PHYTOCHEMICALCONSTITUENTS OFANTIMICROBIAL ACTIVITY AND MARINE GREEN SEAWEED *ULVA LACTUCA*

ABSTRACT

This study was carried out to detect presence of *Ulva lactuca* seaweeds that may have qualitative analysis of seaweed powder extraction and a few selected organic solvents. It belongs to the order Ulvales*Ulvalactuca* is a widespread macro algae growing the Mediterranean coast phylum Chlorophyta, commonly known as "sea lettuce". Collected from the Gulf of Mannar Tamilnadu, India. *Ulva lactuca*.Seaweeds as dried and grounded in to fine powder using inan electric blender. The powder was dried in an oven at 40^{o}c for 24 hourssuch as Alkaloids, Flavonoids, Glycosides, Saponins and Tannins. Nutritional quantity of seaweed *Ulva lactuca*, powder on dry basis rich energy(252.72kCal/mg/gm), followed by Carbohydrates(49.63mg/gm) Protein were low (12.21mg/gm), fat content very (1.04mg/gm), Crude fiber were high (12.71mg/gm) Ash (15.38mg/gm) and Moisture were very high (21.74mg/gm) in the seaweed, UV –Visible spectroscopy that the extracts 200-800nm.TLC analysis 0.925, to 0.477,RF value observed. GC-MS has been one peak value 8.014, and FTIR analysis of seaweeds as *Ulva lactuca*, Peak value 618 to 3525.63 and HPLC has been seven retention time 2.213 to 3.730 were performed.. Antibacterial activity of four concentration were highest activity of *KlebsiellaS.aureus*Methanolic, Ethanolic resistance were respectively. Antifungal activity of the species were *Candida albicans* isolated of four concentration moderately sensitive to Acetone , Ethanol Extract similarly to methanol extract were sensitive to respectivelyand determined the MIC were obtained.

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 in a concentrations much higher than terrestrial plants.**Ambhore**

and Whankatte 2016. Marine macro algae as a source ofbioactive compounds they are produced secondary metabolities characterized by a broad spectrum of biological activities.the order Ulvalesit is used to Asian food condiment. **Raj GA. et al., (2016)**. Seaweed are rich in Polysaccharides, Vitamins, Minerals, bioactive substances like polyphenols, proteins, lipids, and and that possess antibacterial, antifungal, antiviral and other beneficial functions. The phytoconstituents such as Flavonoids,Phenols, and Tannins are present in seaweed and sea grasses. **Shankhadarwar(2015)**Seaweeds have many uses 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.,(2022).** 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 strong**Harroon M. Haniffa(2021).**Metabolities in algae which include polysaccharide, fattyacids, flavonids, terpenoids, alkaloids, quinines, sterols, and peptides lipids. **Viraj Chabake and Sakshi Chaubal(2020).** The nutritional composition of the dried seaweed powder of *Ulva lactuca* utilization in human being are used as carbohydrates it is show that major components and seaweeds of high nutritional value. *Ulva* medicine, biotechnological tools.**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)** 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 contain 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 antimicrobial activity of green, brown, and red algae showing potent activity of belonging to the polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids and halogenated different compounds were analyzed.**Maria Jose Perez, Elena Falque and Herminia Dominguez (2016).** Methanol extract significantly aqueous extract of marine macroalgaeshowed higher activity of against to tested bacterial isolated the minimum inhibitory concentrations of higher activity of gram negative

bacteria **Johnsichristobel et al., (2011).** The present study was screened *Ulva lactuca* seaweed extracts of various organic extract such as Acetone, Ethanol, Ethyl acetate, Methanol,Phytochemical constituents of TLC,GC-MS, FTIR, HPLC, UV-Vis spectroscopyand antimicrobial activity were determined.

