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Research Article

EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES FROM BROWN SEAWEED, *SARGASSUM WIGHTII* (GREVILLE, 1848) AGAINST HUMAN BACTERIAL PATHOGENS

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ABSTRACT

Seaweeds serve as an important source of bioactive natural substances. In the present study, seaweed *Sargassum wightii* was screened for the potential bioactive natural substance against human bacterial pathogens. Crude extracts were made using three solvents (acetone, ethanol and methanol) and then screened for antibacterial activity against human pathogens. The crude extracts were purified by silica gel column chromatography and five fractions obtained from each solvent were collected separately and tested for activity. The 2nd fraction of purified ethanol extract showed maximum activity against seven human bacterial pathogens compared to other fractions of ethanol, methanol and acetone. This was again subjected for purification by silica gel column chromatography and three sub fractions obtained were also tested for the activity. Off the three fractions, 3rd sub fraction of ethanol extract showed the highest zone of inhibition against *Escherichia coli* (25.5±0.71mm) followed, *Staphylococcus aureus* (22.85±0.21mm), *Salmonella paratyphi* (19.1±0.14mm), *Salmonella typhi* (18.5±0.71mm), *Pseudomonas aeruginosa* (18.25±0.35mm), *Vibrio cholerae* (17.5±0.71mm), *Klebsiella pneumoniae* (16.15±0.21mm), *Shigella sonnie* (15.2±0.28mm) and the lowest zone of inhibition was observed against *Proteus Proteus* (12±0mm), *Klebsiella* sp. (8.5±0.71mm) compared to other two sub fractions. The potential 3rd sub fraction was also screened for total antioxidant activity. The results revealed that the potential fraction (100µg/ml) exhibited higher antioxidant activity as compared with standard ascorbic acid with the equivalent concentration between 60-80µg/ml.

Keywords: *Sargassum wightii*, Bioactive compounds, Human bacterial pathogens, Antibacterial activity, Silica gel column chromatography and Total antioxidant activity.

INTRODUCTION

Bacterial diseases are the challenging threat to human population. Human pathogenic bacteria have potential to cause the following diseases such as skin infections, pneumonia, tetanus, typhoid fever, diphtheria, syphilis, meningitis and leprosy [1]. Preventing outbreaks or treating the diseases with drugs or chemicals tackles of these problems [2]. Nowadays the use of antibiotics increases significantly due to heavy bacterial infections. The indiscriminate use of antibiotics has resulted in the accumulation of more resistant pathogenic bacterial strains and also caused some side effects to human. So, the ultimate solution is, development of antibacterial drugs from natural sources against human pathogens [3].

There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, cancer, mutagenesis, arthritis, diabetes, inflammation, aging and genotoxicity [4]. Antioxidant is an inhibitor of ROS. Epidemiological studies have found that intake of antioxidants such as vitamin E and vitamin C reduced the risk of coronary heart disease, stroke and cancer [5]. To overcome these problems a wide range of synthetic antioxidants like butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propylgallate (PG) and butylatedhydroquinone (BHQ) have been used as food preservatives. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, the development of alternative antioxidants from natural origins has drawn more and more attention [6].

Seaweeds are one of the important marine living resources could be termed as the futuristically promising plants and have been a source of food and medicine [7]. It provides a rich source of structurally diverse secondary metabolites. Over 2,400 secondary metabolites have been isolated and described from the divisions Rhodophyta, Phaeophyta and Chlorophyta, many of which have been reported to have excellent biological activity [8]. The functions of these secondary metabolites are defense against herbivores, fouling organisms and pathogens; they also play a role in reproduction, protection from UV radiation and as allelopathic agents [9]. Seaweeds from varied localities have been evaluated for a wide range of biological activities like antibacterial, antiviral, antifungal, antialgal, antitumor, antihypercholesterolemic, anticoagulant and antioxidant activities [10]. Antibacterial and antioxidant activities have been the most widely investigated properties in seaweeds in all around the world [11]. Hence, the present study was aimed to evaluate the bioactive compounds from brown seaweed, *Sargassum wightii* against human bacterial pathogens and also tested for total antioxidant activity. This investigation could scientifically proof the natural products have potent antibacterial agents for use of pharmaceutical industry.

