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In vitro potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes

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Abstract

In this study crude extracts of *Turbinaria decurrens, Padina pavonica, Sargassum muticum* and *Sargassum acinarium* (Phaeophyta); *Ulva lactuca* (Chlorophyta) and *Pterocladia capillacea* (Rhodophyta) seaweeds were tested to evaluate their antioxidant properties and antidiabetic potential on α-amylase and α-glucosidase starch hydrolyzing enzymes. The results showed that all analyzed seaweeds exhibited antioxidant effects using DPPH (2,2-Diphenyl-1-picrylhydrazyl), reducing power and total antioxidant capacity assays in addition to antidiabetic activity that all depended on the species and the extract solvent. Among the tested extracts, acetone extract of *Turbinaria decurrens* showed the highest antioxidant activity and inhibitory effects for α-amylase (96.1%) and α-glucosidase (97.4%), respectively which was related with its total phenolic content and antioxidant activity. *In vitro,* the extract showed no toxicity against fibroblast normal cell lines at lower concentration of 250 µg/ml. Gas Chromatography-Mass Spectrum analysis (GC-MS) of this acetone extract showed the presence of different bioactive compounds mainly cyclotrisiloxane, hexamethyl which could be responsible for its antioxidant and antidiabetic activities. The study results suggest that brown seaweeds especially *T. decurrens* can be used as antioxidant ingredients and as potent reducing drug for postprandial hyperglycemia.

Keywords: seaweeds; antioxidant; antidiabetic; cytotoxicity; GC-MS analysis.

Practical Application: Looking for new natural supplies proved to be safe, economic and eco-friendly such as seaweeds used as food, antioxidant and as a potent drug for diabetes.

1 Introduction

The term of seaweeds refers to the large visible macro-algae growing attaching to rocks and along the sea shore and they are found in a range of aquatic habitats (Raven & Giordano, 2014). They are categorized based on their pigments content, morphological and anatomical attributes into three divisions which are red algae (Rhodophyceae), brown algae (Phaeophyceae) and green algae (Chlorophyceae) (Manivannan et al., 2009). There is an excessive interest for screening helpful medications from marine algae as they contain bioactive compounds, e.g., polysaccharides, pigments, proteins, lipids, peptides, minerals and vitamins (Husni et al., 2016). They have diversified biological activities, such as anti-hypertensive, anti-hyperlipidemic, anti-coagulant, apoptotic activities (Lee & Han, 2012), antioxidant, anti-inflammatory and anticancer (Khalid et al., 2018). They also could play an important role in the control of diabetes (Unnikrishnan et al., 2015) by regulation of glucose-instigated oxidative stress and suppression of starch hydrolyzing enzymes (Husni et al., 2016).

In the body, biochemical pathways of metabolism may produce reactive oxygen species (ROS), as intermediate hazardous products which, upon excess- abundance creation, the body will not be able to neutralize or detoxify these free radicals thus causing oxidative stress impact (Santos-Sánchez et al., 2019). This resulted in oxidative impairment of cell components such as protein denaturation, lipid peroxidation and/or DNA conjugation. Recently, oxidative stress has been related with many diseases like cancer, diabetic, cardiovascular diseases, Parkinson's and Alzheimer disease, post-ischemic and neural degradation disease (Pirian et al., 2017). Antioxidants are compounds that stop or interrupt the oxidation process in cells by scavenging free radicals and hence prevent or mitigate diseases (Kokabi et al., 2013). There are many artificial antioxidant substances, yet with unsafe effects. Thus, exploring new natural antioxidants have been a challenge target. In this concern, marine seaweeds have been known as traditional sources of natural antioxidants (Samaraweera et al., 2012).

Diabetes mellitus is a metabolic disturbance having the feature of hyperglycemia requiring continuous medical care, which occurs primarily because of a relative or absolute absence of insulin, weakened viability of insulin activity or tissue impedance to insulin (American Diabetes Association, 2014). Insulin, a hormone created by the β-cells of pancreas, functions to keep up a strict control of the blood glucose. This hormone enables the tissues and the body cells to consume glucose for energy. Insulin absence or disabling of its activity causes tissues and cells insensitivity, thus can't utilize glucose causing an increased level of glucose in the blood, which leads to diabetes (Akpaso et al., 2017).

Diabetes is basically related with hazardous difficulties including hypertension, nephropathy retinopathy, and other

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cardiac diseases (Pamungkas & Chamroonsawasdi, 2019). Two principle types of diabetes mellitus were known: type 1 and type 2. Type 1 diabetes (or Insulin Dependent Diabetes Mellitus (IDDM)) accounted for around 10% of all diabetes cases and rottenly occurs in individuals under age of 40 years. In this type a normal lack of insulin release occurs which causes damaging of the β-cells in pancreas (Vasudevan & Sreekumari, 2011). Alternatively, type 2 diabetes effects about 90% of persons that are at or more 40 years old. In this type, insensitivity of tissues to insulin stimulating weakened insulin activity and sometimes, high levels of insulin (Vasudevan & Sreekumari, 2011). Enzymes, such as, α-amylase and α-glucosidase in humans body act in harmony to hydrolyze starch by pancreatic α-amylase and to absorb glucose by intestinal α-glucosidase which may swift postprandial hyperglycemia (Unnikrishnan et al., 2015).

The aim of this study was to evaluate, *in vitro,* the antioxidant and antidiabetic activities of six different seaweeds collected from different locations in Egyptian coasts. The study was extended to test the cytotoxicity of the most promising seaweed extracts and to identify the most active compounds that responsible for the antioxidant and antidiabetic activities using GC-MS analysis.

