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Phytochemical Variation, Antimicrobial Activity and Genetic Diversity of Three *Ulva* Species Collected from Mediterranean Seashores of Alexandria, Egypt

Soad M. Mohy El-Din and Amani M.D. El Ahwany

Department of Botany and Microbiology, Faculty of Science, Alexandria University, Egypt.

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Due to wide range of secondary metabolites and their therapeutic properties, seaweeds have natural alternative to the chemical based compounds. This project focuses on phytochemical evaluation and genetic diversity within seaweeds collected from Mediterranean seashores of Alexandria. The seaweeds samples of class Ulvophyceae such as *U. fasciata, U. lactuca* and *U. intestinalis* were extracted using polar solvents such as Methanol, Ethanol, Acetone and Chloroform. Phytochemical screening showed the presence of alkaloids, triterpenoids, steroids, tannins, coumarins, sterpenoids, phytosteroids and flavonoids in marine algae under investigation. Saponins were absent in all *Ulva* sp. The common major compounds like protein, carbohydrate, lipid, phenol, flavonoids, tannins and photosynthetic pigments were extracted using polar solvent methanol. Of the four solvents tested, Methanol was the best solvent for isolation of antimicrobial compounds from the tested marine algae followed by Ethanol. The reducing power, percentage of hydrogen peroxide scavenging and total antioxidant capacity of the various extracts were determined. Among the various extracts methanolic extract was found to have the highest reducing power and total antioxidant capacity, while chloroform extract was found to have highest of hydrogen peroxide scavenging activity. The present study elaborates the bioactive content of seaweeds and demonstrates the effective application of RAPD technique for analyzing the genetic differentiation.

Key words: Algae, Phytochemical screening, Antibacterial activity, RAP.

Seaweeds (Marine algae) belong to a group of eukaryotic known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substance, which can benefit human health.[1] Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae[2, 3]. The environment in which seaweeds grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents but seaweeds seldom suffer any serious photodynamic damage during metabolism. This fact implies that seaweed cell have some protective mechanisms and compounds[4].

Marine algae are rich and varied source of bioactive natural products, so it has been studied as potential biocide and pharmaceutical agents[5]. There have been number of reports of antibacterial activity from marine plants and special attention

* To whom all correspondence should be addressed.
E-mail: dr.soad_mohi@hotmail.com

has been reported for antibacterial and antifungal activities related to marine algae against several pathogens[6]. The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvent for example acetone, methanol, toluene, ether and chloroform-methanol[7]. Using of organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity[8]. Seaweeds or marine macro algae are the renewable living resources, which are also used as food and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food but rich in vitamins, minerals and dietary fibers[9]. In addition to vitamins and minerals, seaweeds are also potentially good sources of proteins, polysaccharides and fibers[10]. The lipids, which are present in very small amounts, are unsaturated and afford protection against cardiovascular pathogens.

Phenolic compounds can act as antioxidants by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system. The term "phenolic compound" describes several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by, at least, one hydroxyl group[3]. Antimicrobial has increased in recent years in order to reduce the use of synthetic forms such as Butylated Hydroxyaniosle (BHA) and Butylated Hydroxytolune (BHT). Natural antioxidants form plant origin can react rapidly with these free radicals and retarder alleviate the extent of oxidative deterioration[11]. Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. The Random Amplified Polymorphic DNA(RAPD) markers are one of the tools to analyze genetic relationships and genetic diversity. The Major advantages of the RAPD technique are suitability for work on anonymous genomes, requirement of low DNA quantity, efficiency, ability to develop a large number of DNA markers in a short time and low expense. As a result, RAPD markers have been extremely useful to analyze population genetics, in addition to parentage analysis, species delimitation and germplasm identification in seaweeds[12,13,14,15]. Hence in the present study is concerned screening of phytochemicals, antioxidantsand antibacterial properties of green algae (*Ulva fasciata, Ulva lactuca* and *Ulva intestinalis*) and second aim to use RAPD technique to analyze seaweeds to determine their taxonomic identify and assess kinship relationships

MATERIALS AND METHODS

Collection and identification of seaweeds

The studied algal species collected from the coastal area of Abu-Ker Alexandria – north Egypt. Algal samples were cleaned of epiphytes and necrotic parts were removed. Then cleaned samples were rinsed with sterile water to remove any associated debris. The cleaned fresh materials wereair- dried as described by Gonzalez del Val [16]. The samples were identified as *Ulva fasciata* (Delile), *Ulva lactuca* (Linnaeus), and *Ulva intestinalis* (Linnaeus).

