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Research Article

ANTI BACTERIAL, ANTI OXIDANT POTENTIAL AND GC/MS APPROACH OF MARINE GREEN ALGAE *ULVA LACTUCA* L. PURIFIED PROTEIN

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ABSTRACT

Seaweeds are good sources of natural anti-oxidant effect of bioactive compounds and can be used as anti-oxidant, controlling bacterial infection in seaweeds as well as pharmacological industries. Present study was analysis of bioactive compounds extracted from *Ulva lactuca L.* HPLC purified protein by the GC-MS study was used to evaluate anti-bacterial and antioxidant effect for controlling bacterial infection in human pathogenic bacteria. ULP protein extract will be novel bioactive compounds with anti-oxidant and radical scavenging possessions divulge the unexploited things of seaweed could be used in the medicinal and food industries.

Keywords: Oxidant, Protein, HPLC, GC-MS and *Ulva*

1. INTRODUCTION

Asian countries broadly use seaweeds for medicinal purposes signifying seaweeds are hopeful sources of novel bioactive compounds and health promoting properties which cannot be found in plants and animals. Some seaweeds compounds purified as alginate are already in the medicinal field as binding agent and carrier material of medical tablets and also wound dressing [1]. Marine algae are widely used in many industries for example food, sweetmeat and textile, pharmacy, dairy and broadsheet mostly as crystallizing, stabilizing and thickening agents. Seaweeds are reservoirs of carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharides. Bioactive elements from marine algae obligate conducted in different parts of the world [2]. Several of the early bioactive components detected in marine algae were polysaccharides and their sulphated derivatives are known as carrageenan and agar, which are used for their therapeutic efficacy [3].

Green, brown and red algae gives an alternative approach to the use of the synthetic antimicrobial agents. However, the potent antimicrobial effect of seaweeds resides in the efficiency of the extraction method [4]. In recent years, there has been an increasing interest in finding natural antioxidants, because these substances can protect the human body from free radical damage and retard the progress of many chronic diseases [5]. Many animal, marine algae and plant sources have been found to possess many biological activities, and the antioxidant activities [6] of some proteins have recently been described.

Several researches have demonstrated that a high dietary intake of natural phenols with the presence of numerous types of antioxidants such as flavonoids commonly found in plants and seaweeds is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, and various types of cancers [7]. Since seaweeds are known to contain an extensive variety of bioactive compounds by itself they are offering a rich source of new drugs with hypothetically lower toxicity [8].

The present study aimed to *in vitro* anti-bacterial effects of marine green seaweed extracted and purified fourth fraction of *Ulva lactuca* L. collected from Mandapam region, Ramanathapuram District Tamilnadu. Fractions were analyzed by GCMS and were tested against some human pathogenic gram positive and gram negative bacterias. In addition of the above, DPPH free radical scavenging and ABTS antioxidant effect of the tested algal sample were also evaluated.

2. MATERIAL AND METHODS

Water, acetonitrile, Helium, Muller Hilton's Agar, Protein solubilizing buffer, ciprofloxacin, DPPH, ethanol, ascorbic acid, D_2O, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS solution, potassium persulphate all kinds of chemical purchased from India | Sigma-Aldrich.

2.1. Sample collection

The collected sample was washed with seawater for two to three times, followed by washing one or two times with distilled water to remove unknown wastes and contamination from other algae. The sample was dried at room temperature; dried sample was homogenated and finally stored at -4 °C further usage [9].

2.2. Extraction of sample

Homogenized algal sample (5 g), comprising distilled water (D_2O, 5 mL), 95 % ethanol (15 mL), and concentrated sulphuric acid (0.70 mL) with uniform shaking for 15 minutes. Once again, 15 mL D_2O and 95 % ethanol (40 mL) were added, and the pH was adjusted to 1.7 using sulphuric acid. The suspension was then filtered using Whattman No-1 filter paper and then again, the pH was adjusted to 3.0 using ammonia solution. To this suspension, 150 mL of 95 % ethanol and 200 mL diethyl ether were added and kept for 12 h at 4°C. After centrifugation at 6,000×g for 20 min, pellet was dissolved in 25% ethanol, final sediment was washed with acetone and di ethyl ether, and pH was adjusted to 8.5 [9-10]. Precipitate was collected for further analysis.

