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Research article

IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY IN MARINE ALGAE *CAULERPA TAXIFOLIA AND CAULERPA RACEMOSA* (C. AGARDH).

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ABSTRACT: The present study was carried out to investigate its antimicrobial potentiality of the algae such as *Caulerpa taxifolia*, *Caulerpa racemosa* (C. Agardh) were studied against both Gram-positive, Gram- negative and fungal pathogens. For microbiological testing of the different crude algal extracts (Hexane, Chloroform, Methanol and water) was determined by the well diffusion method.The zone of inhibition was measured for all the Crude extracts revealed a wide range of antimicrobial activity against tested pathogens. The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of Seaweeds which can be exploited for the production of lead molecules which are use of in pharmaceutical industry.

Keywords: Seaweeds, *Caulerpa taxifolia*, *Caulerpa racemosa*, antimicrobial activity Well diffusion method.

INTRODUCTION

As more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offer a rich source of natural products. Marine environment contains a source of fuctional marerials, including polyunsaturated fatty acids (PUFA), polysaccharides, essential minerals, and vitamins, antioxidants, enzymes and bioactive peptides (Kim *et al*., 2010). Among marine organisms, marine algae are rich sources of structurally 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 could be potential rich sources of natural antioxidants (Matanjun *et al*., 2008). Seaweeds are the most accessible marine resource of the coastal zone that occupies potential importance source of biochemical compound. Pharmaceutical importance of seaweeds are well known all over the world and extensive efforts were given to bring out substances from algae. There are a number of reports regarding the medicinal importance of seaweeds belonging to *Chlorophyceae*, *Phaeophyceae*, *Rhodophyceae* from all over the world (Kolanjinathan *et al*., 2009, Rajasulochana *et al*., 2011). Several studies were made earlier on the antimicrobial activities of marine algae (Battu *et al*., 2011, Selvi *et al*., 2011, Tuney *et al*., 2006, Veeragurunathan and Geetha, 2009, Veeragurunathan *et al*., 2008). Marine macro algae are the most interesting group because of their broad spectrum of biological activities such as antimicrobial (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). They produce a wide variety of chemically active metabolites in their surroundings as an aid to protect themselves against other settling organisms (Bhadury and Wright, 2004). There are numerous reports of macro algae derived chemical compounds that have a broad range of biological activities, some of which have been used in pharmaceutical industries.

MATERIALS AND METHODS

Sample collection

Visakhapatnam located on the east of India (latitudes $17^{0}14^{1}$ 30^{11} and 17^{0} 45^{1} N and the longituteds $83^{0}16^{1}25^{11}$ and $83^{0}21^{1}30^{11}$ E) with luxuriant algal growth. Live and healthy marine algae were collected from the intertidal rocky surfaces of Visakhapatnam coast 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 (Hexane, Chloroform, 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^{o}c until used (Krishnaveni *et al*., 2012).

Bacterial and Fungal pathogens

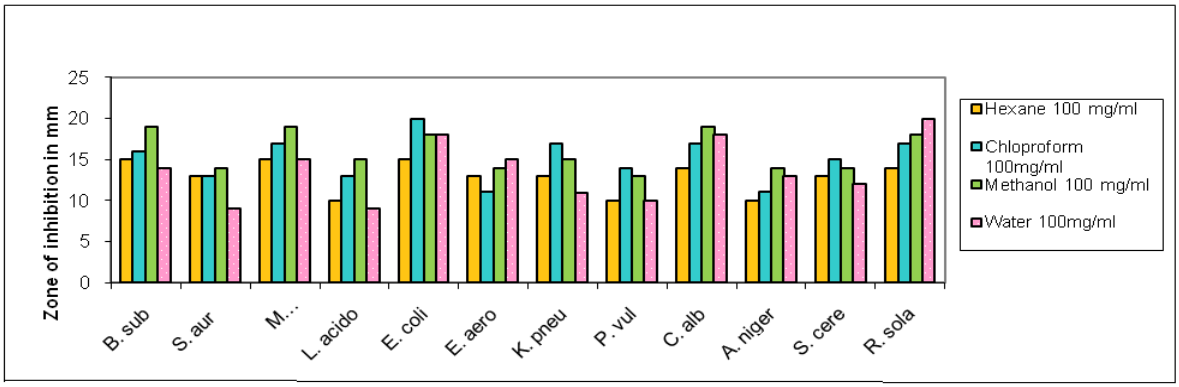
For testing the antibacterial activity, the following Gram positive *Bacillus subtilis* MTCC(2274), *Staphylococcus aureus* MTCC(3160), *Micrococcus luteus* MTCC(106), *Lactobacillus acidophilus* MTCC(447) and Gram negative- *Escherichia coli* MTCC(739), *Enterobacter aerogenes* MTCC(111), *Klebsiella pneumonia* MTCC(4032), *Proteus vulgaris* MTCC(7299), bacterial strains were selected. For antifungal activity, The following fungal strains, *Candida albicans* MTCC (3017), *Aspergillus niger* MTCC (1317), *Saccharomyces cerevisiae* MTCC (3073) *Rhizoctonia solani* MTCC (4634) were used for antifungal activity. They were obtained from the Institute of microbial technology Chandigarh.

