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Ulva lactuca as a cheap and safe biopesticide in fields and its chemical composition *(in vitro)*

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ABSTRACT

Ulva lactuca is a green macro alga called sea lettuce, was isolated from marine environment of Egypt. Its phytochemical constituents were determined by quantitative and qualitative tests using nine solvents different in their polarity. Its chemical structure also was proved by FT-IR spectroscopy. According to its enrichment chemical structure its biological activities as antioxidant, bactericide, fungicide and insecticide against human, plants, fish, poultry and animals pathogens and insects were investigated. Aqueous extract was the best as bactericide and fungicide and also give a good results as insecticide. *Ulva lactuca* showed antioxidant activity with IC_{50} = 9.6 mg/ml. Biological activity of *Ulva lactuca* related to its chemical structure and revealed its importance as natural pesticides.

INTRODUCTION

Nowadays we have several challenges basically on how to get new cheap, safe and natural sources of biologically chemical constituents used as drugs, natural pesticides, fertilizers, feed or food.

Marine environment has huge amount of creatures such as algae. Marine algae called also seaweeds are classified into many families according to their pigments the most common families are green, red and brown algae. These algae in general have many unique phytochemical constituents that have biological activity. Algae have been used as human food in many countries in the world especially in Asia continent, as fertilizers, drugs as antitumor Amin *et al*., (2015) pesticides, biofuel and have a big role in industry (Bilgraml and Saha, 2006) and in food industry products such as fish balls, meat balls, nuggets, broad beans cake and stewed horse beans (Meshhal, 2018).

Ulva lactuca is a green macro algae called also sea lettuce or green laver. The chemical structure of ulva lactuca composed of carbohydrates, proteins, lipids, vitamins, minerals, fibers, phytohormones, phenolic compounds, chlorophylls, carotenoids, flavonoids, alkaloids, terpenes and phytosterols, so their biological activities were as antioxidants, antimicrobial, antitumor, anticoagulant, anti-inflammatory, anti-hyperlipidemic, hypocholesterolemic, hepatoprotective, and insecticidal activity Yu-Qing *et al*., (2016) as animal feed (Abd El-Galil and Amin, 2017) and as prebiotic in food industry (Shalaby and Amin, 2019). Chemical composition of seaweeds varies with environment conditions such as temperature air conditions, season, geographical origin, physiological maturity Yaich *et al*., (2011).

Free radicals and reactive oxygen species (ROS) includes $O^{\cdot}$, $O^{\cdot}H$, $LOO^{\cdot}$ causes damage toward biomolecules, oxidative stress and responsible for diseases such as cancer, diabetes, cardiovascular, atherosclerosis, cataracts, neurological disorders, ageing diseases and other diseases. Antioxidants are very important in controlling oxidative stress that controlling diseases in human. Marine algae showed a strong antioxidant activity and don't have any side effects as some synthetic antioxidants. Antioxidants facilitate as a defense against free radicals that generated as byproducts of biological reactions, pollutants and radiation.

Antioxidants protect human body from oxidative damage by scavenging the free radicals. Natural antioxidants such as vitamins, polysaccharides, phenolic compounds, chlorophylls, carotenoids, alkaloids, flavonoids, terpenes and phytosterols and other phytochemical compounds in plants and algae such as *Ulva lactuca* are more important than synthetic antioxidants because natural antioxidants have less or no side effects as synthetic antioxidants Meenakshi *et al.*, (2012).

Microbes such as bacteria and fungi are responsible for diseases in human, crops, vegetables, fruits, animals, fish, rabbits and poultry. *Ulva lactuca* extracts showed antimicrobial activity against these microbes Alghazeer *et al*., (2013).

Parasites are very harmful for human and plants. *Ulva lactuca* extract was used in controlling parasites such as *Culex pipiens* Abbassy *et al*., (2014).

In this work I hope to spot light on *Ulva lactuca* and determine its chemical composition by quantitative and qualitative tests and its biological activities as antioxidant, bactericide, and fungicide and as insecticide where we should work for exploring cheap, safe and available alternative sources of natural pesticides.

