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In vitro screening of secondary metabolites and evaluationof antioxidant, antimicrobial and cytotoxic properties of *Gelidium sesquipedale* Thuret et Bornet red seaweed from Algeria

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Abstract
In vitro antioxidant, antimicrobial and cytotoxic activities of hydromethanolic extract of red seaweed *Gelidium sesquipedale*Thuret et Bornet and its fraction were investigated. Phytochemical screening, total phenolic and flavonoid contents were also investigated. For phytochemical screening, some common and available standard tests were done. Phytochemical screening revealed the presence of alkaloids, anthocyane, saponins, flavonoids, tannins and *C*-heterosids. The maximum total phenol and flavonoid content was observed in the diethyl ether fraction (101 GAE/g) and in the ether acetate (5.63 QE/g) respectively. The high DPPH radical scavenging was observed in chloroform fraction. The diethyl ether and n-butanol fraction showed good reducing power. The extracts exhibited high antioxidant activity by β-Carotene/linoleic acid bleaching assay during the incubation time. Antimicrobial activity was examined against eight bacteria and one yeasts. Only one bacterial strain (*Enterobacter cloacae*) was not inhibited by seaweed extracts, and chloroform fraction was generally more active than others. Hydromethanolic extract was subjected to brine shrimp lethality bioassay for possible cytotoxicity. Concentration dependent increment of brine shrimp nauplii mortality caused by the extract was indicative of the presence of cytotoxic constituents in this extract.

Keywords: Gelidium sesquipedale, Antioxidant activity, Antimicrobial activity, cytotoxic activity, phytochemical screening.

1. Introduction:
Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents [1-3].The number of new compounds isolated from marine sources has been increasing steadily [4]. Among marine organisms, marine algae are still identified as under-exploited plant resources although they have long been used in food diets as well as traditional remedies in Eastern hemisphere [5]. The term marine algae, as used herein, generally refer to marine macroalgae or seaweeds. The ability of seaweeds to produce secondary metabolites of antimicrobial value, such volatile components as phenols, terpenes [6], steroids [7], phlorotannins [8], lipids [9] and anti-inflammatory value such as retinol which inhibited the phospholipase A2 [10] has already been studied.
The genus *Gelidium* is an excellent sources of Agar [11, 12, 13] .But, also contain many components of therapeutic value [14,15]. *Gelidium* now very popular in developing countries on account of improved knowledge on secondary metabolites (phytochemical), and it has been investigated as a source of medicinal agents.
The *Gelidium sesquipedale* is the main red algae harvested in the word because it is known for being a good raw material for industrial processing and production of agar-agar. The agar-agar produced is used as a food additive

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which plays now an important role in the food industry [16, 17]. It is considered as a good neutral gelling, does not precipitate with proteins, and can manufacture products of high resistance to lactic acid bacteria, etc [18, 19]. It is also used in cosmetics, pharmaceutical products, and in microbiology as nutritional substrates [20, 21].
Until now, however, no screening for antioxidant, antimicrobials activities and Cytotoxic Potentials has been done in Algeria corniche algae, even though the abundance and diversity of algae in the coastal waters of the Algeria corniche are very high [22].
The objective of the present work was to investigate and evaluate the antioxidant, antimicrobial, and cytotoxic activities of the methanol extract and fractions of *Gelidiumsesquipedale*. In addition, we also assessed the total phenolic and flavonoid contents.

2. Material and Methods

2.1. Collection and Extraction of G. sesquipedale Bioactives:

Seaweed specimen was collected from the intertidal habitat of Mediterranean (36°34'N and 1°52'E) area located in middle coast of Algeria (Sidi Brahem, Tipaza). The collection was performed during December 2010 to April 2011 when red algal diversity remains dominant. Living and healthy plants were harvested manually and washed thoroughly in running water to remove epizoones, epiphytes, animal castings, sand, calcareous and other adhering detritus matters. Cleaned plant materials were shade dried under a stream of air flow for two weeks to prevent photolysis and thermal degradation. The completely dried material was weighed and ground coarsely in a mechanical grinder.
The extraction was prepared by pouring 100 ml methanol and distilled water (70%-30%) into the bottle containing 20 g of seaweed powder at room temperature for 48 h under dark condition. The solvent was then removed by filtration and fresh solvent was then added to the residue. This procedure was repeated third. Three extracts of the sample were pooled together, filteredthen evaporated under reduced pressure using rotary flash evaporator. In second extraction, the crude extract was weighed and then dissolved in hot distillated water (100 mL) at room temperature for 12 hours. The aqueous extract was defatted using hexane (50 mL, three times) and further fractionated into different solvent fractions (chloroform, diethyl ether, ethyl acetate and n-butanol (50 mL, three times)). These extracts were dried over anhydrous sodium sulfate, filtered, concentrated under vacuum rotary evaporator and dissolved in methanol. The yields of these fractions were 88 mg, 457 mg, 420 mg and 180 mg respectively. All extracts obtained were stored in colored vials andkept in the dark at +4°C for further analysis.