2. MATERIALS AND METHODS

2.1 Collection of samples Ananthi .G and Dr.Bagyalakshmi 2023

The samples of *Ulva lactuca*(fig-1) were collected form Gulf of Mannar, near Muttom, Kannayakumariregion,Tamilnadu.Samples were collected in sterilized polyethylene bags, and transported to the lab. Samples were washed thoroughly with sea water then sterile distilled water, air dried, cut into small pieces and then ground until a fine powder is obtained.

2.2Preparation of the extracts

5g algal powder into 100ml of distilled water in a conical flask and placed in a hot plate with the magnetic stirred for 15 minutes. The extract was purified using the Buchner funnel and the Whatman No-1 filtered supernatant was used until 4^0c storeditand further process

2.3Qualitative analysis Ananthi .G and Dr.Bagyalakshmi 2023

2.4 Various nutritional content of seaweeds in *Ulva lactuca*performed the test method

Fssai Manual-Cereal And Cereal Product (Clause 8.7) Page No:19:2016

2.5 UV-Visible Spectroscopic analysisJayabarath and Jeyaprakash 2015

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

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

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

HPLC (Shimadzu,LC-10AT VP Series equipped with software class VP series 6.1 (Shimadzu) used were Acetonitrile Water and the column temperature was maintained at 27^0C. Using a fine syringe, 200ul of extract were injected separately and the print out of the Chromatogram was analyzed. Based on the retention time the constituents were identified.

2.8 TLC analysis

Readymade TLC plate was cut to the required size.A sample mixture was dissolved in methanol and spotted at one end of the TLC plate. The plate was kept in the beaker containing the mobile phase in such a way that the end near the sample application should touch the mobile phase

Allow the chromatogram to run about 1-2 hours. (3/4th level for TLC plate)The plate was dried at RT, RF Value of the sample can be determined by using the formula

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

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

3.1 Preparation of sample:

Methanol and Ethanol, Acetone, Ethyl acetate *Ulva lactuca*extracts of seaweed extracts were air dried and 10mg of dry powder was dissolved in 10ml of various solvents .

3.2 Bacterial isolates used :

For testingthe following Gram positive *Staphylococcus aureus*, Gram negative *Klebsiella pneumoniae*, and *Salmonella typhi*. The following fungal strain, *Candida albicans* were used for antifungal activity. Three bacterial colony is one fungal colonyculture collection of Department of Botany VHNSN College,Virudhunagar.

3.3Antifungal activity of seaweeds as *Ulva lactuca* extracts

3.4 Seaweeds extract preparation

This seaweed with different solvent with increasing polarity andplaced into soxhlet apparatus. Each a soxhlet apparatus for 24_{hrs} and afer evaporation in vaccum the extracts were stored at -20^0C until used extracts.

3.5 Antimicrobial Activity of Mininum Inhibitory Concentration sensitivity test:

This test was carried out in Muller- Hinton agar medium. The isolates were inoculated in Nutrient broth and incubated at 37^0C for 18 hours rotary shaker and subcultured in specific Media. Single cell colonies were inoculated in Nutrient broth and incubated for 4hrs at 37^0c . Muller-Hinton agar medium was prepared, and sterilized at 121^0C for 15 minutes. The sterile medium was poured into plates and allowed to solidify. Lawn of the three bacterial isolates was made on agar plates and allowed to dry for 5minutes. Using sterile cork borer, on previously labeled agar plate, five wells were made. Various aliquotes (25_{ul},50_{ul},75_{ul}, and 100_{ul}), Streptomycin (20_{ul}) was used as standared and Methanol, Ethanol, Acetoneand Ethyl acetate extracts of ***Ulva lactuca*** powder were loaded separately into the agar wells using sterile microtip

and incubated at 37^0c for 48hrswas measured and the results were tabulated. The above procedure is allowed for fungal abut expects the media potato dextrose agar insteated of nutrient agar and the antibiotic ketocazole (20_{ul}) was used as standardedincubated at 25^0c of 48hrs, after that was measured.

