2020-10

by Sin War Nawa Sin War Nawa

Submission date: 24-May-2021 10:18PM (UTC+0800)
Submission ID: 1593177311
File name: 2020-10.pdf (351.34K)
Word count: 10416
Character count: 52912

Journal of the Saudi Society of Agricultural Sciences xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of the Saudi Society of Agricultural Sciences

journal homepage: www.sciencedirect.com

Full length article

Bioactivities, heavy metal contents and toxicity effect of macroalgae from two sites in Madura, Indonesia

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ARTICLE INFO

Article history:
Received 15 August 2020
Revised 11 September 2020
Accepted 22 September 2020
Available online xxxx

Keywords:
Macroalgae
Phenolic content
Bioactivities
Heavy metals

ABSTRACT

Sargassum duplicatum and *Padina tetrastromatica* seaweed species collected from oil and a non-oil extraction site at Madura Island were investigated in this study for selected bioactivities, heavy metals and toxicity effects. The collected seaweeds were evaluated for their phytochemical constituents, total phenolic contents (TPC), antioxidant activities, antidiabetic activities, anticancer activities, toxicities and heavy metals using *Folin-Ciocalteus* method, the 2,2-diphemyl-1 picrylhydrazyl (DPPH), α-glucosidase enzyme, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Brine Shrimp Lethality Test (BSLT) and atomic absorption spectrophotometer, respectively. The crude extracts (C), normal hexane (N), ethyl acetate (E), and methanol residue fractions (M) were studied. The highest TPC (589.79 ± 7.14 mg GAE/g) was observed in ethyl acetate fraction of *P. tetrastromatica* from the non-oil extraction site. Meanwhile, the crude extracts and all fractions showed potent antioxidant, antidiabetic, and cytotoxic activities with ethyl acetate fraction of *P. tetrastromatica* from non-oil extraction site displaying the highest effects (IC_{50} 25.25 ± 5.15, 249.12 ± 1.77 and 70.56 ± 2.56 μg/mL: antioxidant, antidiabetic, cytotoxic activities respectively). In the brine shrimp assay, the crude extracts and all fractions of both species from the two sites were non-toxic with exception of the hexane fraction of *P. tetrastromatica* from oil site which was very toxic after 24 h incubation. However, the crude extract of *S. duplicatum* and ethyl acetate fraction of *P. tetrastromatica* from oil extraction site were mildly toxic except the hexane fraction of *P. tetrastromatica* from oil site which was very toxic after 48 h incubation while samples of both species from the non-oil site were nontoxic. In each species, the concentration of Cd from the oil extraction site was higher than those of the non-oil site with the values of *P. tetrastromatica* leading and a similar observation occurred Cu in *P. tetrastromatica* but the same Cu was negligible in *S. duplicatum* as Pb was negligible in both species. According to the findings of this current study, it is safe to conclude that both *S. duplicatum* and *P. tetrastromatica* from Madura Island have antioxidant, antidiabetic and cytotoxic activity, we therefore recommend these species from the non-oil extraction site for drug candidates against the various health ab[illegible]mally they seem to inhibit.

1. Introduction

Oceans cover about 70% of the earth and are a warehouse of a wide biodiversity of marine organisms which can serve as sources of natural products (Palanisamy et al., 2017). Among these organisms, macroalgae otherwise known as seaweeds are part of the natural products which constitute a main source of bioactive compounds. They are highly demanded compared to other marine organisms due to their medicinal importance as well as their applicability in various food processing industries. There are many instances where seaweeds have been used as ingredients in culinary more than other marine organisms in different Asian

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https://doi.org/10.1016/j.jssas.2020.09.007
8-077X/

Please cite this article as: S. War Naw, N. Darli Kyaw Zaw, N. Siti Aminah et al., Bioactivities, heavy metal contents and toxicity effect of macroalgae from two sites in Madura, Indonesia, Journal of the Saudi Society of Agricultural Sciences, https://doi.org/10.1016/j.jssas.2020.09.007

countries such as Japan, China, Thailand, Korea, and Indonesia (Al-enazi et al., 2018). Indonesia, an archipelagic country with 81,000 km long coastline has a great potential for seaweed production with the red and brown seaweeds as the most commonly found species (Mulyati and Geldermann 2016). Seaweeds are diverse in different forms and sizes with approximately 25,000–30,000 known species (Santos et al. 2015). According to nomenclatures, seaweeds can be classified into three major groups notably based on their pigmentation; Rhodopytae (red seaweed), Chlorophytae (green seaweed) and Pheaophytae (brown seaweed) (Mohamed et al., 2012).

The increased cases of oxidative stress have attracted the attention of scholars to explore the importance of antioxidants to resolve various public health concerns. Seaweeds contain antioxidants including carotenoids, vitamin chlorophylls, and polyphenol of ascorbic acid which prevent the oxidative stress caused by reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide, superoxide anions and nitric oxide that are known to react with biomolecules like proteins, DNA and lipids to cause cellular malfunctions that result in tissue damage and cell death (Amorim and López-hernández 2012; Palanisamy et al. 2017).

Diabetes is considered as a major global health threat which can affect people of all ages from different demographic regions. It is commonly known to result from the defects of insulin producing cells (beta cells) or when the body is unable to use the secreted insulin. According to statistics, the number of people who suffered from diabetes in 2017 was 451 million, higher than 2014 which recorded 422 million people. In fact, it is estimated that by 2045, the number of people with diabetes will increase to 693 million (Makinde et al., 2019). Diabetes is among the most life-threatening diseases and it sometimes leads to severe complications such as neuropathy, nephropathy, cardiovascular disease, retinopathy, and lower-limb amputations (Crawford 2017).

Cancer generally refers to the abnormal growth of cells and tissues as a result of over-multiplication of the cells. It can be caused by both genetic and environmental factors such as tobacco, food habits like intake of carbonated beverages, smoke inhalation, and preservatives intake in junk food (Kumar and Adki 2018). The most widely used treatment for cancer presently is chemotherapy. But, it also has a variety of side effects that range from minor cases such as nausea to major health problems such as bone marrow failure and development of multidrug resistance (MDR) (Raguz and Yagu 2008). In order to avoid these side effects, many researchers have focused on cancer research in order to identify potent natural compounds.

On the other hand, heavy metal contaminations in the air, soils and ocean are one of the main issues in need of urgent solutions for the green environmental civilization. Agriculture, the manufacturing industry, and urbanization are major factors which contribute to environmental pollution; pollution of air, land, and water. Virtually on y basis, the rate of emission of chemical contaminants such as aliphatic and aromatic compounds, heavy metals, radionucleot s and phthalate esters into air, water and land is increasing. The pollution by heavy metals in the coastal environment has become a worldwide ecological and health concern because of the environmental persistence, bioaccumulation, and biomagnifications in food chains and toxicity of these elements (Gochfeld, 2003; Kamala-kannan et al., 2008). In order to investigate the distribution rate of heavy metal in marine environment, marcroalgae or seaweeds are most effectively used as bio-monitors in water and sediment (Khaled et al., 2015). In fact, according to Conti and Cecchetti, (2003) the rate of accumulation of trace metal concentration in seaweeds are higher than their corresponding concentration in sea water.

Seaweeds are one of the monetarily significant marine sustainable assets which comprise of bioactive compounds such as peptide, vitamin, polyphenol, polysaccharide, and fatty acids, and have functional properties which are beneficial to human he (Hoon and Bae, 2010; Wijesinghe and Jeon, 2012). Therefore, this study is specifically aimed to evaluate the antioxidant, antidiabetic, anticancer activities, and toxicity of *Sargassum duplicatum* and *Padina tetrastromatica* from a non-oil extraction and an oil extraction site at Madura Island, Indonesia. In addition, the concentration of copper, cadmium, and lead from those species were also determined.