2.2. Phytochemical screening

The first step of our study was the identification of the various bioactive compounds constituents present in the powder, hydromethanolic extract and aqueous extract of this seaweed such as anthraquinones, triterpenes, saponins, flavonoids, tannins, *O*-heterosids, *C*-heterosids, alkaloids, coumarins by preliminary phytochemical screening according to standard phytochemical methods as described by Lespagnol [23]; Harlay et al. [24] and Paris & Moyse [25].

2.3. *Total Phenolic contents*

The total phenolic content (TPC) was determined using the Folin–Ciocalteu assay by spectrophotometry [26]. Briefly, 25 mL of extract was mixed with 3.75 mL of distilled water, added to 0.25 ml of Folin–Ciocalteu phenol reagent, allowed to react for 3 min. Then, added 0.75ml of 20 % sodium carbonate (w/v), incubated for 40 min at 40°C prior to measuring the absorbance at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard Gallic acid graph:
Absorbance = 0.1035 Gallic acid (µg/ml) + 0.1046 (R^2:0.98)

2.4. Total Flavonoid contents

The total flavonoid contents in the extracts were determined by a colorimetric method described by Lamairson and Carnet [27]. 1.5 ml of 2% $AlCl_3.6H_2O$ dissolved in methanol was added to equal volumes of the diluted

extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:
Absorbance = 0.2829 quercitin (μg/ml) – 0.1155 (R^2:0.99)

2.5. *Antioxidant Activity*
2.5.1. DPPH Radical Scavenging Activity Assay
The radical scavenging activity of the extracts and fractions was assessed using DPPH method of Braca et al. [28] Briefly. 1.5 mL of appropriately diluted samples ((5 μg/ml to 1000 μg/ml)) were added to 1.5 mL of DPPH methanol solution (0.004%) freshly prepared . The mixture was allowed to react at room temperature in the dark for 30 min before the absorbance was measured at 517 nm against a methanol blank using the same method as described above. Ascorbic acid, α-tocopherol and BHT were taken as standards.
The percentage (%) inhibition of the DPPH radical was calculated by using the following equation:
$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$
where Ao is the absorbance of the control and A1 is the absorbance of the samples at different concentrations. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration. Studies were conducted in triplicate.

2.5.2. *Reducing Power Assay*
Reducing power of extract obtained red seaweed was determined by the method prescribed by Oyaizu [29]. Briefly, 1.0 ml of distilled water containing different concentration of sample was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50 °C for 20 min. After incubation, 2.5 ml of trichloroacetic acid(10%) was added and centrifuged for 10 min at 3000 rpm. Fromthe upper layer, 2.5 ml solution was mixed with 2.5 ml distiller water and 0.5 ml FeCl3 (0.1%). Absorbance of the reaction mixtures was measured at 700 nm.
Ascorbic acid, α-tocopherol and BHT were used as standards. EC_{50} value (μg ml^{-1}) is the effective concentration at which the absorbance was 0.5 for reducing power. Increased absorbance is indicated increased reducing power.

2.5.3. *β-Carotene/Linoleic Acid Bleaching Assay*
The antioxidant activity of extracts was evaluated by the β-carotene– linoleate model system as described in [30]. Firstly, β-carotene (2 mg) was dissolved in 10 ml of chloroform (HPLC grade). After, 20mg of linoleic acid plus 200 mg of Tween 40 was added at 1 ml of solution. The chloroform was completely removed using a vacuum evaporator. After evaporation chloroform, 50 ml of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. Aliquots of 4.8 ml of this emulsion were transferred into test tubes containing 0.2 ml of extract (2 mg/ml). The tubes were shaken and incubated at 50°C in a water bath for 120min.
As soon as the emulsion was added to each tube, the zero time absorbance (A_0) was measured at 470 nm using a spectrophotometer. An others absorbencies were measured every 30 min for 120 min. A blank, without β-carotene was prepared in a similar way
Ascorbic acid, α-tocopherol and BHT were used as standards. The bleaching rate (R) of β-carotene was calculated according to first-order kinetics, as described in Al-Saikhan et al. [31]:
$R = \text{In } (A_{t=0}/A_{t=t})/t$
Where, ln = natural log, t is the time in minutes, $A_{t=0}$ is the initial absorbance of the emulsion immediately after sample preparation (t = 0 min) and $A_{t=t}$ is the absorbance at time t (30, 60, 90, and 120 min). The percent of antioxidant activity (AA) was calculated using the equation:
$AA = (R_{control} - R_{sample}) / R_{control} \times 100$
Where, $R_{control}$ and R_{sample} are average bleaching rates of the negative control and the antioxidant (plant extract, ascorbic acid or α-tocopherol or BHT), respectively.