MATERIALS AND METHODS

Sample collection and preparation

Live and healthy specimen of seaweed, *Sargassum wightii* (1kg) was collected from intertidal regions of Mandapam coast (Lat. 09° 17.417'N; Long. 079° 08.558'E), Gulf of Mannar, Tamil Nadu, India during low tides. The seaweed was washed thoroughly with seawater to remove the epiphytes and immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. Followed, the specimen was washed with tap water and distilled water so as to remove the salts and other extraneous materials. The sample was shade dried for ten days and ground in an electric mixer for one hour and obtained 300g of seaweed powder.

Preparation of the extracts

The seaweed extracts were prepared following the method of Anushia [12]. The each 100g of powdered sample was weighed and then soaked with 200ml of ethanol, methanol and acetone individually. These three extracts were kept in seven days for room temperature (28ºC). After seven days of incubation, the crude extracts were filtered by using filter paper (Whatman No.1) and the filtrate extracts were concentrated by rotary vacuum evaporator (>45°C) to obtain solid residue and then stored in individual sterile glass container for screen the antibacterial activity.

Pathogens used for the assay

Human bacterial pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Proteus Proteus*, *Vibrio cholerae*, *Salmonella paratyphi*, *Shigella sonnie* and *Salmonella typhi*. The test organisms were

obtained from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, India. The organisms were sub-cultured on nutrient agar medium in test tubes (Slant method). The tubes were incubated at 37°C for 24 hrs and stored in refrigerator (4ºC) to maintain the stock culture.

Antibacterial activity assay of crude extracts

The three solvent extracts of seaweed were tested for antibacterial activity against human pathogens using well - diffusion method [13]. Muller Hinton agar (HIMEDIA, MUMBAI) media was poured on to the sterile petri dishes. After solidification, 18 hrs old tested broth cultures were swabbed by using sterile cotton and allowed up to 10 mins. Later, the wells were made on surface of the agar medium with help of sterile cork borer. 5mg of each solvent crude extract was dissolved in 1ml of respective solvents and 75µl concentration of each sample was placed in different wells (6mm) separately. Controls were maintained with respective solvents alone and the plates were incubated at 37ºC for 24 - 28 hrs and the zones of inhibitions were recorded in millimeters. The experiment was carried out in three replicates.

Purification of crude extracts

The crude extracts (ethanol, methanol and acetone) of seaweed were purified using silica gel column chromatography [14]. The column was packed with 10g of silica gel (60-120 mesh size) using hexane solvent with the maximum height of 20cm. Each solvent crude extract (55g of ethanol, 40g of methanol and 25g of acetone) was weighed and eluted successively with 50 ml of respective solvents and the same were loaded on top of the silica gel separately. The corresponding fractions (1-5) were obtained and each fraction (10ml volume) eluted time taken for 15 - 20 mins. All the fractions were concentrated by rotary vaccum evaporator (>45ºC) and then stored in individual sterile glass container and kept in refrigerator (4ºC) for further use.

Antibacterial activity assay of purified extracts

The purified fractionated samples were screened for antibacterial activity against human pathogens as discussed above. Based on these results, 2nd fraction of ethanol extract having potential bioactive molecules, so the 2nd fraction was again purified with silica gel column chromatography and obtained three sub fractions. The sub fractions were concentrated then screened for antibacterial activity. Penicillin and Ampicillin used as positive controls and ethanol used as a negative control.

Statistical analysis

The experiments were performed in replicated at three times. Statistical differences between three extracts activities were determined using one way ANOVA [15]. Differences were considered statistically significant when $p < 0.05$.

