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Antibacterial and Antioxidant Potential of Ulva and Ectocarpus

KEYWORDS

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ABSTRACT *Crude extracts of Ulva lactuca Lin. and Ectocarpus siliculosus C. Agardh prepared in different organic solvent were screened to determine their antibacterial and antioxidant potentials. DPPH radical scavenging activity, FIC ability, reducing power, H2O2 scavenging activity and TAC were determine in the three organic solvents viz. methanol, ethanol and water. All ethanolic seaweed extracts inhibited the growth of gram negative (Escherichia coli and Proteus vulgaris) and gram positive (Bacillus subtilis, Salmonella typhi and Staphylococcus aureus) bacterial strains. Hexane extract of E. siliculosus exhibited the maximum antibacterial activity against E. coli and S. aureus (25 & 23mm inhibition zone respectively). The methanolic extracts of U. lactuca had an appreciable antioxidant property (FIC, H2O2 scavenging activity and TAC) while aqueous extract of E. siliculosus had the maximum reducing power and DPPH scavenging activity.*

INTRODUCTION

Algae are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated. A number of these compounds possess biological activities such as antibacterial, antifungal, antiviral, antitumour and other specific activity (Canell, 1993). These bioactive compounds include flavonoids (Stafford, 1991), tannins and several others which possess antioxidant properties (Rocha et al., 2007; Serrato et al., 2009), antimicrobial potential (Li, 2009; Saidani et al., 2011) and antiviral activities (Romanos et al., 2002).

Harmful effect of ROS and free radicals can be reduced by the action of antioxidants present in tissues (Halliwell and Cross, 1994). The steady state levels of ROS are maintain in cells by the activity of antioxidant defense system.

Evaluation of antimicrobial and antioxidant potential of two seaweeds along the west coast of Maharashtra is undertaken in the present study. *Ulva lactuca* Lin. is green seaweed and *Ectocarpus siliculosus* C. Agardh is brown seaweed.

MATERIALS AND METHODS

Materials

Fresh and mature thalli of *Ulva lactuca* and *Ectocarpus siliculosus* were collected during low tide from the submerged marine rocks at Kunakeshwar [164'0.120"N latitude and 7328'0.120" E longitude] in Sindhudurg district along the west coast of Maharashtra (India). Algal samples were cleaned with fresh seawater and then in distilled water, dried in shade and ground to form a fine powder. The bacterial cultures were obtained from the department of Microbiology, Rajaram college, Kolhapur and Department of Botany, Shivaji University, Kolhapur.

Extraction of seaweeds

Ten grams of dry algal powder were extracted in 100 ml organic solvent (Hexane/ Petroleum ether/Ethyl acetate/ Methanol/ Ethanol) for 24 hours using an orbital shaker. Extract was filtered through Whatman no.1 filter paper and condensed to half (50ml) of the original volume (Yuvraj et al. 2011). This condensed filtrate was stored in a glass vial in refrigerator until use.

Antibacterial activity

Antibacterial activity of *Ulva* and *Ectocarpus* species was followed through agar well diffusion method using nutrient agar medium (Murray et al. 1995). Bacterial cultures were inoculated on the surface of solid medium and wells, prepared with the help of a sterilized cork borer, were filled with algal extracts. Respective solvent was used as a negative control while antibiotic ampicillin was used as a positive control for comparative efficacy. The plates were incubated at 37^0C for 24h and then zone of inhibition around the well was measured and recorded in each plate.

Antioxidant activity

DPPH radical Scavenging Activity

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) scavenging activity was determined according to the method of Wang et al. (1998). Algal extract and DPPH solution were incubated in dark at R.T and absorbance was measured after 30 min. at 517 nm. DPPH reaction was expressed in terms of percent inhibition of DPPH.

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay was performed as per the method described by Benzine and Strain (1999). The activity was estimated by measuring the increased absorbance caused by generated ferrous ions. The FRAP reagent (0.3M acetate buffer (pH 3.6), 10mM TPTZ (2, 4, 6-Tripyridyl-s-triazine), 40mM HCl and 20mM $Fecl_3$. $6H_2O$ in the ratio of 10:1:1) was mixed with algal extract to initiate the reaction. Absorbance was recorded at 593 nm after 10 min. Antioxidant capacity is expressed as µM/g.

Ferrous Ion Chelating ability (FIC)

Ferrous ion chelating ability was determined as per the method given by Decker and Welch (1990). The reaction mixture contained algal extract, 2mM $FeCl_2$ and 5mM ferrozine solution. It was incubated for 10 min. at room temperature and absorbance was measured at 562 nm. Percentage of chelating ability was calculated.

Reducing Power

Reducing power of the extract was determined according to the method of Yen and Chen (1995) using 1% potassium ferricyanide, trichloroacetic acid (10%) and $FeCl_3$. $6H_2O$ (0.1%). Absorbance was recorded at 700 nm. Results are

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expressed as ascorbic acid equivalents (mgAA/g).

