

# PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF MARINE GREEN SEAWEED *ULVA LACTUCA*

## 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 Ulvales. *Ulva lactuca* is a widespread macro algae growing the Mediterranean coast phylum Chlorophyta, commonly known as “sea lettuce”. Collected from the Gulf of Mannar Tamilnadu, 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<sup>0</sup>c 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 of 0.925, to 0.477, GC-MS RF value of 8.014, 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 extract 11mm, *Candida albicans* 10.5mm were respectively.

**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 2016.** <sup>[13]</sup> Marine macro algae as a source of bioactive compounds that result in secondary metabolites with various biological activities. The order ulvales it is used to asian food condiment. **Raj GA. et al., (2016).** <sup>[5]</sup> Seaweed are rich in bioactive substances such as polysaccharides, vitamins, minerals, poly phenols, proteins, lipids, and has antibacterial, antifungal, and other functions. Seaweed contain plant products such as flavonoids

and tannins<sup>[5]</sup> **Shankhadarwar(2015)**Seaweeds have many uses<sup>[8]</sup>have been used in medicines cosmetics, energy, fertilizers, and industrial agar and alginate biosynthesis of minerals, vitamins, phenols, and other bio actives. **Hossam S.El-Beltagi et al.,(2022)**. Marine algae are one of the most commonly utilized functional food and therapeutic agents in many parts of the world and beneficial secondary metabolites many of which show **Haniffa(2021)**. Metabolites in algae which include polysaccharide, fattyacids, flavonoids, terpenoids, alkaloids, quinines, sterols, and peptides lipids. **Viraj Chabake and Sakshi Chaubal(2020)**.<sup>[7]</sup>The nutritional content of *Ulva* dried seaweed powder in the human body consists of carbohydrates, which indicates that the main ingredient of seaweeds is rich in nutrients. **Abdullah Rasyid (2017)**. 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., (2020)**<sup>[9]</sup>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 (2018)**.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.<sup>[16]</sup> **Maria Jose Perez, Elena Falque and Herminia Dominguez (2016)**. Methanol was found to be more active against Gram negative than water, and Gram-negative bacterial isolated had higher activity at the minimum inhibitory concentrations. **Johnsichristobel et al., (2011)**. This study examined various organic extract such as acetone, ethanol, ethyl acetate, methanol and other Phytochemical components in *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 (Figure-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 (Ananthi G. 2023)**

## **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 Shimadzu spectrophotometer analysis

## **2.6 FTIR analysis (Radhika D. and A. Mohaideen 2015)**

<sup>[6]</sup>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**

<sup>[1]</sup> (Shimadzu,LC-10AT VP Series equipped with HPLC (VP series 6.1 software (Shimadzu) column temperature is maintained at 27<sup>0</sup>C. 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**

<sup>[2]</sup>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  $\frac{3}{4}$  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.

## **2.9 GC-MS analysis (Raubbin et al.,2020)**

## **3. Antibacterial activity of seaweeds as *Ulva lactuca* extracts**

### **3.1 Sample preparation:**

Methanol, ethanol, acetone, ethyl acetate *Ulva* extract, seaweed extract were 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***

#### **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 after evaporating in vacuum store the extracts at  $-20^{\circ}\text{C}$  until the extract is used.

#### **3.4 <sup>[1]</sup> Antibacterial minimum 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  $37^{\circ}\text{C}$  and subcultured in special media. Single - cell colonies were inoculated into nutrient medium and cultured at  $37^{\circ}\text{C}$  for 4h. Prepare Muller-Hinton agar medium and sterilize at  $121^{\circ}\text{C}$  for 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 (  $25_{\mu\text{l}}$ ,  $50_{\mu\text{l}}$ ,  $75_{\mu\text{l}}$ , and  $100_{\mu\text{l}}$ ), ( $20_{\mu\text{l}}$ ) of streptomycin standard and methanol, ethanol, acetone and ethyl acetate extracts used. Use sterile microtip to individually load *Ulva* powder into agar wells and incubate for 48hrs at  $37^{\circ}\text{C}$ . Measure and evaluate the results. The above procedure allows potato dextrose agar the target medium for fungal disease to replace nutrients and the antibiotic ketocazolin ( $20_{\mu\text{l}}$ ) measured after 48hrs, of standard incubation at  $25^{\circ}\text{C}$ .