The samples were shade dried for 7 days and then pulverized into fine powder using pestle and mortar. The extraction was done by taken 10g of air-dried powder with 50ml of solvents viz., methanol, ethanol, acetone and chloroform. The sample was kept in dark for 72 hours with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts) and stored in refrigerator for further use. Each experiment was consists of five replicates.

Estimation of nutritional value of algal species

Protein estimation

The protein fraction (% of DW) was calculated from the elemental N determination using the nitrogen- protein conversion factor of 6.25 according to AOAC[17].

Carbohydrates estimation

The total carbohydrate was estimated by the following phenol-sulphuric acid method of Dubois[18], using glucose as standard.

Lipid estimation

Lipids were extracted with a chloroform methanol mixture (2:1 v/v). The lipids in chloroform were dried over anhydrous sodium sulphate, after which the solvent was removed by heating at 80°C until their vacuum AOAC[19].

Estimation of flavonoids

Total flavonoid content was determined according to the method of Chang [20]. One ml aliquot of each extract was mixed 0.1ml of 10%

aluminium chloride and 0.1ml of 1M potassium acetate. 2.8ml of methanol was added and kept at room temperature for 30 min. The absorbance of reaction mixture was measured at 415 nm. The content of flavonoid was expressed in mg/g. Standard Quercitin.

Estimation of tannins

Total tannin content was determined according to the method of Julkunen-Titto[21].

Briefly, a 50µl of seaweed extract was mixed with 1.5 ml of 40% vanillin (prepared with methanol) and then 750µl of HCl was added. The solution was shaken vigorously and left to stand at room temperature for 20 min in darkness. Absorbance against blank was read at 500nm. Catechin was used as standard.

Estimation of phenols

The total phenol was measured using the Folin-Ciocalteau method of Taga[22]. A 100µl of extract was mixed with 2 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then 100µl of 50% Folin-Ciocalteau phenol reagent was added. After incubation for 30 min in dark, the absorbance was read at 720nm. The total phenol of samples were expressed as mg Gallic acid per gram.

Estimation of chlorophyll

The amount of chlorophyll present in the algae was estimated by the method of Arnon[23]. Absorbance was measured at 645nm and 663nm.

Estimation of carotenoid

The amount of carotenoid was estimated by the method of KirK and Allen[24]. The same chlorophyll extract was measured at 480nm in spectrophotometer to estimate the carotenoid.

Hydrogen peroxide scavenging assay

The free radical scavenging activity of the extract was determined by Hydrogen peroxide assay according to Gulcin[25]. Hydrogen peroxide 10mM solution was prepared in phosphate buffer 0.1M, PH 7.4. One ml of the extract was rapidly mixed with 2ml of H_2O_2. The absorbance was measured at 230nm after 10min of incubation at 37°C against a blank without H_2O_2. The percentage of scavenging of H_2O_2 was calculated using the following formula:

Percentage of scavenging=((A0-Ai) ÷ A0)×100 Where A0 is absorbance of control and Ai is absorbance of sample.

Determination of total antioxidant capacity (TAC)

Total antioxidant activity of seaweed extract was determined according to the method of Mitsuda *et al*[26]. 7.45 ml of sulphuric acid 0.6M, 0.99g of sodium sulfate 28mM and 1.23 of ammonium molybdate 4mM were mixed together in 250ml with distilled water labeled as Total Antioxidant Capacity (TAC). 0.1ml seaweed extract was dissolved in 1ml of TAC and the absorbance was read 695nm after 15min. Ascorbic acid used as standard.

