: Detection, Isolation, and Structural Determination, second ed., CRC Press, Boca Raton, 2007, https://doi.org/10.1016/s0031-9422(97)87094-x.
[18] M. Kumar, U. Kuzhiumparambil, M. Pernice, Z. Jiang, P.J. Ralph, Metabolomics: an emerging frontier of systems biology in marine macrophytes, Algal Res. 16 (2016) 76–92, https://doi.org/10.1016/j.algal.2016.02.033.
[19] Y. Sharifuddin, Y.X. Chin, P.E. Lim, S.M. Phang, Potential bioactive compounds from seaweed for diabetes management, Mar. Drugs. 13 (2015) 5447–5491, https://doi.org/10.3390/md13085447.
[20] N. Mahmood, A review of α-amylase inhibitors on weight loss and glycemic control in pathological state such as obesity and diabetes, Comp. Clin. Path. 25 (2016) 1253–1264, https://doi.org/10.1007/s00580-014-1967-x.
[21] E. Apostolidis, C.M. Lee, *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition, J. Food Sci. 75 (2010), https://doi.org/10.1111/j.1750-3841.2010.01544.x.
[22] S.H. Lee, Y.J. Jeon, Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms, Fitoterapia 86 (2013) 129–136, https://doi.org/10.1016/j.fitote.2013.02.013.
[23] Y. Sharifuddin, Y.X. Chin, P.E. Lim, S.M. Phang, Potential bioactive compounds from seaweed for diabetes management, Mar. Drugs. 13 (2015) 5447–5491, https://doi.org/10.3390/md13085447.
[24] K.Y. Kim, K.A. Nam, H. Kurihara, S.M. Kim, Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*, Phytochemistry 69 (2008) 2820–2825, https://doi.org/10.1016/j.phytochem.2008.09.007.
[25] F.F. Pedroche, A.S. G, Ficología marina mexicana. Diversidad y problemática actual, Hidrobiológica 13 (2003) 23–32.
[26] A.R. Múzquiz-de la Garza, M. Tapia-salazar, M. Maldonado-Muñiz, J. de la Rosa-Millán, J.A. Gutiérrez-Uribe, L. Santos-Zea, B.A. Barba-Dávila, D. Ricque-Marie, E. Cruz-Suárez, Nutraceutical potential of five Mexican brown seaweeds, Biomed. Res. Int. (2019) 1–15, https://doi.org/10.1155/2019/3795160.
[27] C. Landa-Cansigno, L.E. Mateo-Cid, A.C. Mendoza-González, J.A. Guerrero-Analco, Macroalgas marinas del litoral rocoso Neovolcánico de Veracruz, México, Acta Bot. Mex. (126) (2019), https://doi.org/10.21829/abm126.2019.1525.
[28] D. Geissert-Kientz, Regionalización geomorfológica de Veracruz, Investig. Geográficas. 40 (1999) 23–47.
[29] L. Ali, A.L. Khan, M. Al-Broumi, R. Al-Harrasi, L. Al-Kharusi, J. Hussain, A. Al-Harrasi, New enzyme-inhibitory triterpenoid from marine macro brown alga *Padina boergesenii* Allender & Kraft, Mar. Drugs. 15 (2017) 1–8, https://doi.org/10.3390/md15010019.
[30] Y. Ali, D.H. Kim, S.H. Seong, H.R. Kim, H.A. Jung, J.S. Choi, α-Glucosidase and protein tyrosine phosphatase 1b inhibitory activity of plastoquinones from marine brown alga *Sargassum serratifolium*, Mar. Drugs. 15 (2017) 368, https://doi.org/10.3390/md15120368.
[31] L. Ali, A.L. Khan, L. Al-Kharusi, J. Hussain, A. Al-Harrasi, New α-glucosidase inhibitory triterpenic acid from marine macro green alga *Codium dwarkense* Boergs, Mar. Drugs. 13 (2015) 4344–4356, https://doi.org/10.3390/md13074344.