2. Materials and methods

2.1. Seaweed collection

Sargassum duplicatum and *Padina tetrastromatica* were collected from two different locations including Jumiang beach, Pamekasan (a non-oil extraction site) which is located at coordinate position 714′02.5″S 11332′34.6″E Madura and Camplong beach, Kabupaten Sampang (an oil extraction site) with coordinate positio 13′05.4″S 11319′08.5″E Madura Island, Indonesia. Collected seaweeds were washed completely with wa to expel the salts and foreign particles. The samples were dried at room temperature for 7 days and pulverized in a warring blender.

2.2. Sample extraction and percentage yield

P. tetrastromatica and *S. duplicatum* of 400 g and 250 g respectively from the two different locations were extracted twice with 0 mL and 1500 mL of methanol. The filtrates were separately filtered through Whatman No.1 filter paper and then concentrated (at 50C) in rotary vacuum evaporator. The crude extracts were mixed with methanol (100 mL), an partitioned with n-hexane (1:2) three times. It was separated to n-hexane and methanol fractions. The obtained methanol fraction was partitioned with ethyl acetate distilled water (1:2) thrice by using a separating funnel to get ethyl acetate fraction and aqueous mixture (methanol residue). Each fraction was evaporated at 50C using rotary vacuum evaporator. The percentage yields of each extract and fractions of the seaweeds were calculated with the following equation.

$$\text{Percentage yield } (\%) = \frac{\text{Final weight of dried extract}}{\text{Initial weight of powder}} \times 100$$

2.3. Phytochemical screening assay

The crude extracts and fractions of the seaweeds were evaluated for phytochemical constituents including alkaloids (Dragendorff's test), flavonoids (Shinoda's test), Terpenoids and Steroids (Liebermann – Burchard's test) (Kodangala et al., 2010).

2.4. Determination of total phenolic content

The total phenolic content of crude extracts and each fraction was d rmined using Folin Ciocalteu reagents according to Norra et al., (2016). Briefly, 1 mL of s ple was mixed with 1 mL of Folin-Ciocalteu's reagent and 9 mL of distilled wa After 5 min of incubation, 1 mL of 75% Na_2CO_3 was added and incubated for 2 h at room temperature in the dark. The absorbance was measured at 760 nm using a UV spectrophotometer (UV1800ENG240V, SOFT). For the standard calibration curve, gallic acid s used (5, 10, 15, 20, and 25 μg/mL). The TPC was expressed in mg/g of gallic acid equivalent (GAE).

2.5. DPPH radical scavenging activity

The antioxidant activity of each seaweed extract and fractions were determined by using DPPH radical scavenging assay according to the method of Yamaguchi et al. (1998). As a summary, 1 mL of each sample (62.5, 120, 250, 500, and 1000 μg/mL) was added to 1 mL of buffer solution (pH 7.4) and mixed with 0.5 mL of 0.5 mM of freshly prepared DPPH solution. The reaction mixture was shaken energetically and left to stand for 30 min at a room temperature in the dark. After 30 min, the absorbance of the reaction mixture was determined at 523 nm at room temperature. The percentage of the DPPH radical scavenged was calculated by the following equation:

$$\%\ \text{Inhibition of DPPH Radical} = \left(\frac{A_{br} - A_{ar}}{A_{br}}\right) \times 100$$

where A_{br} is the absorbance of control and A_{ar} is the absorbance of sample. The IC_{50} value of the sample was calculated based on 50% inhibition of DPPH concentration by sample concentration using Excel package.

2.6. Cell line and cell culture

Lung cancer cell line A549 were cultured in RPMI-1640 media (Sigma-Aldrich, USA) with 10% (v/v) Fetal bovine serum (FBS; PAA, Pasching, Austria) and 1% penicillin–streptomycin that was incubated for humidification at 37 °C with 5% CO_2.

2.7. MTT assay

Anticancer activity of the seaweed extracts and fractions was evaluated by MTT assay according to the method by Arbiastutie et al., (2017). In summary, A549 cell lines were seeding in 96 well plates at the density of approximately 2×10^4 cells/well in RPMI 1640 media with 10% FBS and consequently incubated at 37 °C in a 5% CO_2. After 48 h, cells were washed and different concentrations of each sample was added in each well and incubated overnight. Thereafter, the cell lines were added 50 μL (5 mg/mL) of MTT solution in each well for 4 h at 37 °C. Cells were reacted with 100 μL DMSO after removal of the MTT solution. The absorbance was measured at 570 nm using an ELISA reader. IC_{50} was calculated based on the sample inhibition of cell growth. The percentage of cell viability was calculated with the following formula.

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of sample} - \text{Absorbance of control media}}{\text{Absorbance of control cell} - \text{Absorbance of control meida}} \times 100$$

2.8. Inhibition of α-glucosidase

Antidiabetic activity of all samples was performed by the use of α-glucosidase in line with Makinde et al., (2019). The sample stock solutions were serially diluted with DMSO into various concentrations from 5000 – 312.5 μg/mL. Ten (10) μL of sample was dissolved with 10 μL of α-glucosidase enzyme (0.4 unit/mL) in 1 mM phosphate buffer (pH 6.9) after which it was incubated in shaker-incubator at 37 °C for 10 min. Next to that was the solution mixture which was added to 50 μL of *p*-nirophenyl α-glucopyranoside (pNPG) and incubated at 37 °C for 20 min, which was discontinued by addition of Na_2CO_3 (100 μL). The inhibition of α-glucosidase was determined at an absorbance of 405 nm. Acarbose was used as a standard while the mixture of 50 μL of 1 mM of phosphate buffer was used as the negative control and the blank sample. IC_{50} was calculated from linear regression based on 50% inhibition of the enzyme by each sample using the following equation;

% Inhibition = $[(A_0 - A_1)/A_0)] \times 100$ where A_0 = absorbance without sample, A_1 = absorbance with sample solution. The experiments were performed in triplicates.

2.9. Brine shrimp assay for toxicity

The toxic effect of seaweed extract was determined in accordance with Meyer et al., (1982). Six (6) mg of each sample was dissolved in 60 μL of ethanol and the volume made up to 3 mL with sea water. The concentration of the stock solution was 3000 ppm. The solution was diluted to 1000, 500, 250, 125, and 62.5 ppm with sea water. Then 0.5 mL of seawater containing 10 nauplii was added to 5 mL of sample solution. After 24 h, the test tube was inspected using a magnifying glass and the number of survived larvae were counted in each tube. The mortality was calculated using Abbott (1925).

$$\%\ \text{Death} = \frac{\text{Death}}{\text{Test}} \times 100$$

2.10. Determination of heavy metals

The concentration of Cd (cadmium), Cu (copper) and Pb (lead) in each sample was determined with an atomic absorption spectrophotometer (Perkin-Elmer AA-700) (Maharana et al., 2010). Briefly, 5 g of each dry sample were added into a 50 mL of Teflon beaker (trice for each sample). The sample was digested by addition of 4 mL of concentrated HNO_3 and heated on a hot plate at 100–200 °C after digestion and cooling, the samples were dissolved with water and the final volume made up to 100 mL in volumetric flask. The results were then reported in mg/kg.

2.11. Data analysis

All the experiments were performed in triplicates (n = 3) and the data was analyzed using one-way ANOVA (SPSS, version 23.0) and the significant difference between means was determined with Duncan's tests at $P \leq 0.05$. IC_{50} were calculated from linear regression with Microsoft Excel program (2007).

3. Results

3.1. Percentage yield of the extracts and fractions

The yield from the crude extract of *S. duplicatum* and *P. tetrastromatica* from the two sites and those of their fractions are as shown in Table 1. The methanol crude extract of *S. duplicatum* and *P. tetrastromatica* from non-oil extraction site had the highest percentage yields of 6.4% and 5% respectively compared to those from the oil extraction site. The highest percentage yield of methanol residue was found in *S. duplicatum* from non-oil extraction site (68.75%). Normal hexane fraction gave the highest percentage yield in *P. tetrastromatica* (40%) from non-oil extraction site. Incidentally the highest percentage yields in ethyl acetate fractions from *S. duplicatum* and *P. tetrastromatica* were recorded from the oil extraction site.

