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Bioprospecting Novel Bioactive Molecules from the Seaweeds in Oman

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Authors' contributions

This work was carried out in collaboration between all authors. Author LL designed the study, performed the statistical analysis, wrote the protocol and wrote the first and the final draft of the manuscript. Authors AAY, IAS, AAK, AAJ, AAW and AAS managed the analyses of the study, managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Seaweeds or marine macro-algae form the base for the marine ecosystems and considered as direct or indirect source of food for people across the world. Today, algae have made their way to almost all the areas of human life like food, feed, fuel, medicines etc. Marine algae provide exceptional diverse storage of bioactive compounds such as antimicrobial elements. 5 different varieties of seaweeds were collected from Salalah and they were: *Ulva fasciata*, *Asparagopsis taxiform*, *Rhizoids of Jolyna laminarioide*, *Jolyna laminarioides* and *Laminaria brasiliensis.* The biochemical composition of these seaweeds were determined by using several analytical techniques such as gas chromatography coupled with mass spectrometry and spectrophotometric methods. The phenolic content, antioxidant of TPC, DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and FRAP analysis were measured. The results have shown a higher antioxidant activity in Brown

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(Rhizoids *of Jolyna laminarioides*) comparing with the other varieties. The antimicrobial activity of *Ulva fasciata*on on *E. coli* (G-) and *Rhizoids of Jolyna laminarioides*) on *S. aureus* (G+) was higher comparing with the control sample and the difference was significant ($p < 0.05$). In conclusion, this study points out the possibility of seaweeds to be used in making different products that can be employed in biotechnological, nutraceutical and pharmaceutical applications even though more investigations are required for separating, purifying and characterizing the varieties of seaweeds in Oman.

Keywords: Seaweeds; antimicrobial; antioxidants; DPPH; FRAP.

1. INTRODUCTION

Edible seaweeds have historically been consumed by coastal populations across the globe. Today, marine harvest is still part of the regular diet in many Asian countries. Seaweed consumption is growing especially in the Western countries, due to the influx of immigrants and Asian cuisine as well as the health benefits associated with consumption. The consumption of seaweeds is generally expended all over the world but more specifically in nations situated by the ocean, including the U.K., Ireland, Norway, the Pacific Islands, African nations, and the Americas, in cutting edge days, they have turned out to be principally connected with Asian cooking [1]. These marine algae have primary and secondary metabolites which cannot be found in other organisms. The studies on seaweeds that have been done around the globe have revealed that they are an important source of food and raw material in cosmetic industries among others [2]. It is known that marine plants can be cultivated in large quantities and or harvested from their natural environment. Several studies on seaweeds have shown the potential these seaweeds have in treatment and prevention of many chronic diseases such as diabetes.

The World Health Organization (WHO) estimates that in the EU alone, more than 20 million individuals' experience the ill effects of diabetes and more than 1 billion individuals on the planet are overweight in addition to more than 300 million individuals are obese, making obesity the most essential element for causing diseases, such as diabetes, cardiovascular disease and cancers [3]. In Europe alone, the monetary expenses connected to cardiovascular disease add up to €169 billion each year [4]. The late acknowledgment that ocean weeds are a rich source of supplements that can prevent and treat these illnesses has started to gain a significant enthusiasm for the utilization of ocean plants as sustenance merchandise [5]. In France, Ireland, Canada, and the United States specifically, there have been a number developments to reintroduce ocean seaweeds into the neighborhood food and to provide job opportunities [6].

Oman has more than 1700 km of coast line and preliminary surveys carried out on the coast of Oman revealed the availability of thousands of tons of seaweeds on the southern coast of Oman along the Arabian Sea [7]. The discovery and development of antibiotics, antioxidants and omega-3 fatty acid are milestone achievements of modern science and technology especially in food technology and drugs industries to control chronic and infectious diseases [8]. The seaweeds act as major sources of omega- 3 for fishes and thus can be exploited for making nutritional supplements for human consumption [9].

The aim of this study was to identify the seaweeds varieties such as: *Ulva fasciata*, *Asparagopsis taxifor, Rhizoids of Jolyna Laminarioides* and *Laminaria brasiliensis* which were collected from the Omani coast of Salalah and to analyze their biochemical compositions and specifically, to determine their antimicrobial, antioxidant , phenolic content, radical scavenging activity and other bioactive molecules on the grounds that biochemical creation relies upon numerous ecological and regular variables [10].