4. RESULTS AND DISCUSSION

*Ulva lactuca*natural habitat is given Plate-1 *Ulva lactuca* is a green macroalga involved in devasting green tides observed world wide. Species of green algae *Ulva lactuca*(Chlorophyta). A marine greenalga, it were collected form Gulf of Mannar, near Muttom, Kannayakumari region,Tamilnadu. Seaweeds possess the broadest spectrum of synthetic activity and have been the source of many useful compounds. The important utilization of algae is as human, food, fodder, fertilizer, drugs in paper production and in various 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. *Ulva lactuca*seaweed extracts.(Plate 4) were collected, air dried seaweed (plate-2) 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. Acetone seaweed extract of the powder was tested for the Phytochemical constituents such as ten tested Alkaloids, Flavonoids Glycosides, saponins, Tannins, etc., The Ethanol, Acetone, Methanol, Ethyl acetate , and seaweed extract was also ten tested for the Phytochemical constituents were analysed. Qualitative tests, pertaining to phytochemical constituents and biomolecules, proves the presence of them

Plate–(1) Natural Habit of

Ulva lactuca

(Plate-2) Dry seaweeds

(Plate:3)Seaweed powder

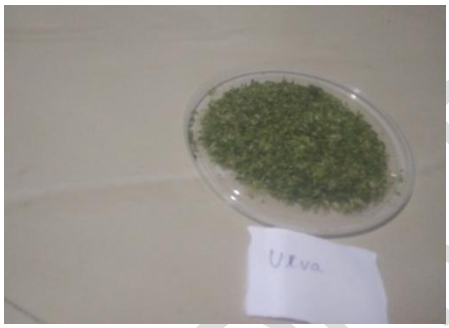


Plate:4 Extraction of *Ulva lactuca* seaweed

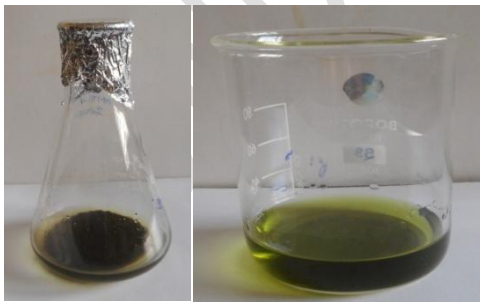


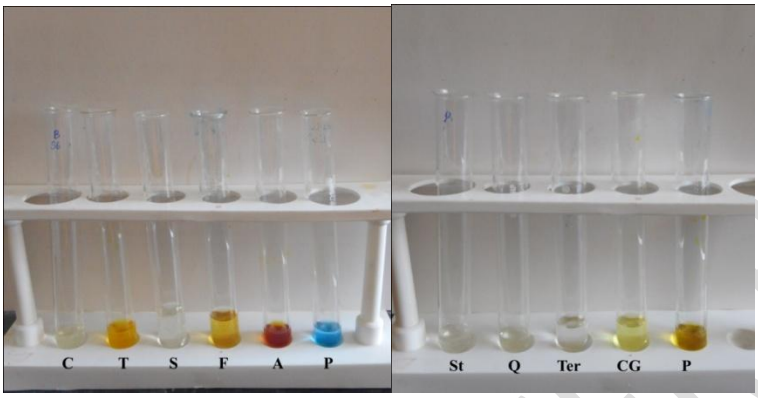
Table 1. Qualitative analysis of *Ulva lactuca* Powder extract

Samples	CONTENTS									
	T	S	F	A	P	St	Q	Ter	CG	Ph

Ulva lactuca	Acetone	-	+	-	+	+	-	-	-	-	-
	Ethanol	-	-	-	+	+	-	-	-	+	-
	E.Acetate	-	-	-	+	-	-	-	+	-	-
	Methanol	-	-	-	-	-	-	-	-	-	-