3.2. Phytochemical contents

Phytohemical screening for four different chemical groups (flavonoids, alkaloids, steroids and terpenoids) was performed in crude extracts and the three fractions of *S. duplicatum* and *P. tetrastromatica* (Table 2). The presence of flavonoids was observed in crude extracts, ethyl acetate and methanol residue fractions, but showed negative results in normal hexane fraction

Table 1
Percentage yields of crude extract and different solvent fractions of *S. duplicatum* and *P. tetrastromatica* from the two sites.

Seaweeds species	Methanol crude extracts	Solvent fractions		
		Normal hexane	Ethyl acetate	Methanol residue
S. duplicatum (non-oil extraction site)	6.4%	6.25%	12.5%	68.75%
S. duplicatum (oil extraction site)	5.2%	15.38%	30.76%	–
P. tetrastromatica (non-oil extraction site)	5%	40%	10%	30%
P. tetrastromatica (oil extraction site)	4.5%	27.77%	22.22%	27.77%

Table 2
Phytochemical analysis of crude extracts and three fractions of *S. duplicatum* and *P. tetrastromatica* from the two different sites.

Constituents	*S. duplicatum* (non-oil extraction site)				*S. duplicatum* (oil extraction site)			*P. tetrastromatica* (non-oil extraction site)				*P. tetrastromatica* (oil extraction site)			
	C	*N*	*E*	*M*	*C*	*N*	*E*	*C*	*N*	*E*	*M*	*C*	*N*	*E*	*M*
Flavonoids	+	–	+	+	+	–	+	+	–	+	+	+	–	+	+
Alkaloids	+	+	–	+	+	+	–	+	+	–	+	+	+	–	+
Steroids	+	–	–	–	+	–	–	+	–	–	–	+	–	–	–
Terpenoids	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue.

of both species from the two locations. The crude extract, normal hexane and methanol residue fractions of both species gave positive result of alkaloids although absent in ethyl acetate fraction from all the samples. Positive result of steroids was observed in crude extract but was negative in the three fractions from two different sites in both species. Terpenoids were absent in all crude extracts and fractions of *S. duplicatum* and *P. tetrastromatica*.

3.3. Total phenolic content (TPC)

TPC values of crude extracts and the three fractions from both species from the two sites are presented in Table 3. All the fractions and crude extract of *S. duplicatum* from the non-oil extraction site (24.66 ± 3.46, 60.81 ± 6.45, 105.17 ± 5.12, and 82.10 ± 3.63 mg GAE/g) ($P \leq 0.05$) were significantly higher than the total phenolic contents of same species from the oil extraction site (15.94 ± 0.44, 44.40 ± 1.17, and 66.20 ± 6.54 mg GAE/g). In *P. tetrastromatica*, the highest values in crude extract, normal hexane, ethyl acetate and methanol residue fractions (102.35 ± 5.77, 70.81 ± 6.54, 589.79 ± 7.14, and 220.04 ± 5.12 mg GAE/g) were observed from non-oil extraction site which were significantly higher than those from oil extraction site with exception of the n-hexane fraction which did not vary significantly (89.28 ± 7.74, 65.68 ± 6.57, 80.56 ± 2.46 and 112.36 ± 4.51 mg GAE/g) ($P \leq 0.05$).

3.4. DPPH radical scavenging assay

The DPPH inhibition by crude extracts and three fractions of *S. duplicatum* and *P. tetrastromatica* from the two sites is tabulated in Table 4. In *S. duplicatum* from non-oil extraction site, the crude extract and all fractions were significantly higher in DPPH inhibition activity (IC_{50} 265.91 ± 1.36, 369.33 ± 4.16, 214.06 ± 16.46, and 320.25 ± 15.27 μg/mL) ($P \leq 0.05$) than those collected from the oil extraction site. In *P. tetrastromatica* from the non-oil site, significantly stronger DPPH inhibitory activity was observed in crude extract, normal hexane, ethyl acetate and methanol residue fractions (IC_{50} 53.44 ± 1.37, 107.43 ± 19.45, 25.25 ± 5.15, and 48.57 ± 0.00 μg/mL) ($P \leq 0.05$) when compared with those from the oil extraction site. Moreover DPPH inhibitory activity of crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from both sites were significantly lower than that of the ascorbic acid used as a standard (IC_{50} 12.33 ± 0.29 μg/mL) ($P \leq 0.05$) except ethyl acetate fractions of *P. tetrastromatica* from non-oil extraction site which did not differ significantly with the standard.

3.5. α-Glucosidase inhibitory activity

The α-glucosidase inhibition activity by crude extracts and three fractions of *S. duplicatum* and *P. tetrastromatica* from the studied sites (Table 5) indicates that *S. duplicatum* from non-oil extraction site had IC_{50} of 787.14 ± 18.91, 881.31 ± 12.60, 712.51 ± 9.44 and 745.25 ± 19.90 μg/mL ($P \leq 0.05$) from the crude extract, normal hexane, ethyl acetate, and methanol residue fraction which were significantly higher in α-glucosidase inhibitory activity compared to those from the oil extraction. The crude extract, normal hexane, ethyl acetate, and methanol residue fractions of *P. tetrastromatica* from non-oil extraction site displayed significantly higher inhibitory activity against α-glucosidase (IC_{50} of 417.56 ± 10.26, 566.94 ± 12.80, 249.12 ± 1.77, and 476.12 ± 9.02 μg/mL) ($P \leq 0.05$) when compared with those from the oil extraction site.

Table 3
Total phenolic content (TPC) of crude extracts and three fractions of *S. duplicatum* and *P. tetrastromatica* from the study sites.

Extracts	Total phenolic contents (mg of GAE/g extract)			
	S. duplicatum (non-oil extraction site)	*S. duplicatum* (oil extraction site)	*P. tetrastromatica* (non-oil extraction site)	*P. tetrastromatica* (oil extraction site)
C	24.66 ± 3.46^{b}	15.94 ± 0.44^{a}	102.35 ± 5.77^{d}	89.28 ± 7.74^{c}
N	60.81 ± 6.45^{d}	44.40 ± 1.17^{c}	$70.81 \pm 6.54^{a,b}$	65.68 ± 6.57^{a}
E	105.17 ± 5.12^{f}	66.20 ± 6.54^{d}	589.79 ± 7.14^{f}	$80.56 \pm 2.46^{b,c}$
M	82.10 ± 3.63^{e}	–	220.04 ± 5.12^{e}	112.36 ± 4.51^{d}

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-f}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

Table 4
IC_{50} values of free radical assay for *S. duplicatum* and *P. tetrastromatica* from the two sites.

Extracts	IC_{50} value of DPPH radical scavenging activity of (μg/mL)			
	S. duplicatum (non-oil extraction site)	*S. duplicatum* (oil extraction site)	*P. tetrastromatica* (non-oil extraction site)	*P. tetrastromatica* (oil extraction site)
C	265.91 ± 1.36^{c}	1208.57 ± 12.13^{g}	53.44 ± 1.37^{b}	120.86 ± 3.13^{c}
N	369.33 ± 4.16^{e}	699.80 ± 25.73^{f}	107.43 ± 19.45^{c}	416.98 ± 5.64^{e}
E	214.06 ± 16.46^{b}	252.55 ± 28.83^{c}	25.25 ± 5.15^{a}	57.77 ± 10.34^{b}
M	320.25 ± 15.27^{d}	-	48.57 ± 0.00^{b}	196.45 ± 2.20^{d}
Ascorbic acid	12.33 ± 0.29^{a}			

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-g}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

Table 5
IC_{50} values of α-glucosidase inhibition assay for the studied *S. duplicatum* and *P. tetrastromatica*.