## **4. RESULTS AND DISCUSSION**

*Ulva* natural area provided Plate-1 <sup>[3]</sup>*Ulva lactuca* is a green macroalgae associated with destructive green tides found world wide. Green algae species *Ulva* (Chlorophyta). It is sea green algae collected from Gulf of Mannar, near Muttom, in Kannayakumari district of Tamil Nadu. 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 *Ulva* grow in saline and waste water and has a higher ability were collected, (Plate-2) air dried seaweed and ground into fine powder. The powder was green in colour( Plate 3 ), Soluble in water and the pH of the powder was 7.2. *Ulva lactuca* seaweed extracts.( Plate 4) seaweed extract was tested for the Phytochemical constituents such as ten tested Alkaloids, Flavonoids Glycosides, saponins, Tannins, etc., Qualitative tests, pertaining to phytochemical constituents and biomolecules, proves the presence of them

**(Figure-1)Natural Habit of *Ulva lactuca***



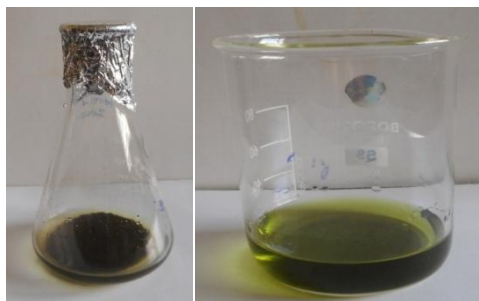
**(Figure-2) Dry seaweeds**



**(Figure-3)Seaweed powder**



**(Figure-4) Extraction of *Ulva lactuca* seaweed**

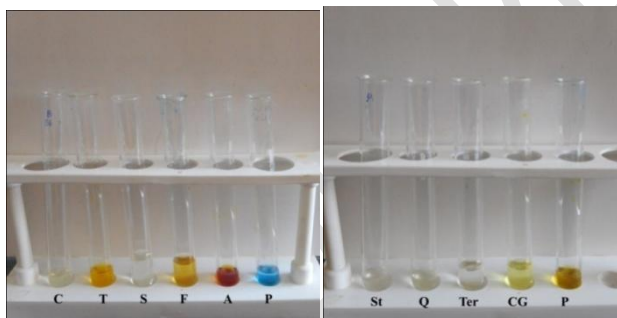


**Table 1. Qualitative analysis of *Ulva lactuca* Powder extract**

Samples		CONTENTS									
		T	S	F	A	P	St	Q	Ter	CG	Ph
<i>Ulva lactuca</i>	Acetone	-	+	-	+	+	-	-	-	-	-
	Ethanol	-	-	-	+	+	-	-	-	+	-
	E.Acetate	-	-	-	+	-	-	-	+	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-

**Note =** <sup>[1]</sup>T-Tannins, S-Saponins, F-Flavonoids, A- Alkaloids, P-Proteins, St-Steroids, Q-Quinones, Ter- terpenoids, CG- Cardiac Glycosides, P-Phenols (+) Positive result (-) Negative result

**(Figure-5) :Qualitative analysis of *Ulva lactuca***



*Ulva lactuca* Seaweed powder tested it was also estimated for the biomolecules such as Ash content, Energy, Carbohydrates, Crude fiber, Fat content, Proteins, Moisture,

