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Antimicrobial activity of marine algae

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Abstract:

The present communication deals with the antimicrobial activity of the seaweeds such as ***Sargassum polycystum*** and ***Sargassum tenerrimum*** against both gram - positive , gram- negative and fungal pathogens . For experimental study different crude seaweeds extracts (Chloroform , Ethanol ,Methanol and water) was determined by the well diffusion method. The inhibition zone was measured for all the Crude extracts revealed a wide range of antimicrobial activity against tested pathogens. Antimicrobial activity indicates that the presence of active constituents in the extractions of marine algae which can be exploited for the production of innovation drugs for the benefit of the humanity.

Keywords: Seaweeds, antimicrobial activity Well diffusion method.

Introduction

Among the marine flora and fauna marine algae are rich sources of diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Wijesekara *et al*., 2010). During the last years, many studies have been made on biological activities of the seaweed and identified as potentiala sources of natural antioxidants (Matanjun *et al.,* 2008). Several authors studied the antimicrobial activities of marine algae in different parts of our country (Battu *et al.,* 2011, Selvi *et al*., 2011, Tuney *et al.,* 2006, Veeragurunathan and Geetha, 2009, Veeragurunathan *et al.,* 2008).In recent year seaweeds are wildly used in several applications such asantimicrobial (Chiheb *et al.,* 2011), antiviral (Bouhlal *et al.,* 2010, Bouhlal *et al.,* 2011, Kim and Karadeniz, 2011), antifungal (De Felicio *et al,* 2010), anti-allergic (Na *et al,* 2005),, anti-coagulant (Dayong *et al.,* 2008), anti-cancer (Kim *et al*, 2011), anti-fouling (Bhadury and Wright, 2004) and antioxidant activities (Devi *et al,* 2011). In the present study an attempt was made on antimicrobial prpperties of some marine occurring at mandapam coast.

Materials and Methods

Sample collection

Mandapam located on the southeast coast of Tamil Nadu (78°08 ' E and 9° 17' N) with luxuriant algal growth throughout the year . At Rameswaram the shore is sandy with boulders and platforms of compressed sandstones with rough end uneven surfaces situated at different level from high water to low water. Live and healthy marine algae were collected in the polythene bags with seawater and brought to the laboratory. Each species was washed with running water to remove epiphytes, animal castings, attached debris and sand particals, the final washings were done with distilled water and dried under shade.

Seaweeds extract preparation

This each Seaweed material mixed with different solvents with increasing polarity (Chloroform,Ethanol, Methanol and water) and placed into the Soxhlet apparatus. Each extraction was carried out in a Soxhlet apparatus for 24 hrs and after evaporation in vaccum the extracts were stored at -20°c until used (Krishnaveni *et ah*, 2012).

Bacterial and Fungal pathogens

For testing the antibacterial activity, the following Gram positive *Bacillus subtilis(*MTCC-441) , *Staphylococcus aureus*(MTCC-96), *Micrococcus luteus((*MTCC-1538),*Staptococcus mutans(*MTCC-890*),Streptococcus anginosus(MTCC-1929), Lactobacillus acidophilus (447)* and Gram negative-*Escherichia coli, Enterobacter aerogenes(MTCC-111), Klebsiella pneumonia(MTCC-432), Pseudomonas aeuroginosa(MTCC-424),Erwinia caratovora* (MTCC-1428)and *Proteus vulgaris* (MTCC-1771*)* bacterial strains were selected. For antifungal activity, The following fungal strains, *Candida albicans*(MTCC-

227), *Aspergillus niger*(MTCC-1344), *Saccharomyces cerevisiae(*MTCC-463*), Rhizoctonia solani*(MTCC-984*) ,Mucor racemosus(*MTCC-6333*)* and *Rhizopus stolonifer*(MTCC-2198) were used for antifungal activity. They were obtained from the Institute of microbial technology Chandigarh. The work was carried out in Department of Microbiology, Andhra University.

Antimicrobial Activity by disc diffusion method:

In the present study, the antimicrobial activity of the seaweeds was studied by agar cup plate diffusion method (Kavangh, 1992). The Chloroform,Ethanol, Methanol and Water extracts of the collected test samples were tested in three dose levels of l00mg/ml, 300mg/ml, and 500mg/ml respectively. The nutrient agar medium prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter petridishes. The medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for about 30 minutes, 5 cups of 6mm diameter were made in each plate at equal distance. Stock solutions of the test residual extract were prepared in l00mg/ml, 300mg/ml, and 500mg/ml. l00mg/ml of each concentration were placed in the cups with sterile pipettes. In each plate one cup was used for control. Antibiotic Chloramphenical (l00mg/ml) was used as standard and respective solvents were used as control. The petridishes were prepared and incubated for 24 hrs at 37° C for bacteria. The above procedure is allowed for fungal assays but expects the media potato dextrose agar instead of nutrient agar and the antibiotic nystatin was used as standard. The plates were incubated at 25^{0c} for 48hrs, after that the zone of inhibition was measured with zonal scale in mm and the experiment was carried out in duplicate.

