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Phytochemical Constituents and Antimicrobial Activity of Marine Green Seaweed *Ulva lactuca*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The aim of this study is to determine the presence of *Ulva* seaweeds, possibly by analyzing the quality of seaweed powder extracts and some organic solvents. It belongs to the order Ulvaales. *Ulva lactuca* is a widespread macro algae growing the Mediterranean coast phylum Chlorophyta, commonly known as "sea lettuce". Collected from the Gulf of Mannar Tamil Nadu, India. Dry the *Ulva* seaweeds and grind it in a food processor until it becomes a fine powder. The powder is dried in an oven at 60^0c for 24 hours. Alkaloids, Flavonoids, Saponins and Tannins. dried seaweed nutrients rich in energy 252.72kcal/mg/gm, carbohydrates 49.63mg/gm, less protein 12.21mg/gm, fat 1.04mg/gm less than gm, high crude fiber, ash15.8mg/g, high moisture 21.74mg/g. in the UV – visible spectrum of seaweed extract TLC analysis 200-800nm found a high value of0.925, to0.477, GC-MS RF value of8.014, and FTIR analysis of seaweeds *Ulva* found a high value of 618 to 3525cm-1 and HPLC showed retention time 2.213- 3.730. The antimicrobial studies maximum inhibition zone, minimum concentrations, minimum inhibition zone and maximum activity of *Klebsiella pneumoniae*, are methanol extract 10mm, *Staphylococcus aureus*, ethyl acetate extract11mm, *Candida albicans* 10.5mm were respectively.

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Keywords: Qualitative analysis; chromatographic technique; antimicrobial activity.

1. INTRODUCTION

Algae are considered as ecologically and biologically important components in the marine ecosystems. Seaweeds make a substantial contribution to marine ecosystems. Seaweeds make a macro and micro trace elements and their concentrations are much higher than terrestrial plants. Ambhore and Whankatte [1]. [2] Marine macro algae as a source of bioactive compounds thatresult in secondary metabolites with various biological activities. The orderulvaales it is used to asian food condiment. Raj GA. et al. [2]. [3] Seaweed are rich in bioactive substances such aspolysaccharides,vitamins, minerals, poly phenols, proteins, lipids, and has antibacterial, antifungal, and other functions. Seaweed contain plant products such as flavonoids and tannins[3] Shankhadarwar [4] Seaweeds have many uses[5]have been used in medicines cosmetics, energy, fertilizers, and industrial agar and alginate biosynthesis of minerals, vitamins, phenols, and other bio actives. Hossam S.EI-Beltagi et al. [6]. Marine algae are one of the most commonly utilized functional food and therapeutic agents in many parts of the world and beneficial secondary metabolities many of which show Haniffa [3]. Metabolities in algae which include polysaccharide, fattyacids, flavonids, terpenoids, alkaloids, quinines, sterols, and peptides lipids. Viraj Chabake and Sakshi Chaubal [7]. Jayabarath[8] the nutritional content of Ulvadried seaweed powder in the human body consists of carbohydrates, which indicates that the main ingredient of seaweeds is rich in nutrients. Abdullah Rasyid [9]. The high concentrations of functional carbohydrates,and dietary fiber content, but has very little lipid content with neutral lipids and glycolipids *Ulva lactuca* comprises on to 3- fatty acids components. Rehana Raj et al. [10,11]Green algae (division Chlorophyta), found nearest the shore in shallow waters and usually growing as thread like filaments, irregular sheets, or branching and nutritional value of great variation. (Alaeldein et al., 2013) Nutrient composition of seaweeds green and red seaweed higher protein contents than brown seaweeds. Proteins are composed of several amino acids and their marine seaweeds has low amount of energy. Most seaweed has more ash contents, seaweeds are important source of metabolic reaction in human animal health, and enzymatic regulation of lipids, carbohydrates and protein metabolism. Lalitha and Dhandapani [12].The antibacterial properties of green, brown, and red algae were evaluated and the effectiveness of different polysaccharides, fatty acids, phyllotannins, pigments, lectins, alkaloids, terpenoids and halogenated compounds were demonstrated[13].Maria Jose Perez, Elena Falque and Herminia Dominguez [14]. Methanol was found to be more active against Gram negative then water, and Gram-negative bacterial isolated had higher activity at the minimum inhibitory concentrations. Johnsichristobel et al.(2011). This study examined various organic extractsuch as acetone, ethanol, ethyl acetate, methanol and other Phytochemical componentsin *Ulva* seaweed extracts using thin layer chromatographic gas chromatography, fourier transform liquid chromatography, UV- visible spectroscopic, Methods were used to determined the activity of bacteria**.**

2. MATERIALS AND METHODS

2.1 Seaweed Collection

The Ulva sample (Fig. 1) was collected from the Gulf of Mannar near muttom kannayakumari district of Tamil Nadu. The samples were washed thoroughly with seawater followed by sterile water, air dried, cut into small pieces, and ground to a fine powder.

