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Full Length Research Paper

Phytochemical studies and their bioactivities of various crude extracts of red alga (*Hypnea boergesenii*)

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ABSTRACT

This study determined the total phenols and flavonoids, antioxidant activity, Preliminary Phytochemical Screening, GC-MS analysis. *Hypnea boergesenii* T. Tanakared seaweed, collected from Chabahar Sea, on the coast of the Gulf of Oman. Powder was extracted using five solvent: (15g) with70% ethanol and (340g) extracted successively with four solvent (Hexane, petroleum ether, dichloromethane and 1445 methanol) using maceration method. All the crude extracts were concentrated under reduced pressure and dried by using freeze dryer. The antioxidant activity of the methanolic extract was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and the IC_{50} is (17.68± 0.33). The total phenolics and total flavonoids content were also determined by Folin-Ciocalteu reagent and aluminum chloride colorimetric methods (TPC =3.42±0.04) and (TFC = 4.79 ± 0.11). In this investigation, was performed to screen, estimate total phenolic, flavonoids, evaluate antioxidant potential of H. boergesenii and investigate Antimicrobial activity of the seaweed extracts. Phytochemical analysis of alcoholic extract showed presence of major classes of phytochemicals, such as: tannins, flavonoids, cardiac glycosides, steroids, etc. Gas chromatography-mass spectrometry results revealed presence phytoconstituents compounds in various extract of *H. boergesenii* alga.

Keywords: *Hypnea boergesenii*, antioxidant, DPPH, Total phenolic, flavonoid.

INTRODUCTION

The ocean environment contains over 80% of world's plant and animal species (Jha and Zi-rong, 2014; Sheeba and Paul, 2016). It is estimated that approximately 90% of the species of marine plant are algae and is nearly 50% of the global photosynthesis is contributed by algae (Srivastava et al., 2010). Marine

organisms are a rich source of structurally novel and biologically active metabolites. So far, many chemically unique compounds of marine origin with different biological activities have been isolated and some of which are under investigation are being developed as new pharmaceuticals (Ely et al., 2004). Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites and their discovery has significantly expanded in the past three decades .An algae synthetize a variety of compounds such as carotenoids, terpenoids, xanthophylls, chlorophyll, vitamins, saturated and polyunsaturated fatty acids, amino acids and acetogenins; antioxidants such as polyphenols, alkaloids, halogenated compounds and polysaccharides such as agar, carrageenan, proteoglycans, alginate, laminaran, rhamnan sulfate, galactosyl glycerol and fucoidan (Almeida et al., 2011; Peng et al., 2013).

Seaweeds have been recognized as potential sources of antibiotic substances. Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds including antimicrobial, antioxidant and anti-inflammatory (Sande-Bruinsma et al., 2008; El Shafay et al., 2016 Tüney et al., 2016). An antimicrobial compound derived from seaweeds consist of diverse groups of chemicals such as macrolides, cyclic peptides, proteins, polyketides, sesquiterpenes, terpenes and fatty acids, which have been shown to have antibacterial activity against both Gram-positive and Gram-negative bacteria (Cavallo et al., 2013). Different diseases were treated with antibiotics, extracted from terrestrial sources that were used as therapeutic agents; new compounds are present in oceans and have commercial value (Smit, 2004). The required number of new antimicrobial agents is higher than ever due to the rapid development of new infections, emergence of multidrug resistance in common pathogens, and the potential for use of multidrug-resistant agents in bioweapons (Peters et al., 2008; Sevindik, 2018; Sevindik, 2019).

In this research project, the study was performed conducted on, Red algae "*H. boergesenii*". The red algal genus *Hypnea* (Gigartinales) has a wide geographical distribution along tropical and subtropical coasts around the world. The relatively simple morphology, often influenced by the conditions of its habitat, complicates the identification of *Hypnea* species. Therefore, the number and status of some species remain in doubt. *H. boergesenii* was at first identified by Tanaka (1941); (Uauer et al., 2014). Even so, the taxonomy of *Hypnea*, based mainly on vegetative characters, is problematic and confusing. One hundred and twelve names have been assigned to Hypnea of which 55 are currently listed as accepted in Algae Base (Guiry and Guiry, 2014; Uauer et al., 2014). In this investigation, was performed to screen, estimate total phenolics, flavonoids, evaluate antioxidant potential of *H. boergesenii* and investigate antimicrobial activity of the seaweed extracts.

