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Antimicrobial activity of *Gelidium pusillum* and *Centroceros clavatum* from Visakhapatnam Coast, India

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Abstract

The present study assessesed the antimicrobial activity of *Gelidium pusillum* and *Centroceras clavulatum* against Gram positive bacteria-*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus, Staptococcus mutans, Streptococcus anginosus, Lactobacillus acidophilus,* Gram negative bacteria-*Escherichia coli, Enterobacter aerogenes, Klebsiella pneumonia, Pseudomonas aeuroginosa, Erwinia caratovora, Proteus vulgaris* and fungal strains- *Candida albicans, Aspergillus niger, Sacharomyces cerevisiae, Rhizoctonia solani, Mucor racemosus* and *Rhizopus stolonifer*. In this investigation, different crude seaweed extracts (Chloroform , Ethanol ,Methanol and water) were determined by the well diffusion method. Among the solvents tested, ethanolic extract of *Centroceras clavulatum* and chloroform extract of *Gelidium pusillum* showed maximum inhibitory activity than other solvents. Ethanolic extract of *Centroceras clavulatum* showed maximum zone of inhibition against *Bacillus subtilis.* The lowest minimum inhibitory concentration (MIC-8mg/ml) value of chloroform extract was observed against bacterial strains *Bacillus subtilis* and the lowest MIC (50mg/ml) value of ethanolic extract was observed against fungal strains *A.niger*. Chloroform extract of *Gelidium pusillum* showed maximum zone of inhibition against *K.pneumonia.* The lowest minimum inhibitory concentration (MIC-12mg/ml) value of chloroform extract was observed against *K.pneumonia*. The lowest MIC (35mg/ml) value of ethanolic extract was observed against fungal strains *R.stolonifer.* The present study confirms that the extractions of marine algae have been screened extensively to isolate life saving drugs for the benefit of the humanity.

Keywords: Seaweeds, well diffusion method, antimicrobial activity.

Introduction

Seaweeds are enormous resource for noval compounds to produce large number of secondary metabolites.Most the of secondary metabolites produced by seaweeds have bacteriostatic properties and to be evaluated for drug activity. Presently seaweeds have been screened extensively to isolate life saving drugs (or) biologically active substances against cancer, microbial infections and inflammations. (Elena *et al*.,2005).Several works have been carried out on the extracts from marine algae. The extractions of major compounds from the different species of seaweeds was depends upon the solvents. Extracts of marine algae were reported to exhibit antibacterial activity (Singh, A. and Chaudhary, B, 2010), antifungal (De Felicio *et al*, 2010), antiviral (Bouhlal *et al.*, 2010, Bouhlal *et al.*, 2011, Kim and Karadeniz, 2011), 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).The present study deals with antimicrobial activity of different extracts of two red algae *Gelidium pusillum* and *Centroceras clavulatum* collected from the Visakhapatnam coast.

Materials and Methods

Sample collection:

Gelidium pusillum (Stackhouse) Lejolis and *Centroceras clavulatum* (C.Agardh) Montagne (1846) were collected along with the substratum without disturbing the holdfast, in bulk quantity from the coastal areas of Visakhapatnam, Andhra Pradesh, India. Seaweeds species exposed on sand and rocks were collected during the low tides and transported to the laboratory in the polythene bags containing sea water. Each species was washed thoroughly under running water to remove epiphytes, animal castings, attached debris and sand particles and the final washing were done by distilled water and later 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 al*, 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*(MTCC227), *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 well 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 250c for 48hrs, after that the zone of inhibition was measured with zonal scale in mm and the experiment was carried out in duplicate.

