Journal of Applied Pharmaceutical Science Vol. 5 (07), pp. 035-040, July, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50706
ISSN 2231-3354

Antimicrobial and Antioxidant Potential of Selected Seaweeds from Kodinar, Southern Coast of Saurashtra, Gujarat, India

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ARTICLE INFO

Article history:
Received on: 16/01/2015
Revised on: 11/04/2015
Accepted on: 09/05/2015
Available online: 27/07/2015

***Key words**:*
Seaweeds, Disc diffusion assay, Total antioxidant, H_2O_2 scavenging assay

ABSTRACT

The antimicrobial and antioxidant activity was evaluated for the marine seaweeds *viz*., *Enteromorpha* sp. *Cystoseria indica, Sargassum swartzii, Gracilaria corticata, Caulerpa taxifolia* and *Caulerpa racemosa* from Kodinar coast, Gujarat. Different solvents *viz*., methanol, ethanol, chloroform and diethyl ether were used for seaweed extraction to envisage the antibacterial activity against both Gram positive and Gram negative bacteria *viz., Escherichia coli, Proteus* sp., *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Staphylococcus aureus*. Hydrogen peroxide scavenging assay and total antioxidant capacity were determined in ethanol extracted seaweeds. The maximum antibacterial activity was observed in the ethanol extract of all the seaweeds except *C. racemosa*. Among the seaweeds, the total antioxidant potential was found to be maximum in the ethanol extract of *S. swartzii* - 19.84±0.14 (19.8 mg of Ascorbic acid/g of seaweed extract) and the greatest H_2O_2 scavenging activity was shown by the ethanol extract of *S. swartzii* (81.63±0.39 % inhibition) compared to the control (ascorbic acid) 95.24±0.22. Hence, from the present study it is evident that the seaweeds collected from Kodinar coast harbors excellent inhibitory activity against various human pathogens and has significant antioxidant potential as well. In particular, the antimicrobial and antioxidant activity of *S. swartzii* was found to be excellent and can be pointed out as the best candidate among the other seaweeds tested.

INTRODUCTION

The requirements for expansion of alternative antibiotic agent were explored since the manifestation of antibiotic resistant microbes. Marine environment is a pool of bioactive natural compounds that are not found in terrestrial natural products (Anandhan *et al.,* 2011). Marine diversity has been the basis of unique chemical compounds with the potential for industrial development. The active metabolites attained from the wide diversity of marine organisms have proved to be the best substitute for conventional pharmaceutical chemicals. Seaweeds are marine macroalgae which are lucrative vital marine living and renewable resources of India. In recent years, there are abundant reports of macro algae derived compounds that have a wide range of biological activities such as antibacterial, antiviral, antifungal, anticancer, etc. Seaweeds act as probable bioactive compounds that can be used for pharmaceutical applications (Del Val *et al.,* 2001). Hodgson (1984) reported that the seaweeds belonging to the family Chlorophyta, Phaeophyta and Rhodophyta exhibits antimicrobial activity. Daoudi *et al.* (2001) isolated acyclic diterpens and sterols from genera *Bifurcaria* and *Bifurcariopsis*. In addition to the antibacterial and antiviral properties of seaweeds, they have also been a good source of antioxidants. Today ready to eat products are in vast demand, but these products are highly susceptible towards pathogenic organisms, hence preservatives that are closely related synthetic antioxidants such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are added to such products (Sherwin, 1990). These preservatives can cause liver damage and are mutagenic and neurotoxic in nature. Phytochemicals like polyphenols, which are broadly appearing in plants, are found to act as free radical scavengers and antimicrobial agents (Gulcin *et al*., 2002; Oktay *et al*., 2003). Marine plants contain high amounts of polyphenols and consequently seaweeds can be used as effective natural antioxidants. Seaweeds are utilized for its rich nutrient content and antioxidant property in treating major degenerative and deficiency diseases.