All tests were carried out in triplicate.

2.6. *Antimicrobial Activity*
2.6.1. *Microbial Strains*
The antimicrobial activities of the algal extracts were tested using pathogenic microbes including three gram positive bacteria (*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (CIP 7625), *Listeria monocytogenes* (CIP 82110)), five gram negative bacteria (*Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (CIP A22), *Enterobacter cloacae* (E13),*Salmonella enterica* (CIP 81.3), *Klebsiella pneumonia*(CIP 82.91)); and one yeast (*Candida albicans* (IPA 200)). All microorganisms were obtained from The Microbiological laboratory, Department of Biology, ENS, Algiers, Algeria. Bacterial strains were cultured in Muller–Hinton agar (Institut Pasteur, Algeria) and yeasts were cultured in Sabouraud dextrose agar (Institut Pasteur, Algeria). All microbial strains were incubated for 24 h at 37°C.

2.6.2. Disc Diffusion Assay
Microbial inoculums were prepared form fresh culture strain and suspended in sterile saline solution (0.9% NaCl). The density of cell was adjusted to 0.5McFarland.Antimicrobial activity was evaluated using the disk diffusion method. The Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar or Sabouraud dextrose agar and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. 10µl of extracts solutions (50 mg/ml) were loaded on the sterile discs (5.5 mm of paper) which were placed on the surface of the solidified agar medium.
Before incubation, all Petri dishes were stored in the dark at +4°C for 1 hour, to allow the diffusion of the extracts from disc to medium without microbial growth. Positive control was prepared using the Levofloxacin (10 µg/disc) for bacteria and nystatin (10 µg/disc) for yeast. The plates were incubated for 24 h at 37°C, the zones of inhibition were recorded in millimeters (diameter of the disc included). The experiment was repeated thrice for concordant results.

2.6.3. *Agar Dilution Method*
The minimum inhibitory concentration (MIC) of marine algae extracts was carried out by the agar dilution method [32]. Appropriate amounts of the extract were added aseptically to sterile medium to produce the concentration range of 25–0.097 mg extract/ml medium. The resulting agar solutions were immediately mixed and poured into Petri plates. The plates were spot inoculated with 1 µl of microorganism. At the end of incubation period, the plates were evaluated for the presence or absence of growth. The MIC was defined as the lowest concentration of the extract needed to inhibit the growth of microorganisms.

2.7. Cytotoxic activity
Brine shrimp cytotoxicity assay was performed according to the standard procedure described by Turker & Camper [33]. 1 g of *Artemia salina* (Linnaeus) cysts (obtained from CNRDPA, Algeria) was aerated in 1 L capacityglass cylinder (jar) containing seawater prepared by dissolving 36 g of sea salt in 1 l of distilled water. The airstone was placed in the bottom of the jar to ensurecomplete hydration of the cysts. After 10-12 hoursincubation at room temperature (27-29°C), newly hatched free-swimming pink-colored nauplii were harvested from the bottom outlet. Two days was allowed for the shrimp to mature as nauplii (shrimp can be used 48-/72h after the initiation of hatching). Since the nauplii are positively phototropic (attracted to light), Illumination was provided on one side to attract newly hatched larvae
The assay system was prepared with 2.5 ml of seawater prepared containing respective concentration of marine extracts algae (10 000, 1000, 100, 10, 1µg/mL). In each, 10 nauplii were transferred and the setup was allowed to remain for 24 h, under constant illumination. After 24 h, the dead nauplii were counted with a hand lens. Based on the percent mortality, the median lethal concentration, LD_{50}value of the plant extract was determined. Three replicates were prepared for each concentration. The same saline solution used to prepare the stock test sample solution was used as a negative control.

2.8. *Statistical Analysis*
The results were expressed as Mean± SD. Statistical analysis was carried out by Analysis of Variance (one way ANOVA) test completed by a Student's test. Differences were considered significant at $p<0.001$. The correlations between methods were determined using analysis of variance (ANOVA) and quantified in terms of the correlation factor. LD_{50} value was obtained by a plot of percentage of dead shrimps against the logarithm of the sample concentration. All statistics analyses were carried out using STATISTICA 6 for Windows. All experiments were carried out in triplicate

3. Results and discussion

3.1. *Preliminary phytochemical screening*
The important phytochemical alkaloids, flavonoids, tannins, anthocyane, saponins, *C*-heterosids, and sugars were screened for their presence and presented in Table-1.