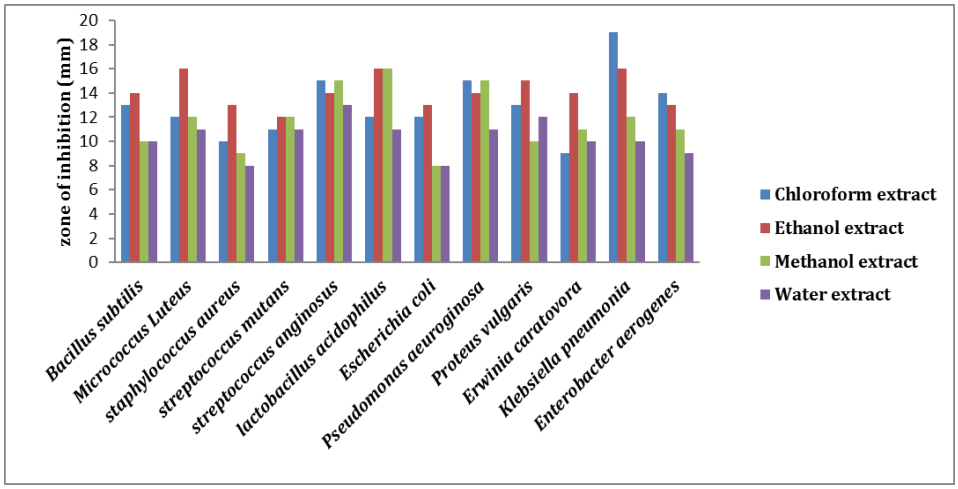
Results

Gelidium pusillum:

Among the four extracts, chloroform extract showed maximum activity when compared to other solvents. The extracts showed considerable activity on tested organisms in the present investigation. The solvent control of chloroform, ethanol, methanol, water, DMSO had no effect on microbial growth of microbes tested.

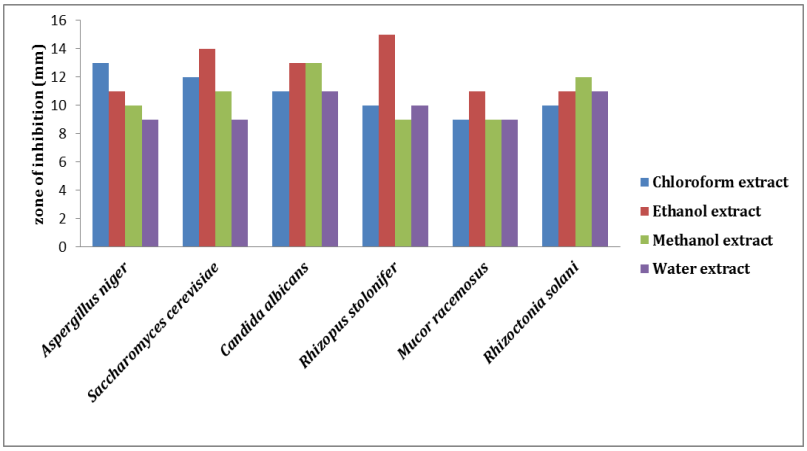
The ethanolic extract of *Gelidium pusillum* showed maximum zone of inhibition against gram negative bacterial strains i.e., *E.aerogenes* (19±0.6), *P.aeuroginosa* (18±0.5), *K.pneumonia* (16±0.2), *P.vulgaris* (16±0.3), *E.coli* (15±0.7), *E.caratovora* (13±0.5), and gram positive bacterial strains,such as *S.aureus* (19±0.4), *L.acidophilus* (18±0.3), *M.luteus* (17±0.7), *S.anginosus* (16±0.4), *B.subitilis* (16±0.8), *S.mutans* (14±0.5),with concentration of 500mg/ml (Fig-A.e). The ethanolic extract of *Gelidium pusillum* showed maximum zone of inhibition against fungal strains,i.e., *R.stolonifer* (17±0.4), *S.cerevisiae* (16±0.4), *C.albicans* (15±0.5), *A.niger* (13±0.5), *R.solani* (13±0.3), *M.racemosus* (11±0.4), with concentration of 500mg/ml (Fig-A.f).

