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Study on quantitative & qualitative assessment on phytochemical activity and in-vitro study on antioxidant & antimicrobial activity from different organic extracts

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ABSTRACT

The present study was to evaluate the phytochemical attributes and overall inhibitory effects of oceanic algal species of UlvaLactua, Gracilaria Corticata, and Ascophyllum Nodossum, collected from the area near Kovalam Beach (fisher's spot), East Coast, Chennai, Tamil Nadu. Algal species are rich in bioactive compounds. Besides, the selected species are a highly diverse group of organisms from secondary metabolites of the natural source are a potential source. Oceanic algae are an interesting group in their broad spectrum of biological activities such as antibacterial, antioxidant, and anticancer. The algal extracts were prepared using a solvent extract approach involving ethanol, acetone, DMS, DCM, and double-distilled water as the solvent of interest for the study. Furthermore, the antibacterial activity and phytochemical activity of Ulva Lactua, Gracilaria Corticata, and Ascophyllum Nodossum. were tested against Candida albicans and Aspergillus niger and Escherichia coli and Proteas Vulgaris by well diffusion method. The phytochemical assay screening of the extracted species of Ulva Lactua, Gracilaria Corticata, and Ascophyllum Nodossum showed a greater degree of phytochemical attributes. In the antibacterial activity the growth of two virulent strains of pathogenic bacteria, E. coli and Proteas Vulgaris, and similarly concerning fungal strains of Candida albicans and Aspergillus niger were observed to exhibit a greater degree of inhibitory effect by the algal extracts, which are observed via the zone of inhibition (in mm). These results showed the investigated oceanic algal species, Ulva Lactua, Gracilaria Corticata, and Ascophyllum Nodossum showcased great biological potential, which could be considered for future uses in pharmaceuticals, food.

Keywords: *Oceanic Algal Species, Antioxidant, Phtochemical Study, and Antibacterial Activity*

1. INTRODUCTION

The practice of plant as a potent antimicrobial agent dates back to ancient times. Their use as curative in treating many communicable diseases has still been under use over rural regions of India. People have developed a series of empirical data concerning therapeutic plants before the practice of orthodox medicine appeared in the picture. Through trial and error the herbalists have gathered a large pool of knowledge regarding medicinal plants (Mitscher et al., 1972).

Natural product research has received greater attention upon the upsurge in green revolution, which impacted upon the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. This made a huge effect over pharmaceutical industry to focus their interest over deriving the essential metabolites which serve as a template of lead species of structure. Their bactericidal nature showed promising interest over synthesizing these metabolites. Among 250,000 species of higher plants, a small percentage range of about 5-10 % have been studied phytochemically and a fraction of it was submitted to biological or pharmacological screenings (Hostettmann, 1991). The plant kingdom still serves as a huge reservoir for new molecules to be discovered. With the rapid evolution of drug resistant strains of bacteria, requirements for new and effective therapeutic agents are of sole importance (Bhavani & Ballow, 2000).

About 90% of the species of marine plants are seaweeds and 50% of the global photosynthesis is from marine algae is that every second molecule of O_2 we inhale comes from marine algae and reuses every second of a molecule of CO_2 we exhale (Nirmala, 1999). Some of them have been potentially used for bioremediation purposes. Seaweeds show enormous descent and have extended potential intrigue. Around 8000 species of seaweed and 30, 000 common algae species were recognized and

sighted across the mundane world. But millions are still undescribed (Guiry, 2012). Algae being chlorophyllous organisms serve as the basis of food chains in water bodies. They are ecologically significant as they influence the biological productivity of water bodies. They may be freshwater or marine and serve as primary producers. Algae are an essential link in the food chain of all aquatic animals and include zooplankton, phytoplankton, diatoms, etc. Phytoplanktons serve as important tools in the environment bio-monitoring of aquatic ecosystems. Exploring the depth is tremendously testing because depending upon the area and the clarity of the water Bathypelagic, Abyssopelagic zones distinguish the unique characteristics and those deep-sea creatures are purposefully incredibly diverse to identify.