Table 1: Phytochemicals detected in *G.sesquipetale*

Phytochemicals	Results
Anthraquinones	-
Alkaloids	+
Anthocyane	+
Saponins	+
Coumarins	-
Flavonoids	+
Tannins	+
O-heterosids	-
C- heterosids	+
Mucilage	-

Key: += present, - = absent

3.2. *Amount of Total Phenolic contents*
Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially their polyphenols have antioxidant activity [34-36]. Therefore, it is worthwhile to determine their total amount in tested extracts.
Based on the absorbance values of the various extract solutions reacted with Folin-Ciocalteu's reagent and compared with the standard solutions of gallic acid equivalents as described above, the total phenolic in the crude extract and their derived fractions of *G. sesquipedale* were determined (Table 2).

Table 2: Total phenolic,flavonoid contents (mean ± SD) of extracts from *G.sesquipedale*.

Extracts	Total phenolic contents[a,b]	Total flavonoid contents[a,c]
Hydromethanolic crude	3.49±0.51	0.85±0.005
Chloroform	28.47±0.82	4.1±0.28
Diethyl ether	101.05±1.30	2.87±0.21
Ethyl acetate	10.34±1.54	5.63±0.32
n-butanol	35.68±0.53	4.37±0.04

[a]Each value is presented as mean ± SD (n = 3)
[b]Total phenolic content was expressed as mg gallic acid equivalents/g dried extract
[c]Total flavonoid content was expressed as mg quercitin equivalents/g dried extract

Results showed that phenolic contents varied significantly as function of solvent nature ($P < 0.001$).As can be seen in table 2, the distribution of phenolic compounds in *G. sesquipedale* demonstrated that the diethyl ether

fraction contained the highest amount 101.05 GAE/g, followed by n-butanol fraction (35.68 GAE/g) and chloroform (28.47 GAE/g). However, the ethyl acetate fraction and the hydromethanolic crude showed weaker polyphenol content (10.34 and 3.49 mg GAE/g respectively), among the solvents used.

3.3. *Amount of Total Flavonoid contents*

Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities [37]. As shown in table 2, the flavonoid content was high in diethyl ether and n-butanol with 5.63 mg QE/g and 4.37 mg QE/g, respectively. The content in hydromethanolic crude was lower one with 0.85 mg QE/g.

Jimenez [38] reported that the decrease of phenolic compounds due to drying and storage was different according to the variety of algae. Earlier, Connan [39] reported that the great variability observed in the phenolic contents in the algae may originate from external environmental factors such as herbivory, light, depth, salinity, nutrients and seasonality as well as from intrinsic factors such as age, length and type of tissues. All these factors could act on the spatio-temporal regulation of phenolic metabolic expressions, inducing marked qualitative and quantitative variations among individuals at very small scale, together with intra-individual variations [40, 41].

3.4. *Antioxidant Activity*

3.4.1. *DPPH Assay (Radical Scavenging Activity)*

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When DPPH is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained, with the simultaneous change in the color of the solution from violet to pale yellow [39]. Hence, DPPH has been used extensively as a free radical to evaluate reducing substances with maximum absorption at 517 nm [42] and is a useful reagent for investigating the free radical-scavenging activities of compounds [43].

The values of percent DPPH scavenging of *G. sesquipedale* hydromethanolic crude extract and four fractions were summarized in figure1. These values were compared with those of the well-known antioxidants such ascorbic acid, α-tocopherol, and BHT.

As can be seen in figure 1, the five extracts of *G. sesquipedale* exhibited a concentration-dependent DPPH radical scavenging activity, which the highest values were observed in the chloroform fraction (61.25%), whilst the other samples, including the hydromethanolic crude, diethyl ether, ethyl acetate and n-butanol fractions showed lower scavenging activity toward DPPH (59.31%, 34.41%, 53.17%, and 51.37%, respectively) at 8 mg/ml.