MATERIALS AND METHODS

Collection of algae (*Ulva lactuca*)

Algae were collected from Mediterranean Sea (Abu Qir) and washed with sea water to remove sand pebbles, epiphytes, and shells, then the algae were brought to the laboratory in plastic bags, washed with diluted solution of sodium chloride then distilled water. The algae were shade dried, grounded in an electric mixer and stored in refrigerator for further using.

Ulva lactuca was identified by professors of microbiology and phycology, Faculty of Science, Zagazig university, as follows

Division: chlorophyta
Subdivision: chlorophytina
Class: cladosiphorophyceae
Family: Ulvaceae
e.g : *Ulva lactuca*

Extraction of *Ulva lactuca* phytochemical

Ten grams of *Ulva lactuca* were dissolved in 100 ml of the solvent (distilled water, acetone, ethanol, ethyl acetate, methanol, benzene, chloroform, hexane or petroleum ether). Stirring for 1hour at 6000 rpm, filtration and the extracts were kept in dark bottles in refrigerator for using (Dhasarathan and Theriappan, 2011).

Qualitative phytochemical analyses by chemical tests (table1) as mentioned by (Sakthieaswari and Srisudha, 2016)

Detection of carbohydrates

Molisch's test

1ml of algal extract and 2 drops of 1% alpha-naphthol were mixed in a test tube and shaked well, then 2 ml of concentrated sulfuric acid were added slowly along the

sides of the test tubes. At the junction of sugar solution and the acid, a purple-violet ring appears indicating the presence of carbohydrate.

Fehling's test

1 ml of algal extract and 2 ml of Fehling reagent were mixed and shaked together in test tube. Then the mixture was boiled for (3:5 min.). any color except the blue is positive and indicated the presence of carbohydrates.

Benedict's test

1 ml of algal extract and 2 ml of Benedict reagent were mixed and shaked together in test tube. Then the mixture was boiled for (3:5 min.). any color except the blue is positive and indicated the presence of carbohydrates.

Detection of Protein

Ninhydrine test

1 ml of algal extract was mixed, shaked and boiled with drops of 0.2% solution of Ninhydrine for 15 min., Appearance of purple color indicated the presence of free amino acids.

Detection of Phytosterols and Triterpenoids

Salkowski test

1 ml of algal extract was mixed with 1 ml chloroform, then 2 ml of concentrated sulfuric acid were added slowly along the sides of the test tubes. Two layers were formed. The upper layer of chloroform turns red indicated the presence of phytosterols and the lower layer of sulphuric acid turns yellow color indicated the presence of triterpenoids.

Detection of Alkaloids

Wagner's test

1 ml of algal extract was mixed and shaked with 1 ml of Wagner's reagent [Solution of iodine in potassium iodide]. a reddish brown precipitate was appeared and indicated the presence of alkaloid.

Detection of Tannins

Ferric chloride test

1 ml of algal extract was mixed and shaked with 1 to 2 drops of diluted ferric chloride 10% solution. A red, blue, green, or purple colors indicated the presence of tannins.

Detection of Saponins

Froth Test

1 ml of algal extract was diluted using distilled water to 20 ml and shaken for 15 minutes. Formation of 1 cm layer of foam indicated the presence of saponins. Persistence of produced foam for ten minutes indicated the presence of saponins.

Test of Gardiac glycosides

Legal’s test

1 ml of algal extract was added to 2 ml of pyridine, a few drops of sodium nitroprusside solutions and drops of 20% sodium hydroxide solution were added. Appearance of pink to red colour indicated the presence of glycosides.

Test of Anthraquinones

Modified Borntrager's test

1 ml of algal extract, 1 ml of $FeCl_3$ and 1 ml of Dil. HCl were mixed and heated for 5 minutes then 1 ml of benzene and 1 ml of $CHCl_3$ were added and shaked well. Separate the organic layer and 2 ml of NH_3 were added. Ammonia layer turns red.