2 Material and methods

2.1 Collection of seaweed samples

Different types of seaweeds were collected from Abou Qir Bay, Alexandria (*Ulva lactuca, Padina pavonica* and *Pterocladia capillacea*) and Hurghada beach, Egypt (*Turbinaria decurrens, Sargassum muticum* and *Sargassum acinarium*) during Summer of 2017. The gathered seaweeds were imparted to the phycology laboratory in plastic bags containing seawater to counteract vaporization. The gathered seaweeds were cleaned utilizing the tap water then with refined water for removing joined epiphytes and non-living network. A portion of the gathered samples were preserved in 0.4% formalin in seawater for taxonomical identification and the remaining portion was dried in the air on retentive papers, under shadow, at room temperature of 25-30 °C. The dried examples were powdery grinded using an electrical mill, then stored until use. The seaweed samples were identified according to Aleem (1993), Jha et al. (2009) and Guiry & Guiry (2019).

2.2 Preparation of seaweed extracts.

The dried seaweed samples were extracted using 80% of various organic solvents such as acetone, ethanol and methanol as well as distilled water for 72 h by using shaking incubators in a dark condition. After being filtered, each filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The samples were finally stocked in a refrigerator at 2-8 °C for use in the subsequent experiments.

2.3 Preliminary phytochemical screening

Typical procedures described by Andima et al. (2014) were used for chemical identification of the phyto-components present in the various extracts.

2.4 Antioxidant assays

DPPH free radical scavenging assay

DPPH free radical scavenging was determined according to the procedures described by Ul-Haq et al. (2012). In a glass tube, 2800 µL of 0.1mM methanolic DPPH (Sigma, St. Louis, USA) solution were mixed with 200 µl of the test sample. The tubes were shaken well and kept away from the light for 60 minutes at 37 °C. The tubes were then centrifuged (Fisher Scientific 225 Centrifuge) at 3000 rpm for 5 min. The absorbance of the reaction mixtures was measured at 517 nm on a spectrophotometer. Ascorbic acid was used as a positive control. The percentage of DPPH free radical scavenging for each sample was calculated by using the following formula (Ul-Haq et al., 2012):

$$\%Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test\ sample}{Absorbance\ of\ control} \times 100$$

The IC_{50} values were also calculated using GraphPad prism 6 software.

Reducing power assay

The method of Mohapatra et al. (2016) was used to assess the reducing power activity of the different seaweed extracts. 1 ml of each extract was mixed with 2.5 ML of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide then incubated in a water bath at 50 °C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL of each) was then diluted with 2.5 mL distilled water and then 0.5 ml of 0.1% ferric chloride solution was added. The intensity of the blue-green color was measured at 700 nm. The results were expressed as mg of ascorbic acid equivalent /g dry weight (mg AAE/g DW). Ascorbic acid was used as standard. Tests were carried out in triplicate.

Total antioxidant capacity assay

The total antioxidant capacity (TAC) was determined following Ahmed et al. (2013). Each test extract (0.3 mL) was mixed with 3 mL phosphomolybdenum reagent in a capped vial and incubated in a boiling water bath at 95 °C for 90 min. After that, the samples were cooled at room temperature and the absorbance was measured at 695 nm. Ascorbic acid was used as a reference and the results were expressed as mg ascorbic acid equivalent /g dry weight of the extract.

2.5 Total phenolic content

The total phenolic concentration was measured using the Folin-Ciocalteau method (Cox et al., 2010). In this procedure, 100 µL of each sample extract were mixed with 2.0 mL of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then, 100 µL of 50% Folin - Ciocalteau's phenol reagent were added and incubated for 30 min at room temperature away from the light. The absorbance was measured at 720 nm. Gallic acid was used as a positive control. The total phenolic contents of the samples were expressed as mg Gallic acid equivalent per gram extract dry weight (mg GAE/g DW).

2.6 Antidiabetic assay

In vitro α-amylase inhibition

The inhibitory activity of α-amylase was determined according to Schomburg & Salzmann (1991). 250 µL of each extract were added to 250 µL of 1 mM phosphate buffer (pH 7.3 with 30 mM $CaCl_2$) containing 0.5 mg/mL α-amylase (porcine pancreatic alpha-amylase Sigma, St. Louis, USA) then, the solution was incubated for 10 min at 25 °C. After incubation, 250 µL of 1% soluble starch solution in 1 mM phosphate buffer (pH 7.3 with 30 mM $CaCl_2$) were added and incubated again at 25 °C for 10 min. The reaction was shut-off by adding 500 µL of dinitrosalicylic acid color reagent (Sigma, St. Louis, USA). The tubes were then incubated in a boiling water bath for 5 min after that, cooled to room temperature. The mixture was then diluted by adding 5 mL of distilled water, and then the absorbance was measured at 540 nm. The tests were done in triplicates, and the inhibitory activity of α -amylase was expressed as (Unnikrishnan et al., 2015):

$$\%Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ extract}{Absorbance\ of\ control} \times 100$$

Concentrations of the most promising extract resulting in 50% inhibition of the enzyme activity (IC_{50}) were determined by GraphPad prism 6 software.