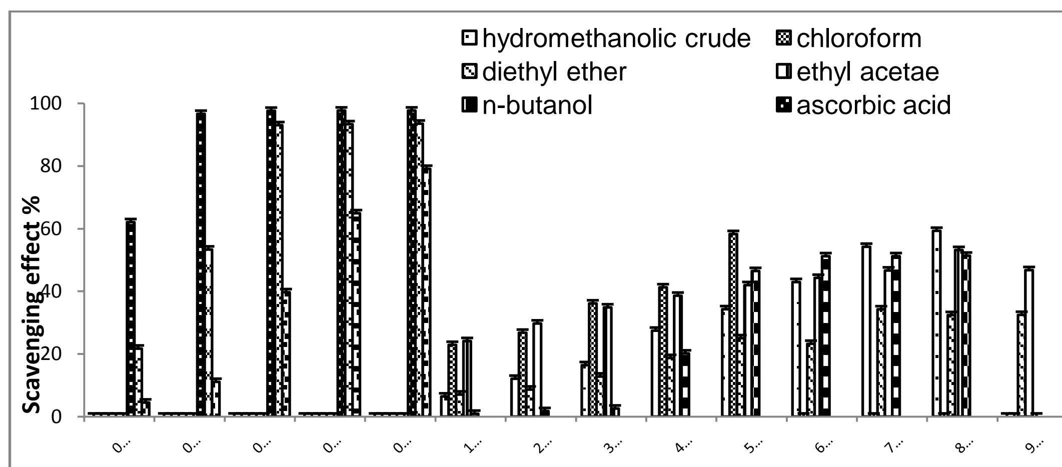

Figure 1: The DPPH radical scavenging activities of ascorbic acid, α-tocopherol, BHT and extracts of *G.sesquipedale*. Each value is expressed as mean ± SD (n = 3).

A lower value of IC_{50} indicates a higher antioxidant activity. As shown in table 3, the highest activity was observed in the chloroform fraction while, hydromethanolic crude and two fraction also showed inhibitory effects in following in order: n-butanol < hydromethanolic crude <ethyl acetate. In addition, diethyl ether fraction showed lower scavenging activity. At a concentration of 9 mg/ml, the value of scavenging activity was under 35 %. For this cause, the value of IC_{50} could not be detected.
When comparing IC_{50} values obtained for standards (BHT: 72.16 µg/ml, α-tocopherol: 9.55 µg/ml and ascorbic acid: 4µg/ml) and all extracts, it was found that these fractions showed a lowed antioxidant potential.
The observations of our study corroborates well with those reported by Ganesan [45] in case of a red seaweed species. Total methanol extract from *Euchemakappaphycus* showed significantly higher scavenging activity of 11.9% followed by *Acanthophoraspicifera* (6.91%) and *Gracilariaedulis* (5.20%). However the extracts of *G. sesquipedale* showed better radical scavenging activity than did the extract of *Palmariapalmata* (dulse) [46]. In other studies of three seaweeds [44, 46], the lowest activity was observed in water extracts while the highest one was recorded for methanol extracts. The maximum radical scavenging activity of methanol extract was found in *Enteromorphacompressa* followed by *Enteromorphatubulosa* and *Enteromorphalinza* (IC_{50} values 1.89±0.04 mg/ml, 2.91±0.05 mg/ml and 3.66±0.05 mg/ml respectively).

Table 3: Antioxidant activities of extracts from *G. sesquipedale* and standards measured by different assays

Plant Extracts	DPPH[a,b]	Reducing power[a,c]	β-Carotene / linoleic acid (%)[a]
hydromethanolic extract	6.44 ± 0.40	(ND)	51.54±0.35
chloroform fraction	4.51±0.005	(ND)	29.70±0.71
diethyl ether fraction	(ND)	2.04 ± 0	83.52±0.25
ethyl acetate fraction	7.52± 0.08	(ND)	87.02±0.02
n-butanol fraction	5.62± 0.32	5.33±0.39	54.82±0.45
ascorbic acid	0.004±0.1	0.047±0.28	09.76±0.02
α-tocopherol	0.009±0.07	0.507±4.16	94.52±0.09
BHT	0.072±0.1	0.633±11.5	96.51±0.51

[a]Each value is presented as mean ± SD (n = 3)
[b]IC_{50} in mg/ml
[c] Concentration at which the absorbance was 0.5 ($EC_{0.5}$)
ND activity no detected

3.4.2. *Reducing Power*

It has been reported that reducing power serves as a significant reflection of antioxidant activity. The presence of reductants in the antioxidant samples cause the reduction of the Fe3+/ferricyanide complex to the ferrous form. The reducing properties were generally associated with the presence of reductones, which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom. Most non-enzymatic antioxidative activity, such as scavenging of free radicals or inhibition of peroxidation, is mediated by redox reaction [48]
Figure 2 shows the reducing power of the various fractions and hydromethanolic crude isolated from *G. sesquipedale*. All of the extracts possessed the ability to reduce iron III and shows there exhibited a dose dependant reducing power at the concentration tested.
The highest amount of reducing power was observed in diethyl ether fraction 0.873 steady by n-butanol 0.548. The extract exhibited a reducing power from 0.14(ethyl acetate) to 0.873 (diethyl ether) at concentration of 6 mg/ml. According to table 3, the result of IC_{50} values the antioxidant assays indicate that diethyl ether and n-butanol fractions of *G.sesquipedale* acts as a best source of antioxidant compounds among the different solvent fraction investigated included hydromethanolic crude but showed significant difference with synthetic antioxidants BHT, α-tocopherol and ascorbic acid ($P< 0.05$). These results are in accordance with the previous published data reported in case of a red seaweed species [38, 45].

