JJBS

Volume 15, Number 3,September 2022
ISSN 1995-6673
Pages 449 – 456
https://doi.org/10.54319/jjbs/150314

Jordan Journal of Biological Sciences

Preliminary Studies on the Potential Antioxidant and Antidiabetic Activities of *Sargassum polycystum* C. Agardh (Phaeophyceae, Ochrophyta)

Eldrin DLR. Arguelles*

Philippine National Collection of Microorganisms (PNCM), National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippine Los Baños, College, Laguna, Philippines, 4031

Received: August 19, 2021; Revised: December 25, 2021; Accepted: January 19, 2022

Abstract

Seaweeds are considered natural sources of chemical compounds with notable potent antioxidant and antidiabetic activities. The study aims to know the total polyphenolic content (TPC) and assess the antioxidant and antidiabetic activities of *Sargassum polycystum* for potential pharmacological use. The seaweed has a TPC of 1.149 ± 0.22 mg GAE/g. Antioxidant activity of *S. polycystum* is characterized by having potent scavenging activity against $ABTS^{+}$ radical and high copper reduction capacity with IC_{50} value of 49.50 µg GAE/ml and 20.40 µg GAE/ml, respectively, more effective than ascorbic acid (control). Assessment of the antidiabetic properties of *S. polycystum* was done *in vitro* via α-amylase and α-glucosidase inhibition assay. Results of the analysis proved that *S. polycystum* has potent α-amylase (IC_{50} of 0.264 µg/ml) and α-glucosidase (IC_{50} of 120 µg/ml) inhibition activities in collation to that of acarbose (antidiabetic drug) with IC_{50} values of 4.81µg/ml and 6771 µg/ml, respectively. This study is a pioneering research investigation in the Philippines describing the bioactive properties of *S. polycystum* as renewable source of bioactive substances for inhibition of important carbohydrate degrading enzymes.

Keywords: biological activities; diabetes; marine; phenolic compounds; seaweeds

1. Introduction

Diabetes is a systemic long-term disease known for the occurrence of hyperglycemia in which there is an elevated concentration of sugar in the blood because the pancreas fails to produce enough insulin (Arguelles & Sapin, 2020a). It causes glycation (non-enzymatic) of serum proteins which leads to generation of glycation end products (Ulrich & Cerami, 2001). These end products cause major complications of diabetes such as kidney failure, stroke, and heart attack (Ulrich & Cerami, 2001). In general, medical treatments available for patients with diabetes include insulin injections and oral antidiabetic drugs. However, prolonged use of these treatments leads to lower therapeutic capacity and several side effects (Arica *et al.,* 2017). In addition, leading carbohydrate degrading enzyme inhibitors, miglitol, and acarbose, are recently documented to produce intestinal side effects like diarrhea, bloating, and abdominal pain occurring simultaneously (Ulrich & Cerami, 2001; Arica *et al.,* 2017). Thus, preventive antidiabetic substances with minimum side effects are needed. A therapeutic method in treatment of diabetes is to lessen the occurrence of hyperglycemia via inhibition of α-amylase (enzyme for the degradation of long-chain starch) and α-glucosidase (an enzyme responsible for oligosaccharide and disaccharide breakdown) (Zhao *et al.,* 2017). These enzymes are responsible for regulating the postprandial blood sugar levels and are considered target enzymes for the development and synthesis of natural hypoglycemic drugs (Ulrich & Cerami, 2001). Inhibitors of these hydrolyzing enzymes impede carbohydrate degradation causing a lower absorption of glucose preventing diabetes (Ulrich & Cerami, 2001; Zhao *et al.*, 2017).

Naturally derived antidiabetic drugs are currently becoming popular because of high financial burdens and side effects that are coupled with allopathic therapy strategies for the treatment of diabetes. Antioxidants are substances that inhibit oxidation and are considered to have medicinal value in treatment of several diseases like diabetes (Arguelles *et al.,* 2017; Rajendiran *et al.,* 2018). Diabetes treatment using antioxidants (such as thioctic acid, tocopherol, and vitamin C) protects beta-cells against oxidative stress-induced apoptosis and prevents complications caused by the disease. Studies using streptozotocin-induced diabetic rats showed that antioxidant therapy (diet supplementation) using tocopherol and vitamin C lowers the concentration of lipid peroxide and significantly increases superoxide dismutase (SOD) activity improving the health condition of the animal (Seven *et al.,* 2004). In addition, vitamin C therapy in diabetic rats lowers the erythrocyte sorbitol levels and helps in improving the insulin resistance of the animal (Rajendiran *et al.,* 2018). These findings suggest that dietary supplementation of antioxidants may reduce the complication of diabetes and are beneficial for diabetes treatment (Seven *et al.,* 2004; Rajendiran *et al.,* 2018).

* Corresponding author. e-mail: edarguelles@up.edu.ph.

Seaweeds are marine organisms that have diverse medicinal value and are currently being tapped as candidate natural resources for bioactive substances that can be used for diabetes treatment (Arguelles, 2018; Zhao *et al*., 2017). Among seaweeds, brown macroalgae are reported as promising sources due to the therapeutic potential of polyphenolic compounds isolated from these organisms that exhibit antidiabetic activity (Arica *et al.,* 2017). *Sargassum polycystum* C. Agardh is a brown seaweed that is taxonomically classified under the phylum Ochrophyta, and class Phaeophyceae which are commonly observed in Philippine coastal areas. This seaweed is characterized by having a large and brownish thallus with oblanceolate leaves that is usually attached to a solid substratum. The primary branch is terete with secondary branches that are arranged irregularly alternate with several proliferations. The leaves of this seaweed have percurrent midrib and serrated margins usually with vesicles that are ovoid or spherical (Trono, 1997). Phenolic compounds from this seaweed such as phlorotannins, gallic acids, ellagic acids, and phloroglucinol can activate insulin secretion and possess inhibitory properties against carbohydrate degrading enzymes which can be utilized for pharmaceutical research applications (Hwang *et al.,* 2014; Zhao *et al.,* 2017).