Experimental

General experimental procedures

Quercetin, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, ascorbic acid, were purchased from Sigma-Aldrich, USA. Folin- Ciocalteu reagent and Muller-Hinton agar, Sabouraud Dextrose Agar, Muller Hinton Broth and Sabouraud Dextrose Broth medium were obtained from Merck, Germany. All other chemicals and solvents used in the study were obtained from Merck, Germany.

Sample collection

H. boergesenii red seaweed was collected from coastal region of Chabahar Sea, Sistan and Baluchestan Province, Iran in May and June 2015, and washed several times with seawater to remove sand, mud, and attached fauna and then washed by fresh water.

The alga was dried in the shade at ambient temperature for 1 week. The dried algal materials were ground using kitchen blender to obtain the coarse and kept in a refrigerator till further use.

Preparation of extracts

The dried powdered of red alga (340g) was taken and extracted successively with four different solvents (Hexane, petroleum ether, dichloromethane and methanol) using maceration method at room temperature for one week. The crude extracts were concentrated under reduced pressure at first and then all of then the extracts were dried by using freeze dried. The methanolic extract was further subjected to antioxidant assays and determination of total phenolics, flavonoid content. Dried powdered of red alga (15g) was taken and extracted with 60 mL ethanol 70% (ethanol and distilled water 70:30) by maceration method at room temperature and for a 72 h on heather stirrer. The crude extracts were concentrated under reduced pressure and dried by using freeze dryer. The ethanolic extract was further subjected to Phytochemical Screening test.

Test for alkaloids

One half a gram of the plant extract was dissolved in 5 mL HCl (5%) on a steam bath. The filtrate (1mL) was treated with few drops of Dragendorff's reagent (potassium iodide 0.11 M, bismuth nitrate 0.6M in acetic acid 3.5M). Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for flavonoids

Ethanol extract (5mL) was added to a concentrated sulphuric acid (1mL) and 0.5g of Mg. A pink or red coloration that disappears on standing (3min) indicated the presence of flavonoids.

Test for glycosides

The extract (approximately 0.5g of extract) was dissolved in 2mL glacial acid containing 1 drop of 1% $FeCl_3$. Brown ring at the interface indicated the presence of glycosides. A violet ring may form just above ring and gradually spreads through this layer.

Test for steroids

The extract (about 0.5g) was dissolved in $CHCl_3$ (3 mL) and then filtered. To the filtrate was added concentrated H_2SO4 to form a lower layer. A reddish brown color was taken as positive for steroids.

Test for tannins

About 1 ml of the ethanol extract was added to 2mL of water in a test tube. A few drops (2 to 3 drops) of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or blue-black (Gallic tannins) coloration.

Test for terpenoids

The extract was mixed with 2mL of chloroform and concentrated H_2SO_4 (3mL) was carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.

Test for phenols

One half g of extract was dissolved in 5ml distilled water a few drops of %5 aqueous ferric chloride solution was added. Formation of blue or green color indicated the presence of phenols (Harbourne, 1983; Trease and Evans, 1983; Abioye et al. 2013; Mir et al. 2013).

GC-MS analysis

GC-MS analysis of various crude organic extracts of *H. boergesenii* was performed on HP- 5MS GC System (30m length, 0.25µm inner diameter, 0.25µm film thicknesses; maximum temperature, 300°C, coupled to HP- 5MS GC MS. Ultra-high purity helium 99.99% was used as carrier gas at a constant flow rate of 1.0mL/min. The injection, transfer line and ion source temperatures were 150°C). The ionizing energy was 70eV. Electron multiplier voltage was obtained from auto tune. The oven temperature was programmed from 40°C (hold for 4min) to 200°C at a rate of 5°C/min (hold 10min). And then to 280°C at a rate of 4°C/min (hold 10min) and 300°C at a rate of 8°C/min (hold 10min).The crude samples were diluted with appropriate solvent (1/100, v/v) and filtered. The particle-free diluted crude extracts (1 elk) were taken in a syringe and injected into injector with a split ratio 30:1. The percentage composition of the crude extract constituents was expressed as a percentage by peak area. The identification and characterization of chemical compounds in various crude extracts was based on GC retention time. The mass spectra were computer matched with those of standards available in mass spectrum libraries.