Estimation of reducing power

Reducing power of the extract was determined by following method of Yamaguchi[27]. 0.75ml of extract was mixed with 0.75ml phosphate buffer pH 6.6 and 0.75ml of 1% potassium ferricyanide was added. The mixture was incubated at 50°C for 20min. 0.75ml of 10% trichloroacetic acid was added and centrifuged at 3000rpm for 10min 1.5ml of the supernatant was mixed with 1.5ml of water and 0.1% ferric chloride. The absorbance was read at 700nm after 10min of incubation.

Preliminary phytochemical tests

Phytochemical analysis was performed according to the standard protocol described by Sadasivam and Manickam[28]. All the prepared seaweed extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, triterpenoids, steroids, tannins, saponins, coumarins, terpenoids, quinines, phytosteroids, flavonoids and glycosides.

Effect of Algal extracts on *Vibrio fluvialis*

Determination of antibacterial effect

The used microorganisms for the antimicrobial activity assay was *Vibrio fluvial*is obtained from National Institute of Oceanography and fisheries, Alexandria.

These were all cultured in an LB broth at 37°C and maintained on LB agar (Luria Bertani; in g/l:Tryptone, 5; Yeast extract, 5; NaCl, 10; sterilized by autoclaving for 25 min at 121°C). The pH was adjusted to 7 with 1 N NaOH or 1 N HCl prior to sterilization. After sterilization media were supplemented with 175mg/mL mycostatin to inhibit fungal growth. All cultures were supplemented with 15% glycerol (w/v) and stored frozen at 4°C. The assay was performed as previously described by Senthil and Kamaraj[29]. Each pathogen was grown on its medium of isolation and incubated at 30° C

until visible growth. Bacterial suspension of each indicator pathogen was then plated with the LB media in the agar plates. A well was cut in the middle of the plate and 0.5mL of extract was added in it. The plate was incubated at 30 ° C for 24h. Inhibition zones were scored as antibacterial activity measured in cm.

Random Amplified Polymorphic DNA (RAPD) technique

DNA extraction Procedure for total genomic of three green algae species according to manufacturer protocol of Omega Co. (USA.LMt.), according to Baghel[30]

Preparation of the PCR master mixture

Illustrate Ready-To-Go RAPD analysis kit (GE Healthcare Life Science) was applied to perform RAPD molecular fingerprinting technique according to manufacturer protocol (Welsh[31]). Preparation of the amplification reaction was done under the biosafety cabinet in a separate room rather than that in which the amplification and the extraction were done.

Random Amplified Polymorphic DNA (RAPD) primers under study

Primers	Sequence
RAPD analysis primer 1	(5'-d[GGTGCGGGAA]-3')
RAPD analysis primer 2	(5'-d[GTTTCGCTCC]-3')
RAPD analysis primer 3	(5'-d[GTAGACCCGT]-3')
RAPD analysis primer 4	(5'-d[AAGAGCCCGT]-3')
RAPD analysis primer 5	(5'-d[AACGCGCAAC]-3')

RAPD-PCR amplification

Total genomic DNA was amplified through GeneAmp Polymerase Chain Reaction (PCR) system cycler. PCR for amplified genomic DNA was carried out. Initial step was performed for 5 min at 95°C. The reaction consists of 45 cycles; each cycle consisted of denaturation at 95°C for 1 min followed by annealing at 36°C for 1 sec and extension at 72°C for 2 min. There was an initial delay for 15 min at 95°C at the beginning of the first cycle and 10 min delay at 72°C at the end of the last cycle as a post extension step (Lopera-Barrero[32] .The product was stored at –20°C or 4°C.

RESULTS AND DISCUSSION

Phytochemical screening of marine Collected Algae

The quantitative phytochemical screening of different chemical compounds was tested in four different extracts of crude powder of *Ulva* species carried out in order to assess the presence of bioactive compounds, which might have antibacterial potency. The results were illustrated in Table 1. The presence of alkaloids, terpenoids, triterpenoids, steroids, Phytosteroids, tannins, flavonoids, quinines, coumarins and glycosides in all extracts except quinines and glycosides are absent in acetone and chloroform. Also saponins absent in all the extracts. The seaweeds known as medicinal are rich in secondary metabolites, which includes alkaloids, glycosides, flavonoids, tannins, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry[33]. Presence of flavonoids and alkaloids in most tested algae is interesting because of their possible use as natural additive emerged from a growing tendency to replace synthetic antioxidant and antimicrobials with natural ones[34]. Our results were in agreement with previous findings, which showed presence of alkaloids, flavonoids and steroids in most algae[35,36].