The Philippines diverse seaweed natural resource is considered underutilized and have not been widely studied as a source of bioactive ingredients for medicinal use. This study serves as a pioneering research investigation in the country assessing the bioactive properties of *S. polycystum* against carbohydrate degrading enzymes (Arguelles & Sapin, 2020a). The current investigation aims to determine the TPC and assess the antioxidant and antidiabetic properties of *S. polycystum* extract for pharmacological use. The antidiabetic activities of the algal extract were assessed using the α-amylase and α-glucosidase inhibition assays while antioxidant activities of *S. polycystum* were analyzed using copper reduction antioxidant capacity and $ABTS^{+}$ radical scavenging assay. In addition, correlation analysis between phenolic concentration of the macroalgal extract and the antioxidant and antidiabetic activities were established.

2. Materials and Method

2.1. Seaweed collection and sample preparation

S. polycystum was harvested on 19 January, 2020 in shallow waters in the coastal area of Laiya, (Lat. 13° 40' 26.364" N; Long. 121° 23' 48.948" E), Batangas, Philippines (Figures 1 and 2). Mature dark brown thalli of *S. polycystum* were collected in the sampling area. The seaweed was scrubbed using brush bristles. The seaweed was rinsed several times using sterile tap water to remove sand particles, epiphytes, and other necrotic parts present in the thalli of the alga. The cleaned thalli of *S. polycystum* was securely packed in polythene bags (kept inside a chilled plastic cooler) and immediately transported to the laboratory for further processing. The washed seaweed was oven-dried and pulverized before solvent extraction (Arguelles *et al.,* 2019; Arguelles, 2020). The seaweed was identified using relevant morphotaxonomic features and taxonomic monographs of Trono (1997) and Algae Base (Guiry & Guiry, 2021).

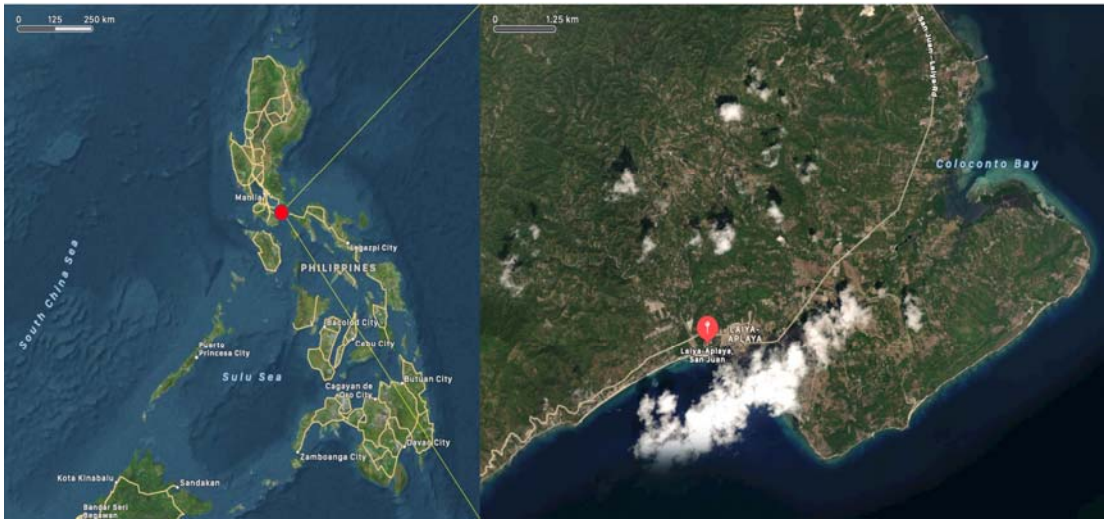

Figure 1. Location map of sampling site of *Sargassum polycystum* from Batangas, Philippines.

Figure 2. Thallus morphology of *Sargassum polycystum* C. Agardh.

2.2. Seaweed Extract Preparation

Powdered *S. polycystum* biomass (1 g) was immersed with 30 ml of the extraction solvent (acidified methanol, 1:80:10 v/v, HCl: CH_3OH: H_2O) and stirred for 1 h using an ultrasonic bath following the extraction protocol of Gao et al. (2002). Using a rotary evaporator (BUCHI™ Rotavapor™ Scholar System), the liquid soluble constituent was concentrated at 40 ºC. The concentrated algal crude extract was placed in a sterile screw-capped tube and kept at 4 ºC until use (Gao *et al.,* 2002; Arguelles & Sapin, 2020a). The yield extract of *S. polycystum* was calculated using the equation:

$$\text{Yield (\%)} = \frac{\text{Weight of the algal extract (g)}}{\text{Weight of the dried algal biomass (g)}} \times 100$$

2.3. Determination of the total phenolic content

The TPC of *S. polycystum* was estimated using the Folin-Ciocalteu method (Nuñez-Selles *et al.,* 2002). Initially, 0.5 ml of *S. polycystum* extract was mixed with 0.5 ml of 10% sodium carbonate and Folin-Ciocalteu's reagent. The solution was mixed via a vortex mixer until homogenous and was set aside for 5 min. The volume of the reaction mixtures was adjusted by adding 5 ml of sterile distilled water. Absorbance reading of each sample were taken using an Ultraviolet-Visible spectrophotometer at 720 nm wavelength. The TPC of *S. polycystum* was estimated using a standard gallic acid calibration curve ($y = 0.0682x - 0.0214$, $R^2 = 0.997$). The TPC of *S. polycystum* is reported as milligrams of gallic acid equivalent per gram of the seaweed sample (Nuñez-Selles *et al.,* 2002).