Results

Antimicrobial activity of *sargassum polycystum*

Fig: 1.1 Shows the antimicrobial activity ***Sargassum polycystum*** at 100 mg/ml conc. of chloroform, ethanol, methanol and water extracts. In these four extracts ethanol and methanol showed moderate activity. Methanol extract showed a maximum activity against pathogens like *Proteus vulgaris (*18mm), *K. pneumonia (*18mm) and fungal strains methanol extract of *A.niger*(19mm*), R. stolonifer*(19mm*),* ethanol extract of *R. stolonifer*(19mm) showed promising activity.

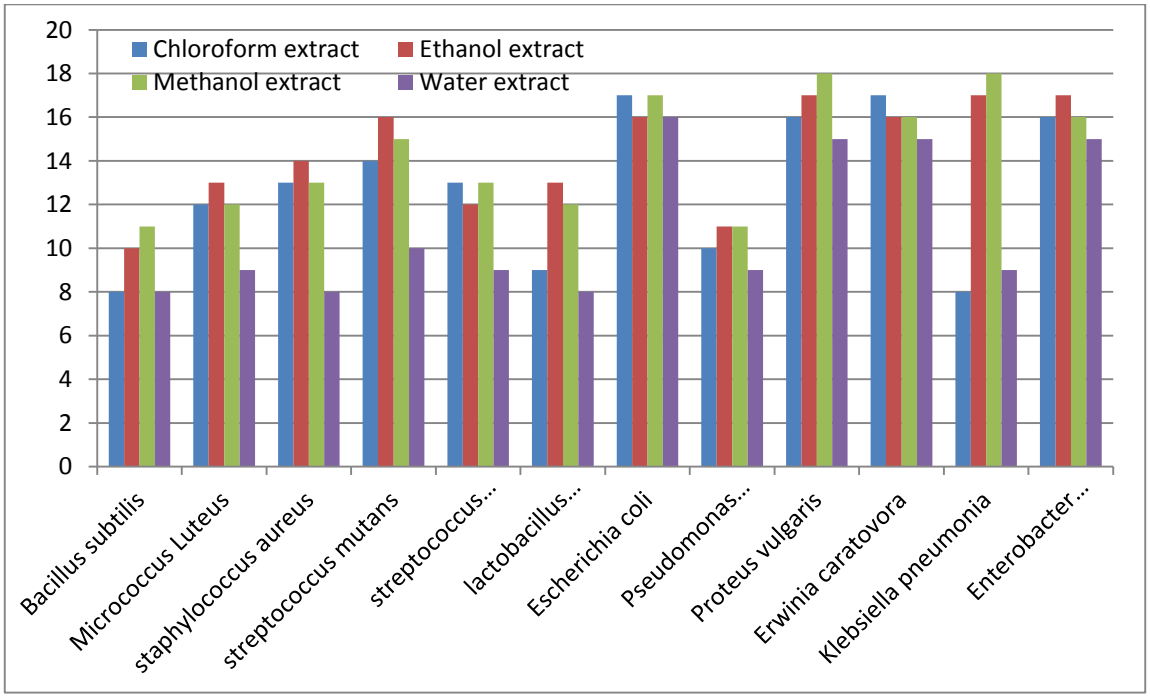

Fig: 1.1 Antibacterial activity of *SARGASSUM POLYCYSTUM* 100mg/ml