2. MATERIALS AND METHODS

2.1 Biomass Sampling and Preparation

Five samples of seaweeds (*Ulva fasciata* / Green, *Asparagopsis taxiform* / Red, *Rhizoids of Jolyna laminarioides* / Brown, *Laminaria brasiliensis* / Brown, *Jolyna laminarioides* / Brown) were collected from the Salalah coast between August and October 2014. About 2 Kg of wet biomass were put in cool box to keep them fresh. Algal biomass sample (the whole plant) was washed with distilled water and their

epiphytes removed. The fresh seaweed was placed in a freezer (at −20°C) 8h after collection. The washed seaweed was then dried at room temperature 38°C for 3 days and then transformed to fine powder and stored in a freezer (at −20°C) in airtight containers. Column and flash column chromatography were used for Soxhlet extraction. It was performed using Soxhlet apparatus for 6 h by using ethanol solvent than we make stock to use in each experiment [11].

2.2 Antimicrobial Properties Extraction

In twenty five mL of N.A (nutrient agar) it was placed in each plate, 100 µL / mL of culture of sample from 5 different concentrations. Symbol s was given for control for (20 µL / mL, 40 µL / mL,60 µL / mL, 80 µL / mL,100 µL / mL, control (ethanol), 5 different concentration from the stock (20 µL / mL, 40 µL / mL,60 µL / mL, 80 µL / mL,100 µL / mL). Cup-plate agar diffusion method were used and two bacterial culture *E. coli* (G-) and *S. aureus* (G+) nutrient agar was prepared, inoculated with bacterial cultures, and transferred to sterile 15-cm diameter Petri dishes. The medium in the plate was allowed to set at room temperature for 10 minutes and solidify for 30 minutes. Three wells (6-mm inner diameter) were made in each plate. Stock solutions of the test residual extract were prepared at concentrations of 200, 400,600, 800, 1000 mg/ mL. In each concentration 700 microliters was placed in the well with sterile pipettes. In each plate, a well was used as control. The respective solvent was used as the control. The Petri dishes were incubated for 24 h at 37ºC and examined with regard to size of the zones of inhibition. The length of the inhibition zone was measured in millimeters from the edge of the well to the edge of inhibition zone, and the results were tabulated.

2.3 GC-MC Extract

The seaweed samples were shade dried, and pulverized to powder using a mechanical grinder. 5 g of this powder is transferred to a flask, treated with 100% ethanol until the powder was fully immersed, incubated overnight and filtered through a Whatmann No. 41 filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulphate must be wetted with 100% alcohol. The filtrate is then concentrated to 1 µL by bubbling nitrogen gas into the solution. The extract contains both polar and nonpolar components of the plant material, and 2 µL of the sample of the solutions was employed in GC-MS for analysis of different compounds. As long as the injector temperature is kept at 250°C the liquid samples can be injected directly.

2.4 Total Phenol Content (TPC)

Antioxidant activity through TPC was determined according to the method of Musa et al. [10] with minor modification. About 100 µL of each samples was added to 0.4 mL of distilled water and 0.5 mL of diluted Folin-Ciocalteu reagent. Samples with the reagent were left for 5 min, and then 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was measured at 765 nm using a spectrophotometer after 2 h. Calibration curve of gallic acid was plotted to evaluate the activity capacity of the samples. Result was expressed as milligram of gallic acid equivalents per 100 gram of fresh sample (mg GA/100 g of FW).

2.5 Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was performed according to Musa et al. (12) with minor modification. FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, 16 mL glacial acid made up to 1:1 with distilled water), 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. Approximately 100 µL of samples was added to 1 mL FRAP reagent, and the absorbance was measured at 595 nm wavelength using a spectrophotometer after 30 min. Calibration curve of Trolox was set up to estimate the activity capacity of samples expressed as milligram of Trolox equivalents per 100 gram of fresh samples (mg TE/100 g of FW).