2.4. ABTS⁺ radical scavenging assay

The extract of *S. polycystum* was evaluated for $ABTS^+$ radical scavenging property using the protocol of Re *et al.,* (1999). An aliquot (40 µl) of the algal extract at varying phenolic concentrations and 40 µl of the control were mixed with 3 ml of $ABTS^+$ free radical mixture (with initial optical density of 0.72±0.05 at 734 nm). The reaction mixtures were mixed and stored for 5 min at normal room temperature. The absorbance of each test samples was taken at 734 nm. The effective concentrations (IC_{50}) for *S. polycystum* and the ascorbic acid (control) were determined during the analysis. The percent inhibition was computed using the equation:

$$ABTS^+ \text{ Inhibition (\%)} = \frac{Abs_{734}\text{ (control)} - Abs_{734}\text{ (sample)}}{Abs_{734}\text{ (control)}} \times 100$$

2.5. CUPRAC assay

The antioxidant capacity of *S. polycystum* extract for copper reduction was evaluated following the colorimetric method of Alpinar *et al.,* (2009). Initially, 1 ml each of 0.01 M copper (II) chloride ($CuCl_2$), 1 M ammonium acetate buffer (pH 7), and 0.0075 M neocuproine solutions were mixed until homogenous in a test tube. An aliquot (0.5 ml) of *S. polycystum* extract prepared at different phenolic concentrations as well as ascorbic acid were added from the initial mixture. The total volume of the reaction mixture was adjusted up to 4.1 mL for each concentration using fresh distilled water. The reaction mixtures were placed for 30 min at ambient temperature condition. The absorbance readings of each test sample were noted at 450 nm. The effective concentrations (IC_{50}) for *S. polycystum* extract and the control (ascorbic acid) were determined during the analysis of copper reduction antioxidant capacity (Alpinar *et al.,* 2009; Arguelles & Sapin, 2020c).

2.6. Alpha-amylase inhibition assay

The capacity of *S. polycystum* extract to inhibit α-amylase was assayed *in vitro* conditions using the methods of Phoboo *et al.,* (2015) with modifications. Solutions of alpha-amylase from porcine pancreas (0.5 mg/ml, Sigma A3176), 0.02 M Sodium-phosphate buffer (pH 6.9) with 0.006M NaCl and 1% starch solution were prepared. The samples of *S. polycystum* with different phenolic concentrations were prepared by dilution with water. In a 50 µl of the alpha-amylase solution, 25 µl of the sample (*S. polycystum* at varying phenolic concentrations) were mixed in test tubes. As for the case of the control, 25 µl of buffer was mixed with 50 µl of alpha-amylase solution. The volume was made up to 250 µl by adding 175 µl phosphate buffer. Then, 250 µl starch solution was added at timed intervals. After 20 min of incubation, the reaction was halted by adding 400 µl DNS color reagent also at timed intervals. The blank to zero the instrument consisted of 500 µl buffer and 400 µl DNS reagent. The reaction tubes were subjected to a boiling hot water bath for about 5 minutes, cooled, and further diluted with 5 ml fresh distilled water. Absorbance reading of the test mixture was noted at 540 nm wavelength. Acarbose served as the control (anti-diabetic drug) in the analysis. The inhibition was determined using the equation:

$$\text{α-Amylase inhibition (\%)} = \frac{Abs_{540}\text{(control)} - Abs_{540}\text{(sample)}}{Abs_{540}\text{(control)}} \times 100$$

2.7. Alpha-glucosidase inhibition assay

The capacity of *S. polycystum* to inhibit α- glucosidase was done *in vitro* using the methods of Nair *et al.,* (2013). A mixture consisting of 75 µl of α – glucosidase (2.5 U/ml) and 100 µl of the different prepared phenolic

concentrations of *S. polycystum* or 100 µl of 0.1 M phosphate buffer pH 6.8 (in case of the control) were prepared and thoroughly mixed in sterile test tubes. The volume of the test sample was adjusted to 500 µl by adding 295 µl buffer and 30 µl of 10mM p-nitrophenyl-α-D-glucopyranoside (Sigma N1337). The mixtures were kept at 37 ºC for 12 min after which 3 ml of 50 mM NaOH were added. The absorbance reading of the mixtures was taken at 410 nm. Acarbose served as the control (an α-glucosidase inhibitor and anti-diabetic drug) in the analysis. The α – glucosidase inhibition was determined using the equation:

$$\text{α-Glucosidase inhibition (\%)} = \frac{Abs_{410}(control) - Abs_{410}(sample)}{Abs_{410}(control)} \times 100$$

2.8. Statistical Data Analyses

The assays used in this study were done in three replicates (means ± standard deviations). The correlation analysis among antidiabetic and antioxidant activities as well as the phenolic concentrations of *Sargassum polycystum* extract were done using Pearson's correlation (R) coeeficient via Microsoft (MS) Office Excel 2021 (Arguelles & Sapin, 2020b).