In vitro α-glucosidase inhibition

The effect of the seaweed extracts on the activity of *α*-glucosidase was determined as described by Kazeem et al. (2013) using *α*-glucosidase enzyme derived from *Saccharomyces cerevisiae*. Aliquot of 100 µL of *α*- glucosidase (1.0 U/mL, Sigma, St. Louis, USA) was preincubated with 50 µL of seaweed extracts at different concentrations for 10 min. Then, 50 µL of 3.0 mM *p*-nitrophenyl glucopyranoside (pNPG) (Sigma, St. Louis, USA) as a substrate dissolved in 20 mM phosphate buffer (pH 6.9) was added to begin the reaction. The reaction mixture was incubated at 37 °C for 20 min then stopped by adding 2 ml of 0.1 M Na_2CO_3 solution. The *α*-glucosidase activity was determined by measuring the yellow-colored *p*-nitrophenol released from pNPG at 405 nm on a spectrophotometer. The results were expressed as inhibition percentage of the blank control as (Kazeem et al., 2013):

$$\%Inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ extract}{Absorbance\ of\ control} \times 100$$

Concentrations of the most promising extract resulting in 50% inhibition of the enzyme activity (IC_{50}) were determined by GraphPad prism 6 software

2.7 Cytotoxicity (MTT) assay

This assay was performed according to Kohler et al. (2005) procedures. A human prostatic stromal myofibroblast normal cell line (WPMY-1) was maintained in a standard medium consisting of DEMEM (Dulbecco's Modified Eagle Medium) with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin and incubated at 37 °C in a 5% CO_2 prior to use. Then, cells were seeded into 96-well cell culture plates at a concentration of 1×10^4 cells/mL and incubated for 24 h at standard condition. After that, cells were treated with different concentration (250 to 1000 µg/mL) of *T. decurrens* acetone extract. After 48 h incubation period, the medium was removed and 5 mg/mL of MTT reagent was added to each well and re-incubated for 3-4 h. The developed formazan crystals were dissolved in 100 µL acidified isopropanol and measured at 630 nm using ELISA microplate reader (Bio-Rad micro plate reader, Japan). Each concentration was repeated in triplicates and cell viability was calculated as (Kohler et al., 2005):

$$Cell\ viability(\%) = \left(1 - \frac{Absorbance\ of\ control - Absorbance\ of\ extract}{Absorbance\ of\ control}\right) \times 100$$

2.8. Gas chromatography-mass spectrum (GC-MS) analysis of Turbinaria decurrens acetone extract

T. decurrens acetone extract was subjected to GC-MS Spectrometer (Perkin Elmer model: Clarus 580/560 S) for identification of its different components. GC-MS analysis was performed by injecting 1µL of sample on the column (Rxi-5Sil MS column 30m, 0.25mmID, 0.25 df) under Helium as carrier gas. Oven temperature was programed as: initial temp. 50 °C for 2.50 min, 8 °C/min to 250 °C, hold 5 min, 5 °C/min to 280 °C, hold 2 min; injector temp.: 280 °C, with solvent delay time: 4.00 min. Transfer temp.: 280 °C; source temp.: 200 °C; split ratio: 20:1; scan: 50 to 600 Da. The identification of components was based on comparing their mass spectra with the database of the National Institute Standard and Technology (NIST) software applied to the GC-MS instrument.

2.9 Statistical analysis

Statistical analysis was performed using two-way ANOVA (SPSS 25, 2017) software and all the results were expressed as mean ± standard deviation (n=3).

3 Results and discussion

3.1 Identification of the collected seaweeds

Collected seaweed species were identified to be: *Turbinaria decurrens* (Bory), *Padina pavonica (*Linnaeus) Thivy, *Sargassum muticum* (Yendo) Fensholt and *Sargassum acinarium* (Linnaeus) Setchell (as Phaeophyta); *Ulva lactuca* Linnaeus (as Chlorophyta) and *Pterocladia capillacea* (Gmelin) Barnet et Thuret (as Rhodophyta) (Figure 1).

3.2 Phytochemical analysis of different seaweed extracts

Phytochemical analysis of different seaweed extracts (Table 1) showed presence of carbohydrates and valuable secondary metabolites such as phenols, flavonoids, alkaloids, terpenoids, steroid and glycosides in nearly all tested extracts. Meanwhile, tannins and saponins were rarely detected. Presence of these compounds were apparently dependent on the solvent and the tested seaweed species.

The presence of these bioactive compounds in the various extracts might be responsible for their biological activities as being acted synergistically to pursue antioxidant and antidiabetic

Figure 1. Photos of the studied seaweeds (a) *Turbinaria decurrens* (b) *Ulva lactuca* (c) *Padina pavonica* (d) *Pterocladia capillacea* (e) *Sargassum muticum* (f) *Sargassum acinarium* (Taken by Omnia Abdel-Karim).

Table 1. Qualitative phytochemicals screening of different seaweed extracts.

Seaweed	*T. decurrens*				*P. pavonica*				*S. muticum*				*S. acinarium*				*U. lactuca*				*P. capillacea*			
Phytochemicals/solvents	W	A	E	M	W	A	E	M	W	A	E	M	W	A	E	M	W	A	E	M	W	A	E	M
Alkaloid	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Tannins	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-	+	+	-	-
Glycoside	-	+	+	+	+	+	+	+	-	-	+	+	-	-	+	-	+	+	+	+	+	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-
Steroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Abbreviations: W: water; A: acetone; E: ethanol; M: Methanol; +: present, −: absent.

functions. Similar compounds were reported from *Gracilaria verrucosa, Enteromorpha compressa, Ulva fasciata* and *Turbinaria conoides* extracts (Mohapatra et al., 2016). Different extracts of *Jania rubens*, *Corallina mediterranea* and *Pterocladia capillacea* exhibited several biological activities as antimicrobial and antioxidant activities as have been reported by Mohy El-Din & El-Ahwany (2018).

