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Analysis of Proximate Composition and *In-Vitro* Antibacterial Activity of Selected Green Seweeds from South Andaman Coast of India

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A B S T R A C T

The present study was aimed to analyse the nutritional composition and antibacterial activity of selected green macroalgae from Andaman Islands. Proximate composition was estimated for the selected four green macroalgae species namely, *Halimeda macroloba, Halimeda tuna, Enteromorpha* sp. and *Acetabularia acetabulum.* Methanol and Dimethyl Sulphoxide (DMSO) dissolved extracts of these macroalgae were tested against human and fish pathogens such as *Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli* and *Streptococcus pneumoniae*. The results revealed that maximum protein content was observed in *Enteromorpha sp*. (16.56 ± 2.45) while minimum was observed in *A. acetabulum* (8.123 ± 0.89). All the analysed samples were high in ash content which ranged from 18.56 ± 0.12 to 33.67 ± 0.23. Among all the analysed macroalgae species, *Enteromorpha* sp. showed highest zone of inhibition against all the pathogens tested and *S. aureus* showed sensitivity to the methanolic extracts of all the four macroalgae species. Considerable level of protein, dietary fibre and high ash content were observed in all the analysed macroalgae species. The methanolic extract of *Enteromorpha* sp. was found active against all the tested pathogens. Thus macro algae can serve as a source of food protein and an ingredient of high nutritional value in human and animal diet and can even be used against pathogenic infection in human and animal treatment.

Introduction

Marine ecosystem is a complex system of diverse group of organisms living in close association with each other under constant pressure of competition, predation and hostile environment. Macroalage are also continually in contact with various dangerous microbes which they have seemingly defended, through the production of various secondary metabolites which have broad spectrum of biological activity such as anti-oxidative, anti-microbial, cytotoxic, anti-fungal and anti-helminthic (Kolanjinathan *et al.*, 2009 and Radhika *et al.*, 2012). Antibacterial agents found in the seaweeds include amino acids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides and fatty acids (Mtolera and Semesi, 1996). This aids in limiting the growth and development of potentially dangerous bacteria, viruses, fungi and epibionts on the macroalgae (Perez and Avila, 1990). The bioactive compounds

present in the macrolage serve as a compound of great interest in the pharmaceutical industry. The ability of the pathogens to develop resistance against the existing drugs and the side effects caused by those drugs has led to the extensive screening of new antimicrobial compounds for the development of novel drugs which may serve as an effective alternative to overcome the emergence of multi-drug resistant pathogens and the infectious diseases caused by these organisms (Kayalvizhi *et al.,* 2012).

Antibacterial activity of macroalgae belonging to all the three classes has been reported by various authors. Hellio, Brrener, Pons, Cotlenceau and Borgongman (2000) have reported the antimicrobial activity of macroalgae extracts on fungi and bacteria. Kolanjinathan *et al.,* (2009) has reported highest antibacterial activity in class Rhodophyceae (80%) followed by Chlorophyceae (62.5%) and Phaeophyceae (61.9%).

Andaman and Nicobar Islands are rich in macroalgae beds covered with around 105 different marine macroalgae or seaweed species, of which 39.38 % is dominated by Rhodophyceae, 35.71 % by Chlorophyceae and 25 % by Phaeophyceae (Mohanraju and Tanushree, 2012). Karthick, Mohanraju, Ramesh, Murthy and Narayana (2013) reported that South Andaman water serves as home for more macroalgae species compared to North Andaman, which comprises of around 29 species belonging to Chlorophyceae, 23 species of Phaeophyceae family and 20 species belonging to Rhodophyceae. According to Palanisamy (2012) *Caulerpa sp., Halimeda sp., Enteromorpha sp., Ulva sp., Chaetomorpha sp.,* and *Acetabularia sp.,* are the major genera proliferating along the South Andaman coast. The present investigation thus focuses on assessing the nutritional composition and antibacterial property of selected green macroalage from South Andaman Island.