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 Hexane, Chloroform, Methanol and Water extracts of the collected test samples were tested in three dose levels of 100mg/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 100mg/ml, 300mg/ml, and 500mg/ml. 100ug/ml of each concentration were placed in the cups with sterile pipettes. In each plate one cup was used for control. Antibiotic Chloramphenical (100ug/ml) was used as standard and respective solvents were used as control. The petridishes were prepared and incubated for 24 hrs at 37^{0} 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.

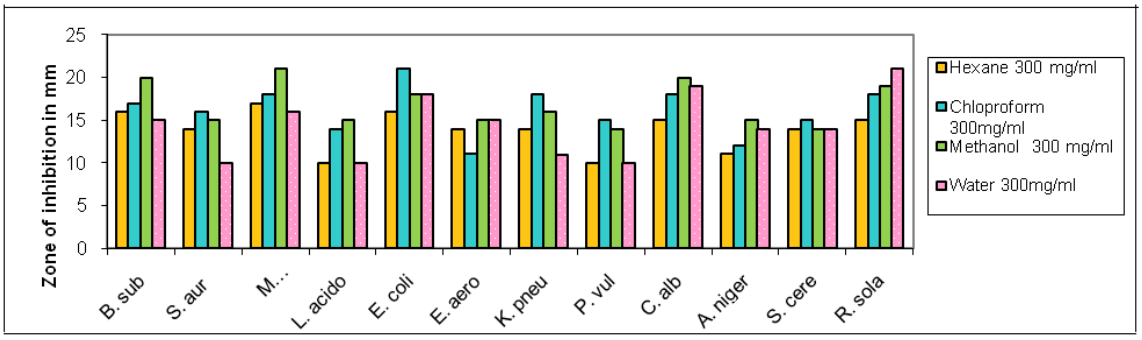
RESULTS

Graph 1.represents that comparison between four solvents of *Caulerpa taxifolia* at 100mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts methanol and water extracts showed moderate activity. Bacterial strains of chloroform extract of *E.coli*(20mm) and water extract of *Rhizoctonia solani*(20mm)showed moderate activity.



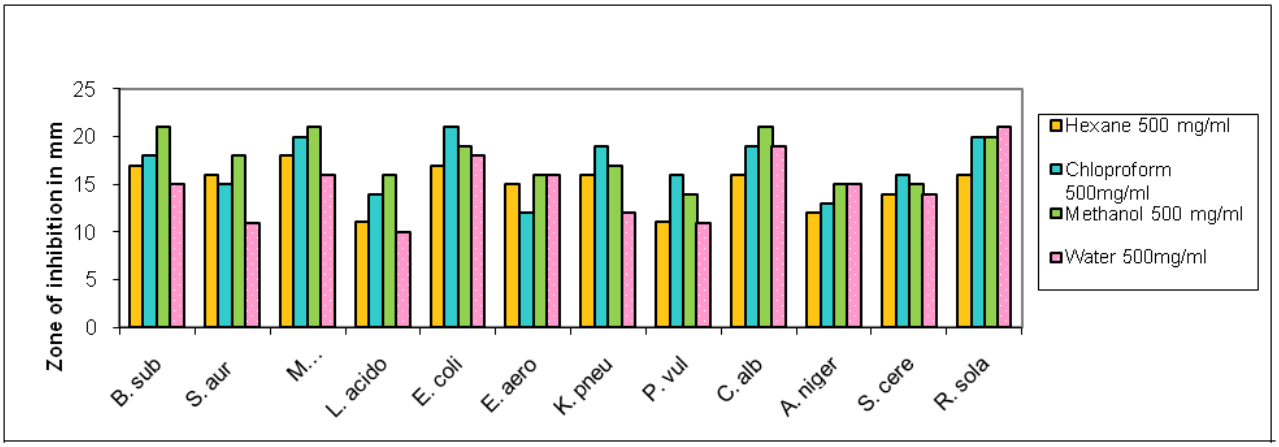
Graph1.1. Antimicrobial Activity of *Caulerpa taxifolia* 100mg/ml

Graph: 1.2. Represents that comparision between four solvents of *Caulerpa taxifolia* at 300mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts hexane extract showed moderate activity. Bacterial pathogens of chloroform extract of *E.coli*(21mm), methanol extract of *M.luteus*(21mm) and water extract of fungal strain *R.solani* (21mm)showed more activity.