Total antioxidant activity assay

The antioxidant activity of potential fraction from ethanol extract was determined by phosphomolybdenum method [16]. The assay is based on the reduction of Mo(VI) - Mo(V) by the extract and subsequent formation of a green phosphate / Mo(V) complex at acid pH. 5mg of sample was mixed with 1ml of ethanol and made up to different concentrations (20-100µg/ml) were combined with 1ml of reagent solutions (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) separately. In the case of blank, ethanol was used in the place of sample. The tubes containing samples were capped and incubated in water bath at 95ºC for 90mins. After the samples were cooled to room temperature, the absorbance was measured at 695nm using a spectrophotometer against blank. The antioxidant activity was expressed as an equivalent of standard ascorbic acid. All the measurements were measured in triplicates.

RESULTS

Antibacterial activity of crude extracts

The crude extracts (ethanol, methanol and acetone) of seaweed were screened for antibacterial activities against human pathogens are shown in Fig 1. Ethanol showed highest zone of inhibition against *Escherichia coli* (19.8±0.25mm) followed, *Staphylococcus aureus* (17.5±0.5mm), *Klebsiella pneumoniae* (17.2±0.36mm), *Vibrio cholerae* (16.3±0.15mm), *Salmonella paratyphi* (15±0.25mm) and moderate zone of inhibition was observed against *Pseudomonas aeruginosa* (14.7±0.17mm), *Proteus Proteus* (12.5±0.5mm), *Klebsiella* sp. (12.2±0.2mm), *Salmonella typhi* (11±0.5mm) and lowest zone of inhibition was observed against *Shigella sonnie* (10.5±0.28mm). Methanol showed highest zone of inhibition against *Escherichia coli* (17.3±0.15mm), *Staphylococcus aureus* (15.5±0.25mm), *Klebsiella* sp. (15.2±0.20mm) and moderate zone of inhibition was observed against *Pseudomonas aeruginosa* (14.8±0.20mm), *Shigella sonnie* (14±0.12 mm), *Salmonella typhi* (11.7±0.51mm) and lowest zone of inhibition was observed against *Klebsiella pneumoniae* (10±0.00mm), *Proteus Proteus* (9±0.12mm), *Vibrio cholerae* (8.2±0.25mm) and *Salmonella paratyphi* (7.5±0.28 mm). Acetone showed highest zone of inhibition against *Escherichia coli* (17.7±0.21mm), *Staphylococcus aureus* (15.5±0.25 mm) and moderate zone of inhibition was observed against *Vibrio cholerae* (14.9±0.15 mm), *Pseudomonas aeruginosa* (14.3±0.25mm), *Shigella sonnie* (14.2±0.2mm), *Klebsiella pneumoniae* (11.6±0.23mm), *Klebsiella* sp. (11.5±0.5mm) and lowest zone of inhibition was observed against *Proteus Proteus* (9.5±0.28mm), *Salmonella paratyphi* (9±0.23mm) and *Salmonella typhi* (6±0.12mm). The zones of inhibitions made by three solvent crude extracts were significant ($p > 0.05$). Among three crude extracts, ethanol exhibited the powerful antibacterial activity against five pathogens out of ten.