2.6 DPPH (DPPH. 2,2-Diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity

The method of Musa et al. [12] with minor modification was used to evaluate antioxidant activity through DPPH scavenging system. To prepare the stock solution, 40 mg was dissolved in 100 mL methanol. The solution was then stored at -20°C until use. By mixing 350 mL of the stock solution with 350 mL methanol, an

absorbance of 1.0 ± 0.01 unit was obtained using a spectrophotometer (Epoch, Biotek, USA) at 517 nm wavelength. Approximately 100 µL of each seaweed samples extract with 1 mL methanolic DPPH solution was prepared and kept in the dark for 2 h to allow scavenging reaction to occur. The percentage of DPPH scavenging activity was determined based on the following equation: DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100, where A is the absorbance.

2.7 Statistical Analysis

All experiments were conducted in triplicate (n = 3) and one-way ANOVA (using SPSS statistical software) was used to compare the mean values of each treatment. Significant differences between the means of parameters were determined by using the posthoc=Duncan alpha (0.05).

3. RESULTS

3.1 Antimicrobial Extraction

In this study, the antimicrobial activity of 5 ethanolic seaweeds extracts (*Ulva fasciata* /Green, *Asparagopsis taxiform* / Red, *Rhizoids of Jolyna laminarioides* / Brown, *Jolyna laminarioides*/ Brown, *Laminaria brasiliensis* / Brown was assessed. Two different types of bacteria which were: *E. coli* and *Staphylococcus* were used. The results indicated the ethanolic seaweeds extracts had noticeable antimicrobial activity against bacteria. The Zone of Inhibition (ZOI) of *Ulva fasciata* (S2) (100 microlitre) on *E. coli* was 6 mm and this value was better than control which produced ZOI of 4 mm similarly the ZOI of S1 and S2 against *S. aureus* was 5.4 mm and 5.3 mm respectively which was higher than the control ZOI (i.e, 4 mm). The lowest antimicrobial activity was in *Asparagopsis taxiform* (S3) on *E. coli* and *S. aureus*. The results are shown in Table 1.

3.2 GC-MC Extract

In this study, analyses of each sample of seaweed have shown many compounds. Fig. 1 shows the GC-MS chromatogram of *Rhizoids of Jolyna laminarioides (S1)*, which has highest amount of Oleylaldehyde (33.54%) from the total compounds.

In sample *Asparagopsis taxiform (*S3), analysis have shown that the highest amount were Oleamide (5.43%), Oleamide (39.42%) whereas for *Jolyna laminarioides* (S4) the highest amounts were for Oleic acid (24.91%). The results are demonstrated in Figs. 2, 3 and 4 respectively.

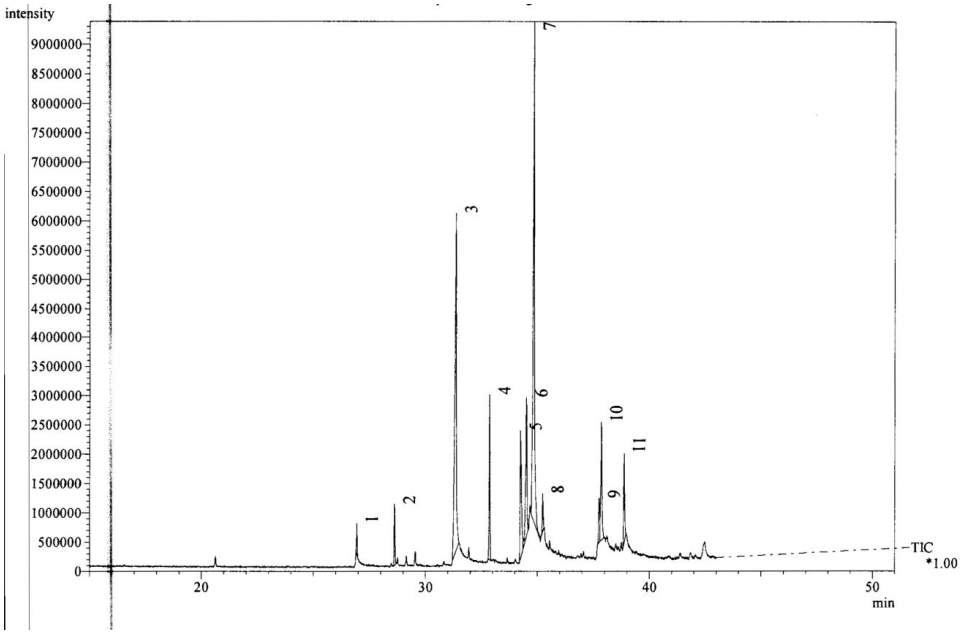

Fig. 1. The GC-MC extracts of *Rhizoids of Jolyna laminarioides* (S1)