Note = 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

Picture 1 :Qualitative analysis of *Ulva lactuca*



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

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

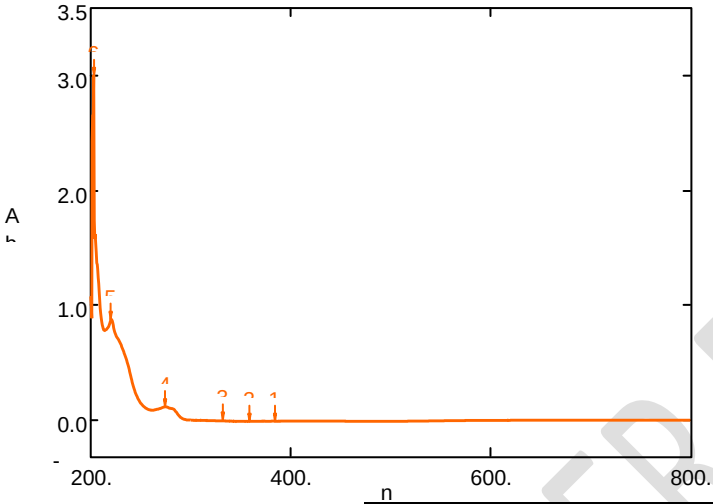
Seaweed (On dry basis)	**Ash content**	**Energy**	**Carbohydrates**	**Crude fiber**	**Fat content**	**Protein**	**Moisture**
Ulva lactuca	15.38	256.72 Kcal/100g	49.63	12.71	1.04	12.21	21.74

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 biomolecules, Fat content were low (1.04mg/gm), Crude fiber were high (12.71 mg/gm) in the seaweeds. Ash content of this seaweeds 15.38(mg/gm), and Moisture were very high 21.74 (mg/gm) respectively. The powder was extracted with andAcetone, Ethyl acetate, Ethanol, Methanol. The extracts were evaporated and powder form was suspended to water and used for UV- visible, Spectrophotometric , FT-IR,HPLC. Phytochemical screening of various extracts of seaweed.

Spectrophotometric analysis of seaweed as *Ulva lactuca*

UV-visible spectroscopy reveals that the *ulvalactuca* 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 .

Picture 2 :**Spectrophotometric analysis of seaweed as *Ulva lactuca***



N	P/	Wavelen	Ab	Descri
1		384	-	
2		359	-	
3		332	-	
4		274	0.1	
5		220	0.8	
6		203	3.0	

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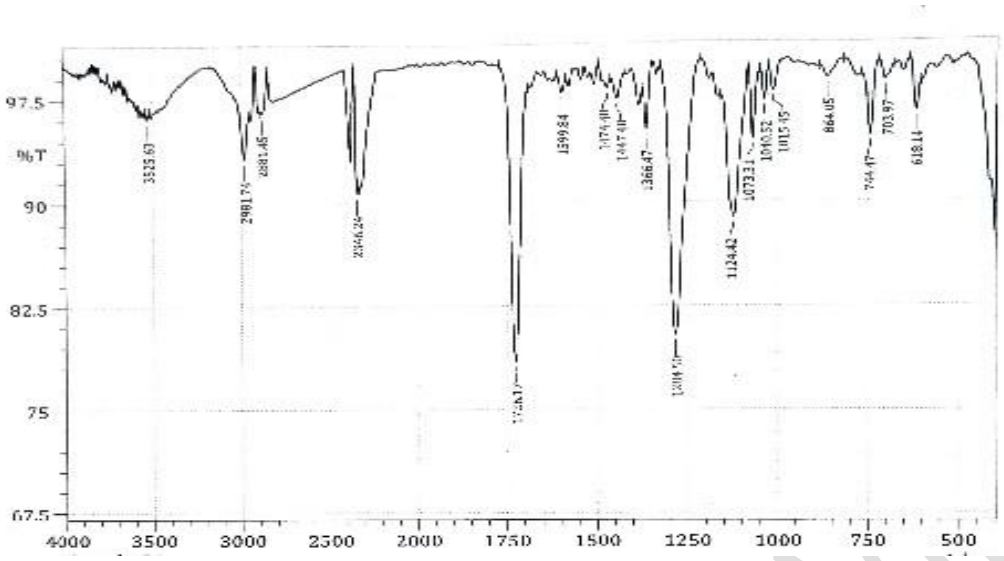
Picture 3 :FTIR Analysis of seaweeds as *Ulva lactuca*