3. Results and Discussion

3.1. Extraction yield and TPC

The crude extract of *S. polycystum* obtained from this study is brownish, which can be attributed to algal pigments such as xanthophylls, violaxanthin, carotenoids, fucoxanthin, and chlorophyll present in the crude extract (El-Sheekh *et al.,* 2021). Extraction yield is the percentage of the crude extract that can be utilized from the sample (El-Sheekh *et al.,* 2021). *Sargassum polycystum* crude extract have an extraction yield of 12.47± 0.31% which is higher as compared to that observed by Sivagnanam *et al.,* (2015) from ethanol extracts of *S. japonica* (1.22± 0.12%) and *S. horneri* (1.36±0.14%) from the coastal area of Korea. However, this extraction yield is lower than that obtained by El-Sheekh *et al.,* (2021) from methanol and cold aqueous extracts of *Taonia atomaria* from Alexandria, Egypt with an extraction yield of 16.5 ± 0.2% and 13.5 ± 0.1%, respectively. The results obtained in the current investigation are consistent with earlier studies (Sivagnanam *et al.,* 2015; El-Sheekh *et al.,* 2021), which documented that extraction yield is highly dependent on the polarity of the solvent. Generally, sample extracts that are prepared using polar solvents are observed to have the highest amount of crude extractable substances (Sivagnanam *et al.,* 2015). Variation in the extraction yield of *S. polycystum* reported in this investigation can be ascribed to several factors such as sample particle size, method of extraction, and temperature used in the extraction protocol (Sivagnanam *et al.,* 2015). Hence, optimization of the extraction condition is recommended for large-scale production of the active compound from *S. polycystum*.

Table 1. Extraction yield and TPC of *Sargassum polycystum* acidified methanolic extract.

Sample	Extract	Extract Yield (%)	Total Phenolic Content (mg GAE/g)
Sargassum polycystum	Acidified Methanol	12.47± 0.31	1.149 ± 0.22

Seaweeds are considered natural sources of biologically active chemical compounds with therapeutic uses. Polyphenols from marine macroalgae are characterized to possess potent antioxidant and antidiabetic activities. The TPC of *S. polycystum* is 1.149±0.22 mg GAE/g extract (Table 1). This result was comparable with previous research done by Chakraborty *et al.,* (2017) and Fu *et al.,* (2015) for *S. polycystum* with TPC of 0.37 mg GAE/g and 8.71 mg GAE/g, respectively. However, Arguelles (2020) showed that the green seaweed (*Codium intricatum*) has a higher TPC, which was 40.79±0.015 mg GAE/g. Cikoš *et al.,* (2018) also reported that strains of *Ascophyllum nodosum* extracted using methanol showed TPC ranging from 0.51 mg GAE/g to 1.40 mg GAE/g. The differences in the TPC of several algal species could be attributed to several intrinsic and extrinsic factors such as seasonal variations, age or maturity of the alga, geographic location, tidal cycles, and salinity that can affect the metabolic profile of the macroalga (Arguelles and Sapin, 2020b,c; Arguelles, 2021a,b).

3.2. Antioxidant activities of S. polycystum extract

The antioxidant properties of *S. polycystum* were evaluated using CUPRAC and $ABTS^+$ radical scavenging assays. Results showed that *S. polycystum* has potent antioxidant activity, more effective than ascorbic acid (control). As presented in Table 2, scavenging efficiency of the algal extract was observed to cause a dose-dependent inhibition of $ABTS^+$ free radicals. The computed effective concentration (IC_{50}) of *S. polycystum* extract is 49.50 µg GAE/ml which is more effective than ascorbic acid (control) with IC_{50} value of 147.90 µg/ml (Table 2). In comparison with other known seaweeds, *S. polycystum* was able to exhibit potent $ABTS^+$ radical scavenging activity more potent than those obtained from methanol extracts of *Acetabularia acetabulum* (IC_{50} of 6.30 mg/ml) and *Halimeda tuna* (IC_{50} of 16.1 mg/ml) (Sivaramakrishnan *et al.,* 2017). However, Arguelles and Sapin (2020a) reported that methanol extract of a brown macroalga, *Turbinaria decurrens*, has a more potent antioxidant activity than *S. polycystum* having extract with an IC_{50} value of 49.31 ug GAE/ml. Several phenolic compounds derived from seaweeds are antioxidants with excellent scavenging activity against free radical (Fu *et al.,* 2015). These substances are capable of terminating autoxidation of free radicals by hydrogen atom donation from phenolic hydroxyl (OH) groups present in the compound (Fu *et al.,* 2015; Arguelles *et al.,* 2019; Arguelles & Sapin, 2020b).

Table 2. $ABTS^+$ free radical scavenging activity and IC_{50} value of phenolics from *S. polycystum* and ascorbic acid.

Sample	Phenolic concentration (µg GAE/ml)					IC_{50} * (µg/ml)
	12.5	25.0	37.5	50.0	62.5	
	$ABTS^+$ Inhibition (%)					
Sargassum polycystum	15.58 ± 1.22	28.74 ± 0.04	41.92 ± 0.31	50.43 ± 0.51	59.02 ± 1.02	49.50
	Concentration (µg/ml)					
	37.5	75.0	112.5	150.0	187.5	
	$ABTS^+$ Inhibition (%)					
Ascorbic Acid	11.70 ± 1.54	24.56 ± 0.62	36.70 ± 0.51	50.87 ± 0.82	64.53 ± 1.64	147.90

*IC_{50} is the concentration capable of inhibiting the activity of $ABTS^+$ radical by 50%. Computed via interpolation.