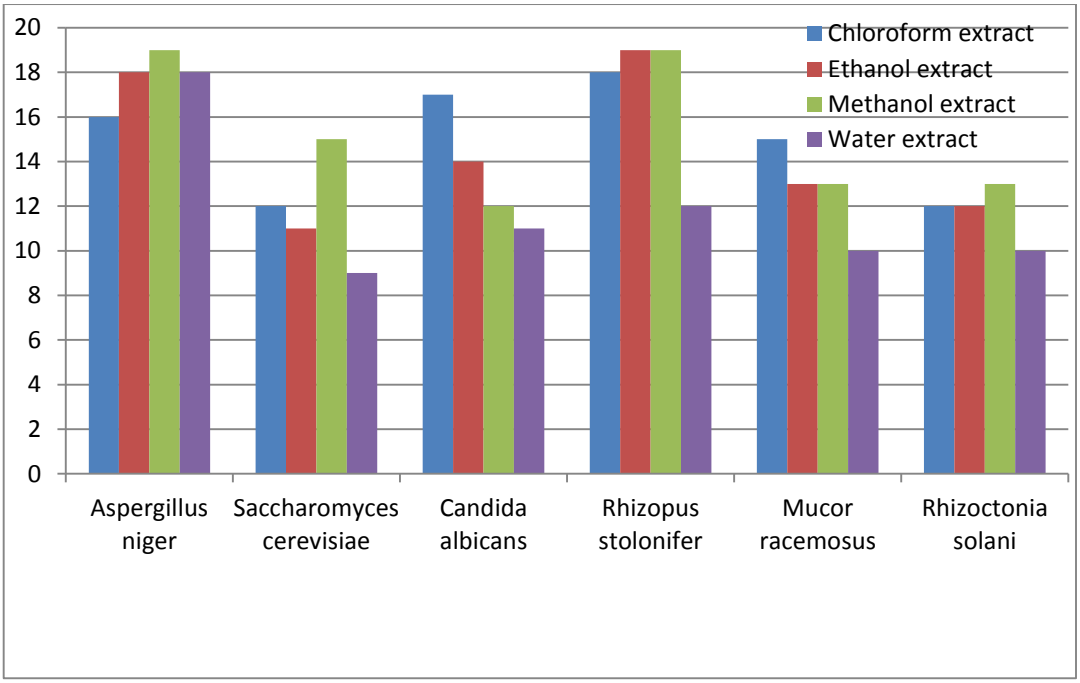

Fig: 1.1a Anti fungal activity of *SARGASSUM POLYCYSTUM* 100mg/ml

Fig:1.2 Shows the antimicrobial activity ***Sargassum polycystum*** in 300mg/ml conc. of chloroform,ethanol,methanol and water extracts. In these four extracts methanol and ethanol extract showed moderate activity. Methanol extract of *E.coli(19mm), P.vulgaris (*19mm), *K. pneumonia (*19mm) , ethanol extract *P.vulgaris (*19mm), *E.aerogenes(19mm)* showed promising activity and fungal strain of methanol extract *R. stolonifer(21mm)* showed high activity and Water extract of *S.cerevisiae* showed low activity .