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The presence of antioxidant substances in seaweeds is found to be an endogenous defense mechanism as a protection against oxidative stress due to tremendous environmental conditions (Aguilera *et al.,* 2002). The Gulf of Mannar situated along the east coast of India and Sri Lanka, possesses abundant growth of about 680 species of seaweed. Ten seaweeds isolated from the Thondi, Southern Coast of Tamil Nadu, India were evaluated for antioxidant and antimicrobial activity (Pandima Devi *et al*., 2008). Hence an attempt was undertaken to evaluate the antioxidant and antibacterial activity of selected seaweeds from the Kodinar coast.

MATERIALS AND METHODS

Collection and Extraction of Seaweeds

The Fresh seaweeds were collected from the intertidal regions of the Kodinar, situated in the southern coastal region of Saurashtra peninsula (200 41‘ N, 700 46‘ E), India. The six seaweed species *Enteromorpha* sp., *Cystoseira indica, Sargassum swartzii, Gracilaria corticata, Caulerpa taxifolia* and *Caulerpa racemosa* were collected and immediately brought to the laboratory in plastic bags containing water in order to prevent evaporation.

The collected samples were washed thoroughly with tap water to remove extraneous materials. The samples were cut into small pieces and shade dried until the constant weight obtained and ground to make fine powder. The powdered samples (15 g) were extracted with different solvents (250 ml) like diethyl ether, chloroform, ethanol and methanol in the soxhlet extractor. The extracts were collected and concentrated under reduced pressure by using the rotary vacuum evaporator and the obtained crude extracts were stored in the refrigerator for the further experiments.

ANTIMICROBIAL ACTIVITY

The test pathogens of different bacterial cultures *viz*., *Escherichia coli, Pseudomonas aeruginosa, Proteus* sp., *Klebsiella pneumoniae* and *Staphylococcus aureus* were procured from Gujarat Adani Institute of Medical Sciences, Bhuj – Kachchh, Gujarat. The cultures were grown on the plates containing Mueller Hinton Agar medium.

Disc Diffusion Method

The antibacterial activity of the test samples was determined by the standard disc diffusion method proposed by Bauer *et al.* (1966). The 6 mm diameter sterile discs were prepared using Whatman filter paper no.1 and sterilized using an autoclave. Bacteria were grown in Mueller Hinton broth overnight at 37°C. The petri plates were poured with 20 mL of Muller Hinton Agar and allowed to solidify for the use in susceptibility test bacteria. The enriched cultures were used to seed the plates with the help of a sterile swab and the plates were uniformly swabbed.

Then the plates were allowed to dry for 5 minutes. After drying the extract impregnated discs were placed on the plate with the help of sterilized forceps and gently pressed to ensure contact with the media. The plates were incubated at 37°C for 24 hours. The zone of inhibition was quantified in millimeters (mm).

ANTIOXIDANT ACTIVITY

Total Antioxidant Assay

The total antioxidant capacity for all the seaweed extracts was evaluated by the phosphomolybdenum method according to the procedure given by Prieto *et al*. (1999). A reagent solution was prepared by combining 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. Ethanol extracts with different concentration was combined with 3 ml of reagent solution. Then the tubes containing the reaction solution were incubated at 95°C for 90 min in a water bath. The tubes were kept for cooling at room temperature. At 695 nm, the absorbance was measured using a UV-VIS spectrophotometer against blank after cooling to room temperature. Ethanol was used in the place of extracts as the blank. The total antioxidant activity is articulated as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid with ethanol (100, 80, 60, 40 and 20 µg/ml).

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide radical scavenging assay was performed with ethanol extract of all the seaweed samples. The ability of the seaweed extracts to scavenge H_2O_2 was revealed, according to the method of Ruch *et al*. (1989) with slight modification. About 40 mM H_2O_2 was prepared in phosphate buffer (pH 7.4) and the H_2O_2 concentration was determined spectrophotometrically. Ethanol extracts with different concentration in distilled water and ascorbic acid (20 – 100 µg/ml) were added to 0.6 ml of 40 M H_2O_2 solution and the absorbance of H_2O_2 was determined at 230 nm after 10 min incubation against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 was calculated using the following formula:

$$H_2O_2 \text{ radical scavenging activity } [\%] = [(A_0 - A_1) / A_0] \times 100$$

(Where A_0 – Absorbance of control; A_1 – Absorbance of sample)

Statistical Analysis

All the data were expressed as means ± standard deviation (SD). The experiments were carried out in triplicates.