Since ancient times, macroalage served as a source of food for coastal communities around the globe and in various Asian countries such as Japan, China, Korea, Indonesia, Vietnam and Taiwan (Dere *et al.,* 2003) they are still consumed as food and food additive in fresh as well as dried form suggesting that they are prominent source of vitamins, minerals, carbohydrates, dietary fibres and considerable level of proteins. The appealing anti-cancer, anti-thrombotic, anti-coagulant, anti-inflammatory and anti-viral properties recorded in the macroalage extracts is attributed to the presence of nutritive compounds such as fucoidan, xylans, ulvans (polysaccharides), appreciable levels of a ω3 and ω6 fatty acids and other micronutrients such as vitamins (vitamin $B_{12,}$ C and E) and minerals such as iodine, calcium, iron, etc. (Angstwurm, 1995 and Charreau, 1997). These nutritional constituents helps in preventing cardiovascular disease, cancer, anaemia, chronic fatigue syndrome and it also strengthens immune system, which serves as the major reason for their demand in the pharmaceutical and therapeutic industries. Hydrocolloids such as agar, alginin and carrageenan are also extracted from seaweeds, for which there are great demands in the Indian market (Ramalingam *et al.,* 2000).

Materials and Methods

Collection and preparation of sample

Four green macroalgae species such as *H. tuna, H. macroloba, Enteromorpha* sp. and *A. acetabulum* were collected along the South Andaman coast at three different locations during low tide and GPS coordinates for each sampling station was recorded. The samples were thoroughly washed with seawater to remove epiphytes, sand and pebbles and

immediately transported to the laboratory in ice box, where they were washed with freshwater to remove salt. The samples were shade dried at room temperature for 3-4 days. The dried samples were then homogenised with pestle and mortar and stored at 4 °C for further analysis.

Analysis of proximate composition

Proximate composition of the samples was determined by adopting the methods of Association of Official Analytical Chemists (AOAC, 1995). Moisture content of the sample was estimated using hot air oven at 100±2 °C. Total ash content was determined by dry ashing method using Muffle furnace at 550-650 °C. Micro Kjeldahl technique was used to determine the elemental nitrogen content, from which the protein fraction was calculated using the nitrogen-protein conversion factor (6.25).

Preparation of Extract

One gram each dried macroalage samples was dissolved in 25 ml of 80% methanol and kept for overnight incubation in orbital shaking incubator. The samples were then centrifuged at 4500 rpm for 10 minutes, and then the supernatant was filtered with Whattman no. 1 filter paper. The filtrate was evaporated at 50º C in a vacuum rotary evaporator. The concentrated extracts were transferred to petri plates and kept at 50º C in hot air oven for complete evaporation of the solvent and based on the yield; the extract was diluted in 80% methanol and DMSO separately, to acquire a concentration of 10 mg/ml. The extracts were then tested for their antibacterial activity against the selected pathogens.

Bacterial strains

The extracts were tested against four pathogenic bacteria namely, *Staphylococcus aureus* (ATCC 25923), *Aeromonas hydrophila* (ATCC 35654), *Escherichia coli* (ATCC 4157) and *Streptococcus pneumonia* (ATCC 49619) received from Microbiologics, USA.

Antibacterial assay

The antibacterial susceptibility test was carried out by agar well diffusion method (Holder and Boyce, 1994). Bacterial strains were inoculated in nutrient broth and also in Luria Bertani broth and incubated at 37º C for 18-24 hours. Mueller Hinton agar plates were prepared and 50 µl of bacterial culture was evenly spread throughout the plate using sterile glass L- rods. A total of 4 wells were made in each plate by using a sterile cork borer. Both methanol and DMSO dissolved macroalage extracts (50 µl each) were loaded separately into the wells in duplicate. Likewise, negative control (solvent alone) and positive control (streptomycin) were also prepared in duplicate. The plates were incubated at 37˚C for 24 hours. The zone of inhibition was measured after 24 hours from the periphery of the well.

Statistical analysis

Data were expressed as mean ± standard error in three replicates. All the statistical analysis was carried out using SPSS 16.0. To determine whether there were any difference among the means, one way analysis (ANOVA) and the Duncan's multiple range test were applied to the result and P values < 0.05 regarded to be significant.

