International Journal of Engineering Research and General Science Volume 2, Issue 5, August-September, 2014
ISSN 2091-2730

Antimicrobial Activity of Three Ulva Species Collected from Some Egyptian Mediterranean Seashores

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Abstract:-Members of the class Ulvophyceae such as *Ulva fasciata Delile*,*Ulva intestinalis Linnaeus* and *Ulva lactuca Linnaeus* were collected from tidal and intertidal zone of Mediterranean sea shores during April 2011 and extracted in ethanol. The total summation of the recorded total protein increase in the order: *Ulva fasciata* < *Ulva intestinalis* < *Ulva lactuca*, with percentage; 28.7, 27 and 17.6%, respectively. The total summation of the recorded total carbohydrate increase in the order: *Ulva lactuca* < *Ulva intestinalis* < *Ulva fasciata*, with percentage; 55.6, 49.63 and 47.93%, respectively. The total summation of the recorded total ash increase in the order:*Ulva lactuca* < *Ulva fasciata* < *Ulva intestinalis* with percentage; 17.6, 17 and 14.6 %, respectively. The total summation of the recorded total moisture increase in the order: *Ulva intestinalis* < *Ulva fasciata* < *Ulva lactuca*, with percentage; 9.93, 9.28 and 8.50% respectively. The total summation of the recorded total crude fat increase in the order *Ulva lactuca* < *Ulva fasciata* < *Ulva intestinalis*, with percentage; 0.7, 0.60 and 0.54 % respectively. Phytochemical screening showed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes and traces of tannins in all marine algae under investigation, the presence of both free flavonoids and/or combined flavonoids in all marine algae under investigation, Saponins are absent in all Ulva sp. under investigation, Cardiac glycosides, anthraquinones and alkaloids are absent in all Ulva species under investigation and volatile substances are also absent. Antimicrobial activity of Ulva sp. was tested against (10 Gram +ve bacteria, 10 Gram –ve bacteria and 10 unicellular Filamentous fungi). The antimicrobial activities were expressed as zone of inhibition and minimum inhibitory concentration (MIC). Identification of compounds from crude extract of Ulva sp. carried by LC/MS technique. Finally Ulva sp. could serves as useful source of new antimicrobial agents.

Keywords:-Marine algae, *Ulva fasciata*, *Ulva lactuca*, *Ulva intestinalis*, Minimum inhibitory concentration (MIC), LC/MS (Liquid chromatography/Mass spectroscopy) and Phytochemical screening.

INTRODUCTION

Seaweeds (Marine algae) belong to a group of eukaryotic known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances 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]. 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 cells have some protective mechanisms and compounds [3].

Marine algae are rich and varied source of bioactive natural products, so it has been studied as potential biocide and pharmaceutical agents [4]. There have been number of reports of antibacterial activity from marine plants and special attention has been

reported for antibacterial and antifungal activities related to marine algae against several pathogens [5]. The antibacterial activity of seaweeds is generally assayed using extracts in various organic solvent for example acetone, methanol-toluene, ether and chloroform-methanol [6]. Using of organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity [7].

In recent years, several marine bacterial and protoctist forms have been confirmed as important source of new compounds potentially useful for the development of chemotherapeutic agents. Previous investigations of the production of antibiotic substances by aquatic organisms point to these forms as a rich and varied source of antibacterial and antifungal agents. Over 15,000 novel compounds have been chemically determined. Focusing on bioproducts, recent trends in drug research from natural sources suggest that algae are a promising group to furnish novel biochemically active substances [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 fibres [9]. In addition to vitamins and minerals, seaweeds are also potentially good sources of proteins, polysaccharides and fibres [10]. The lipids, which are present in very small amounts, are unsaturated and afford protection against cardiovascular pathogens.

2. MATERIALS AND METHODS

2.1. Collection and identification of seaweeds

The studied algal species collected from the inter-tidal region of Mediterranean Sea shores between Ras elbar and Baltim. Seaweeds were identified as *Ulva lactuca*, *Ulva fasciata* and *Ulva intestinalis* (Green algae). The identification of the investigated marine algae was kindly verified by Prof. Dr. Ibrahim Borie and Prof. Dr. Neveen Abdel-Raouf, Botany Department Faculty of Science, Beni-sweif University, Egypt.

2.2. Preparation of seaweed extracts

The collected seaweeds *Ulva lactuca*, *Ulva fasciata* and *Ulva intestinalis* were cleaned and the necrotic parts were removed hundred gram of powdered sea weeds were extracted successively with 200 mL of solvent (Ethanol 70%) in Soxhelet extractor until the extract was clear. The extracts were evaporated to dryness reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

2.3. Collection of test microbial cultures

Twenty different bacterial cultures and ten fungal cultures were procured from Biotechnological Research Center, AL-Azhar University (for boys), Cairo, Egypt. ten different fungal isolates were used in this present study. The fungal cultures were procured from Biotechnological Research Center, AL-Azhar University (for boys), Cairo, Egypt.

2.4. Determination of Antibacterial activity of *Ulva* species.

2.4.1. Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 M.C. Farland standards and then used for the determination of antibacterial activity.

2.4.2. Well diffusion method

The antibacterial activities of investigated *Ulva* species were determined by well diffusion method proposed by Rahman et al., (2001) [11]. The solution of 50 mg/ml of each sample in DMSO was prepared for testing against bacteria. Centrifuged pellets of bacteria from a24 h old culture containing approximately 104 -106 CFU (Colony forming Unit) per ml were spread on the surface of Nutrient agar (typetone 1%, Yeast extract 0.5%, agar 1%, 100 ml of distilled water, PH 7.0) which autoclaved under 12oC for at least

20 min.Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45oC.The activity was determined by measuring the diameter of the inhibition zone (in mm).100µl of the tested samples (100mg / ml) were loaded into the wells of the plates. All samples was prepared in Dimethyl Sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 37oC for 24h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Penicillin G and Streptomycin were used as antibacterial standard drugs.

2.4.3. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of investigated sea weeds against bacterial isolates were tested in Mueller Hinton broth by Broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Muller Hinton broth for bacteria to get a concentration of 80, 40, 20, 10, 5, 2.50 and 1.25 mg/ml for investigated sea weeds extracts and 50ml of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of investigated *Ulva* species. The culture tubes were incubated at 37oC for 24 hours. The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

2.5. Determination of Antifungal activity

2.5.1. Well diffusion method

The antibacterial activities of investigated *Ulva* species were determined by well diffusion method proposed by Rahman et al. (2001) [12]. Petri plates were prepared by Sabourad dextrose agar plates: A homogenous mixture of glucose-peptone-agar(40:10:15) was sterilized by autoclaving at 121oC for 20 min.The sterilized solution (25ml) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate .These plates were inverted and kept at 30oC in incubator to remove the moisture and check for any contamination. Antifungal assay: Fungal strain was grown in 5mL Sabourad dextrose broth (glucose: peptone; 40:10) for3-4 days to achieve 105 CFU/ml cells. The fungal culture (0.1ml) was spread out uniformly on the Sabourad dextrose agar plates. Now small wells of size (4mm×20mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8 % soft agar to prevent the flow of test sample at the bottom of the well.100µl of the tested samples (10mg/ml) were loaded into the wells of the plates .All Samples was prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30oC for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin B was used as antifungal standard drugs.

2.5.2. Minimum inhibitory concentration

Minimum inhibitory concentrations (MIC) of investigated *Ulva* species extracts against fungal isolates were tested in Sabouraud's dextrose broth by Broth macro dilution method. The *Ulva* species extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth for fungi to get a concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for *Ulva* species extracts and 50ml of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 28oC for 48 hours (yeasts) and 72 hours (molds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as Minimum inhibitory concentration (MIC).

2.6. Estimation of nutritional value of algal species

2.6.1. 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 (1995) [13].

2.6.2. Carbohydrates estimation

The total carbohydrate was estimated by following the phenol-sulphuric acid method of Dubois et al. (1956) [14], using glucose as standard.

2.6.3. 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 under vacuum AOAC (2000) [15].

2.6.4. Moisture estimation

The moisture content was determined by oven method at 105°C until their constant weight was obtained.

2.6.5. Moisture estimation

Ash content was acquired by heating the sample overnight in a furnace at 525°C and the content was determined gravimetrically.

2.7. Preliminary Phytochmical Tests

Preliminary phytochmical tests for identification of alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and terpenes were carried out for all the extracts using standard qualitative methods that have been de- scribed previously [16-20].

2.8. Liquid chromatography / Mass spectroscopy (LCMS)

High resolution mass spectrometric data were obtained using a Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDAautosampler, and Accela pump).The following conditions were applied: capillary voltage 45 V, capillary temperature 260°C, auxiliary gas flow rate 10-20 arbitrary units, sheath gas flow rate 40-50 arbitrary units, spray voltage 4.5 kV, mass range 100_2000 amu (maximum resolution 30 000). The exact mass obtained for eluted peaks was used to deduce the possible molecular formulae for such mass, and these formulae were searched in Dictionary of Natural Products, CRC press, online version, for matching chemical structures.

3. RESULTS AND DISCUSSION

3.1. Identification of the marine Algae.

Seaweeds were identified as *Ulva lactuca, Ulva fasciata* and *Ulva intestinalis* **(Green algae: Chlorophyta)**.The identification of the investigated marine algae was kindly verified by Dr. Ibrahim Borai Ibrahim, Professor of Phycology, Botany & Microbiology Department Faculty of Science, Beni-suef University, Egypt and Prof. Dr. Nevein Abdel-Rouf Mohamed, Professor of Phycology and Head of Botany & Microbiology Department, Faculty of Science, Beni-suef University.

3.2. Antimicrobial activity.

No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from (28.7 ± 0.2 mm to 16.4 ± 0.3 mm) against the Gram positive bacteria pathogens.

3.2.1 Antimicrobial activity of *Ulva lactuca*

3.2.1.1 Antimicrobial activity of *Ulva lactuca* against Gram +ve bacteria

Ulva lactuca showed highest mean zone of inhibition (22.0±0.8) against the Gram positive bacteria *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* (19.8±0.3mm), *Streptococcus mutans* (17.8±0.9mm), *Bacillus subtilis* (17.5±0.3mm), *Streptococcus pyogenes* (14.2±0.5mm), *Bacillus cereus* (12.6±0.1mm) *and Staphylococcus epidermidis* (10.5±0.4).*Gram positive*

bacteria, *Streptococcus pneumonia*, *Enterococcus faecali* and *Corynebacterium diphtheria* showed highly resistance against *Ulva lactuca* crude extract.