[32] W.R. Taylor, Marine Algae of the Eastern Tropical and Subtropical Coasts of the Americas, 1st ed., University of Michigan Press, Ann Harbor, 1960.
[33] D.S. Littler, M.M. Littler, Caribbean reef plants, An Identification Guide to the Reef Plants of the Caribbean, Bahamas, Florida and Gulf of Mexico, 1st ed., Offshore Graphics, Inc, Washington, DC, 2000.
[34] C.F.D. Gurgel, S. Fredericq, J.N. Norris, Gracilaria apiculata, G. flabelliformis, (Gracilariaceae, Rhodophyta): restoring old names for common tropical western Atlantic species, including the recognition of three new subspecies, and a replacement name for "*G. lacinulata*,", Cryptogam. Algol. 25 (2004) 367–396.
[35] L.M.D. Gestinari, S.M.B. Pereira, Y. Yoneshigue-Valentin, Distribution of *Cladophora* species (Cladophorales, Chlorophyta) along the Brazilian coast, Phytotaxa 14 (2010) 22, https://doi.org/10.11646/phytotaxa.14.1.2.
[36] F.J. Gella, G. Gubern, R. Vidal, F. Canalias, Determination of total and pancreatic α-amylase in human serum with 2-chloro-4-nitrophenyl-α-D-maltotriosid as substrate, Clin. Chim. Acta 259 (1997) 147–160, https://doi.org/10.1016/S0009-8981(96)06481-9.
[37] J. Kellogg, M.H. Grace, M.A. Lila, Phlorotannins from Alaskan seaweed inhibit carbolytic enzyme activity, Mar. Drugs. 12 (2014) 5277–5294, https://doi.org/10.3390/md12105277.
[38] M.J. Crawley, The R Book, second ed., John Wiley & Sons Ltd Publications, Chichester, 2013, https://doi.org/10.1086/431086.
[39] C. Ritz, F. Baty, J.C. Streibig, D. Gerhard, Dose-response analysis using R, PLoS One 10 (2015) 1–13, https://doi.org/10.1371/journal.pone.0146021.
[40] D. Borcard, F. Gillet, P. Legendre, Numerical Ecology with R, 1st ed., Springer, New York, 2011, https://doi.org/10.1007/978-1-4419-7976-6.
[41] K.Y. Kim, T.H. Nguyen, H. Kurihara, S.M. Kim, α-Glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia*, J. Food Sci. 75 (2010), https://doi.org/10.1111/j.1750-3841.2010.01629.x.
[42] H.E. Moon, M.N. Islam, B.R. Ahn, S.S. Chowdhury, H.S. Sohn, H.A. Jung, J.S. Choi, Protein tyrosine phosphatase 1B and α-glucosidase inhibitory phlorotannins from edible brown algae, *Ecklonia stolonifera* and *Eisenia bicyclis*, Biosci. Biotechnol. Biochem. 75 (2011) 1472–1480, https://doi.org/10.1271/bbb.110137.
[43] S. Lakshmana Senthil, T.V. Kumar, D. Geetharamani, T. Maruthupandi, Screening of seaweeds collected from southeast coastal area of India for α-amylase inhibitory activity, antioxidant activity and biocompatibility, Int J Pharm Pharm Sci 5 (2013) 240–244.
[44] S. Murugesan, S. Bhuvaneswari, V. Sivamurugan, Evaluation of *in vitro* antidiabetic activity of red seaweed *Portieria hornemannii* (Lyngbye) (Silva) and Spyridia fusiformis (Wulfen), World J. Pharm. Sci. 4 (2016) 415–419.
[45] Y.X. Chin, P.E. Lim, C.A. Maggs, S.M. Phang, Y. Sharifuddin, B.D. Green, Antidiabetic potential of selected Malaysian seaweeds, J. Appl. Phycol. 27 (2015) 2137–2148, https://doi.org/10.1007/s10811-014-0462-8.