Determination of total phenols by Folin-Ciocalteu reagent method

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC) of the various organic crude extracts. Gallic acid was used as a reference standard (0.01- 0.1 mg/mL) for plotting calibration curve. A volume of 0.5mL of the plant extract (2-50mg/mL) was mixed with 2.5mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and were neutralized with 2.5mL of sodium carbonate solution (7.5%, w/v), was shaken. The reaction mixture was incubated at 45°C for 45min. The absorbance of the resulting blue color was measured by double beam UV-Vis spectrophotometer at fixed wavelength of 765nm. The TPCs were determined by linear regression equation obtained from the standard plot of Gallic acid. The content of total phenolic compounds was calculated as mean ± SD (n=3) and expressed as mg/g gallic acid equivalent (GAE) of 1g dry extract (Stanković, 2011; Talole et al., 2013)

Estimation of total flavonoid content (TFC) by aluminum chloride colorimetric method

TFC in crude extracts at various concentrations (2-50mg/mL) was determined by quercetin as a standard to construct the calibration curve. Briefly, 10 mg of quercetin was dissolved in 100 mL Absolute methanol and then diluted to 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 and 0.09mg/mL. The diluted standard solutions of quercetin or plant extracts (0.5mL) of different concentration were separately mixed with 0.1 mL of 10% aluminum chloride, 0.1mL of 1mol/L potassium acetate and 2.8mL of distilled water in a test tube. The test tubes were incubated for 30min at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at 415nm with double beam UV-Vis spectrophotometer against blank. A typical blank (0.5mL distilled water) solution contained all reagents except aluminum chloride was replaced by equal amount of distilled water. The amount of flavonoid was calculated from linear regression equation obtained from the quercetin calibration curve. The flavonoid content was calculated as mean ± SD (*n*=3) and expressed as mg/g of quercetin equivalent (QE) of 1g dry extract (Stanković, 2011; Pallab et al., 2013; Bag et al., 2015)

Assay of free radical scavenging activity by DPPH method

DPPH is a stable, free radical which has an unpaired valence electron at one atom of Nitrogen Bridge and the Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay, the reduction of the radical is followed by a decrease in the absorbance at 517nm.The free radical scavenging activity of different concentrations of crude extracts of *H. boergesenii* and of standard ascorbic acid was evaluated by DPPH radical scavenging method. various crude extracts (4, 6, 8, 10, 20, 30, 40 and 50mg/mL) or standard ascorbic acid solution (0.2- 1mM) of 2 mL at different concentrations were taken in separate test tubes. Two milliliter of 0.5mmol/L DPPH radical solution, prepared at methanol was added to each test tube. The solution was rapidly mixed and allowed to stand in dark at 37°C for 30min. The blank was prepared in a similar way without extract or ascorbic acid. The absorbance of each solution was measured at 517nm using UV-Vis spectrophotomer. The percentage of radical scavenging activity of tested extracts and positive control ascorbic acid was calculated by the following equation:

DPPH Scavenged (%) = $A_c - A_s / A_c \times 100$

Where A_c=Absorbance of control (methanol + DPPH) at 517nm and A_s=Absorbance of sample.