3.2.1.2 Antimicrobial activity of *Ulva lactuca* against Gram -ve bacteria

Concering about extract of *Ulva lactuca* against Gram negative bacteria, maximum zone of inhibition was recorded against *Slamonella typhimurium* (22.1±0.5mm) followed by *Serratia marcescens* (20.8±0.6mm), *Escherichia coli* (20.2±0.2mm) and *Neisseria meningitides* (15.9±0.6mm). *Ulva lactuca* showed lowest mean zone of inhibition (12.2±0.7mm) against *Klebsiella pneumonia* followed by *Haemophilus influenza* (13.2±0.8mm). Gram negative bacteria, *Pseudomonas aeruginosa*, *Proteous vulgaris*, *Yersinia enterocolitica* and *Shigella flexneria* showed highly resistance against *Ulva lactuca* crude extract.

3.2.1.3 Antimicrobial activity of *Ulva lactuca* against Unicellular & Filamentous fungi

Ulva lactuca showed highest mean zone of inhibition (23.2±0.3mm) against the pathogenic fungi *Geotricum candidum* followed by *Candida albicans* (22.5±0.7mm), *Aspergillus clavatus* (21.6±0.7mm), *Aspergillus fumigatus* (19.9±0.8mm), *Rhizopus oryzae* (19.7±0.7mm) and *Mucor circinelloides* (15.8±0.3mm).*Ulva lactuca* showed lowest mean zone of inhibition against *Penicillium marneffei* (10.3±0.1mm). Pathogenic fungi, *Syncephalastrum racemosum*, *Absidia corymbifera* and *Stachybotrys chartarum* showed highly resistance against *Ulva lactuca* crude extract.

3.2.2 Antimicrobial activity of *Ulva intestinalis*

3.1.2.1 Antimicrobial activity of Ulva lactuca against Gram +ve bacteria

Ulva intestinalis showed highest mean zone of inhibition (17.9±0.3 mg/ml) against the Gram positivebacteria *Staphylococcus saprophyticus* followed by *Streptococcus mutans* (16.5±0.1 mg/ml), *Bacillus subtilis* (15.5±0.7 mg/ml), *Streptococcus pyogenes* (11.8±0.1 mg/ml), *Bacillus cereus* (10.9±0.2 mg/ml) *and Staphylococcus epidermidis* (8.7±0.2 mg/ml). *Gram positive bacteria*, *Streptococcus pneumonia*, *Enterococcus faecali and Corynebacterium diphtheria* showed highly resistance against *Ulva intestinalis* crude extracts.

3.2.2.2 Antimicrobial activity of *Ulva intestinalis* against Gram -ve bacteria

Ulva intestinalis showed the highest activity against *Slamonella typhimurium* (20.8± 0.9 mg/ml) followed by *Serratia marcescens* (18.9±0.5 mg/ml), *Escherichia coli* (18.2±0.9 mg/ml), *Neisseria meningitides* (14.2±0.5 mg/ml), *Haemophilus influenza* (10.2±0.1 mg/ml) and *Klebsiella pneumonia* (10.2±0.1 mg/ml). Gram negative bacteria, *Pseudomonas aeruginosa*, *Proteous vulgaris*, *Yersinia enterocolitica* and *Shigella flexneria* showed highly resistance against *Ulva intestinalis* crude extract.

3.2.2.3 Antimicrobial activity of *Ulva intestinalis* against Unicellular & Filamentous fungi

Ulva intestinalis showed highest mean zone of inhibition (21.7±0.1 mg/ml) against the pathogenic fungi *Geotricum candidum* followed by *Aspergillus clavatus* (20.1±0.3 mg/ml), *Candida albicans* (19.3±0.5 mg/ml), *Aspergillus fumigatus* (17.8± 0.7 mg/ml), *Rhizopus oryzae* (16.4±0.5 mg/ml), *Mucor circinelloides* (13.7±0.2 mg/ml) *and Penicillium marneffei* (10.3±0.1mg/ml). Pathogenic fungi, *Syncephalastrum racemosum*, *Absidia corymbifera and Stachybotrys chartarum* showed highly resistance against *Ulva intestinalis* crude extract.

3.2.3 Antimicrobial activity of *Ulva fasciata*

3.2.3.1 Antimicrobial activity of *Ulva fasciata* against Gram +ve bacteria

Ulva fasciata showed highest mean zone of inhibition (22.2±0.6 mg/ml) against the Gram positive bacteria *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* (19.6±0.4 mg/ml), *Bacillus subtilis* (17.9±0.9 mg/ml), *Streptococcus mutans* (17.9±0.1 mg/ml), *Streptococcus pyogenes* (14.7±0.3 mg/ml), *Bacillus cereus* (12.9±0.1mg/ml) and *Staphylococcus epidermidis*

(10.8±0.1 mg/ml). Gram positive bacteria, *Streptococcus pneumonia*, *Enterococcus faecali* and *Corynebacterium diphtheria* showed highly resistance against *Ulva fasciata* crude extract.

3.2.2.2 Antimicrobial activity of *Ulva fasciata* against Gram -ve bacteria

Maximum zone of inhibition was recorded in *Ulva fasciata* crude extract against *Slamonella typhimurium* (22.4±0.5mg/ml) followed by *Serratia marcescens* (21.2±0.6mg/ml), *Escherichia coli* (20.6±0.5mg/ml) and *Neisseria meningitides* (16.2±0.3mg/ml), *Haemophilus influenza* (13.7±0.5mg/ml) and *Klebsiella pneumonia* (12.6±0.7mg/ml). *Pseudomonas aeruginosa*, *Proteous vulgaris*, *Yersinia enterocolitica* and *Shigella flexneria* showed highly resistance against *Ulva fasciata* crude extract.

3.2.2.3 Antimicrobial activity of *Ulva fasciata* against Unicellular & Filamentous fungi

Ulva fasciata showed highest mean zone of inhibition (23.4±0.6mg/ml) against the pathogenic fungi *Geotricum candidum* followed by *Candida albicans* (22.9±0.4 mg/ml), *Aspergillus clavatus* (21.1±0.7 mg/ml), *Aspergillus fumigatus* (20.1±0.6 mg/ml), *Rhizopus oryzae* (20.1±0.8 mg/ml) and *Mucor circinelloides* (16.4±0.5 mg/ml) and *Penicillium marneffei* (10.7±0.3 mg/ml). Pathogenic fungi, *Syncephalastrum racemosum, Absidia corymbifera, and Stachybotrys chartarum* showed highly resistance against *Ulva fasciata* crude extract.

3.3 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of reference antibiotic (Ampicillin) ranged from (0.03 to 15.63 mg/ml). Ampicillin is highly sensitive against *staphylococcus epidemidis, staphylococcus aureus, staphylococcus saprophyticus, Bacillus* cereus, *Bacillus subtilis, Streptococus pneumonia, Streptococcus pyogenes, Streptococcus mutans* and *Enterococcus faecali* (0.03, 0.06, 0.06, 0.06, 0.12, 0.25, 0.98 & 1.95 mg/ml) respectively. Ampicillin showed less activity against *Corynebacterium diphtheria* (15.63 mg/ml).

3.3.1. MIC of *Ulva lactuca*

3.3.1.1 MIC of *Ulva lactuca* against Gram +ve bacteria

The Minimum inhibitory concentration (MIC) value of *Ulva lactuca* showed MIC against the Gram positive bacteria was ranged between (0.98 mg/ml to 250 mg/ml). The lowest MIC (0.98 mg/ml) value was recorded against *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* (3.9 mg/ml), *Streptococcus mutans*, *Bacillus subtilis* which have the same MIC (7.81 mg/ml), *streptococcus pyogenes* (31.25 mg/ml), *Bacillus cereus* (125 mg/ml) and *Staphylococcus epidermidis* (250 mg/ml).

3.3.1.2 MIC of *Ulva lactuca* against Gram -ve bacteria

The Minimum inhibitory concentration (MIC) value of *Ulva lactuca* against the Gram negative bacteria was ranged between (0.98 mg/ml to 125 mg/ml). The lowest MIC (0.98 mg/ml) value was recorded against *Slamonella typhimurium* followed by *Escherichia coli* and *Serratia marcescens* which have the same MIC (1.95 mg/ml), *Neisseria meningitides* (15.36mg/ml), *Haemophilus influenza* (62.5mg/ml) and *Klebsiella pneumonia* (125 mg/ml).

3.3.1.3 MIC of *Ulva lactuca* against Unicellular & Filamentous fungi

MIC value of *Ulva lactuca* against the Unicellular & Filamentous fungi was ranged between (0.49 mg/ml to 250 mg/ml). The lowest MIC (0.49 mg/ml) value was recorded against *Geotricum candidum* followed by *Candida albicans* (0.98 mg/ml), *Aspergillus clavatus* (1.95mg/ml), *Aspergillus fumigatus* and *Rhizopus oryzae* which have the same MIC value (3.9 mg/ml), *Mucor circinelloides* (15.63 mg/ml) and *Penicillium marneffei* (250 mg/ml).

3.3.2. MIC of *Ulva intestinalis*

3.3.2.1 MIC of *Ulva intestinalis* against Gram +ve bacteria

MIC value of Ulva intestinalis against the Gram positive bacteria was ranged between (3.9mg/ml to 500 mg/ml). The lowest MIC (3.9 mg/ml) value was recorded against *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* and *Streptococcus mutans* which have the same MIC value (7.81 mg/ml), *Bacillus subtilis* (15.63mg/ml), *streptococcus pyogenes* (125 mg/ml), *Bacillus cereus* (250 mg/ml) and *Staphylococcus epidermidis* (500 mg/ml).

3.3.2.2 MIC of *Ulva intestinalis* against Gram -ve bacteria

The Minimum inhibitory concentration of *Ulva intestinalis* against the Gram negative bacteria was ranged between 1.95 mg/ml to 250 mg/ml. The lowest MIC (1.95 mg/ml) value was recorded against *Slamonella typhimurium* followed by *Serratia marcescens* (3.9 mg/ml), *Escherichia coli* (7.81 mg/ml), *Neisseria meningitides* (31.25 mg/ml), *Haemophilus influenza* (125 mg/ml) and *Klebsiella pneumonia* (250 mg/ml).

3.3.2.3 MIC of *Ulva intestinalis* against Unicellular & Filamentous fungi

Concering *Ulva intestinalis* showed an excellent MIC ranged between (0.95mg/ml to 250 mg/ml). The lowest MIC (0.95 mg/ml) value was recorded against *Geotricum candidum* followed by *Candida albicans* and *Aspergillus clavatus* which have the same MIC value (3.9 mg/ml), *Aspergillus fumigatus* and *Rhizopus oryzae* which have the same MIC value (7.81 mg/ml), *Mucor circinelloides* (62.5 mg/ml) and *Penicillium marneffei* (250 mg/ml).