2.2 Extraction of Seaweeds

Pour 5g of seaweed powder into a 100ml distilled Erlanmar flask, place on a warm plate and stir magnetically for 15 minutes. Purify the extract using a Buchner funnel and remove the supernatant using Whatman no.1 to 40c for storage and processing.

2.3 Qualitative Analysis [15]

The green seaweed samples were used for the phy tochemical quality analysis of the presence of significant phytochemical studies.

2.4 Various Nutritional Properties of Seaweeds in *Ulva* were Tested by

According to fssai cereals and Cereal Product Handbook (Section 8.7) Page No:19:2016.

2.5 UV-Visible Spectrophotometry

The extracts were centrifuged at 3000rpm for 10min. 200-900nm Shimazdu spectrophotometer analysis.

2.6 FTIR Analysis [16]

Hossam et al. [6] infrared reflectance vibration spectra were carried out on powdered samples using a spectrometer with instrument resolution of about (1/cm)in the wave number region(4000-400/cm) at room temperature were performed.

2.7 HPLC Separation of Phytochemical Constituents

Abdullah et al. [9] (Shimadzu,LC-10AT VP Series equipped with HPLC (VP series 6.1 software (Shimadzu) column temperature is maintained at 27^0C. using acetonitrile Water. Using a fine syringe, 200µl is injected as extract and excellent spectrum analysis is published. The product according to the storage time define.

2.8 TLC Analysis

Alaedein et al. [17] cut the prepared TLC paper to size. The sample mixture was dissolved in methanol and dropped onto one end of the TLC plate. Place the plate in the beaker containing the mobile phase with the end closest to the application sample in contact with the mobile phase and allow the chromatography to run for approximately 1-2 hours. (Level ¾ on the TLC board). The plate is dried at room temperature and the RF value of the sample can be determined using the formula below.

$$RF = \frac{\text{Distance moved by the solute (a-g)}}{\text{Distance moved by the solvent (A)}}$$

A-Solvent front, B-Sample spotted, a,b,c,d,e,f, and g Samples moved

2.9 GC-MS Analysis (Raubbin et al.,2020)

Gas chromatography mass spectrometry analysis of the ethyl acetate extract of *Ulva* seaweed extract was performed using a Shimadzu instruments gas chromatography to a one peak were recorded 8.014

3. ANTIBACTERIAL ACTIVITY OF SEAWEEDS A*sulva lactuca* EXTRACTS

3.1 Sample Preparation

Methanol, ethanol, acetone, ethyl acetate *Ulva* extract, seaweed extractwere air dried and 10mg of dry powder was dissolved in 10ml of various solvents. For the detection of bacterial isolates of Gram- positive *Staphylococcus aureus*, Gram-negative *Klebsiella pneumoniae*, and *Salmonella typhi*. The following types of fungi are used for *Candida albicans* prophylaxis. Three bacterial strains and one fungal group were obtained from VHNSN College culture collection of Department of Botany,Virudhunagar.

3.2 Antifungal Activity of Seaweeds *Ulva*

Inhibition tests of the fungal culture *Candida albicans* used in the examination of the green seaweed *Ulva* extract were performed on potato sucrose agar plates. MIC records the lowest content that inhibits microbial activity and the least fungal content.

3.3 *Ulva* Extract Preparation

This seaweed in a soxhlet extractor along with different solvents of increasing polarity, place each a soxhlet extractor for 24hours and afer evaporating in vaccum store the extracts at -20^0Cuntil the extract is used.

3.4 [9] Antibacterial Mininum Inhibitory Concentration Sensitivity Test

The test was performed on Muller- Hinton agar medium. The isolates were inoculated into nutrient medium and placed on a rotary shaker for 18h at 37^0C and subcultured in specal media. Single - cell colonies were inoculated into nutrient medium and cultured at 37^0c for 4h. Prepare Muller-Hinton agar medium and sterilize at 121^0Cfor 15 minutes. Pour sterile medium into the plate and test for 5minutes. Using pre-labeled sterile mushroom stopper agar plates make five wells on different aliquots (25µl,50µl,75µl, and 100µl), (20µl) of streptomycin standared and methanol, ethanol, acetone and ethyl acetate extracts used. Usesterile microtip to individually load *Ulva*powder into agar wells and incubate for 48hrs at 37^0c. Measure and evalute the results. The above procedure allows potato dextrose agar the target medium for fungal disease toreplacenutrients and the antibiotic ketocazolin (20µl) measuredafter 48hrs, of standard incubation at 25^oc.