Test of Flavonoids

Alkaline Reagent Test

1 ml of algal extract, a few drops of diluted sodium hydroxide were mixed. An intense yellow colour was produced in the algal extract, which become colorless and addition of a few drops of diluted acid indicated the presence of flavonoids.

Quantitative phytochemical tests

Primary metabolites (% of DW) (Table 2)

Determination of Carbohydrates

Total carbohydrate was estimated by phenol-sulphuric acid method of Dubois *et al*., (1956) using glucose as standard.

Determination of Proteins

Total protein was calculated using the elemental N determination by the nitrogen-protein conversion factor of 6.25 according to (AOAC, 1995).

Determination of Lipids

Total lipids were estimated according to (AOAC, 2000).

Determination of Moisture

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

Determination of Ash

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

Determination of Fiber

ANKOM AOCS approved procedure Ba 6a-05& instrument instruction manual of ANKOM 2000 fiber analyzer

Determination of Minerals

Determination of Minerals was achieved by using Flame Atomic Absorption. Savant AA (GBC Scientific Equipment) in the central lab of faculty of Science Ain Shams university, Egypt. (Table 3).

Determination of Calories

It was calculated by the following equation:

Calories (kcal.100g $^{-1}$) = 4 • protein (%) + 9 • lipids (%) + 4 • carbohydrate (%)

Secondary metabolites (Table 4)

Alkaloids determination by (Harborne, 1973).

2.5 g of *Ulva lactuca* and 100 ml of 10% acetic acid in ethanol were added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium Hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed by diluted ammonium hydroxide and then was filtered. The residue is the alkaloid.

Saponins determination by (Obdoni and Ochuko, 2001)

20 g_s of *Ulva lactuca* and 100 cm_3 of 20% aqueous ethanol were mixed. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The combined extracts to were reduced 40 ml over water bath at about 90 °c. The concentrate was transferred into a reparatory funnel and 20 ml of diethyl ether were added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After

evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage which was dried and weighed.

Total terpenoids determination

Total terpenoids content were determined by (Ebrahimzadeh and Niknam, 1998) 5ml of extract was dried in an oven at 100 °C for 1 h. After cooling; 5 ml freshly prepared vanillin reagent (0.7% in 65% H_2SO_4) were added. Then were heated at (60±1)°C in a water bath for 1 h. after cooling in crushed ice bath the absorbance was measured at 473 nm within 1 minute against blank prepared by using distilled water instead of terpenoid solution. saponin used for preparation of standard curve.

Total pigments determination

Determination of chlorophyll a, chlorophyll b and total carotenoids according to Balamurugan *et al*., (2013) as follows

0.5 g of dry weight of *Ulva lactuca* was homogenized manually using pestle and morter and the pigments were extracted in 100% acetone and stored in the dark at -20 ºC for 18 hrs. The pellet was discarded and the supernatant was separated which contains the pigments. Equations for calculating concentration of pigments were as follows:

Ca (chlorophyll a) = $11.75[A_{662}] - 2.35\ [A_{645}]$;

Cb (chlorophyll b) = $18.61\ [A_{645}] - 3.96\ [A_{662}]$,

total carotenoids = $(1000\ [A_{450}] - 2.270 \times Ca - 81.4 \times Cb)/227$

Phenolic compounds determination (fig.1)

Total phenolic compounds content of nine solvents of nine extracts of *Ulva lactuca* were determined by the colorimetric method of (Shahidi and Naczk, 1995) Using 0.5 ml of each extract, 0.5 ml Folin reagent and 8 ml of distilled water then shaking for 2 minutes then 1 ml Na_2CO_3 was added, the blue color developed was determined after 1 hour at 725 nm against blank. Results were expressed in g of Gallic acid / $100g_s$ of dry weight of *Ulva lactuca*.

Total flavonoids determination (Fig.2)

Total flavonoids contents of nine solvents of nine extracts of *Ulva lactuca* were determined by the aluminum chloride colorimetric assay according to Marinova *et al*., (1995) An aliquot (0.5 ml) of each extract was added to 4 ml distilled water and 0.3 ml of 5% $NaNO_2$ was added. After 5 min. 0.3 ml of 10% $AlCl_3$ was added and after 6 minutes, 2 ml 1M NaOH were added and the total volume was made up to 10 ml with distilled H_2O. The solution was mixed well and the absorbance was measured against the blank at 510 nm. Total flavonoids were expressed as g quercetin equivalent/100 g_s dry weight of *Ulva Lactuca*.