Seaweeds are an important source of food, phycocolloids, pharmaceuticals, and more recently biomass for energy. Seaweeds have valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products, neurotoxins, and suspending agents in radiological preparations (Bibi et al., 2020). Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of antibiotics (Arioli et al., 2015; Rouphael & Giordano 2018). Marine natural products are considered as one of the important sources of antimicrobial agents. Drugs derived from unmodified natural products or semi-synthetic drugs constitute 78% of the new drugs and are approved by the Food and Drug Administration (FDA) between 1983 and 1994.

The importance of screening natural products from this point of view is quite evident (Tuney et al., 2006). Amongst approximately 50, 000 known marine plant species and 30, 000 species of marine algae, only a small percentage are screened for use of potentially bioactive compounds. Seaweeds are nowadays considered as promising alternative source for Biofuel and industrially important chemicals in addition to having considerable potentials in reducing greenhouse gas emissions and global warming (Milledge 2014; Wijesekara, 2010; Sahoo, 2012).

2. MATERIALS AND METHODS

2.1. Materials

The reagents and chemicals used were of standard analytical grade and bought them from Sd-fine Chemicals. Ltd., Mumbai, India and Hi-media Laboratories Pvt. Ltd., Mumbai, India.

2.1.1. Collection of plant: The samples of algal flora were collected from the water bodies of East Coastal region. Green Alga *Ulva Lactuta* was collected from Sublittoral Zone in Kovalam Beach, Chennai, Tamil Nadu, India at monsoon in October 2019. Brown alga *Gracilaria Corticata* was collected from Sublittoral Zone under calm tide condition in Kovalam Beach, Chennai, Tamil Nadu, India at in October 2019. The samples were then stored in a cool dry place to prevent deterioration. The dried samples were then and made into a powder using amixer grinder. The powdered samples (*Ulva Lactuta* and *Gracilaria Corticata*) were filtered to get fine particles, and then stored in air-tight bag for future use at (–20ºC) . *Ascophyllum Nodossum* was preserved properly in the imported package.

2.1.2. Preparation of extracts: Three seaweed extracts were prepared individually by crushing and soaking in the respective solvents at an average of 50 grams (50g). Further, the sample of ground seaweeds were extracted with six various solvents which includes Ethanol (ETOH - C_2H_5OH), Dichloromethane (DCM-CH2CL2), Diethylether (DL- $(C_2H_5)_2O$), Dichloromethane (DC-CH_2CL_2), Acetone (C_3H_6O), Distilled Water – H2O. They were extracted by sequential and maceration. The extracts were derived via sequential extraction and macertation extraction technique

2.1.3. Phytochemical screening: Concentrated extracts were subjected to preliminary phytochemical screening for the presence of secondary metabolites (Mir et al., 2013).

2.1.4. Antibacterial activity: By using the technique called well diffusion, antibacterial activity was performed (Ramakrishnan et al., 2011).

2.1.5. Well Diffusion technique: Well diffusion method performed over the inoculated solidified Mueller Hinton Agar plate (Himedia, India). Wells of 6mm diameter ranges were made using a sterile cork borer. The controls and extracts were placed in the 6mm diameter well. Accurately 100 µl of 25, 50, 75 and 100 µg/ml crude extracts were pipetted in every well. The plates were incubated at 37°C in incubator for 24 h. Erythromycin and sterile water were used as both positive & negative controls respectively. Further, the plates were observed for inhibition zones formed in terms of mm[16].