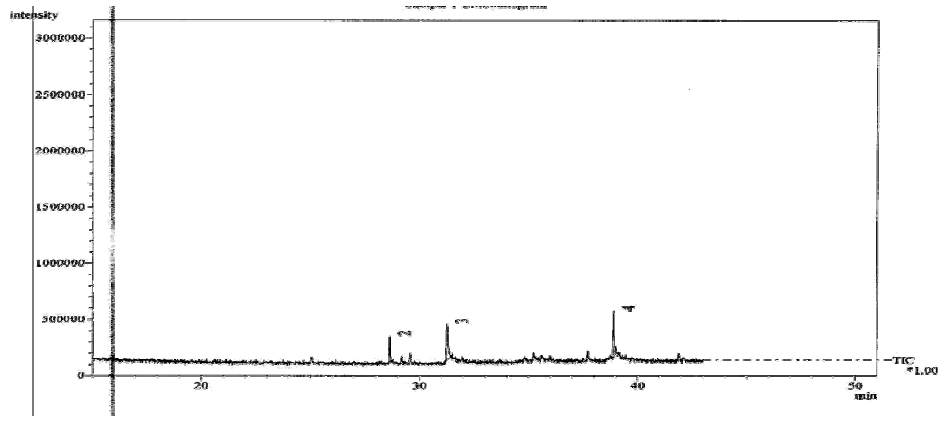

Fig. 2. The GC-MC extracts of *Ulva fasciata* (S2)

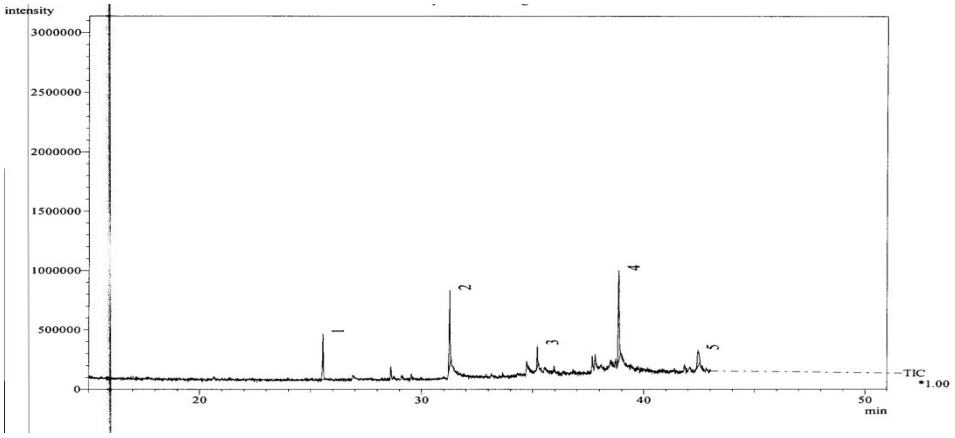

Fig. 3. The GC-MC extracts of rhizoids of *Asparagopsis taxiform* (S3)