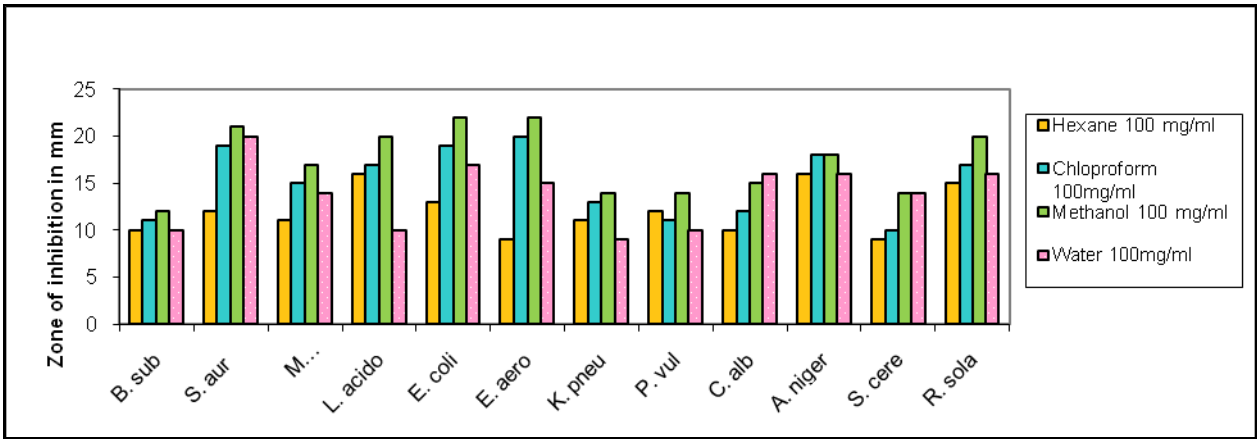
Graph1. 2.Antimicrobial Activity of *Caulerpa taxifolia* 300mg/ml

Graph: 1.3. Represents that comparision between four solvents of *Caulerpa taxifolia* at 500mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts bacterial strains of methanol extract of *B.subtilis*(21mm),*M.luteus*(21mm) and fungal strains *C.albicans*(21)mm showed high activity.



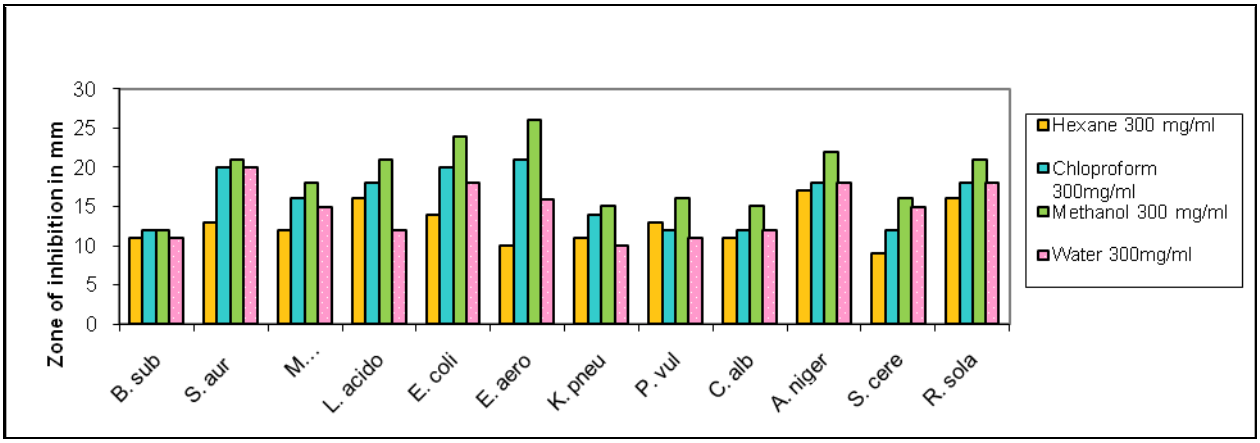
Graph 1. 3 Antimicrobial Activity of *Caulerpa taxifolia* 500mg/ml

Graph: 2.1. Represents that comparision between four solvents of *Caulerpa racemosa* at 100mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts hexane showed moderate activity. Bacterial strains of methanol extract of *E.coli* (22mm), *E.aerogenes* (24mm) showed high activity,