2.3. Purification through RpHplc

Ulva lactuca L. extracted sample was analyzed by HPLC on a (Waters, Australia) reverse phase Column-18 300A (5.0 µm particle size) Phenomenex - 250 × 4.6 mm column. The mobile phase was water/acetonitrile (50:50 v/v) mixture supplied at the rate of 0.0-0.20 mL/min, using a sample temperature of 25°C during the analysis. The analytical HPLC was performed using Empower 2 software following isocratic method. The purified protein/peptide fractions were freeze-dried and stored at -20°C and used for the further study [9] following Purified characterizations of protein.

2.4. GC-MS Spectroscopic approaches

The GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. One (1.0) µL aliquot of sample was injected into the column using a PTV injector whose temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 °C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the samples were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

2.5. Antibacterial assay

The antimicrobial activities of samples were checked by following Disc diffusion method. The MHA (Muller Hilton's Agar) plates were spread inoculated with 100µl of log cultures (adjust with Mcfarland unit (0.5) concentration) of all the bacteria (*Bacillus subtilis* (MTCC1133), *S. aureus, Pseudomonas aeruginosa* (MG650162), *Serratiamarcescen s*(MG650161) and *E. coli* (MTCC40) followed by placing the discs containing a volume (100µl) of the antibacterial agent or extract solution containing concentration 100µg/ml of sample (ULP). One disc was loaded with solvent (Protein solubilizing buffer) alone which served as vehicle control or negative control and ciprofloxacin solution (20µg/well) was taken as positive control for bacteria. The plates were incubated at 30°C for 12-24 h. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested and halo zones created around the well were measured and recorded.

2.6. Anti-oxidant activity

2.6.1. DPPH radical scavenging activity

DPPH radical scavenging activity of the ULP was evaluated with some modifications [11]. 0.1 mM of DPPH in 95% ethanol (v/v) was prepared and 2ml sample (1.00 mg/L) was added to 3.0 ml of such solution. The mixture was allowed to stand in dark for 35 min in room temperature. The absorbance of 517nm in the blank control, the sample was relieved with D_2O. In the positive control, the sample was relieved with ascorbic acid. The DPPH scavenging activity was calculated by the following equation:

$$\text{Scavenging effect (\%)} = (1 - A_{\text{sample517}} / A_{\text{control517}}) \times 100$$

2.6.2. ABTS free radical scavenging assay

Free radical scavenging assay (modified) was used [12]. ABTS radical cations ($ABTS^+$) were produced by reacting ABTS solution (7mM) with 2.45mM potassium persulphate. The mixture was incubated at room temperature in the dark for 12 to 16 hrs to yield a dark-

colored solution containing $ABTS^{\bullet+}$ radicals and diluted for an initial absorbance of about 0.700 (±0.02) at 734 nm. Aliquots (10µl) of the different concentrations of extract were added to 1ml of ABTS solution. The absorbance was read at 734nm after 6 minutes in a spectrophotometer. L-Ascorbic acid was used as the standard. Appropriate solvent blanks were run in each assay. All determinations were carried out in triplicate and the percent of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

The results were P<3.00 expressed as mean of three triplicates ± SD and bacterial and anti oxidant were statistically analyzed by using one way analysis of variance ANOVA (SPSS 16.0).

3. RESULTS AND DISCUSSION

Extracted *Ulva lactuca* L., sample using RpHplc on a (Waters, Australia) reverse phase Column-18 300A (5.0 µm particle size) Phenomenex - 250 × 4.6 mm column. The mobile phase was water/acetonitrile (50:50 v/v), taken four type of fraction such as 2.407, 5.217, 6.362, and 8.702 (Fig.1). In this fraction large amount of only fourth fraction was taken because of minimum below 10 have been low molecular weight proteins that protein used for various diseases and encapsulation of medicinal fields.