Fourier-transform infrared spectroscopy (FT-IR) of *Ulva lactuca*. (Fig. 3), (Table 5).

The chemical composition of active groups of *Ulva lactuca* were determined by NICOLET- 6700- FT/IR- Thermo Scientific in the central lab of faculty of Science Ain Shams university, Egypt and the spectra were recorded in the wavelength interval of 4000 to 400nm.

Antioxidant activity of *Ulva lactuca*

Antioxidant activity of *Ulva lactuca* According to the method of Gulluce *et al*., (2004) Adding100,200,300,400,500,600,700,800,900,1000 µl of *Ulva lactuca* extract of distilled water solvent (1g/10 ml solvent) and raised to 1 ml by ethanol and was added to 4 ml of DPPH (0.1mM of 2.2'- biphenyl picryl hydrazyl (DPPH) . After 30 min. of incubation period at room temperature in the dark, the absorbance was read against the blank at 517 nm. Inhibition of free radical DPPH was calculated according to the following equation:

% Scavenging activity = (A control –A sample /A control) × 100

Antioxidant activity of the nine extracts of nine solvents of *Ulva lactuca* (Fig. 4)

1000 µl of each one of the nine extracts of *Ulva lactuca* was added to 4 ml of DPPH (0.1mM of 2.2'- biphenyl picryl hydrazyl (DPPH). After 30 min. of incubation period at room temperature in the dark, the absorbance was read against the blank at 517 nm. Inhibition of free radical DPPH was calculated according to the following equation:

% Scavenging activity = (A control –A sample /A control) × 100

Antimicrobial activity of *Ulva* lactuca by Kirby-Bauer Method (Table 2).

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method Bauer, *et al.*, (1966). Briefly, 100 µl of the test bacteria was grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria 10^5 Pfaller, *et al*., (1988). 100 µl of bacterial suspension was spread onto agar plates corresponding to the broth in which they were maintained.

Of the many media available, (NCCLS, 1997) recommends Mueller-Hinton agar due to: it results in good batch-to-batch reproducibility.

Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the (NCCLS, 2003).

Plates inoculated with the Gram (-) bacteria *Desulfomonas pigra* ATCC 29098T and were incubated at 35-37^{o}C for 24-48h then the diameters of the inhibition zones were measured in millimeters Bauer *et al.,* (1966).

Standard discs of Ampicillin (Antibacterial agent), served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (distilled water, chloroform, DMSO) were used as a negative control.

The agar used is Meuller-Hinton agar that is rigorously tested for composition and pH. Further the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10µl of tested concentration of the stock solutions.

When a filter paper disc impregnated with a tested chemical is placed on agar the chemical will diffuse from the disc into the agar. This diffusion will place the chemical in the agar only around the disc. The solubility of the chemical and its molecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or" Clear zone".

For the disc diffusion, the zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards (NCCLS, 1993).

Agar-based methods such as E-test and disk diffusion can be good alternatives because they are simpler and faster than broth-based methods Liebowitz, *et al*., (2001), Matar, *et al*., (2003).

Insecticidal activity of *Ulva lactuca*

Insecticidal activity of *Ulva lactuca* extracts was investigated against *Culex pipiens* according to (WHO, 1981) Batches of 25 early 3rd instar larvae of *Culex pipiens* in 100 ml of each concentration (1, 2, 5, 10, 30 and 50 mg/ml) and distilled water (control) contained in beakers. Three replicates were used for each concentration. Swimming activity was observed and recorded after 24h of treatment.

Lethal percentage was calculated and lethal concentrations required to produce 50% mortality (LC_{50}) for larvae was concluded.

