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Bioactive Chemical Constituent Analysis, *in vitro* Antioxidant and Antimicrobial Activity of Whole Plant Methanol Extracts of *Ulva lactuca* Linn

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Authors' contributions

This work was carried out in collaboration between all authors. Author VTA designed the study and wrote the protocol. Author RNV conducted the study, managed the literature searches and wrote the first draft of the manuscript. Author KDR managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To analyse the bioactive chemical constituents and evaluate *in vitro* antioxidant and antimicrobial activities of whole plant methanol extracts of *Ulva lactuca* Linn.
Study Design: Preliminary phytochemical screening, Gas Chromatography-Mass Spectrometry (GC-MS) analysis, evaluation of antioxidant and antimicrobial activities.
Place and Duration of Study: Veterinary University Training and Research Centre, Ramanathapuram-623503, Department of Botany, A.V.V.M Sri Pushpam college, Poondi, Thanjavur-613503 and K.M.C.H college of pharmacy, Kovai Estate, Kalapatti Road, Coimbatore-641048. The studies were carried out during August-December 2016.
Methodology: Phytochemical constituents were identified by qualitative and GC-MS analysis.

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In vitro antioxidant activity of the extracts were studied using DPPH radical scavenging assay, total phenol and total flavonoid content determination assays. Antimicrobial activity was investigated by disc diffusion technique.

Results: Phytochemical screening of methanol extracts showed positive results for alkaloids, flavonoids, phenols, tannins, terpenoids, glycosides, steroids, proteins and negative results for saponins and cardiac glycosides. GC-MS analysis revealed the presence of 10 compounds in *Ulva lactuca* methanolic extracts and some of the phytocomponents screened were terpenoids, fatty acid derivatives and aliphatic hydrocarbons and many of them have antioxidant, anti-inflammatory, antimicrobial and anticancer actions. Methanolic plant extract showed significant antioxidant activity under DPPH free radical scavenging activity, total phenol and total flavonoid content determination assay. In disc diffusion technique among six bacterial species, methanol extract showed potent activity against three Gram positive (*Bacillus subtilis*, *Corynebacterium diphtheriae* and *Staphylococcus aureus*) and three Gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella paratyphi*). The extracts were more potent towards fungal isolates (*Aspergillus niger* and *Aspergillus fumigatus*) and no activity reported in *Candida albicans.*

Conclusion: The results demonstrate that methanolic extracts of whole plant of *U. lactuca* can be used as a potential source of antioxidant and antimicrobial agent.

Keywords: Ulva lactuca Linn; GC-MS; antioxidant; phenolic; flavonoids; DPPH; antimicrobial.

1. INTRODUCTION

Seaweeds are one of the important marine living resources and are excellent source of Vitamins (A, B, B12, C, D & E), riboflavin, niacin, panthothanic acid and folic acid as well as minerals such as Ca, P, Na and K, bioactive substances such as polyunsaturated fatty acids, sterols, proteins, polysaccharides, antioxidants and pigments [1]. Seaweeds known as macroalgae produce many biologically active phytochemicals such as carotenoids, terpenoids, xanthophylls, chlorophylls, phycobilins, polyunsaturated fatty acids, polysaccharides, vitamins, sterols, tocopherols and phycocyanins [2]. Nowadays seaweeds are used as dietary supplements in daily life to animals and human and are easily metabolized [3]. Seaweed dietary fibres perform varied range of functions such as antioxidant, antimicrobial, antimutagenic, anticoagulant, antitumor etc., [4]. Nature provides varied antimicrobial compounds that defence against all kinds of living organisms [5]. Marine organisms are emerging as good alternate source for bioactive substances. [6] surveyed the occurrence of organic compounds from marine organisms that have been reported to possess antimicrobial activities. Metabolites from microorganisms is produced as a result of biochemical process largely obtained from algae and invertebrates may be produced by associated microbes. Several researchers have made attempts to identify marine organisms producing bioactive substances and met with success [7,8]. In recent years, many microorganisms are developing resistance against to synthetic and semi-synthetic drugs used for microbial infections treatment. However, the synthetic antimicrobial agents have the side effects. Thus, numerous scientists supposed that there is an urgent need to develop or discover new antimicrobial substances [9,10]. The results of numerous studies indicated that seaweed extracts had selective and effective antimicrobial activities against bacteria, fungi and virus in the parts of the world [11]. There are reports that seaweeds are also rich source of antioxidant compounds [12,13]. Lipids, proteins and nucleic acids involve in reactive oxygen species that cause oxidative damage. It may trigger various chronic diseases such as coronary heart disease, atherosclerosis, cancer and aging [14]. Epidemiological studies have indicated that intake of certain vitamins, minerals and other nutrients help to protect the body against heart disease, cancer and aging process. Antioxidants may have a protective effect in preventing or reducing the severity of these diseases [15]. The synthetic antioxidants, such as butylated hydroxyanisol and butylated hydroxytoluene have been suspected to use theirs effects of carcinogenesis and liver damage [16]. Concerns about the reliability of synthetic antioxidants have increased the interest in plants and algae commonly present in natural antioxidants [17,18]. Hence in the present study is concerned screening of phytochemicals by qualitative analysis and GC-MS study to identify the components responsible for antioxidants and antimicrobial property in *Ulva lactuca* Linn. (Family: Ulvaceae), commonly called as green