RESULTS

Antimicrobial Activity

Antimicrobial activities of seaweed extracts in various solvents were tested against five human bacterial pathogens *viz*., *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus* sp. and *Staphylococcus aureus.* The ethanol extract exhibited a higher zone of inhibition against both gram-

positive and gram-negative bacteria when compared to other solvent extracts in all the species of seaweeds.

The Fig. 1 clearly depicts that among the different solvent extract, ethanol extract of *Enteromorpha* sp displayed highest inhibitory activity against *P. aeruginosa* (11±0.23 mm)*, K. pneumonia* (19±0.45 mm), *S. aureus* (13±0.12 mm) *, Proteus* sp. (13±0.31 mm)*, E. coli* (10±0.28 mm) followed by methanol, chloroform and diethyl ether. The maximum inhibition zone was obtained in the ethanol extract of *C. indica* against *P. aeruginosa* (14±0.12 mm)*, K. pneumonia* (16±0.31 mm), *S. aureus* (9±0.11 mm) *, Proteus* sp. (15±0.22 mm) and *E. coli* (13±0.25 mm) followed by chloroform, diethyl ether and methanol (Fig. 2). Figure 3 reveals that the ethanol extract of *S. swartzii* showed remarkable inhibitory activity against *P. aeruginosa* (10±0.32 mm)*, K. pneumonia* (14±0.14 mm), *S. aureus* (7±0.21mm) *, Proteus* sp. (22±0.35 mm)*, E. coli* (16±0.15 mm) followed by chloroform, methanol and diethyl ether. The highest zone of inhibition was established by ethanol extract of *G. corticata* against *P. aeruginosa* (10±0.20 mm)*, K. pneumonia* (14±0.43 mm), *S. aureus* (7±0.28mm)*, Proteus* sp. (22±0.31 mm), *E. coli* (16±0.19 mm) followed by methanol and chloroform (Fig. 4).

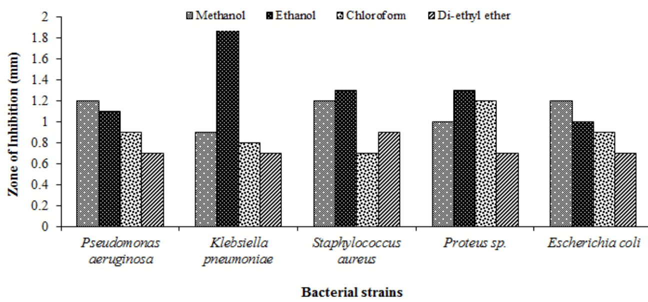

Fig. 1: Antibacterial activity of *Enteromorpha* sp against human pathogens.

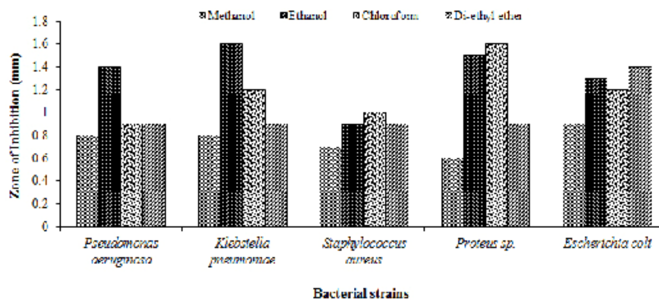

Fig. 2: Antibacterial activity of *Cystoseira indica* against human pathogens.