3. QUANTITATIVE DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS

3.1. Estimation of tannin

2 gram of *Ulva lactuta*, *Gracilaria corticata* and *Ascophyllum Nodossum* extracts was mixed with 100ml of distilled water in 500ml conical flask separately and was heated delicately and boiled it for half an hour. Then, the extracts underwent for the process of centrifugation for 20 mins at 2000rpm and the the supernatant was collected in the volumetric flask of 100ml and made up to 100ml. 1ml of the sample extract was transferred to a 100ml volumetric flask containing 100ml of water. 5ml of Folinciocalteu phenol reagent was added followed by 10ml of sodium carbonate solution and diluted to 100ml with distilled water and shaken well. The intensity of blue colour was measured in a spectrophotometer. The absorbance was read at 700 nm after 30minute and the 30 times dilution of the samples was distilled water and standard graph by using 0 – 100 µg gallic acid. The tannin content of the extract samples was calculated as gallic acid equivalents from the standard graph expressed as mg/100mg.

3.2. Total phenolic content

The estimation of total phenolic content was measured by the Folin-Ciocalteu (FC) method as reported by Boonchum et al. (2011). 10 µl of six different extracts namely (DSM, , Acetone, ethanol, and distilled (H_2O)) of the three Algae extracts were mixed separately with sodium carbonate(50 µl) and FC reagent (20µl). It was further made up to 1000µl by adding the required amount of Distilled H2O and incubated in dark for 1 hour. The absorbance was recorded at 725 nm using Biophotometer. The gallic acid was used as a standard. Phenolic content was expressed as gallic acid equivalents in mg/g of dried seaweeds.

3.3. Determination of Total phenolic content

The concentration of phenolics in all three algae extracts (UlvaLactuta, Gracilariacorticata and AscophyllumNodossum) was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1g of Ulva Lactuta, Gracilaria Corticata, Ascophyllum Nodossm extracts and 9ml of distilled water was taken in a volumetric flask (25ml) separately. The mixture and Folin-Ciocalteu phenol reagent (1ml) were treated with each other and shaken it well. 7% Sodium carbonate (Na_2CO_3) solution (10ml) and the mixture were treated well after 5 minutes. The volume was made up to 25ml. As already described, a set of gallic acid (20, 40, 40, 60,

80 and 100 µg/ml) standard solutions were made up in the same way. Then, it was incubated for one and a half hours at room temperature and the absorbance for standard solutions and test were found in contradiction to the reagent blank at 550nm with an Ultraviolet (UV)/ Visible spectrophmeter. Total phenol content was expressed as mg of GAE/gm of extracts (Singleton et al., 1999; Rizman et al., 2011; Stankovic et al., 2011).

3.4. Determination of Reducing Sugar

The quantification of reducing sugars was carried out by using Dinitrosalicyclic acid method described by Bernfeld (1955). The samples before and after hydrolysis were both assessed. The hydrolysis was proceeded with concentrated HCl with 2 hour intervals at 100˚C and neutralized with 20% NaOH. The following experiments were carried out in tripilicate (Bernfeld, 1955).

3.5. Determination of neutral sugar

As described by Dubois et al., (1956), using phenol sulphuric acid, the content of sugars was determined. The following experiments were carried out in triplicate.

3.6. Thin Layer Chromatography (TLC)

Using silica gel plates, the chromatography of the extracts was performed. Acetone/n-butanol/water (80: 10: 10: v/v/v) was the elution system used for monosaccharides and was revealed with 85% orthophosphoric acid (5: 5: 1v/v/v) and 2% diphenylamine prepared in acetone. Glucose, Arabinose Sucrose and Galatose were the used standards. Additionally, thin layer chromatography of the Algae extracts (Ulva Lactuta, Gracilaria Corticata, Ascophyllum Nodossum) found the sugars (monosaccharides).

3.7. Determination of total flavonoid content

The determination of total flavonoid content was carried out by the modified method of Juan et & Chou (2010). Concisely, 25 µl of each Algae extracts was added to potassium acetate (25 µl 25mM), double distilled water (700 µl), ethanol (350 µl) and Aluminium chloride (25 µl). It was then incubated in dark condition for 30 minutes. The sample mixture absorbance was then recorded at 415 nm. Quercetin was used as a standard. The obtained results are represented as the equivalents of Quercetin mg(QE)/g extracts Cox et al (2010).