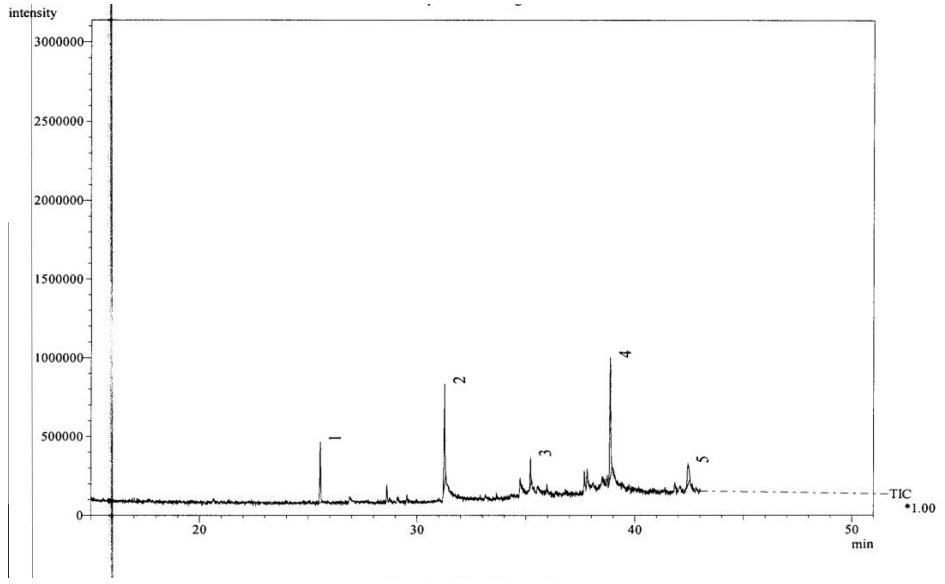

Fig. 4. The GC-MC extracts of *Rhizoids of Jolyna laminarioides* (S4)

Table 1. Anti-microbial activity for five extracts *Rhizoids of Jolyna laminarioides (S1),* *Ulva fasciata* (S2), *Asparagopsis taxiform* (S3), *Jolyna laminarioides* (S4), *Laminaria brasiliensis* (S5)

Bacteria	G- (*E. coli*)					G+ (*S. aureus*)				
Seaweed	Concentration					Concentration				
sample	20 µL	40 µL	60 µL	80 µL	100 µL	20 µL	40 µL	60 µL	80 µL	100 µL
S1	2 mm	2.8 mm	3.2 mm	3.8 mm	4.4 mmd	2.83 mm	3.6 mm	4 mm	4.5 mm	5.4 mma
S2	3 mm	2 mm	4 mm	5 mm	6 mma	2 mm	3 mm	3 mm	4 mm	5.3 mmb
S3	2 mm	3.1 mm	3.8 mm	4 mm	4.6 mme	1.1 mm	1.4 mm	2 mm	4.2 mm	4.4 mme
S4	1 mm	1.7 mm	2 mm	3 mm	5 mmc	1.2 mm	1.6 mm	2.4 mm	3.2 mm	3.8 mmd
S5	2.4 mm	3.4 mm	3.5 mm	4.4 mm	5.2 mmb	2 mm	2.6 mm	3 mm	3.2 mm	4.7 mmc
Control	3.26 mm					3.88 mm				

** Different characters denotes significant difference p<0.05*

3.3 Antioxidant Activity Assays

3.3.1 The total phenolic content (TPC)

The Total phenolic content (TPC) of these seaweed samples: (*Ulva fasciata* / Green, *Asparagopsis taxiform* / Red , *Rhizoids of Jolyna laminarioides* / Brown , *Jolyna laminarioides/* Brown ,*Laminaria brasiliensis/*- Brown along with the standard gallic acid is shown in Fig. 5. *Rhizoids of Jolyna laminarioides (S1)* exhibited higher activity than*, Asparagopsis taxiform* (S3), *Laminaria brasiliensis* (S5), *Jolyna laminarioides* (S4), and *Ulva fasciata* (S2). Total phenolic content was the highest in (S1) followed by (S3) and then (S5). The difference was significant among these samples whereas the difference was not significant between (S2) and (S4) which has the lowest TPC among the 5 samples.

3.3.2 Ferric reducing antioxidant power (FRAP)

High variability in antioxidant activity (as mg TE/100 g of FW extract) is shown in (Table 3). The highest activity (656.6667 mg TE/100 g) was found in the *Rhizoids of Jolyna laminarioides* extract whereas in *Ulva fasciata* (S2) the lowest antioxidant activities were found (168 mg TE/100 g).