FTIR spectrum of *Ulvalactuca*by 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) 703.97 peaks 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 the C-CI 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 peaks shows the C-F stretching Vibrations. 1447.48, 1474.48 peaks shows the Alkanes NO_2 Stretch, 1599.84 peaks shows the –C=C- Stretching Vibrations. 1726.17 peaks shows the Ketones C=O Stretching Vibrations. 2346.24, 2881 .74, 2981.74 peaks shows the Aldehydes C-H Stretch H-C=O 2981.74 peaks shows the Alkanes C-H Stretching vibrations 3525.63 peaks shows the Amides N-H Stretching vibrations 1^0 and 2^0 bonds.

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

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

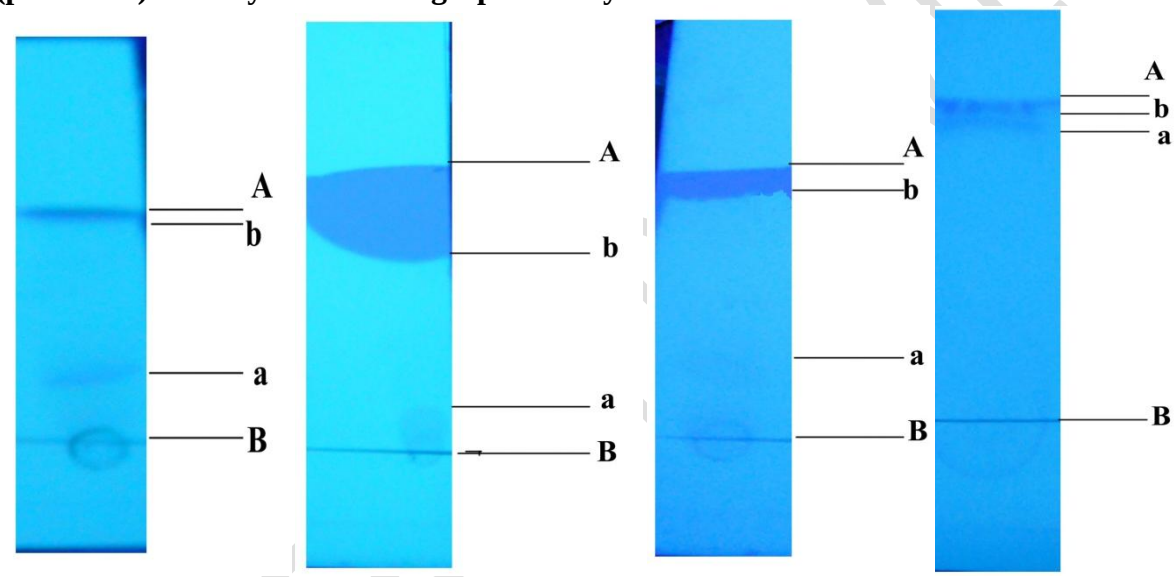
The chromatographic techniques such as thin layer chromatography (TLC) analysis was used to separate and isolate from the organic extract Acetone, Ethyl acetate , Ethnol, Methanol of Ulva lactuca. The solvent system of TLC was Chloroform: Methanol (19:1) was used and its RF value was detected.

(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	Ethanol	4.4	3.1	0.704

	a			
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

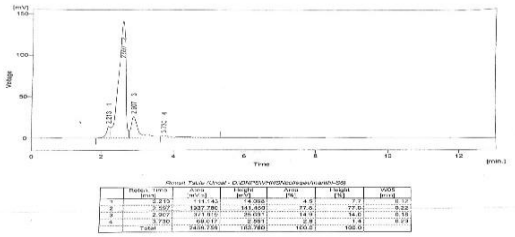
(picture -4)Thin layer chromatographic analysis of seaweed as *Ulva lactuca*



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.