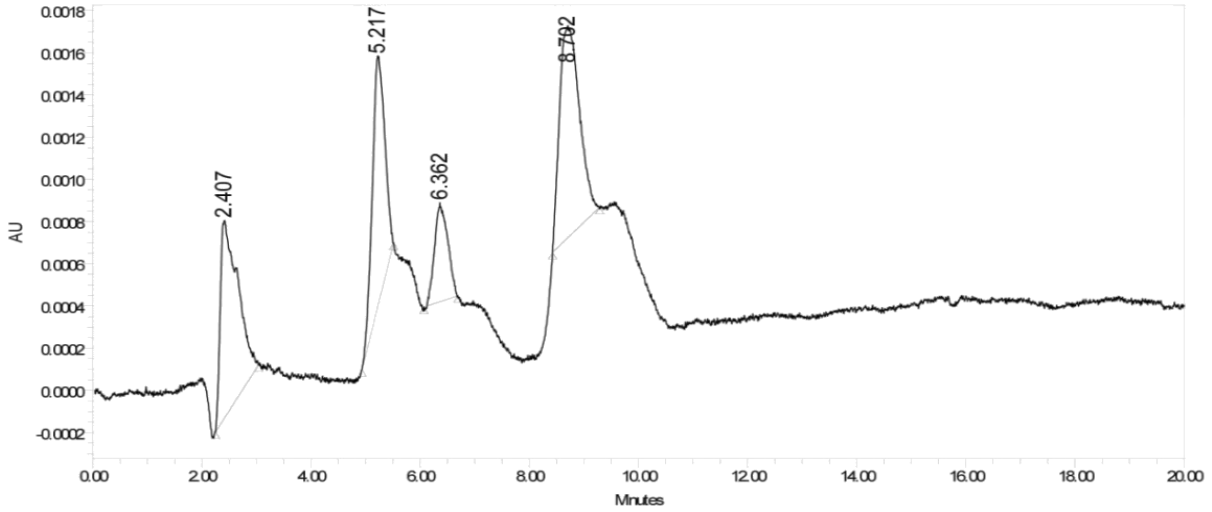


Fig. 1: Hplc chromatogram of *Ulva lactuca*L. extracted sample

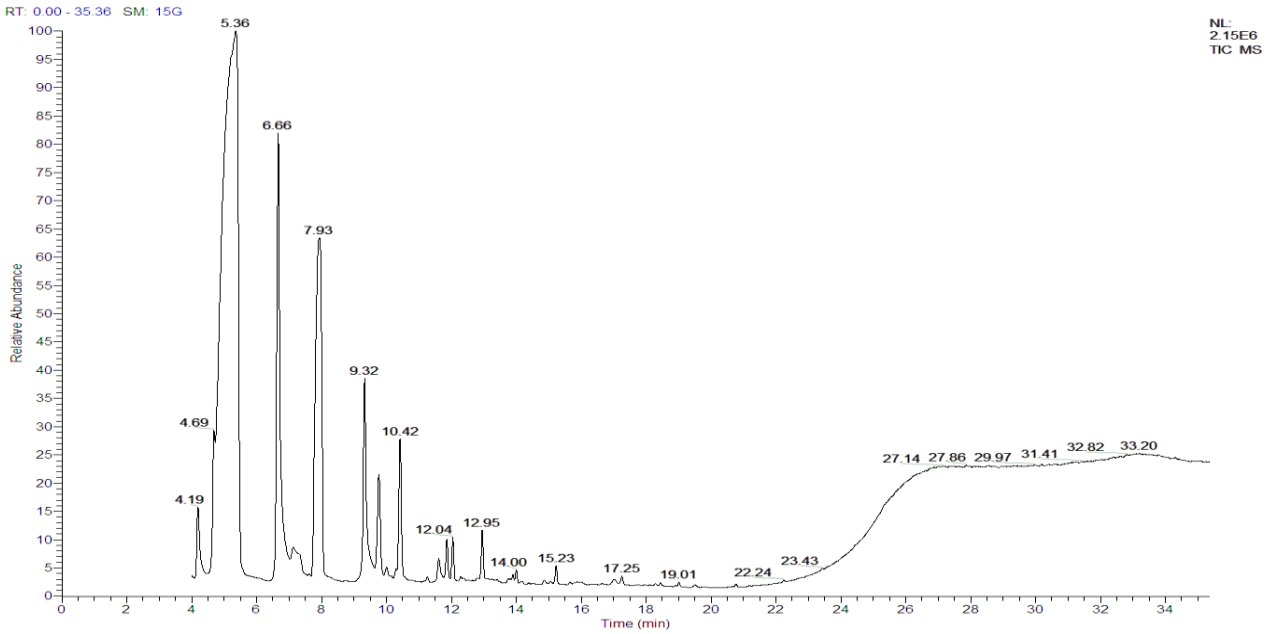
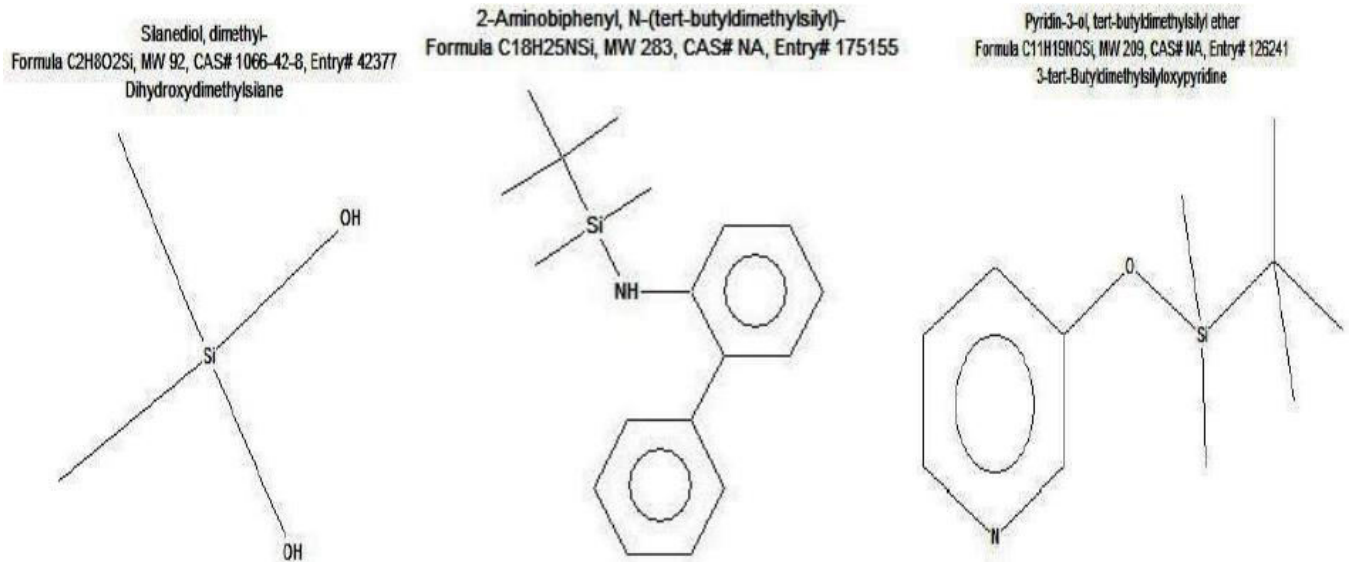


Fig.2: GCMS Spectroscopy of purified sample fraction

The high resolutions of mass spectrum equipped with a data system in combination with gas chromatography was used for the chemical analysis of active marine green seaweed *Ulva lactuca* L. extracted crude. Based on the spectral data GC-MS analysis, it was found to be a mixture of volatile compounds and eight major peaks were observed with retention time as presented in fig.2 and table 1. 4,6- Bis (1,1- Dimethyllethyl) -2,2- Dimethoxy-1,1, 3,3-Tetraphenyl 2-Ol acid is high abundant (RT 12.04) molecular weight of 418 and molecular formula $C_{28}H_{34}O_3$ and retention time of 4.19 molecular weight 92, formula of $C_2H_8O_2S$ first peak of spectroscopy analysis.

Table.1. Main components from crude extract of *Ulva lactuca* L.