Statistical analysis

The statistical package SPSS (version 20) was used for statistical analysis. Propit analysis was performed to calculate the medium lethal concentration (LC_{50}) for determining insecticidal activity. The medium effective concentration (EC_{50}) for determining bactericidal and fungicidal activity and the medium inhibition concentration (IC_{50}) for determining antioxidant activity were calculated by linear regression analysis.

RESULTS AND DISCUSSION

In this work new sources of biologically active compounds were examined in algae. Marine environment is rich in more creatures that should be studied to explore new natural bactericides, fungicides, insecticides and antibiotic and cause no side effects for human or environment. Bacteria and fungi are pathogens that cause diseases for plants, animals, fish and human and cause harmful effects in our environment. Green macro algae such as *ulva lactuca* are safe, easy getting and have many phytochemicals.

In this study nine solvents varying in their polarity for making extracts were used such as Acetone, Benzene, Chloroform, Distilled water, Ethanol, Ethyl acetate, Hexane, Methanol and Petroleum ether. Using these solvents to identify the best solvent which has most of biologically active components of *Ulva lactuca* by doing many qualitative and quantitative tests. Many qualitative organic tests were summarized in Table 1. Results of Table 1 indicate the presence of many phytochemical constituents in *Ulva lactuca*.

Table 1: Qualitative organic tests of *Ulva lactuca*

	Acetone	Benzene	Chloroform	Distilled water	Ethanol	Ethyl acetate	Hexane	Methanol	Petroleum ether
Mulish	+	+	+	+	+	+	+	+	+
Fehling	+	+	+	+	+	+	+	+	+
Benedict	+	+	+	+	+	+	+	+	+
Ninhydrine	+	-	+	+	+	+	-	+	-
Cardiac Glycosides	+	-	-	+	+	-	-	+	-
Saponines	++++	++	+	++++	++++	+++	++	++++	++
Phytosterols	+	-	-	+	+	-	-	+	-
Terpenoids	+	-	-	+	+	-	-	+	-
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoids	++	+	+	+6	++	++	+	++	+
Anthraquinones	+	+	+	+	+	+	+	+	+
Tannins	-	-	-	-	-	-	-	-	-

Presence = (+) Absence = (-)

The chemical composition of primary metabolities of *Ulva lactuca* was summarized in Table 2. Minerals content of *Ulva lactuca* was summarized in Table 3. Calories (kcal.100g $^{-1}$) = 4 • protein (%) + 9 • lipids (%) + 4 • carbohydrate (%) = 176.78.

Table 2: Primary metabolities of *Ulva lactuca*

Component	Moisture	Ash	Carbohydrates	Protein	Lipids	Fiber
%(w-w)	7.9%	42.2%	26.41%	17.2%	0.26%	6.03%

Table 3: Minerals in *Ulva lactuca*

Mineral	Cupper	Zinc	Cadmium	Lead	Ferrous	Manganese	Nickel	Cobalt	Chromium	Magnesium	Calcium	Sodium	Potassium
%w-w	0.0009	0.0012	0.00011	0.0013	0.0411	0.0021	0.0009	0.0004	0.0002	1.2880	5.7608	3.2017	1.2895

Atomic absorption spectrophotometer was used to detect the presence of several minerals where these elements play important roles in the metabolism of plants and human.

The chemical composition of Secondary metabolities of *Ulva lactuca* was summarized in Table 4.

Table 4: Secondary metabolities of *Ulva lactuca*

Component	Terpines	Alkaloids	Saponines	Chlorophyll a	Chlorophyll b	Carptenoids	Phenolic compounds	Flavonoids
%(w-w)	0.00666	4.4	10	0.468449	0.2975508	0.116722403	2.38	0.717

Phenolic compounds were evaluated using nine extracts of *Ulva lactuca* and extract of distilled water exhibited the highest percent of phenolic compounds (Fig. 1).