**Table-2 Quantity of phytochemicals and biomolecules in *Ulva lactuca* Seaweed powder.**

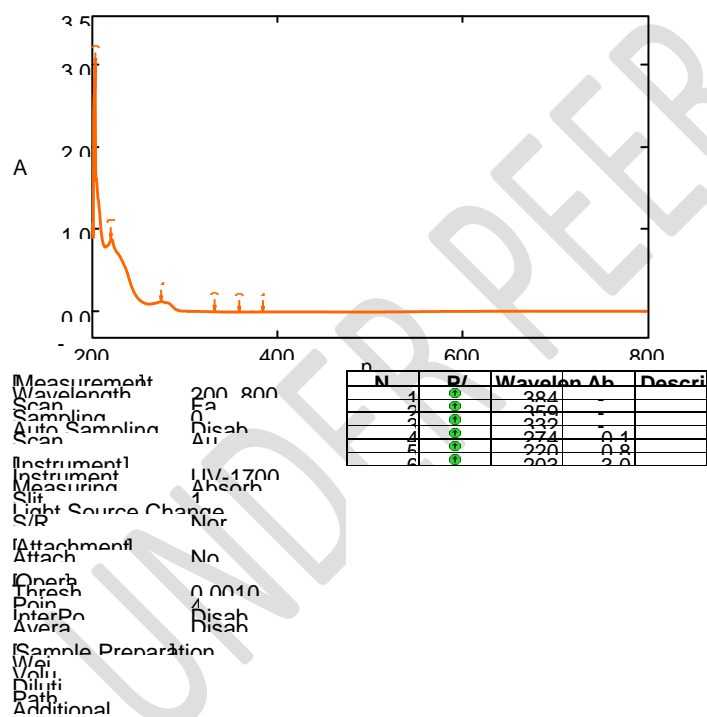
Seaweed (On dry basis)	Ash content	Energy	Carbohydrates	Crude fiber	Fat content	Protein	Moisture
<i>Ulva lactuca</i>	15.38	256.72 Kcal/100g	49.63	12.71	1.04	12.21	21.74

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.

### Spectrophotometric analysis of seaweed as *Ulva lactuca*

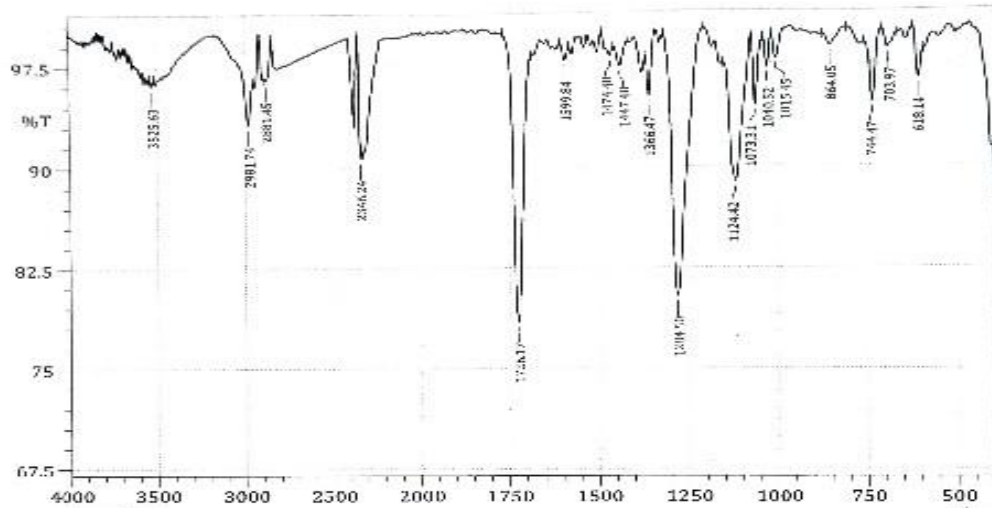
UV-visible spectroscopy reveals that the *Ulva lactuca* extract was taken at the 200-800nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra profile showed the six peaks from 384, 359, 332.50, 274.50, 220.50, 203 with the absorption .