S.No	Compounds Name	MW	RT	Formula	Biological uses	Reff.
1	Silanediol, Dimethyl- Dihydroxydimethyisilane	92	4.19	$C_2H_8O_2Si$	-	-
2	2- Aminobiphenyl	289	5.36	$C_{18}H_{25}NSi$	Cosmetics and coloring agent	[18]
3	Pyridin-3-Ol, Tert-Butyldimethylsilyl Ether	209	6.66	$C_{11}H_{19}NOSi$	Anti-Proliferative activity	[19]
4	1,1- Biphenyl, 4,2,3,4- Tetramethoxy-5- Methyl- Methylaminomethyl	331	7.93	$C_{19}H_{25}NO_4$	Insecticides	[20]
5	Phenanthridin-6, 10-Diol 10 Acetyl-2 Methyl-7,8,9,10-Tetrahydro	271	9.32	$C_{16}H_{17}NO_3$	Alkaloids	[21]
6	Silane, Diethyl (2-Pentyloxy) Tetradecyloxy	386	10.42	$C_{23}H_{50}O_2Si$	-	-
7	Bhenzophenone, 2-Hydroxy-4methoxy, (2-Carboxyphenyl) Hydrazone	362	12.95	$C_{21}H_{18}N_2O_4$	-	-
8	4,6- Bis (1,1- Dimethyllethyl) -2,2- Dimethoxy-1,1, 3,3 -Tetraphenyl 2 -Ol	418	12.04	$C_{28}H_{34}O_3$	-	-