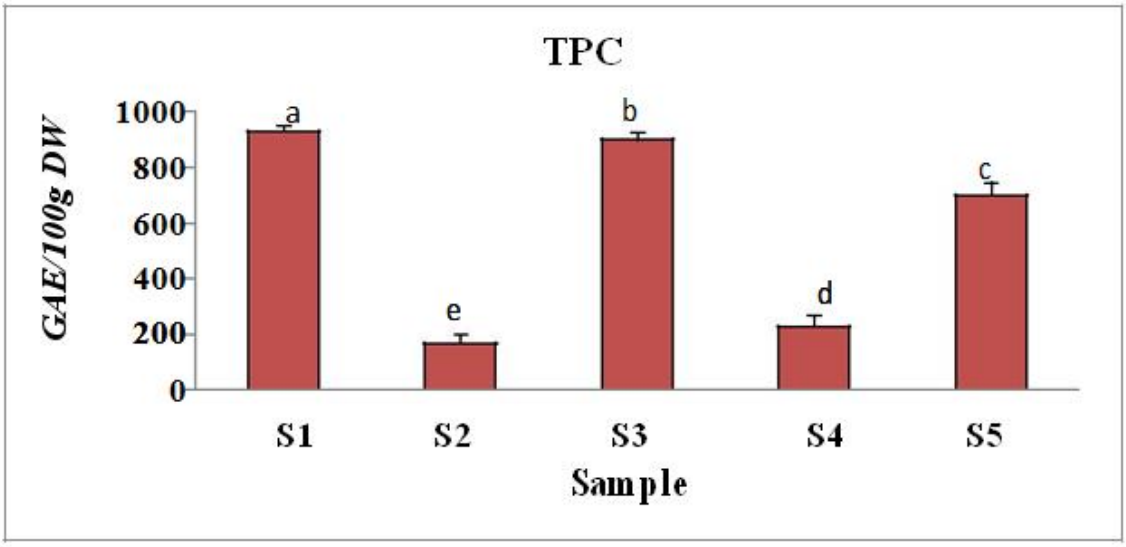

Fig. 5. Total phenolic content (TPC) of *Rhizoids of Jolyna laminarioides (S1)*

Table 2. Total phenolic content (TPC) of *Rhizoids of Jolyna laminarioides (S1), Ulva fasciata* (S2), *Asparagopsis taxiform* (S3), *Jolyna laminarioides* (S4), *Laminaria brasiliensis* (S5)

TPC

Duncan

Antioxidant	N	Subset for alpha = 0.05				
		1	2	3	4	5
S2	3	167.1400				
S4	3		227.6900			
S5	3			699.7967		
S3	3				899.1200	
S1	3					927.6900
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed. a. Uses Harmonic Mean Sample Size = 3.000. Ulva fasciata (S2), Asparagopsis taxiform (S3), Jolyna laminarioides (S4), Laminaria brasiliensis (S5)

3.3.3 DPPH (2,2-Diphenyl-1-Picrylhydrazyl) assay

The free radical scavenging activity of ethanol extract of seaweed was assessed by the DPPH assay. Fig. 6 shows a significant difference in the values of DPPH radical due to scavenging ability of the seaweeds. The results obtained from this study have shown that *Rhizoids of Jolyna laminarioides (S1)* had the highest DPPH• scavenging activity (86.9467%) among the seaweeds. Followed by S3 whereas S2, S4, and S5 had the lowest values of DPPH. This indicates that *Rhizoids of Jolyna laminarioides (S1)* as a good source of natural antioxidants.

The scavenging activity of the samples was higher in S1 and S3 between these samples and S2, S4 and S5and the difference was significant as shown in Table 4.

4. DISCUSSION

Reactive oxygen species such as hydroxyl, super oxide and peroxyl radicals produced in human tissue cells are responsible for extensive oxidative damage that leads to age related degenerative conditions, cancer and a wide range of other human diseases. several researchers have found different type of antioxidants in many species of higher plants further, seaweeds represents as interesting source of antioxidant, antimicrobial and active compounds [12,13]. In fact many studies have reported the antioxidant properties of both brown and red seaweeds from across the globe. Moreover, the evidence available in the literature suggests potential protective effects of seaweeds against oxidative stress, lipids oxidation and against bacteria growth. A free radical is a molecule with one or more unpaired electron whereas natural antioxidant has less or no electrons in the outer orbital. Many of these free radicals side effects are oxidative stress which can be fought by seaweed consumption. Seaweeds have received special attention due to these ingredients fighting oxidative stress and as a source of natural antioxidants [14]. Seaweeds by the imbalance of the bodily antioxidant defense are known source of pharmacological and food system and free-radical formation. Oxidative stress has additives with potential health effects like anti-oxidative been linked to cancer, aging, ischemic injury, inflammation and anti-carcinogenic [15]. The study has shown the potential of the seaweeds found on the Omani coasts. They are rich in anti-microbial activities as their extracts showed Zone of Inhibition against different bacteria especially in the following samples S1, S3, S5. Omani seaweeds have shown to have reasonable oleamide values in addition to their anti-oxidants activities.