3.8. Determination of total antioxidant activity

Here, the Phosphomolybdenum method (Raghu et al (2011)) determined the overall antioxidant activity of *Ulva Lactuta, Gracilaria, Ascophyllum Nodossum*. Mo (IV) can be reduced by the antioxidant to Mo (V). further, Mo (V) compounds/the green phosphate exhibits a peak at 695 nm. The sample (10 µl) is assorted with the reagent solution of 1ml in separate test tubes (Sulphuric acid (10ml) + sodium phosphate (10 ml) + Ammonium molybdate (10 ml)). Further, it underwent incubation at 98ºC for 90 minutes in boiling water bath. With the help of Bio Photometer, the sample mixture absorbance was read at 675 nm. As a standard, ascorbic acid was used. Thus, the activity was expressed as the number of ascorbic acid equivalence in extracts of mg/g.

3.9. Determination of Vitamin E

By using 3 centrifuge tubes (test, standard and blank), pipette out 1. 5ml of Distilled water, 1. 5ml of each Algae sample extracts and 1.5ml of the standard. Ethanol (1.5 ml) and standard water (1.5ml) were added to the blank and test. Then, xylene (1.5) was added to every tube and then it was stoppered, mixed well and centrifuged well.

The, xylene layer (1.5ml) was transferred into some other stoppered tube and then it was taken care and not included and any protein or ethanol that added 2'2 – dipyridyl reagent (1ml) to every tube, then mixed and stoppered. Mixtures (1. 5ml) were pipetted into cuvettes of spectrophotometer and the standard and test absorbance was read opposite to the blank at 460nm. Further, added ferric chloride solution (0.33%) with the blank. Then, tilted it well and standard and test was again read in opposite to the blank at the absorbance of 520nm after 15 minutes. By using the below formula, the amount of vitamin E can be easily measured.

$$(AA520nm - AA460nm \times conc. \times 0.29) * Total\ Volume$$

$$Vitamin\ E\ (u.\ g\ /\ g) = (AA520nm * Volume\ for\ experiment * wt.\ of\ the\ sample)$$

3.10 Antifungal activity (Disc diffusion method):

As explained by Murugeasan Subbaiah, et al; 2015, the method called disc diffusion agar method performed the antifungal activity of MEGC and MEHM. Sterilized MHAM medium filled in the Sterile Petri dishes and it was chilled to the temperature of 40˚C. Algae extracts (2. 5 mg/5 mg/10 mg) with different concentrations were loaded in the discs of sterile filter paper with the diameter of 6 mm (what man No. 42) and then let it dry for 10 minutes. Flucanozole (100 µg/ml) contained discs was used as a positive control. With the sterile cotton pads of fungal pathogens, the Petri dishes were cleansed and inoculated. To sterilized medium of MHAM, the dried discs fortified with crude drug were transferred and they underwent for the process of incubation for 2 days (48 hours) at room temperature.

3.11. Measurement of zone of inhibition

Scale measured the standard and the clear zone of inhibition around the extract. The formula then corrected the reading, after measuring the diameter of inhibition zone.

Inhibition Zone= Diameter of Disc (Control/Sample/Standard)

4. RESULTS AND DISCUSSIONS

4.1. Phytochemical analysis

In order to identify the characteristic of extract synthesized from organic solvents of ethanol, methanol and acetone were subjected to phytochemical study which showed the presence and absence of the metabolites out of which ethanol and acetone extracts provides predominantly with a wider range of metabolites from the thorough analysis. This showed us the extraction efficiency as well as the essential metabolites present in the algal species. Quantitative determination was carried out for the following study. It was found that From the investigation so far with regards to flavonoids, phenol, reducing and non-reducing sugars, neutral sugar, tannins, alkaloids and vitamin-E were quantitatively determined. From the investigation, ethanol extract of the algal sp exhibited slightly higher significant quantitative data compared to aqueous extract.