Results and Discussion

Proximate composition of macroalage

Biochemical composition of all the green macroalage in terms of moisture, ash, crude fat, crude protein and crude fibre are given in

Table 1 and Figure 1. The moisture content of the macroalage ranged from 71.94±1.14 to 82.35 ±1.19. Among the four species of macroalgae studied, *A. acetabulum* showed the highest moisture content (82.35±1.19) followed by *Enteromorpha sp.* (78.78±0.41), *H. macroloba* (73.01±1.42) and *H. tuna* (71.94±1.14). The ash content varied from 18.56±0.12 to 33.67±0.23. The total lipid content was low in all macroalgae samples (0.69±0.58 to 3.22±0.09) in which *A. acetabulum* showed the maximum content and minimum was observed in *H. tuna.*

The range of crude protein (%) varied from 8.123±0.89 to 16.56±2.45 which was highest in *Enteromorpha* sp. (16.56 ± 2.45) and lowest in *A. acetabulum* (8.123±0.89). The crude fibre content varied from 3.54±0.62 to 7.12±0.25. The highest value of crude fibre was observed in *A. acetabulum* followed by *H. macroloba, H. tuna* and *Enteromorpha sp.*

Antibacterial activity

Methanolic extracts of four green macroalage namely *H. tuna, H. macroloba, Enteromorpha* sp. and *A. acetabulum* dissolved in methanol (Figure 2) and DMSO (Figure 3) was tested against four pathogenic bacteria namely *A. hydrophila, S. aureus, E. coli* and *S. pneumoniae* (Table 2).

Aeromonas hydrophila

DMSO dissolved extract of *H. macroloba* showed the maximum zone of inhibition (4.25±0.75 mm), followed by *H. tuna* (4.15±0.15 mm). Moderate activity was observed in *Enteromorpha* sp. (3.5±0.5 mm). In the methanol dissolved extracts of *H. macroloba* and *Enteromorpha* sp. an inhibition zone of 2±0.1 mm and 1.5±0.5 mm was recorded respectively. Methanol dissolved extract of *H. tuna* did not show any inhibition against the pathogen, while methanol and DMSO dissolved extracts of *A. acetabulum* did not show any zone of inhibition against the pathogen.

Staphylococcus aureus

Extracts of all the four tested seaweeds demonstrated anti-bacterial activity against *Staphylococcus aureus*. The maximum zone of inhibition was recorded in methanol dissolved extract of *A. acetabulum* (10.5±0.5 mm) followed by DMSO dissolved extract of the same seaweed (10.5±1.5 mm). Methanol and DMSO dissolved extracts of *Enteromorpha* sp. showed an inhibition activity of 10±2 mm and 9.75±2.25 mm. *H. tuna* and *H. macroloba* showed moderate activity against the tested pathogen.

Escherichia coli

DMSO dissolved extract of all the tested macroalage except *Enteromorpha* sp, was found ineffective against the pathogen. DMSO dissolved extract of *Enteromorpha* sp. showed maximum zone of inhibition (16±1 mm) whereas, the methanol dissolved extract of the same species showed the least zone of inhibition (2.5±0.5 mm). Inhibition activity of 9.5±0.5 mm and 4.5±0.5 mm was observed in the methanol dissolved extract of *H. macroloba* and *H. tuna*. Both methanol and DMSO dissolved extract of *A. acetabulum* was found ineffective against the pathogen.

Streptococcus pneumoniae

The maximum zone of inhibition was observed in the methanol dissolved extract of *A. acetabulum* (11±3 mm) followed by DMSO (9.5±0.5 mm) and methanol (7±1 mm) dissolved extract of *Enteromorpha* sp respectively. Methanol and DMSO dissolved extract of *H. tuna* was ineffective whereas *H. macroloba* showed moderate activity against the test pathogen.