seaweed/ sea lettuce/ sea grass which is an abundant marine organism.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

The whole plants of *Ulva lactuca* Linn. (Family: Ulvaceae) were collected in Mannar Coast, Rameshwaram, Ramanathapuram District (Latitude and Longitude are 9° 23' 0" N and 78° 50' 0" E) respectively, Tamilnadu, India. The seaweed were collected in a single location site during spring season (Aug-Nov 2016). The collected seaweeds were identified and a voucher specimen (No: VUTRC 001) has been deposited for future investigation. The whole plants were thoroughly washed with water and placed into a dryer having a good air circulating system and a temperature controlling thermostat. Then all parts were dried in hot air oven at 60℃. The dried whole plants were ground to coarse powder with a mechanical grinder (Grinding Mill). The weight of the total dry powder was 1000 g. 500 g powdered plant materials were used for extraction. Extraction was performed with solvent, methanol as per the method stated [19]. The powders were kept in 800 mL of methanol for 5 days in sealed container accompanying occasional shaking and string. Then extracts were filtered through fresh cotton bed. Then solvent was completely removed by heating in a water bath at temperature of 40 ± 2ºC. After methanol extraction the powders were dried and the yield obtained was 40.5 g and stored at 4ºC until use.

2.2 Phytochemical Screening

The phytochemical screening was assessed as per standard method [20]. Phytochemical screening was performed using methanol solvents to identify the major natural chemical groups such as alkaloids, flavonoids, phenols, tannins, terpenoids, glycosides, cardiac glycosides, steroids, proteins and saponins.

2.3 GC-MS Analysis

Based on the preliminary phytochemical results, methanolic extract of *Ulva lactuca* Linn. was chosen for the analysis of possible bioactive compounds by GC and MS technique. The Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited, were engaged for analysis [21]. The instrument was set as follows, Injector port temperature set to 250ºC, Interface temperature set as 250ºC, and source kept at 200ºC. The oven temperature programmed as a variable, 70ºC for 2 minutes, 150ºC @ 8ºC/min, up to 260ºC @ 10ºC/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS Nonpolar column was used whose dimensions were 0.25 mm OD x 0.25 µm ID x 30 metres length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200ºC and < 40 motor vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

2.4 Antioxidant Activity Evaluation

2.4.1 DPPH free radical scavenging assay

DPPH free radical scavenging assay was determined as described by Rajesh and Natvar [22]. The hydrogen donating ability was examined in the presence of DPPH stable radical. One millilitre of 0.3 mM DPPH methanol solution was added to 1 mL of plant extracts (1000 µg/mL) at different concentration and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm. Methanol solution was used as a blank and DPPH solution (1.0 mL, 0.3 mM) with 1 mL methanol served as negative control. Ascorbic acid (1000 µg/mL) was taken as the positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ inhibition} = \frac{A_{control} - A_{test}}{A_{control}} X\ 100$$

Where '$A_{control}$' was the absorbance of the control reaction and 'A_{test}' was the absorbance in the presence of the extract/standard. The mean values were obtained from triplicate analysis. The antioxidant activity of the extract was expressed as IC_{50}.

2.4.2 Determination of total phenol content

The total phenolic content of the extract was determined using the Folin-Ciocalteu assay

method described by Kokate et al. [23]. An aliquot (1 ml) of extract or standard solution of gallic acid (20, 40, 60, 80 and 100 µg/ml) were mixed with 0.2 ml of Folin-Ciocalteu reagent. After 5 min, 1 ml of 15% Na_2CO_3 solution and 2 ml of distilled water were added to the above mixture. Reagent blank using distilled water was prepared. After incubation for 90 min at room temperature, the absorbance of the mixture was measured against prepared reagent blank at 750 nm. Samples were analysed in triplicate. The mean of three readings was used and the total phenolic contents were expressed as milligrams of Gallic equivalents per gram extract.

