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Research Article

INVITRO ANTIBACTERIAL AND ANTIOXIDENT ACTIVITY OF A BROWN ALGAE *SARGASSUM TENERRIMUM*

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Sargassum tenerrimum, Antioxident activity, DPPH radical scavenging, TPC, TAA, Deoxy-ribose radical scavenging, *S. aureus, B.subtilis, K. pneumonia,* E. *coli*, *P. putida and Antibacterial activity*

A B S T R A C T

The antibacterial activities of brown algae namely *Sargassum tenerrimum* from rameshwaram. The methanolic extracts of *S. tenerrimum* was tested for its an antibacterial activity against five human bacterial pathogens *(S. aureus, K. pneumonia, E.coli, P. putida* and *B. subtilis*) by using three different concentrations viz., 0.25, 0.50, 0.75µg/mL. by Well-Diffusion method. The standard drug as commercial antibiotic Ampicillin. The maximum inhibition of antibacterial activity was observed in *B. subtilis* (75µg/mL). The antioxidant activity was determined by means of the Total phenolic content, Total antioxidant (TAA), De-oxyribose radical scavenging activity and DPPH radical scavenging test using different fractions of methanol extract. Result from three methods indicate that the antioxidant activity of *S. tenerrimum* of methanol extract were time and concentration dependent. TPC exhibited higher activity in the methanolic extraction (6.26±0.45 mg GAE/g). TAA of methanolic extract were noted higher activity (0.77±0.17) .The Deoxy-ribose radical scavenging activity (3.02±0.02% inhibition) of total methanolic extract and fractions of *S. tenerrimum.* The results were highest DPPH scavenging activity (1.53±0.44 %) hexane.

INTRODUCTION

In Biomedicine much attention has been paid to natural antibacterial and their association with health benefits (Kalim *et al.,* 2010). Seaweeds are marine macro algae that are multi cellular, non-vascular, non flowering plants. The plant body consists of thallus, there is no differentiate into root, stem, leaves. About 8000 species of seaweeds have been identified and are grouped into different classes viz., green (chlorophyta), brown (phaeophyta) and red (rhodophyta) algae. Red and brown algae are mainly used as human food sources. It is one of the commercially important marine living renewable prosperity. Seaweeds are the extra-ordinary sustainable resources in the marine ecosystem which have been used as a source of food, feed and medicine (Dhargalkar *et al.,* 2005).

The total global seaweed production of the year 2004 was 15 million metric tones of which nearly 15-20% is contributed by Indian Ocean region. Seaweed harvest across Indian coast is about 100000 metric tones (wet weight) (Dhargalkar and Pereira, 2005). Seaweeds provides for an excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals (Bhaskar and Miyashita, 2005; Fleurence, 1999; Nisizawa, 1988). In Asian countries, Japanese are the main consumers of seaweed with an average of 1.6kg (dry weight) per year per capita (Fujiwara-Arasaki, *et al*., 1984). However in India seaweeds are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan and not as culinary item or for recovering beneficial biomolecules.

Seaweeds have been screened extensively to isolate life saving drugs or biologically active substances all over the world. (Padmini 1995). Bacterial infection causes high rate of mortality in human population and aquaculture organisms. Nowadays the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming to resistant to drugs is common due to indiscriminate use of antibiotics. Decreased efficiency and resistance of pathogen to antibiotics have necessitated the development of new alteration. Approximately 2500 new metabolites were reported from a variety of marine organisms during the years from 1977 to 1987.

It has been used as food, fertilizer and for medicinal purpose for a long time. Like other plants, seaweeds, contain various kinds of inorganic and organic substances which probably benefit human health. It has been reported that seaweeds contain high levels of minerals, vitamins, essential aminoacids, indigestible carbohydrates, and dietry fiber (Jimenez Escrig

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and Goni, 1999). In food manufacturing, seaweeds have been developed as raw or semi processed food products (Mabeau and Fleurrence, 1993). The aim of the present study was to determine the *invitro* antibacterial activities of brown alga *Sargassum tenerrimum*.