Extracts	IC_{50} (μg/mL)			
	S. duplicatum (non-oil extraction site)	*S. duplicatum* (oil extraction site)	*P. tetrastromatica* (non-oil extraction site)	*P. tetrastromatica* (oil extraction site)
C	787.14 ± 18.91^{d}	1018.70 ± 28.57^{g}	417.56 ± 10.26^{c}	600.08 ± 4.20^{f}
N	881.31 ± 12.60^{e}	1130.34 ± 25.63^{h}	566.94 ± 12.80^{e}	654.83 ± 24.03^{g}
E	712.51 ± 9.44^{b}	954.65 ± 17.02^{f}	249.12 ± 1.77^{b}	419.32 ± 9.91^{c}
M	745.25 ± 19.90^{c}	-	476.12 ± 9.02^{d}	577.75 ± 9.79^{e}
Acarbose	154.02 ± 4.84^{a}			

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-h}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

In addition, the crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from both sites had lower inhibitory activity on α-glucosidase as a compared to the positive control acarbose (IC_{50} 154.02 ± 4.84 μg/mL) ($P \leq 0.05$).

3.6. Cytotoxicity assay

IC_{50} values of crude extract and all fractions of *S. duplicatum* and *P. tetrastromatica* from both extraction sites against A549 cells are detailed in Table 6. After 48 h incubation, higher cytotoxicity on A549 were found in the crude extract, normal hexane, ethyl acetate, and methanol residue fractions of *S. duplicatum* from non-oil extraction site with lower inhibitory concentrations (IC_{50} of 182.41 ± 13.27, 214.98 ± 1.33, 178.98 ± 1.15, and 227.78 ± 2.36 μg/mL) which were significantly lower than (241.49 ± 3.83, 361.22 ± 3.20, 236.24 ± 7.24 μg/mL) ($P \leq 0.05$) from the oil extraction site. Similarly, the crude extract, normal hexane, ethyl acetate and methanol residue fraction of *P. tetrastromatica* from the non-oil extraction site with IC_{50} of 80.44 ± 12.88, 165.46 ± 0.66, 70.56 ± 2.56, and 77.50 ± 0.43 μg/mL ($P \leq 0.05$) were equally significantly lower than the IC_{50} of the same samples of the same species sourced from the oil extraction site (136.43 ± 7.12, 169.94 ± 1.19, 125.10 ± 0.51 and 134.30 ± 1.14 μg/mL).

3.7. Brine shrimp assay for toxicity

The result of toxicity effects against *Artemia salina* nauplii larvae at different concentrations and incubation time of crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from the two sites categorized as non-toxic (<50% death), mildly toxic (>50% but > 75% death) and highly toxic (>75% death) are as shown in Tables 7 and 8. According to exposure period, between 24 and 48 h incubation time, there was no 100% mortality rate found at any concentration. In *S. duplicatum*, the crude extract from the oil extraction site at 1000 μg/mL concentration recorded the highest mortality rate which was 36 ± 6% at 24 h, and 70 ± 10% at 48 respectively compared to the same sample from the non-oil extraction point which had 23 ± 6% at 24 h, and 40 ± 10% at 48 h. In *P. tetrastromatica*, normal hexane fraction from oil extraction site at 1000 μg/mL concentration appeared highest in mortality rate with 90 ± 10% at 24 h with same mortality rate at 48 h compared to the same fraction from non-oil extraction site (0% at 24 h and 30 ± 0% mortality at 48 h) and the rest samples (See Table 8).

3.8. Determination of heavy metals

The significantly highest value of Cd in *S. duplicatum* was observed from the oil extraction site (0.251 ± 0.16 mg/kg) as com-

Table 6
IC_{50} values of *S. duplicatum* and *P. tetrastromatica* from two different sites on A549 (lung cancer cells).

Extracts	IC_{50} (μg/mL)			
	S. duplicatum (non-oil extraction site)	*S. duplicatum* (oil extraction site)	*P. tetrastromatica* (non-oil extraction site)	*P. tetrastromatica* (oil extraction site)
C	182.41 ± 13.27^{a}	241.49 ± 3.83^{d}	80.44 ± 12.88^{b}	136.43 ± 7.12^{d}
N	214.98 ± 1.33^{b}	361.22 ± 3.20^{e}	165.46 ± 0.66^{e}	169.94 ± 1.19^{e}
E	178.98 ± 1.15^{a}	236.24 ± 7.24^{c,d}	70.56 ± 2.56^{a}	125.10 ± 0.51^{c}
M	227.78 ± 2.36^{c}	-	77.50 ± 0.43^{b}	134.30 ± 1.14^{d}

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-e}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

Table 7
Toxicity result after 24 and 48 h Brine shrimp exposure to *S. duplicatum* from the study sites.

% of mortality after 24 h							
Concentrations (μg/mL)	*S. duplicatum* (non-oil extraction site)				*S. duplicatum* (oil extraction site)		
	C	*N*	*E*	*M*	*C*	*N*	*E*
62.5	0	10 ± 0	0	0	0	0	0
125	10 ± 0^{b}	0	10 ± 0^{b}	10 ± 0^{b}	13 ± 6^{b}	0	0
250	13 ± 6^{b}	0	0	0	16 ± 6^{b}	0	0
500	16 ± 6^{c}	10 ± 0^{b}	0	10 ± 0^{b}	26 ± 6^{d}	10 ± 0^{b}	0
1000	23 ± 6^{c}	0	20 ± 0^{c}	10 ± 0^{b}	36 ± 6^{e}	27 ± 6^{d}	10 ± 0^{b}
% of mortality after 48 h							
Concentrations (μg/mL)	*C*	*N*	*E*	*M*	*C*	*N*	*E*
62.5	0	30 ± 0^{d}	0	20 ± 0^{c}	20 ± 10^{c}	10 ± 0^{b}	10 ± 0^{b}
125	26 ± 5^{b}	13 ± 5^{a}	10 ± 0^{a}	13 ± 5^{a}	40 ± 10^{c}	10 ± 0^{a}	10 ± 0^{a}
250	20 ± 10^{b}	20 ± 10^{b}	0	20 ± 10^{b}	50 ± 10^{c}	20 ± 10^{b}	13 ± 5^{b}
500	30 ± 0^{d}	23 ± 5^{d}	0	13 ± 5^{b}	60 ± 10^{c}	26 ± 0^{d}	30 ± 0^{d}
1000	40 ± 10^{b}	23 ± 5^{a}	26 ± 5^{a}	30 ± 0^{a}	70 ± 10^{a}	53 ± 0^{c}	30 ± 0^{a}

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-e}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

Table 8
Toxicity result after 24 and 48 h Brine shrimp exposure to *P. tetrastromatica* from the study sites.

% of mortality after 24 h								
Concentrations (μg/mL)	*P. tetrastromatica* (non-oil extraction site)				*P. tetrastromatica* (oil extraction site)			
	C	***N***	***E***	***M***	***C***	***N***	***E***	***M***
62.5	0	0	0	0	0	0	0	0
125	0	0	0	0	13 ± 0^{b}	0	0	0
250	0	0	0	0	17 ± 0^{b}	0	0	0
500	10 ± 0^{b}	0	0	0	20 ± 0^{c}	10 ± 0^{b}	0	0
1000	13 ± 0^{b}	0	0	0	30 ± 10^{c}	90 ± 10^{d}	10 ± $0^{a,b}$	10 ± $0^{a,b}$
% of mortality after 48 h								
Concentrations (μg/mL)	*C*	*N*	*E*	*M*	*C*	*N*	*E*	*M*
62.5	0	10 ± 0^{b}	10 ± 0^{b}	20 ± 0^{c}	13 ± 6^{b}	37 ± 6^{d}	10 ± 0^{b}	23 ± 0^{c}
125	10 ± 0^{a}	10 ± 0^{a}	13 ± 6^{a}	27 ± 6^{c}	23 ± $6^{b,c}$	43 ± 6^{d}	17 ± $6^{a,b}$	23 ± $6^{b,c}$
250	13 ± 6^{a}	17 ± 6^{a}	20 ± 0^{a}	30 ± 0^{b}	33 ± 5^{b}	56 ± 6^{e}	20 ± 0^{a}	33 ± 5^{b}
500	23 ± $5^{a,b}$	17 ± 6^{a}	20 ± 0^{a}	30 ± $0^{b,c}$	36 ± $5^{c,d}$	76 ± 5^{e}	20 ± 0^{a}	40 ± 0^{d}
1000	37 ± $6^{b,c}$	30 ± $0^{a,b}$	20 ± 0^{a}	37 ± $6^{b,c}$	57 ± 6^{d}	90 ± 10^{e}	50 ± 0^{d}	47 ± $12^{c,d}$