Nutritional value of collected marine algae

Also in the present study, comparative nutritive value screening was carried out on investigated marine algae. Results depicted in the Table (2), the total summation of the recorded total protein increase in the order: *U.fasciata*> *U.intestinalis*> *U.lactuca*, with percentage; 24.40, 23.17, and 15.65 % respectively. The total protein content is as higher as compared to all other substances like, carbohydrates, lipids and phenols. Hence, the species can serve as functional food with vital nutritional and biological values. Similar observation was made in the present studies. Mohamed[37]observed that *Kappahycusalvarezii* is rich in protein (16.24%). Dhamotharan[38], investigated the level of protein content is high in *Stoehosperummargintum*(16.6%) as compared to *Padinatetrstromatica*(9.95%).The variations for proteins are attributed due to seasonal and environmental variations, which are transitory.The total summation of the recorded total carbohydrate increase in the order: *U.lactuca*> *U.intestinalis*> *U.fasciata*, with percentage; 29.60, 18.65 and 17.42% respectively. Similar observation was made in the present studies[36]. The total summation of the recorded total lipids increase in the order: *U.lactuca*>*U.intestinalis*> *U.fasciata*, with percentage; 0.82, 0.54 and 0.53% respectively.

Table 1. Preliminary phytochemical screening of the green seaweed extracts

Solvent	Seaweed	Alkaloids	Triterpenoids	Steroids	Tannins	Saponins	Coumarins	Terpenoids	Quinines	Phytosteroids	Glycosides	Flavinoids
Methanol	*U.fasciata*	+	+	+	+	-	+	+	+	+	+	+
	U.lactuca	+	+	+	+	-	+	+	+	+	+	+
	U.intestinalis	+	+	+	+	-	+	+	+	+	+	+
Ethanol	*U.fasciata*	+	+	+	+	-	+	+	+	+	+	+
	U.lactuca	+	+	+	+	-	+	+	+	+	+	+
	U.intestinalis	+	+	+	+	-	+	+	+	+	+	+
Acetone	*U.fasciata*	+	+	+	+	-	+	+	-	+	-	+
	U.lactuca	+	+	+	+	-	+	+	-	+	-	+
	U.intestinalis	+	+	+	+	-	+	+	-	+	-	+
Chloroform	*U.fasciata*	+	+	+	+	-	+	+	-	+	-	+
	U.lactuca	+	+	+	+	-	+	+	-	+	-	+
	U.intestinalis	+	+	+	+	-	+	+	-	+	-	+

Table 2. Pigment, Biochemical composition, Phenol, Flavonoid and Tannin in selected seaweeds.

	Protein %	Carbohydrate%	Lipid%	Flavonoids mg/g	Tannins mg/g	Phenol mg/g	Chlorophyll a mg/g f.w	Chlorophyll b mg/g f.w	Total chlorophyll mg/g f.w	Carotenoids mg/g f.w.
U.fasciata	24.40	17.42	0.53	13.77	15.30	2.26	0.461	0.315	0.741	0.085
U. lactuca	15.65	29.60	0.82	11.42	17.02	5.03	0.457	0.393	0.849	0.111
U.intestinalis	23.17	18.65	0.54	2.70	5.23	12.45	0.296	0.438	0.738	0.081

The total flavonoids increase in the order: *U.fasciata> U. lactuca> U.intestinalis,* with amount; 13.77, 11.42 and 2.70 mg/g respectively. Flavonoids are important in plant defense mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet radiation[39]. The total summation of the recorded total tannins increase in the order: *U.lactuca>U. fasciata> U. intestinalis*, with amount; 17.02, 15.30 and 5.23mg/g respectively. Tannins are defined as naturally occurring plant polyphenolic compounds and are widespread among terrestrial and marine plants[40]. Tannin compounds, which have been found only in marine algae, which have been reported to possess strong antioxidant activity, which may be associated with their unique molecular skeleton[41,42]. Tannins has been also found to have antiviral, antibacterial, anti-inflammatory and antioxidant property for possible therapeutic applications[43,44]. The total summation of the recorded total phenols increase in the order: *U. intestinalis>U.lactuca> U. fasciata*, with amount; 12.45, 5.03 and 2.26mg/g respectively. The maximum chlorophyll "a" (0.461 mg/g fresh wt) was recorded in *U. fasciata* whereas chlorophyll "b"(0.438 mg/g fresh wt) was highest in *U.intestinslis.* While the highest values of total chlorophyll and carotenoids(0.849 and 0.111mg/g