Fig.1 Proximate composition of the selected green macroalgae: a. *H. tuna*, b. *H. macroloba*, c. *Entermorpha sp.* d. *A. acetabulum*

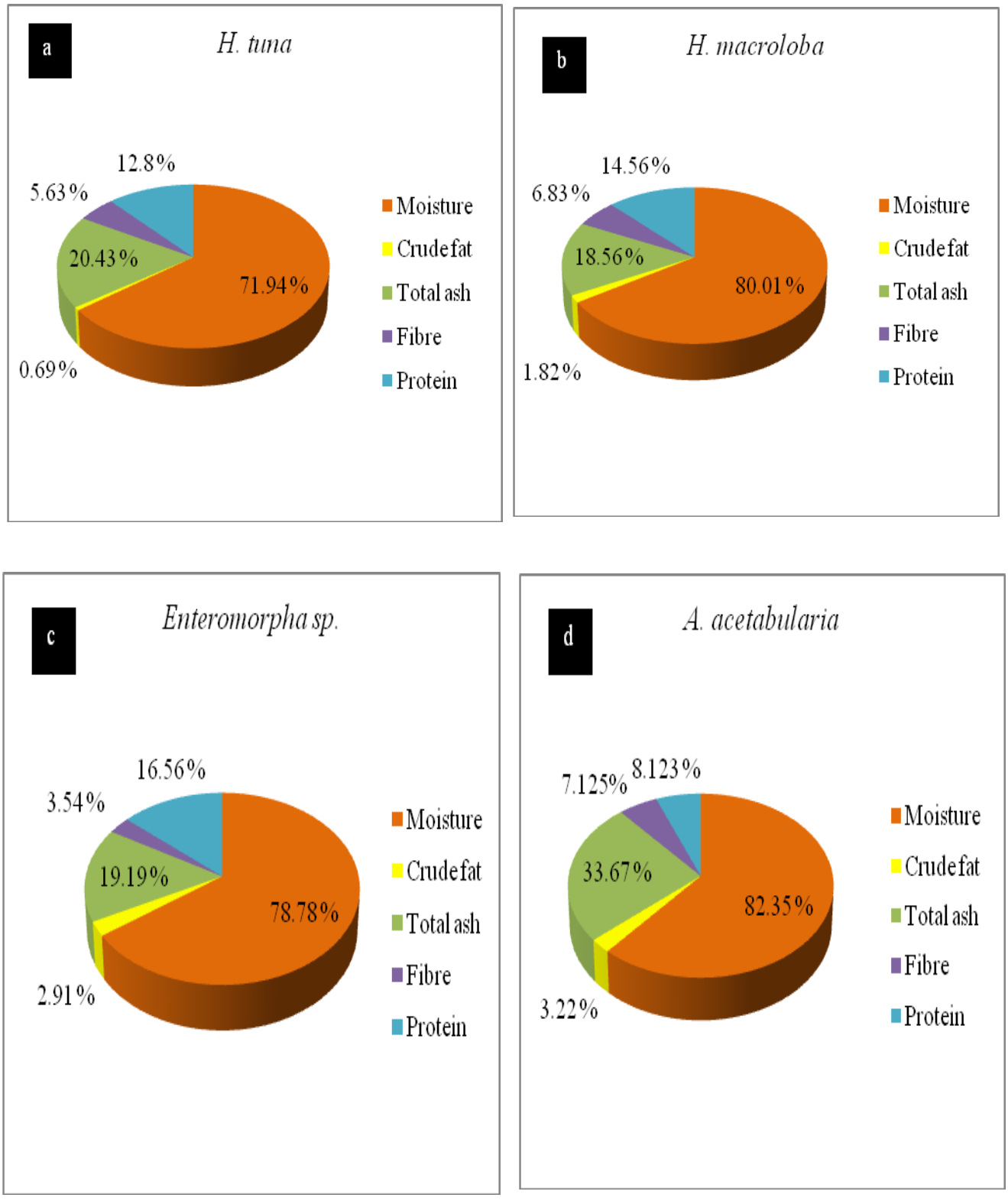

Fig.2 Antibacterial activity of methanol dissolved (methanolic) extract of green macroalage