Note: C = crude extract; N = normal hexane; E = ethyl acetate; M = methanol residue. The values are expressed as mean ± S.D of three replicates with $^{a-e}$ nomenclature with the different superscripts indicating significant difference ($P \leq 0.05$).

pared to that from the non-oil extraction site (0.133 ± 0.01 mg/kg). The concentration of Cd was significantly higher in *P. tetrastromatica* from oil extraction site (0.382 ± 0.09 mg/kg) than that of non-oil extraction site (0.156 ± 0.05 mg/kg) ($P \leq 0.05$). In terms of Cu, *P. tetrastromatica* from the oil-extraction site recorded the highest Cu value (0.740 ± 0.21 mg/kg) which was statistically higher compared to that of the non-oil extraction site (0.054 ± 0.01 mg/kg) ($P \leq 0.05$). Howbeit, Cu content in *S. duplicatum* from both sides was negligible. In the same manner, the Pb contents of both macroalgae from the two study sites were all negligible (Table 9).

4. Discussion

4.1. Total phenolic content (TPC)

Seaweeds have been proven to contain a wide variety of bioactive compounds with applications in pharmaceutical and biological studies such as anti-inflammatory, antiangiogenic, gastroprotecitve, antibacterial, anticoagulant, antiviral, immunomodulatory, anti-diabetic, antioxidant and anticancer activities (Yang et al., 2019). The percentage yield of extraction is often influenced by

Table 9
Concentration of heavy metals (mg/kg dry weight) in *S. duplicatum* and *P. tetrastromatica* from two different sites.

Metal	*S. duplicatum* (non-oil extraction site)	*S. duplicatum* (oil extraction site)	*P. tetrastromatica* (non-oil extraction site)	*P. tetrastromatica* (oil extraction site)
Cd	0.133 ± 0.01^{a}	0.251 ± $0.16^{a,b}$	0.156 ± 0.05^{a}	0.382 ± 0.09^{b}
Cu	Negligible	Negligible	0.054 ± 0.01^{a}	0.740 ± 0.21^{c}
Pb	Negligible	Negligible	Negligible	Negligible

The values are expressed as mean ± S.D. $^{a-c}$ nomenclature with the different superscripts indicates significant difference ($P \leq 0.05$).

various variables such as the physical qualities of the sample, amount of the sample, chemical composition, extraction time, temperature, solvent polarities and solvent–solute ratio (Herodez et al., 2003). Therefore, the occurrence of high or low percentage yield in the extraction with a solvent does not affect the biological activities of the sample. The solubility of the bioactive compound is one of the factors that influence variations in biological activities (Dellavalle et al. 2011). Based on the result of this current study, all fractions of *P. tetrastromatica* from non-oil extraction site had the highest total phenolic content compared to those from the oil extraction site. Since brown seaweeds are known to contain phlorotannins and bipolar polyphenols that act as antioxidants (Ashraf et al., 2011), the total phenol content (TPC) from this study aligns with such pervious study. According to the result of Chia et al., (2015b), the methanol extract of *P. tetrastromatica* from the West Coast of Malaysia gave TPC values of 97.5 ± 1.51 mg GAE/g. At another location; Port Dickson, Malaysia, Chia et al., (2015a) observed that that the TPC of *P. tetrastromatica* methanol extract was 69.5 ± 1.74 mg GAE/g. On the other hand, Vinayak et al., (2011) who also evaluated the total phenolic content of *P. tetrastromatica* and *S. marginatum* methanol extract from India obtained 25.29 ± 0.445 and 13.19 ± 0.32 mg GAE/g respectively. Apart from the 97.5 ± 1.51 mg GAE/g from the West Coast of Malaysia which lies in same range with our *P. tetrastomatica* methanol crude extract TPC values, the rest of those TPC values are lower than the values from methanol crude extract of *P. tetrastomatica* from both the oil and non-oil extraction sites of this study, such differences are probably linked to the locations and/or processing methods of these studies. In addition, *S. duplicatum* extracted with various solvents in the study by Johnson et al., (2019) obtained total phenolic contents of 700 ± 33.33 mg GAE/g from methanol which is higher than the methanol crude extracts and methanol residues of *S. duplicatum* from this study, indicating that same species of seaweeds extracted with the same solvent in different locations can vary in total phenolic contents.

4.2. DPPH free radical scavenging activity

DPPH free radical is a stable free radical, which has been extremely useful as one of the principal tools for determination of antioxidant properties of bioactive compounds (Mohsin et al., 2013). DPPH has a deep-purple color, which changes from violet to yellow upon the donation of electron from paired electron by a sample to an unpaired electron in DPPH (Abootalebian et al., 2016). In this study, crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from the non-oil extraction site had the highest inhibition effect against DPPH compared to the extract and fractions from the oil extractions site. Interestingly, ethyl acetate fraction exhibited the best DPPH inhibitory activity of both *S. duplicatum* and *P. tetrastromatica* from the two different sites (Table 3). In a likewise manner, the previous study by Khaled et al., (2012) who evaluated the antioxidant activity of ethyl acetate and other fractions of *P. pavonica* and *S. vulgare* equally reported higher free radical scavenging activity from the ethyl acetate fraction compared to others. In brown seaweed, phenolic compounds such as phlorotanins and fucoxanthin are known to highly aid the antioxidant activities (Lim et al., 2018; Chandini et al., 2008). Based on the results of this study, the phenolic content and antioxidant activities of *S. duplicatum* and *P. tetrastromatica* from the two different sites are highly correlated, this same relationship was previously reported by Johnson et al., (2019). Chia et al. (2015b) who studied the DPPH radical scavenging activity of *P. tetrastromatica* from the West Coast of Malaysia obtained an IC_{50} value of 45.57 ± 1.63 μg/mL from methanol extract, and this value is lower than that of *P. tetrastromatica* in this study from both extraction sites (IC_{50} 53.44 ± 1.37 and 120.86 ± 3.13 μg/mL). Such a variation may have occurred as a result of different factors like processing methods, seasons of the two studies, etc. since some reports have linked such variations in natural products to study seasons. Meanwhile, the ethyl acetate extract of *P. tetrastromatica* from Port Dickson located in Malaysia exhibited DPPH inhibitory activity with IC_{50} value of 171.67 ± 2.89 μg/mL (Chia et al. 2015a). The IC_{50} values of the study (Chia et al., 2015a) appear higher than our findings from *P. tetrastromatica* from both sites in this study (IC_{50} 25.25 ± 5.15 and 57.77 ± 10.34 μg/mL) which shows higher antioxidant activity from our samples. Observed difference in antioxidant activity could be related with difference in sample and DPPH concentrations used in these two studies.