4.2. Antioxidant activity

The Antioxidant activity by DPPH scavenging activity represented graphically from the Table 2 shows that the extract possess a good antioxidant activity with respect to ascorbic acid as the standard sample. From the following graphical representation the extracts showed an increase in DPPH inhibition with the increase in the concentration (µg) of the sample. According to reports provided by Beg et al[3] representing the importance of antioxidants in reducing the hypertension which in turn leads to certain other complexities such as stroke, heart disease, and kidney failure. From assessing the overall % of the savenging activity it could be inferred that the ethanolic

extract of all the algal species possesses similar antioxidant activity and even the study also showed to possess a good antioxidant activity.

4.3. Total Tannin Content

For the following estimation, tannic acid was employed as standard and the total tannin content could be expressed in the form of Tannic Acid Equivalents (TAE). Absorbance spectra were furthermore subjected to spectrometric readings in ranges of 700nm.

Table-1: Quantitative assessment of Total Tannin Content

Concentration of Tannic Acid (µg/ml)	Mean Absorbance
100	0.07 ± 0.0002
200	0.12 ± 0.0002
300	0.15 ± 0.0003
400	0.21 ± 0.0001
500	0.27 ± 0.0005
Ulva Lactuca;	0.095±0.0002
Gracilaria Cortcata	0.053±0.0002
Ascophllum Nodossum	0.082±0.0001

These studies showed that the ethanol extract *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum* having statistically significant amount of tannin content as compared to aqueous extract.

Table-2: Total Tannin Content of different extracts of *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum*

Oceanic algal sp.	Tannin content -Tannic acid equivalent (mcg/ml)	
	Aq Extract (100 µg/mL)	Et. Extract (100 µg/mL)
Ulva Lactuca	40.01 ± 0.020	60.22± 0.010
Gracilaria Cortcata	38.72±0.0002	39.81±0.0001
Ascophllum Nodossum	32.14±0.0002	44.33±0.0001

4.4. Phenols content

Phenols content from the oceanic algal extracts were determined by Folin- Cuocalten reagent method. A standard for the preparation involving standard graph alongside with absorbance were recorded at 630 nm. Rutin was utilized as standard graph and absorbance were recorded for the same. Absorbance for the estimation of phenols shoed that the highest phenols were in the ethanolic extracts of - 0.778 mg/g and the lowest phenols in the case of aqueous extract of- 0.321 mg/g, as presented in following table and figure.

Table-3: Absorbance of standard phenols (mg/g) Rutin

Concentration (100 µg /mL)	Absorbance
0.2	0.065
0.4	0.121
0.6	0.171
0.8	0.237
1.0	0.289

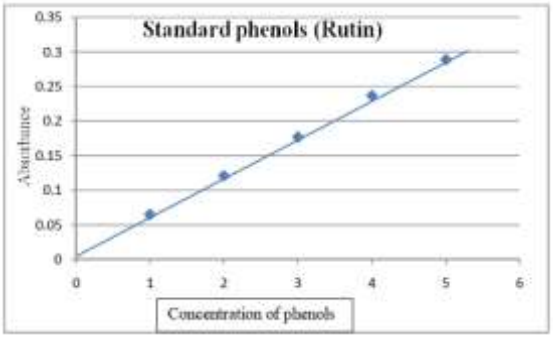

Fig. 1: Standard graph of phenols (mg/g) (Rutin)

Table-4: Quantitative estimation of phenols (mg/g)

Oceanic algal sp.	Aq Extract	Et. Extract
Ulva Lactuca;	0.587	0.778
Gracilaria Cortcata	0.349	0.646
Ascophllum Nodossum	0.321	0.443

4.5. Quantitative estimation of reducing & non-reducing sugar

The following table represents distribution of total sugar, reducing sugar, non-reducing sugar/ neutral sugar from the oceanic algal species of *Ulva Lactuca; Gracilaria Cortcata* & *Ascophllum Nodossum* were as follows.