Antioxidant activity has become a hot topic and the subject of intensive investigation due to the ever increasing demand by the food and pharmaceutical industries to develop natural bioactive anti-aging and anti-carcinogenic compounds that demonstrate measurable health benefits. Antioxidant from biosources have created deep interest among researchers, food manufactures, and cosumers due to their protective role against dreadful diseases such as coronary heart disease and cancer (Loliger 1991). The search for novel antioxidants biomolecule with high phenolic content has become of their important issue, become of their inhibitor role in on mutagenesis and carcinogenesis in human beings. Antioxidative substances obtained from natural source , such as seed oil, grains, beans, vegetables, fruits, leafwaxes, burk, roots, species and hulls, have already been investigated (Fujimoto *et al.,* 1985; Guiry and Blunden 1991; Gordon *et al*.,1993). However, there are very few studies in the literature on antioxidant activity associated to sulfated polysaccharides from seaweeds.

Recently oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and result in extensive oxidative damage which can lead to age related degenerative condition, cancer and a wide range of other human diseases (Reaven and Witzum, 1996; Aruoma, 1999). Phenolic compounds can act as antioxidant by chelating metal ions, preventing radical formation and improving the antioxidant endogenous system (Al-Azzawie and Mohamed- saiel, 2006).

The term phenolic compounds describes several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by at least, one hydroxyl group (Manach *et al.,*2004). Polyphenols represent a diverse class of compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavonols, chalcones and flavan-3-ols), ligins, tocopherols, tannis and phenolic acids (Shukla *et al*., 1997). The aim of the present study was to determine the *invitro* antioxidant activities of brown alga *Sargassum tenerrimum*.

MATERIALS AND METHODS

Collection and Processing of Seaweeds

The collected seaweeds were washed with seawater to remove all the epiphytes and sand particles then washed thoroughly in fresh water (3 - 4 times) to remove the salts and extraneous materials. Morphologically distinct thallus of seaweeds was placed separately in new polythene bags and were kept in an icebox containing slush ice and transported to the laboratory. Then the seaweeds were spread on blotting paper to remove excess water and shadow dried for few days and then in an oven at 60ºC until constant weight obtained. Then they were cut into small pieces and made into powder for analysis for activities.

Preparation of the Seaweed Extracts

Five hundred grams of powdered *S. tenerrimum* seaweed sample was taken and extracted successively with methanol (90%) using a soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to obtain their corresponding residues. The methanolic extracts were further subjected to antioxidant assays in triplicate.

Antibacterial Activity

Bacterial strains used

Bacterial strains were obtained from the P.G. & Research Department of Biotechnology, Edayathangudy G.S. Pillay Arts & Science College, Nagappattinam. The bacterial stock cultures were maintained on Muller-Hinton Agar medium at 4°C. The bacterial strains used were Gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* Gram negative bacteria (*Klebsiella pneumonia, Escherichia coli* and *Pseudomonas putida).*

Bacterial medium and its preparation

Composition of Muller Hinton Agar

Beef infusion	- 30.0 g
Casein acid hydrolysate	- 17.5 g
Soluble Starch	- 1.5 g
Agar	- 17.0g

To 38 g of Muller Hinton Agar were suspended in 1000 ml of distilled water and the pH was adjusted to 7.3 ± 0.2. The medium was boiled to dissolve completely and sterilized by autoclaving at 15 Lbs (121^0c) for 15 minutes.

Antibacterial Assay: (Well Diffusion Assay)

Agar diffusion assay is used to determine the anti-bacterial activity of crude extract. The technique works well with defined inhibitors. The well diffusion assay is suitable for aqueous extracts because they are difficult to dry on paper discs.

1. Muller – Hinton agar prepared and poured in the petridish and allowed for solidification.
2. After complete solidification of media, 24 hrs growing bacterial cultures were swabbed on the agar plate and then grouped accordingly.
3. The wells (8 mm diameter) were made by using cork borer.
4. The different concentrations (25-75 µg/mL) of the crude extract were loaded in the wells.
5. The plates were then incubated at 37^0c for 24 hrs.
6. After the incubation the zone of inhibition in diameter was measured against control group.

Antioxidant Activity

Preparation of seaweed extracts and fractions

First extraction of seaweed was prepared by pouring methanol into the bottle containing 50 g of seaweed powder at the ratio of 10:1 (v/w), the mixture was flushed with kept under orbital shaking incubator at room temperature (29 ± 2 ºC) for 24 h under dark condition. Then the methanol extract were purified using different solvents like hexane, chloroform, acetone and aqueous extract. Then the methanol extract and their fractions of were pooled together and evaporated under reduced pressure using rotary flash evaporator. The fraction of each sample was weighed and then dissolved in 90% aqueous methanol for fractionation. Methanol fraction was further fractionated into different solvent fractions .Briefly, first fractionation was carried out with 100 ml hexane. Methanol fraction was collected and chloroform fraction finally water, methanol, ethanol ratio from (1:6:3) phase was evaporated

under reduced pressure to give a semisolid. Then semisolid portion was dissolved in 200 ml distilled water and further fractionated with Resulting fractions including aqueous were evaporated to dryness. Dried fractions were dissolved in methanol and stored in colored vials for further analysis. Extracts used for all experiments were at the concentration activity estimations.