4. RESULTS AND DISCUSSION

Ulva natural area provided fig-1[1]*Ulva lactuca* is a green macroalgae associated with destructivegreen tides found world wide. Green algae species *Ulva* (Chlorophyta). It is sea green algaecollected form Gulf of Mannar, near Muttom, in Kannayakumari district ofTamilNadu. Seaweeds has the wide range of synthetic products and is a source of many important elements. The main use of algae are human

medicine, food, fodder, fertilizer, paper and other industries. *Ulva lactuca* in addition, to the knowledge of the chemical constituents of seaweeds would further be valuable in discovering the actual medicinal value. This study is undertaken to analyses the phytochemical constituents, and to assess the antibacterial and antifungal potential a source of essential amino acids, to eat *Ulva* from green tide is safe, and its high content of proteins and unsaturated fat with a low ratio and also has the U*lva* grow in saline and waste water and has a higher ability were collected, fig-2 air dried seaweed and ground into fine powder. The powder was green in colour fig-3, Soluble in water and the pH of the powder was 7.2. *Ulva lactuca* seaweed extracts fig-4 seaweed extract was tested for the Phytochemical constituents such as ten tested Alkaloids, FlavonoidsGlycosides,saponins, Tannins,etc.,Qualitative tests, pertaining to phytochemical constituents and biomolecules, proves the presence of them.

Fig. 1. Natural Habit of *Ulva lactuca*

Fig. 2. Dry seaweeds

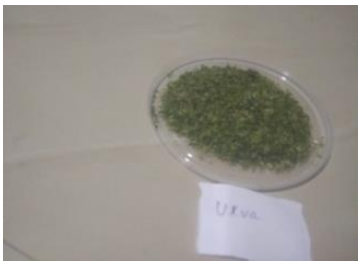

Fig. 3. Seaweed powder

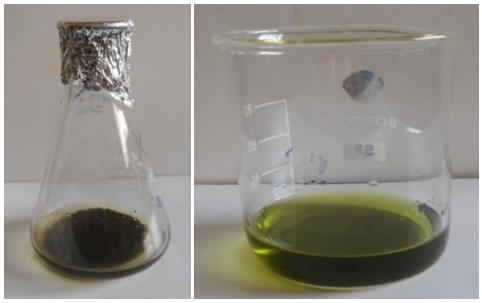

Fig. 4. Extraction of *Ulva lactuca* seaweed

Ulva lactuca Seaweed powder tested it was also estimated for the biomolcules such as Ash content, Energy, Carbohydrates, Crude fiber, Fat content, Proteins, Moisture,that the phytochemical constituents varied in the seaweed powder of the 7 constituents, Energy were high (256.72kcal/mg/gm) followed by Carbohydrates (49.63 mg/gm), Protein were low (12.21mg/gm), in the seaweed. Among the bio molecules in seaweed Fat content is low (1.04mg/gm), and the fiber content is high (12.71 mg/gm) in the seaweed has ash content is high 15.38(mg/gm), and high Moisture content 21.74 (mg/gm) respectively.The powder was extracted with and acetone, ethyl acetate, ethanol, methanol. The extracts was evaporated and the powder form was suspended in water and used for UV- visible,Spectrophotometric, FT-IR,HPLC. Phytochemical evaluation of various seaweed extracts.

Table 1. Qualitative analysis of *Ulva lactuca* Powder extract

Samples		Contents									
		T	S	F	A	P	St	Q	Ter	CG	Ph
Ulva lactuca	Acetone	-	+	-	+	+	-	-	-	-	-
	Ethanol	-	-	-	+	+	-	-	-	+	-
	E.Acetate	-	-	-	+	-	-	-	+	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-

Note =[9]T-Tannins,S-Saponins, F-Flavonoids, A- Alkaloids, P-Proteins, St-Sterioids, Q-Quinones, Ter-terpenoids, CG- Cardiac Glycosides, P-Phenols(+) Positive result(-) Negative result

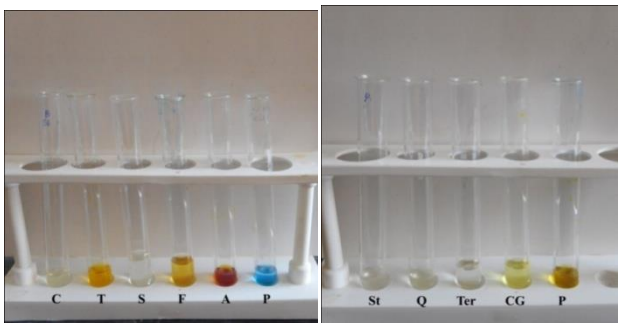

Fig. 5. Qualitative analysis of *Ulva lactuca*

Table2. Quantity of phytochemicals and biomolecules in *Ulva lactuca* Seaweed powder