Table 3. Copper reduction antioxidant capacity and IC_{50} value of phenolics from *S. polycystum* and ascorbic acid.

Sample	Phenolic concentration (µg GAE/ml)					IC_{50}* (µg/ml)
	5	10	15	20	25	
	CUPRAC (Absorbance at 450 nm)					
Sargassum polycystum	0.154 ± 0.001	0.278 ± 0.008	0.402 ± 0.001	0.486 ± 0.001	0.599 ± 0.011	20.40
	Concentration (µg/ml)					
	10	20	30	40	50	
	CUPRAC (Absorbance at 450 nm)					
Ascorbic acid	0.112 ± 0.002	0.213 ± 0.007	0.328 ± 0.004	0.429 ± 0.012	0.542 ± 0.011	46.30

*IC_{50} is the concentration that shows a CUPRAC value of 0.5 at a wavelength of 450 nm. Computed via interpolation.

S. polycystum extract also exhibited copper ion reduction ability in a concentration-dependent manner (Table 3). The seaweed extract exhibited more effective antioxidant activity than ascorbic acid with IC_{50} of 20.40 µg/ml and IC_{50} = 46.30 µg/ml, respectively. The observed trend in this analysis is homologous to that observed from $ABTS^+$ scavenging assay in which at 62.5 µg/ml concentration the highest $ABTS^+$ free radical inhibition of 59.02% was noted. The copper reduction antioxidant capacity of *S. polycystum* is more effective than that of *Turbinaria ornata* (IC_{50} value of 24.34 µg GAE/ml) but is less potent than that of *Sargassum siliquosum* with IC_{50} of 18.50 µg GAE/ml (Arguelles & Sapin, 2020b,c). The results obtained in this antioxidant assay show that *S. polycystum* is capable of inhibiting oxidation via a metal chelation which can be attributed to phenolic compounds like phloroglucinols, phlorotannins, fucoxanthin, and bromophenols that are found in the algal extract (Ponnan *et al.,* 2017; Arguelles & Sapin, 2020b,c).

3.3. Correlation analysis of antioxidant activities and phenolic content

The correlation analysis between phenolic concentration of *S. polycystum* extract and antioxidant activities using $ABTS^+$ free radical scavenging and CUPRAC assay is shown in Figure 3. The analysis exhibited positive correlations among antioxidant ($ABTS^+$ radical scavenging and CUPRAC assays) capacity and the phenolic concentrations of the seaweed extract with R=0.993741 and R=0.997816, respectively. It is clear from these results that phenolic compounds may serve an important function in the metal ion chelating and free-radical scavenging abilities exhibited by the algal extract. This finding is further supported by previous studies showing positive correlations between antioxidant activities and phenolic contents in several *Sargassum* species such as *S. acinarium*, *S. ilicifolium, S. muticum*, and *S. vulgare* (Arguelles *et al.,* 2019; Ismail *et al.,* 2020; Arguelles, 2021a).

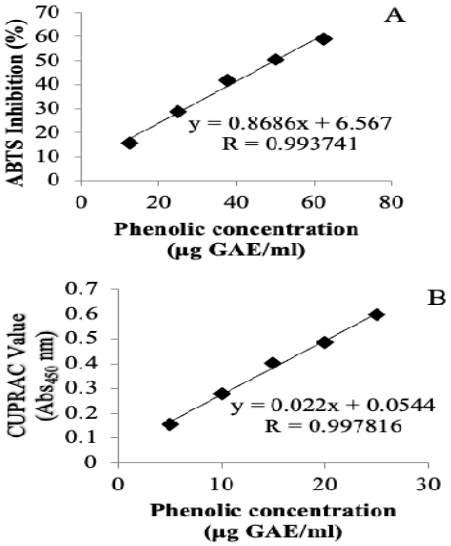

Figure 3. Correlation among phenolic concentration and antioxidant activities via (A) $ABTS^+$ radical scavenging and (B) copper reduction antioxidant capacity of *S. polycystum*.

3.4. Antidiabetic activities of S. polycystum extract

The most common way of treating patients with diabetes is by preventing the occurrence of hyperglycemia. This is made possible by suppressing key carbohydrate hydrolyzing enzymes in the digestive system such as α-glucosidase and α-amylase (Pirian *et al.,* 2017; Ismail *et al.,* 2020). The antidiabetic activities of *Sargassum polycystum* were evaluated by measuring the ability of the algal extract to cause inhibition of these carbohydrate hydrolyzing enzymes. The result of the α-amylase inhibitory effect of *S. polycystum* extract is presented in Table 4. The seaweed extract showed a more potent α-amylase inhibition activity as compared to acarbose (standard antidiabetic drug) with IC_{50} values of 0.264 µg/ml and 4.81 µg/ml, respectively. The α-glucosidase inhibition activity of *S. polycystum* extract is shown in Table 5. The inhibition of various concentrations of the seaweed extract also exhibited a concentration-dependent reduction in enzyme activity. *S. polycystum* extract showed high inhibitory activity of α-glucosidase with an effective concentration (IC_{50}) of 120 µg/ml. This IC_{50} value is considered more potent than that of acarbose, which gave an IC_{50} of 6771 µg/ml.

Table 4. α-amylase inhibition and IC_{50} of phenolics from *S. polycystum* in comparison to acarbose.