3.4.3. *β-Carotene-linoleic Acid Bleaching Assay*

The mechanism of bleaching of β-carotene assay is a free radical-mediated phenomenon resulting from the hydroperoxides formed from linolic acid by air oxidation. The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atom from diallylic methylene groups of linoleic acid, attacks the highly unsaturated of β-carotene molecules [49]. As β-carotene molecules lose their double bonds by oxidation in this model system, in the absence of an antioxidant, the compounds loses its chromophore and characteristic orange color which can be monitored spetrophotometrically [50].

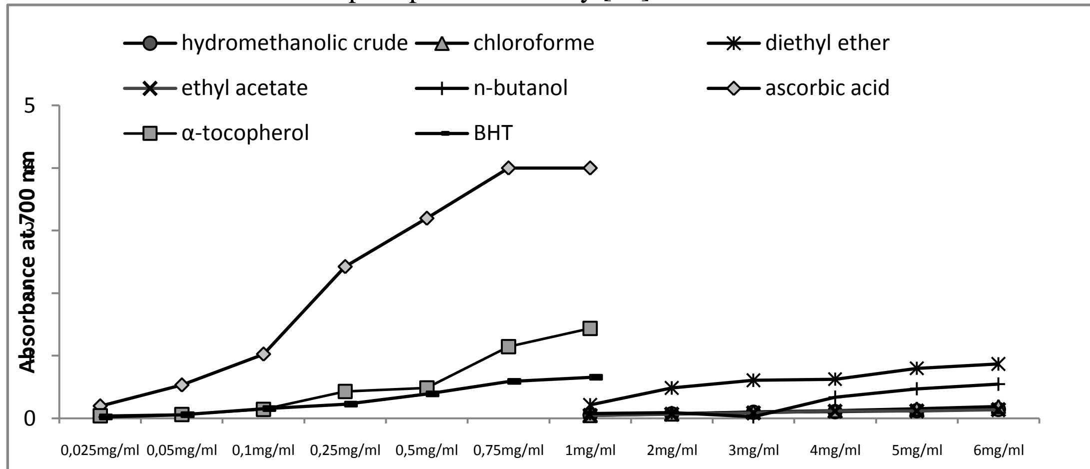

Figure 2: Reducing powers of various concentrations of hydromethanolic crude extract and its fractions from *G. sesquipedale*. Each value is expressed as mean ± SD (n = 3).

The antioxidant activities of hydromethanolic crude and four fractions derived from *G. sesquipedale* as well as the positive controls, BHT, ascorbic acid and α-tocophenol, as measured by the bleaching of β-carotene, are presented in figure 3. All of the extracts were able to reduce the rate of degradation of β-carotene by scavenging linoleate-derived free radicals.

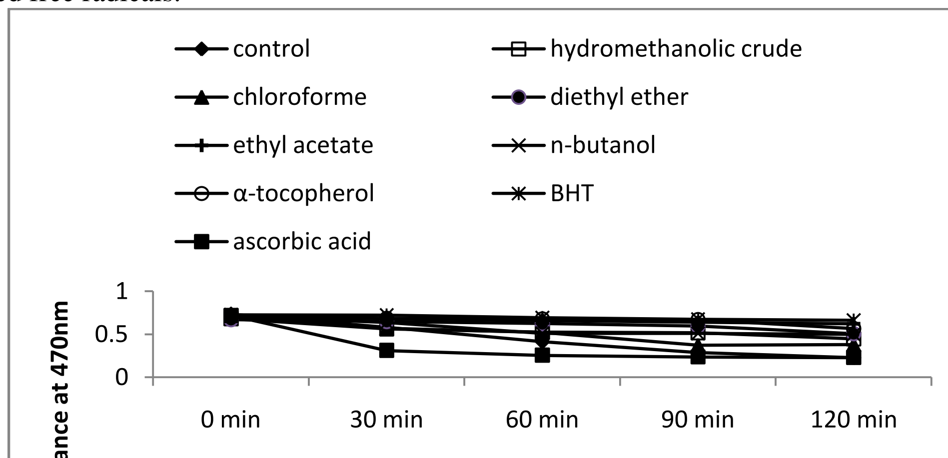

Figure 3: Inhibition of bleaching of β-carotene–linoleic acid emulsion by the extracts of *G. sesquipedale*. Each value is expressed as mean ± SD (n = 3).