2.4.3 Determination of total flavonoid content

The total flavanoid content of *Ulva lactuca extract* was determined using the aluminium chloride colorimetric method described by [20]. Quercetin was used to make the calibration curve. Ten milligrams of Quercetin was dissolved in 80% methanol and then diluted to 20, 40, 60, 80 and 100 µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of methanolic extract was reacted with aluminum chloride for determination of flavonoid content as described above. All samples were analysed in triplicate. The mean of three readings was used and the total flavonoid contents were expressed as milligrams of quercetin equivalents per gram extract.

2.5 Antimicrobial Activity Evaluation

2.5.1 Microorganisms

Three Gram positive *Bacillus subtilis, Corynebacterium diptheriae and Staphylococcus aureus,* three Gram negative *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella paratyphi* and three fungal isolates *Aspergillus fumigatus, Aspergillus niger* and *Candida albicans* were used for this investigation. The bacterial and fungal strains were collected as pure cultures from the Department of Pharmacy, K.M.C.H college of Pharmacy, Coimbatore.

2.5.2 Antimicrobial activity by disc diffusion technique

The inoculums for the experiment were prepared in fresh nutrient broth for determination of antibacterial activity and fresh sabouraud's broth for determination of antifungal activity from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture may be adjusted by the addition of sterile saline or broth (if excessive) or by further incubation to get required turbidity [23]. Cotton wool swab on wooden applicator or plastics were prepared and sterilized by autoclaving or dry heat (only for wooden swabs) by packing the swabs in culture tubes, papers or tins etc. Sterilize forceps by dipping in alcohol and burning off the alcohol**.** The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60º after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed. Each petri dish is divided into 2 parts, in one part sample disc *Ulva lactuca* (100 µg) (discs are soaked overnight in sample solution) and another part standard Ciprofloxacin (10 µg) in case of antibacterial activity and standard Clotrimazole (10 µg) in case of antifungal activity, were placed with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4ºC or at room temperature for 1 hour for diffusion. Incubate at 37ºC, 24 hours for antibacterial activity and incubate at 28º C, 48 hours for antifungal activity. Observe the zone of inhibition produced by different samples. Measure it using a scale and record the average of two diameters of each zone of inhibition.

2.5.3 Determination of minimum inhibitory concentration

Serial 2 fold dilutions of the test antimicrobial agent were made in 1ml of Muller Hinton Broth. Series of 10-15 dilutions to final concentrations of 100-1.56 µg/ml are prepared. Overnight culture are grown at 37ºC Kirby Bauer procedure [24] and diluted to Muller Hinton Broth. This overnight culture was diluted to 10^{-2}. The sterile tubes were labelled 1-8 and 8^{th} tube was taken as control. 1

ml of Muller Hinton Broth was transferred to all tubes, except 6th & 7th. 0.5 ml of broth was transferred to 6th & 7th tubes. 1 ml of drug solution was added to 1st tube and mixed well. From the 1st tube transfer 1ml of solution to the 2nd tube and was repeated up to 6th tube. From the 6th tube 0.5 ml of solution was taken and transferred to 7th tube. 0.01 ml of culture was added to all the test tubes. All the tubes were incubated at 37ºC for 18-24 hrs for antibacterial and 25ºC for 24-48 hrs for antifungal activity. After incubation observe the turbidity by visually or OD value by Spectrophotometric method.

2.6 Statistical Analysis

Linear regression analysis was used to calculate the IC_{50} values. Pearson's correlation coefficient was calculated using Microsoft excel 2007.

3. RESULTS

3.1 Phytochemical Screening

In the preliminary phytochemical analysis of methanolic extracts of *Ulva lactuca* Linn showed (Table 1) the strong positivity of flavonoids, terpenoids and steroids and positivity of alkaloids, phenols, tannins and proteins. However, cardiac glycosides and saponins are absent in the study.

Table 1. Qualitative phytochemical screening of *Ulva lactuca*

S. no	Test	Methanol extract
1.	Alkaloids	+
2.	Flavonoids	++
3.	Phenols	+
4.	Tannins	+
5.	Terpenoids	++
6.	Glycosides	+
7.	Cardiac glycosides	-
8.	Steroids	++
9.	Proteins	+
10.	Saponins	-

++: Strong positive; +: Positive; -: Negative

3.2 GC-MS Analysis

The GC-MS analysis revealed the presence of ten compounds in the methanolic sea weed extracts by comparing their retention time and by interpretation of their mass spectra (Table 2 and Fig. 1). The compounds identified such as a´-PINENE, dl – Limonene and Nonacosane (CAS) exhibited antimicrobial property. a´-OCIMENE exhibited antioxidant property. Compounds such as Neophytadiene, Phytol exhibited both antioxidant and antimicrobial activity studied.