Sample	Phenolic concentration (µg GAE/ml)					IC_{50}* (µg/ml)
	0.1	0.2	0.3	0.4	0.5	
	α-glucosidase inhibition (%)					
Sargassum polycystum	11.48 ± 0.20	31.79 ± 0.25	60.30 ± 0.20	72.48 ± 0.15	79.27 ± 0.25	0.264
	Concentration (µg/ml)					
	1.5	3.0	4.5	6.0	7.5	
	α-amylase inhibition (%)					
Acarbose**	24.01 ± 0.87	38.85 ± 2.32	47.90 ± 0.93	58.24 ± 0.43	65.43 ± 0.43	4.81

* IC_{50} is the effective concentration that inhibits α-amylase activity by 50%.

**Reference α-amylase inhibitor and anti-diabetic drug.

Table 5. α-glucosidase inhibition and IC_{50} of phenolics from *S. polycystum* in comparison to acarbose.

Sample	Phenolic concentration (µg GAE/ml)					IC_{50}* (µg/ml)
	25	50	75	100	125	
	α-glucosidase inhibition (%)					
Sargassum polycystum	5.81 ± 0.14	14.48 ± 0.00	24.61 ± 0.00	38.61 ± 0.10	52.77 ± 0.12	120
	Concentration (µg/ml)					
	2000	4000	6000	8000	10000	
	α-glucosidase inhibition (%)					
Acarbose**	17.96 ± 1.36	31.69 ± 1.22	45.32 ± 1.90	57.26 ± 0.49	62.35 ± 0.49	6771

* IC_{50} is the effective concentration that inhibits α-glucosidase activity by 50%.

**Reference α-glucosidase inhibitor and anti-diabetic drug.

The extract of *S. polycystum* was significantly effective in inhibiting carbohydrate hydrolyzing enzymes being more potent than acarbose (standard antidiabetic drug). The IC_{50} values of *S. polycystum* acidified methanol extract showed that the seaweed is an effective and potent inhibitor for both enzymes. Thus, showing that *S. polycystum* can be utilize in the control of postprandial hyperglycemia and treatment of diabetes. This result is further supported by previous studies from seaweed extracts of *Turbinaria decurrens* (IC_{50} of 2.84 µg/ml), *Turbinaria ornata* (IC_{50} of 535.6 µg/ml), and *Sargassum glaucescens* (IC_{50} of 8.9 mg/ml) with potent enzyme inhibition activities that can be utilized as antidiabetic agents (Unnikrishnan *et al.,* 2014; Payghami *et al.,* 2015; Arguelles & Sapin, 2020a). Polyphenolic compounds have the ability of chelating α-amylase and α-glucosidase, causing these key enzymes to chemically precipitate and have structural changes in combination with loss of biological activities. This confirms the potent carbohydrate hydrolyzing enzyme inhibition exhibited by *S. polycystum* extract in the current study. In addition, the diverse kinds of polyphenolic compounds (such as bromophenols, phlorotannins, p-coumaric acid, and dihydrobenzoic acid) found in the algal extract may have expressed synergistic effects, causing higher effectiveness in α-amylase and α-glucosidase inhibition (Firdaus *et al.,* 2015). The promising carbohydrate hydrolyzing enzyme inhibition properties of *S. polycystum* extract show the possible use of this macroalga as an alternative source of natural antidiabetic drugs that can be used in the regulation of hyperglycemia in the body via slow digestion and absorption (Arguelles & Sapin, 2020a).

In this study, *S. polycystum* extract exhibited a stronger inhibition activity in α-amylase than α-glucosidase. A possible reason for this might be that the strength of enzyme inhibition may not only depend on the number of phenolic compounds included in the algal extract but also on the composition. Previous findings have reported positive correlation between the polyphenol concentration (in the seaweed extracts) and their potent ability to cause inhibition of the carbohydrate digestive enzymes; however, high concentration of phenolic compounds of seaweed extracts are not always correlated or associated with potent α-amylase than α-glucosidase inhibition (Pirian *et al.,* 2017; Ismail *et al.,* 2020). This suggests that the interaction (among the chemical components of the extract) and the type of phenolic compounds present in seaweed extract may be potent to α-amylase but not so for α-glucosidase or vice versa. Thus, showing that *S. polycystum* extract may have a phenolic profile with specific phenolic compounds that have greater enzyme inhibition for α-amylase than for α-glucosidase.

3.5. Correlation analysis of antidiabetic activities and phenolic content

The correlation analysis among antidiabetic activities and phenolic concentration of *S. polycystum* extract via enzyme inhibition activities are shown in Figure 4. The analysis exhibited a positive correlation between antidiabetic (enzyme inhibition) activities and phenolic concentrations of the seaweed extract with R=0.93999 and R=0.99415, respectively. Such correlation supports the potential function of polyphenols in the antidiabetic properties of the algal extract. This correlation is homologous to that observed by Firdaus *et al.,* (2015) where they attributed the carbohydrate hydrolyzing enzyme inhibition activities of different *Sargassum* species extracts to their high phenolic content.

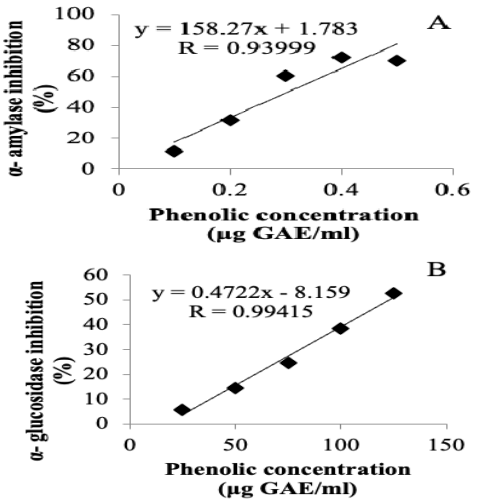

Figure 4. Correlation between phenolic concentration and antidiabetic properties via (A) α-amylase inhibition and (B) α-glucosidase inhibition activities of *S. polycystum*.