Fig:A.a Antibacterial activity of *Gelidium pusillum* (100mg/ml)



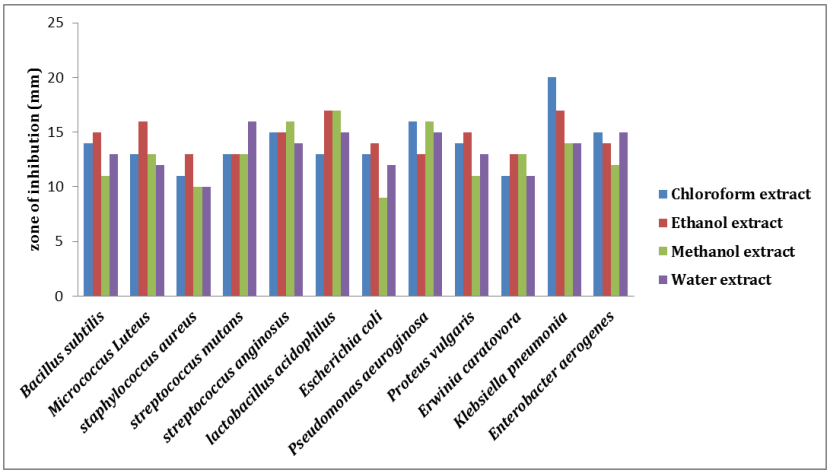
Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:A.b Antifungal activity of *Gelidium pusillum* (100mg/ml)



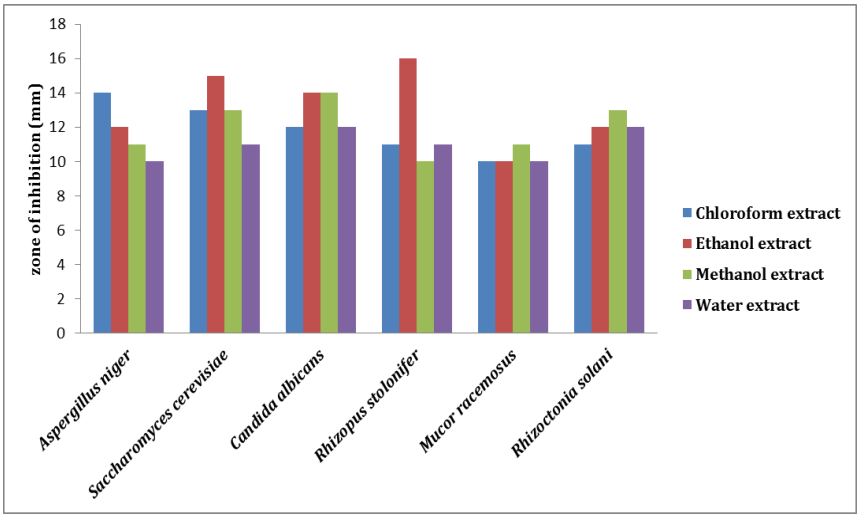
An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

Fig:A.c Antibacterial activity of *Gelidium pusillum* (300mg/ml)



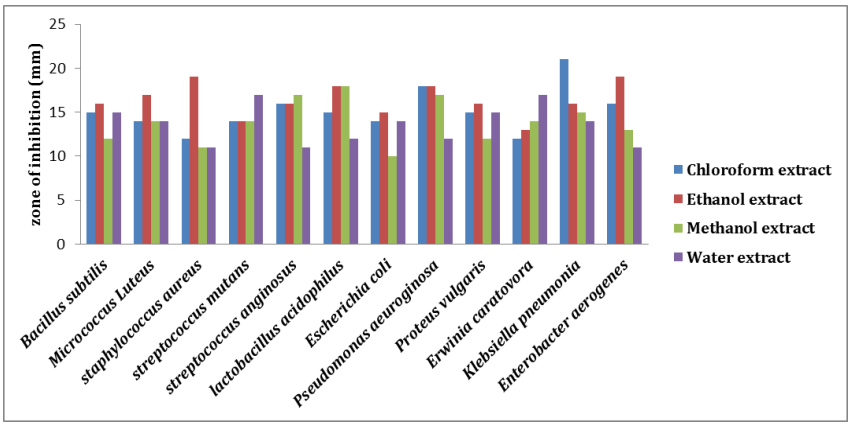
Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:A.d Antifungal activity of *Gelidium pusillum* (300mg/ml)