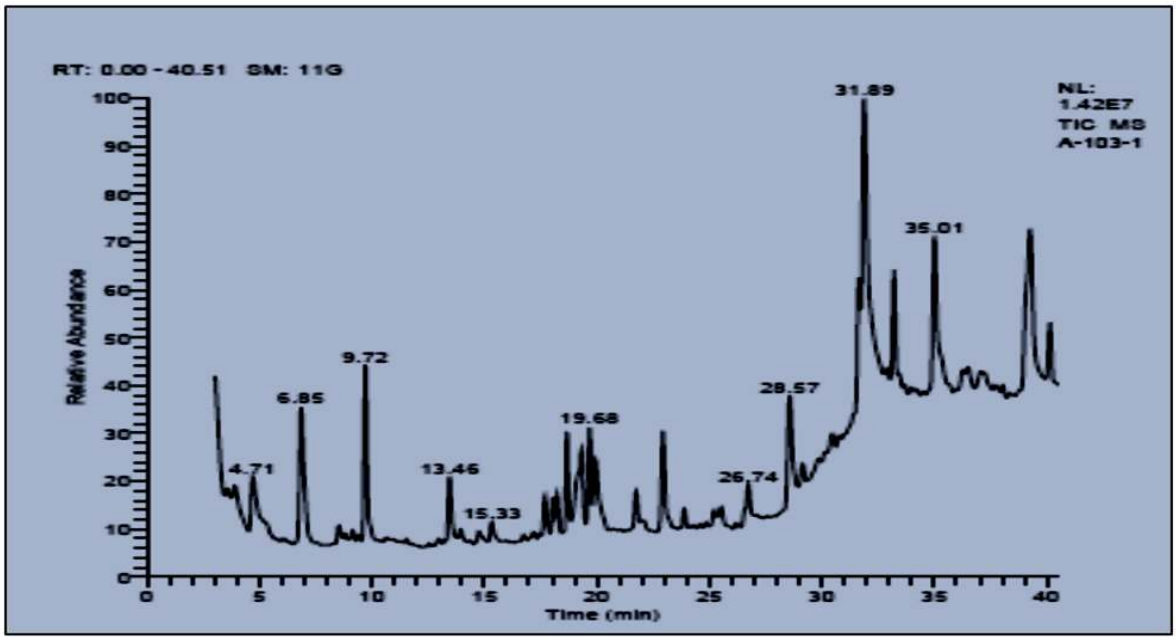

Fig. 1. GC-MS chromatogram of *Ulva lactuca*

Table 2. Activity of phytocomponents in *Ulva lactuca*

S. no	RT	Chemical compound	Nature of compound	Medicinal uses**
1.	4.71	a´-PINENE, (-)-	Terpene derivative	Antimicrobial
		dl – Limonene	Terpene derivative	Antimicrobial
		a´-OCIMENE	Terpenoids	Antioxidant
2.	6.85	1- Dodecanol (CAS)	Alcoholic compound	Antimicrobial
3.	9.72	Tetradecene	Long chain fatty acid	Antifungal
4.	13.46	1- Hexadecanol (CAS)	Aliphatic hydrocarbon	Antibacterial, antioxidant
5.	15.33	8- Heptadecene	Long chain fatty acid	Antibacterial
6.	19.68	2-Hexadecen-1-ol, 3,7,11,15- tetramethyl-, [R-[R*,R*-(E)]] (CAS)	Diterpene	Antioxidant, Antimicrobial, Anthelmintic, Anticancer, Anti-inflammatory, Diuretic and Antidiabetic
		Neophytadiene	Diterpene	Antioxidant, Antimicrobial
		Phytol, acetate	Diterpene	Antioxidant, Antimicrobial
		3- Eicosyne	Saturated aliphatic hydrocarbon	Antibacterial
7.	26.74	Octadecanoic acid, ethylester (CAS)	Fatty acid derivative	Anti-inflammatory, Hypocholesterolemic, Antibacterial, Hepatoprotective, Antihistaminic, Antiarthritic, Anticoronary
		Tetradecanoic acid, ethylester (CAS)	Fatty acid derivative	Anti-inflammatory, Antibacterial
8.	28.57	Nonacosane (CAS)	Aliphatic hydrocarbon	Antibacterial
		Docosane (CAS)	Aliphatic hydrocarbon	Antibacterial
		Heptacosane (CAS)	Aliphatic hydrocarbon	Antibacterial
9.	31.89	Methyl (12E) – 12- [(2,4-Dinitrophenyl) Hydrazon O] Dodecanoate	Aromatic compound	Antimicrobial
10.	35.01	Nonacosane (CAS)	Aliphatic hydrocarbon	Antibacterial
		Octacosane	Aliphatic hydrocarbon	Antibacterial
		Heptacosane – 9- hexyl- (CAS)	Aliphatic hydrocarbon	Antibacterial