Total phenolic content

The total phenolic content of the extract was estimated by the Folin-ciocalteu method. Two hundred microlitres of diluted sample were added to 1ml of 1:10 diluted Folin-ciocalteu reagent. After 4 minutes, 800µl of saturated sodium carbonate (75g) was added after 2 h of incubation at room temperature. The absorbance at 765 nm was measured using the perkin Elmer lamba 25UV – Vis spectrophotometer. The results were expressed as milligrams of gallic acid equivalents (GAE)/g dry weight of *S. tenerrimum*, and calculated as mean value±SD (n=3).

Determination of total antioxidant activity (Prieto et al. 1992)

Briefly 0.3ml of sample solution (0.1 mg /ml) was mixed with 3.0 ml of reagent solution (0.6M sulfuric acid 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95˚C for 90 min. under water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalent of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid equivalents /g of sample on a dry weight basis.

De-oxyribose radical scavenging activity

De-oxyribose non- site specific hydroxyl radical scavenging activity of crude extracts was determined according to the method, briefly 2.0ml aliquots of sample were added to the test containing reaction mixture of 2.0 ml $FeSO_4$, $7H_2O$ (10mM), 0.2ml EDTA (10mM) and 0.2ml de-oxyribose (10mM). The volume was made upto 1.8ml with phosphate buffer (0.1M, PH7.4) and to that 0.2ml H_2O_2(10mM) was added. The mixture was incubated at 37^0c under dark for 4 h. After Incubation 1ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then left to stand under boiling water bath for 10 min. After treatment absorbance was measured at 532nm. If the mixture was turbid, the absorbance was measured after filtration, scavenging activity (1%) was calculated using the equation given by Heo *et al.,* 2005.

DPPH scavenging activity determination

The free radical scavenging activity of *S. tenerrimum* was determined by Bloi's method (Blois, 1958). One ml of the extract solution were added into 0.1mM of 1,1-diphenyl-2-picryl-hydrazil (DPPH) methanol solution. After 30 min of incubation, the absorbance was measured at 517 nm.

RESULTS AND DISCUSSION

Antibacterial Activity of crude extract

In the present investigation, the methanolic extracts of *S. tenerrimum* was tested for its an antibacterial activity against five human bacterial pathogens *(Staphylococcus aureus, Klebsiella pneumonia, Eschericia coli, Pseudomonas putida* and *Bacillus subtilis*) by using three different concentrations viz., 0.25, 0.50, 0.75µg/mL. by Well-Diffusion method. The standard drug as commercial antibiotic Ampicillin.

The maximum inhibition of antibacterial activity was observed in
B. subtilis (75µg/mL) and the low inhibition was observed in *P. putida* (75µg/mL) Show on the **Table 1.** and **Plate 1.**

Table 1 Antibacterial Activity of Methanolic Extract of *S. tenerrimmum.*

Pathogens	Concentration, µg/mL *Zone of Inhibition (mm dia)*			
	25	50	75	Standard (Amp)
K. pneumoniae	-	5	8	19
E. coli	-	3	11	21
S. aureus	3	3	7	14
B. subtilis	-	-	13	20
P. putida	-	-	3	14

(-) = Antibacterial acivity was not detected; **Amp** = Ampicillin.

K. pneumonia

E. coli

S. aureus

B. subtilis

P. putida

Plate .1 Antibacterial activity Well diffusion assay of *Sargassum tenerrimum* (methanolic extract).

Antioxidant Activity

The methanolic extract of *S. tenerrimum* were purified with different fractions like chloroform, acetone and aqueous. The Total phenolic content (TPC) exhibited higher activity than methanol extraction maximum (6.26±0.45 mg GAE/g) and minimum value hexane fraction of (0.81±0.05mg GAE/g) following order by methanol, acetone, chloroform, aqueous and hexane fractions of different based on their polarity the results were observed. Fig- 1.

The Total antioxidant activity of methanolic extract were noted higher activity (0.77±0.17) and of aqueous fraction (0.15±0.01)were recorded the minimum activity Fig- 2.