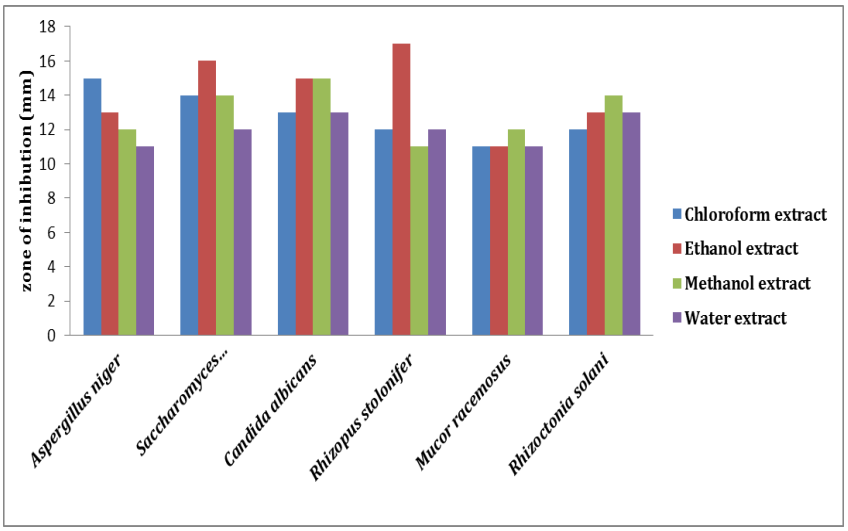
An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

Fig:A.e Antibacterial activity of *Gelidium pusillum* (500mg/ml)



Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:A.f Antifungal activity of *Gelidium pusillum* (500mg/ml)



An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

The methanolic extract of *Gelidium pusillum* showed maximum zone of inhibition against gram positive bacterial strains, i.e., *L.acidophilus* (18±0.3), *S.anginosus* (17±0.9), *S.mutans* (14±0.9), *M.luteus* (14±0.5), *B.subitilis* (12±0.2), *S.aureus*(11±0.4), and gram negative bacterial strains,i.e., *P.aeuroginosa* (17±0.2), *K.pneumonia* (15±0.3), *E.caratovora* (14±0.3), *E.aerogenes* (13±0.3), *P.vulgaris* (12±0.2), *E.coli* (10±0.5), with concentration of 500mg/ml (Fig-A.e). The methanolic extract of *Gelidium pusillum* showed maximum zone of inhibition against fungal strains *C.albicans* (15±0.6), *R.solani* (14±0.3),*S.cerevisiae* (14±0.9), *M.racemosus* (12±0.4), *R.stolonifer* (11±0.4), *A.niger* (12±0.6),with concentration of 500mg/ml (Fig-A.f).

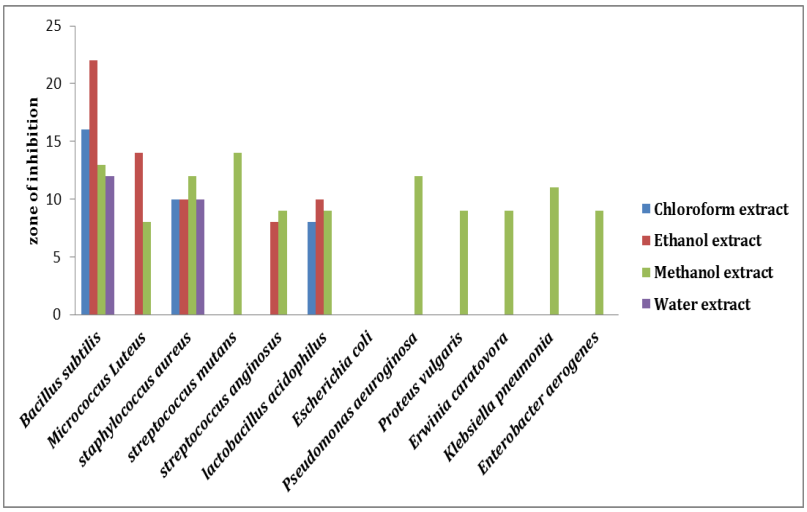
The chloroform extract of *Gelidium pusillum* showed maximum zone of inhibition against gram positive bacterial strains such as *S.anginosus* (16±0.9), *B.subitilis* (15±0.2), *L.acidophilus* (15±0.9), *M.luteus* (14±0.8), *S.mutans* (14±0.4), *S.aureus* (12±0.4), and gram negative bacterial strains such as *K.pneumonia* (21±0.3), *P.aeuroginosa* (18±0.2), *E.aerogenes* (16±0.3), *P.vulgaris* (15±0.2), *E.coli* (14±0.5), *E .caratovora* (12±0.3), with concentration of 500mg/ml (Fig-A.e). The chloroform extract of *Gelidium pusillum* showed maximum zone of inhibition against fungal strains, *R.stolonifer* (15±0.4), *A.niger* (15±0.6), *S.cerevisiae* (14±0.4), *C.albicans* (13±0.6), *R.solani* (11±0.3), *M.racemosus* (11±0.4), with concentration of 500mg/ml (Fig-A.f).