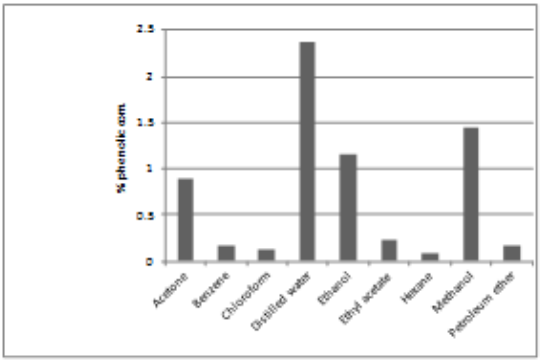

Fig. 1: Phenolic compounds of nine extracts of *Ulva lactuca*.

Flavonoids were evaluated using nine extracts of *Ulva lactuca* and extract of distilled water exhibited the highest percent of Flavonoids (Fig. 2).

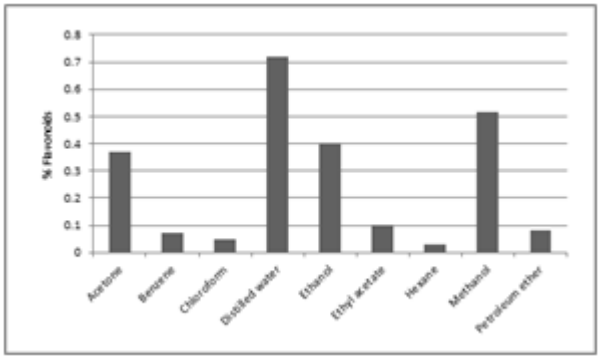

Fig. 2: Flavonoids of nine extracts of *Ulva lactuca*

Tables and figures indicated the chemical composition of *Ulva lactuca* and proved that *Ulva lactuca* is a macro algal rich in many primary and secondary metabolities that have biological activity especially in polar solvents especially in distilled water as obtained in Barot *et al*., (2016).

FT-IR spectroscopy of *Ulva lactuca* showed more active groups that previous indicated by quantitative and qualitative tests as follows in (table 5 & fig.3) and these

results emphasized the results obtained from qualitative and quantitative tests of phytochemical components of *Ulva lactuca* and revealed the presence of many primary and secondary metabolites.

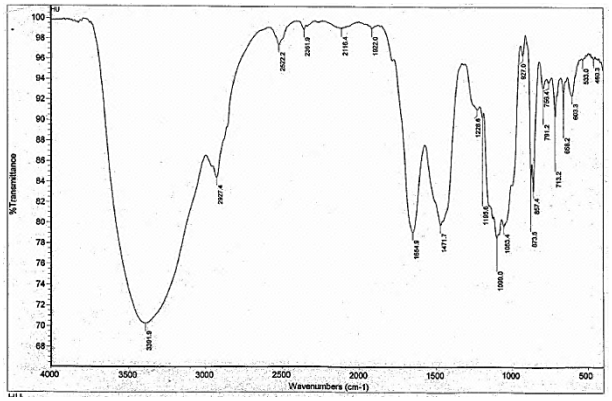

Fig. 3: FT-IR spectroscopy of *Ulva lactuca*.

Table 5: FT-IR spectroscopy of *Ulva lactuca*

Frequency (cm^{-1})	Bond/ stretching	Functional groups
3391.9	O-H stretch & N-H stretch	Alcohols , Phenols, Carbonyl compounds
2927.4 & 2522.2	C-H stretch	Alkanes
2361.9	N=C=O stretch	Unsaturated nitrogen compounds(Isocyanates)
2116.4	C-H stretch	Alkynes
1654.9	C=O stretch	Carbonyl compounds containing N-O bond (Amides) and aldehydes and ketones
1471.7	C-C stretch	Aromatics
1228.6	C-N stretch	Aliphatic amines
1195.6	C-O stretch	Esters
1099	C-O stretch	Secondary alcohols and ethers
927	O-H bending	Carboxylic acids
756.4	C-Cl stretch	Alkyl halide
713.2	C-Br stretch	Alkyl halide
658.2	S=O stretch	Sulfonic acid

Biological activity of *Ulva lactuca*

Antimicrobial activity of *Ulva lactuca*

Most of extracts of *Ulva lactuca* showed antibacterial and antifungal activities, but the strongest and most effective extract was extract of distilled water which showed an excellent results in quantitative and qualitative tests. Antimicrobial of *Ulva lactuca* was with higher inhibition zone than the used standard in most tested pathogens.