Table 3. Reducing power assay, Percentage of hydrogen peroxide scavenging assay and Total antioxidant capacity of selected tested seaweed

Parameters	Solvent	*U.fasciata*	*U.lactuca*	*U.intestinalis*
Reducing power	Methanol	*4.335*	4.194	4.249
	Ethanol	4.456	3.987	4.127
	Acetone	2.233	2.140	3.138
	Chloroform	1.202	1.339	1.478
Percentage of H_2O_2scavenging	Methanol	29.753	36.537	38.536
	Ethanol	19.776	18.757	8.521
	Acetone	42.405	29.370	40.963
	Chloroform	51.696	37.033	47.102
Total antioxidantcapacity(TAC)	Methanol	1.550	1.788	1.545
	Ethanol	0.701	0.433	0.417
	Acetone	0.556	0.496	0.534
	Chloroform	0.986	1.416	1.335

Table 4. Antibacterial activity of three seaweed species

Algal Species	Methanol	Ethanol	Acetone	Chloroform
U. fasciata	0.2 cm	0.3 cm	NA	NA
U. lactuca	0.4cm	0.2cm	NA	NA
U. intestinalis	0.5cm	NA	NA	NA

NA-no activity

Table 5. Illustrate Random Amplified Polymorphic DNA (RAPD) technique for three samples under study

Primers	Total amplified bands	Polymorphic bands	Monomorphic bands	Polymorphism %
RAPD analysis primer 1	19	14	5	74
RAPD analysis primer 2	15	6	9	40
RAPD analysis primer 3	12	8	4	67
RAPD analysis primer 4	18	8	10	44
RAPD analysis primer 5	16	12	4	75

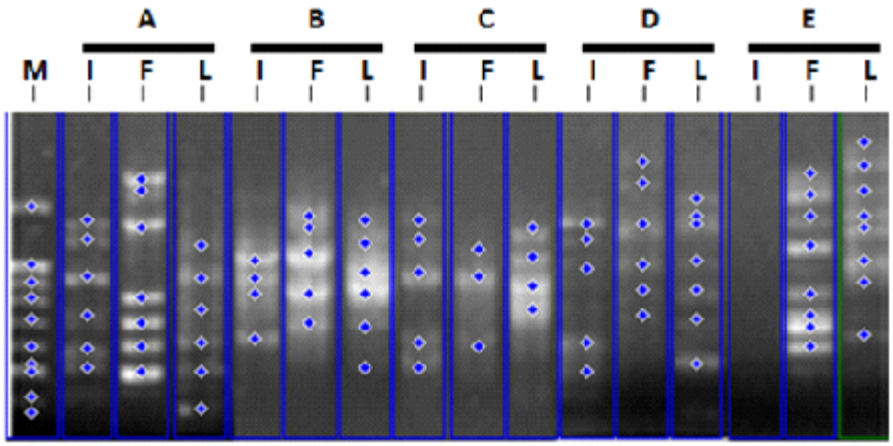

Fig. 1. Computerized band detection of Random Amplified Polymorphic DNA (RAPD) products for three green algae species. Five RAPD primers (A, B, C, D). M-DNA ladder, I-*U. intestinalis*, *F- U. fasciata and L- U. lactuca*