Water extracts of *Gelidium pusillum* showed maximum zone of inhibition against gram positive bacterial strains i.e., *S.mutans* (17±0.4), *B.subitilis* (15±0.2), *M.luteus* (14±0.4), *S.aureus* (11±0.2), *L.acidophilus* (12±0.3), *S.anginosus* (11±0.4), and gram negative bacterial strains, i.e., *E.caratovora* (17±0.4), *P.vulgaris* (15±0.1), *K.pneumonia* (14±0.4), *E.coli (*14±0.3), *P.aeuroginosa* (12±0.2), *E.aerogenes* (11±0.6) with concentration of 500mg/ml (Fig-A.e). water extracts of *Gelidium pusillum* showed maximum zone of inhibition against fungal strains, *R.solani* (13±0.3), *C.albicans* (13±0.3), *S.cerevisiae* (12±0.4), *R.stolonifer* (12±0.4), *M.racemosus* (11±0.4), *A.niger* (11±0.2), with concentration of 500mg/ml (Fig-A.f).
Minimum inhibitory concentration of (MIC) values of *Gelidium pusillum* against bacteria was ranged between 12 to 85 mg/ml.The lowest MIC (12 mg/ml) value of chloroform extract recorded against *K.pneumonia*. Minimum inhibitory concentration of (MIC) values of *Gelidium pusillum* against fungus was ranged between (35to 85 mg/ml. The lowest MIC (35mg/ml) value of ethanol extract against *R.stolonifer*.
Bouhlal Rhimon *et al*., (2010) reported that methanolic extract of *Gelidium pusillum* showed maximum activity against *S.aerues, E.coli, E.faecalis* and *K.pneumonia*. In present study methanolic extracts showed maximum activity against *L.acidophilus.* Chloroform extract showed highest inhibition against *K.pneumonia* and ethanolic extract showed maximum activity against *S.aerues, E.aerogenes* where as methanolic extract showed moderate activity against *M.luteus, S.aerues.*

Centroceras clavulatum:

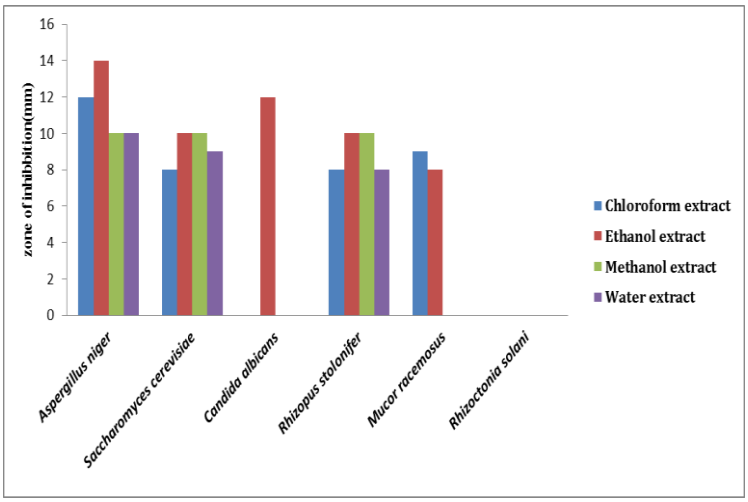
Among the four extracts, ethanol extract showed maximum activity when compared to other solvents. The extracts showed considerable activity on tested organisms in the present investigation The solvent control of chloroform, ethanol, methanol, water, DMSO had no effect on microbial growth. The ethanol extract of *Centroceras clavulatum* showed highest zone of inhibition against gram positive bacterial strains i.e., *B.subitilis* (30±0.2), *L.acidophilus* (13±0.9), *S.aureus* (12±0.2), *M.luteus* (11±0.8), *S.anginosus* (10±0.4), and gram negative bacterial strains i.e., *P.aeuroginosa* (21±0.2), *K.pneumonia* (15±0.3), *E .caratovora* (14±0.3), *P.vulgaris* (11±0.2), with concentration of 500mg/ml (Fig-B.e). The ethanol extract of *Centroceras clavulatum* showed maximum zone of inhibition against fungal strains such as *A.niger* (20±0.4), *R.stolonifer* (14±0.4), *C.albicans* (13±0.3), *S.cerevisiae* (12±0.4), *M.racemosus* (12±0.4), with concentration of 500mg/ml (Fig-B.f).