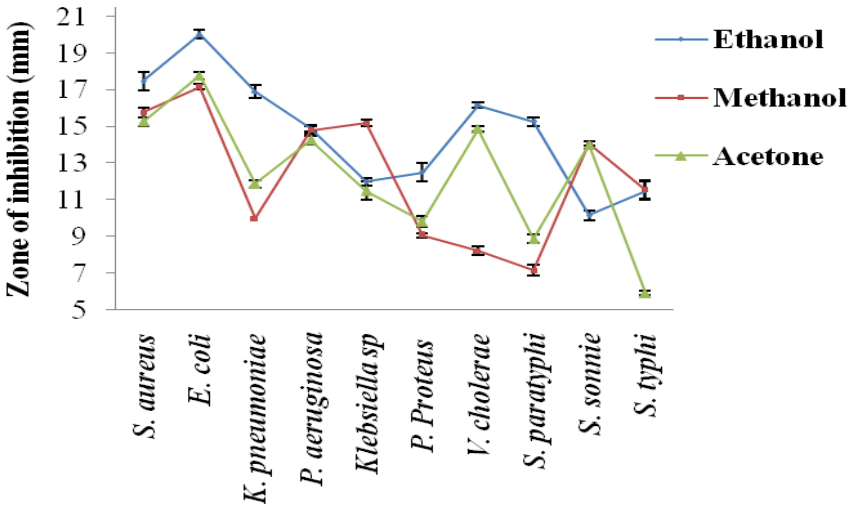

Fig. 1: Antibacterial activities of three crude extracts of *S. wightii* against human pathogens

Antibacterial activity of purified extracts

The purified five fractions with ethanol extract were screened for antibacterial activities against human pathogens are mentioned in Fig 2. Among the five fractions, 2nd fraction showed maximum zone of inhibition against *Escherichia coli* (22.15±0.21mm) followed, *Staphylococcus aureus* (20.25±0.35mm), *Klebsiella pneumoniae* (18.5±0.21mm), *Vibrio cholerae* (18.25±0.35mm), *Salmonella typhi* (17.5±0.71mm), *Pseudomonas aeruginosa* (17.2±0.28mm), *Salmonella paratyphi* (16.8±0.28mm) and moderate zone of inhibition was observed against *Klebsiella* sp. (13.1±0.14mm), *Shigella sonnie* (12±0mm) and *Proteus Proteus* (11.25±0.35mm) compared to other fractions. The zones of inhibitions made by different purified fractions were significant (p>0.05).

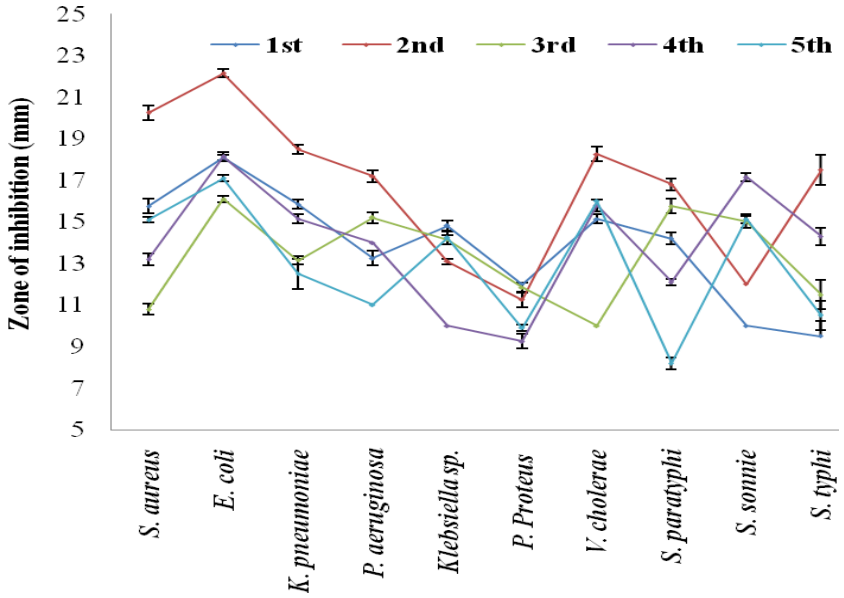

Fig. 2: Antibacterial activities of purified five fractions with ethanol extract against human pathogens

The purified five fractions with methanol extract were screened for antibacterial activities against human pathogens are mentioned in Fig 3. Among the five fractions, 3rd fraction showed maximum zone of inhibition against *Staphylococcus aureus* (16.2±0.28mm) followed, *Shigella sonnie* (15.85±0.21mm), *Escherichia coli* (15.1±0.14mm), *Vibrio cholerae* (15±0mm) and moderate zone of inhibition was observed against *Proteus Proteus* (14.3±0.42mm), *Salmonella paratyphi* (14±0mm), *Klebsiella* sp. (12±0mm), *Salmonella typhi* (11.9±0.14mm) and lowest zone of inhibition was observed against *Klebsiella pneumoniae* (7.25±0.35mm) and *Pseudomonas aeruginosa* (5.5±0.71mm) compared to other fractions. The zones of inhibitions made by different purified fractions were non-significant (p>0.05).