The Deoxy- ribose radical scavenging activity (3.02±0.02% inhibition) of total methanolic extract and fractions of brown seaweed (*S. tenerrimum)* were presented in Fig - 3. The deoxyribose assay is that it involves the hydroxyl radical which is the most active reactive. Lower inhibition rate of 1.32±0.06% was observed in Hexane fraction of *S. tenerrimum*.

S. tenerrimum were assessed by the DPPH assay the activity shown in the Fig- 4. The results were highest DPPH scavenging activity (1.53±0.44 %) hexane fraction and the minimum activity were represented the aqueous fraction (0.94±0.11%).

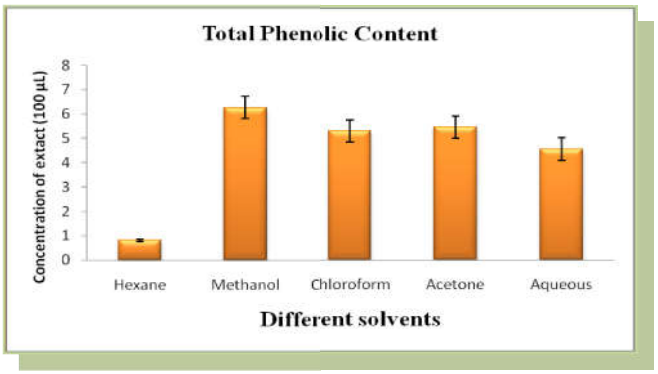

Fig 1 Total Phenolic content

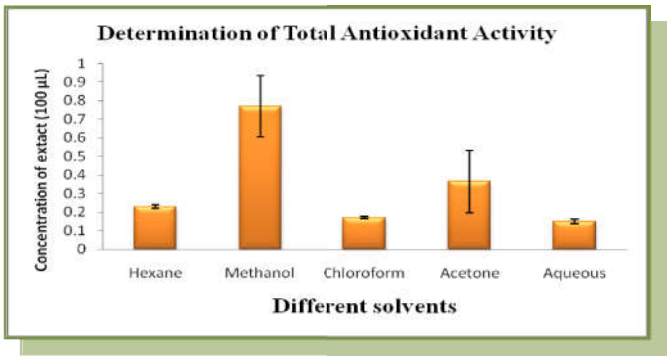

Fig 2 Determination of total antioxidant activity

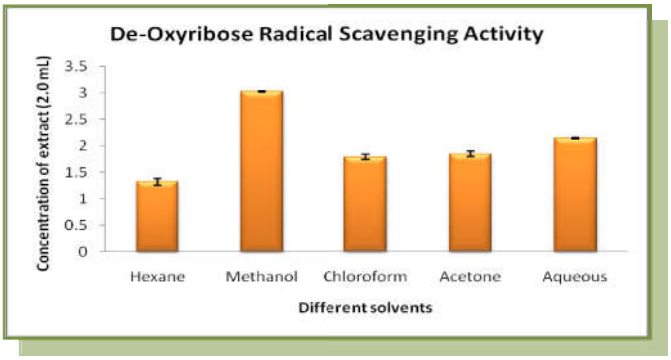

Fig 3 De-oxyribose radical scavenging activity

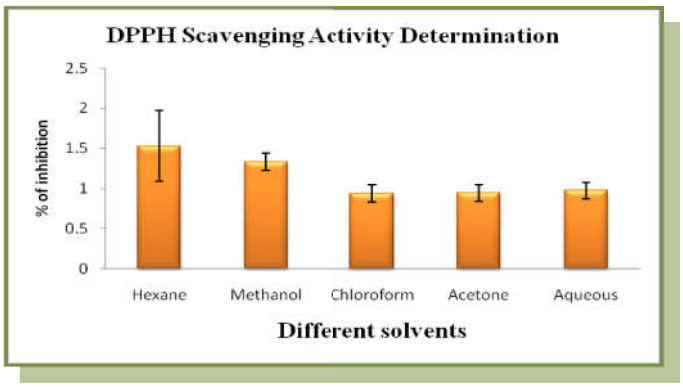

Fig 4 DPPH scavenging activity determination

DISCUSSION

In the present study pilot screening of methanolic extracts of Brown seaweed *Sargassum tenerrimum* were found to show specific activity against the human bacterial pathogens such as Gram positive (*Staphylococcus aureus, Bacillus subtilis)* and Gram negative *(Klebsiella pneumonia, Eschericia coli, Pseudomonas putida*) by using three different concentrations viz., 0.25, 0.50, 0.75 µg/mL. The details of screening of antibacterial activities of methanolic extracts of marine algae

against the pathogens are presented. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds.