4. Conclusions

Sargassum polycystum C. Agardh contains a high amount of polyphenols and exhibited antioxidant activity characterized by having potent $ABTS^{+}$ radical scavenging activity and high copper reduction capacity more effective than ascorbic acid. In addition, analysis of the antidiabetic properties of *S. polycystum* showed that the seaweed has potent α-amylase and α-glucosidase inhibition properties in collation to that of acarbose (antidiabetic drug) proving the potential application of *S. polycystum* for treatment of diabetes. This study is a pioneering research investigation in the Philippines that reported the potential antioxidant and antidiabetic properties of *S. polycystum* as potential source of active substances for inhibition of target carbohydrate degrading enzymes. It is recommended that investigation on the isolation, identification, and mass production of the target active substances found in *S. polycystum* extract should be done to further understand the mechanisms involved in other biological activities of the alga *in vivo*. In addition, the use of other food-grade solvents such as aqueous ethanol (at different percentages) and water as the extraction solvents in the assay is also recommended to further support safety concerns regarding the use of seaweed for pharmaceutical application.

Acknowledgement

The author acknowledges the support given by the PNCM-BIOTECH, UPLB for this research study. The author is also thankful to Mrs. Arsenia B. Sapin (Food Laboratory) for her technical assistance in the conduct of the assays needed in the study.

References

Alpinar K, Özyurek M, Kolak U, Guclu K, Aras Ç, Altun M, Celik SE, Berker KI, Bektasoglu B and Ampal R. 2009. Antioxidant capacities of some food plants wildly grown in Ayvalik of Turkey. *Food Sci Technol Res.,* **15(1)**: 59-64.

Arguelles EDLR. 2021a. Evaluation of antioxidant capacity, tyrosinase inhibition, and antibacterial activities of brown seaweed, *Sargassum ilicifolium* (Turner) C. Agardh 1820 for Cosmeceutical Application. *J Fish Environ.*, **45(1)**: 64-77.

Arguelles EDLR. 2021b. Biochemical composition and bioactive properties of *Chlorella minutissima* (Chm1) as a potential source of chemical compounds for nutritional feed supplement and disease control in aquaculture. *Curr Appl Sci Technol.,* **21(1)**: 65-77.

Arguelles EDLR. 2020. Evaluation of nutritional composition and *in vitro* antioxidant and antibacterial activities of *Codium intricatum* Okamura from Ilocos Norte (Philippines). *Jordan J Biol Sci.,* **13(3)**: 375-382.

Arguelles EDLR and Sapin AB. 2020a. *In vitro* antioxidant, alpha-glucosidase inhibition and antibacterial properties of *Turbinaria decurrens* Bory (Sargassaceae, Ochrophyta). *Asia-Pacific J Sci Technol.,* **25(3)**: https://so01.tci-thaijo.org/index.php/APST/article/view/240714/165247.

Arguelles EDLR and Sapin AB. 2020b. Bioprospecting of *Turbinaria ornata* (Fucales, Phaeophyceae) for cosmetic application: antioxidant, tyrosinase inhibition and antibacterial activities. *J ISSAAS*., **26**(2): 30-41.

Arguelles EDLR and Sapin AB. 2020c. Bioactive properties of *Sargassum siliquosum* J. Agardh (Fucales, Ochrophyta) and its potential as source of skin-lightening active ingredient for cosmetic application. *J Appl Pharm Sci.*, **10(7)**: 51-58

Arguelles EDLR, Monsalud RG, and Sapin AB. 2019. Chemical composition and *in vitro* antioxidant and antibacterial activities of *Sargassum vulgare* C. Agardh from Lobo, Batangas, Philippines. *J ISSAAS.*, **25(1)**:112-122.

Arguelles EDLR. 2018. Proximate analysis, antibacterial activity, total phenolic content and antioxidant capacity of a green microalga *Scenedesmus quadricauda* (Turpin) Brébisson. *Asian J Microbiol Biotechnol Environ Sci.,* **20(1)**:150-158.

Arguelles EDLR, Laurena AC, Martinez-Goss MR and Monsalud RG. 2017. Antibacterial sctivity, total phenolic content, and antioxidant capacity of a green microalga *Desmodesmus* sp. (U-AU2) From Los Baños, Laguna (Philippines). *J Nat Stud.,* **16(2)**: 1-13.

Arica SC, Ozyilmaz A, and Demirci S. 2017. A study on the rich compounds and potential benefits of algae: A review. *Pharm Innov J.*, **6(12)**: 42-51.

Chakraborty K, Maneesh A and Makkar F. 2017. Antioxidant activity of brown seaweeds. *J Aquat Food Prod Technol.,* **26**:406–419.

Cikoš A-M, Jokic´ S, Šubaric´ D and Jerkovic´ I. 2018. Overview on the application of modern methods for the extraction of bioactive compounds from marine macroalgae. *Marine Drugs,* **16**: 348.

El-Sheekh MM, El-Shenody RAEK, Bases EA and El-Shafay SM. 2021. Comparative assessment of antioxidant activity and biochemical composition of four seaweeds, Rocky Bay of Abu Qir in Alexandria, Egypt. *Food Sci Technol.,* **41(Suppl. 1)**: 29-40.