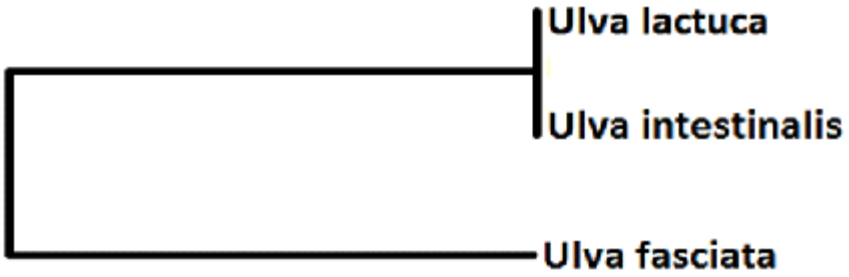

Fig. 2. Genetic similarity among three green algal species based on RAPD data

fresh wt) were recorded *in U. lactuca*. Carotenoids in seaweeds have beneficial effects in cancer chemo prevention by acting as either an antioxidant or pro-oxidant depending on the environment[45].

Antioxidant Activity of selected seaweeds

Reducing Power assay

In this, assay the yellow color of the test solution changes to shades of green and blue depending on the reducing power of each compound. The presence of reducer (antioxidants) causes the reduction of the Fe^{+3}/ ferric cyanide complex to the ferrous form. The reducing activity of various solvent extract of marine algae (*U.fasciata, U. lactuca and U. intestinalis*)was evaluated according to the method of Yamaguchi[27]. Results depicted in the Table (3). The highest amount of reducing power was observed in methanol extract of the tested algae, which recorded as follow: *U. fasciata*> *U. intestinalis*> *U. lactuca*, with value; 4.456, 4.249 and 4.194 respectively. Followed by ethanol extract, *U. fasciata*> *U. intestinalis*> *U. lactuca*, with value; 4.335, 4.127 and 3.987 respectively. The minimum reducing power exhibited by chloroform extract of all tested algae, which recorded in order: *U.intestinalis*> *U. lactuca*> *U. fasciata*, with the value 1.478, 1.339 and 1.20 respectively. Similar observation was made in the present studies[46,47].

Hydrogen peroxide scavenging assay

The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2. It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise of hydroxyl in the cells. The inhibitive effect of seaweed extract of selected algae subjected to hydrogen peroxide scavenging assay. The data was observed in Table (3). Among the four different extracts, the chloroform extract has showed the

greater ability to decrease the pro-oxidant H_2O_2 which recorded increase in order: *U. fasciata> U.intestinalis> U lactuca,* with percentage; 51.696, 47.102 and 37.033%, respectively, followed by acetone extract, which had recorded an increase in order: *U. fasciata> U. intestinalis> U. lactuca,* with percentage; 42.405, 40.963 and 29.370%, respectively. Which, followed by the methanol extract. The minimal activity was determined by ethanol extract elevated in the order: *U.fasciata>U. lactuca> U. intestinalis,* with percentage; 19.776, 18.757 and 8.521%, respectively. Similar observation was made in the present studies[46,47]

Total Antioxidant Capacity (TAC)

The reducing properties are generally associated with the presence of reductions. Reductions were reported to be terminators of free radical chain reactions by donating a hydrogen atom. In most cases, irrespective of the stage in the oxidative action is assessed, most non-enzymatic anti-oxidative activity mediated by redox reactions. In the present study the reducing power of the seaweed, extract of tested algae with different solvents. The strongest reducing power was concentrated in the methanol extract of the three *Ulva* species, which recorded as follows: *U.lactuca> U. fasciata> U. intestinalis,* with absorbance values; 1.788, 1.550 and 1.545, respectively, followed by chloroform extract, which recorded an increase in the order: *U. lactuca> U. intestinalis> U. fasciata,* with absorbance values; 1.416, 1.335 and 0.986 at the same concentration. The minimum reducing capacity was found in ethanol extract of *U. intestinalis*(0.417). Similarly, Viswanathan[3] was recorded. Literature reports indicated that the manifestation of total antioxidant capacity of sample is based on single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms.