[46] T. Cavalier-Smith, Intron phylogeny: a new hypothesis, Trends Genet. 7 (1991) 145–148, https://doi.org/10.1016/0168-9525(91)90377-3.
[47] D.B. Stengel, S. Connan, Z.A. Popper, Algal chemodiversity and bioactivity: sources of natural variability and implications for commercial application, Biotechnol. Adv. 29 (2011) 483–501, https://doi.org/10.1016/j.biotechadv.2011.05.016.
[48] P. Unnikrishnan, K. Suthindhiran, M. Jayasri, Alpha-amylase inhibition and antioxidant activity of marine green algae and its possible role in diabetes management, Pharmacogn. Mag. 11 (2015) 511, https://doi.org/10.4103/0973-1296.172954.
[49] V.L. Teixeira, F.D. Rocha, P.J. Houghton, M. Auxiliadora, C. Kaplan, R.C. Pereira, α -amylase inhibitors from Brazilian seaweeds and their hypoglycemic potential, 78 (2007) 35–36, https://doi.org/10.1016/j.fitote.2006.09.017.
[50] V.. Mohanapriya, N. Murugesan, S. Sivamurugan, In vitro α -amylase and α -Glucosidase inhibitory activity of methanol extract of *Tolypiocladia glomerulata*, (C. Agardh) F. Schmitz, Saudi J. Biomed. Res. 1 (2016) 59–63, https://doi.org/10.21276/sjbr.2016.1.3.1.
[51] J.P.J. Paul, Screening of anti-diabetic properties of fucoidan extraced from *Padina distromatica* Hauck (brown seaweed) from Hare Island, Thoothukudi, Tamil Nadu, India, Innov. Drug Discov. 4 (2014) 164–169.
[52] M.H. Park, J.S. Han, Hypoglycemic effect of *Padina arborescens* extract in streptozotocin-induced diabetic mice, Prev. Nutr. Food Sci. 17 (2012) 239–244, https://doi.org/10.3746/pnf.2012.17.4.239.
[53] C.W. Lee, J.S. Han, Hypoglycemic effect of *Sargassum ringgoldianum* extract in STZ-induced diabetic mice, Prev. Nutr. Food Sci. 17 (2012) 8–13, https://doi.org/10.3746/pnf.2012.17.1.008.
[54] E.A. MacGregor, Š. Janeček, B. Svensson, Relationship of sequence and structure to specificity in the α-amylase family of enzymes, Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1546 (2001) 1–20, https://doi.org/10.1016/S0167-4838(00)00302-2.
[55] A. Faridmoayer, C.H. Scaman, Binding residues and catalytic domain of soluble *Saccharomyces cerevisiae* processing alpha-glucosidase I, Glycobiology 15 (2005) 1341–1348, https://doi.org/10.1093/glycob/cwj009.
[56] S.H. Lee, M.H. Park, S.J. Heo, S.M. Kang, S.C. Ko, J.S. Han, Y.J. Jeon, Dieckol isolated from *Ecklonia cava* inhibits α-glucosidase and α-amylase *in vitro* and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice, Food Chem. Toxicol. 48 (2010) 2633–2637, https://doi.org/10.1016/j.fct.2010.06.032.
[57] S.H. Eom, S.H. Lee, N.Y. Yoon, W.K. Jung, Y.J. Jeon, S.K. Kim, M.S. Lee, Y.M. Kim, α-Glucosidase and α-amylase inhibitory activities of phlorotannins from *Eisenia bicyclis*, J. Sci. Food Agric. 92 (2012) 2084–2090, https://doi.org/10.1002/jsfa.5585.
[58] A.C. Murugan, M.R. Karim, M.B.M. Yusoff, S.H. Tan, M.F.B.F. Asras, S.S. Rashid, New insights into seaweed polyphenols on glucose homeostasis, Pharm. Biol. 53 (2015) 1087–1097, https://doi.org/10.3109/13880209.2014.959615.