Seaweed (On dry basis)	Ash content	Energy	Carbohydrates	Crude fiber	Fat content	Protein	Moisture
Ulva lactuca	15.38	256.72 Kcal/100g	49.63	12.71	1.04	12.21	21.74

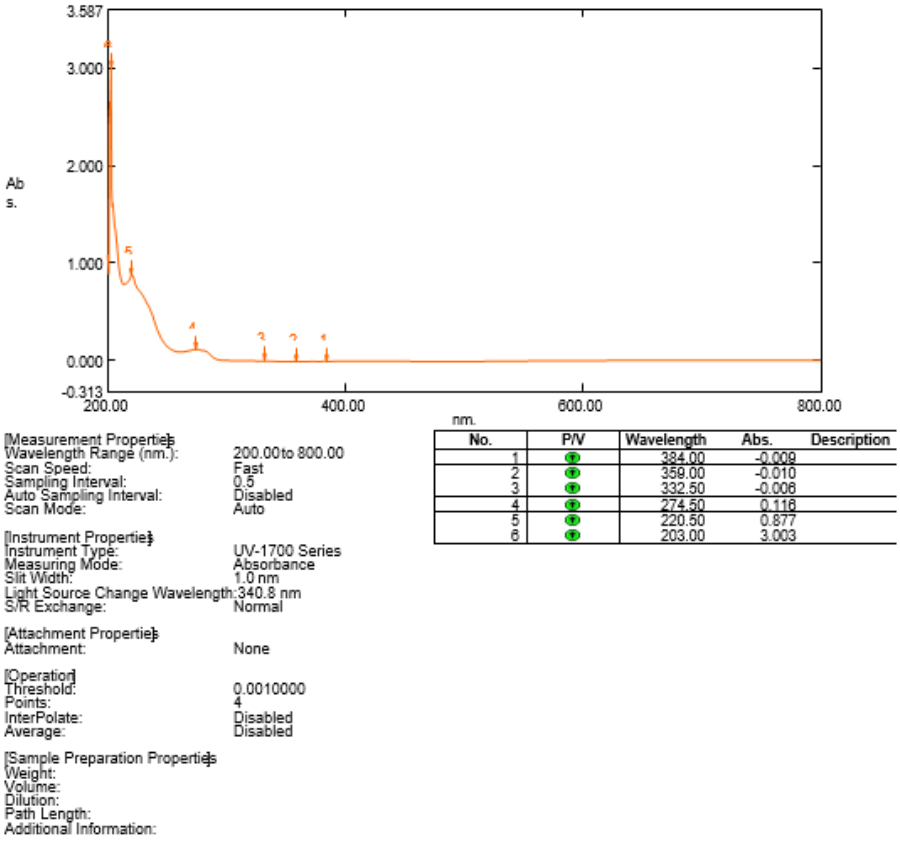

Fig. 6. Spectrophotometric analysis of seaweed as *Ulva lactuca*

4.1 Spectrophotometric Analysis of Seaweed as *Ulva lactuca*

UV-visible spectroscopy reveals that the *Ulva lactuca* extract was taken at the 200-800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectraprofile showed the six peaks from 384, 359, 332.50, 274.50, 220.50, 203 with the absorption.

FTIR spectrum of various organic solvents Acetone, Ethanol, Ethyl acetate, Methanol spectra for *Ulva lactuca* by various organic solvents the adsorption peaks are noted in 618.14 to 3525.65cm-1 the 618.14 peaks shows the Halogen compound (C-I) 703.97peaks shows the Alkyl and Aryl Halides C-Br stretching vibrations 744.47 peaks shows the OH group, N-H stretching Vibrations $1^0$and 2^0 bonds. 864.01 peak shows theC-CI stretching Vibrations,1015.45 peaks shows C-F stretching Vibrations, 1040.52 peaks shows thealcohols, also absorb in the region due to the C-O Stretch Vibrations,1073.31 peaks shows theCarboxylic Acids and Anhydrides. Stretching Vibrations 1124.42 shows the peaks C-OH Stretching vibrations, 1284.5 peaks shows the Alkyl ketones, 1366.47 peaks shows the alkenes C-H bending Vibrations. 1366.47peak 1366.47 indicates C-F stretching Vibrations. Peak 1447.48, and 1474.48 indicate alkanes NO_2 Stretching, and peak 1599.84 indicate –C=C- Stretching Vibration. 1726.17 indicate the Ketones C=O Stretching Vibration. The beaks at2346.24, 2881 .24, and 2981.74indicate the aldehydes H-C=O. The peak at2981.74 indicates the C-H Stretching vibration of alkanes. The peak at 3525.63 indicates Stretching of N-Hydehydes.