Table 3. Ferric-Reducing Antioxidant Power (FRAP) (μmol Trolox g−1 extract) of extracts obtained from *Rhizoids of Jolyna laminarioides (S1), Ulva fasciata* (S2), *Asparagopsis taxiform* (S3), *Jolyna laminarioides* (S4), *Laminaria brasiliensis* (S5)

FRAP

Duncan

Antioxidant	N	Subset for alpha = 0.05				
		1	2	3	4	5
S2	3	168.0000				
S4	3		264.0000			
S5	3			472.6667		
S3	3				559.3333	
S1	3					656.6667
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

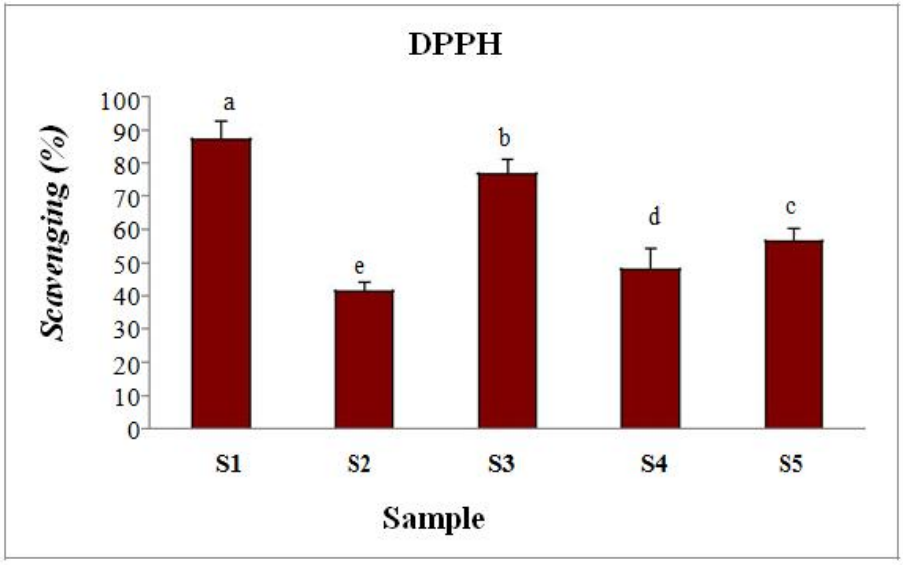

Fig. 6. Free radical scavenging activity (DPPH) of *Rhizoids of Jolyna laminarioides (S1), Ulva fasciata* (S2) *Asparagopsis taxiform (S3), Jolyna laminarioides (S4), Laminaria brasiliensis (S5)*

Comparison of free radical scavenging activity of methanolic extract of seaweeds (100 µg/mL)
Results are mean ± SD (n = 3)

Table 4. The scavenging activity of extracts Rhizoids *of Jolyna laminarioides (S1), Ulva fasciata* (S2), *Asparagopsis taxiform* (S3), *Jolyna laminarioides* (S4), *Laminaria brasiliensis* (S5)

DPPH

Duncan

Antioxidant	N	Subset for alpha = 0.05				
		1	2	3	4	5
S2	3	41.3567				
S4	3		47.9400			
S5	3			56.5267		
S3	3				76.8067	
S1	3					86.9467
Sig.		1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.0