Table-5: Quantitative estimation of reducing & non-reducing sugar

Oceanic algal sp.	Total sugar mg/ g	Reducing Sugar mg/ g	Non-Reducing Sugar mg/ g
Ulva Lactuca;	38.32	9.31	29.01
Gracilaria Cortcata	12.24	2.81	9.43
Ascophllum Nodossum	35.32	4.43	30.89

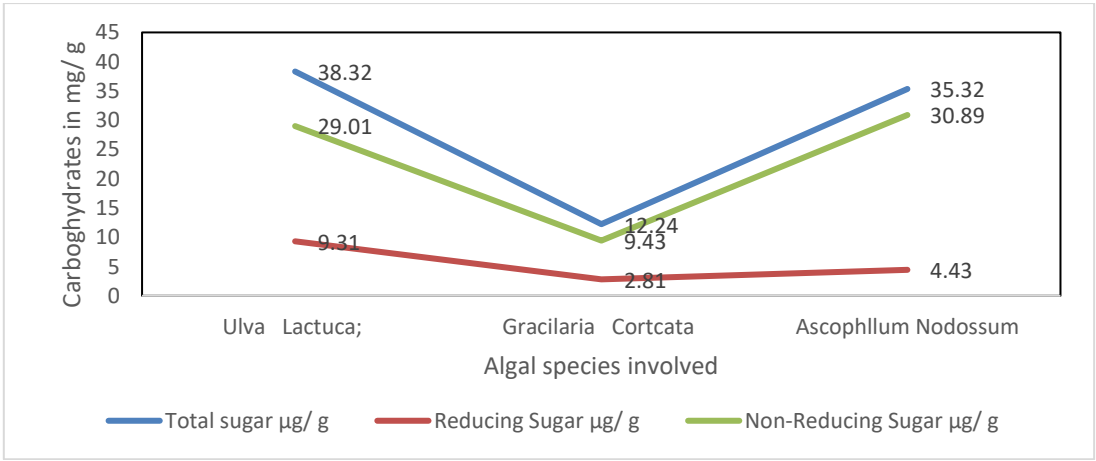

Fig. 2: Distribution of Carbohydrates

(Sugar, Red. Sugar & Non-Red. Sugar for Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum)

4.6. Estimation of neutral sugars

The separation involving six neutral sugars was evaluated via TLC systems. The mechanism involved in the separation of sugars cannot be presented with assurance and it is a subject for further investigation required.

Table-6: TLC estimation of neutral sugars (R_f)

Compound	Relative Mobility (Rf x 100)		
	Ulva Lactuca	Gracilaria Cortcata	Ascophllum Nodossum
L-arabinose	48	34	56
L-rhamnose	67	45	73
D-galactose	42	28	48
D-xylose	58	40	64
L-fucose	39	-	29
D-mannose	44	30	57

4.7. Quantitative estimation of Flavonoids

The maximum flavonoids were observed (0.590 mg/g) in ethanolic extract of *Ulva Lactuca* and minimum (0.319 mg/g) in aqueous extract of *Gracilaria Cortcata*, as represented in

Table-7: Quantitative estimation of flavonoids

Oceanic algal sp.	Aq Extract (mg/g)	Et. Extract (mg/g)
Ulva Lactuca	0.542	0.590
Gracilaria Cortcata	0.319	0.339
Ascophllum Nodossum	0.402	0.432

4.8. Quantitative estimation of Alkaloids

The estimation of alkaloids was performed via precipitation of extracts (both ethanol and aqueous extracts) via utilizing ammonium hydroxide solution. From the investigation, it could be inferred that the maximum estimated alkaloids quantified was observed 4.402 in ethanolic extract for *Ulva Lactuca* and minimum of 1.871 from the aq. extract of *Gracilaria Cortcata*