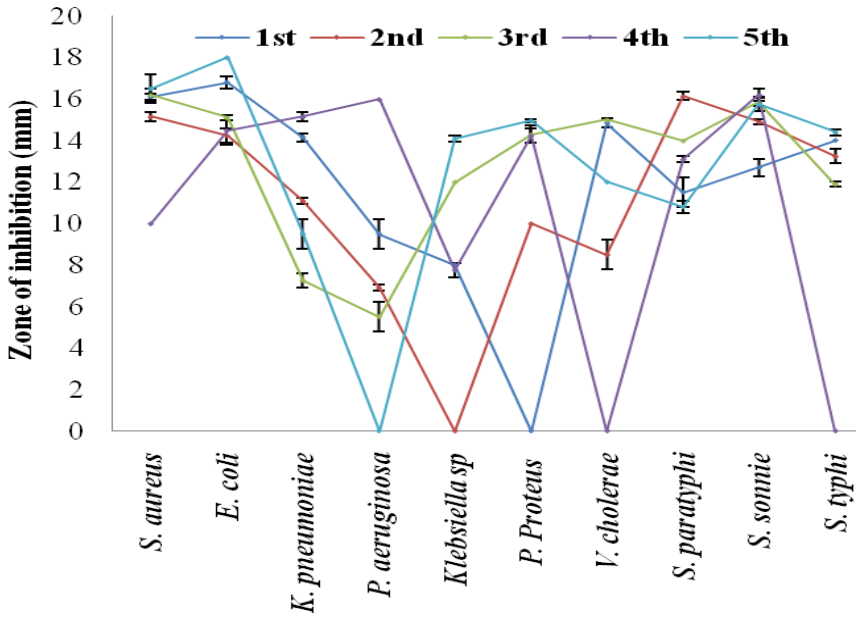

Fig. 3: Antibacterial activities of purified five fractions with methanol extract against human pathogens

The purified five fractions with acetone extract were screened for antibacterial activities against human pathogens are mentioned in Fig 4. Among the five fractions, 1st fraction showed maximum zone of inhibition against *Klebsiella* sp. (16±0mm) followed, *Shigella sonnie* (15.85±0.21mm), *Salmonella paratyphi* (15.15±0.21mm), *Staphylococcus aureus* (15.1±0.14mm) and moderate zone of inhibition was observed against *Salmonella typhi* (14.3±0.42mm), *Escherichia coli* (14±0mm), *Pseudomonas aeruginosa* (12±0mm) and lowest zone of inhibition was observed against *Klebsiella pneumoniae* (10.25±0.35mm), *Proteus Proteus* (9.3±0.42mm) and no activity was observed against *Vibrio cholerae* (0±0) compared to other fractions. The zones of inhibitions made by different purified fractions were non-significant (p>0.05).