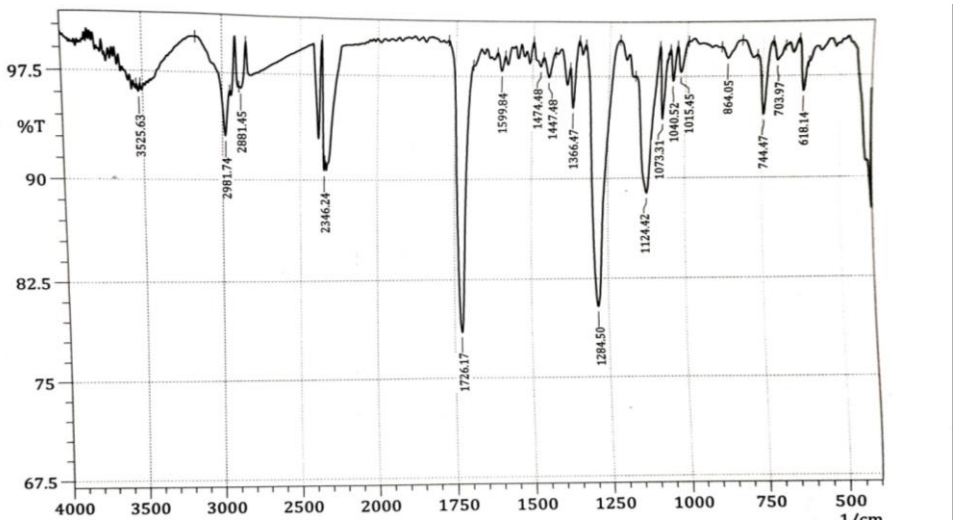

Fig. 7. FTIR Analysis of seaweeds as *Ulva lactuca*

Table 3. FTIR Analysis of seaweeds as *Ulva lactuca*

[10]S.NO	Peak	Intensity	Corr. intensity	Base (H)	Base(L)	Area	Corr.area
1	618.14	96.402	2.883	635.5	602.71	0.278	0.182
2	703.97	98.617	1.239	719.4	677.93	0.146	0.113
3	744.47	94.68	4.81	768.58	719.4	0.49	0.383
4	864.05	98.793	0.964	886.23	818.73	0.16	0.112
5	1015.45	97.819	1.977	1028.95	993.27	0.169	0.144
6	1040.52	97.171	2.373	1052.1	1028.95	0.162	0.116
7	1073.31	94 .399	4.848	1085.85	1059.81	0.351	0.263
8	1124.42	88.881	9.735	1163.96	1085.85	2.206	1.727
9	1284.5	80.373	19.51	1328.86	1217	4.165	4.124
10	1366.47	95.236	3.628	1377.08	1350.08	0.297	0.187
11	1447.48	97.421	1.586	1464.83	1426.26	0.286	0.122
12	1474.48	98.192	0.947	1499.55	1464.83	0.186	0.093
13	1599.84	97.902	1.142	1614.31	1589.23	0.164	0.062
14	1726.17	78.485	20.538	1772.46	1691.46	3.22	2.917
15	2346.24	90.67	3.308	2361.67	2339.49	0.593	0.154
16	2881.45	96.496	0.903	2893.02	2849.63	0.527	0.164
17	2981.74	93.151	3.637	3167.86	2952.81	2.226	0.413
18	3525.63	96.114	0.52	3538.17	3515.03	0.37	0.025

Table 4.Thin layer chromatographic technique Rf Vaue

S.NO	Sample fractions	Distance moved by the solvent (A) (CM)	Distance moved by the solute (CM)	RF (B/A)
1	Acetone a	4	3.7	0.925
2	Acetone b	4	1.1	0275
3	Ethanola	4.4	3.1	0.704
4	Ethanol b	4.4	0.9	0.204
5	Ethyl acetate a	4.4	3.6	0.818
6	Ethyl acetate b	4.4	2.1	0.477
7	Methanol a	4	3.7	0.925
8	Methanol b	4	1.1	0275