3.3.3. MIC of *Ulva fasciata*

3.2.3.1 MIC of *Ulva fasciata* against Gram +ve bacteria

The lowest concentration of *Ulva fasciata* crude extract that will inhibit the visible growth of Gram positive bacteria was ranged between (1.95 mg/ml to 250 mg/ml). The lowest MIC (1.95 mg/ml) value was recorded against *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* (3.9 mg/ml), *Streptococcus mutans* (7.81 mg/ml), *Bacillus subtilis* (15.63 mg/ml), *Streptococcus pyogenes* (62.5 mgml), *Bacillus cereus* (125 mg/ml) and *Staphylococcus epidermidis* (250 mg/ml).

3.3.3.2 MIC of *Ulva fasciata* against Gram -ve bacteria

The Minimum inhibitory concentration (MIC) value of Ulva fasciata against the Gram positive bacteria was ranged between (1.95 mg/ml to 250 mg/ml). The lowest MIC (1.95 mg/ml) value was recorded against *Staphylococcus aureus* followed by *Staphylococcus saprophyticus* (3.9 mg/ml), *Streptococcus mutans* (7.81 mg/ml), *Bacillus subtilis* (15.63 mg/ml), *Streptococcus pyogenes* (62.5 mg/ml), *Bacillus cereus* (125 mg/ml) and *Staphylococcus epidermidis* (250 mg/ml).

3.3.3.3 MIC of *Ulva fasciata* against Unicellular & Filamentous fungi

Ulva fasciata showed MIC ranged between (0.98 mg/ml to 250 mg/ml). The lowest MIC (0.98 mg/ml) value was recorded against *Geotricum candidum* followed by *Candida albicans* and *Aspergillus clavatus* which have the same MIC value (1.95mg/ml), *Aspergillus fumigates* (3.9 mg/ml), *Rhizopus oryzae* (7.81 mg/ml), *Mucor circinelloides* (31.25 mg/ml) and *Penicillium marneffei* (250 mg/ml).

Table (3.1): Anti-bacterial activity of *Ulva species* (Gram Positive).

Marine algae	Inhibition zone diameter(mm/sample)									
	Streptococcus pneumoniae	*Streptococcus pyogenes*	*Streptococcus mutans*	*Bacillus cereus*	*Bacillis subtilis*	*Enterococcus faecali*	*Corynebacterium diphtheriae*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Staphylococcus saprophyticus*
AM	23.8± 0.2	22.7± 0.2	21.6± 0.1	27.9±0.1	26.4± 0.3	20.3± 0.3	16.4± 0.3	28.3± 0.1	28.7± 0.2	28.4± 0.2
Ulva lactuca	NA	14.2± 0.5	17.8± 0.9	12.6±0.1	17.5± 0.3	NA	NA	22.0± 0.8	10.5± 0.4	19.3± 0.3
Ulva intestinalis	NA	11.8± 0.1	16.5± 0.1	10.9±0.2	15.5± 0.7	NA	NA	20.1± 0.4	8.7± 0.2	17.9± 0.3
Ulva fasciata	NA	14.7± 0.3	17.9± 0.1	12.9±0.1	17.9± 0.5	NA	NA	22.2± 0.6	10.8± 0.1	19.6± 0.4

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample, The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 µl Was tested), *NA : No activity and AM: Reference antibiotic Ampicillin (30µ/disk).

Table (3.2): Anti-bacterial activity of *Ulva species* (Gram Negative).

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample, The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 µl Was tested), *NA : No activity and GT: Reference antibiotic Gentamicin (30µ/disk).

Marine algae	Inhibition zone diameter(mm/sample)									
	Pseudomonas aeruginosa	*Escherichia coli*	*Salmonella typhimurium*	*Proteous vulgaris*	*Klebsiella pneumoniae*	*Yersinia enterocolitica*	*Serratia marcescens*	*Neisseria meningitides*	*Haemophilus influenzae*	*Shigella flexneri*
GT	**17.3± 0.1**	**19.9± 0.3**	**27.3± 0.7**	**20.4±0.6**	**29.3± 0.3**	**18.7± 0.2**	**19.3± 0.2**	**17.6± 0.1**	**21.4± 0.1**	**23.7± 0.3**
Ulva lactuca	**NA**	**20.2± 0.2**	**22.1± 0.5**	**NA**	**12.2± 0.7**	**NA**	**20.8± 0.6**	**15.9± 0.6**	**13.2± 0.8**	**NA**
Ulva intestinalis	**NA**	**18.2± 0.9**	**20.8± 0.9**	**NA**	**10.2± 0.1**	**NA**	**18.9± 0.5**	**14.2± 0.5**	**11.2± 0.4**	**NA**
Ulva fasciata	**NA**	**20.6± 0.5**	**22.4± 0.9**	**NA**	**12.6± 0.7**	**NA**	**21.2± 0.6**	**16.2± 0.3**	**13.7± 0.5**	**NA**

Table (3.3): Anti-fungal activity of *Ulva species*.

Marine algae	*Inhibition zone diameter(mm/sample*									
	Penicillium marneffei	*Aspergillus clavatus*	*Aspergillus fumigatus*	*Syncephalastrum racemosum*	*Mucor circinelloides*	*Absidia corymbifera*	*Rhizopus oryzae*	*Geotricum candidum*	*Candida albicans*	*Stachybotrys chartarum*
AMP	**20.6± 0.2**	**22.4± 0.1**	**23.7± 0.1**	**19.7± 0.2**	**17.9± 0.1**	**19.8± 0.3**	**18.3± 0.4**	**28.7± 0.2**	**25.4± 0.1**	**18.9± 0.3**
Ulva lactuca	**10.3± 0.1**	**21.6± 0.7**	**19.9± 0.8**	**NA**	**15.8± 0.3**	**NA**	**19.7± 0.7**	**23.2± 0.3**	**22.5± 0.7**	**NA**
Ulva intestina	**11.5± 0.8**	**20.1±**	**17.8±**	**NA**	**13.7± 0.2**	**NA**	**16.4±**	**21.7±**	**19.3±**	**NA**

lis		**0.3**	**0.7**				**0.5**	**0.1**	**0.5**	
Ulva fasciata	**10.7± 0.3**	**22.1± 0.7**	**20.1± 0.6**	**NA**	**16.4± 0.5**	**NA**	**20.1± 0. 8**	**23.4± 0.6**	**22.9±0 . 4**	**NA**

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample,The test was done using the diffusion agar technique, Well diameter:6.0 mm(100 µl Was tested), *NA: No activity and AMP: Reference ibioticAmphotericin B (30µ/disk).

Table (3.4): MIC of *Ulva species* crude extract against Gram positive bacteria.

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample,The test was done using the diffusion agar technique, Well diameter:6.0 mm (100 µl Was tested), *NA : No activity and AM: Reference antibioticAmpicillin (30µ/disk).

Marine algae	**Inhibition zone diameter(mm/sample**									
	Streptococcus pneumoniae	***Streptococcus pyogenes***	***Streptococcus mutans***	***Bacillus cereus***	***Bacillis ubtilis***	***Enterococcus faecali***	***Corynebacterium diphtheriae***	***Staphylococcus aureus***	***Staphylococcus epidermidis***	***Staphylococcus saprophyticus***
AM	**0.25**	**0.98**	**1.95**	**0.06**	**0.12**	**1.95**	**15.63**	**0.06**	**0.03**	**0.06**
Ulva lactuca	**NA**	**31.25**	**7.81**	**125**	**7.81**	**NA**	**NA**	**0.98**	**250**	**3.9**
Ulva intestinalis	**NA**	**125**	**7.81**	**250**	**15.63**	**NA**	**NA**	**3.9**	**500**	**7.81**
Ulva fasciata	**NA**	**62.5**	**7.81**	**125**	**15.63**	**NA**	**NA**	**1.95**	**250**	**3.9**

Table (3.5): MIC of *Ulva species* crude extract against Gram negative bacteria.

Marine algae	**Inhibition zone diameter(mm/sample**									
	Pseudomonas aeruginosa	***Escherichia coli***	***Salmonella typhimurium***	***Proteus vulgaris***	***Klebsiella pneumoniae***	***Yersinia enterocolitica***	***Serratia marcescens***	***Neisseria meningitides***	***Haemophilus influenzae***	***Shigella flexneri***
GT	**7.81**	**3.9**	**0.06**	**1.95**	**0.015**	**3.9**	**3.9**	**7.81**	**0.98**	**0.25**
Ulva lactuca	**NA**	**1.95**	**0.98**	**NA**	**125**	**NA**	**1.95**	**15. 63**	**62.5**	**NA**
Ulva intestinalis	**NA**	**7.81**	**1.95**	**NA**	**250**	**NA**	**3.9**	**31.25**	**125**	**NA**
Ulva fasciata	**NA**	**1.95**	**0.98**	**NA**	**250**	**NA**	**3.9**	**15.63**	**125**	**NA**

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample,The test was done using the diffusion agar technique, Well diameter:6.0 mm (100 µl Was tested), *NA : No activity and AMP: Reference antibiotic Amphotericin B (30µ/disk).

Table (3.6): MIC of *Ulva species* crude extract against Unicellular & Filamentous fungi.

Marine algae	Inhibition zone diameter(mm/sample									
	Penicillium marneffei	***Aspergillus clavatus***	***Aspergillus fumigatus***	***Syncephalastrum racemosum***	***Mucor circinelloides***	***Absidia corymbifera***	***Rhizopus oryzae***	***Geotricum candidum***	***Candida albicans***	***Stachybotrys chartarum***
AMP	**1.95**	**0.98**	**0.49**	**3.9**	**7.81**	**3.9**	**7.81**	**0.03**	**0.12**	**3.9**
Ulva lactuca	**250**	**1.95**	**3.9**	**NA**	**15.63**	**NA**	**3.9**	**0.49**	**0.98**	**NA**
Ulva intestinalis	**250**	**3.9**	**7.81**	**NA**	**62.5**	**NA**	**7.81**	**0.95**	**3.9**	**NA**
Ulva fasciata	**250**	**1.95**	**3.9**	**NA**	**31.25**	**NA**	**7.81**	**0.98**	**1.95**	**NA**

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range clinically pathogenic microorganisms using (50 mg/ml) concentration of tested sample,The test was done using the diffusion agar technique, Well diameter:6.0 mm (100 µl Was tested), *NA : No activity and AMP: Reference antibiotic Amphotericin B (30µ/disk)

3.4. Phytochemical screening of marine Collected Algae

The qualitative phytochemical screening of the crude powder of Ulva species was carried out in order to assess the presence of bioactive compounds which might have anti-bacterial potency. The presence of the alkaloids, flavonoids, tannins, steroids and saponins. The absence of anthraquinones, Crystalline sublimate, steam volatile substances, Carbohydrates/glycosides and Cardiac glycosides was investigated (**Table 3.7**). Alkaloids and Flavonoids were present in moderate amounts (++) in 3 marine algae. Sterols and triterpenes were present in higher amounts (+++).Carbohydrates, Tannins were present in low amounts(+). Presence of flavonoids and alkaloids in most tested algae is interesting because of their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidant and antimicrobials with natural ones [21]. Our results were in agreement with previ- ous findings which showed presence of flavonoids and alkaloids in most of marine algae [22-24].