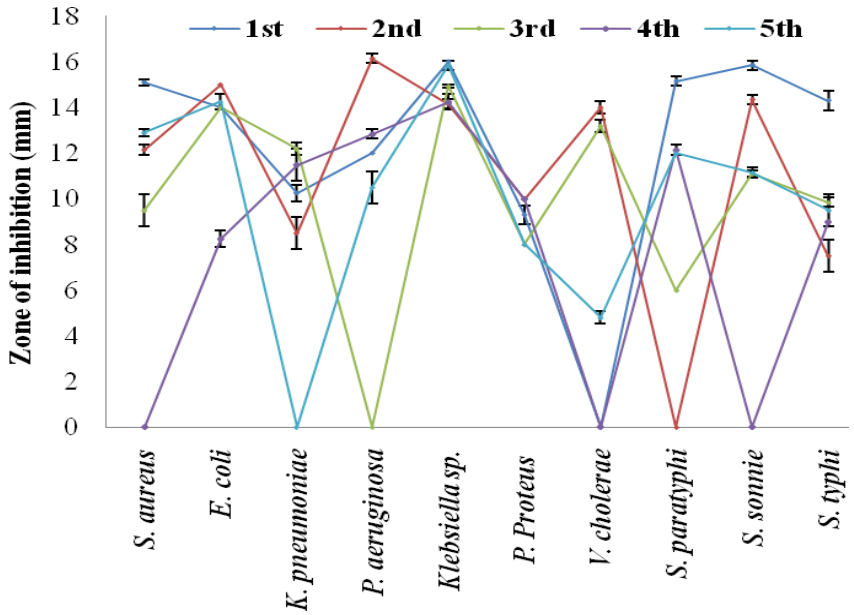

Fig. 4: Antibacterial activities of purified five fractions with acetone extract against Human pathogens

Based on the results, 2nd purified fraction of ethanol extract exhibited the powerful antibacterial activity against seven pathogens out of ten compared to other fractions of ethanol, methanol and acetone extracts.

The results of purified three sub fractions of ethanol extract, positive and negative controls were screened for antibacterial activities against human pathogens are shown in Fig 5. Among three sub fractions, 3rd fraction showed highest zone of inhibition against *Escherichia coli* (25.5±0.71mm) followed, *Staphylococcus aureus* (22.85±0.21mm), *Salmonella paratyphi* (19.1±0.14mm), *Salmonella typhi* (18.5±0.71mm), *Pseudomonas aeruginosa* (18.25±0.35mm), *Vibrio cholerae* (17.5±0.71mm), *Klebsiella pneumoniae* (16.15±0.21mm), *Shigella sonnie* (15.2±0.28mm) and lowest zone of inhibition was observed against *Proteus Proteus* (12±0mm), *Klebsiella* sp. (8.5±0.71mm) compared to other two sub fractions.

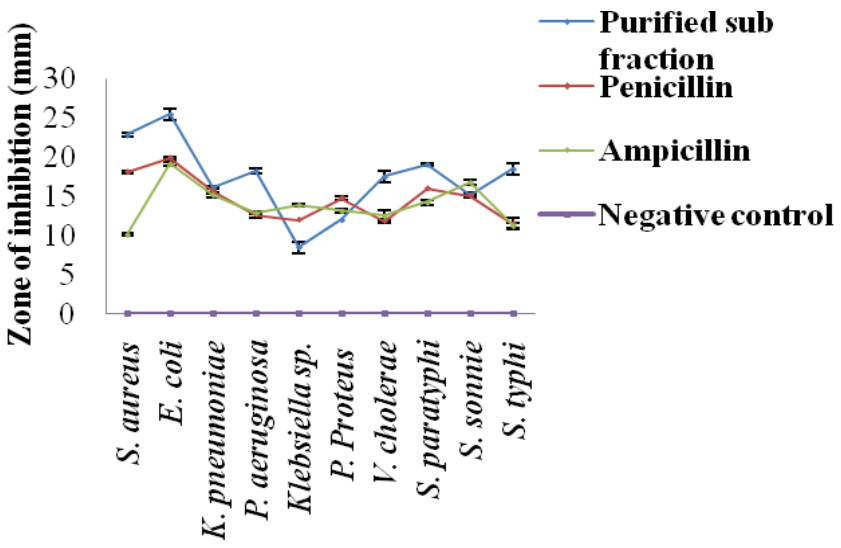

Fig. 5: Antibacterial activities of purified 3rd sub fraction, positive and negative controls against human pathogens