Ulva lactuca showed higher antimicrobial activity against human pathogens such as *Candida ablicans* and all tested bacteria and against fish pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeuroginasa*, but moderate antimicrobial activity against *Klebsiella* sp. *Ulva lactuca* also showed higher antimicrobial activity against animal pathogens such as *Candida ablicans, Aspergillus fugigatus* and *Bacillus subtilus*. It also showed antimicrobial activity against plant pathogens such as *Fusarium oxysporum*, *Penicellium* sp., *Alternaria alternate*, *Pseudomonas aeuroginasa* and *Bacillus subtilus*. The results of antimicrobial activity of *Ulva lactuca* were summarized in table 6 where the control was DMSO, but the standard of antibacterial activity was Ampicillin and the standard of antifungal activity was Amphotericin B.

Table 6: Antimicrobial activity of *Ulva lactuca*

Microorganism		Standard	Inhibition zone diameter (mm/mg)
Bacillus subtilus	+G	32	38
Escherichia coli	G-	30	30
Klebsiella sp.	G-	18	17
Pseudomonas aeuroginasa	G-	28	36
Staphylococcus aureus	G+	26	26
Streptococcus faecalis	G+	30	30
Neisseria gonorrhoeae	G-	25	25
Alternaria alternate	F	-	55
Aspergillus fugigatus	F	14	60
Candida ablicans	Y	16	60
Fusarium oxysporum,	F	-	60
Penicellium sp.	F	-	48

F = Fungus /G+ = Gram positive bacterium/ G- = Gram negative bacterium/ Y = Yeast/- = not determined

Antioxidant activity

Natural antioxidants are available and have less or non-side effects as some synthetic antioxidants. All extracts of *Ulva lactuca* showed antioxidant activity at 1000 µl of each extract of each solvent of *Ulva lactuca* (Fig. 4), but the highest antioxidant activity was by the extract of distilled water with IC_{50} = 9.6 mg/ml.

The higher antioxidant activity of *Ulva lactuca* is sure due to its high content of primary and secondary metabolites as determined in Yang *et al.*, (2006) where presence of polysaccharides, phenolic compounds, chlorophylls, carotenoids, flavonoids, alkaloids, terpenes and phytosterols is responsible for antioxidant activity of *Ulva lactuca* Meenakshi *et al.*, (2012).

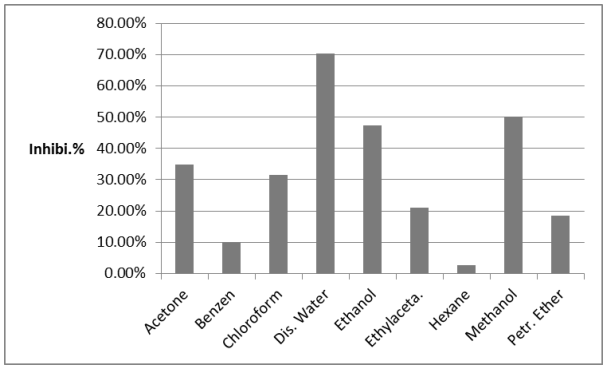

Fig. 4: Antioxidant activity of nine extracts of *Ulva lactuca*

Insecticidal activity

Last decades researches using chemical control for fighting pests by using chemical compounds such as organophosphorous, chlorinated, carbamate and pyrethroid pesticides. Unfortunately these chemical compounds have bad effects on plants, animals, human and environment. On the other hand pests gained resistance against theses chemical compounds. Controlling mosquitoes needs more researches that based on natural sources that added easily to water and can't cause any side effects on plants, human and environment. Distilled water extract of *Ulva lactuca* showed insecticidal effect on mosquito larva and prevent any birth of new mosquitoes. Many researchers made many insecticides using algae extracts such as Abbassy *et al.*, (2014). Hsiao *et al.*, (2004) mentioned that AChE plays an important role in neurotransmission at cholinergic synapses by rapidly hydrolyzing the excitatory neurotransmitter acetylcholine into choline and acetic acid. (Pradhan and