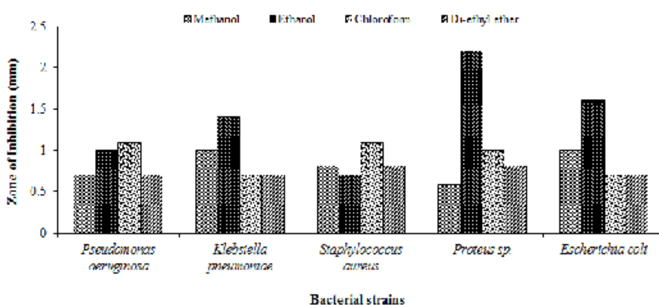

Fig. 3: Antibacterial activity of *Sargassum swarztii* against human pathogens.

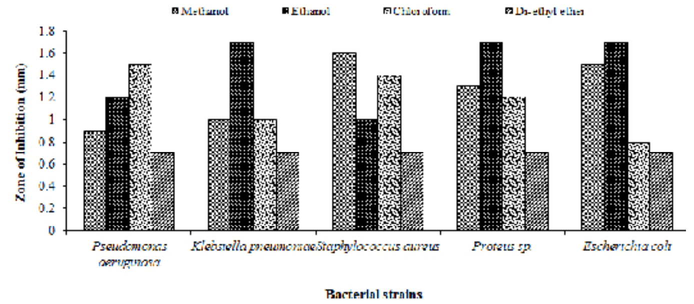

Fig. 4: Antibacterial activity of *Gracilaria corticarta* against human pathogens.

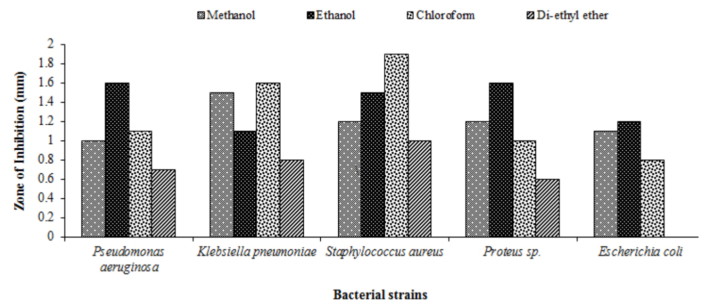

Fig. 5: Antibacterial activity of *Caulerpa taxifolia* against human pathogens.

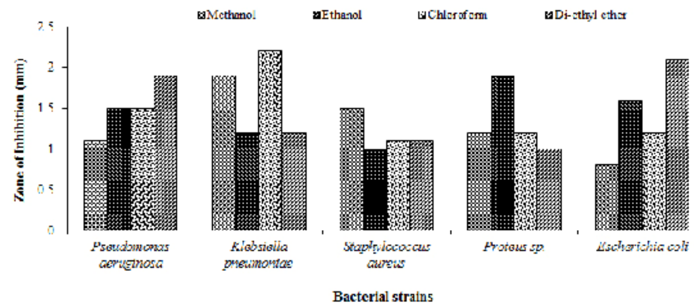

Fig. 6: Antibacterial activity of *Caulerpa racemosa* against human pathogens

The results represented in Figure 5 shows that the greatest zone of inhibition was ascertained by ethanol extract of *C. taxifolia* against *P. aeruginosa* (16±0.13 mm)*, K. pneumonia* (11±0.24 mm), *S. aureus* (15±0.30mm) *, Proteus* sp. (16±0.21 mm)*, E. coli* (12±0.11 mm). The chloroform extract exhibited notable inhibitory activity against *K. pneumonia* (16±0.42 mm), *S. aureus* (19±0.18 mm) followed by methanol. In diethylether extract, the inhibitory activity was noticed to be sparse and also no activity was found against *E. coli.* The diethyl ether extract of *C. racemosa* generated maximum zone of inhibition against *P. aeruginosa* (19±0.14 mm) and *E. coli* (21±0.25 mm) followed by chloroform, ethanol and methanol. The chloroform extract exhibited prominent inhibitory activity against *K. pneumonia* (22±0.43 mm). In ethanol extract, a remarkable inhibitory activity was found against *Proteus* sp. (19±0.21 mm) whereas methanol extract exhibited significant inhibitory activity against *S. aureus* (15±0.18 mm) (Fig. 6).