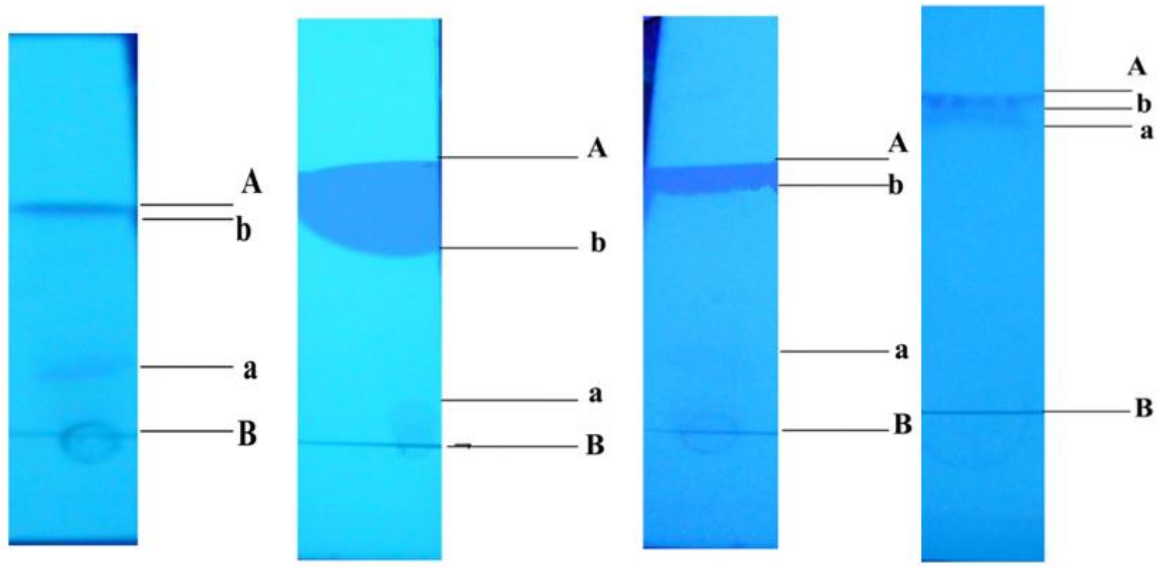

Fig. 8. Thin layer chromatographic analysis of seaweed as *Ulva lactuca*

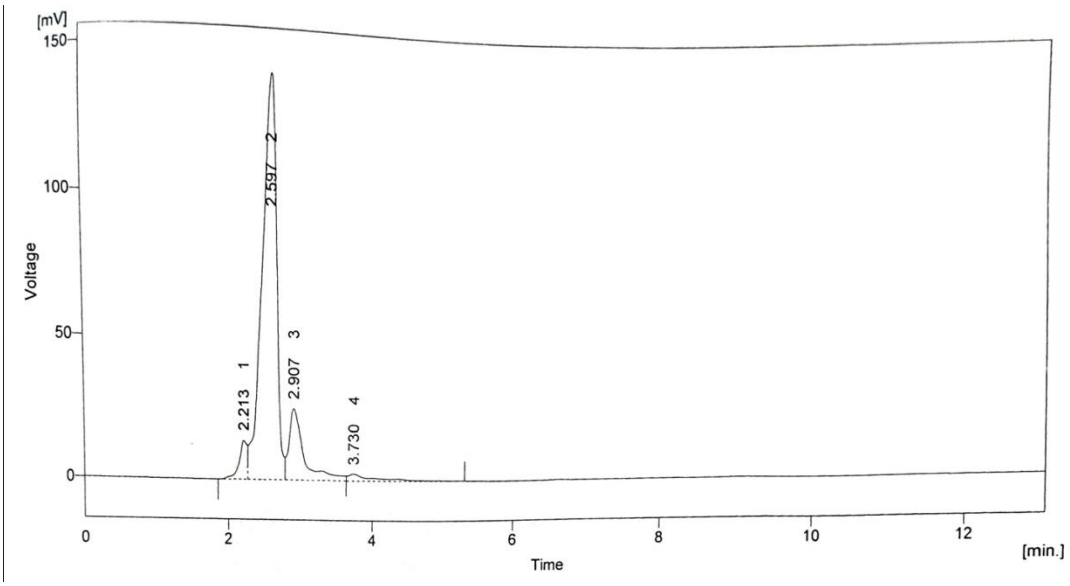

Fig. 9. HPLCChromatogram analysis ofseaweed as *Ulva lactuca*

4.2 GC-MS Analysis

GC-MS Chromatogram for used asmethanol extract of *Ulva lactuca* is were identified one peak value is were obtained 8.014.

4.3 TLC Analysis

The [12]chromatographic techniques such as thin layer chromatography (TLC) analysis was used to separate and isolate from the organic extractAcetone, Ethyl acetate, Ethnol, Methanol of Ulva lactuca. The solvent system of TLC was Chloroform: Methanol (19:1) was used and its RF value was detected.