Table (3.7): Phytochemical screening of *Ulva species.*

Test	***Ulva fasciata***	***Ulva lactuca***	***Ulva intestinalis***
Crystalline sublimate	-	-	-
Steam volatile substances	-	-	-
Carbohydrates and/or glycosides	+	+	+
Tannins	+	+	+
Flavonoids ***aglycones**	++	++	++
***glycosides**	+	+	+
Saponins	-	-	-
Sterols and/or triterpenes	+++	+++	+++

Alkaloids	++	++	++
Anthraquinones ***aglycones**	-	-	-
***combined**	-	-	-
Cardiac glycosides: **-Killer Killiani** **-Baljet** **-Kedde**	- -	- -	- -
	-	-	-

(+++): present in higher amounts (++): present in moderate amounts (+): lower amounts

3.5. Nutritional value of collected marine Algae

Also in the present study, Comparative nutritive value screening was carried out on investigated marine algae (*Ulva fasciata*, *Ulva lactuca*and *Ulva intestinalis*) from Ras elbar, Baltim and Gamasa sea shores. Results depicted in the Table (3.8), the total summation of the recorded total protein increase in the order: *Ulva fasciata* < *Ulva intestinalis* < *Ulva lactuca*, with percentage; 28.7, 27 and 17.6%, respectively. The total summation of the recorded total carbohydrate increase in the order: *Ulva lactuca* < *Ulva intestinalis* < *Ulva fasciata* with percentage; 55.6, 47.93 and 44.2%, respectively. The total summation of the recorded total ash increase in the order: *Ulva lactuca*< *Ulva fasciata* < *Ulva intestinalis, with percentage;* 17.6, 17 and 14.6%,respectively.The total summation of the recorded total moisture increase in the order: *Ulva intestinalis*< *Ulva fasciata* < *Ulva lactuca,* with percentage;9.93, 9.28 and 8.50% respectively.The total summation of the recorded total crude fat increase in the order *Ulva lactuca* < *Ulva fasciata* < *Ulva intestinalis* with percentage; 0.7, 0.60 and 0.54%respectively.

Table (3.8): Nutritive value of *Ulva species*

Item **Type of analysis**	***Ulva fasciata***	***Ulva lactuca***	***Ulva intestinalis***
Total protein (as % of dry weight)	**28.7**	**17.6**	**27**
Total crude fat (as % of dry weight)	**0.6**	**0.7**	**0.54**
Total ash (as % of dry weight)	**17**	**17.6**	**14.6**
Total carbohydrates (as % of dry weight, by difference)	**44.2**	**55.6**	**47.93**
Total moisture (as % of fresh weight)	**9.28**	**8.50**	**9.93**

3.6. LC/MS of collected marine Algae.

The combination of high-performance liquid chromatography and mass spectrometry (LC/MS) has had a significant impact on drug development over the past decade. Continual improvements in LC/MS interface technologies combined with powerful features for structure analysis, qualitative and quantitative, have resulted in a widened scope of application. These improvements coincided with breakthroughs in combinatorial chemistry, molecular biology, and an overall industry trend of accelerated development. New technologies have created a situation where the rate of sample generation far exceeds the rate of sample analysis. As a result, new paradigms for the analysis of drugs and related substances have been developed. The growth in LC/MS applications has been extensive, with retention time and molecular weight emerging as essential analytical features from drug target to product. LC/MS-based methodologies that involve automation, predictive or surrogate models, and open access systems have become a permanent fixture in the drug development landscape. An iterative cycle of "what is it?" and "how much is there?" continues to fuel the tremendous growth of LC/MS in the pharmaceutical industry. During this time, LC/MS has become widely accepted as an integral part of the drug development process.

3.6.1. LC/MS of *Ulva fasciata*

In the present study, the data recorded in the Table (3.9) & Figs (3.1-3.11), demonstrated that only twenty eight compounds from the crude extract of *Ulva fasciata* can be determined. These compounds were determined and compared to previous isolated compounds using different libraries data bases. The identified compounds were found to be 4-hexahydroxy flavoneacetylB glucopyranosid, Formycin-A, Adenosine, 5'-Deoxyguanosine and n-Alkenylhydroquinol dimethyl ether.

3.6.2. LC/MS of *Ulva lactuca*

The data recorded in the Table (3.10) & Figs (3.12-3.14), demonstrated that only six compounds from the crude extract of *Ulva lactuca* can be determined. These compounds were determined and compared to previous isolated compounds using different libraries data bases. No identified compounds were matched with any previous isolated compounds which may be novel compounds.

3.6.3. LC/MS of *Ulva intestinalis*

It demonstrated that only nine compounds from the crude extract of *Ulva intestinalis* can be identified as had shown in Table (3.11) & Figs (3.15-3.16). These compounds were determined and compared to previous isolated compounds using different libraries data bases (Dictionary of Natural Products; an online version and AntiMarin 2012).The identified compounds were found to be n-Alkenylhydroquinol dimethyl ether only.

Table (3.9): LC/MS data of *Ulva fasciata* crude extract with their suspected formula and suggested identified compounds.

No.	R_t	M_{Wt}	C_f	Identification
1	4.32	507.1147	$C_{24}H_{18}O_9N_4$	No hits
			$C_{23}H_{22}O_{13}$	4- hexahydroxyflavoneacetylBglucopyranosid
2	6.22	236.1494	$C_{10}H_{21}O_5N$	No hits
		471.2911	$C_{21}H_{38}O_6N_6$	No hits
			$C_{20}H_{42}O_{10}N_2$	No hits
3	8.50	236.1494	$C_{10}H_{21}O_5N$	No hits
		333.1294	$C_{13}H_{20}O_8N_2$	Shinorine
4	9.56	268.1044	$C_{10}H_{13}O_4N_5$	Formycin-A,Adenosine,5'-Deoxyguanosine
5	12.16	204.0867	$C_8H_{13}O_5N$	No hits
		384.1500	$C_{14}H_{25}O_{11}N$	No hits
			$C_{15}H_{21}O_7N_5$	No hits
		477.1578	$C_{16}H_{25}O_{11}N_6$	No hits
		546.2031	$C_{21}H_{31}O_{12}N_5$	No hits
6	16.40	376.2330	$C_{18}H_{33}O_7N$	No hits
7	20.40	236.1493	$C_{10}H_{21}O_5N$	No hits
		534.3804	$C_{27}H_{47}O_4N_7$	No hits
		666.4223	$C_{25}H_{59}O_{13}N_7$	No hits
8	25.45	236.1481	$C_{10}H_{22}O_5N$	No hits
		507.2537	$C_{22}H_{38}O_{11}N_2$	No hits
		593.5108	$C_{33}H_{64}O_3N_6$	No hits
		734.5291	$C_{39}H_{73}O_8N_3Na$	No hits
			$C_{40}H_{69}O_4N_7Na$	No hits
9	26.89	474.3774	$C_{25}H_{49}O_2N_5Na$	No hits

			$C_{22}H_{47}O_4N_7$	No hits
10	28.51	474.3806	$C_{35}H_{62}O_3$	n-Alkenyl hydroquinol dimethyl ether
		581.5161	$C_{37}H_{64}ON_4$	No hits
		722.5358	$C_{45}H_{71}O_6N$	No hits
			$C_{44}H_{69}O_2N_5Na$	No hits

R_t: Retention time, MW: Molecular weight, C_f: Compound formula

Table (3.10): LC/MS data of *Ulva lactuca* crude extract with their suspected formula and suggested identified compounds.

No.	R_t	M_{Wt}	C_f	Identification
1	16.16	341.0514	$C_{15}H_8O_6N_4$	No hits
			$C_{14}H_{12}O_{10}$	No hits
		363.0334	$C_{15}H_8O_6N_4Na$	No hits
2	26.91	677.3722	$C_{31}H_{58}O_{14}Na$	No hits
			$C_{29}H_{52}O_{12}N6$	No hits
			$C_{44}H_{50}O_3N_2Na$	No hits

R_T: Retention time, MW: Molecular weight, C_F: Compound formula

Table (3.11): LC/MS data of *Ulva intestinalis* crude extract with their suspected formula and suggested identified compounds.