[59] W.K. Lee, L.L. Wong, Y.Y. Loo, S. Kasapis, D. Huang, Evaluation of different teas against starch digestibility by mammalian glycosidases, J. Agric. Food Chem. 58 (2010) 148–154, https://doi.org/10.1021/jf903011g.
[60] V. Lavelli, P.S.C. Sri Harsha, M. Laureati, E. Pagliarini, Degradation kinetics of encapsulated grape skin phenolics and micronized grape skins in various water activity environments and criteria to develop wide-ranging and tailor-made food applications, Innov. Food Sci. Emerg. Technol. 39 (2017) 156–164, https://doi.org/10.1016/j.ifset.2016.12.006.
[61] C.H. Su, C.H. Hsu, L.T. Ng, Inhibitory potential of fatty acids on key enzymes related to type 2 diabetes, BioFactors 39 (2013) 415–421, https://doi.org/10.1002/biof.1082.
[62] W.H. Gerwick, W. Fenical, N. Fritsch, J. Clardy, Stypotriol and stypoldione; ichthyotoxins of mixed biogenesis from the marine alga, *Stypopodium zonale*, Tetrahedron Lett 20 (1979) 145–148, https://doi.org/10.1016/S0040-4039(01)85907-4.
[63] C. Francisco, B. Banaigs, L. Codomier, A. Cave, Cystoseirol a. a novel rearranged diterpene of mixed biosynthesis from the brown alga *Cystoseira mediterranea*, Tetrahedron Lett. (1985), https://doi.org/10.1016/S0040-4039(00)94985-2.
[64] A. Slobodnick, B. Shah, M.H. Pillinger, S. Krasnokutsky, Colchicine: old and new, Am. J. Med. 128 (2015) 461–470, https://doi.org/10.1016/j.amjmed.2014.12.010.
[65] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, Brine shrimp: a convenient general bioassay for active plant constituents, Planta Med. 45 (1982) 31–34, https://doi.org/10.1055/s-2007-971236.
[66] J.L. Carballo, Z.L. Hernández-Inda, P. Pérez, M.D. García-Grávalos, A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural

products, BMC Biotechnol. 2 (2002) 1–5, https://doi.org/10.1186/1472-6570-2-17.
[67] F.Q. Alali, K. Tawaha, T. El-Elimat, R. Qasaymeh, C. Li, J. Burgess, Y. Nakanishi, D.J. Kroll, M.C. Wani, N.H. Oberlies, Phytochemical studies and cytotoxicity evaluations of *Colchicum tunicatum* Feinbr and *Colchicum hierosolymitanum* Feinbr (Colchicaceae): two native Jordanian meadow saffrons, Nat. Prod. Res. 20 (2006) 558–566, https://doi.org/10.1080/14786410500183381.
[68] J. Ara, V. Sultana, S. Ehteshamul-Haque, R. Qasim, V.U. Ahmad, Cytotoxic activity of marine macro-algae on *Artemia salina* (brine shrimp), Phyther. Res. 13 (1999) 304–307, https://doi.org/10.1002/(SICI)1099-1573(199906)13:4<304::AID-PTR439>3.0.CO;2-9.
[69] Hira Ayesha, V. Sultana, J. Ara, S. Ehteshamul-Haque, *In vitro* cytotoxicity of seaweeds from Karachi coast on brine shrimp, Pakistan J. Bot. 42 (2010) 3555–3560.
[70] S.K. Meenakshi, M. Nagarajan, M. Sundararaman, Brine shrimp cytotoxicity was not warrant for teratogenicit : comparative analysis of seaweed methanolic extracts collected from Tamil Nadu coastal region on brine shrimp and zebra fish embryos, Res. Rev. A J. Life Sci. 8 (2018) 24–32.
[71] E. Chmielewská, J. Medved, Bioaccumulation of heavy metals by green algae *Cladophora glomerata* in a refinery sewage lagoon, Croat. Chem. Acta 74 (2001) 135–145.