3.3 Antioxidant assays

The antioxidant potential of different seaweeds with diverse solvents was studied through its scavenging ability of the DPPH radical. Most of the tested seaweed extracts were able to reduce the steady DPPH radical to the yellow colored diphenylpricrylhydrazine depending on the solvent and species. The acetone extract of *T. decurrens* exhibited the highest DPPH scavenging activity of 61.6%; followed by the water extract of *P. pavonica* with 55.2% inhibition capacity. Likewise, the ethanol extract of *S. muticum and S. acinarium* showed significant inhibition activity with 53.89% and 49.42%, respectively (Figure 2). The IC_{50} value recorded 40.66 mg/mL for the acetone extract of *T. decurrens* which was comparable to the estimated IC_{50} value of ascorbic acid 5.21 mg/mL. In accordance with these results Unnikrishnan et al. (2014) reported the scavenging activity of *Turbinaria oranta* acetone extract against DPPH with 65%. Aqueous extracts of *Cystoseira crinita* and *Cystoseira compressa*

(Mhadhebi et al., 2014) and ethyl acetate extract of *Anthophycus longifolius* (Chakraborty et al., 2017) exhibited an excellent scavenging activity against DPPH radical estimated as IC_{50} value. Also, the acetone extract of *Sargassum wightii* and the benzene extract of *Sargassum polycystum* exhibited DPPH antioxidant activity with 43% and 22%, respectively (Unnikrishnan et al., 2015). In a recent study, the Celluclast (a preparation used to degrade cellulose into glucose) enzymatic extract of *Sargassum polycystum* revealed a strong DPPH scavenging activity estimated to be 57.23% (Shanura Fernando et al., 2018).

As shown in (Table 2), acetone extract of the tested *T. decurrens* and water extract of *P. pavonica* showed the highest reducing ability of 2.72 and 1.58 mg AAE/g DW, respectively followed by the ethanol extract of *S. muticum and S. acinarium* with 1.37 and 1.34 mg AAE/g DW, respectively. However, the methanolic extracts recorded the lowest reducing activity compared to the other extracts.

Results in (Table 2) showed that in the total antioxidant capacity assay, all seaweeds presented significant (at $P \leq 0.05$) antioxidant activity using different solvents. The acetone extract of *T. decurrens* recorded the maximum activity of 4.3 mg AAE/g DW. Also, the water extract of *P. pavonica* and the ethanol extract of *S. muticum* showed significant potent activity with 3.26 and 3.09 mg AAE/g DW, respectively. On the contrary, the methanol extract of *T. decurrens* gave the lowest result with 0.52 mg AAE/g DW. The antioxidant activity of these seaweeds might be due to their ability to act as free radicals' scavengers or by providing a hydrogen atom to the molecule (Boonchum et al., 2011). The obtained results were in accordance with those reported by Mohy El-Din & El-Ahwany (2018) for the reducing power and total antioxidant capacity of *Pterocladia capillacea* methanolic extract with recorded values of 4.007 and 0.940 mg AAE/g DW, respectively.

3.4 Total Phenolic Content (TPC)

The total phenolic contents of various tested seaweed extracts ranged from 0.38 to 4.32 mg GAE/g DW (Figure 3). The acetone extract of *T. decurrens* exhibited the highest phenolic content value of 4.32 mg GAE/g DW followed by the water extract of *P. pavonica*, while the ethanol extract of *S. muticum* and *S. acinarium* recorded close total phenolic contents values with 3.31 and 2.88mg GAE/g DW, respectively. Collectively, the correlation between total phenolic content of different extracts and their antioxidant activity was evident as shown in (Table 3), especially for *T. decurrens, P. pavonica* and *S. muticum* seaweed extracts, respectively. The findings reported by Wang et al. (2009) confirmed that amounts of polyphenols in brown seaweeds were higher than that in red and green ones and might be the reason

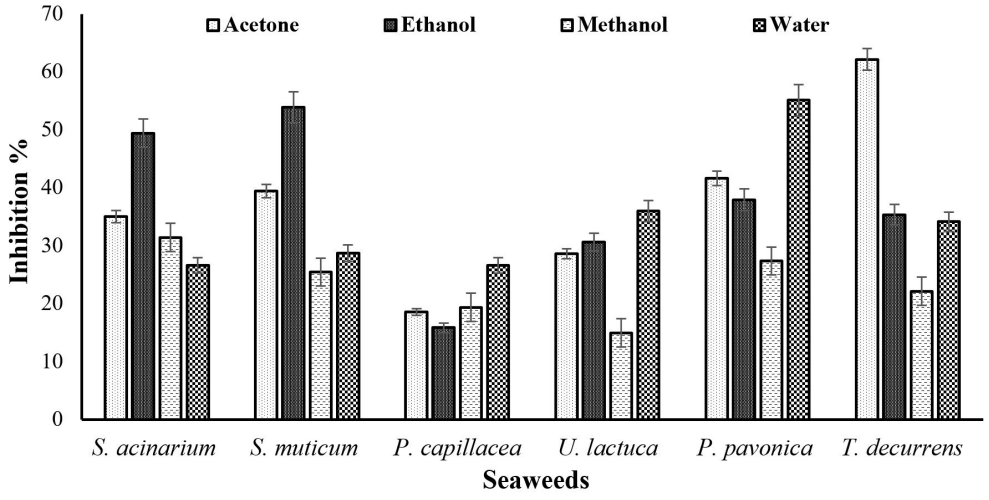

Figure 2. DPPH radical scavenging activity of different seaweed extracts.

Table 2. Total Antioxidant capacity and reducing power activity in the studied seaweeds.