The concentration of sample required to scavenge 50% of DPPH free radical (IC50) was determined from the curve of percent inhibitions plotted against the respective concentration (Pallab et al., 2013; Waheed et al., 2014; Bag et al., 2015)

Total reduction capacity assay

Total antioxidant activity was measured by ferric reducing antioxidant power Assay. This method is based on the chemical reduction of ferricyanide (Fe^{3+}) complex to ferrous (Fe^{2+}) form. In this assay, the yellow color of the test solution changes to various shades of green and blue, that exhibits a broad light absorption at 700nm. Calibration curve was prepared from ascorbic acid Standard solutions (1, 0.8, 0.6, 0.4, and 0.2mMol L^{-1}). The various crude extracts (10, 20, 30, 40 and 50mg/mL) or standard ascorbic acid solutions 1mL was pipetted into separate tubes, and t 2.5mL of phosphate buffer and then 2.5mL of potassium ferricyanide solution was added. Solutions were kept at 50°C for 20mins. Then 2.5mL of trichloroacetic acid was added and solution was centrifuged for 15mins at 3000rpm. Supernatant was collected. 2.5mL of the supernatant was taken into separate tubes and to each 2.5mL distilled water and then 0.5mL of ferric chloride solution was added. The absorbance of each solution was determined at 700nm against the blank and absorbance vs. concentration graph was plotted (Sherikar et al., 2015).

Determination of Antimicrobial activity of seaweed's extracts

Evaluation of Antibacterial properties of extracts by disk-diffusion method- Microbial strains and antimicrobial evaluation

Antibacterial activity was determined against, Gram negative bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumoniae*; Gram positive bacterial strains *Staphylococcus aureus* and compared with Standard antibiotics table 1 using the paper disk assay method. (Table 1)

Whatman No. 1 filter paper disk of 6-mm diameter was sterilized by autoclaving for 30 min at 121°C. The sterile disks were impregnated with different extracts and Standard antibiotics (400µL). The impregnated disks were placed on the medium suitably spaced apart and then M. H agar plates were surface inoculated uniformly from the Mueller-Hinton agar culture of the test microorganisms. In all cases the suspensions of

Table 1. Pathogens and standard Antibiotics for comparison of antibacterial effect

Pathogen	Standard antibiotics
P. aeruginosa	Imipenem, Gentamicin, Meropenem
E. salmonella	Ciprofloxacin, Imipenem, Gentamicin, Meropenem
K. pneumoniae	Ciprofloxacin, Imipenem, trimethoprim
E. coli	Ciprofloxacin, trimethoprim, Vancomycin
S. aureus	Vancomycin, Gentamicin, Meticillin, Streptomycin
P. vulgaris	Ciprofloxacin, Imipenem, trimethoprim

Table 2. Pathogen and standard Antibiotics for comparison Antifungal effect

Pathogen	Standard antibiotics
C. albicans	Canazole, Metronidazole, Miconazole
M. gypseum	Canazole, Metronidazole, Miconazole
T. rubrum	Canazole, Metronidazole, Miconazole

microorganisms were initially adjusted with sterile distilled water to a density equivalent to the 0.5 McFarland (10^6CFU/mL) standards. The plates were incubated at 37 ̊C for 24h. The diameter (mm) of the growth inhibition zone caused by the 4 extracts of marine organisms and Antibiotic disks was measured.

Determination of the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC)

MIC was determined by the serial two –fold in 12 numbered tubes add to each one of them 2mL of Broth medium and then700 /1000µL (respectively for bacteria and fungi) of the tested extracts was added to the first test tube and mixed well. 1 mL of content of tube 1 was added to tube 2 then it was stirred and 1mL was withdrawn and transferred for tube 3, this serial add was repeated until tube 10, added to each tube from 1 to 11, 100µL of bacterial suspension, the 11 tube was Negative control and the 12 tube was Positive control, all the tubes were incubated at 35°C -37°C / 26°C -28°C for 24 hrs (respectively for bacteria and fungi). The MIC endpoint was considered as the lowest concentration of the extract inhibiting the total growth of microorganisms. MIC was detected by lack of visual turbidity (matching the negative growth control).

Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Subcultures were made from the clear wells which did not show any growth after incubation during the MIC assay, the higher and lower dilutions of MIC assays results on Mueller-Hinton agar for bacteria and Sabouraud agar for fungi and algae in order to achieve the Minimal Bactericidal Concentration (MBC) the Minimal Fungicidal Concentration (MFC), all the plates were incubated at 35°C -37°C / 26°C -28°C for 24hrs (respectively for bacteria and fungi). The lowest concentration that yielded no growth after this sub-culturing was taken as (MBC) and (MFC).