4.3. Antidiabetic activity by α-Glucosidase assay

α-Glucosidase is an enzyme found in the intestine which is responsible for the breakdown of carbohydrate (carbohydrate metabolism). Therefore, inhibition of this enzyme may lead to decreased postprandial hyperglycemic levels (Makinde et al., 2019; Ademiluyi and Ganiyu, 2013). Phenolic compounds with good antioxidant activities play a vital role as one of the best inhibitors of this enzyme (Shobana et al., 2009). The result of this study indicates that crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from non-oil extraction site exhibited greater α-glucosidase inhibitions compared to those from the oil extractions site. As presented in result section (Table 4), the crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from non-oil extractions site gave high levels of total phenolic content, expectedly, these extracts equally revealed higher antidiabetic activity. Park and Han, (2012) earlier reported the antidiabetic activity of methanol extract of *Padina arborescens* against-amylase and α-glucosidase where their result indicated that the inhibition activity of *P. arborescens* on α-amylase and α-glucosidase gave IC_{50} values of 0.23 ± 0.03 mg/mL and 0.26 ± 0.05 mg/mL with higher inhibitory effects compared to acarbose. Zaharudin et al., (2019) who investigated the antidiabetic activity of methanol, acetone and water extracts of *Sargassum polycystum* on α-glucosidase enzyme observed that the methanol and water extracts inhibited α-glucosidase with IC_{50} values of 3.8 ± 0.3 and 1.5 ± 0.2 mg/mL, however acetone extract demonstrated no inhibitory activity against α-glucosidase. Interestingly, the results of this study differ from that of Zaharudin et al., (2019) as most of our IC_{50} from antidiabetic activity are lower than the previous study, indicating higher antidiabetic activity from our study. The difference could be as a result of difference in study locations and consequential different phytochemical contents, sample preparation, concentrations used as well as species variations.

4.4. Cytotoxicity test

The result of cytotoxicity test demonstrated that crude extract and all fractions of *S. duplicatum* and *P. tetrastromatica* from non-oil extraction site had the best inhibition activity on the viability of A549 human lung cancer cells as compared to same species obtained from the oil extraction site. Many works have been published on the cytotoxicity of different algae species of the genus *Sargassum*, for example the hexane and ethyl acetate fraction from *S. swartzii* exhibited cytotoxic effect on Caco-2, with IC_{50} values of 99.9 ± 1.38 and 501.18 ± 23.45 μg/mL respectively (Khanavi et al. 2010). In addition, Tannoury et al., (2016) reported that the extracts of water: ethanol and chloroform: ethanol from *S. vulgare* had cytotoxic effect against Jurkat cancer cell line where the result revealed the water: ethanol group with IC_{50} value of 49.056 ± 3.2 μg/mL while chloroform: ethanol with IC_{50} of 136.907 ± 5.2 μg/mL respectively after 72 h of treatment. According to Mashjoor

et al., (2016), the ethyl acetate and methanol fractions of the seaweed *P. antillarum* and *P. boergeseni* from the Persian Gulf exhibited cytotoxicity against MCF7, HeLa and Vero cancer cell lines as they obtained the effective cytotoxicity against MCF7, HeLa and Vero cells from ethyl acetate fraction of *P. boergeseni* to be 83.89, 59.26, and 79.23 μg/mL. Incidentally, this study also observed that the ethyl acetate fractions of *S. duplicatum* and *P. tetrastromatica* from the two study locations had the most effective cytotoxicity on A549 cells among the other fractions. The similarity could be connected with the same solvent used in both studies since certain solvents are known with the ability to pull out selected bioactive analysts (Altemimi et al., 2017). However, a previous study by Chia et al., (2015a) reported that the dichloromethane, ethyl acetate, acetone, methanol and hexane extract of *P. tertrastromatica* from Malaysia gave low cytotoxic effect against MCF-7 cell line as only hexane extract gave an IC_{50} value of 130.0 ± 1.72 μg/mL, which was higher than the rest extracts in cytotoxic effect. Interestingly, the IC_{50} of cytotoxicity recorded from hexane extract in the study by Chia et al., (2015a) is lower than our findings from hexane (165–170 μg/mL) from *P. tertrastromatica*, however, the IC_{50} from ethyl acetate (70–125 μg/mL) from our study is lower than that of Chia et al., (2015a). The differences could be linked with different cancer cell lines used and perhaps the study locations.

4.5. Brine Shrimp Lethality test (BSLT)

The brine shrimp assay is often used due to its simple, inexpensive and straightforward nature that facilitates the assessment of toxicity from extracts of natural products, pure and synthetic-organic compounds. Many researchers have suggested the testing of toxicity of terrestrial plant and macroaglage with brine shrimp assay (Mwangi et al., 2014; Vinayak et al., 2011). The crude extracts and all fractions of *S. duplicatum* and *P. tetrastromatica* from the two study locations in this study were assessed for toxicity using the brine shrimp assay and toxicity effect on *A. salia* was found to be both concentration and incubation time dependent. Ayesha et al., (2010) evaluated the toxicity effect of ethanolic extract from nine seaweeds species using brine shrimp assay and found that *Sargassum lanceolatum* had the lowest cytotoxic effect among the other species. Our present study agrees with Ayesha et al., (2010) since we equally found that the toxicity of *S. duplicatum* is the lowest on *A. Salia* nauplii compared to *P. tetrastromatica*. In another previous study, Vinayak et al., (2011) reported that the methanol extract of *Dictyopteryis australis, Dictyopteryis delicatula, Padina tetrastromatica Sargassum duplicatum, Spatoglossum aspermum, Spatoglossum variable*, and *Stoechospermum marginatum* which were tested for cytotoxic effect by the brine shrimp assay with different incubation times and concentrations revealed that *Sargassum duplicatum* and *Padina tetrastromatica* are non-cytotoxic to *A. Salina* nauplii in all of the tested conditions. This study partly agrees with that of Vinayak et al., (2011) since *S. duplicatum* had a low toxicity effect on the same shrimp species. However the normal hexane fraction of *P. tetrastromatica* from oil extraction was highly toxic at high concentration during 48 h treatment in this research, hence, this variation may be certainly due to the oil extraction activity at this site since the habitat has a high probability of becoming polluted by the oil extraction process at that location which could be the reason behind the toxicity of macroalgae from the location.

4.6. Heavy metal test

In the marine environment, anthropogenic and natural sources of metals are known to highly impact the concentration of heavy metals. Cu, Mn, Fe and Zn are vital micronutrients for the growth of organisms, but can be toxic at higher concentrations. However, even at very low concentrations, Cd, Hg and Pb are toxic to living organisms (Nies, 1999; Wood, 1974; Maharana et al., 2010). The results of heavy metal concentration (Cd, Cu, and Pb) in this study as obtained from *S. duplicatum* and *P. tetrastromatica* from non-oil extraction and oil extraction sites indicates that the concentration of Cd is significantly higher in both *S. duplicatum* and *P. tetrastromatica* from the oil-extraction site than the non-oil extraction site. Cu was similarly significantly higher in *P. tetrastromatica* but not in *S. duplicatum* as the element was negligible in the latter, supporting the wide utilization of brown seaweeds for bio-monitoring of heavy metals. Maharana et al., (2010) determined Cd and Pb concentrations in *P. tetrastromatica* from three different locations in India and obtained lower concentrations compared to our values for Cd. The variation in these studies may be connected to the different locations and the level of industrial activity since our study covered an oil-extraction site. In addition, Qari (2015) reported Hg, Fe, Mn, Cu, Ni, Zn, Cr, Pb, Co, Cd, and Hg values in *P. pavonia* and *P. tetrastromatica* brown seaweed at different beaches of Karachi Coast during different seasons of the year with values lower than those of Cd, Cu and Pb in this study with the exception of Pb which we found negligible in both species and sites. The difference in those values is not a surprise since this research studied both an industrial and non-industrial location unlike that of Qari (2015) which focused on beaches.

In general, our findings on the bioactivities of *S. duplicatum* and *P. tetrastromatica* from the oil and non-oil extraction sites at Madura have shown that the industrial activity of an area can affect the surrounding aquatic life including macroalgae since the bioactivities of both seaweeds were higher from the non-oil extraction site. These findings have proven that macroalgae can be used as bio-monitors for sustainable management of aquatic environments, notwithstanding, the findings are also indications of an intermediate level of sea pollution brought about by human (industrial) activity at one of the study sites (oil extraction site) which calls for a prompt correction in order to avoid sever pollution, contamination and damage of aquatic biota at the site.