Assay	Total Antioxidant Capacity (mg AAE/g DW)				Reducing Power Activity (mg AAE/g DW)			
	Extract							
Seaweed	Acetone	Ethanol	Methanol	Water	Acetone	Ethanol	Methanol	Water
S. acinarium	$1.68^{a,A}$±0.006	$2.76^{a,B}$±0.015	$2.10^{a,C}$±0.008	$0.71^{a,D}$±0.003	$0.35^{a,A}$±0.021	$1.34^{a,B}$±0.099	$0.36^{a,A}$±0.027	$0.52^{a,A}$±0.005
S. muticum	$2.29^{b,A}$±0.007	$3.09^{b,B}$±0.022	$0.86^{b,C}$±0.002	$0.67^{b,D}$±0.003	$0.96^{b,A}$±0.008	$1.37^{a,B}$±0.010	$0.24^{b,C}$±0.014	$0.71^{b,D}$±0.009
P. capillacea	$1.01^{c,A}$±0.001	$0.86^{c,B}$±0.002	$0.79^{c,C}$±0.001	$0.98^{c,D}$±0.002	$0.25^{c,A}$±0.013	$0.27^{c,A}$±0.001	$0.16^{c,B}$±0.009	$0.65^{c,C}$±0.019
U. lactuca	$1.17^{d,A}$±0.004	$1.08^{d,B}$±0.003	$1.01^{d,C}$±0.001	$1.18^{d,A}$±0.004	$0.36^{d,A}$±0.018	$0.45^{d,B}$±0.009	$0.24^{b,C}$±0.006	$0.71^{c,e,D}$±0.016
P. pavonica	$2.50^{e,A}$±0.074	$1.17^{e,B}$±0.038	$1.13^{e,B}$±0.034	$3.26^{e,C}$±0.010	$0.85^{d,A}$±0.026	$0.68^{e,B}$±0.02	$0.67^{d,C}$±0.013	$1.58^{e,D}$±0.097
T. decurrens	$4.34^{f,A}$±0.003	$0.58^{f,B}$±0.002	$0.52^{f,C}$±0.002	$1.19^{d,D}$±0.002	$2.72^{e,A}$±0.014	$0.48^{d,B}$±0.005	$0.32^{a,C}$±0.017	$0.75^{d,D}$±0.020

Values were mean ± SD of three replicates (n =3). Small Values in the same column with different small superscripts are significantly different, and capital values in the same row with different capital superscripts are significantly different at $p \leq 0.05$ in the two-sided test of equality for row means.

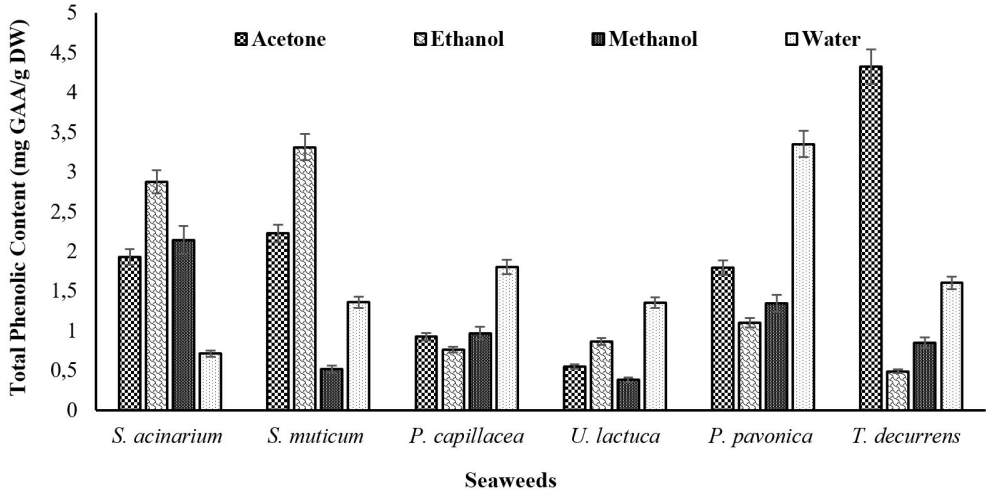

Figure 3. Total phenolic content of different seaweed extracts.

Table 3. Correlation values between total phenolic content and antioxidant activity in different seaweeds.

Algal extract / Antioxidant activities	Total Phenolic content					
	S. acinarium	*S. muticum*	*P. capillacea*	*U. lactuca*	*P. pavonica*	*T. decurrens*
DPPH radical scavenging activity	0.890	0.942*	0.897	0.727	0.867	0.981*
Reducing Power Activity	0.738	0.990*	0.951*	0.876	0.983*	0.993*
Total Antioxidant Capacity	0.931	0.935	0.981*	0.969*	0.919	0.988*

*Correlation is significant $p \leq 0.05$.

of their antioxidant activity. In addition, phenolic compounds were ordinarily found in brown, red and green seaweeds and could act as antioxidants by chelating metal ions, hindering radical formation and stimulating the endogenous defense system of antioxidant (Al-Azzawie & Alhamdani, 2006). Our results also indicated a strong correlation (at $P \leq 0.05$) between the antioxidant activity using DPPH, reducing power and TAC with the estimated total phenolic content of seaweeds which were in adherence to studies by Ismail et al. (2016) and Ismail (2017). In addition, some studies reported the antioxidant activities of the seaweeds are associated with the phenolic algal content (Shanura Fernando et al., 2018). Nowadays, there is a growing concern on the discovery of natural antioxidants mainly because biologically active phytochemicals are largely more secure than manufactured synthetic analogies and for their conservative role in the improvement of disease like cancer, atherosclerosis, arthritis, diabetes, alzheimer's and aging (Santos-Sánchez et al., 2019).