No.	R_t	M_{Wt}	C_f	Identification
1	24.96 - 29.72	553.4584	$C_{35}H_{62}O_3Na$	n-Alkenylhydroquinol dimethyl ether
2		609.2718	$C_{22}H_{38}O_9N_{10}Na$	No hits
			$C_{38}H_{38}O_4N_2Na$	No hits
3		734.5917	$C_{43}H_{77}O_3N_5Na$	No hits
			$C_{44}H_{79}O_7N$	No hits
4		941.6046	$C_{47}H_{82}O_{10}N_8Na$	No hits
			$C_{60}H_{80}O_7N_2$	No hits
			$C_{48}H_{84}O_{14}N_4$	No hits
			$C_{46}H_{86}O_{14}N_4Na$	No hits

R_T: Retention time, MW: Molecular weight, C_F: Compound formula

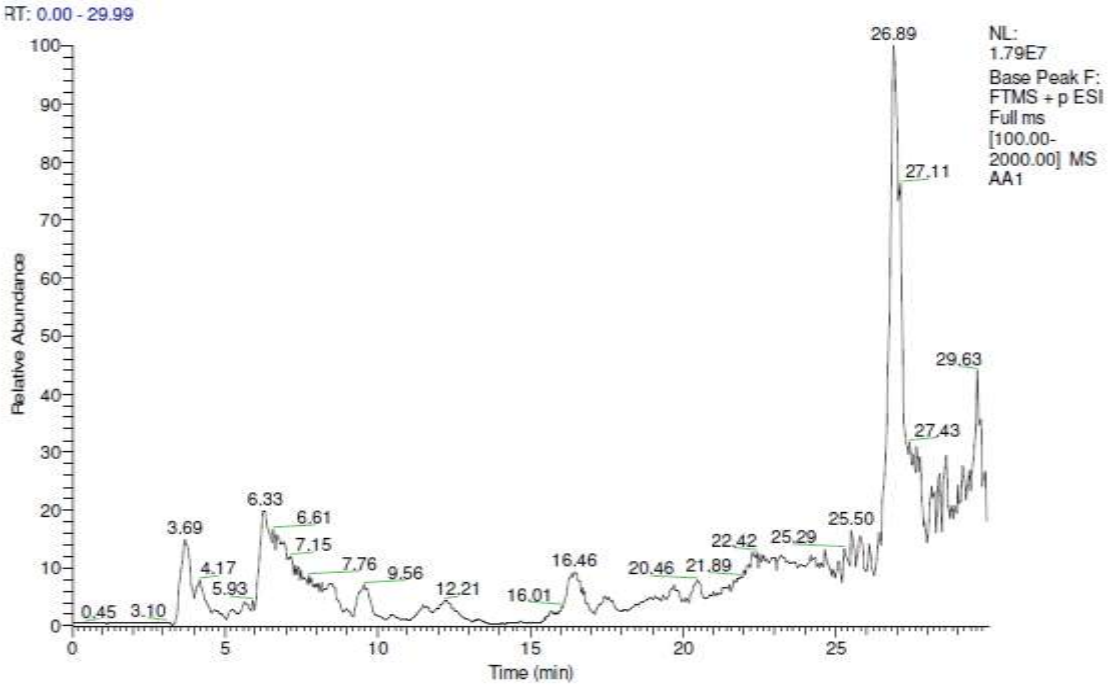

Figure (3.1) LC/MS of *Ulva fasciata* crude extract

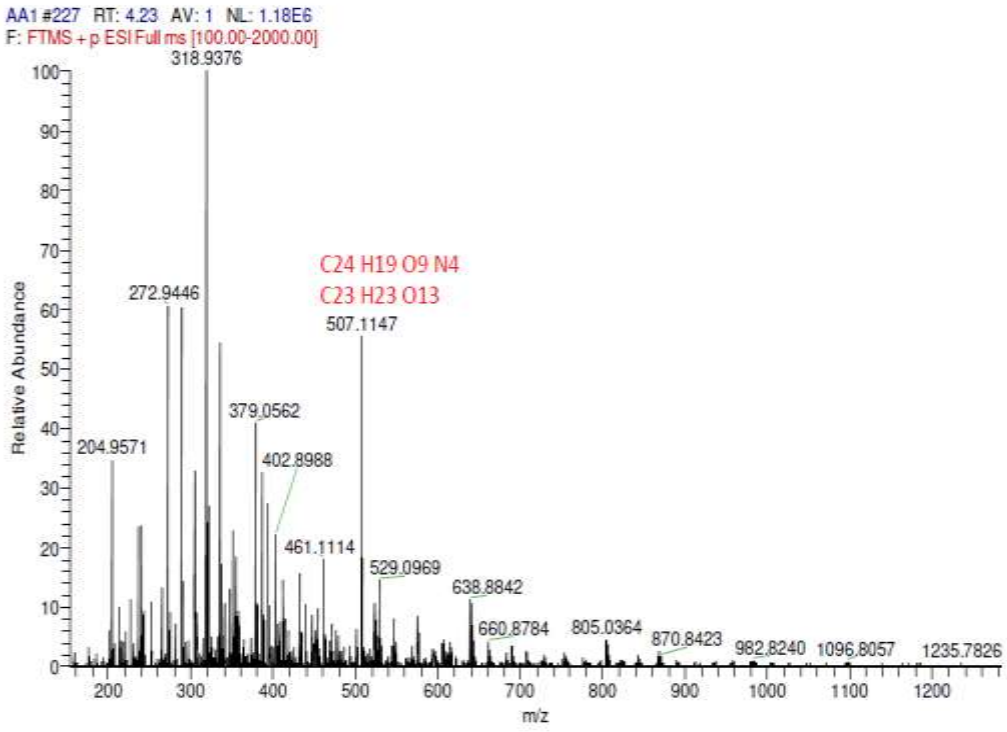

Figure (3.2) HRESIMS spectrum of compound 1 (*Ulva fasciata*)

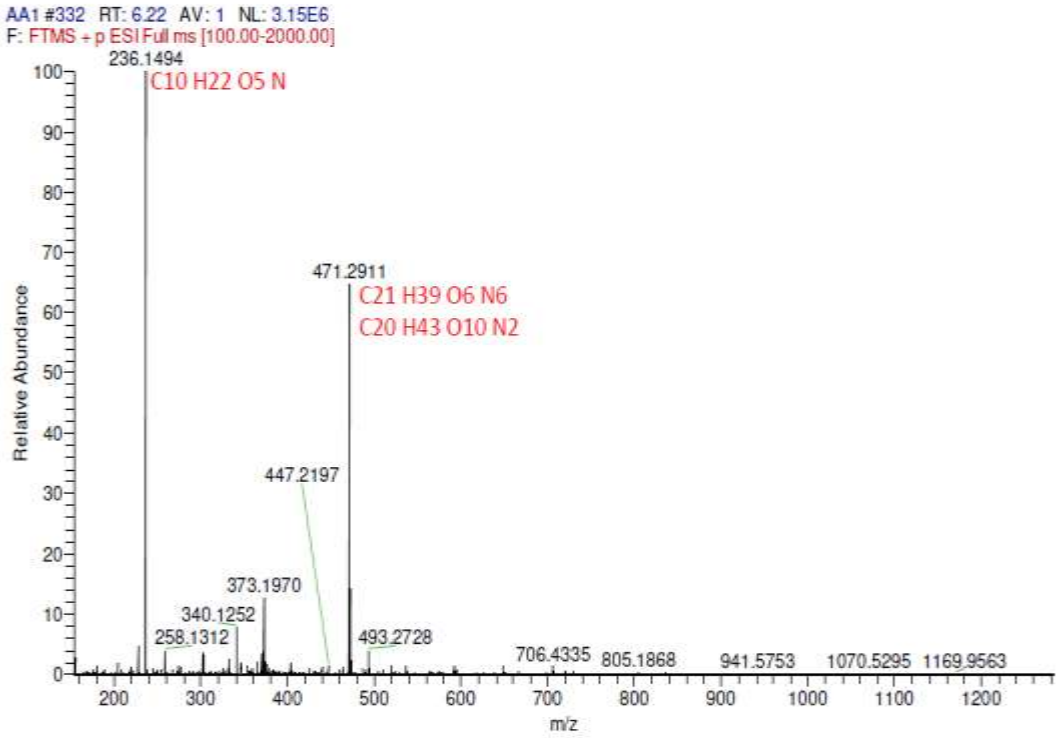

Figure (3.3) HRESIMS spectrum of compound 2 (*Ulva fasciata*)

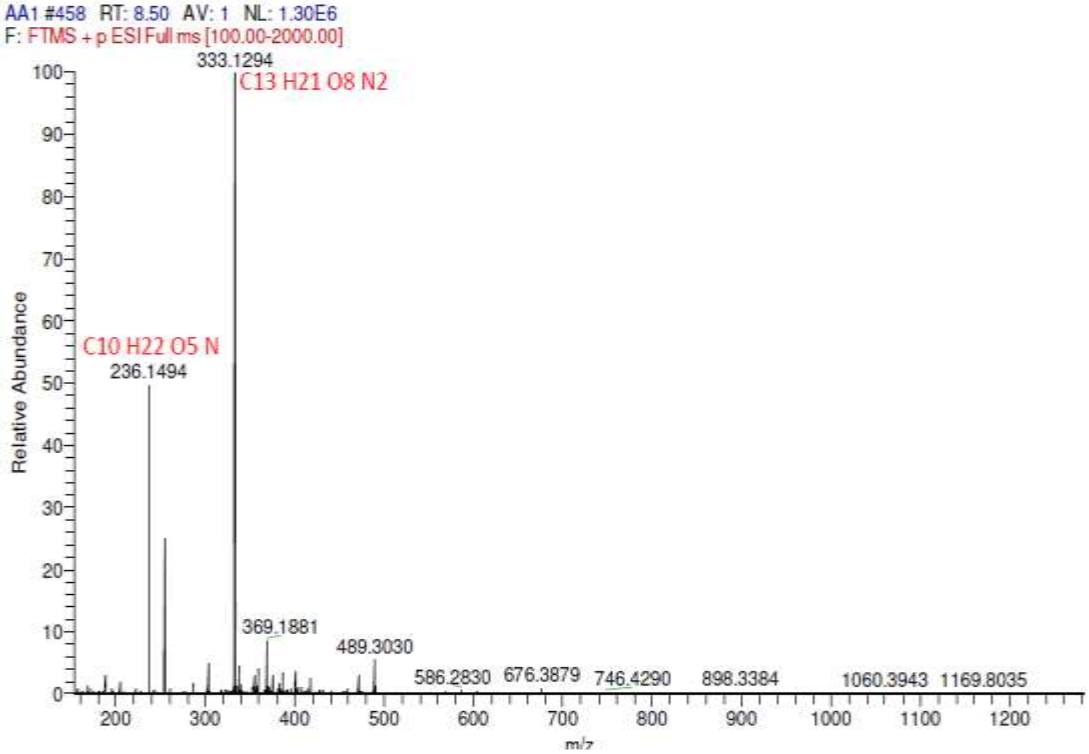

Figure (3.4) HRESIMS spectrum of compound 3 (*Ulva fasciata*)