### Spectrophotometric analysis of seaweed as *Ulva lactuca* (Figure-6)



### FTIR Analysis of seaweeds as *Ulva lactuca* (Figure-7)

FTIR spectrum of various organic solvents Acetone, Ethanol, Ethyl acetate, Methanol



Wavenumber, cm<sup>-1</sup>

spectra for *Ulva lactuca* by various organic solvents the adsorption peaks are noted in 618.14 to 3525.65cm<sup>-1</sup> the 618.14 peaks shows the Halogen compound (C-I) 703.97 peaks shows the Alkyl and Aryl Halides C-Br stretching vibrations 744.47 peaks shows the OH group, N-H stretching Vibrations 1<sup>0</sup> and 2<sup>0</sup> bonds. 864.01 peak shows the C-Cl stretching Vibrations, 1015.45 peaks shows C-F stretching Vibrations, 1040.52 peaks shows the alcohols, also absorb in the region due to the C-O Stretch Vibrations, 1073.31 peaks shows the Carboxylic 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. 1366.47 peak 1366.47 indicates C-F stretching Vibrations. Peak 1447.48, and 1474.48 indicate alkanes NO<sub>2</sub> Stretching, and peak 1599.84 indicate -C=C- Stretching Vibration. 1726.17 indicate the Ketones C=O Stretching Vibration. The beaks at 2346.24, 2881.24, and 2981.74 indicate the aldehydes H-C=O. The peak at 2981.74 indicates the C-H Stretching vibration of alkanes. The peak at 3525.63 indicates Stretching of N-Hydehdes.

**(Table -3): FTIR Analysis of seaweeds as *Ulva lactuca***

[14] 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

### GC-MS analysis

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

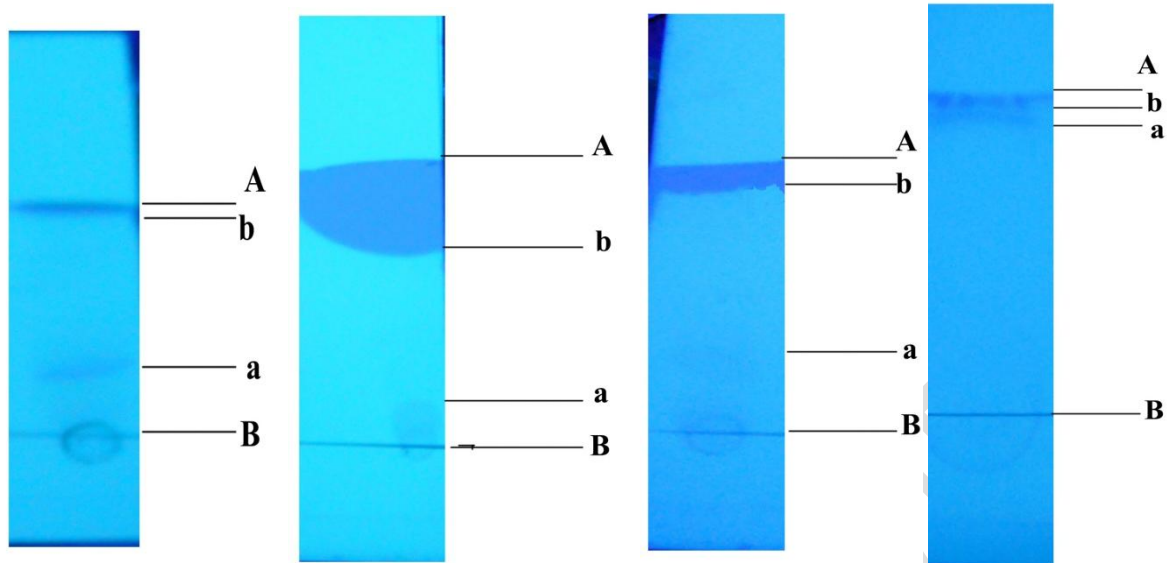
### TLC analysis

The <sup>[10]</sup>chromatographic techniques such as thin layer chromatography (TLC) analysis was used to separate and isolate from the organic extract Acetone, Ethyl acetate , Ethanol, Methanol of *Ulva lactuca*. The solvent system of TLC was Chloroform: Methanol (19:1) was used and its RF value was detected.