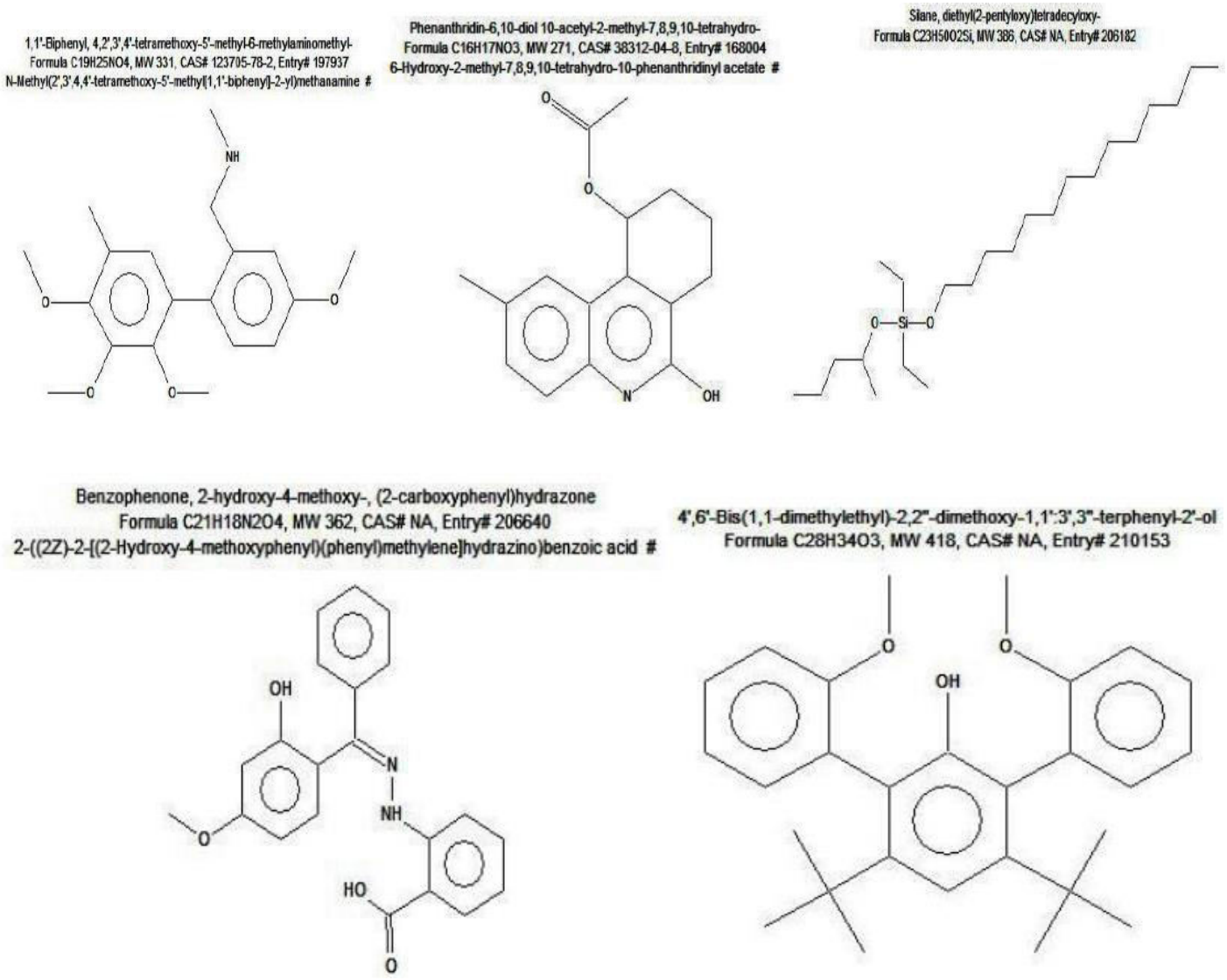


Fig.3: GC-MS Volatile compounds structure help of NIST tool

3.1. Anti-bacterial effect

Fractions of protein were screened against five human pathogenic bacteria by determining maximum inhibitory concentration (MAC results shown Table 2, Fig. 4). Purified sample's growth inhibitory effect against *Basillus subtilis* gram pasitive bacteria, *E.coli* gram negative bacteria, *Staphylococcus aureus* gram pasitive bacteria, *Pseudomonas aeruginosa* gram negative bacteria and finally *Serratia marcescens* gram negative bacteria was observed.

Table 2: Measurement of zone of inhibition anti-bacterial effect of ULP

Concentration of sample	*B. subtilis*	*E. coli*	*S.aureus*	*P. aeroginosa*	*S. marcescens*
Sample (ULP fraction) (100µg/ml)	**40.33±0.881**	**36.66±1.201**	**32±0.55**	**43±1.154**	**37.33±0.881**
-Ve control	**1.33±0.33**	**2.66±0.33**	**7.33±1.45**	**3.33±0.881**	**10.66±0.881**
+Ve control	**11.66±0.881**	**6.33±0.881**	**25±2.306**	**17.33±2.33**	**13.66±3.71**

Anti-bacterial MAC determination indicated the purified fraction inhibits the microorganisms tested. The *U. lactuca* fraction exhibited broad spectrum anti-bacterial activity, even on drug resistant *E.coli*.

The protein solubilizing buffer used as the negative control in this test indicated activity on some microorganisms tested. Ciprofloxacin solution (20µg/well) was also used as positive control. Results of

the anti-bacterial effects of *Ulva lactuca* purified fraction IV showed most resistance in this effect when compared to positive control and negative control. ULP sample exhibited good effect on *Basillus subtilis*, *Pseudomonas aeruginosa* moderate effect on *E.coli*, *Serratiamarcescens* least effect on *Staphylococcus aureus*.

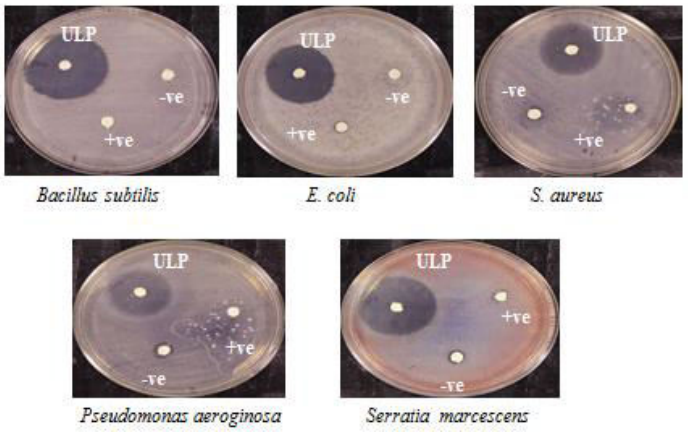


Fig. 4: Antibacterial activity of purified ULPfrom marine green seaweed

+ve control-ciprofloxacin; -ve control-protein solubilizing buffer; ULP-Ulva Lactua Protein

3.2. Anti oxidant activity

The DPPH radical scavenging assay is accredited to hydrogen donating ability of the anti oxidant. radicals scavenging ability of the ULP fraction are shown in fig. 5.