Among two positive controls, Penicillin showed highest zone of inhibition against *Escherichia coli* (19.9±0.14mm) followed, *Staphylococcus aureus* (18.1±0.14mm), *Salmonella paratyphi* (16±0mm), *Klebsiella pneumoniae* (15.75±0.35mm), *Shigella sonnie* (15.1±0.14mm) and moderate zone of inhibition was observed against *Proteus Proteus* (14.8±0.28mm), *Pseudomonas aeruginosa* (12.7±0.42mm), *Klebsiella* sp. (12±0mm), *Vibrio cholerae* (11.85±0.21mm) and *Salmonella typhi* (11.5±0.71mm). Ampicillin showed highest zone of inhibition against *Escherichia coli* (19.15±0.21mm) followed, *Shigella sonnie* (16.8±0.28mm), *Klebsiella pneumoniae* (15.2±0.28mm) and moderate zone of inhibition was observed against *Salmonella paratyphi* (14.25±0.35mm), *Klebsiella* sp. (13.9±0.14mm), *Proteus Proteus* (13.15±0.21mm), *Pseudomonas aeruginosa* (12.9±0.14mm), *Vibrio cholerae* (12.5±0.71mm), *Salmonella typhi* (11.25±0.35mm) and *Staphylococcus aureus* (10.15±0.21mm). The zones of inhibitions made by 3rd sub fraction and positive controls were non-significant (p>0.05). No activity was observed against negative control.

The present study results described that purified 3rd sub fraction of ethanol extract showed tremendous antibacterial activity against eight pathogens compared with two positive controls. Penicillin showed maximum antibacterial activity against five pathogens and Ampicillin showed against three pathogens out of ten. So, the present study concluded that this fraction was selected as a potential bioactive compounds containing fraction against diseases causing human pathogens.

Total antioxidant activity of potential fraction

The total antioxidant activity of *Sargassum wightii* was measured. The antioxidant activity increases with increasing concentrations of the sample. At the concentration of 100µg/ml, the potential fraction exhibited higher antioxidant activity as compared with standard ascorbic acid with the equivalent concentrations between 60-80µg/ml, which is shown in Fig 6.

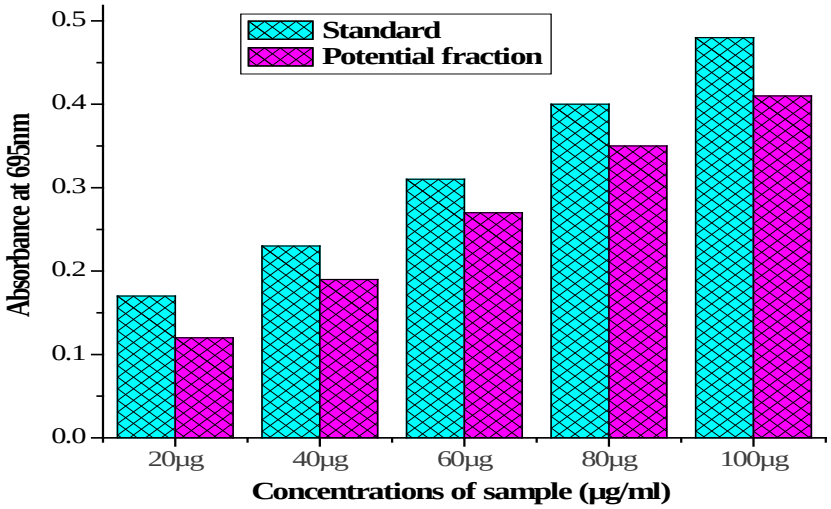

Fig. 6: Total antioxidant activity of potential fraction compared with Standard ascorbic acid

DISCUSSION

It is a crucial stage where it is essential to explore new strategies in order to combat human infectious diseases. The marine environment representing approximately half of the global biodiversity, is an enormous resource for new compounds. Seaweeds are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents [17]. Many studies were reported about the biological activities of algal extracts from different coastal regions around the world [18].

Antibacterial activities of seaweeds also varied with the species division. Brown seaweeds showed greater antibacterial activity than green and red seaweeds [19]. Salvador [20] reported that species of Rhodophyta showed highest antibacterial activity. Battu [21] found that highest antibacterial activity was exhibited by species of Phaeophyta. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to definite conclusion. In the present study, species of Phaeophyta showed highest antibacterial activity against human bacterial pathogens.