***Sources: Dr. Dukes´s phytochemical and ethnobotanical databases*

3.3 Antioxidant Activity of *Ulva lactuca* Extracts

3.3.1 DPPH free radical scavenging assay

The results of DPPH free radical scavenging activity of plant extracts and standard were summarized in Table 3 and Fig. 2. DPPH free radical scavenging activity of methanol extract of *Ulva lactuca* was found to be increased with the increase of concentration of the extracts. IC_{50} values of the methanol extracts was 81.36 µg/ mL and standard 56.95 µg/ mL respectively. The IC_{50} values differ significantly ($p< 0.05$) from one another.

3.3.2 Determination of total phenol content

Results of total phenol content was presented in Table 4 and Fig. 3. Total phenol content activity was found to be increased with the increase of concentration of the extracts (µg/ mL) as compared to the standard GAE (mg/g).

Table 3. DPPH free radical scavenging activity of *Ulva lactuca*

S. no	Concentration (µg/ ml)	Percentage inhibition	
		Ascorbic acid (Standard)	Methanolic plant extract (Test)
1.	10	27.71	4.849
2.	20	30.2	18.01
3.	40	34.87	34.87
4.	60	55.65	38.80
5.	80	59.81	51.03
6.	100	66.87	56.12
IC_{50} values		**59.65**	**81.36**

Table 4. Determination of total phenol content

S. no	Concentration (µg/ ml)	Absorbance value	
		Gallic acid (Standard)	Methanolic plant extract (Test)
1.	10	0.418	0.134
2.	20	0.641	0.173
3.	40	0.871	0.220
4.	60	1.137	0.492
5.	80	1.339	0.738
6.	100	1.418	0.881

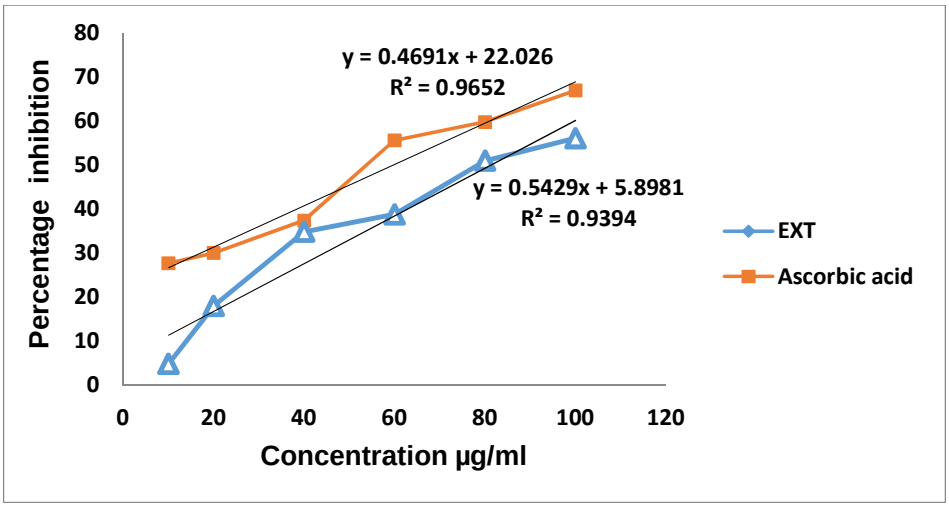

Fig. 2. Determination of antioxidant activity using DPPH free radical scavenging assay

3.3.3 Determination of total flavonoid content

Results of total flavonoid content was presented in Table 5 and Fig. 4. Total flavonoid content activity was found to be increased with the increase of concentration of the extracts (µg/ mL) as compared to the standard QE (mg/g). The IC_{50} values for standard was 44.31 µg/ mL and test extracts was 92.84 µg/ mL.