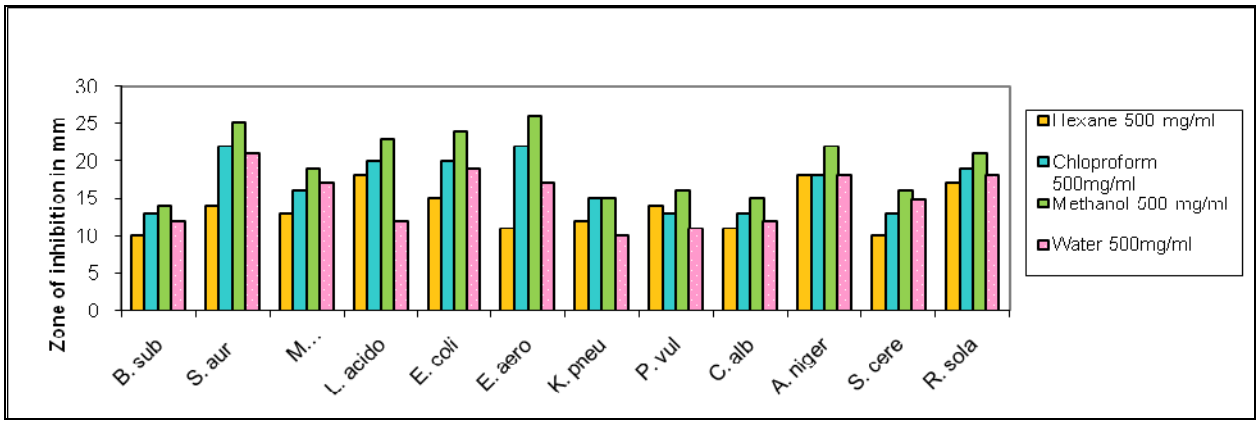
Graph :2.1. Antimicrobial Activity of *Caulerpa racemosa* 100mg/ml

Graph: 2.2. Represent that comparision between four solvents of *Caulerpa racemosa* at 300mg/ml conc of hexane, chloroform, methanol and water extracts. In these four extracts methanol extract showed high activity. Bacterial strains of methanol extract of *S.aureus*(22mm),*E.coli*(24mm),*E.aerogenes*(26mm) and fungal strains *A.niger*(22mm) showed high activity.



Graph: 2.2. Antimicrobial Activity of *Caulerpa racemosa* 300mg/ml

Graph: 2.3. Represent that comparision between four solvents of *Caulerpa racemosa* at 500mg/ml conc of hexane, chloroform, methanol and water extracts. In these four chloroform and water showed moderate activity. Bacterial strains of methanol extract of *S.aureus*(24mm), *E.coli*(24mm), *E.aerogenes*(26mm) showed high activity.



Graph: 2.3 Antimicrobial Activity of *Caulerpa racemosa* 500mg/ml

DISCUSSION

Genovese *et al.,* reported that the marine biodiversity and associated chemical diversity constitute an unlimited reserve of bioactive substances in the field of bioactive products. Seaweeds provide a rich source of structurally diverse secondary metabolite. Several studies (Rodriguez *et al*., 2010, Bhacuni and Rawat, 2005, Priyadharshini *et al*., 2011) have demonstrated that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities. Hediat *et al.*, reported that different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent. In this present study also supported that optimizes their antimicrobial activity by selecting the best solvent to extract the active compound from seaweeds. So this suggests that Seaweeds should be extracted in different solvent system in order to optimize their antibacterial activity by selecting the best solvent system. In our study chloroform and methanol extract maximum inhibition was produced by *Caulerpa taxifolia* and *Caulerpa racemosa*. In our results contrast with Manilal *et al*., and Rangaiah *et al*., showed that methanol extraction yielded higher antimicrobial activity than n-hexane and ethyl acetate which in supported to our results. Methanol extract of *Caulerpa taxifolia* showed more activity against *Bacillus subtillis* and *Micrococcus luteus* and fungal organism *Candida albicans*. Methanol extracts of *Caulerpa racemosa* showed high activity against test pathogens *Staphylococcus aureus*, *Escherichia coli* and *Enterobacter aerogenes*. All the four extracts hexane extract appears to have less antibacterial and antifungl activity than the chloroform, methanol and water extracts.

CONCLUSION

Finally it can be concluded from the study that extracts of algal species used in the present investigation showed better antibacterial activity against pathogens used. They are potential sources of bioactive compounds and should be investigated for natural antibiotics. But further research should be made to identify and purify these antibacterial substances.

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