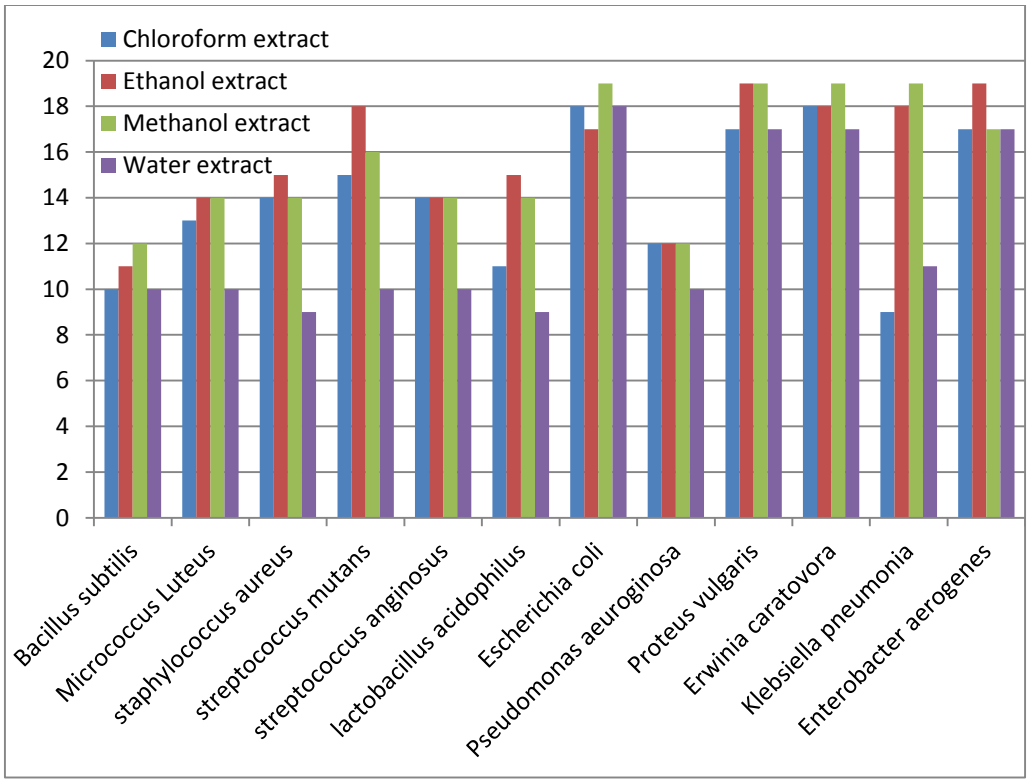

Fig:1.2 Antibacterial activity of *SARGASSUM POLYCYSTUM* 300mg/ml

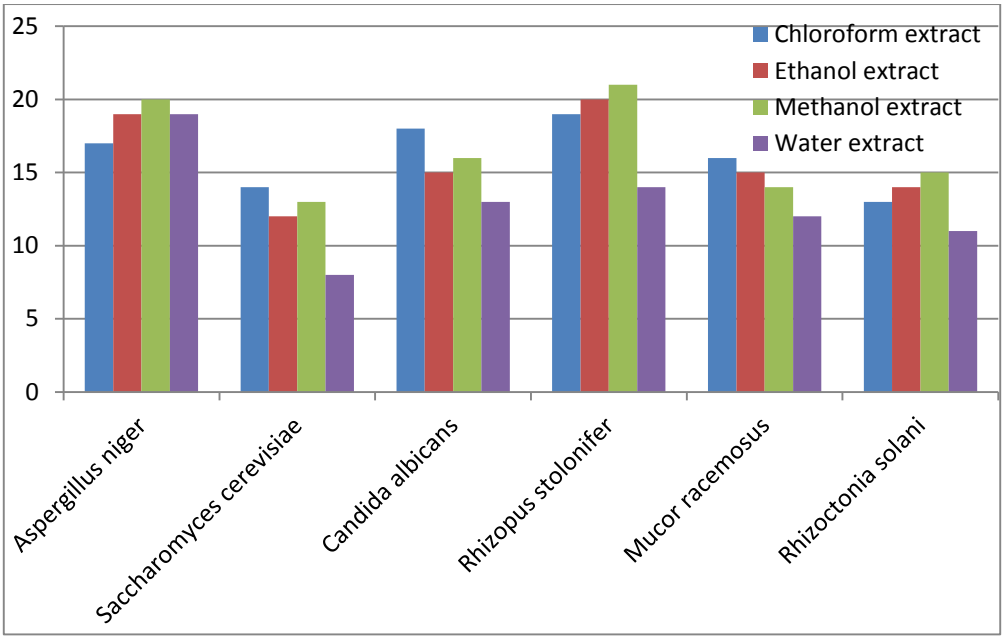

Fig:1.2aAnti fungal activity of *SARGASSUM POLYCYSTUM* 300mg/ml

Fig: 1.3. Shows of antimicrobial activity ***Sargassum polycystum*** at 500mg/ml conc. of chloroform,ethanol, methanol and water extracts. In these four methanol extract showed high activity. The extract obtained using chloroform showed maximum activity against pathogens like *E.coli(*19mm), *E. caratovora(*19mm *)*, *E.aerogenes(*19mm) and showed low activity against *K. pneumonia (*11mm). Fungal stains *A.niger(20mm), R. stolonifer*(21mm) and *Candida albicans*(20mm) showed high activity. Ethanol extract observed the highest activity against pathogens like *S. aureus (*20mm), *K. pneumonia (*21mm) *P.vulgaris (*20mm),*E. caratovora(*20mm), *E.aerogenes(*20mm) and *A.niger(20mm), R. stolonifer*(21mm), *Candida albicans*(20mm) showed high activity. The extracts obtained using methanol showed highest activity against pathogens like *E.coli(*21mm),*P.vulgaris (*20mm), *E. caratovora(*21mm), *K. pneumonia (*21mm), *A.niger(22mm), R. stolonifer*(22mm). The extracts obtained using water showed highest activity against pathogens like *E.coli(*19mm),*P.vulgaris (*19mm), *E. caratovora(*19mm), fungal stains *A.niger(20mm)* showed highest activity and extract of *S.cerevisiae* showed mild activity .