The present investigation has also proved that dry algal samples possess antimicrobial activity to achieve excellent results by inhibiting the growth of maximum pathogenic bacteria. This may be due to the masking of antibacterial activity by the presence of some inhibitory compounds of the crude extracts (Sastry and Rao, 1994). Padmini Sreenivasa Rao *et al.,* (1986) have reported extracts from shade dried samples exhibited best antibacterial activity.

In the present study better and higher activity was noted in *B. subtilis*,
(208 observed the evaluation of invitro antibacterial property of seaweed of southeast coast of India recorded from the Methanolic extracts of *S. tenerrimum* against *K. Pneumoniae* (8 mm), *E. coli* (12 mm), *S. aureus* (12 mm), *B. subtilis* (15 mm). In the present study showed that the methanolic extracts of *S. tenerrimum* showed against *B. subtilis* (13 mm) and *E. coli* (11 mm).

The difference between the results of the present investigation and results of other studies may be due to the production of bioactive compounds related to the seasons, method, organic solvent used for the extraction of bioactive compounds and differences in assay methods. Finally it can be concluded form this study that the methanol extract of alga, *Sargassum tenerrimum* used in the present investigation showed good antibacterial activity against five bacterial pathogens used. It may form the potential source of bioactive compounds and should be investigated for natural antibiotics.

The Total phenolic content (TPC) exhibited higher activity than methanol extraction maximum (6.26±0.45 mg GAE/g) and minimum value hexane extract of (0.81±0.05mg GAE/g). Karthikai devi *et al* (2011) reported the activities were relatively lower than that of standard compound. *T. conoides* in brown alga south east coast of India higher TPC of 1.231±0.173 mg GAE/g in methanol extract, when compared to considerably value, Natural antioxidants are not limited to terrestrial sources and reports have revealed seaweeds to be rich sources of natural antioxidant compounds (Lim *et al.*, 2002; Duan *et al.*, 2006; Kuda *et al.*, 2007) phenolic compounds are commonly found in plants, including seaweeds, and have been reported to have a wide range of biological activities including antioxidant properties.

The total antioxidant activity of total methanol extract the higher activity of (0.77±0.17) and Lowest value of aqueous extract (0.15±0.01). the antioxidant activity early reported was higher activity of 39.62, 9.79 and 9.65 mg ascorbic acid equivalent/g extract (or 0.31, 0.08 and 0.17 mg ascorbic acid equivalent/g of seaweed on dry weight basis) was observed in EA fraction of *S. marginatum*, DCM fraction of *Padina tetrastomatica* and aqueous fraction of *T. conoides*, respectively (Kumar Chandini *et al .,*2008). The total antioxidant activity of total methanolic extracts was significantly different between the three seaweeds. Kumaran and Karunakaran (2007) have reported total antioxidant activity in the range of 245 to 376 mg higher plant extracts. Higher activity was observed in fractions as compared to total methanolic extract. Higher activity in fractions may be due to the interferences of other compounds present in crude (methanolic) extract and it has also been reported that solvents used for extraction have dramatic effect on the chemical species (Yuan, Bone, & Carrington, 2005).

These results also supported by the study of Duffy & Power (2001) who described different samples in different solvents to give different antioxidant potentials. Previous studies reported that ethanolic extracts of licorice samples displayed high antioxidant potential compared to water extracts. However, other ethanolic Chinese plant extracts showed little antioxidant potential (Duffy & Power, 2001) Marinova & Yanishlieva (1997), antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential. In addition, a polar solvents were found to be the most suitable solvents for extracting polyphenols from water (Moure *et al*., 2001).

DPPH reagent has been used extensively for investigating the free radical scavenging activities of compounds. In the DPPH test, the dried extracts are potentially able to reduce the stable DPPH radical to the yellow coloured diphenyl pricryl hydrazine. The assay is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction .DPPH results are often interpreted as the "efficient concentration" or EC50 value, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity.

The results indicate that different solvent fractions obtained from total (methanolic) extract exhibit higher antioxidant activities as compared to the total extract. The findings of this work are useful to further research to identify, isolate and characterize the specific compound which is responsible for higher antioxidant activity. Bioactive compounds found in seaweeds await a major breakthrough for a variety of food/medical applications as they have the potential for application of such compounds as natural antioxidants in different food/ pharmaceuticals products.

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