The previous study reported that antibacterial activities of six species of seaweeds tested against human pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis* [22]. In this study acetone was the best solution for extracting effective antibacterial materials from algal species used in this experiment. The result could be related to the presence of bioactive metabolites are not soluble in ethanol and methanol, but they can be soluble in acetone. Raghavendra and Mahadevan [13] studied that the *in vitro* antimicrobial activity of various plant latex against resistant human pathogens. Battu [21] investigated that *in vitro* antibacterial activity and phytochemical screening of three algal species. In this study, the three algae extracts were showed good antibacterial activity against tested bacterial species. Similarly our results showed that ethanol extract of *Sargassum wightii* effectively inhibited the eight human bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella paratyphi*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Klebsiella pneumoniae* and *Shigella sonnie.* Based on the obtained experimental results, ethanol caused better halo-zones than methanol and acetone.

Although a variety of solvents have been employed in screening seaweeds for antibacterial activity, it is still uncertain what kind of solvent is most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening antimicrobial activity of seaweeds and made comparisons. Kannapiran [23] described that benzene and diethyl ether were suitable solvents for extracting the antibiotic principle. Manivannan [24] reported that extracts obtained with acetone, ethyl alcohol and ether showed maximum antibacterial activity than that of extracts obtained with chloroform. The results from present study revealed that highest antibacterial activity was exhibited by ethanol extract and the least by methanol and acetone extracts, which may suggest that particular solvent is required to extract some antibacterial substances within the algal plant.

The remarkable differences between our results and the results obtained in previous studies may be due to several factors. First of all, this can be because of intraspecific variability in production of secondary metabolites, occasionally related to seasonal variations [25]. Secondly, there may also be differences in capability of extraction protocols to recover active metabolites and differences in assay methods that would result in different susceptibilities of target strains [26]. This is an inevitable fact for all biochemical research because test materials have trace impurities [27].

Reactive Oxygen Species (ROS) are molecules or ions formed by incomplete one electron reduction of oxygen. Nonetheless, excessive production of ROS by various endogenous and exogenous factors may lead to oxidative stress, loss of cell function and ultimately apoptosis or necrosis in humans [28]. The defense against ROS or free radical damage is presence of antioxidants. Seaweeds are considered to be a rich source of antioxidants. Recently, active antioxidant compounds were identified as fucoxanthin in *Hijikia fusiformis* and phlorotannins in *Sargassum kjellamanianum* [29]. Polyphenolic antioxidants have been known to play a similar role as endogenous antioxidants and are abundantly found in brown seaweeds [30]. Previous studies are reported that aqueous extract of brown seaweed, *Padina minor* showed an ability to reduce free radicals or oxidative damage. Meenakshi [31] evaluated that antioxidant activities are higher in two seaweeds, *Ulva lactuca* and *Sargassum wightii.* Vijayabaskar [32] described that high amount of potential antioxidant properties are present in brown seaweed, *Turbinaria ornata.* Similarly, the present study established that brown seaweed, *Sargassum wightii* have a rich source of antioxidant compounds. The antioxidative constituents possibly play a complimentary role by delaying or preventing the oxidation of cellular oxidizable substrates and selectively inhibiting the ROS cascade of events in humans.

CONCLUSION

The seaweed, *Sargassum wightii* possessed highest antibacterial activity and also have a rich source of antioxidant property. It is evident from present study; ethanol extract of this seaweed could be utilized as a good source of antibacterial agent in pharmaceutical industry. However, the active components responsible for antibacterial and antioxidant activities need to be evaluated. Therefore it is suggested that further works may be performed on isolation and identification of these active components for its pharmaceutical application. Finally it is concluded that macro-algae from south east coast of India have the potential sources of bioactive compounds and should be investigated for natural antibiotics and also conservation of these natural sources from pollution threats.

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