Firdaus M, Nurdiani R and Prihanto AA 2015. Antihyperglycemic of *Sargassum* sp. extract. In: Kim SK, Chojnacka K. (Eds.). **Marine algae extracts: Processes, products and applications**. Wiley, Weinheim, pp 381–394.

Fu CWF, Ho CW, Yong WTL, Abas F and Tan CP. 2015. Effects of phenolic antioxidants extraction from four selected seaweeds obtained from Sabah. *PeerJ PrePrints,* **3**: e1249v1.

Gao L, Wang S, Oomah BD, and Mazza G. 2002. Wheat quality: Antioxidant activity of wheat millstreams. In: Ng P, Wrigley CW. (Eds.). **Wheat Quality Elucidation**. AACC International, St. Paul, Minnesota, USA, pp 219 – 233.

Google. (n.d.). [Google Maps location of sampling site in Laiya, Batangas, Philippines]. Retrieved December 18, 2021, from https://www.google.com/maps/place/13%C2%B040'26.4%22N+121%C2%B023'49.0%22E/@13.729365,121.3579252,12.22z/data=!4m5!3m4!1s0x0:0x3b38fcb0886aa22!8m2!3d13.67399!4d121.39693

Guiry M D, in Guiry M D, and Guiry G M. 2021. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Retrieved July 19, 2021, from http://www.algaebase.org.

Hwang PA, Hung YL, Tsai YK, Chien SY and Kong ZL. 2014. The brown seaweed *Sargassum hemiphyllum* exhibits α-amylase and α-glucosidase inhibitory activity and enhances insulin release *in vitro*. *Cytotechnology,* **67**: 653–660.

Ismail GA, Gheda SF, Abo-Shady AM, and Abdel-Karim OH. 2020. *In vitro* potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. *Food Sci Technol.,* **40(3)**: 681-691.

Iwai K. 2008. Antidiabetic and antioxidant effects of polyphenols in brown alga *Ecklonia stolonifera* in genetically diabetic KK-Ay mice. *Plant Foods Hum Nutr.,* **63(4)**: 163–169.

Nair SS, Kavrekar V and Mishra A. 2013. *In vitro* studies on alpha amylase and alpha-glucosidase inhibitory activities of selected plant extracts. *Eur J Exp Biol.,* **3(1)**:128-132.

Nuñez-Selles A, Castro HTV, Aguero JA, Gonzalez JG, Naddeo F, De Simone F and Pastrelli L. 2002. Isolation and quantitative analysis of phenolic antioxidants, free sugars and polyols from mango (*Mangifera indica* L.) stem bark aqueous decoction used in Cuba as a nutritional supplement. *J Agric Food Chem.,* **50**: 762-766.

Payghami N, Jamili S, Rustaiyan A, Saeidnia S, Nikan M and Gohari AR. 2015. Alpha-amylase inhibitory activity and sterol composition of the marine algae, *Sargassum glaucescens*. *Pharmacog Res.,* **7**:314-321.

Pirian K, Moein S, Sohrabipour J, Rabiei R and Blomster J. 2017. Antidiabetic and antioxidant activities of brown and red macroalgae from the Persian Gulf. *J Appl Phycol*., **29(6)**: 3151-3159.

Phoboo S, Shetty K and Abdelkhalig TEE. 2015. *In vitro* assays of anti-diabetic and anti-hypertensive potential of some traditional edible plants of Qatar. *J Med Act Plants*, **4(3-4)**: 22-29.

Ponnan A, Ramu K, Marudhamuthu M, Marimuthu R, Siva K and Kadarkarai M. 2017. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh) Kuetz. *Clin Phytoscience,* **3(1)**:1–10.

Rajendiran D, Packirisamy S and Gunasekaran K. 2018. A review on role of antioxidants in diabetes. *Asian J Pharm Clin Res.*, **11(2)**:48-53.

Re R, Pellegrine N, Proteggente A, Pannala A, Yang M and Rice-Evans C. 1999. Antioxidant activity applying an improved $ABTS^{+}$ radical cation decolorization assay. *Free Radic Biol Med.*, **26**:1231-1237.

Sivagnanam SR, Yin S, Choi JH, Park YB, Woo HC and Chun BS. 2015. Biological properties of fucoxanthin in oil recovered from two brown seaweeds using supercritical CO_2 extraction. *Marine Drugs*, **13**:3422-3442.

Sivaramakrishnan T, Swain S, Saravanan K, Kiruba SR, Roy SD, Biswas L and Shalini B. 2017. *In vitro* antioxidant and free radical scavenging activity and chemometric approach to reveal their variability in green macroalgae from South Andaman Coast of India. *Turk J Fish Aquat Sci.,* **17**: 639-648.

Seven A, Guzel S, Seymen O, Civelek S, Bolayirli M, Uncu M and Burcak G. 2004. Effects of Vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma. *Yonsei Med J.,* **45(4)**:703-710.

Trono G C. 1997. **Field Guide and Atlas of the Seaweed Resources of the Philippines**, First ed. Makati City: Bookmark Inc.

Ulrich P and Cerami A. 2001. Protein glycation, diabetes, and aging. *Recent Prog Horm Res.,* **56**:1-21.

Unnikrishnan P, Suthindhiran K and Jayasri M. 2014. Inhibitory potential of *Turbinaria ornata* against key metabolic enzymes linked to diabetes. *BioMed Res Int.,* 1-10.

Zhao C, Yang C, Liu B, Lin L, Sarker SD, Nahar L, Yu H, Cao H and Xiao J. 2017. Bioactive compounds from marine macroalgae and their hypoglycemic benefits. *Trends in Food Sci Technol.*, **72**:1-12.