Mishra 1998) added that insecticides inhibit cholinesterase (ChE) activity leading to accumulation of acetylcholine (ACh) at the synapses with consequence disruption of nervous activity in different animals. (Hansch and Leo, 1995) and Francis *et al*., (2006) indicated that the bioactivity of insecticides depends on two factors; first, the compound hydrophopicity that models the compound ability to penetrate the organism biophase to reach the bioreceptor and facilitates the hydrophobic interaction with the active site, and second, the electrophilicity of the compound phosphorus atom that model the compound reactivity towards the enzyme active site. All nine extracts

showed insecticidal activity, but extracts of nonpolar solvents showed higher insecticidal activity than extracts of polar solvents and that was due to that Acethylcholine esterase is hydrophobic in insects, but hydrophilic in human where nonpolar extracts can easily penetrate into tissue and reach to AChE and inhibit AChE more effectively than polar extracts. Polar extracts especially aqueous extract should be preferred than nonpolar extracts where it's safe, cheap and surely doesn't cause any side effects on surrounded environment or human. (LC_{50} of aquous extract = 7.5 mg/ml). Distilled water was the most suitable solvent for extraction phytochemical compounds of *Ulva lactuca* such as carbohydrates, proteins, phenolic compounds, flavonoids and saponines where carbohydrates and proteins are water soluble while phenolic compounds, flavonoids and saponines are polar compounds. On the other hand there were alkaloids and terpenoids in the extract of distilled water however they are not soluble in water. Presence of alkaloids and terpenoides may be due to the moment dipole of polar solvents that make induction to the non-polar compounds that haven't dipole and so non-polar compounds can dissolve in polar solvents (Prasetyo, 2013).

In this research many extracts of *Ulva lactuca* were used as insecticides and all of them are good. Non polar exracts of *Ulva lactuca* are better than polar extracts of *Ulva lactuca*, but I think using aqueous extract is safe for human and environment as insecticides on the other hand polar extracts especially extract of distilled water was the best as antimicrobial.

In these days we should search about new alternative sources that could be used as biopesticides and should be used in agriculture. *Ulva lactuca* has many biological activities due to its phytochemical constituents such as polysaccharides, protein, lipids, pigments, polyphenols, saponines, flavonoids, alkaloids, terpenes, chlorophylls, carotenoids that could be used as antimicrobial and antioxidant. Manchu *et al*., (2014), (Sakthieaswari and Srisudha, 2016), (Pradhan and Mishra, 1998) and Chojnacka *et al*., (2012).

Qualitative, quantitative tests and FT-IR spectroscopy analysis of Ulva lactuca showed the presence of many primary and secondary metabolites that called phytochemical components and showed a good antimicrobial, insecticides and antioxidant activity. Extract of distilled water was the best extract that has many of these phytochemical components and was the best extract as antimicrobial, but not the best extract as insecticide compared to used nonpolar solvents. My view is to find a solution for low and lower income countries that face physical problems. They should get pesticides during growing economic crops and also using pesticides during the periods between planting one crop and another otherwise they will face problems of snakes and mices attack during those periods.

CONCLUSION

In this paper I hope to make a spot light on *Ulva lactuca* as a green macro algal has a good chemical structure. Its aqueous extracts should be used as biopesticide against bacteria, fungi and insects that cause diseases in human, plants, animals, fish and poultry without any harmful effects as an alternative source of natural pesticides comparing to other synthetic pesticides which exhibit side effects on environment.

In the near future the researcher is looking forward to series of marine organisms that have many phytochemical constituents and showe many biological activities and on the other hand safe and not toxic such as *Ulva lactuca*. Also the researcher is looking forward of making application of using *Ulva lactuca* as natural pesticide in the field against plant pathogens and may be in Fish farms against fish pathogens, poultry farms against poultry pathogens and rabbit farms against rabbits pathogens. Also using extracts of *Ulva lactuca* in food industry as supplement for fighting spoilage of foods.

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