Antioxidant Activity

The Total antioxidant activity was remarkable in ethanol extract. Total antioxidant activity is expressed as the number of

equivalents of ascorbic acid in milligram per gram of extract. Figure 7 clearly point up that among the six seaweeds, the highest antioxidant potential was exhibited by the ethanol extract of *S. swartzii* - 19.84±0.14 (19.8 mg of Ascorbic acid/g of seaweed extract). Very low activity was displayed in *C. racemosa.* The H_2O_2 scavenging assay in Figure 8 depicts that the maximum H_2O_2 scavenging activity was shown by the ethanol extract of *S. swartzii* (81.63±0.39 % inhibition) compared to the control 95.24±0.22 (ascorbic acid) followed by ethanol extract of *C. taxifolia,* which showed 79.32±0.17 % inhibition.

The lowest scavenging activity was recorded in *C. racemosa* (52.32±0.24%). Finally, it was concluded that among the six seaweeds, *S. swartzii* displayed excellent antioxidant activity in both Total antioxidant and H_2O_2 scavenging assay.

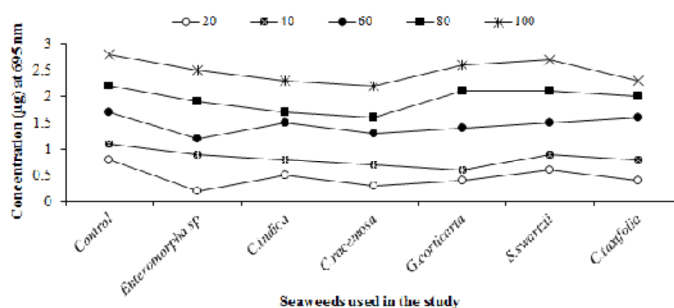

Fig. 7: Total antioxidant activity of six seaweeds.

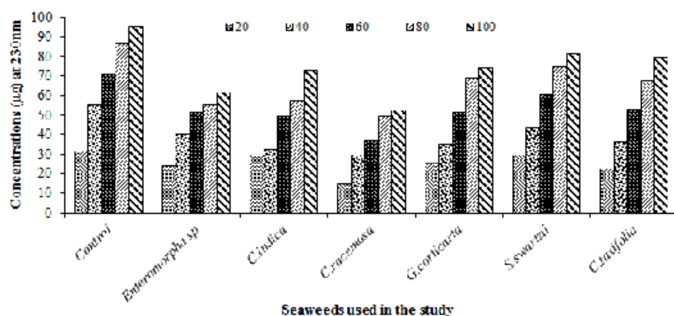

Fig. 8: Hydrogen peroxide scavenging activity of six seaweeds.

DISCUSSION

Seaweeds are broadly screened to isolate drugs or bioactive substances all over the world (Bhakuni and Silva, 1974; Caccamese *et al*., 1980; Martinez-Nadal *et al*., 1963; Padmini Sreenivasa Rao, 1991). Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Cox *et al.* 2010). *In vitro* antibacterial activity of six selected marine algae (seaweeds), have been selected and their extracts have been tested as an alternative to commonly used antibiotics (Lavanya and Veerappan, 2011; Rizvi 2010). Accordingly, the present study was focused to screen six seaweeds *viz*., *Enteromorpha* sp. *C. indica, S. swartzii, G. corticata, C. taxifolia* and *C. racemosa* using four different solvents for evaluating the potential of antibacterial activity. Seaweed extracts in various solvents exhibited different antimicrobial activities against five human bacterial pathogens viz., *P. aeruginosa, E. coli, K. pneumoniae, Proteus* sp. and *S. aureus.*

The present study coincides with Karthikaidevi *et al*. (2009), that among the solvents, ethanol extract of seaweeds exhibited a higher zone of inhibition against both gram–positive and gram–negative bacteria. In the present study, among the different solvent extract, ethanol extract of *Enteromorpha* sp displayed highest inhibitory activity against the test pathogens. Jeyanthi *et al.* (2013) reported that the methanol extract of *Enteromorpha flexuosa* showed maximum activity against *Klebsiella* (21 mm) and in diethyl ether extract of *Enteromorpha clathrata,* the inhibitory activity was found to be very low. Similarly, in this study, methanol extract showed maximum activity only against *P. aeruginosa* (12±0.11 mm) and *E. coli* (12±0.34 mm) and also in diethyl ether extract, the inhibitory activity was found to be stumpy. Only a moderate inhibition was noticed in chloroform extract.