Fig:B.a Antibacterial activity of *Centroceras clavulatum* (100mg/ml)



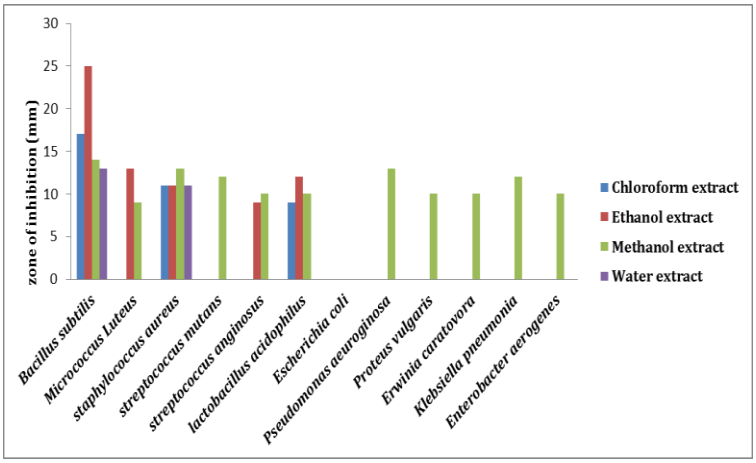
Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:B.b Antifungal activity of *Centroceras clavulatum* (100mg/ml)



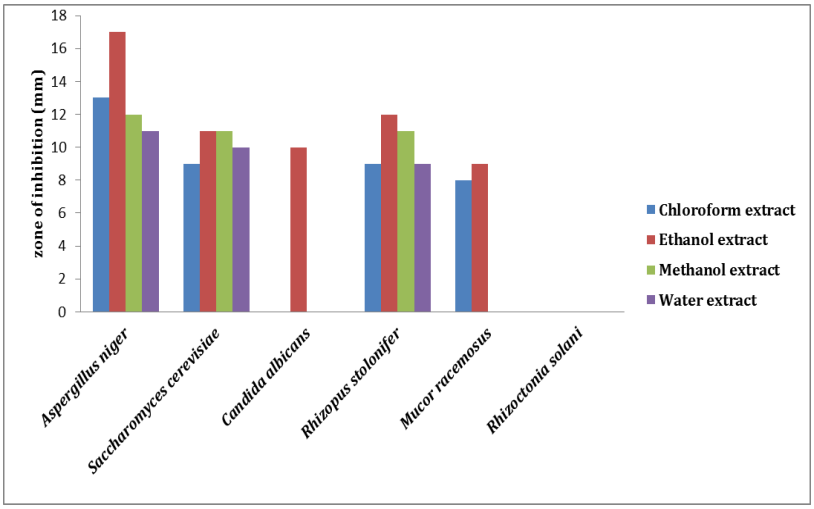
An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

Fig:B.c Antibacterial activity of *Centroceras clavulatum* (300mg/ml)