Hydrogen Peroxide Scavenging Activity

The ability of the seaweed extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). Extract was added to a hydrogen peroxide (40mM) solution prepared in 0.2M phosphate buffer (pH 7.4)). Absorbance was measured against a blank solution at 230 nm.

Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined according to Prieto et al. (1999). The extract was mixed with reagent (0.6 M H_2SO_4, 28mM sodium phosphate and 4mM ammonium molybdate) and incubated at 95^{0}C for 90 min. Absorbance was measured at 695 nm using ascorbic acid as the standard. TAC is expressed as ascorbic acid equivalents (mg AA/g).

RESULTS

Antibacterial activity

Hexane, ethyl acetate and ethanol extracts of *Ulva lactuca* exhibited a significant antibacterial activity against *Bacillus subtilis* (18-21mm zone) (Table 1). Petroleum ether extract of *Ectocarpus siliculosus* produced the largest zone of inhibition against *Salmonella typhi* (27.46mm). *Staphylococcus aureus* was the resistant organism which had a minimum zone in methanol extract of *U. lactuca* (9.50mm).

Ethanolic extract of *U. lactuca* exhibited a high antibacterial index against *B. subtilis* and *P. vulgaris* (79.62 and 77.08% respectively). The growth of gram positive bacteria *B. subtilis* (ethanol) and *S. typhi* (petroleum ether) was highly inhibited by *E. siliculosus* showing more than 95% inhibition (Fig. 1).

Antioxidant activity

DPPH radical scavenging activity

DPPH has been used extensively as a free radical to evaluate reducing substances (Cotelle et al. 1996). The maximum DPPH radical scavenging effect was shown by the water extract of *E. siliculosus* (83.47%) and ethanol extract of *U. lactuca* (64.36%). The inhibition was lowest in water extracted *U. lactuca* (36.11%).

Ferric Reducing Antioxidant Power (FRAP):

The FRAP assay revealed a maximum antioxidant activity in aqueous extract of *U. lactuca* and *E. siliculosus* (about 6 µM/g). In methanol extract of *U. lactuca* also FRAP value was significant (5 µM/g).

Ferrous Ion Chelating ability (FIC)

Ferrous ion chelating activity ranged between 18-75% in both seaweeds and methanol and ethanol extracts of *U. lactuca* gave the maximum values (> 70%). The activity was greater than 45% in all samples of *E. siliculosus.*

Reducing Power

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound or sample (Nakayama et al. 1999). Presence of reductants causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. In the present study water extract of *E. siliculosus* possessed a good reducing power (0.919 mg/g). Methanol extracted samples of both the seaweeds also exhibited a better reducing power.

Table 1: Effect of *Ulva lactuca* and *Ectocarpus siliculosus* on bacterial growth

Solvent / Pathogen	Zone of inhibition (Diameter in mm)									
	Ulva lactuca					Ectocarpus siliculosus				
	E.coli	P. vulgaris	B. subtilis	S. typhi	S. aureus	E.coli	P. vulgaris	B. subtilis	S. typhi	S. aureus
Hexane	14.16 ± 0.28	10.33±0.57	**18.20** ±0.34	13.30±0.26	10.40±0.36	**25.33±**0.30	10.83±0.76	12.5±0.50	16.16± 0.28	**23.30±**0.26
Petroleum ether	12.50± 0.50	16.50± 0.50	14.50 ± 0.50	10.36± 0.32	13.13±0.11	20.50± 0.50	12.50±0.50	22.23±0.25	**27.46**± 0.41	13.30±0.26
Ethyl acetate	11.50±0.50	10.50±0.50	**20.33** ±0.30	12.36±0.32	14.36±0.32	**22.33**±0.28	11.56±0.49	24.36±0.32	19.50± 0.50	15.23±0.20
Methanol	13.16±0.28	12.50 ± 0.50	11.16 ± 0.15	11.16 ± 0.28	9.50 ± 0.50	20.10± 0.17	09.43±0.38	10.53± 0.50	14.50± 0.50	16.30±0.26
Ethanol	15.50±0.50	18.50±0.50	**21.50** ±0.50	14.43±0.37	16.30±0.26	14.40±0.40	**18.33**±0.28	**26.30**±0.26	24.53± 0.50	17.50±0.50
Ampicillin (Std.)	34.66 ± 0.15	24.00 ± 0.00	27.00 ± 0.00	27.33 ± 0.15	30.00 ± 0.00	34.66 ± 0.15	24.00±0.00	27.00± 0.00	**27.33±** 0.15	30.00± 0.00