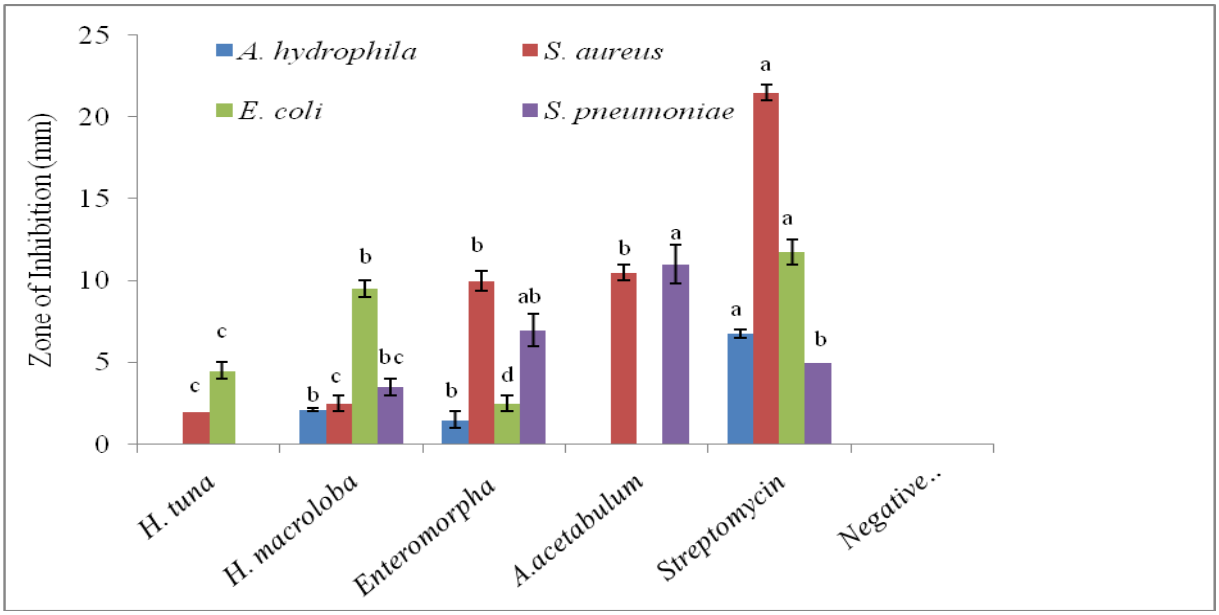

Fig.3 Antibacterial activity of DMSO dissolved extract of green macroalgae

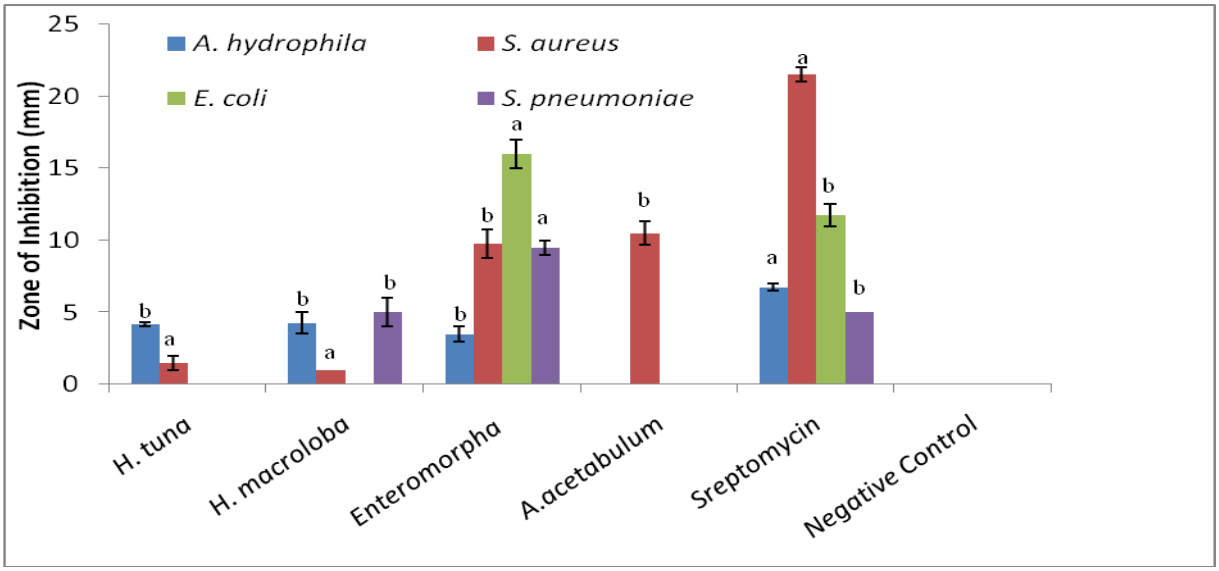

Table.1 Proximate composition of green macroalage

Seaweeds	Moisture (%)	Total Ash (%)	Crude Fat (%)	Crude Protein (%)	Crude Fibre (%)
H. tuna	71.94 ± 1.14^{b}	20.43 ± 0.11^{b}	0.69 ± 0.58^{c}	12.8 ± 1.25^{b}	5.62 ± 0.75^{a}
H. macroloba	73.01 ± 1.42^{b}	18.56 ± 0.12^{b}	1.82 ± 0.50^{b}	14.56 ±1.76^{a}	6.83 ± 0.27^{a}
Enteromorpha sp.	78.78 ± 0.41^{a}	19.19 ± 2.22^{b}	2.91 ± 0.10^{a}	16.56 ± 2.45^{a}	3.54 ± 0.62^{b}
A. acetabulum	82.35 ± 1.19^{a}	33.67 ± 0.23^{a}	3.22 ± 0.09^{a}	8.123 ± 0.89^{c}	7.12 ± 0.25^{a}

***** Each value is expressed as the mean ± standard error (n=3)

Table.2 Antibacterial activity of methanol and DMSO dissolved macroalage extract