Antifungal assay

Antifungal activity was determined against: *Candida albicans*, *Microsporum gypseum, Trychophyton rubrum* and Miconazole, Metronidazole and Canazole as standard antibiotics by well diffusion method table 2.

Fungal suspensions of microorganisms were initially adjusted with sterile distilled water to a density equivalent to the (10^3CFU/mL) standards. In this method a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cylinder and a volume 100µL of the extract solution and antibiotics into the well. The agar plate's surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface and then, the plates were incubated at 28 ̊C for 24h. The diameter (mm) of the growth inhibition zone caused by the 4 extracts of marine organisms and Antibiotic was examined (Seenivasan et al., 2010; Qiao, 2010; Alghazeer et al., 2013; Dantas et al., 2015; Neut et al., 2015).

RESULT AND DISCUSSION

Preliminary phytochemical screening

The present study carried out on the Red alga "*H. boergesenii*" and Preliminary phytochemical analysis metabolites such as tannins, flavonoids, cardiac glycosides in alcoholic extract Table 3 and etc. alkaloids were found to be absent in the extracts.

Table 3. Phytochemical screening of H. boergesenii extracts

Chemical Test	Result
Alkaloids	-
Flavonoids	+
Cardiac glycosides	+
steroids	+
tannins	+
Phenolic compounds	+
terpenoids	+

Table 4. Total phenolic and flavonoid content of methanolic extract of H. boergesenii (n=3) Total phenolic: mg of GAE/g of 1g dry extract; Total flavonoids: mg of QE/g of 1 dry extract; Values are mean ± SD

Extracts	Total phenolic	Total flavonoids
methanol	3.42±0.04	4.79±0.11
Ethanol 70%	2.12±0.02	5.09±0.17

Table 5. IC_{50} (mg/ ml) of DPPH free radical by Alcoholic extracts/ ascorbic acid at 517 nm (n=3)

ascorbic acid	Methanol	Ethanol 70%
0.27±0.00	17.68± 0.33	20.390±0.54

showed the presence of major classes of secondary

TPCs

The TPC of the alcoholic extract is expressed in terms of GAE and presented in Table 4. The TPCs were calculated by the following linear regression equation obtained from the standard plot of Gallic acid:
$y = 14.389x - 01798$, $R^2 = 0.9681$ from the standard curve for methanolic extract and
$y = 13.012x - 0.0471$, $R^2 = 0.9947$ from the standard curve for Ethanolic extract. *(y* is absorbance and *x* is the amount of Gallic acid in µg.).

TFCs

The TFCs of the alcoholic crude extracts are expressed in terms of QE and are presented in Table 4. The TFCs were calculated using the following linear regression equation obtained from the standard plot of quercetin:
$y = 10.09x - 0.0502$, $R^2 = 09935$ from the standard curve for methanolic extract and
$y = 8.9964x - 0.0466$, $R^2 = 0.9965$ from the standard curve for Ethanolic extract. *(Y* is absorbance and *x* is the amount of quercetin in µg.)

Methanol extract was found to contain higher quantity of phenolic compounds, but no significant difference in the phenolic contents of two extracts ($P > 0.05$) and ethanol extract was found to contain higher quantity of phenolic compounds, but no difference in the phenolic contents of two extracts ($P > 0.05$).

In vitro antioxidant activity

The antioxidant activity of methanolic and Ethanolic crude extracts of H. boergesenii were investigated by commonly used radical scavenging methods such as DPPH and Total reduction capacity assay the scavenging effect of the extract on the DPPH free radicals was expressed as IC_{50} of DPPH and they were compared with standard antioxidant, ascorbic acid.