Table-8: Quantitative estimation of alkaloids (mg/g) from the ethanolic and aqueous extracts of *Ulva Lactuca; Gracilaria Cortcata* & *Ascophllum Nodossum*

Oceanic algal sp.	Aq Extract	Et. Extract
Ulva Lactuca;	2.288	4.402
Gracilaria Cortcata	1.871	3.981
Ascophllum Nodossum	2.119	3.873

4.9. Antioxidant activity of *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum* involving DPPH assay

1, 1-Diphenyl- 2- picryl hydrazine (DPPH) radical scavenging activity was increased with increase in the concentration of ethanolic and aqueous root, bark and leaf extracts of selected elite germplasms of *S. anacardium* from (50 - 250 µg/mL). The standard wad used Ascorbic acid it is compared with the ethanolic and aqueous root, bark and leaf extracts of selected oceanic algal species- *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum* involving DPPH assay.

Table-9: DPPH antioxidant assay

Oceanic algal species	Conc.	Aqueous extract		Ethanolic extract	
		Absorbance of sample	% Scavenging activity	Absorbance of sample	% Scavenging activity
Ulva Lactuca	50	0.236	16.90	0.232	18.31
	100	0.225	20.78	0.224	21.13
	150	0.223	21.48	0.212	26.05
	200	0.219	22.89	0.204	28.17
	250	0.207	27.11	0.202	28.87
Gracilaria Cortcata	50	0.182	35.91	0.171	39.79
	100	0.174	38.73	0.164	42.25
	150	0.166	41.55	0.152	46.48
	200	0.152	46.48	0.142	50.00
	250	0.148	47.89	0.134	52.82
Ascophllum Nodossum	50	0.218	23.24	0.211	25.70
	100	0.210	26.06	0.189	33.45
	150	0.198	30.28	0.187	34.15
	200	0.181	36.27	0.178	37.32
	250	0.169	40.50	0.161	43.31

4.10. Quantitative distribution of Vitamin E

The following table provides a quantitative distribution of Vitamin E for the algal sp. *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum* as follows

Table-10: Quantitative determination of Vitamin E

Oceanic algal sp.	Tannin content -Tannic acid equivalent (mcg/ml) Aq Extract (100 µg/mL)
Ulva Lactuca	16.1
Gracilaria Cortcata	7.0
Ascophllum Nodossum	N.F.

N.F. - Not Found

4.11. Anti-microbial assay The resultant extracts were further subjected to antibacterial and antifungal activity performed using well-diffusion technique for determining their maximum zone of inhibition. The study concluded that there is a greater degree of ZOI observed for almost all the extracts pertaining to

antibacterial activity. However with respect to antifungal activity, the lowest concentration of 25µl/well showed zone of inhibition against all the tested pathogens of both species, ranging between 14 and 43 mm whilst, the highest concentration of 100 µl/well showed zone of inhibition ranged between 30 to 50 mm. *Candida albicans* showed resistance towards lower concentration but showed minute inhibitory effects at higher concentrations of 75µl & 100 µl. However, when on comparison with other extracts of Acetone, DCM, DMS, DE & DH_2O having No zones or slight zone of inhibition. From the quantitative estimation of the aqueous and ethanol extract of *Ulva Lactuca; Gracilaria Cortcata* & *Ascophllum Nodossum.* Extracts were shown the dose dependent activity against both gram positive and gram-negative bacteria. The zone of inhibition in different concentration were determined and shown in the **Table No 11, Table 12.**