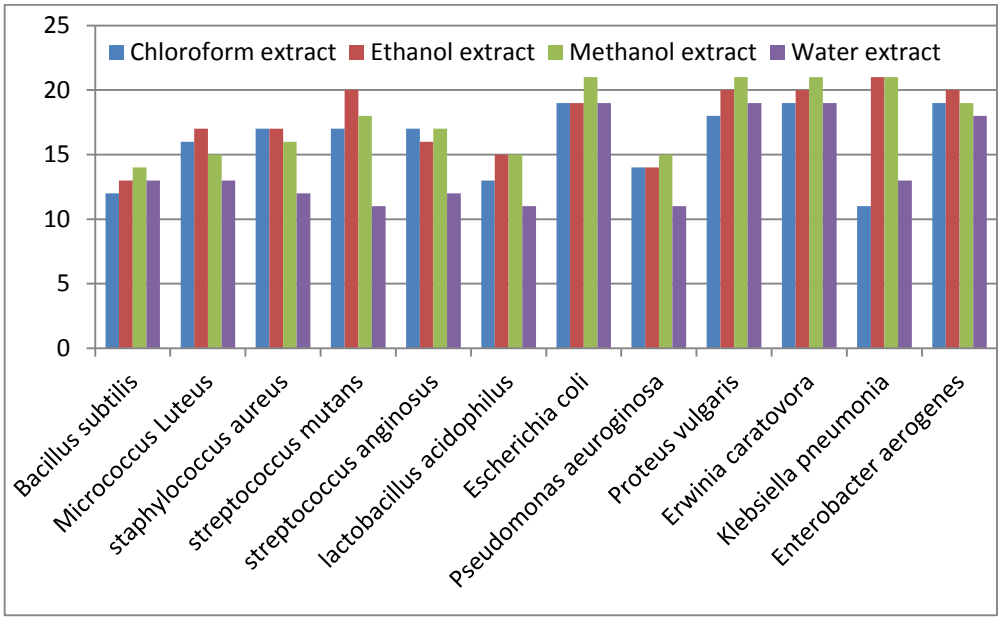

Fig: 1.3 Antibacterial activity of *SARGASSUM POLYCYSTUM* 500mg/ml

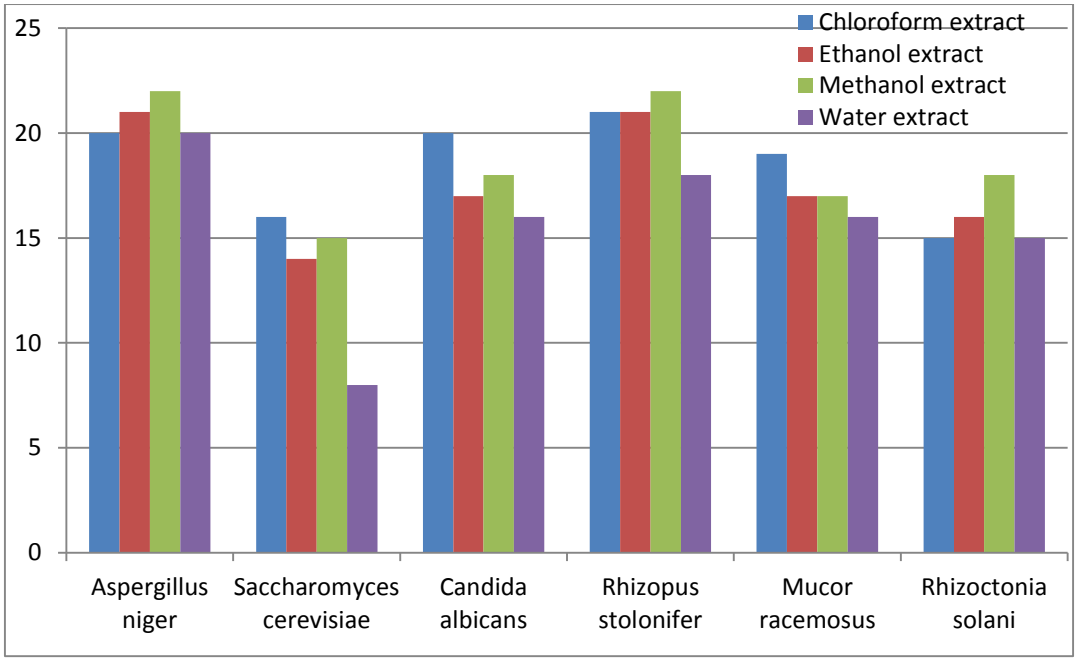

Fig: 1.3a Anti fungal activity of *SARGASSUM POLYCYSTUM* 500mg/ml

Antimicrobial activity of *Sargassum tenerrimum*

Fig: 2.1 .Shows the antimicrobial activity of *Sargassum tenerrimum* at 100 mg/ml conc. of chloroform ethanol, methanol and water extracts. In these four extracts ethanol and chloroform showed moderate activity. Ethanol extract shows promising acyivity against pathogen like of *S. aureus*(15mm) and fungal strains A.niger(10mm), *R. stolonifer*(10mm)and *M.racemosus*(10mm) showed mild activity.