Picture 5 : HPLC Chromatogram analysis of seaweed as *Ulva lactuca*

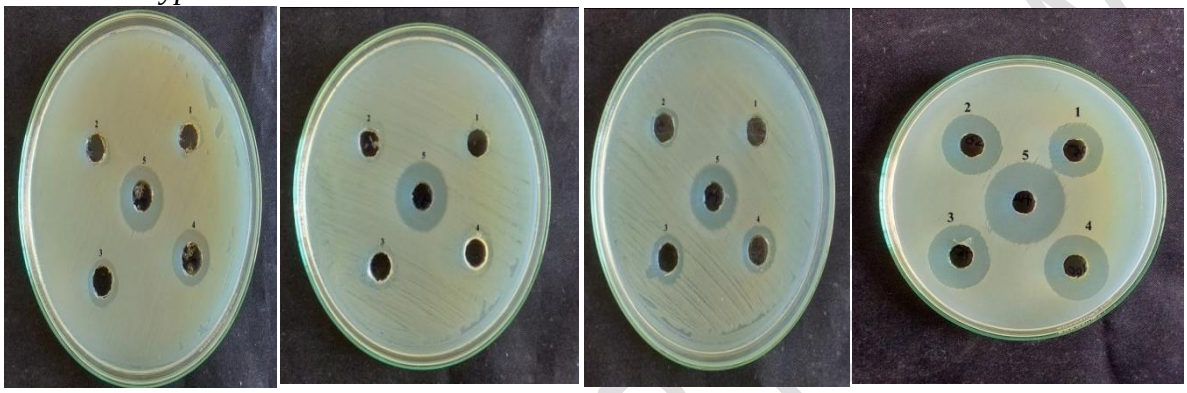


Antibacterial activity of a*Ulva lactuca*

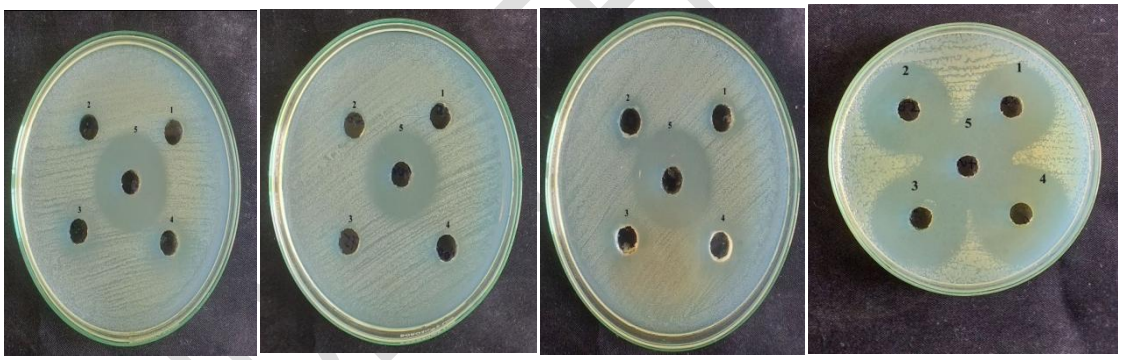
Fig:5:1,2,3 Shows the antimicrobial activity *Ulva lactuca*Ethanol, Acetone, Methnol, Ethyl acetate. In these four extracts of various organic extracts was assayed by using bacteria isolated from different solvents (Acetone, ethanol, Ethyl acetate) were added in the concentration of (25µl, 50µl, 75µl and 100 µl), Streptomycin (20 µl) was loaded in respective wells. These plates were incubated for 24 hrs at 37ºC .Then the microbial growth was determined by measuring the diameter of zone of inhibition it is clear from the result that the, inhibition was proportional to the quantity of Acetone, Ethanol, Ethyl acetate, Methanol crude extract added in the agar well of the 3 isolates tested, isolates 1 and 3 showed maximum zone of inhibition , maximum zone of inhibition, i,e 11.0 mm and 12.0 mm respectively and with reference to methanolic extracts of *Ulva lactuca*, the trend in inhibition was similar to that of Ethyl acetate extract. Similarly, maximum inhibitory effect, i,e 10.0 mm and 11.0 mm, was seen respectively in isolates 2 and 3. But, minimum inhibitory effect, i,e 4.0 mm, 5.0mm , 6.0 mm was noted .The antibacterial effect of Ethyl acetate extract concentration of lesser than Ethanolic extracts of*Ulva lactuca* . This finding is in agreement of with that of highest activity of against *S.aureus, Klebsiella*and *Salmonella typhi*Ethanolic resistant were respectively.The antibacterial effect of acetone extractmoderately three isolates organism. The antifungal activity of seaweeds are summarized in and figure-3.6-4. Ethyl acetate without