5. Conclusion

The findings from this study have demonstrated that the ethyl acetate fractions derived from *Sargassum duplicatum* and *Padina tetrastromatica* from the non-oil extraction site have the highest inhibition activity on DPPH free radical. Moreover, the same fraction equally demonstrated the highest total phenol contents which might be a major contributor to the antioxidant activities of the two seaweed species. Additionally, ethyl acetate fractions of *Sargassum duplicatum* and *Padina tetrastromatica* from the non-oil extraction site has a great potential for cytotoxicity against A549 cancer cell line, it also had the best inhibition activity on α-glucosidase enzyme. The toxicity result in general indicated that *S. duplicatum* and *P. tetrastromatica* from both locations are not very toxic, which can be a good reason to recommend the seaweeds for applications in the food and drug industries. *S. duplicatum* and *P. tetrastromatica* from the oil extraction site had higher levels of heavy metal concentration compared to those obtained from non-oil extraction site hence the two seaweeds have proven their suitability to serve as bio-monitors for assessment and monitoring of the marine environment. Based on the results of the studied bioactivities, seaweeds from non-oil extraction site at Madura Island have shown higher antioxidant, antidiabetic and anticancer activities, hence we recommend the seaweeds from that site for antioxidant, antidiabetic and anticancer drug candidates.

6. Contribution of authors

S.W.N collected the samples, performed the laboratory work including DPPH, anti-diabetic, TPC, BSLT and drafted the manuscript, N.D.K collected samples, performed DPPH and Heavy metals. N.S.A supervised the research, participated in result discussion and corrected the manuscript, M.A.A supervised the research, participated in result discussion and did correction of the report writing, A.N.K performed anti-diabetic and anticancer, A.S.N performed DPPH test, fixed the final manuscript and handed submission, H.T.A participated in literature review and result discussion. Data analysis was done by S.W.N and A.S.N.

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.

Acknowledgement

The paper is part of the M.Sc. thesis of the first and second authors. The authors are grateful to Universitas Airlangga and Ministry of Education and Culture of Indonesia for financially supporting this research. We are also thankful to Dr. Khun Nay Win Tun and Ei Ei Aung for their various assistances during the research.

References

Abbott, W., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18 (2), 265–267. PMID: 3333059.

Aboutalebian, M., Keramat, J., Kadivar, M., Ahmadi, F., Abdinian, M., 2016. Comparison of total phenolic and antioxidant activity of different *Mentha spicata* and *M. longifolia* accessions. Ann. Agric. Sci. 61 (2), 175–179. https://doi.org/10.1016/j.aoas.2016.10.002.

Ademiluyi, A.O., Ganiyu, O., 2013. Soybean phenolic-rich extracts inhibit key enzymes linked to type 2 diabetes (-amylase and -glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. Exp. Toxicol. Pathol. 65 (3), 305–309. https://doi.org/10.1016/j.etp.2011.09.005.

Al-enazi, N.M., Awaad, A.S., Zain, M.E., Alqasoumi, S.I., 2018. Antimicrobial, Antioxidant and Anticancer Activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extracts. Saudi Pharmaceutical Journal, 26 (1) 44–52. https://doi.org/10.1016/j.jsps.2017.11.001.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., 2017. Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6 (4), 1–42. https://doi.org/10.3390/plants6040042.

Amorim, K., Lage-Yusty, M., López-hernández, J., 2012. Changes in bioactive compounds content and antioxidant activity of seaweed after Cooking Processing. CyTA J. Food 10 (4), 321–324. https://doi.org/10.1080/19476337.2012.658871.

Ardiastutie, Y., Marsono, D., Hartati, M.S., Purwanto, P., 2017. The potential of Understorey plants from Gunung Gede Pangrango National Park (West Java, Indonesia) as cervixs anticancer agents. Biodiversitas 18 (1), 109–115. https://doi.org/10.13057/biodiv/d180115.

Ashraf, M.A., Maah, M.J., Yusoff, I., Mahmood, K., Wajid, A., 2011. Study of antioxidant potential of Tropical fruit. Int. J. Biosci. Biochem. Bioinformat. 1 (1), 35–57. https://doi.org/10.7763/IJBBB.2011.V1.10.

Awesha, H., Sultana, V., Ara, J., Haque, S.E., 2010. *In vitro* cytotoxicity of seaweeds from Karachi coast on brine shrimp. Pak. J. Bot. 42 (5), 3555–3560.

Chandini, S.K., Ganesan, P., Bhaskar, N., 2008. *In vitro* antioxidant activities of three selected brown seaweeds of India. Food Chem. 107 (2), 707–713. https://doi.org/10.1016/j.foodchem.2007.08.081.

Chia, Y.Y., Kanthimathi, M.S., Khoo, K.S., Rajarajeswaran, J., Cheng, H.M., Yap, W.S., 2015a. Antioxidant and cytotoxic activities of three species of Tropical seaweeds. BMC Complement. Alternat. Med. 15 (1), 339. https://doi.org/10.1186/s12906-015-0867-1.

Chia, Y.Y., Kanthimathi, M.S., Rajarajeswaran, J., Khoo, K.S., Cheng, H.M., 2015b. Antioxidant, antiproliferative, genotoxic and cytoprotective effects of the methanolic extract of *Padina tetrastromatica* on human breast adenocarcinoma and embryonic fibroblast cell lines. Front. Life Sci. 8 (4), 411–418. https://doi.org/10.1080/21553769.2015.1051245.

Conti, M.E., Cecchetti, G., 2003. A Biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. Environ. Res. 93 (1), 99–112. https://doi.org/10.1016/s0013-9351(03)00012-4.

Crawford, K., 2017. Review of 2017 Diabetes standards of care. Nurs. Clin. North Am. 52 (4), 621–663. https://doi.org/10.1016/j.cnur.2017.07.010.

Dellavalle, P.D., Cabrera, A., Alem, D., Larrañaga, P., Ferreira, F., Rizza, M.D., 2011. Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria Spp*. Chilean J. Agric. Res. 71 (2), 231–239. https://doi.org/10.1038/sj.bjc.6604510.

Gochfeld, M., 2003. Cases of mercury exposure, bioavailability, and absorption. Ecotoxicol. Environ. Saf. 56 (1), 174–179. https://doi.org/10.1016/S0147-6513(03)00060-5.

Hernández, S.S., Hadolin, M., Skerget, M., Kenz, Z., 2003. Solvent extraction study of antioxidants from Balm (Melissa officinalis L.) leaves. Food Chem. 80, 275–282. https://doi.org/10.1016/S0308-8146(02)00411-5.

Johnson, M., Kanimozhi, S.A., Malar, T.R.J.J., Shibila, T., Freitas, P.R., Tintino, S.R., Menezes, I.R.A., da Costa, J.G.M., Coutinho, H.D.M., 2019. The antioxidative effects of bioactive products from *Sargassum polycystum* C. Agardh and *Sargassum duplicatum* J. Agardh against inflammation and other pathological issues. Complement. Therap. Med. 46, 19–23. https://doi.org/10.1016/j.ctct.2010.03.045.

Kamala-kannan, S., Batvari, B.P.D., Lee, K.J., Kannan, N., Krishnamoorthy, R., Shanthi, K., Jayaprakash, M., 2008. Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and seaweed (*Ulva lactuca*) in the Pulicat Lake, South East India. Chemosphere 71 (7), 1233–1240. https://doi.org/10.1016/j.chemosphere.2007.12.004.

Khaled, A., Hessein, A., Abdel-Halim, A.M., Morsy, F.M., 2015. Distribution of heavy metals in seaweeds collected along Marsa-Matrouh Beaches, Egyptian Mediterranean Sea. The Egyptian J. Aquat. Res. 40 (4), 363–371. https://doi.org/10.1016/j.ejar.2014.11.007.