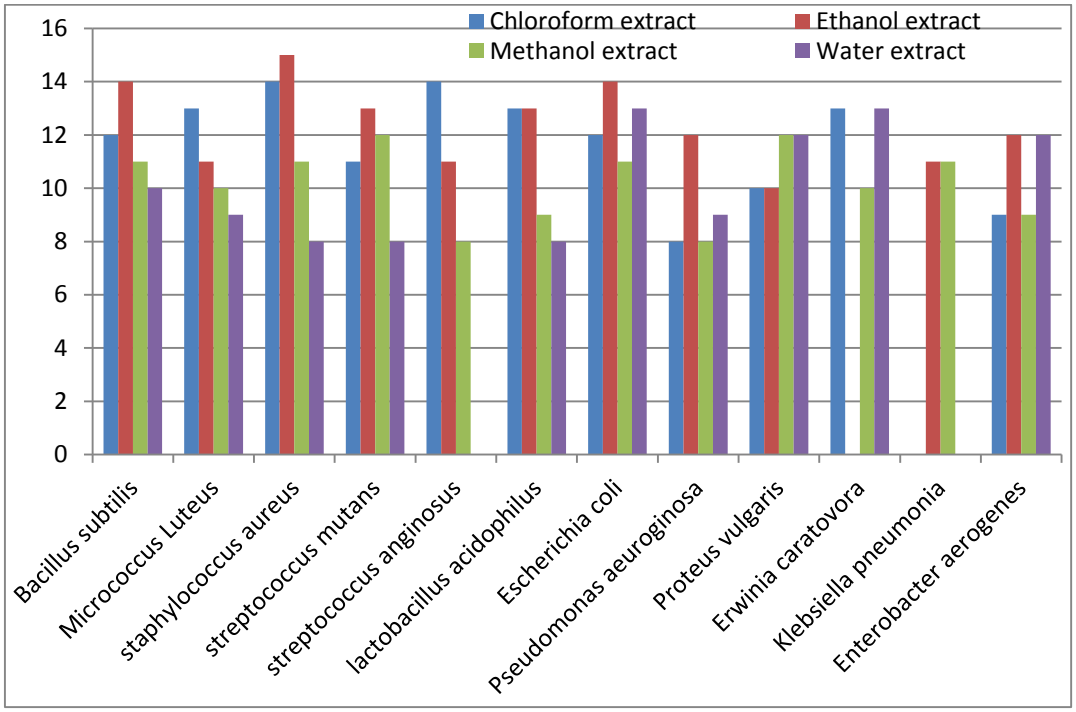

Fig: 2.1 Antibacterial activity of *SARGASSUM TENERRIMUM* 100mg/ml

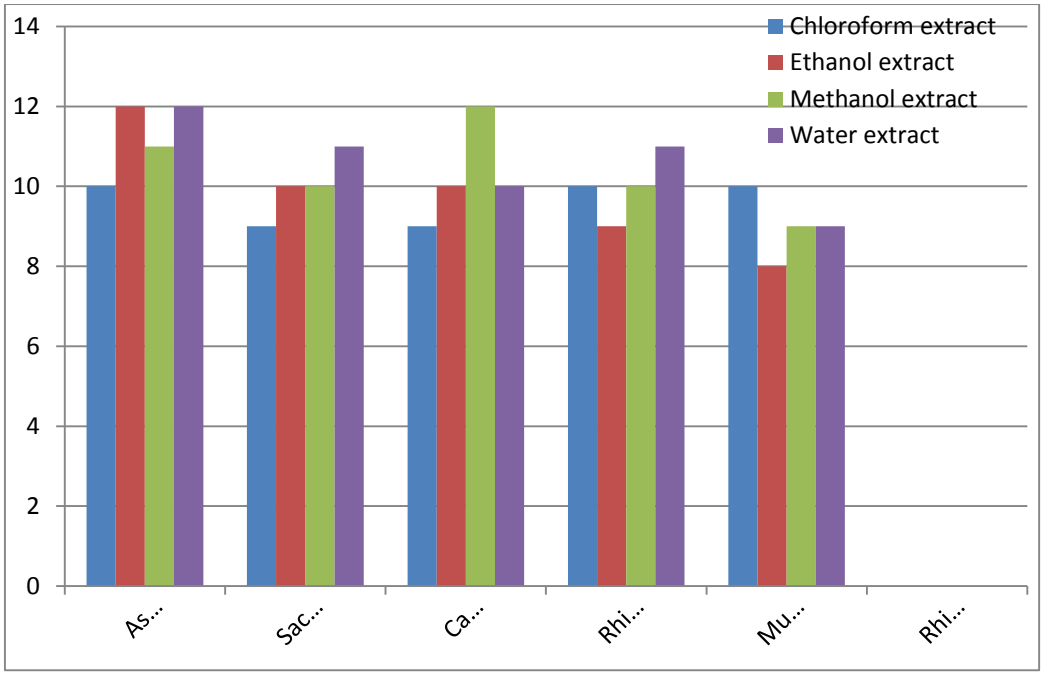

Fig: 2.1a Antifungal Activity of *SARGASSUM TENERRIMUM* 100mg/ml

Fig: 2.2 Shows the antimicrobial activity of *Sargassum tenerrimum* at 300mg/ml conc. of chloroform,ethanol,methanol and water extracts. Among these four extracts ethanol extract showed highest activity against pathogen like *S. aureus* (19mm) and fungal strain of water extracts of A.niger(14mm) , methanol extract of *Candida albicans*(13mm)and ethanol extract of A.niger(13mm) showed moderate activity