algae extract was used as negative control no antifungal this case. *Candida albicans* isolated Extracts of the of green algae showed antifungal activity against every fungal strain test in this study. Methanolic extract showed the lowest activity against Acetone extracts. Sample Stock Concentration – 100 mg/ml. The study was to evaluate antifungalactivity. Four concentration were made along with *Ulva lactuca* extract of alga in different solvents to test for their actions against the fungi species which are pathogenic to cause various diseases. A control with other match able concentration was also tested in experiments were excellent on the ability of antifungal activity. The most strickingresults 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 *Candidaalbicans* it was also noted thatantifungal activities of seaweeds methanolic extracts are summarized in and Figure-3:6-4 Ethanol without algae extract was used as negative control, no antifungalof fungal cultures isolates of *Ulva lactuca* algae showed antifungal activity against everyfungal strain tested in thisstudy. Methanolic extract of *Ulva*

lactuca (Chlorophyceae) showed the lowest activity against *Candida albicans* 25ul, 50ul, 75ul, the same strain was moderately sensitive to extract of *Candida albicans* 25ul, 50ul, lowest activity against to the 75ul. The same strain was moderately sensitive to extract of Ethyl acetate extract, *Salmonella typhi*, Acetone extract *S.aureus*, Methanol extract*Klebsiellapnemoniae*, was most sensitive strain against all the extracts. Streptomycin Standards.

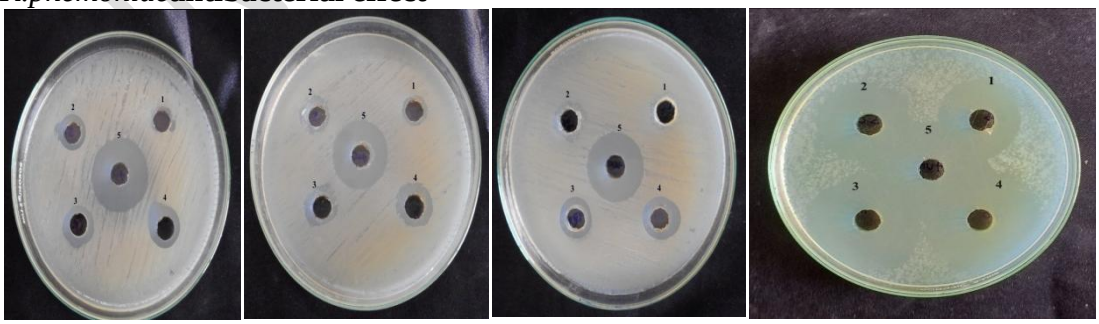
(fig-1) *Ulva lactuca* seaweedsare Acetone,Ethyl acetate, Ethanol, Methanol extract in the *Salmonella typhi*antibacterial effect