4.4 HPLC Profile of *Ulva lactuca*

The qualitativeextracts of *Ulva lactuca* were 254nm baseline. Methanol *Ulva lactuca*fourpeak value were separated at different retention time viz., 2.213,2.597, 2..907, 3.730,were respectively.

4.5 Antibacterial Activity ofa *Ulva lactuca*

(Figs. 10-13) *Ulva* Shows the antibacterial properties ethanol, acetone, methanol, ethyl acetate. In these four extraction streptomycin (20µl) was added for various organic extracts (25µl, 50µl, 75µl and 100 µl),using bacteria separated by different solvents (Acetone, ethanol, Ethyl acetate) was measured according to water. The plates were incubated at 70c for 24hrs. The growth was of these bacteria was determined by measuring the area of the zone. It is clear from the result that the, inhibition was proportional to the amount of acetone, ethanol, ethyl acetate, methanol crude extract on theagar well of the 3 separations. In addition to water isolates tested, isolates1 and 3 showed highest zone of inhibition; the highest inhibition zone were 11.0 mm and 12.0 mm respectively. Similarly, the highest inhibitory effect was observed in isolates 2 and 3 i.e., 10.0 mm and 11.0 mm, respectively. However, the minimum impact was recorded to be 4.0 mm, 5.0mm and 6.0 mm(Table5). The antibacterial effect of ethyl acetate extract concentration is lower than that of *Ulval* ethanol extract This finding is consistent with the effects of*Staphylococcus aureus*, *Klebsiella species* against *Salmonella typhi* acetone extract has neutral antibacterial properties against three isolates. The antibacterial properties of seaweeds were recorded, and ethyl acetate without seaweed extract was used as negative control; this was a control without antibiotics. *Candida albicans* isolated Extracts of the of green algae showed antifungal activity against all fungal species tests in this study. Methanol extract was least effective against acetone extracts.Product concentration – 100 mg/ml. The study was designed to measure immune function. In order to test the effects of *Ulva* extracts on various diseases-causing fungi, four quantities were prepared in different solvents. Other controls with similar concentration were also tested in the experiment and appeared to be highly protective. The most stricking results were shown by Ethyl acetate extract against *Candida albicans* it shows zone of inhibition highestconcentration andcompare with Ethanol extract moderately growth were obtained. Methanol extract against*Candida albicans* it was also noted that antifungal activities of seaweeds methanolic extracts are summarized in and ethanol without algae extract was used as negative control, no antifungal of fungal cultures isolates of *Ulva lactuca* algae showed antifungal activity against every fungal strain tested in this study. Methanolic extract of *Ulva lactuca* (Chlorophyceae) showed the lowest activity against *Candida albicans* 25µl, 50µl, 75µl, the same strain was moderately sensitive to extract of *Candida albicans* 25µl, 50µl, lowest activity against to the 75µl.The same strain was moderately sensitive to extract of Ethyl acetate extract, *Salmonella typhi*, Acetone extract *Staphylococcus aureus*, Methanol extract *Klebsiella pnemoniae*, was most sensitive strain against all the extracts.Streptomycin Standards.

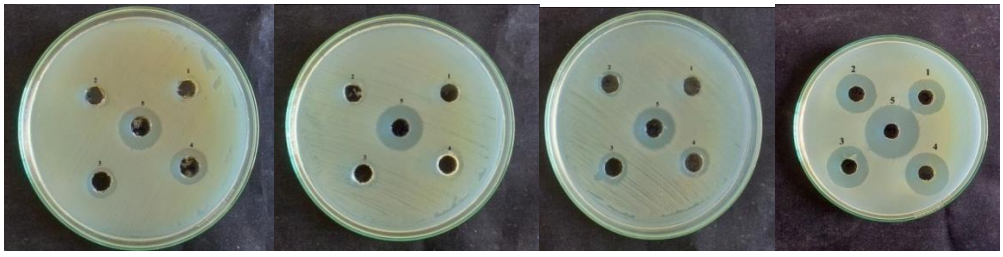

Fig. 10.*Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Salmonella typhi*

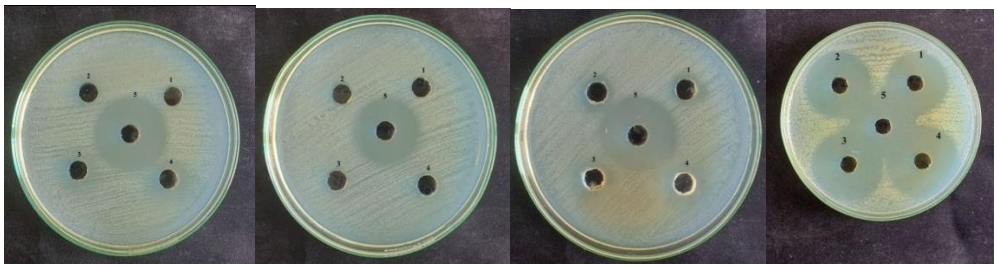

Fig. 11. *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Staphylococcus aureus*