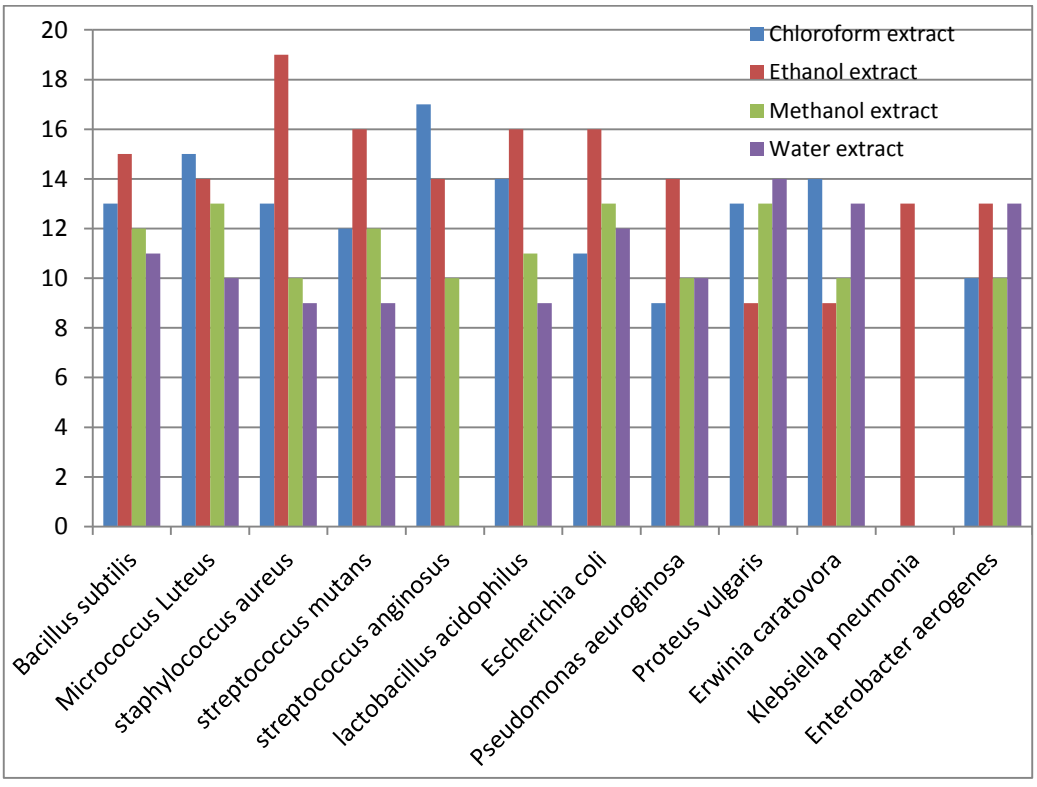

Fig: 2.2 Antibacterial activity of *SARGASSUM TENERRIMUM* 300mg/ml

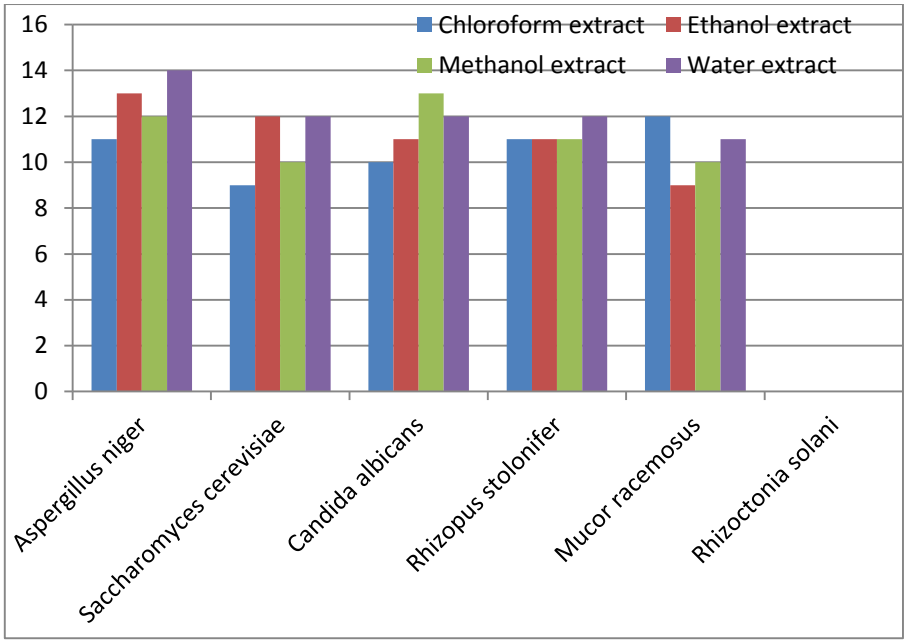

Fig: 2.2a Antifungal Activity of *SARGASSUM TENERRIMUM* 300mg/ml

Fig:2.3. Shows the antimicrobial activity of *Sargassum tenerrimum* at 500mg/ml conc. of chloroform, ethanol, methanol and water extracts. In these four extracts ethanol and chloroform extracts showed moderate activity. Chlorofrom etract showed promising activity against pathogens like *S. aureus* (17mm) , *S.anginosus(18mm),*and *M.racemosus*(13mm) showed mild activity.Ethanol extract proved the highest activity against pathogens like *S. aureus* (19mm) , *E.coli(*18mm) and fungal strain *A.niger*(14mm), *S.cerevisiae* (13mm) showed mild activity. Methanol extract observed the promising activity against pathogens like *E.coli(*15mm), *P.vulgaris (*15mm), *Candida albicans*(14mm) and*R. Solani(10mm)* showed low activity. Water extract observed the promising a activity against pathogens like *E.coli(14mm), P.vulgaris (*14mm), *E.aerogenes(*14mm) and *A.niger(15mm), R. stolonifer*(14mm), showed moderate activity .