The two extracts showed scavenging activity of DPPH and were less than to standard antioxidant. The IC_{50} value (in mg/mL) of the extracts was found in the order of Ethanol > methanol extract (Table 5). It was interesting to note that the scavenging ability of organic extracts was greater than ascorbic acid although IC_{50} value of ascorbic acid (0.27mg/mL) was much less than the alcoholic extracts.

The Total reduction capacity assay of the alcoholic extracts and compared with ascorbic acid has been shown in (Table 6). The reducing power of H.

Table 6. Determination of total reduction capacity assay of alcoholic extracts

extract	mg/g of standard equivalent (A.A) of 1 g dry extract
Ethanol 70%	4.81
Methanol	5.00

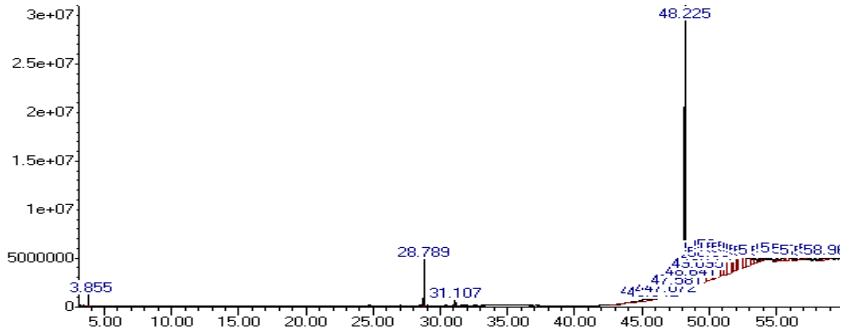

Figure 1. GC-MS chromatogram of hexane extract

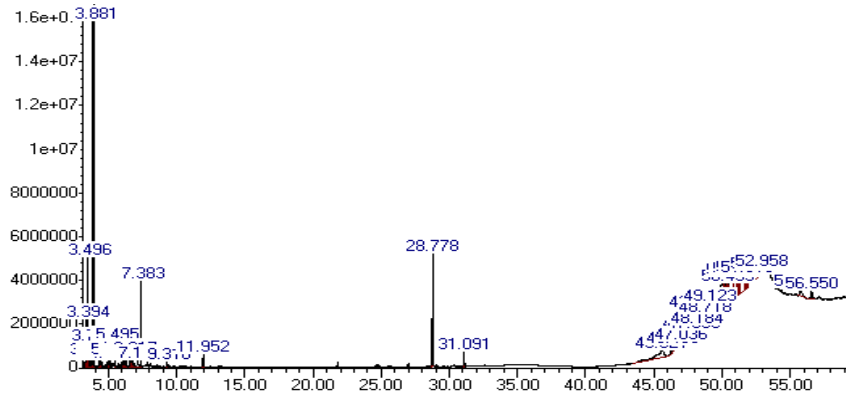

Figure 2. GC-MS chromatogram of p. ether extract

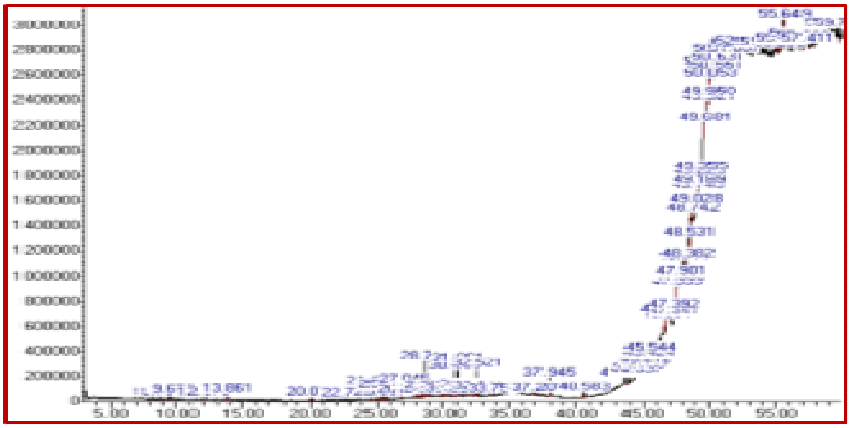

Figure 3. GC-MS chromatogram of dichloromethane extract

boergesenii was found to be remarkable, which increased gradually with a rise in the concentration.