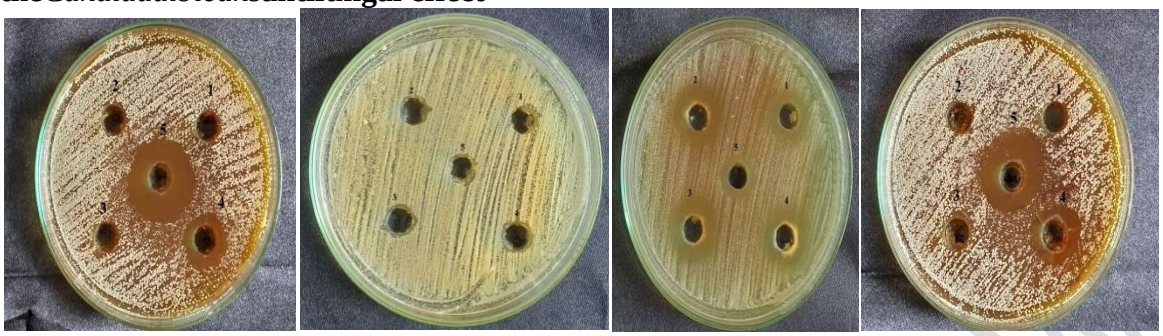
(fig-2)*Ulva lactuca* seaweeds are Acetone,Ethyl acetate, Ethanol, Methanol extract in the *S.aureus*antibacterial effect



(fig-3) *Ulva lactuca*seaweeds are Acetone,Ethyl acetate, Ethanol, Methanol extract in the *K.pnemoniae*antibacterial effect



(fig -4) *Ulva lactuca* seaweeds are Acetone,Ethyl acetate, Ethanol, Methanol extract in the*Candidaalbicans*antifungal effect



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

Human pathogens	**Concentration**	**Inhibition Zone (mm)**			
		Organic solvents			
Klebsiella pneumoniae	Volume of extract (ul)	Acetone	Ethyl acetate	Ethanol	Methanol
	25ul	4	5	6	7
	50ul	6	6	7	8
	75ul	7	7	8	9
	100ul	8	9	9	10
Staphylococcus aureus	25ul	7.5	8.5	7	6.5
	50ul	8.3	9.5	7.5	7.5
	75ul	9	10	8	8.5
	100ul	10	11	9	9.5
Salmonella typhi	25ul	6.9	7.5	6	7
	50ul	7.5	8.5	7	8.5
	75ul	8.5	9.5	8	9
	100ul	9.5	11	9.5	9.7
Candida albicans	25ul	5.5	6	8	7
	50ul	6.7	8	9	7.6
	75ul	7.5	9	9.8	8.5
	100ul	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*. The ethanol, extract showed maximum inhibition*S.aureus* have reported red algae showed higher activity than the **Sujatha Ravi et al., 2019.**

5. CONCLUSIONS

Qualitative analysis of acetone extract showed theAlkaloids, Proteins and Ethanol extract presence of Alkaloids, Proteins, Cardiac glycosides, Terpenoids. Nutritional analysis like rich amount of energy, crude fiber, moderate amount of Carbohydrates, protein,Ash low amount of moisture, and fat. *Ulva lactuca* seaweed extract various organic solvent were analyzed for different technique involved in TLC, HPLC, GC-MS,FTIRUV-Visspectroscopy. Antimicrobial activity of four organic solvent used and three isolates bacterial pathogens, one fungal pathogens maximum zone of inhibition 11.0 mm and 12.0 mm and minimum inhibitory effect 4.0 mm, 5.0mm , 6.0 mm was noted. The highest activity of against *S.aureus*, *Klebsiella* and *Salmonella typhi* Ethanolic resistant were respectively. Ethyl acetate without algae extract was used as negative control no antifungal activity was observed. Methanolic extract showed the lowest activity against the Acetone extracts.This seaweeds utilized forbeneficial of mankind.

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