3.5 Antidiabetic assay

One of the most effective ways for treating diabetic patients is by controlling of postprandial hyperglycemia. This can be done by inhibiting the hydrolyzing enzymes of carbohydrate in the digestive system such as α-amylase and α- glucosidase, thus decreasing the absorption of glucose (Pirian et al., 2017). The inhibitory effect of seaweed extracts using different solvents were assayed for α-amylase enzyme (Figure 4). All tested extracts showed significant (at $P \leq 0.05$) α-amylase inhibition activity depending on the solvent. Organic solvents were of pronounced effect than aqueous solvent. The acetone extract of *T. decurrens* was of the maximum inhibition capacity recording 96.1% with an IC_{50} value of 4.37 mg/ml compared to positive control (Acarbose) with 1.59 mg/ml followed by the ethanol extract of *S. muticum* with an inhibition activity of 94.3%. Similarly, the ethanol extract of *S. acinarium* presented 91.8% inhibition of α-amylase enzyme.

p-nitrophenyl *α*-D-glucopyranoside compound was used as a substrate to determine the inhibitory activity of different seaweed extracts on *α*-glucosidase enzyme (Figure 5). The acetone and ethanol extracts were significantly effective. At a concentration of 90 mg/mL, the acetone extract of *T. decurrens* showed the maximum inhibition record of 97.4% with an IC_{50} value of 2.84 mg/mL, while acarbose, which considered to be positive control, was with an IC_{50} value of 1.03 mg/ml. Similar findings were found by Unnikrishnan et al. (2014) who reported that *Turbinaria oranta* in acetone showed the highest α-glucosidase inhibition activity (87.6%), while the same seaweed methanol extract recorded the maximum percentage inhibition on α-amylase (96.5%). In addition, the percentage inhibition and IC_{50} values showed that the acetone extract of *T. decurrens* was an effective inhibitor for both carbohydrate analyzing α-amylase and α-glucosidase enzymes and thus played a key role in controlling of postprandial hyperglycemia. Furthermore, many studies reported the feasibility of brown seaweed extracts such as *Ishige okamurae* and different *Sargassum spp.* as antidiabetic agents and their potent activity as an inhibitor of α-glucosidase and α-amylase (Lee & Han, 2012; Unnikrishnan et al., 2015; Yang et al., 2019). However, moderate inhibition of α-amylase may be preferable to avoid side effects that can be appear by the abnormal bacteria

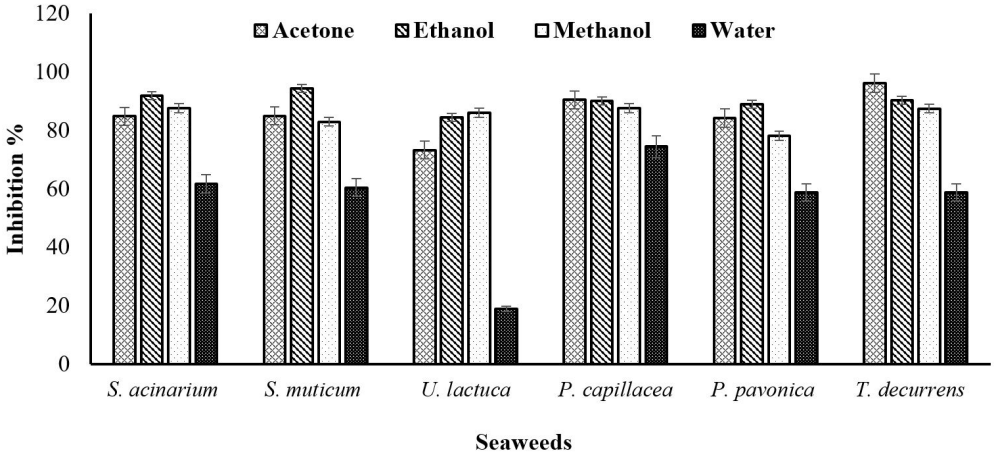

Figure 4. *In vitro* α-amylase inhibitory activity (at 540 nm) of different seaweed extracts.

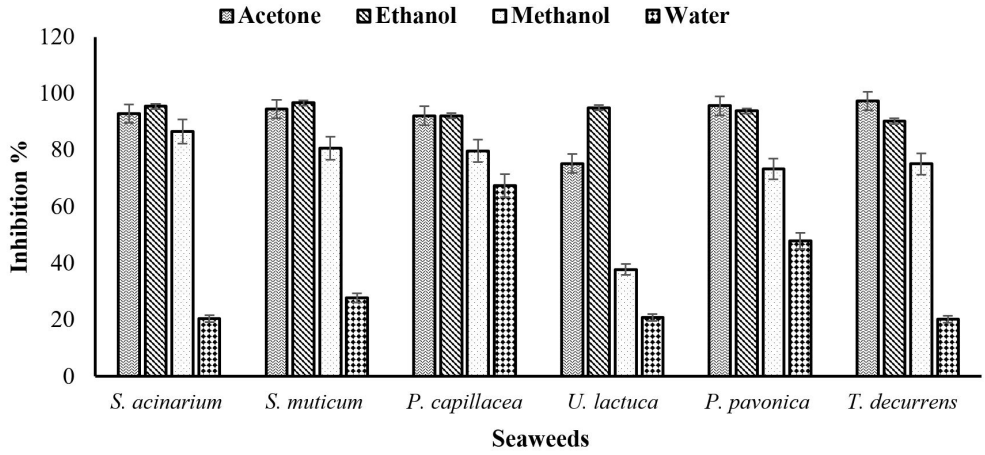

Figure 5. *In Vitro* α-glucosidase inhibitory activity (at 405 nm) of different seaweeds extracts.