The absorbance of the control at 470 nm decreased to a minimal value of 0.228 after 120 min, while those of the extracts were still between 0.381 and 0.621. As shown in table, amongst the five extracts, the ethyl acetate fraction presented the highest activity (87.02 %) steady by the diethyl ether (83.52%), which indicated that compounds with the strongest antioxidant activity in the β-carotene-linoleate assay system were also of medium polarity. Although, the percentage of inhibition of both fraction (ethyl acetate and diethyl ether) is high, but it presents a significant difference compared to the positive control (BHT and α-tocopherol) ($p<0.001$ and $p<0.05$ respectively).

Duan [43] found that mean antioxidant activity of n-butanol fraction and ethyl acetate fraction from a red alga, *Polysiphoniaurceolata* was 56.9 % and 85.6% respectively which according with our results for the same solvent of extraction. Wang [51] have also reported that ethyl acetate fraction of the marine red alga, *Rhodomelaconfervoides*show strong antioxidant activity (96 %). The finding, in the study of Zubia [52] of 10 Phaeophyta species from Brittany coasts four Fucales, *Bifurcaria bifurcata, Cystoseira tamariscifolia, Fucus ceranoides* and *Halidrys siliquosa,* displayed a high antioxidant activity by bleaching of β-carotene.

3.5. *Correlation between Antioxidant Capacity and Total Phenolic Contents*

The polyphenols of seaweeds such as phlorotannins [53] could assist the algae to overcome oxidative stress as well as play a putative adaptive role in defense against grazers, such as marine herbivores. Correlation between the content of phenolic compounds and antioxidant activity has been described [43, 51, and 54].In our study, a high correlation between the total phenolic content and DPPH radical scavenging (R2= 0.70) was found in different extracts from *G. sesquipedale*.According to Novaczek [55], a correlation was found between the total phenolic contents and IC_{50} of DPPH. When the total phenolic content was high, the IC_{50} was low what is observed in our study in regard to diethyl ether fraction with 101.05 mg GAE/g. this is due to the high amount of polyphenolic constituents present in the seaweed, which were capable of functioning as free radical scavengers. However, this assay was not specific to any particular antioxidants [56]. A positive correlation was also observed between the DPPH radical scavenging activity and β-carotene linoleate assay (R2=0.93). In addition, a weak correlation between the total phenolic contents and the antioxidant activity was also observed for the β-carotene-linoleic acid assay (R^2=0.36). This result is in accordance with the most studies in case of relation between total phenol content and β-carotene bleaching assay. [57-60].Although, a positive correlation has been reported [43, 61].Data analysis revealed that the absorbance of reducing power at 6mg/ml also showed a high correlation with total phenolic content (R2= 0.94).

3.6. *Antimicrobial Activity*

The inhibitory effects of crude hydromethanolic and fractions of *G. sesquipedale* on the growth of various gram positive and negative bacteria using agar diffusion method are shown in table 4. The chloroform fraction appeared to be the most active extract, as they displayed activity against three Gram positive, three Gram negative bacteria and yeast with MIC values ranging between 3.125 to 25 mg/ml. but the diethyl fraction showed the weak antibacterial activity against one bacteria only *Staphylococcus aureus* (zone inhibition = 9mm , MIC 6.25mg/ml). *Staphylococcus aureus* was found to be more sensitive (inhibition zone: 28 mm) among the Gram negative bacteria (*Enterobacter cloacae*) was found to be more resistant. Concerning gram positive bacteria *Salmonella enterica* and gram negative bacteria *Bacillus subtilis* were able to be inhibit by only hydromethanolic crude and chloroform fraction respectively. On the other hands, *Candida albicans* exhibited resistance to all extract except the chloroform fraction with 28 mm. The extracts showed a significant antibacterial activity against gram positive as well Gram negative bacteria that confirm previous findings [62-66]. Some species of the *Gelidium* genus have been subjected to antimicrobial activity evaluation [67-7

3.7. *Cytotoxic activity*

Methanolic extracts of *Gelidium sesquipedale* showed prominent result in brine shrimp cytotoxicity assay. The LD_{50} value was 2.22 µg/ml (Fig.4). In addition, the degree of lethality was found to be directly proportional to the concentration of the extract. The present study supports that brine shrimp bioassay as a reliable method for the assessment of bioactivity of seaweeds and lends support for their use in pharmacology.

Many studies of cytotoxic activity of other red algae is already reported. Zubia [52] reported that *A. armata* had strong cytotoxic activities against cancer cell lines, Daudi and Jurkat cells. Similarly, Manilal [73] reported the cytotoxicity of active fraction of *Laurencia brandenii* showed value of 93µg/ml for the LC_{50} from brine shrimp lethality.