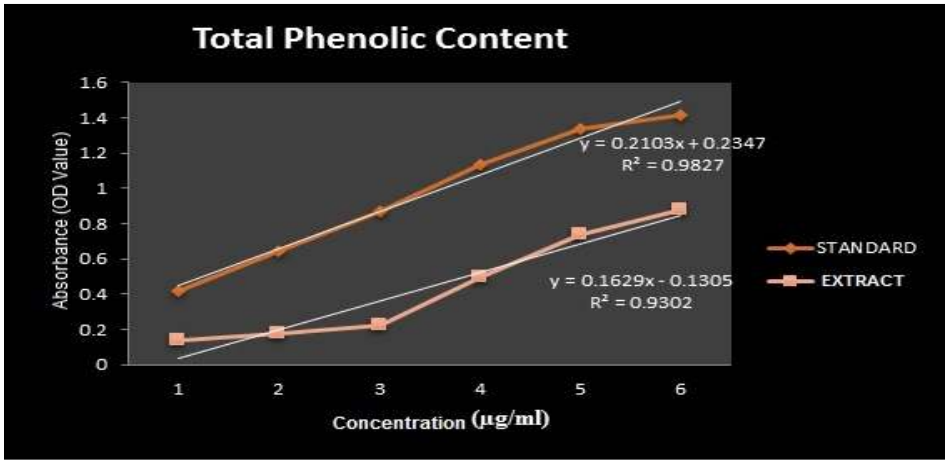

Fig. 3. Determination of total phenol content

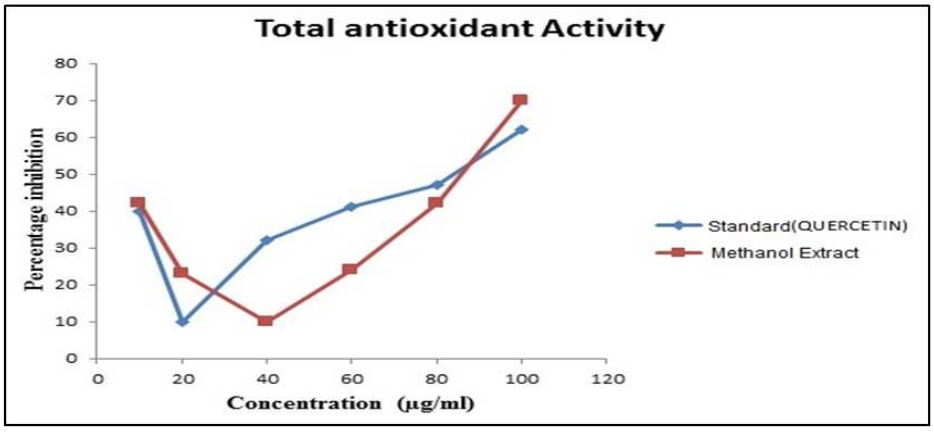

Fig. 4. Estimation of total flavonoid content

Table 5. Determination of total flavonoid content

S. no	Concentration µg/ ml	Standard (Quercetin)		Test (Methanolic extract)	
		O.D value	Percentage inhibition	O.D value	Percentage inhibition
1.	10	0.673	40	0.683	42
2.	20	0.432	10	0.592	23
3.	40	0.328	32	0.432	10
4.	60	0.284	41	0.368	24
5.	80	0.257	47	0.282	42
6.	100	0.182	62	0.142	70
IC_{50} values		**44.31 µg/ml**		**92.84 µg/ml**	

3.4 Antimicrobial Activity of *Ulva lactuca* Extracts

3.4.1 Antibacterial screening

The result of disc diffusion method of *U. lactuca* extracts for bacterial isolates are shown in Table 6 and the Minimum Inhibitory Concentration (MIC) for the isolates are presented in Table 7. Among the six bacterial species methanol extracts of *U. lactuca* showed strong activity against all bacterial isolates as compared to standard ciprofloxacin. The MIC values was found to be 250 µg/ mL for *Bacillus subtilis, Staphylococcus aureus* and *Salmonella pratyphi* and 500 µg/ mL for *Corynebacterium diphtheria, Escherichia coli* and *Pseudomonas aeruginosa.*

3.4.2 Antifungal screening

The result of disc diffusion method of *U.lactuca* extracts for fungal isolates are shown in Table 7 and the Minimum Inhibitory Concentration (MIC) for the isolates are presented in Table 9. Among the three fungal species methanol extracts of *U.lactuca* showed strong activity against two organism *Aspergillus fumigatus* and *Aspergillus niger* and no action against *Candida albicans* when compared to the standard clotrimazole. The MIC values was found to be 125 µg/ mL for *Aspergillus fumigatus and Aspergillus niger.*