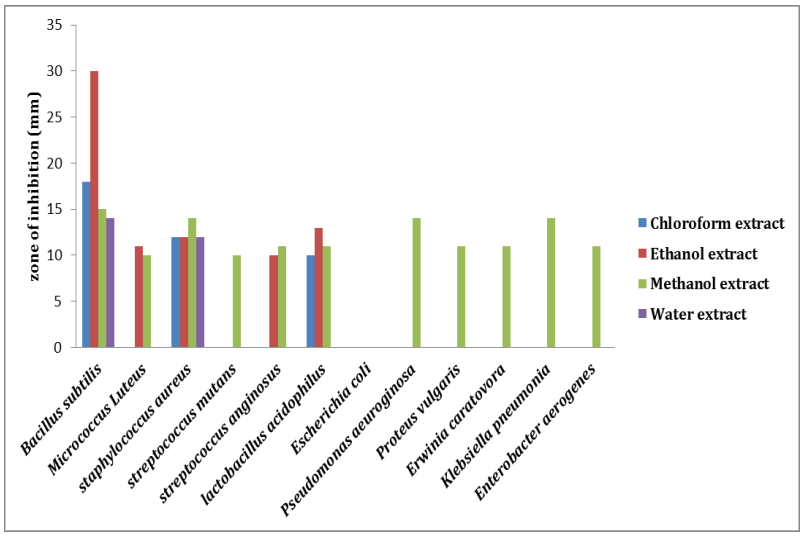
Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:B.d Antifungal activity of *Centroceras clavulatum* (300mg/ml)



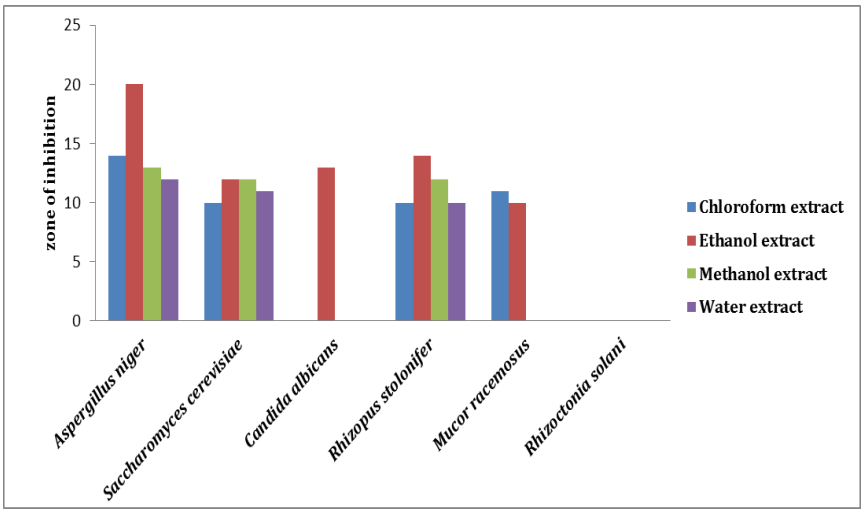
An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

Fig:B.e Antibacterial activity of *Centroceras clavulatum* (500mg/ml)



Bs=Bacillus subtilis, Ml=Micrococcus luteus, Sa=Staphylococcus aureus, Sm=Streptococcus mutans, San=Streptococcus anginosus, La=Lactobacillus acidophilus, Ec=Escherichia coli, Pa=Pseudomonas aeuroginosa, Pv=Proteus vulgaris, Ec=Erwinia caratovora, Kp=Klebsiella pneumoniae, Ea=Enterobacter aerogenes

Fig:B.f Antifungal activity of *Centroceras clavulatum* (500mg/ml)



An=Aspergillus niger, Sc=Saccharomyces cerevisiae, Ca=Candida albicans, Rst=Rhizopus stolonifer, Mr=Mucor racemosus, Rs=Rhizoctonia solani

The chloroform extract of *Centroceras clavulatum* showed highest zone of inhibition against gram positive bacterial strains i.e., *B.subitilis* (18±0.2), *L.acidophilus* (10±0.9), *S.aureus* (12±0.2), and gram negative bacterial strains, *K.pneumonia* (15±0.4), *P.aeuroginosa* (14±0.2), *P.vulgaris* (12±0.2), *E.aerogenes* (10±0.3), with concentration of 500mg/ml (Fig-B.e). The ethanol extract of *Centroceras clavulatum* showed maximum zone of inhibition against fungal strains, *A.niger* (14±0.4), *R.stolonifer* (10±0.4), *S.cerevisiae* (10±0.4), *M.racemosus* (11±0.4), with concentration of 500mg/ml (Fig-B.f).