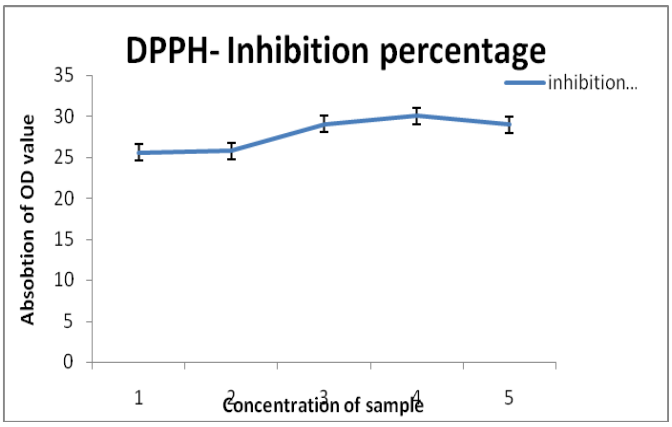


Fig. 5: DPPH activity of sample compared with Standard

Table 3: DPPH activity of sample

Samples	% of inhibitions				
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
Sample	25.55±6.53	25.77±4.16	29.05±2,31	30.08±1.64	28.97±4.66
Std. (Ascorbic acid)	26.88 ± 1.88	58.44 ± 4.09	77.33 ± 5.41	91.33 ± 6.39	94.68±5.66

Table.4. ABTS activity of sample

Samples	% of inhibitions				
	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	100 (µg/ml)
Sample	20.66 ± 1.44	43.55 ± 3.04	72.22 ± 5.05	83.55 ± 5.84	78.33±4.78
Std. (Ascorbic acid)	26.88 ± 1.88	58.44 ± 4.09	77.33 ± 5.41	91.33 ± 6.39	94.68±5.66

Values expressed as Mean ± SD for triplicate

IV fraction possesses strong DPPH scavenging activity among different concentrations (fig.5, table 3). ULP fraction exhibited the highest DPPH radical scavenging effect, followed by the IV fraction (25.55±6.53 at 20µg/ml, to 30.08±1.64 at 80µg/ml and 28.97±4.66 at 100 µg/ml) Furthermore, the scavenging effects increased.

The virtual antioxidant effect of radical scavenging ABTS+ have been compared to the standard ascorbic acid is an admirable way for determination of anti-oxidant activity of hydrogen donating anti-oxidant and molecular chain breaking of anti-oxidants.

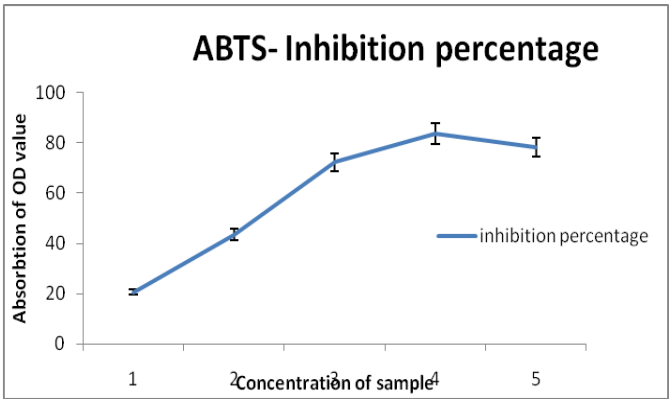


Fig. 6: ABTS activity of sample compared with Standard

Fig. 6, table 4 represents action of ABTS+ radical scavenging of ULP fraction at four different concentrations. High Radical scavenging of ABTS for sample was observed at 80 µg/ml. High concentration of extract was more effective in appeasing free radicals in this activity therefore following anti-oxidant effect [16, 17].

4. CONCLUSION

Marine green algae have several active chemicals such as antioxidant and antibacterial compounds. This investigation underwent detailed investigations with the objective of isolating biologically active molecules along with the search for new compounds. Moreover, it was specified that the Mandapam region Ramanathapuram (Pamban) is a probable source of a multiplicity of naturally active marine organisms. In this research it was reported first time that ULP purified fraction possess anti-oxidant and antibacterial activities and some essential identified compounds the identified chemical compounds are not working properly in bioactive tests. It is concluded that fractionated purified sample can be used as a source of natural antibacterial and antioxidant agents for food poisoning and human health, furthermore, characterizations of active principle is needed to understand the mechanisms of action and therapeutic value *in-vivo* and *in-vitro* and *in-silico*.

5. ACKNOWLEDGEMENT

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Conflict interest

The author declared no conflict of interest

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