in the colon due to fermentation (Lordan et al., 2013). Disposed that acetone extract of *T. decurrens* has stronger inhibitory effect against α-glucosidase than α-amylase enzyme, implying a potent effective medication for treatment of postprandial hyperglycemia with low side effects. According to Apostolidis et al. (2011); Nwosu et al. (2011) and Pirian et al. (2017), the antidiabetic activity of seaweeds might be due to the presence of different phytochemical compounds such as polyphenols which bind to the active sites of the diabetic enzymes and alter their catalytic activity, so that correlated to the antioxidant activity. The same correlation was observed in this study where the acetone extract of *T. decurrens* had the highest phenolic content between the other tested seaweeds.

3.6 Cytotoxicity assay

The cytotoxic effect of *T. decurrens* acetone extract at different concentrations on WPMY-1 normal cell line was shown in Figure 6 at 24 and 48 hours. By increasing the concentration, the cell inhibition percentage increased too. At lower concentrations of 250 µg/mL, 97.62% and 96.18% of cell viability was recorded at 24 and 48 hours of incubation, respectively. The recorded values for the extract were above the maximum tested concentration (1000 µg/mL) that supporting the safety of the extract. The obtained results were in contract with Unnikrishnan et al. (2014) who reported the safety of *Turbinaria oranta* acetone extract on J774 cell line at lower concentrations.

3.7 GC-MS analysis of T. decurrens acetone extract

GC/MS analysis of *T. decurrens* acetone extract identified different compounds (Figure 7) which might be responsible for its hypoglycemic properties. The major bioactive compounds and their bioactive properties were listed in Table 4. The cyclic, unsaturated cyclotrisiloxane, hexamethyl (23.002%) has been reported as antimicrobial agents (Keskın et al., 2012), antibacterial activity (Musini et al., 2013) and antioxidant activity and play a critical role in scavenging of free radicals (Prakash & Vuppu, 2014). The a cyclic diterpene 3,7,11,15-tetramethyl-2-hexadecen-1-ol (known as phytol alcohol) (15.42%) has been known to act as a good preventive for the reactive oxygen species and as precursor for vitamin E and vitamin K1 (Ganesh & Mohankumar, 2017), as antimicrobial, diuretic, and chemo-preventive properties and was used in vaccine formulations (Krishnamoorthy & Subramaniam, 2014) as well as anti-cancer properties (Gautam et al., 2018), o-Xylene (12.251%), and cyclotetrasiloxane, octamethyl (6.625%)

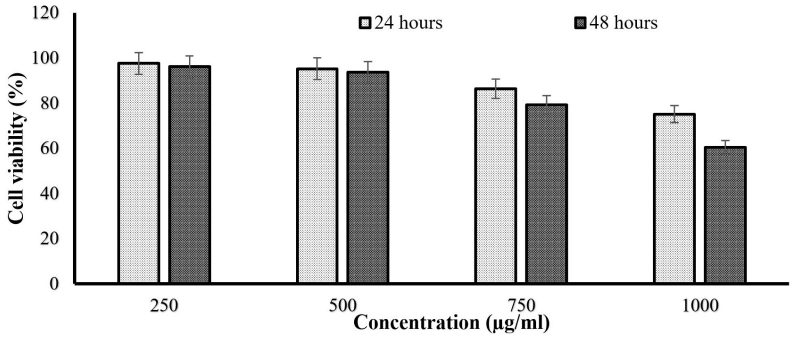

Figure 6. Cytotoxicity on WPMY-1 normal cell line treated with different concentrations of acetone extract of *Turbinaria decurrens*.

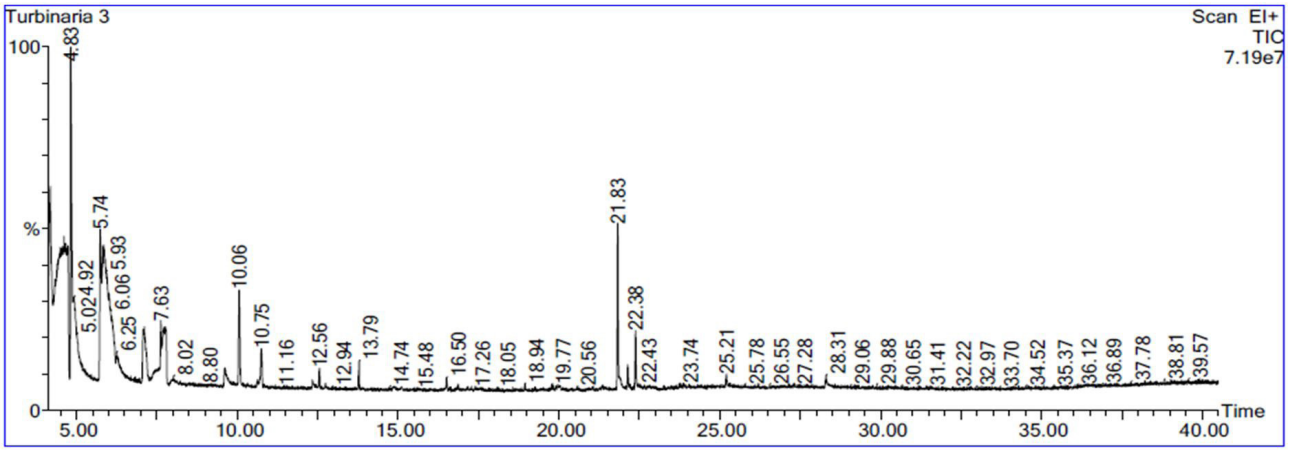

Figure 7. GC-MS chromatogram of *Turbinaria decurrens* acetone extract.