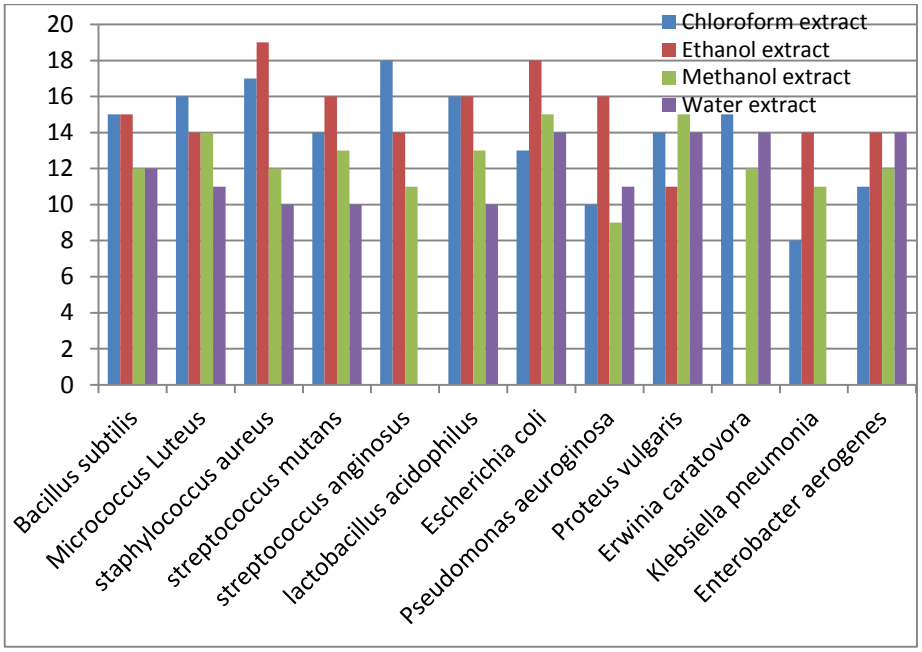

Fig:2.3 Antibacterial activity of *SARGASSUM TENERRIMUM* 500mg/ml

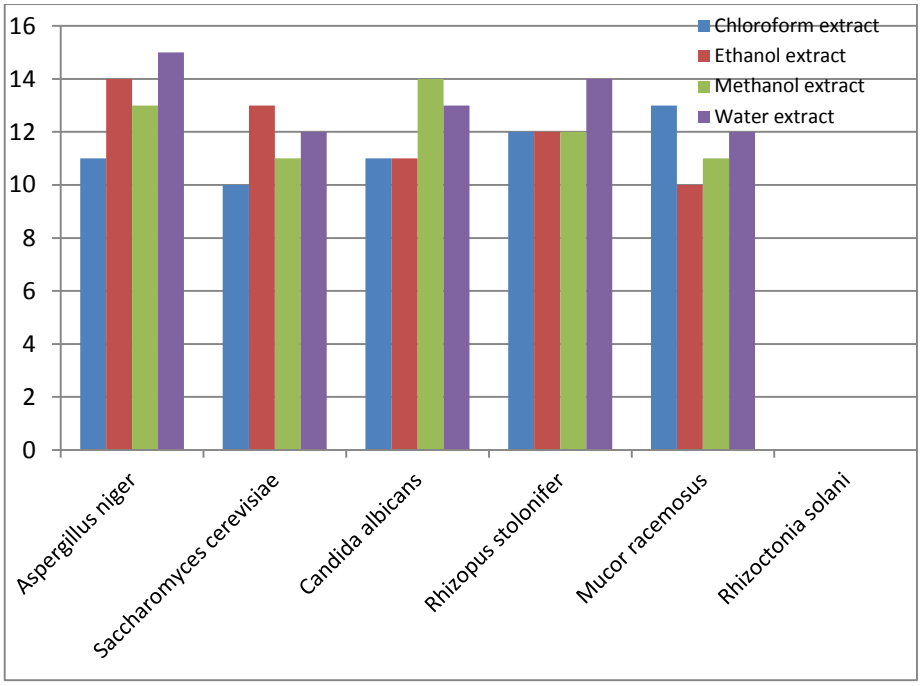

Fig: 2.3a Antifungal Activity of *SARGASSUM TENERRIMUM* 500mg/ml

Discussion

The present study was to evaluate the antimicrobial activity of the different macroalgae for its bioactive potentials.Rodriguez *et al., (*2010), Bhacuni and Rawat, (2005), Priyadharshini *et al,(*2011) have reported that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities. Depending upon their solubility and polarity, different solvents shows the different antimicrobial activity. So chemical compounds should be extracted from different seaweeds in order to optimize their antibacterial activity by selecting the best solvent system (Hediat *et al.,).*The results from the present study revealed that the strongest antibacterial activity exhibited by the methanol extract by *Sargassum polycystum* .Methanol have higher antibacterial activity than that of extracts obtained with other organic solvents (Febles et al.,1995;Sidharata et al.,1997;Kumar et al.,2008;Seenivasan et al.,2010;Lavany and veerappan,2011).Devi et al.,2008;Meenakshi et al.,2009;Cox et al.,2010;Srivastava et al.,2010,reported that the methanol extract of seaweeds contains phenolics,alkaloids and amino acids which may responsible for the antimicrobial activity. Manilal *et al.,* and Rangaiah *et al.,* exlained that the methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate . Methanol extract of *Sargassum polycystum* showed more activity against *E.coli, P.vulgaris ,E. caratovora, K. pneumonia* and fungal strain of methanol extract of *A.niger* and *R. stolonifer.* Chloroform extract showed moderate activity with *Sargassum tenerrimum.*Ethanol extract of *Sargassum tenerrimum* showed highest activity against *S.aureus* and water extract of *A.niger* showed moderate activity . Water extract shows less activity Hodgson(1944). The result shows the activity of four solvent extracts in the order methanol > ethanol > chloroform > aqueous extracts agreeing with Vizaya parthasarathy et.al.,(2004). The present study reveals that solvents are always better for extraction when compared with water.

Conclusion

The results of this present study on *sargassum polycystum* and *sargassum ternerrimum* using four different solvent extract against eighteen different pathogens showed significant antimicrobial activity .However, more research has to be done on isolation ,purification and identification of the active ingredients in order to understand their bio prospects.

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