Macroalage species	Solvent used	Bacteria			
		A.hydrophila	***S. aureus***	***E. coli***	***S. pneumoniae***
H. tuna	Methanol	-	2± 0^{c}	4.5± 0.5^{c}	-
	DMSO	4.15± 0.15^{b}	1.5± 0.5^{a}	-	-
H. macroloba	Methanol	2.1± 0.1^{b}	2.5± 0.5^{c}	9.5± 0.5^{b}	3.5± 0.5^{bc}
	DMSO	4.25± 0.75^{b}	1± 0^{a}	-	5± 1^{b}
Enteromorphasp.	Methanol	1.5± 0.5^{b}	10± 2^{b}	2.5± 0.5^{d}	7± 1^{ab}
	DMSO	3.5± 0.5^{b}	9.75± 2.25^{b}	16± 1^{a}	9.5± 0.5^{a}
A. acetabulum	Methanol	-	10.5± 0.5^{b}	-	11± 3^{a}
	DMSO	-	10.5± 1.5^{b}	-	-
Positive control	Methanol	6.75± 0.25^{a}	21.5± 0.5^{a}	11.75± 0.75^{a}	5± 0^{b}
	DMSO	6.75± 0.25^{a}	21.5± 0.5^{a}	11.75± 0.75^{b}	5± 0^{b}
Negative control	Methanol	-	-	-	-
	DMSO	-	-	-	-

***** Each value is expressed as the mean ± standard error (n=3); - indicates No zone of inhibition; Positive control- Streptomycin and Negative control- Methanol.