Based on the above facts, Seaweeds are known to be a rich source of antioxidant compounds based of this result. Moreover, the (*Ulva fasciata* / Green, *Asparagopsis taxiform* / Red, *Rhizoids of Jolyna laminarioides* / Brown, *Jolyna laminarioides/* Brown, *Laminaria brasiliensis* / Brown extract have antimicrobial activity and defiant fatty acid as Omega-9, Oleamide and Omega-7. Antimicrobial compounds have great advancements in antimicrobial surface technology that can help reduce patient and staff exposure to dangerous microorganisms [16]. On the anther hand Omega-9, Oleamide and Omega-7 could be useful to reduce fasting glucose**,** treatment for mood and sleep disorders and reduce risk of type II diabetes respectively [17]. Finally, this seaweeds able to open a new field in Omani industrial, to produce different kind of medical treatment [18]. Adding to that, this study could be extended in order to further indentify nutritive value of seaweeds and other bioactive compounds that can be very import sources for different industries including food industries [19].

Table 5. Shows the Descriptive of this three test (TPC, FRAP and DPPH) of antioxidant activity extracts of *Rhizoids of Jolyna laminarioides (S1), Ulva fasciata* (S2), *Asparagopsis taxiform* (S3), *Jolyna laminarioides* (S4), *Laminaria brasiliensis* (S5)

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TPC	S1	3	927.6900	1.17779	.68000	924.7642	930.6158	926.33	928.37
	S2	3	167.1400	2.04000	1.17779	162.0724	172.2076	165.10	169.18
	S3	3	899.1200	2.35559	1.36000	893.2684	904.9716	897.76	901.84
	S4	3	227.6900	3.11615	1.79911	219.9491	235.4309	224.29	230.41
	S5	3	699.7967	3.53916	2.04333	691.0049	708.5884	695.71	701.84
	Total	15	584.2873	337.50657	87.14382	397.3824	771.1922	165.10	928.37
FRAP	S1	3	656.6667	2.30940	1.33333	650.9298	662.4035	654.00	658.00
	S2	3	168.0000	2.00000	1.15470	163.0317	172.9683	166.00	170.00
	S3	3	559.3333	1.15470	.66667	556.4649	562.2018	558.00	560.00
	S4	3	264.0000	3.46410	2.00000	255.3947	272.6053	262.00	268.00
	S5	3	472.6667	3.05505	1.76383	465.0775	480.2558	470.00	476.00
	Total	15	424.1333	188.58945	48.69359	319.6960	528.5707	166.00	658.00
DPPH	S1	3	86.9467	1.69500	.97861	82.7360	91.1573	85.25	88.64
	S2	3	41.3567	.69515	.40134	39.6298	43.0835	40.76	42.12
	S3	3	76.8067	1.27017	.73333	73.6514	79.9619	75.34	77.54
	S4	3	47.9400	1.29294	.74648	44.7282	51.1518	46.53	49.07
	S5	3	56.5267	.84500	.48786	54.4276	58.6258	55.68	57.37
	Total	15	61.9153	17.92980	4.62945	51.9861	71.8445	40.76	88.64

5. CONCLUSION

The present study was a pioneer study in Oman to study the antioxidant property of five seaweeds. This study suggested that, among the five seaweed samples, the (*Ulva fasciata* /Green, *Asparagopsis taxiform* / Red, *Rhizoids of Jolyna laminarioides/* Brown, *Jolyna laminarioides/* Brown, *Laminaria brasiliensis/-* Brown extract possesses high antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress related disorders. It can be concluded that *Asparagopsis taxiform* / Red, *Rhizoids of Jolyna laminarioides/-* Brown, in appropriate combination, can act as an effective and safe food preservative which could enhance the quality and nutritive value of food items and could also be used as a natural drugs of several diseases that are caused as a result to oxidative stress aging and cancers. In addition, the antimicrobial properties of these five seaweed samples would have promising future in enhancing the food safety. There are few reports on the antioxidant capacity of seaweed and the mechanism of seaweeds as anti-oxidative agents is still not fully understood.

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DISCLAIMER

Some part of this manuscript was previously presented in the following conference.
Conference name: **Nordic Seaweed Conference 2016**
Dates: October 12th and 13th 2016
Location: Kystvejens Hotel and Conference Centre in Grenaa, Denmark
Web Link of the proceeding:
http://www.algecenterdanmark.dk/media/14147/invitation_and_programme_nordic_seaweed_conference_2016.pdf"

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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