**(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	0.275
3	Ethanol a	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	0.275

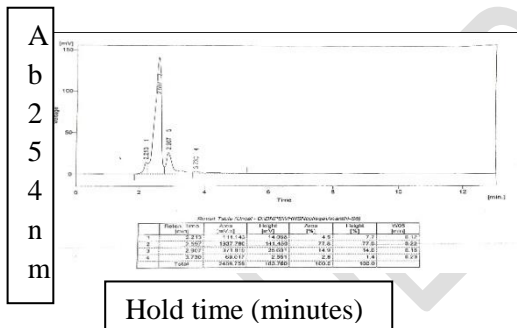
**Thin layer chromatographic analysis of seaweed as *Ulva lactuca* (Figure-8)**



**HPLC profile of *Ulva lactuca***

The qualitative extracts of *Ulva lactuca* were 254nm baseline. Methanol *Ulva lactuca* four peak value were separated at different retention time viz., 2.213, 2.597, 2.907, 3.730, were respectively.

**HPLC Chromatogram analysis of seaweed as *Ulva lactuca* (Figure-9)**

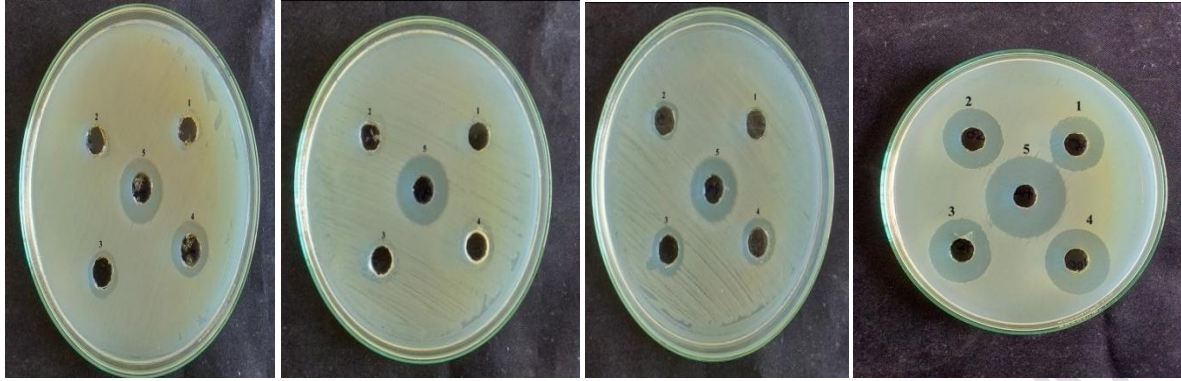


**Antibacterial activity of *Ulva lactuca***

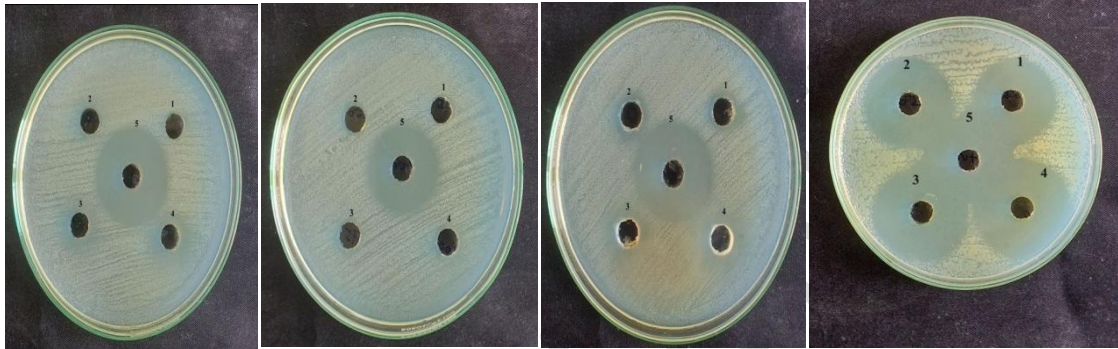
(Figure-10-13) *Ulva* Shows the antibacterial properties ethanol, acetone, methanol, ethyl acetate. In these four extraction streptomycin (20µl) was added for various organic extracts (25µl, 50µl, 75µl and 100 µl), using bacteria separated by different solvents (Acetone, ethanol, Ethyl acetate) was measured 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 the agar well of the 3 separations. In addition to water isolates tested, isolates 1 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 (Table-5). The antibacterial effect of ethyl acetate extract concentration is lower than that of *Ulva* 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 striking results were shown by Ethyl acetate extract against *Candida albicans* it shows zone of inhibition highest concentration and compare 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 the 75µl. The same strain was moderately sensitive to extract of Ethyl acetate extract, *Salmonella typhi*, Acetone extract *Staphylococcus aureus*, Methanol extract *Klebsiella pneumoniae*, was most sensitive strain against all the extracts. Streptomycin Standards.