Table 6. Antibacterial activity of methanol extract of *Ulva lactuca*

S. no	Microorganism	Inhibition zone (mm)	
		Standard	Test
		Ciprofloxacin (10 µg/ disc)	*Ulva lactuca* (100 µg/ disc)
1.	*Bacillus subtilis*	33	12
2.	*Corynebacterium diphtheriae*	15	11
3.	*Staphylococcus aureus*	31	13
4.	*Escherichia coli*	15	10
5.	*Pseudomonas aeruginosa*	16	09
6.	*Salmonella paratyphi*	30	15

Table 7. Minimum inhibitory concentration value against bacterial pathogens

S. no	Microorganism	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	MIC value
1.	*Bacillus subtilis*	-	-	-	+	+	+	+	250 µg/ml
2.	*Corynebacterium diphtheria*	-	-	+	+	+	+	+	500 µg/ml
3.	*Staphylococcus aureus*	-	-	-	+	+	+	+	250 µg/ml
4.	*Escherichia coli*	-	-	+	+	+	+	+	500 µg/ml
5.	*Pseudomonas aeruginosa*	-	-	+	+	+	+	+	500 µg/ml
6.	*Salmonella paratyphi*	-	-	-	+	+	+	+	250 µg/ml

Table 8. Antifungal activity of methanol extract of *Ulva lactuca*

S. no	Microorganism	Inhibition zone (mm)	
		Standard	Test
		Clotrimazole (10 µg/ disc)	*Ulva lactuca* (100 µg/ disc)
1.	*Aspergillus fumigatus*	32	10
2.	*Aspergillus niger*	31	09
3.	*Candida albicans*	20	-

Table 9. Minimum inhibitory concentration value against fungal pathogens

S. no	Microorganism	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	MIC value
1.	*Aspergillus fumigatus*	-	-	-	-	+	+	+	125 µg/ml
2.	*Aspergillus niger*	-	-	-	-	+	+	+	125 µg/ml

4. DISCUSSION

The chemical constituents present in sea weeds or crude extracts are known to be biologically active ingredients. Some chemical components are considered as secondary metabolites. They are responsible for different activity such as antioxidant, antibacterial and antifungal [25-32]. This study was designed to make logical and authentic approach in ascertaining the mentioned pharmacological properties of *Ulva lactuca.* Phytochemical constituents found in methanol extract of whole plant is consistent with the previous findings [25,33] shown the presence flavonoids, terpenoids, steroids, alkaloids, phenols, tannins and proteins. However, cardiac glycosides and saponins are absent in the study. This slight variation may be due to difference in solvent extraction, local climate, soil composition and the harvest time of the collected plant.

Marine environment has been described as a source of novel chemical diversity for drug discovery [34] because many bioactive substances are isolated from marine organisms, including phytoplankton, algae, sponges, tunicates and mollusks [24,35]. Algae play a vital role in the aquatic ecosystem. They provide food and shelter for other organism and are important in the process of absorbing nutrients and toxins. Marine algae are considered as excellent source of bioactive compounds which has a broad range of biological activities including antibacterial and antioxidant. Different types of solvents such as acetone, ethanol, methanol, ethyl acetate, diethyl ether, petroleum ether, hexane, chloroform, aqueous, benzene were used earlier to extract bioactive principles from seaweeds [36,37]. The present investigation was carried out in methanol extracts. Using organic solvents always provides a higher efficiency in extracting compounds for antimicrobial activity [38]. Marine algae are considered to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activated green algae belonging to the genus *Ulva* contain 18-26% protein [39,40]. Compounds with cytotoxic, antiviral, anthelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae [41,42]. The present investigation report the presence of Phytoconstituents in methanol extract of *Ulva lactuca* for its antibacterial and antifungal property. Antimicrobial effects of crude methanol extracts obtained from *Ulva lactuca* harvested from Gulf of Mannar coast was found to be effective against pathogenic bacteria namely *Bacillus subtilis, Corynebacterium diphtheria, Escherichia coli, Pseudomona aeruginosa, Salmonella paratyphi* and *Staphylococcus aureus*, fungal pathogens *Aspergillus fumigatus* and *Aspergillus niger* by disc diffusion method. The extract released only on the active principles but it may change due to the assay methods, incubation temperature and culture media. This study revealed that all the extracts showed antimicrobial activity against variety of pathogens except *Candida albicans* and evaluation of the potential usefulness of natural products in the development of new antimicrobials.