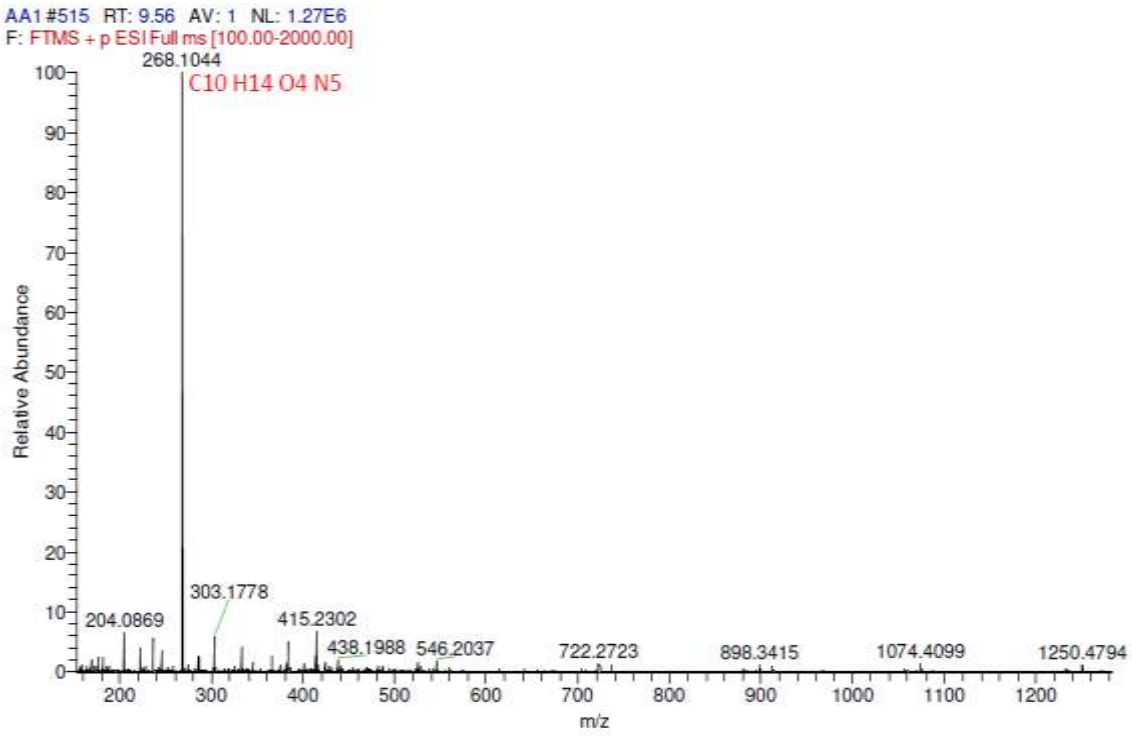

Figure (3.5) HRESIMS spectrum of compound 4 (*Ulva fasciata*)

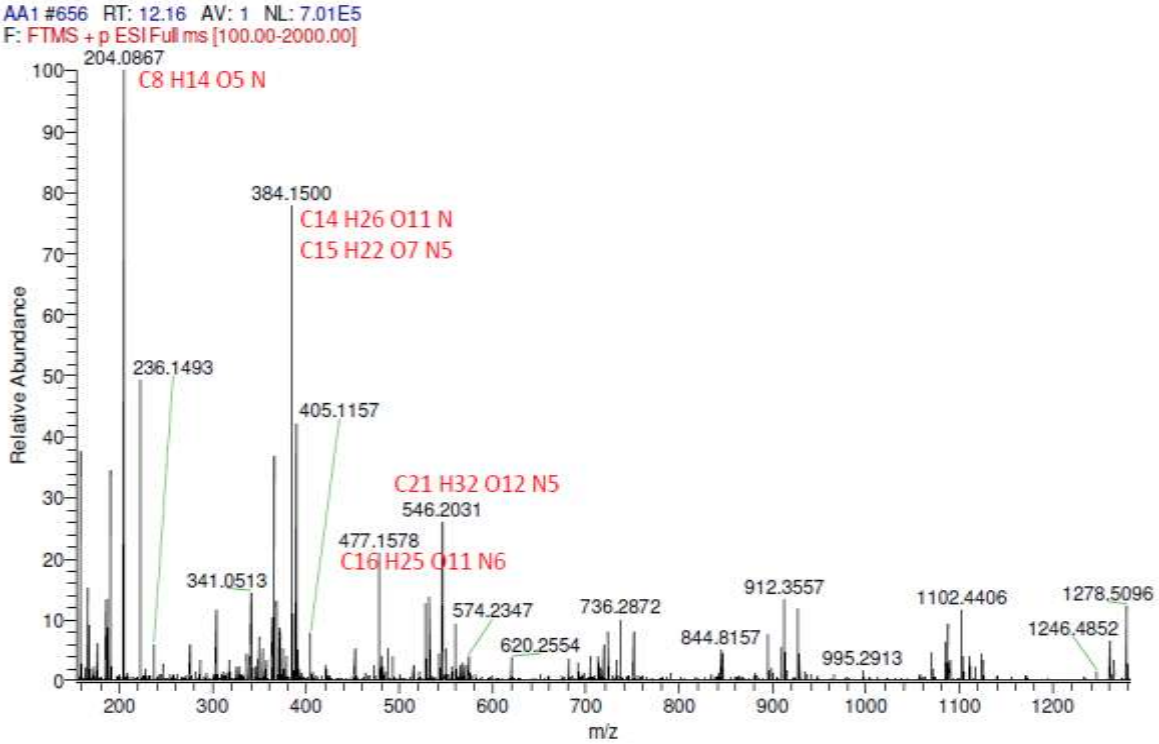

Figure (3.6) HRESIMS spectrum of compound 5 (*Ulva fasciata*)

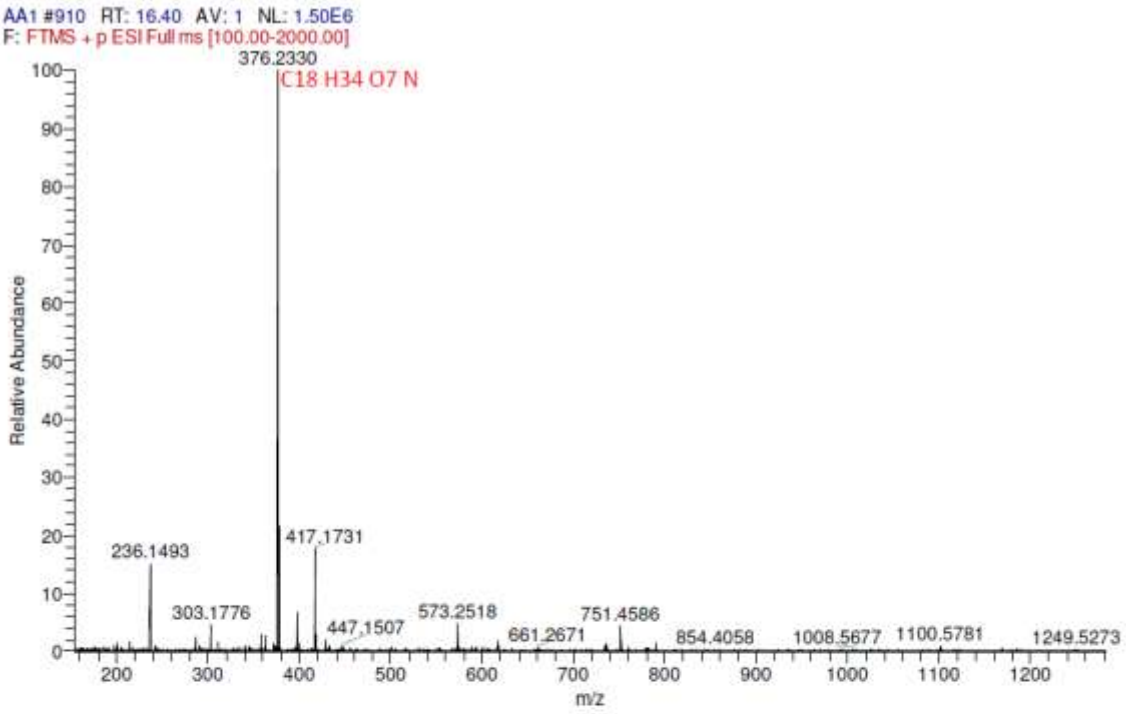

Figure (3.7) HRESIMS spectrum of compound 6 (*Ulva fasciata*)

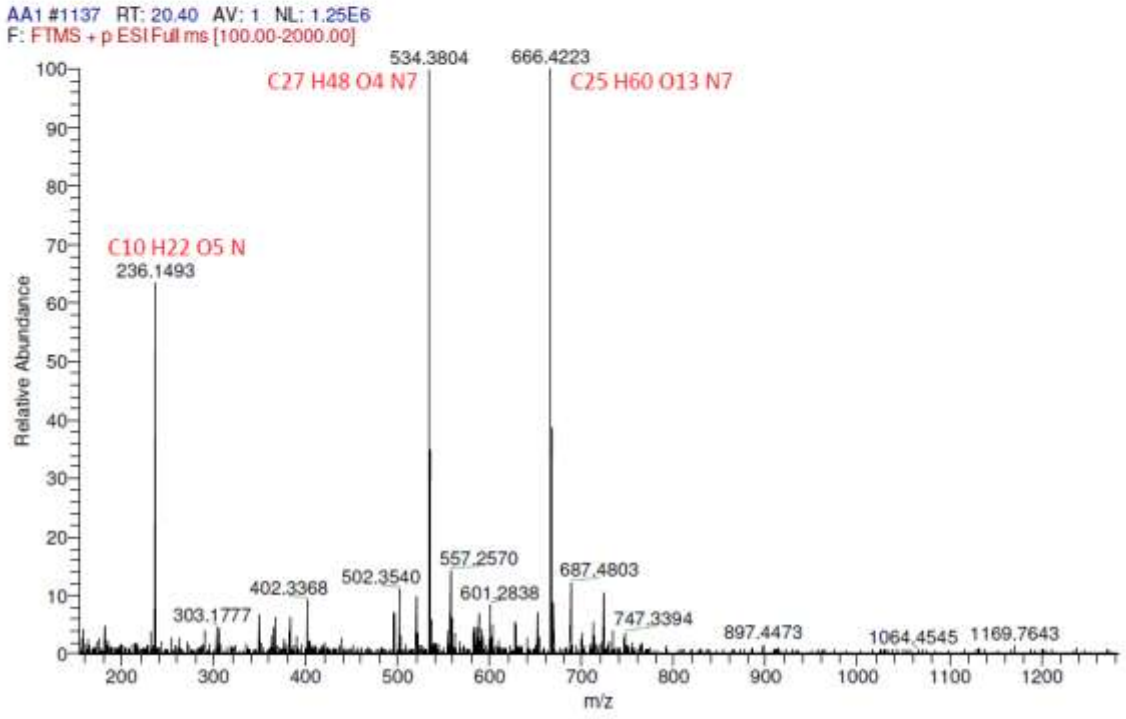

Figure (3.8) HRESIMS spectrum of compound 7 (*Ulva fasciata*)