The result of the total reduction capacity assay was calculated by the following linear regression equation

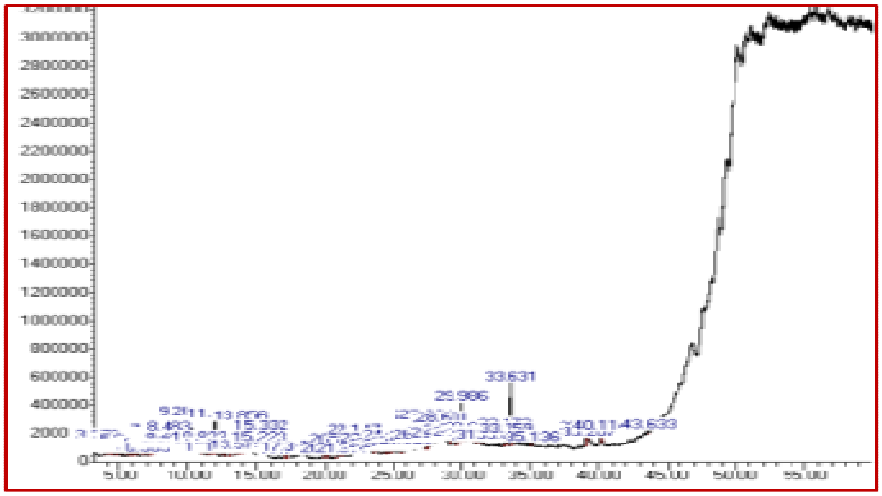

Figure 4. GC-MS chromatogram of methanol extract

Table 7. Chemical composition of various extracts of H. boergesenii alga

Extracts	C. No.	Compound name	Retention time (min)	% content
	1	Octane	3.85	72
Hexane	2	Heptadecane	28.79	99
	3	2-Pentadecanone, 6,10,14-trimethyl	31.11	95
	4	Hexane, 2,3-dimethyl	3.33	91
Petroleum	5	Heptane, 2-methyl	3.40	91
ether	6	Cyclohexane, 1,3-dimethyl	3.60	91
	7	Cyclohexane, ethyl	4.14	94
	8	p-Xylene	5.07	97
	9	Octane, 2,6-dimethyl	6.11	94
	10	Decane	7.38	97
	11	Heptadecane	28.77	99
	12	alpha.-Ionone	22.78	97
	13	2,4-Di-tert-butylphenol	25.51	95
Dichloromethane	14	5-Bromo-1H-indole	28.01	90
	15	Heptafluorobutyric acid, pentadecyl ester	28.62	93
	16	Tridecanoic acid, 12-methyl-, methyl ester	29.16	97
	17	2-Pentadecanone, 6,10,14-trimethyl	31.06	93
	18	7,9-Ditert-butyl-1 oxaspiro (4,5) deca-6,9-diene-2,8-dion	32.64	99
	19	Phytol	37.94	91
	20	Hexadecanamide	40.58	94
	21	2-Furanmethanol	5.05	87
	22	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl	10.82	64
Methanol				
	23	Benzeneacetic acid	15.33	94
	24	2(4H)Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	25.66	95
	25	Dodecanoic acid	26.66	94
	26	Thiosulfuric acid, S-(2-aminoethyl) ester	29.73	93
	27	2methoxycarbonylbenzothiazole	30.59	90
	28	9-Hexadecenoic acid	33.17	98
	29	Octadecanoic acid	40.11	91

obtained from the standard plot of ascorbic acid: $y = 0.9819x - 0.153$, $R^2 = 0.9927$ from the standard curve for methanolic extract and.

$y = 0.715x - 0.1855$, $R^2 = 0.9978$ from the standard ascorbic acid curve for Ethanolic extract.