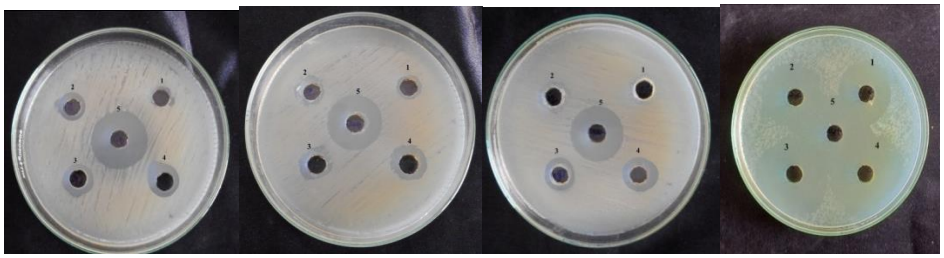

Fig. 12. *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Klebsiella pneumoniae*

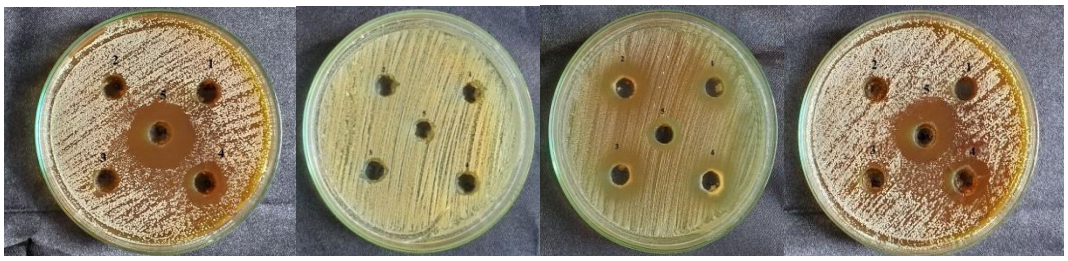

Fig. 13. *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Candida albicans*

Table 5. Antibacterial, antifungal effect of *Ulva lactuca* seaweeds

Human pathogens	Concentration	Inhibition Zone (mm)			
		Organic solvents			
Klebsiella pneumoniae	Volume of extract (µl)	Acetone	Ethyl acetate	Ethanol	Methanol
	25µl	4	5	6	7
	50µl	6	6	7	8
	75µl	7	7	8	9
	100µl	8	9	9	10
Staphylococcus aureus	25µl	7.5	8.5	7	6.5
	50µl	8.3	9.5	7.5	7.5
	75ul	9	10	8	8.5
	100µl	10	11	9	9.5
Salmonella typhi	25µl	6.9	7.5	6	7
	50µl	7.5	8.5	7	8.5
	75µl	8.5	9.5	8	9
	100µl	9.5	11	9.5	9.7
Candida albicans	25µl	5.5	6	8	7
	50µl	6.7	8	9	7.6
	75µl	7.5	9	9.8	8.5
	100µl	8.5	10	10.5	9.5

Agar well diffusion method was carried out to test the antibacterial activities of four different organic extracts of marine green algae *Ulva lactuca*. Ethanol, extract showed the best inhibitory effect. *Staphylococcus aureus* reportedhigher red algae activity Sujatha Ravi et al. [18].

5. CONCLUSIONS

A qualitative analysis of acetone extracts showed the presence of alkaloids, proteins, cardiac glycosides and terpenoids in protein and ethanol extracts. Nutritional analysis: Rich in energy, crude fiber, moderate carbohydrates, protein, ash, water content, and fat. Various organic solvent were analyzed using different methods such as TLC, HPLC, and GC-MS. Antibiotic activity of four organic solvents, three bacterial isolates and one fungal isolates was observed. The maximum blocking range is 11.0 mm and the minimum blocking range is 4.0 mm, 5.0mm, and 6.0 mm. The highest activity is against *Staphylococcus aureus*, *Klebsiella pnemoniae* and *Salmonella typhi* respectively. Which are resistant to ethanol. No immune suppression was seen when ethyl acetate without algae extract was used as a negative control. Methanol extract was least effective against the acetone extract. This seaweed is used humans.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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