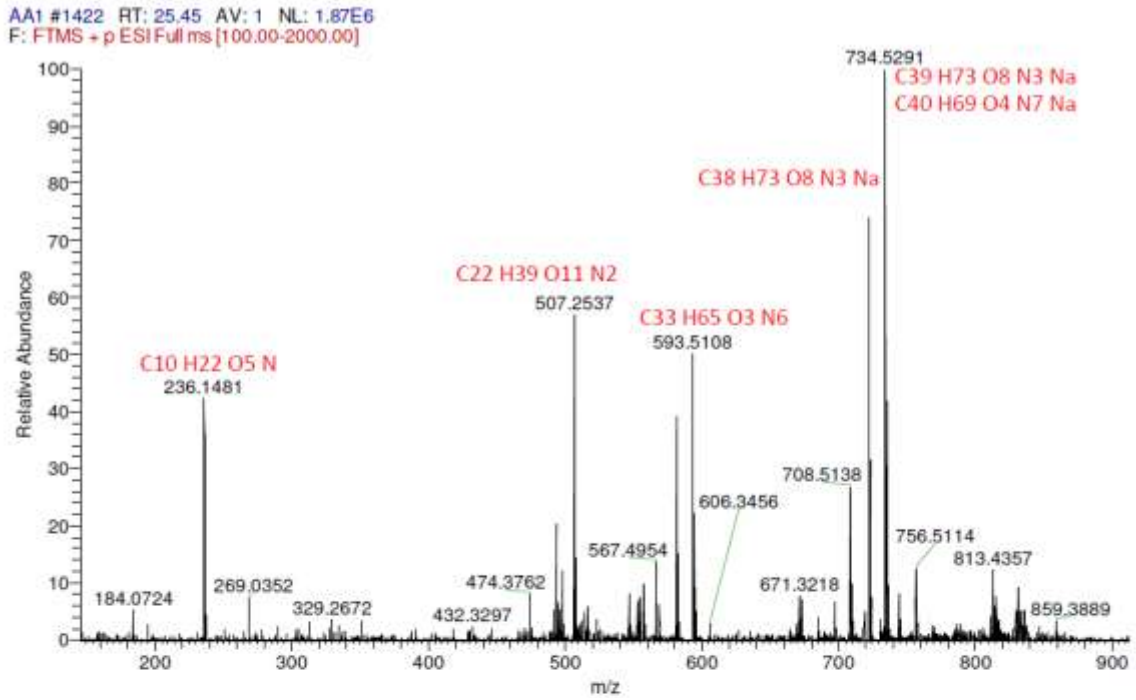

Figure (3.9) HRESIMS spectrum of compound 8 (*Ulva fasciata*)

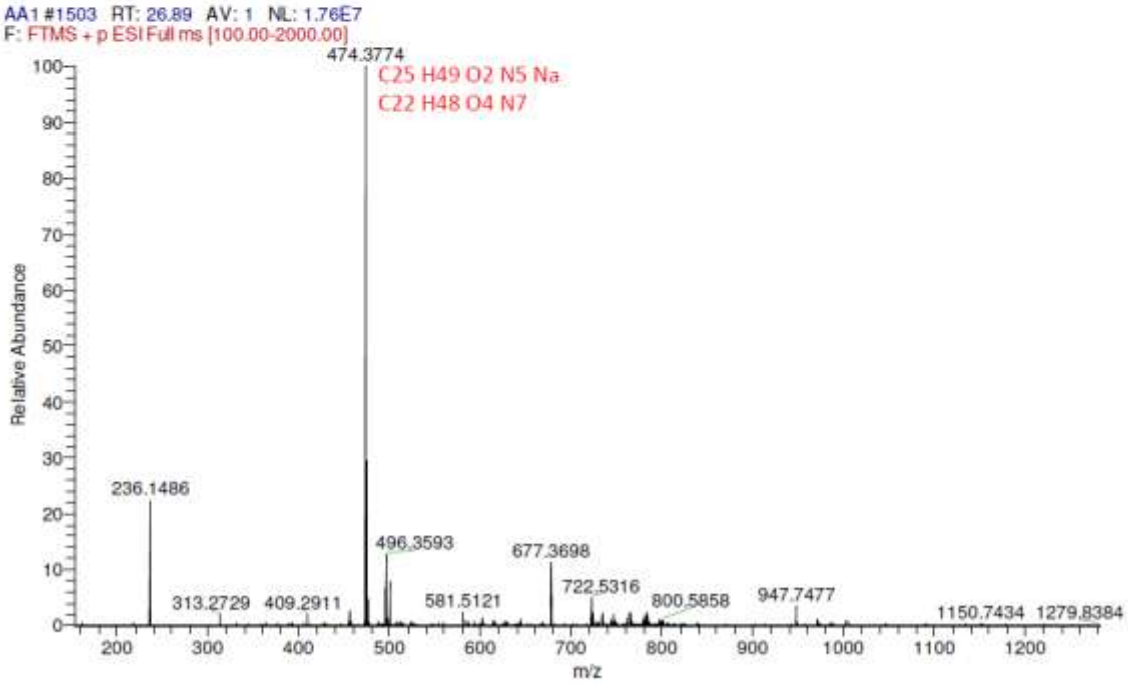

Figure (3.10) HRESIMS spectrum of compound 9 (*Ulva fasciata*)

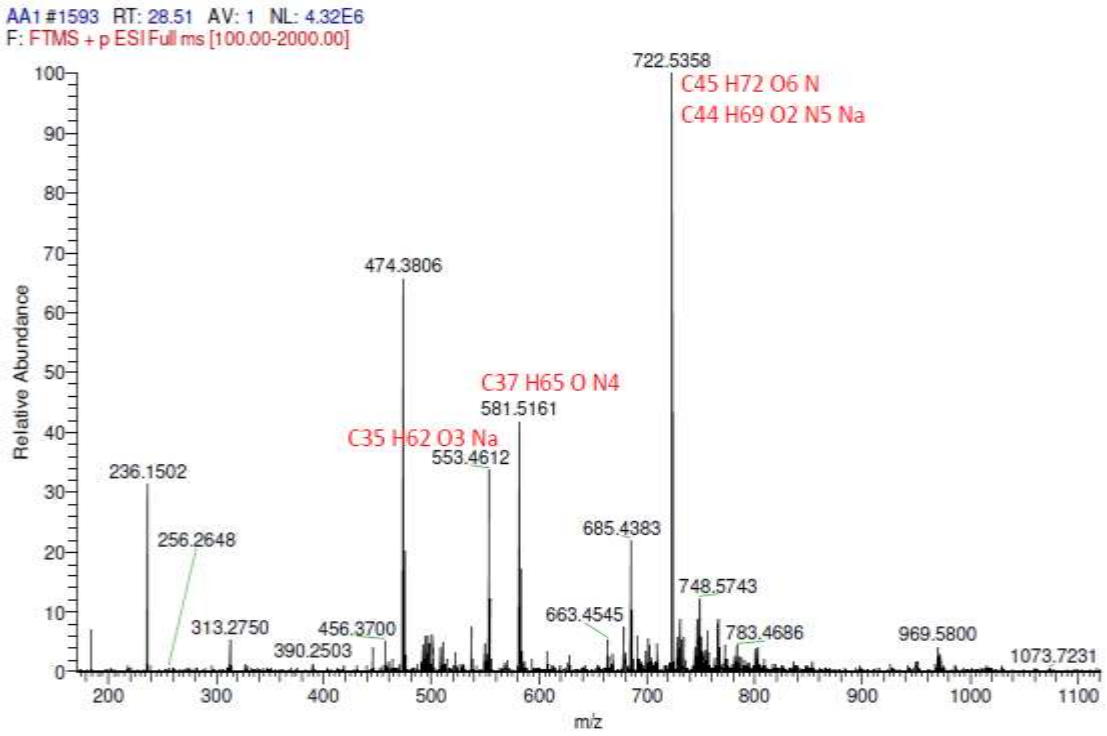

Figure (3.11) HRESIMS spectrum of compound 10 (*Ulva fasciata*)