Table-11: Screening of antibacterial effect in Oceanic Algae

Algae Extracts	Zone of Inhibition in mm																								
	Ethanol (µg/ml)				Acetone (µg/ml)				DCM (µg/ml)			DMS (µg/ml)				DE (µg/ml)				D. H2O (µg/ml)				Control (Ampicillin)		
	25	50	75	100	25	50	75	100	25	50	75	100	25	75	50	100	25	50	75	100	25	50	75	100	100
Ulva Lactuca	28	21.1	30	33	17	20	36	47	13	15	34	52	4	14	19	30.1	11	22	21.1	31	SL	NZ	17	29	65
	14	18	9	18	12	20.7	39	42	20	28	21.1	40	6	18	28	21	6	29	30.2	40.1	SL	NZ	NZ	30.1	56
Gracilaria Cortcata	17	21.1	33	6	12	23	32	49	10	20	37	47	49	30	25	44	18	28	SL	69	NZ	NZ	SL	12	58
	14	21.4	29	49	16	27	41	61	12	27	31	49	6.1	6	18	18	20	19	9	52	NZ	NZ	SL	*11*	63
Ascophllum Nodossum	30	31.2	40.5	41.4	20.8	21.2	30.6	30.8	20.8	20.9	21.3	31.1	21.1	21.3	30.1	30.8	30.8	31.1	31.4	40.5	NZ	NZ	SL	SL	52
	40.3	47	31.1	50.7	30.9	31.1	40.1	41.2	NZ	NZ	SL	NZ	20.9	21.4	41.1	40.9	30.4	30.7	40.0	40.7	SL	21.9	21.7	*31.1*	62

Nz- No zone; SL – Slight Zone; Control- Fluconazole

	Escherichia coli
	Proteas Vulgaris

4.12. Anti-fungal activity

The extracts have shown dose dependent antifungal activity against *Candida albicans* and *Aspergillusniger*. The diameter of zone of inhibition of Standard of different concentration were determined and shown in the Table 12.

Table-12: Screening of antifungal effect in Oceanic Algae

Algae Extracts	Zone of Inhibition in mm																								
	Ethanol (µg/ml)				Acetone (µg/ml)				DCM (µg/ml)			DMS (µg/ml)				DE (µg/ml)				D. H2O (µg/ml)				Control	
	25	50	75	100	25	50	75	100	25	50	75	100	25	75	50	100	25	50	75	100	25	50	75	100	100
Ulva Lactuca	20	20.7	20.9	40.4	8	16	SL	21	16	28	SL	29	12	0.9	SL	31	1	8	SL	31.1	SL	NZ	NZ	NZ	42
	31	30.5	9	31.1	10	13	19	20	9	12	SL	NZ	13	23	24	16	16	11	20.7	40	11	SL	NZ	*NZ*	32
Gracilaria Cortcata	14	30.8	19	31.0	12	20	16	40.2	9	12	SL	10	11.1	22	49	61	12	SL	SL	23	SL	NZ	SL	SL	55
	206	30.3	21	30.8	14	17	31	31.2	SL	12	15	16	19	27	32	49	2	15	27	32	SL	NZ	SL	SL	42
Ascophllum Nodossum	21.4	30.3	31.2	40.8	40.6	50.4	50.8	50.6	NZ	SL	SL	NZ	21.1	30.6	30.4	31.4	21.1	30.3	31.4	40.5	NZ	NZ	SL	SL	38
	43	47	31.1	50.7	30.9	31.1	40.1	41.2	NZ	NZ	NZ	NZ	SL	SL	SL	SL	SL	21.1	10.9	NZ	20.7	11.3	SL	*30.2*	45

Nz- No zone; SL – Slight Zone.

	Candida Albicans
	Aspergillus Niger

5. CONCLUSION

Algal communities are some of the unexplored species to date, with regards to investigation on phytochemical & secondary metabolites. From our investigation, it could be inferred that oceanic algal species namely- *Ulva Lactuca; Gracilaria Cortcata & Ascophllum Nodossum* showed efficacious phytochemical attributes. Also from the antibacterial and antifungal activities performed showcased greater inhibitory effects on microbial growth, thus indicating their potent antimicrobial activity, and could pose as an alternative medication over numerous ailments. The prospects aim towards assessing various *in vitro* investigations that could answer numerous investigations to date.

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