Rizvi (2010) found that the inhibitory activity of methanol extract of the seaweed *C. indica* obtained from the Karachi coast of Pakistan showed 16mm for *K. pneumonia.* No inhibitory activity was found in *E. coli, Proteus mirabilis*, *P. aeruginosa*. In the present study, the activity was petite in methanol and diethyl ether. The ethanol extract of *C. indica* showed antifungal activity (Ambreen *et al*., 2012). About 100% inhibition activity was obtained by ethanolic extracts of *C. tamaricifolia* (Souhaili *et al*., 2004). In the antibacterial activity of the present study, the maximum inhibition zone was obtained in the ethanol extract of *C. indica* against *K. pneumonia* (16±0.31 mm), *E. coli* (13±0.25 mm), *Proteus* sp. (15±0.22 mm) and *P. aeruginosa* (14±0.12 mm). The chloroform extract showed good activity against *S. aureus* (10±0.11 mm) and *Proteus* sp. (16±0.22 mm).

Matloub and Awad (2012) found that the different extracts of *Sargassum* spp. *viz*., *Sargassum asperifolium, Sargassum dentifolium* and *Sargassum linifolium* have various antimicrobial activities and can act as promising natural sources of bioactive products. The maximum inhibition was noticed with ethanol extract of *S. lanceolatum* (Ambreen *et al*., 2012) and *S. ilicifolium* (Jeyanthi *et al*., 2012) against different bacterial strains. Consequently, in the present study the ethanol extract of *S. swartzii* showed remarkable inhibitory activity against the test pathogens but the activity in chloroform extract was noteworthy against *P. aeruginosa* (11±0.34 mm) and *S. aureus* (11±0.19 mm). The inhibitory activity was found to be low in methanol and diethyl ether.

Shanmugapriya *et al*. (2008) revealed that the tested seaweed *G. corticata* was highly active against Gram negative bacteria than Gram positive bacteria. Kolanjinathan et al. (2009) found that ethanolic extract of *G. edulis* inhibited *E. coli, P. aeruginosa, S. aureus* and *S. faecalis.* Similarly, in the present work, the highest zone of inhibition was established by ethanol extract of *G. corticata* against *S. aureus* (7±0.28mm), *K. pneumonia* (14±0.43 mm), *P. aeruginosa* (10±0.20 mm) and

E. coli (16±0.19 mm). The seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *G. corticata*, maximum inhibitory activity was observed with methanol (Kolanjinathan and Stella, 2011) and minimum with chloroform extracts (Rangaiah *et al.,* 2010). In present study, the chloroform extract of *G. corticata* exhibited remarkable inhibition against *P. aeruginosa* (10±0.36 mm) and methanol extract showed significant activity against *S. aureus* (7±0.23mm). In diethyl ether extract, the activity was found to be skimpy.

The present study contradicts with the result of Rizvi (2003), that the antibacterial activity of *C. taxifolia* showed no zone of inhibition against *P. aeruginosa, E. coli, S. aureus, B. subtilis* and *S. typhi.* But in the present study, the greatest zone of inhibition was ascertained by ethanol extract of *C. taxifolia* test pathogens. The present study supports with Pushparaj *et al.* (2014) that the seaweed *C. sertularioides* showed the maximum antibacterial activity in ethanol extract. The chloroform extract exhibited notable inhibitory activity against *K. pneumonia* (16±0.42 mm), *S. aureus* (19±0.18 mm). Low activity was recorded in methanol extract. In diethylether extract, the inhibitory activity was noticed to be sparse and also no inhibitory activity for *E. coli.*