Table 4. GC-MS analysis of the acetone extract of *Turbinaria decurrens*.

RT*	Compounds name	PA* %	MF*	Biological activity
4.188	Cyclotrisiloxane, hexamethyl	20.42	$C_6H_{18}O_3Si_3$	Antimicrobial, antioxidant, antibacterial
4.619	Cyclotrisiloxane, hexamethyl	100	$C_6H_{18}O_3Si_3$	As mentioned
4.829	2-Pentanone, 4-hydroxy-4-methyl	73.56	$C_6H_{12}O_2$	A plant metabolite, solvent
5.744	o-Xylene	21.95	$C_6H_4(CH_3)_2$	Antifungal, antibacterial and antioxidant
5.849	o-Xylene	94.01	$C_6H_4(CH_3)_2$	As mentioned,
6.249	Oxime-, methoxy-phenyl	10.72	$C_8H_9NO_2$	Antimicrobial, antioxidant
7.115	Phenyl-pentamethyl-disiloxane	16.34	$C_{11}H_{20}OSi_2$	Phenolics aldehyde
7.455	Octamethylcyclotetrasiloxane	2.68	$C_8H_{24}O_4Si_4$	Antimicrobial, antioxidant, antibacterial
7.555	Cyclotetrasiloxane, octamethyl	3.19	$C_8H_{24}O_4Si_4$	As mentioned,
7.630	Cyclotetrasiloxane, octamethyl	8.79	$C_8H_{24}O_4Si_4$	As mentioned,
7.770	Cyclotetrasiloxane, octamethyl	19.07	$C_8H_{24}O_4Si_4$	As mentioned,
7.990	Benzene, 1,2,3-trimethyl	1.21	C_9H_{12}	----
9.611	Cyclotrisiloxane, hexamethyl	5.10	$C_6H_{18}O_3Si_3$	Antimicrobial, antioxidant, antibacterial
10.061	N6-(4-Methoxybenzoyl) adenosine	11.35	$C_{18}H_{19}N_5O_6$	-----
10.751	Cyclopentasiloxane, decamethyl	6.02	$C_{10}H_{30}O_5Si_5$	Antimicrobial, antioxidant, antibacterial
12.342	Cyclotetrasiloxane, octamethyl	1.03	$C_8H_{24}O_4Si_4$	As mentioned
12.557	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-(trimethylsiloxy) hexasiloxane	1.47		-----
13.787	Cyclohexasiloxane, dodecamethyl	2.06	$C_{12}H_{36}O_6Si_6$	Antimicrobial, antioxidant, antibacterial
16.513	Cyclotetrasiloxane, octamethyl-	1.39	$C_8H_{24}O_4Si_4$	As mentioned
21.831	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.42	$C_{20}H_{40}O$	Anti-inflammatory, antioxidant, antimicrobial, anticancer, anti-diuretic
22.146	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.27	$C_{20}H_{40}O$	As mentioned,
22.381	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5.56	$C_{20}H_{40}O$	As mentioned,
25.207	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.44	$C_{20}H_{40}O$	As mentioned,

*RT: Retention Time;*PA: Peak Area; *MF: Molecular Formula.

were known for their antifungal, antibacterial and antioxidant activity (Rizwana et al., 2016), Oxime-, methoxy-phenyl (1.964%) has been reported for its antimicrobial (Patil et al., 2012) and antioxidant properties (Özen & Taş, 2009). Although there was no information confirming the presence of antidiabetic effects of any of the previously mentioned compounds, but still they may act synergistically to exert the estimated significant inhibition of diabetic enzymes. The over-production of reactive oxygen species or impaired antioxidant defense system resulted in diabetes which leads to oxidative damage of β-cells of pancreas (Baynes, 2003). A lot of reports detected that reactive oxygen species (ROS) led to several diseases including diabetes; thus, involving efficient quantities of antioxidant compounds in dietary meals may be valuable in controlling diabetic complications (Mohapatra et al., 2016). In this connection, marine seaweeds are great provenance of dietary fibers, polysaccharides, polyphenols, polyunsaturated fatty acids, minerals, and vitamins which have different biological activities like antidiabetic, anti-inflammatory and antioxidant activity (Ismail et al., 2016; Ismail, 2017; Yang et al., 2019).

4 Conclusion

In this study different organic and water extracts of six studied seaweeds belonging to different divisions showed great variation in their antioxidant potential particularly in acetone, water and ethanol extracts. This antioxidant capacity was significantly correlated to their phenolic content, especially for the brown seaweeds: *T. decurrens, P. pavonica, S. muticum* and *S. acinarium* These seaweeds were also exhibited a potent inhibitory activity, *in vitro,* on α-amylase and α-glucosidase starch hydrolyzing enzymes. The acetone extract of *T. decurrens* showed maximum inhibitory activity combined with a highest antioxidant capacity. *In vitro* toxicological parameter indicated that *T. decurrens* acetone extract was greatly below the cytotoxicity levels. The GC/MS analysis confirmed the presence of bioactive compounds responsible for the antioxidant and antidiabetic activities which recommended this extract ingredients for suppressing hyperglycemia. *In vivo* future studies are in need to evaluate the feasibility of *T. decurrens* extract for developing potent antioxidant and/or antidiabetic drugs.

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