Khaled, N., Hiba, M., Asma, C., 2012. Antioxidant and antifungal activities of *Padina pavonica* and *Sargassum vulgare* from the Lebanese Mediterranean coast. Adv. Environ. Biol. 6 (1), 42–48.

Khanavi, M., Nabavi, M., Sadati, N., Ardekani, M.S., Sohrabipour, J., Nabavi, S.M.B., Ghaeli, P., Ostad, S.N., 2010. Cytotoxic activity of some marine brown algae against cancer cell lines. Biol. Res. 43 (1), 31–37. PMID: 21157630.

Kodangala, C., Saha, S., Kodangala, P., 2010. Phytochemical studies of aerial parts of the plant *Leucas lavandulae*. Schol. Res. Libr. 2 (5), 434–437.

Kumar, M.S., Adki, K.M., 2018. Marine natural products as multi-targeted cancer treatment: a future insight. Biomed. Pharmacother. 105, 233–245. https://doi.org/10.1016/j.biopha.2018.05.142.

Lim, S., Choi, A.H., Kwon, M., Joung, E.J., Shin, T., Lee, S.G., Kim, N.G., Kim, H.R., 2018. Evaluation of antioxidant activities of various solvent extract from *Sargassum serratifolium* and its major antioxidant components. Food Chem. 278, 178–184. https://doi.org/10.1016/j.foodchem.2018.11.058.

Maharana, D., Jena, K., Pise, N.M., Jagtap, T.G., 2010. Assessment of oxidative stress indices in a marine macro brown alga *Padina tetrastromatica* (Hauck) from comparable polluted coastal regions of the Arabian Sea, West coast of India". J. Environ. Sci. 22 (9), 1413–1417. https://doi.org/10.1016/S1001-0742(09)60268-0.

Makinde, E.A., Ovatlamporn, C., Adekoya, A.E., Nwabor, O.F., Olatunji, O.J., 2019. Antidiabetic, antioxidant and antimicrobial activity of the aerial part of *Tiliacora triandra*. S. Afr. J. Bot. 125, 337–343. https://doi.org/10.1016/j.sajb.2019.08.012.

Mashjoor, S., Yousefzadi, M., Esmacili, M.A., Rafice, R., 2016. Cytotoxicity and antimicrobial activity of marine macro algae (Dictyotaceae and Ulvaceae) from the Persian Gulf. Cytotechnology 68 (5), 1699–1708. https://doi.org/10.1007/s10616-015-9921-6.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., Mclaughlin, J.L., 1982. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45 (5), 31–34. https://doi.org/10.1055/s-2007-971236.

Mohamed, S., Hashim, S.N., Rahman, H.A., 2012. Seaweeds: a sustainable functional food for complementary and alternative therapy. Trends Food Sci. Technol. 23 (2), 83–96. https://doi.org/10.1016/j.tifs.2011.09.001.

Mohsin, S., Mahadevan, R., Muraleedhara, G.K., 2013. Free-radical-scavenging activity and antioxidant effect of ascophyllan from marine brown algae *Padina tetrastromatica*. Biomed. Prevent. Nutr. 4 (1), 75–79. https://doi.org/10.1016/j.bionut.2013.08.006.

Mulyati, H., Geldermann, J., 2016. Managing risks in the Indonesian seaweed supply chain. Clean Technol. Environ. Pol. 19 (1), 175–189. https://doi.org/10.1007/s10098-016-1219-7.

Mwangi, G.G., Wagacha, J.M., Nguta, J.M., Mbaria, J.M., 2014. Brine shrimp cytotoxicity and antimalarial activity of plants traditionally used in treatment of malaria in Msambweni district. Pharm. Biol. 53 (4), 588–593. https://doi.org/10.3109/13880209.2014.935861.

Nies, D.H., 1999. Microbial heavy-metal resistance". Apply Microb. Biotechnol. 51, 730–750. https://doi.org/10.1007/s002530051457.

Norra, I., Aminah, A., Suri, R., 2016. Effects of drying methods, solvent extraction and particle size of Malaysian brown seaweed, *Sargassum Sp.* on the total phenolic and free radical scavenging activity. Int. Food Res. J. 23 (4), 1558–1563.

Palanisamy, S., Vinosha, M., Marudhupandi, T., Rajasekar, P., Prabhu, N.M., 2017. Isolation of fucoidan from *Sargassum polycystum* brown algae: structural characterization, *in vitro* antioxidant and anticancer activity. Int. J. Biol. Macromol. 102, 405–412. https://doi.org/10.1016/j.ijbiomac.2017.03.182.

Park, M.H., Han, J.S., 2012. Hypoglycemic Effect of *Padina arborescens* extract in Streptozotocin-induced diabetic mice. Prevent. Nutrit. Food Sci. 17 (4), 239–244. https://doi.org/10.3746/pnf.2012.17.4.239.

Qari, R., 2015. Heavy metals concentrations in brown seaweed *Padina pavonia* (L.) and *P. tetrastromatica* at different beaches of Karachi Coast. Ind. J. Geo-Marine Sci. 44 (8), 1200–1206.

Raguz, S., Yagu, E., 2008. Resistance to chemotherapy: new treatments and novel insights into an old problem. Br. J. Can. 99 (3), 387–391. https://doi.org/10.1038/sj.bjc.6604510.

Santos, S.A.O., Vilela, C., Freire, C.S.R., Abreu, M.H., Rocha, S.M., Silvestre, A.J.D., 2015. Chlorophyta and Rhodophyta macroalgae: a source of health promoting phytochemicals. Food Chem. 183, 122–128. https://doi.org/10.1016/j.foodchem.2015.03.006.

Shobana, S., Sreerama, Y.N., Malleshi, N.G., 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: mode of inhibition of α-glucosidase and pancreatic amylase. Food Chem. 115 (4), 1268–1273.

Tannoury, M.Y., Elia, J.M., Saab, A.M., Makhlouf, H.Y., Abboud, J.S., Daou Chabo, R.J., Diab-Assaf, M., 2016. Evaluation of cytotoxic activity of *Sargassum vulgare* from the Lebanese coast against Jurkat cancer cell line. J. Appl. Pharmaceut. Sci. 6 (6), 108–112. https://doi.org/10.7324/JAPS.2016.60619.

Vinayak, R.C., Sabu, A.S., Chatterji, A., 2011. Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. Evidence Based Complement. Alternat. Med. 1–9. https://doi.org/10.1093/ecam/neq024.

Wijesinghe, W.A.J.P., Jeon, Y.J., 2012. Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds: a review. Fitoterapia 83 (1), 6–12. https://doi.org/10.1016/j.fitote.2011.10.016.

Wood, J.M., 1974. Biological cycles for toxic elements in the environment. Science 183 (4129), 1049–1052. https://doi.org/10.1126/science.183.4129.1049.

Yamaguchi, T., Takamura, H., Matoba, T., Terao, J., 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl 2-picrylhydrazyl. Biosci. Biotechnol. Biochm. 62 (6), 1201–1204. https://doi.org/10.1271/bbb.62.1201.

Yang, C.F., Lai, S.S., Chen, Y.H., Liu, D., Liu, B., Ai, C., Wan, X.Z., Gao, L.Y., Chen, X.H., Zhao, C., 2019. Anti-diabetic effect of oligosaccharides from seaweed *Sargassum confusum* via JNK-IRS1/PI3K signalling pathways and regulation of gut microbiota. Food Chem. Toxicol. 131,. https://doi.org/10.1016/j.fct.2019.110562 110562.

Zaharudin, N., Staerk, D., Dragsted, L.O., 2019. Inhibition of α-glucosidase activity by selected edible seaweeds and fucoxanthin. Food Chem. 270, 481–486. https://doi.org/10.1016/j.foodchem.2018.07.142.

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