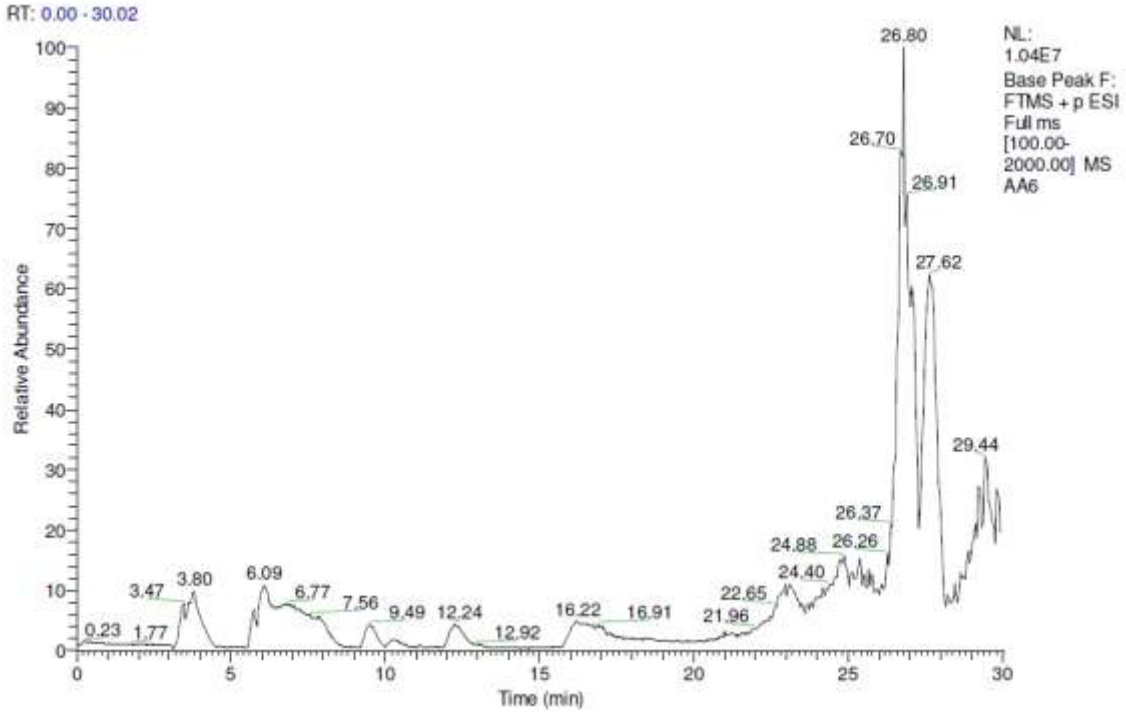

Figure (3.12) LC/MS of *Ulva lactuca* crude extract

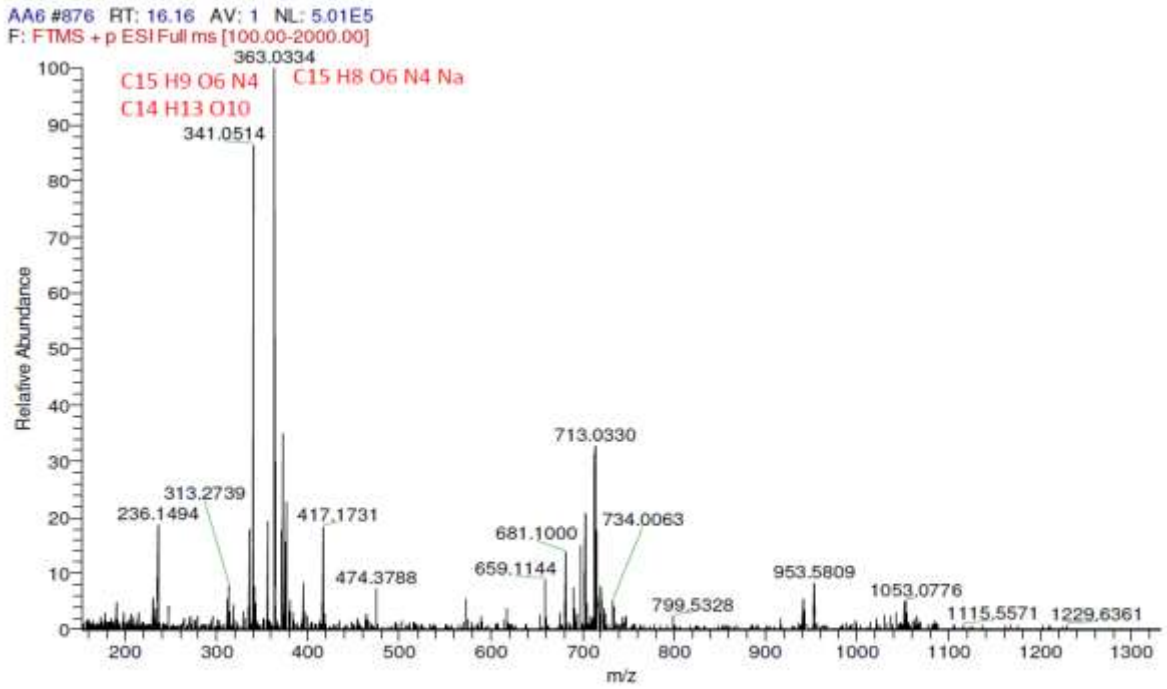

Figure (3.13) HRESIMS spectrum of compound 1 (*Ulva lactuca*)

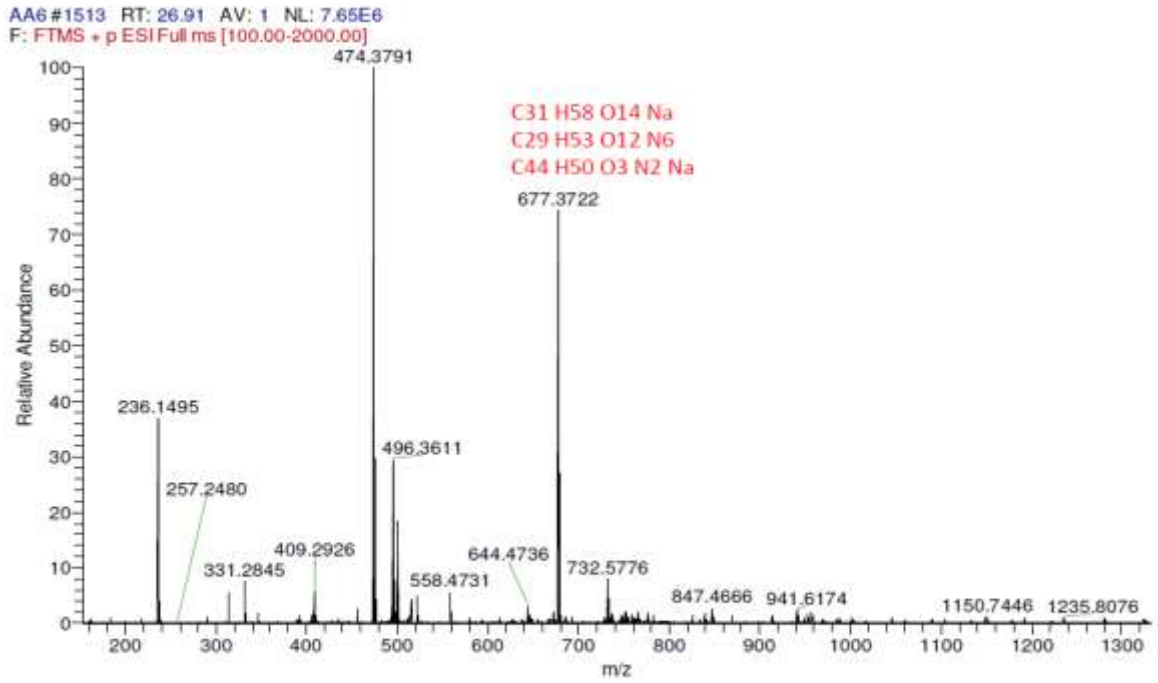

Figure (3.14) HRESIMS spectrum of compound 2 (*Ulva lactuca*)

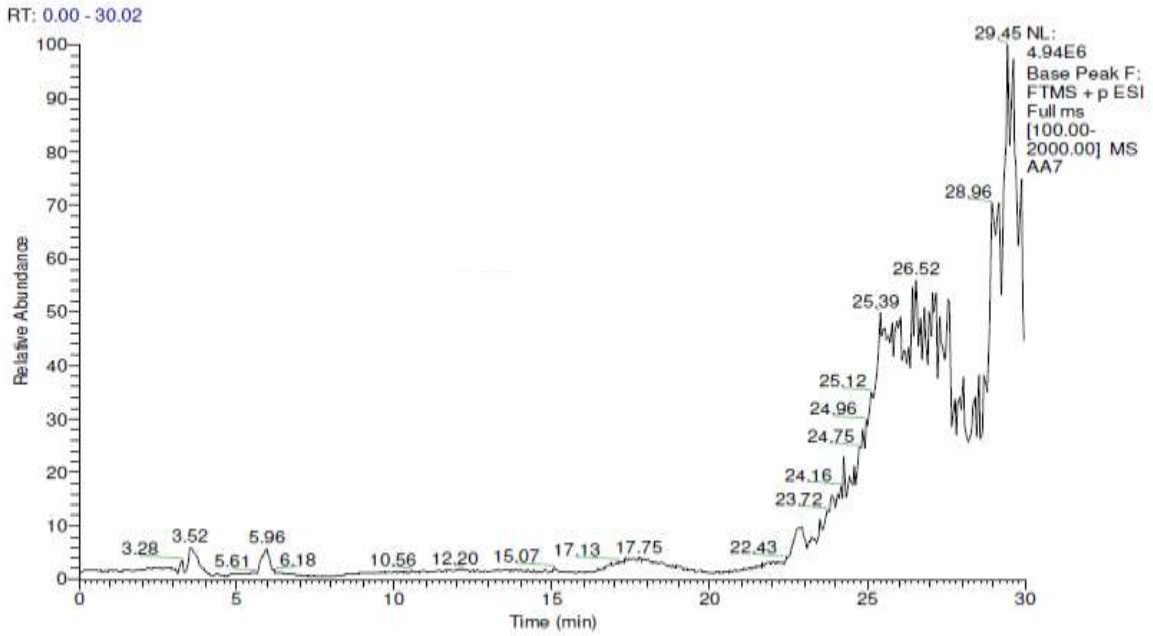

Figure (3.15) LC/MS of *Ulva intestinalis* crude extract

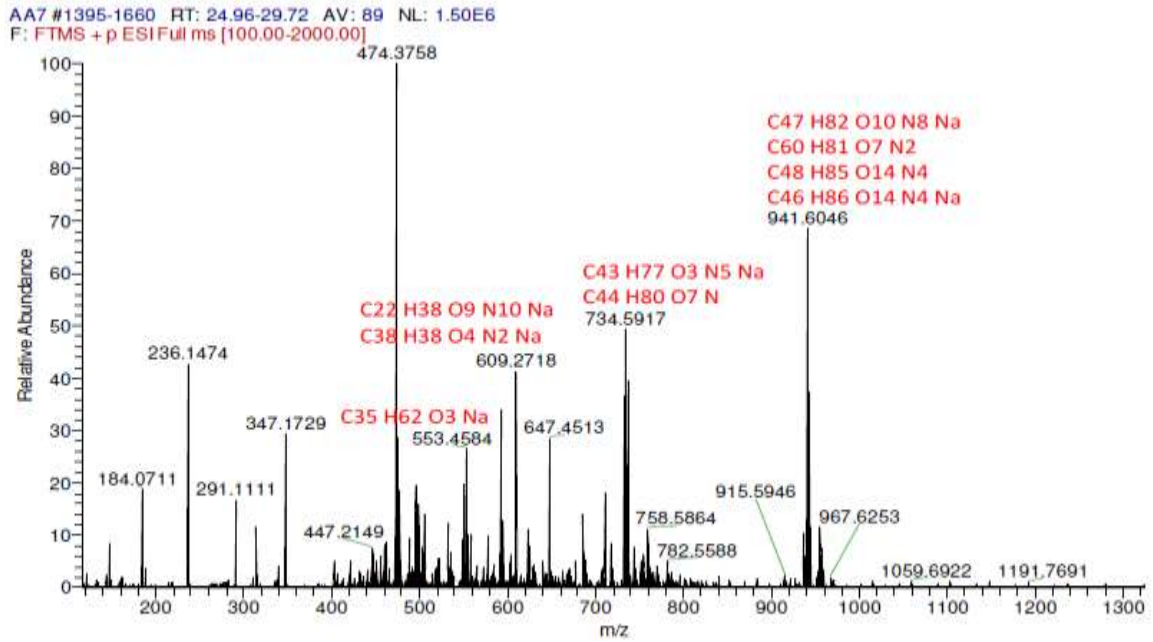

Figure (3.16) HRESIMS spectrum of compound 2 (*Ulva intestinalis*)

ACKNOWLEDGMENT

I would like to express my deepest gratitude and appreciation to Dr. Ibrahem Borie Ibrahem and Dr. Nevein Abdel-Raouf Mohammed, Prof. of Phycology, Faculty of Science, Beni-Suef University for his continuous help, careful guidance, and helpful discussion.

CONCLUSION

Our results indicated that, these species of seaweeds collected from Mediterranean Sea shores showed variety of antimicrobial activities, 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.

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