Macroalgae are known as a rich source of structurally diverse bioactive secondary metabolites which may function as anti-oxidative, antimicrobial, cytotoxic and anti-inflammatory agents. Present study aims at exploring the nutritional composition and antibacterial activities of selected green seaweeds (*H. tuna, H. macroloba, Enteromorpha sp.* and *A. acetabulum*) from South Andaman. Proximate composition of the four green macroalage collected from different locations of South Andaman was assessed. Burkholder, Burkholder and Almodovar (1971) reported that macroalgae contain 68-88% moisture which is in agreement with the results obtained in the present study. The ash content ranged from 18.56±0.12 to 33.67±0.23 which is nearly similar to the earlier studies in various green macroalgae (Arunkumar *et al.,* 2014; Khairy and El-Shafay, 2013; Rohani-Ghadikolaei *et al.,* 2012). Higher ash content is a general feature of seaweeds which indicates the presence of significant quantity of mineral compounds. In general, macroalgae contains less amount of crude lipid fibre. Burtin (2003) and Polat & Ozogul (2008) reported that macroalgae are relatively low (1-5% of dry weight) in lipid content. In the present study, crude lipid content observed was in the range of 0.69 - 3.22%. Manivannan *et al.,* (2008) analysed different group of macroalgae from Vedalai coastal waters and estimated lipid contents in *Enteromorpha intestinalis* (1.33%) and *Enteromorpha clathrata* (4.6%) which is in line with the results obtained in the present study. *Enteromorpha* sp. had a lipid content of 2.91% which was very low when compared to the results obtained by Chackroborty and Santra (2008) for *Enteromorpha intestinalis* (7.1%). Parthiban *et al.,* (2013) also recorded high crude lipid content in the green seaweed *Enteromorpha intestinalis* (5.30%). The range of protein content determined in the present study (8.123 -16.56%) was similar to the findings reported by Rohani-Ghadikolaei *et al.,* (2012); Manivannan *et al.,* (2008); Parthiban *et al.,* (2013); Kasimala, Mebrahtu *et al.,* (2015) but slightly lower when compared to the reports of Murugaiyan and Narasimman (2013); Dere *et al.,* (2003); Patarra *et al.,* (2011). Macroalgae are widely used as a source of dietary fibre. The fibre content in the present study varied from 3.54-

7.12% which is very low when compared to the previous studies conducted by Patarra *et al.,* (2011) and Ahmad, Sulaiman *et al.,* (2012) on green macroalgae. The differences found in the present study may be due to the seasonal, spatial and temporal variations (Dawes, 1998 and Jimenez-Escrig and Cambrondon, 1999) and also due to the difference in the competence of the extraction technique followed (Tuney *et al.,* 2007).

Antibacterial activity of the macroalgae depends upon the algal species and the solvent used for extraction. Organic solvents are commonly used for extraction of active compounds from algae. In the present study, methanol was used for extraction. According to Sangeetha *et al.,* (2014) and Tuney *et al.,* (2006) methanolic extracts gives higher antibacterial activity than the other extracts. Various species of marine algae have been collected and analysed for their antibacterial activity from different parts of the world. Reichelt and Borowitzka (1984) and Salvador *et al.,* (2007) reported that members of red algae exhibited high antibacterial activity. On contrary, Khandhasamy and Arunachalam (2008) reported that members of green algae were more active when compared to other groups of algae. Karthick *et al.,* (2015) also reported that green algae exhibited antibacterial activity against both gram positive and gram negative bacteria whereas brown and red algae showed moderate activity.

According to the study conducted by Karthikaidevi *et al.,* (2009) methanolic extract of *Halimeda tuna* showed antibacterial activity against both *E. coli* and *S. aureus* and no activity was observed against *Streptococcus* sp. which is in agreement with the results obtained in the present study. Mtolera and Semesi (1996) investigated various green macroalage including two species of *Halimeda* and reported activity against *S. aureus* and *E. coli.* Tuney *et al.,* (2007) found that the methanolic extract of *Enteromorpha* sp. was ineffective against *E. coli*. Glombitza (1969) established that *Enteromorpha intestinalis* was ineffective against *S. aureus* and Ibrahim and Lim (2015) reported the species to be ineffective against both *S. aureus* and *E. coli*, where as in the present study potential antibacterial activity against the respective pathogens were observed in *Enteromorpha* sp. Similarly, Seenivasan, Indu, Archana and Geetha (2010) reported prospective antibacterial activity in *Ulva fasciata* against *E. coli.* In the present study, *A. acetabulum* was found with the highest activity against *S. aureus* and no activity against *E. coli*, which is contradictory with the report of Karthick *et al.,* (2015) for *Acetabularia* sp. against *E. coli*.

This study concludes that the methanolic extract of four different macroalage possess significant antibacterial activity against the tested pathogens, among which the crude extract of *Enteromorpha* sp. was found active against all the four pathogens. However, further study is required to isolate and characterize the bioactive compounds responsible for the activity.

Considerable levels of protein, dietary fibre and high ash content were observed in the studied macroalage. Thus macroalage can serve as source of food protein in human and animal diet and can even be used in industries as an ingredient of high nutritional value. However, further study is required to isolate and characterize the bioactive compounds responsible for the activity.

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