Antioxidant in biological system have multiple fractions, including defending against oxidative damage in the major signalling pathways of cells. Several synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxyquinones and tertbutyl hydroquinone are commercially available and are currently in use. However their use is now restricted due to their side effects. Marine algae are also being studied as a source of antioxidants [43]. Meenakshi et al. [43] in their study evaluated *in vitro* antioxidant activity of two sea weeds *Ulva lactuca* and *Sargassum wightii* [44]. Antioxidant activity of methanolic extract of these present study showed significant activity under DPPH free radical scavenging activity, total phenol and flavonoid activity.

In the present study certain compounds present in *Ulva lactuca* identified by GC-MS chromatogram showed ten peaks and mass spectrum detected the compounds present in the respective peak areas. Tetradecene (9.72), Methyl (12E) – 12- [(2, 4-Dinitrophenyl) Hydrazon O] Dodecanoate (31.89) and Nonacosane (35.01) was more abundant with retention time.

Among the volatile compounds alkanes, alkenes, esters, alcohols and terpenoid compounds were present. GC-MS analysis indicated most of the Phytoconstituents could be responsible for antimicrobial and antioxidant activity and further isolation of active bioactive components could be warranted as outcome for this study.

It is obvious that climatic factors, geographic distribution, locality of collection sites, environmental factors like growing conditions and salinity type contribute for varied phenolic contents, antioxidant and antimicrobial activity of the sea weed. Kumar [45] described the phenolic content and antioxidant activity in *Kappaphycus alvarezii* varied and differences were noticed in location of collected sea weed especially from Langkawi and Sabah. Furthermore, the antioxidant content in foods depends on environmental factors such as growing season, geographical origin and agricultural practises. Manivannan et al. [46] stated the variability within the species growing conditions, time of maturity, climatic and environmental conditions, the bioactivities and chemical compositions in sea weeds will vary. The chemical composition of the algae and the antimicrobial activities vary with species, physiological status, the region of the thallus, environmental aspects (climate, location, salinity, temperature), pollution, growth conditions, collection time and epiphytic organisms [46,47]. Different studies confirmed the variation in chemical compositions and the antimicrobial action according to season. Most authors detected maximal antimicrobial potential in spring, probably due to the predominance of some active compounds in this period. However, a different variation of the phenolic composition and antibacterial/antioxidant activities was observed, with the highest inhibitory activity in spring and summer, and phenolic content and antioxidant activity in late winter [48]. The effect of the latitude and related environmental factors on the phenolic content and phlorethol type in *Sargassum muticum* collected in different European coasts was reported [49]. Over a long evolutionary period, the marine organisms sharing a common environment have established associations [50]. In the present study the weed was collected in single point in the mannar coast, of Arabian Sea, Rameshwaram coastal belts. The collection was carried out during the spring season (Aug- Nov 2016).

Extraction techniques play a tremendous role in antimicrobial acitivity. The bioactive yield can be affected by the method of conditioning and extraction. The drying stage is important, since loss of volatile antimicrobials present in fresh algae (hydrogen peroxide, terpenoid and bromo-ether compounds and volatile fatty-acids) could occur at high temperatures [51]. These authors observed increased permeability of cell membranes and reported that extracts from dried algae processed at high temperatures presented wider inhibition zones for *Salmonella enteritidis, Pseudomonas aeruginosa, Listeria innocua,* and on both clinical and food isolates of *Staphylococcus aureus.* Various methods have been proposed to extract the bioactive compounds from seaweed, using organic solvents. The yield of extractables and antimicrobials from the different species of seaweed is solvent dependent. Systematical evaluation and optimization of the solvent is necessary for accurate and reproducible preparation of extracts. Several studies aimed at selecting the best solvent, which was usually one of the following: water, methanol, ethanol, acetone, ethyl acetate, dichloromethane, chloroform, diethyl ether and hexane [19,52]. In the present study, shade drying and extraction using solvent methanol was applied for the purification and estimation of samples.

5. CONCLUSIONS

On the basis of the findings of the present study it may be assumed that total phenolic, flavonoid content and DPPH free radical scavenging activity were showed significant antioxidant activity by the methanol extract. Methanol extract exhibited antimicrobial activity against all bacterial isolates as comparable to standard ciprofloxacin and 2 fungal isolates except *Candida albicans, which* is comparable to the standard drug clotrimazole. The potential of these extracts as antioxidant and antimicrobial agents may be due to the presence of different Phytoconstituents which are confirmed under GC-MS analysis. However, extensive further researches are necessary to search for active principles responsible for these activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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