Antibacterial activity

Challenge of crude algal extracts against *Vibrio fluvialis*

The antibacterial activity of *U. fasciata, U. lactuca and U. intestinalis* against *Vibrio fluvialis* presented in Table (4). The agar well diffusion method was used to evaluate the antibacterial activity by measuring the zone of inhibition against *V. fluvialis.* The methanol extract of all tested algae has exhibited the prominent antibacterial activity against *V. fauvialis.* The ethanol extract of *U.fasciata and U. lactuca* also resemble the same activity as methanol extract, except with *U. intestinalis* did not show any effect against *V. fluvialis*. Acetone and chloroform extracts of all three seaweeds were not effective against *V. fluvialis*. In the present study. *Ulva* species extracts reduced and inhibited the growth of *V.fluvialis*.

Emergence of microbial disease in aquaculture industries implies serious loses. Usage of commercial antibiotics for fish disease treatment produces undesirable side effects. Marine organisms are a rich source of structurally novel biologically active metabolites[48]. Cell extracts and active constituents of various algae may be potential bioactive compounds of interest in the pharmaceutical industry[49].

In winter samples, *Ulva* species such *as Ulva lactuca* showed the strongest antibacterial activity against Gram-negative bacteria, while. in summer exhibited the higher activity against Gram positive bacteria[50].

This difference is likely due to the structural differences between the two bacterial types[51]. The cell wall of Gram-negative bacteria is more complex, which decreases susceptibility to antimicrobial substances[52], or may be due to seasonal variation in environmental conditions, which affect the metabolites.

Previous studies have shown that Gram negative bacteria such as pathogenic bacteria (e.g., Vibrios) are often associated with algae[53].

In Mari culture, diseases of microbial origin can cause significant economic losses worldwide; the evolution of microorganism resistance to antibiotics has resulted in a growing need for new antibacterial compounds that are effective in veterinary medicine and characterized by limited undesirable side effects. Increased attention has recently been turned to seaweeds as a promising source for metabolites with antimicrobial activity. Vibriosis is a common disease, caused by bacteria of the genus *Vibrio,*that can result in high mortality in aquaculture.

Chloroform/methanol extracts of seaweed species including *Ulva* species were tested for their antibacterial activities against six fish pathogenic *Vibrio* species using the disc diffusion method[54].

The preliminary phytochemical characterization and antimicrobial efficacy of macro

algae *U. fasciata* were studied against *V. hareryi*[55]. Priyadharshini[56] observed that aqueous and solvent based extracts of *U.fasciata* showed inhibition against bacteria and fungi pathogens. Abdel-Khaliq[36], has observed that *U. fasciata, U. lactuca, U.intestinalis* extracts are inhibitory to human pathogenic bacteria and fungi.

Random Amplification Polymorphic DNA

This project focuses on evaluation of genetic diversity within seaweeds and its relationship with their bioactive compounds. To relocate taxonomical position of three algal species, genetic

Similarity Random amplified polymorphic DNA (RAPD) technique was performed to detect the differences among three *Ulva* species through five arbitrary primers. Using primer (A) with the genomic DNA from three algal species reflected nineteen genomic bands. The numbers of polymorphic bands were 74% polymorphism. Using primer (B) recorded fifteen bands; six bands were as polymorphic with 40% polymorphism. Primer (C), twelve bands were obtained. In addition, eight polymorphic bands were recorded with 67% polymorphism. Using primer (D), eighteen bands were recorded with eight bands as polymorphic with 44% of polymorphism. Primer (E), presented sixteen genomic bands, the number of polymorphic bands were twelve with 75% of polymorphism. The data represented in Figure (1) and table(5). As expected, individual from genus were clustered together in the dendrogram(Figure 2). The genetic similarity coefficient between *U. lactuca* and *U. intestinalis* was found to be 0.96.

CONCLUSION

The results of the present study indicated that the collected seaweeds showed successfully displayed of phytochemical analysis, antioxidant and antimicrobial activity, which make them interesting for programs of screening for natural products. This ability not restricted to one order or division within the macro algae but all of them offer opportunities for producing new types of bioactive compounds. The chemical and nutritional composition of the seaweeds depends on many factors, including species, geographical origin or area of cultivation, seasonal, environmental and physiological variations, and time of harvest, water temperature and processing methods. Seaweed content of proteins, carbohydrates, lipids, fiber, metabolites, ect. can be influenced by their growing parameters. For this reason, seaweeds can be natural bioreactors, able to provide different types of compounds at different quantities.

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