Kandhasamy and Arunachalam (2008) reported that *C. racemosa,* green algae were more active compared to other groups of algae screened for their antibacterial activity. The methanol extract showed maximum zone of inhibition against *K. pneumonia* (15±0.41), *P. aeruginosa* (14±0.26) and *S. aureus* (16±0.36). In the present study, methanol extract showed 15±0.18 mm of inhibitory activity against *S. aureus* and contrast to other seaweed extract, diethyl ether extract of *C. racemosa* generated maximum zone of inhibition against *P. aeruginosa* (19±0.14 mm) and *E. coli* (21±0.25 mm). The chloroform extract exhibited 22±0.43 mm of inhibitory activity against *K. pneumonia*. In *C. racemosa*, all the extracts are found to show astounding activity against the test pathogens.

The maximum total antioxidant activity was shown by the *Sargassum* sp. compared to the other algae (Kumar *et al*., 2008; Meenakshi *et al*. 2009). The maximum antioxidant activity was exhibited by the ethanol extract of *S.wightii*- 12.8±0.11 (12.81 mg of Ascorbic acid/g of seaweed extract) and the lowest activity was recorded in the green algae *Chaetomorpha linum* (Indu and Seenivasan, 2013). Similarly, the present findings also corroborated that the total antioxidant activity was remarkable in ethanol extract. The highest antioxidant potential was exhibited by the ethanol extract of *S. swartzii* was 19.84±0.14 followed by *G. corticata, Enteromorpha* sp., *C. taxifolia, C. indica* and *C. racemosa.* Hence it can be confirmed that the *S. swartzii* posses good antioxidant property compared to other algae and the lowest activity was recorded in *C. racemosa.*

S. swartzii collected from Asaloye-Niband marine protected area of the Persian Gulf and found could be a potential rich source of natural antioxidants antioxidants (Sadati *et al*., 2011). Heo *et al*. (2005) reported that the different species of *Sargassum viz*, *S. flvelum, S. horneri, S. coreanum, S. thunbergii* possess strong antioxidant activity, H_2O_2 radical scavenging activity and reducing power. The scavenging activity was increased with increasing concentrations and the maximum value was obtained from the methanol extract of *S. dentifolium* (82 & 86% inhibition) compared to green and red algae (Matsukawa *et al*. 1997). In the present study, maximum H_2O_2 scavenging activity was shown by the ethanol extract of *S. swartzii* (81.63±0.39 % inhibition) compared to the control 95.24±0.22 followed by *C. taxifolia* (79.32±0.17 % inhibition). The result suggests that *S. swartzii* can be a better antioxidant for removing H_2O_2.

CONCLUSION

Marine seaweeds have numerous bioactive components such as antioxidant and antimicrobial compounds. The antimicrobial and antioxidant assay in the study are more significant and thus the study suggests that the active components are responsible for antibacterial and antioxidant metabolites in seaweeds and the results are found to be interesting. Thus exploration of such biological agents might be a probable resource of an array of biologically active compounds and the present results will ensure a starting point for exploiting natural bioactive substances present in the extracts of algae. Hence the thorough investigations with the objectives of isolation and identification of the antioxidant and antimicrobial components in *Sargassum swartzii* must be carried out for clinical application.

Further work is in progress which aimed at the investigation of detailed phytochemical screening of seaweeds to find the potential of natural secondary metabolites. Also, detailed studies on quantification, purification and evaluation of such compounds can take this to a large scale application in pharmaceutical industries.

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How to cite this article:

Kannan Karthikeyan, Kewlani Shweta, Ganapathi Jayanthi, Kolanthasamy Prabhu, Ganapathy Thirumaran. Antimicrobial and Antioxidant Potential of Selected Seaweeds from Kodinar, Southern Coast of Saurashtra, Gujarat, India. J App Pharm Sci, 2015; 5 (07): 035-040.