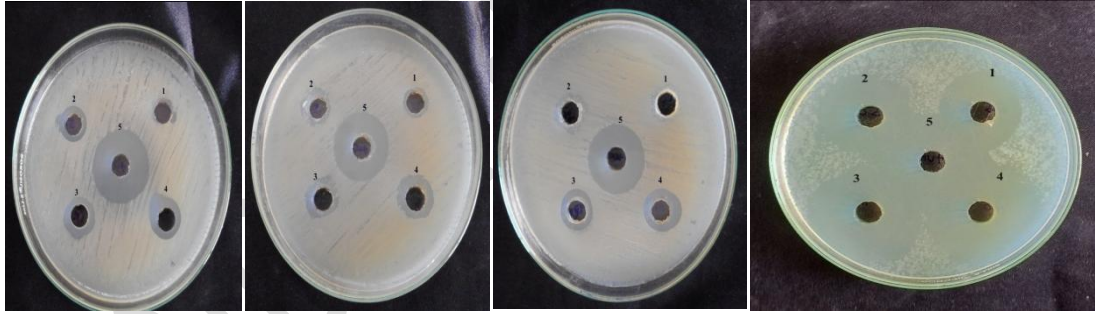
**(Figure-10) *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Salmonella typhi***



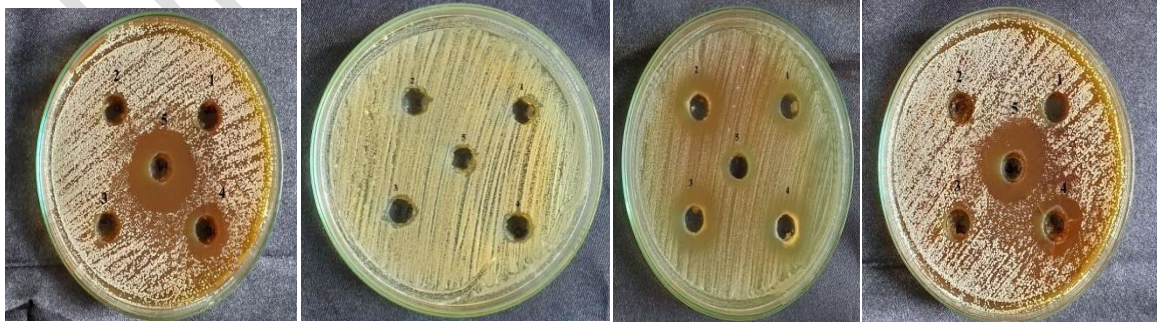
(Figure-11) *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Staphylococcus aureus*



(Figure-12) *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Klebsiella pneumoniae*



(Figure-13) *Ulva* seaweed contains extract of acetone, ethyl acetate, ethanol, and methanol antibacterial properties against *Candida albicans*



**Antibacterial, Antifungal effect of *Ulva lactuca* seaweeds (Table -5)**

Human pathogens	Concentration	Inhibition Zone (mm)			
		Organic solvents			
	Volume of extract (µl)	Acetone	Ethyl acetate	Ethanol	Methanol
<i>Klebsiella pneumoniae</i>	25µl	4	5	6	7
	50µl	6	6	7	8
	75µl	7	7	8	9
	100µl	8	9	9	10
<i>Staphylococcus aureus</i>	25µl	7.5	8.5	7	6.5
	50µl	8.3	9.5	7.5	7.5
	75µl	9	10	8	8.5
	100µl	10	11	9	9.5
<i>Salmonella typhi</i>	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
<i>Candida albicans</i>	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* reported higher red algae activity **Sujatha Ravi et al., 2019**.

## 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 pneumoniae* 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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