Tabel 8. Inhibition Zone of the organic extracts against bacteria (mm in diameter)

pathogen extract	Hexane	Petroleum ether	Dichromethan	Methanol
K. pneumoniae	13.5	16.5	17	15
S. aureus	20	19	15	20
P. aeruginosa	23.5	22.5	20	15.5
E. coli	17	13	16	10
P. vulgaris	17	17	17.5	20
S. typhimurium	25	20	22	20

Table 9. Inhibition Zone of standard antibiotics against bacteria (mm in diameter)

pathogen S. A.B	Ciprofloxacin	Trimethoprim	Imipenem	Gentamicin	Meropenem	Vancomycin	Streptomycin	Meticillin
K. pneumoniae	28	-	24	*	*	*	*	*
S. aureus	*	*	*	22	-	20	15	-
P. aeruginosa	*	*	16	10	11	*	*	*
E. coli	29	25	*	*	*	-	*	*
P. vulgaris	21	15	16	*	*	*	*	*
S. typhimurium	32	*	25	18	33	*	*	*

Table 10. Concentrations for determination the (MIC) and (MBC) of bacteria

Number of the test tube	1	2	3	4	5	6	7	8	9	10
Cµg/mL	350	175	87.5	43.75	21.87	10.94	5.47	2.73	1.37	0.68

Table 11. Determination the (MIC) and (MBC) of bacterias

pathogen	) µg/mL(MIC	)µg/mL(MBC
K. pneumoniae	175	350
S. aureus	175	350
P. aeruginosa	87.5	350
E. coli	175	350
P. vulgaris	175	350
S. typhimurium	350	350

Tabel 12. Inhibition Zone of the organic extracts against fungi (mm in diameter)

pathogen extract	Hexane	P.ether	Dichromethan	Methanol	Ethanol 70%
C. albicans	19	19.5	16.5	17	20
T. rubrum	15.5	18	15	18	14
M. gypseum	23	24	21	21	21

Chemical composition of extracts by GC-MS analysis

The major chemical compound from GC-MS analysis of curd extracts (hexane, petroleum ether, dichloromethane and methanol) were identified in Figure1- 4 above and Table 7 above these compounds mainly comprised of hydrocarbons, esters, alcohols and ketones.

Antimicrobial activity of seaweed's extracts

Table 8 and 9 show the result of the in vitro testing of extracts against pathogenic bacteria which is comparable to the standard antibiotics. The (MIC) and (MBC) of bacterias and the used concentration show in table 10 and 11. In the present study the highest inhibition for bacteria was showed in hexane extract. *Pseudomonas* showed the highest sensitivity against

Table 13. Inhibition Zone of standard antibiotics against fungi (mm in diameter)

pathogen	S. A.B	Miconazole	Metronidazole	Canazole
C. albicans		25	26.5	25
T. rubrum		29.5	33	29
M. gypseum		30	30	34.5

Table 14. Concentrations for determination the (MIC) and (MFC) of fungi

Number of the test tube	1	2	3	4	5	6	7	8	9	10
C (µg/mL)	500	250	125	62.5	31.25	15.62	7.81	3.90	1.95	0.97

Table 15. Determination the (MIC) and (MFC) of fungi

Pathogen	µg/mL(MIC)	µg/mL(MFC)
C. albicans	125	500
T. rubrum	125	125
M. gypseum	125	125

methanolic extract (MIC = 87.5µg/mL) and Salmonella showed the highest resistance against methanol extract (MIC = 350µg/mL). Minimum Bactericidal Concentration (MBC) for all of the pathogenic bacteria was equal (MBC = 350µg/mL). The organic extract from *H. boergesenii* exhibited strong activity against each of the bacteria and fungi tested. Table 12 and 13 show result of the in vitro testing of extracts against pathogenic fungi. And all of the organic extracts show strong antifungal activity that is comparable to the standard antibiotics. Table 14 and 15 showed the concentrations that were used for Determination the (MIC) and (MFC) of the fungi. All of the tested fungi showed the same Minimum Inhibitory Concentration (MIC = 125µg/mL). Minimum fungicidal concentration (MFC) for the *T. rubrum* and *M. gypseum* was (125µg/mL) and the (MFC) for *C. albicans* was (500 µg/mL).

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