The methanol extract of *Centroceras clavulatum* showed highest zone of inhibition against gram positive bacterial strains i.e., *B.subitilis* (15±0.2), *S.aureus* (14±0.2), *S.anginosus* (11±0.4), *L.acidophilus* (11±0.9), *M.luteus* (10±0.8), *S.mutans* (10±0.4), and gram negative bacterial strains such as *P.aeuroginosa* (14±0.2), *K.pneumonia* (14±0.3), *E .caratovora* (11±0.3), *P.vulgaris* (11±0.2), *E.aerogenes* (11±0.3), with concentration of 500mg/ml (Fig-B.e).The ethanol extract of *Centroceras clavulatum* showed maximum zone of inhibition against fungal strains *A.niger* (13±0.4), *R.stolonifer* (12±0.4), *S.cerevisiae* (12±0.4), (Fig-B.f).

The water extract of *Centroceras clavulatum* showed highest zone of inhibition against gram positive bacterial strains i.e., *B.subitilis* (14±0.2), *S.aureus* (12±0.2), and gram negative bacterial strains such as *P.aeuroginosa* (13±0.2), *K.pneumonia*

(14±0.3), *P.vulgaris* (10±0.2), *E.aerogenes* (10±0.3), with concentration of 500mg/ml (Fig-B.e).The ethanol extract of *Centroceras clavulatum* showed maximum zone of inhibition against fungal strains such as *A.niger* (12±0.4), *R.stolonifer* (10±0.4), *S.cerevisiae* (11±0.4), with concentration of 500mg/ml (Fig-B.f). No zone of inhibition noticed against *R. Solani* and *E.coli* with concentration of (100mg/ml, 300mg/ml and 500mg/ml).

Minimum inhibitory concentration of (MIC) values of *Centroceras clavulatum* against bacteria was ranged between 8 to 85 mg/ml. The lowest MIC (8 mg/ml) value of ethanol extract recorded against *B.subitilis*. Minimum inhibitory concentration of (MIC) values of *Centroceras clavulatum* against fungus was ranged between 45 to 85 mg/ml. The lowest MIC (50 mg/ml) value of ethanol extract against *A.niger*.

Luis Villarreal - Gomez *et al., (*2010) evaluated the antibacterial and anticancer activities of extracts from the seaweeds *like Egregia meniesii, Codium fragile, Sargassum muticum, Endarachne binghamiae, Centroceras clavulatum and Laurencia pacifica.* They obtained the organic extracts from bacteria, free algae and from surface–associated bacteria. Pathogenic strains *S.aureus, K.pneumonia, P.mirabilis* and *P.aeruginosa* were used to test antibacterial activity. The strains *Centroceras clavulatum, Sargassum muticum and Endarachne binghamiae* showed anticancer with IC50 Valuesof 6.492,5.531,and 2.843µgml-1 repectively. The extracts from the seaweed-associated bacteria inhibited the growth of the gram negative bacterium *Proteus mirabilis*. Similary present finding showed that *Centroceras clavulatum* inhibited the growth of the gram negative bacterium *P.aeruginosa, P.vulgaris, K.pneumonia.* Bouhlal Rhimou *et al., (*2010) reported that methanolic extract of *Centroceras clavulatum* exhibited activity against *S.aureus*. Similary in the present study methanolic extract showed maximum activity against *S.aureus.*

Conclusion

The present study concluded that the organic solvent extraction was suitable to verify the antimicrobial properties of *Gelidium pusillum* and *Centroceras clavulatum*.However, more research has to be done on isolation ,purification and identification of the active ingredients in order to probe this hypothesis.

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