Table 4: Antimicrobial activities of hydro-methanolic crude extract and its fractions from *G. sesquipedale* against tested microbial strains.

	hydro-methanolic crude extract		chloroform fraction		Diethyl ether fraction		ethyl acetate fraction		n-butanol fraction		Positive controls[C]	
Test microorganisms	DD[a]	MIC[b]	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-negative bacteria												
Pseudomonas aeruginosa	10.66±0.20	12.5	19.66±0.15	3.125	-	-	-	-	-	-	24.16±0.76	0.024
Escherichia coli	-	-	11.66±0.15	6.25	-	-	11.66±0.15	25	-	-	29±1.00	0.024
Klebseilla pneumonia	-	-	17.33±0.25	6.25	-	-	-	-	9.33±0.15	25	13.33±0.57	0.097
Salmonella enterica	7.33±0.10	50	-	-	-	-	-	-	-	-	19.33±0.57	0.048
Enterobacter cloacae	-	-	-	-	-	-	-	-	-	-	20±0.00	0.048
Gram-positive bacteria												
Bacillus subtilis	-	-	12.00±0.2	25	-	-	-	-	-	-	36±1.00	0.006
Staphylococcus aureus	28.00±0.2	6.25	9.00±0.1	6.25	11.66±0.26	6.25	-	-	6.00±0.1	25	32±1.00	0.012
Listeria monocytogenes	-	-	10.00±0.1	25			7.83±0.10	50	-	-	34.33±1.15	0.012
Yeasts												
Candida albicans	-	-	28±0.26	25	-	-	-	-	-	-	33	0.125

A dash (–) indicate no antimicrobial activity

a: Inhibition zone in diameter (mm) around the impregnated discs and each value is presented as mean ± SD (n = 3)

b: Minimal inhibition concentrations;values given as mg/ml

c: positive controls: levofloxacin for bacteria, nystatin for yeast

Many of the secondary metabolites produced by the marine red algae are well known for their cytotoxic property. As noted by Harada [74], the extract from a red alga, *Amphiroa zonata* exhibited strong cytotoxicity to human leukemic cell line. El-Baroty[75] demonstrated the cytotoxic activities of powdered *Asparaguses taxiformis* and its water extract on *Daphna magna*.

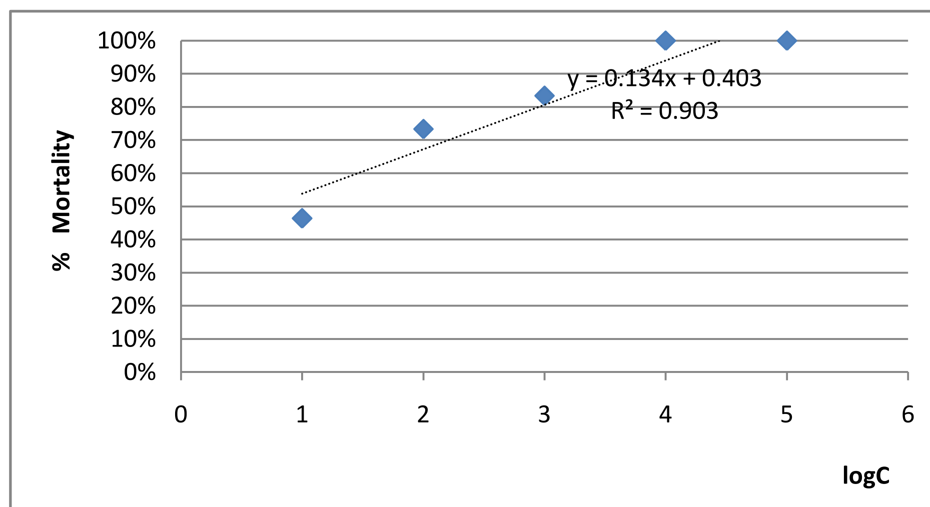

Figure 4: Determination of LC_{50} of hydromethanolic extractsof *Gelidium sesquipedale*against brine shrimp nauplii.

Conclusion

In conclusion, seaweeds or marine algae are a valuable source of natural antioxidant compounds as their crude extracts and fractions exhibit antioxidant activity. The results indicate also the potent antimicrobial and cytotoxic activities. Higher levels of total phenolics are probably responsible for its biological activities observed. These findings of this work are useful for further research to identify, isolate and characterize the specific compound which is responsible for these activities. Bioactive compounds found in seaweeds await a major breakthrough for a variety of food/medical application as they have the potential for application as natural antioxidants in different food/pharmaceutical products.

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