Values are mean of three replicates. ± values represent SD

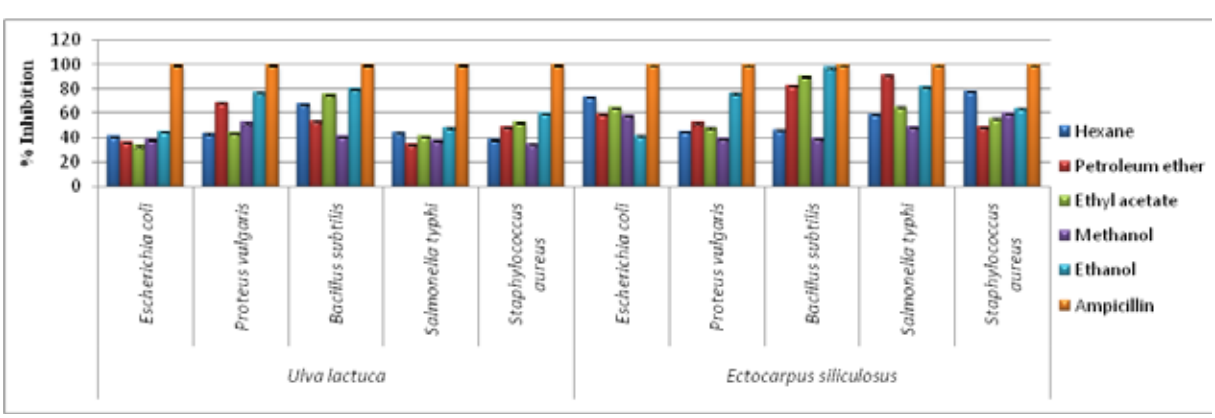

Fig. Antibacterial effect of *Ulva lactuca* and *Ectocarpus siliculosus* with respect to standard antibiotic (Ampicillin)

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Hydrogen Peroxide scavenging activity

Many species of seaweed possess scavenging ability of hydrogen peroxide (Siriwardhana et al. 2003) which can cross membranes and may slowly oxidize a number of compounds. The scavenging activity was moderate (about 30%) in methanol, ethanol and aqueous extracts of *U. lactuca* and low (less than 15%) in *E. siliculosus.*

Total Antioxidant Capacity

The total antioxidant activity was maximum in methanol extract of *U. lactuca* (163.01mg/g) and minimum in the ethanol extract of *E. siliculosus* (64.52 mg/g).

Table 2: Antioxidant activity of *Ulva lactuca* and *Ectocarpus siliculosus*

Activity	Ulva lactuca			Ectocarpus siliculosus		
	Methanol	Ethanol	Water	Methanol	Ethanol	Water
DPPH scavenging activity (%)	55.65±0.10	64.36±0.05	36.11±0.002	37.20± 0.15	63.16±0.10	83.47±0.001
FRAP (µM/g)	3.29±0.002	3.63±0.045	6.04±0.36	05.22±0.002	03.51±0.062	05.97±0.35
FIC (%)	75.73±0.25	73.40±0.00	18.90±0.52	45.60±0.10	55.63±1.38	49.30±0.10
Reducing Power (mg/g)	0.729±0.0015	0.165±0.0006	0.263±0.05	0.696±0.002	0.270±0.005	0.919±0.0005
H_2O_2 scavenging activity (%)	31.05±0.025	29.35±0.07	31.05±0.060	11.17±0.020	12.51±0.075	14.45±0.045
TAC (mg/g)	163.01±0.20	76.02±0.37	73.64±0.84	110.83±1.72	64.52±1.61	104.59±0.51

Values are mean of three replicates with standard deviation.

DISCUSSION

Marine organisms are expected to possess new pharmaceutical compounds with novel activities that will provide new drugs to combact a number of microbial pathogens which are resistant to conventional antibiotic therapies (Ramalingam and Amutha, 2013).

Natural antioxidants are found in some vegetables, fruits and a variety of other foods (Moon and Shibamoto, 2009). Seaweeds produce various types of antioxidant to counteract environmental stresses (Lesser, 2006). Therefore, seaweed is a potential source of novel antioxidants. In addition, natural antioxidants are more acceptable than synthetic antioxidants as this antioxidant do not contain chemical contaminants and display a variety of beneficial functions such as, improvement in consumer health, reducing the effect of harmful diseases and other broader aspects of immune system function (Shahidi, 2009).

Kandhasamy and Arunachalam (2008) have reported that *U. lactuca* extract inhibited all of test organisms except *E. coli*. Similarly methanolic extract of *U. rigida* and *U. fasciata* were effective against both *S. aureus* and *E. coli* (Ibtissam et al. 2009 and Priyadharshini et al. 2012). Vallinayagam et al. (2009) screened *U. lactuca* against several human bacterial pathogens and reported a minimum activity against *Pseudomonas aeruginosa.* On the contrary Perez et al. (1990) and Tuney et al. (2006) could not found any relevant activity against pathogenic bacterial strains in *U. lactuca*.

In the present study ethanol, petroleum ether, hexane and ethyl acetate extracts of *U. lactuca* revealed a better antibacterial activity and effectively controlled *B. subtilis*. Other organisms were moderately inhibited.

In the brown alga *E. siliculosus* it was significant. Yan et al. (1999) and Wang et al. (2009) also reported a high DPPH activity in brown seaweed *Hijikia.*

CONCLUSION

The results of the present study displayed a moderate antibacterial potential in *U. lactuca* while in *E. siliculosus* antibacterial action was significant. The extraction of antimicrobials from different species of seaweeds was solvent dependent. In methanol and ethanol a better extraction of antibacterials was observed. The macroalgal species investigated also had a promising antioxidant potential indicating their use in medicine in future.

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