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Challenges in Biochemical Engineering and Biotechnology for Sustainable Environment

Biopotential Activity of Marine Microalgae Extracts

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Abstract : Microalgae are the simple photosynthetic microorganisms existing in the fresh water, salt water and in land. Microalgae have been an attractive source of potential compounds due to presence of phytochemicals such as terpenoids, alkaloids, amino acids, flavonoids etc. In this study the marine microalgae have been isolated from the marine water by serial dilution. The ethanolic and acetone extract of the marine microalgae were prepared and checked for their biological activity. The antibacterial activity of the extract was studied against test organisms like *Escherichia coli, Salmonella typhi, Klebseilla pneumonia, Bacillus subtilis* etc. The antifungal activity was studied against the fungal species *Metarhizium anisopliae, Penicillium sp.* The isolated microalgae elicited antibacterial activity against all the tested bacteria showing maximum activity against the *Bacillus subtilis*. Similarly antifungal activity was observed against all test organisms with maximum activity against both *Metarhizium anisopliae, Penicillium sp.*
Keywords: microalgae, antibacterial, antifungal activity, potential compounds

Introduction

Algae are the simple, diverse group of photosynthetic organism which exist in vast ecosystem such freshwater, marine water and in soil. Algae are the major producer of the water ecosystem. Based on the size algae can be a microalgae and macroalgae[1,2]. Both micro and macroalgae have been exploited due the presence of significant molecules. Algae are the major source of amino acids, terpenoids, phlorotannins, steroids, phenolic compunds which found to have biological activity such as antibiotics, fungicidal, antivirals, antitumorals etc.[3,4,5]. In this study, the marine microalga was isolated from the marine water. The preliminary phytochemical analysis was found and the potential activity such as antibacterial, antifungal activity was tested by disc diffusion method.

Materials and Methods

Isolation of microalgae by serial dilution

For the isolation of microalgae, marine water from the Kerala coastal region, Tamil Nadu was collected during the month of August 2013 and used. The water was filtered using the nitrocellulose membrane with 0.45µm pore size. Deposit on the surface was used as the inoculum for the serial dilution. And test tubes were kept in the 10hrs light incubation for 7days. The cells growth was monitored continuously.

Preparation of extracts

For the preparation of the extracts the cells pellet was collected. The ethanolic and acetone extract was prepared by adding the solvent to the cell pellet and kept in agitation for 2days. The supernatant was collected and used for the study.

Preliminary phytochemical analysis of the extracts

The preliminary phytochemical analysis was done for the extracts of the isolated sample. The presence of the phytochemical constituents was studied by following procedure[6,7]

Bradford's Test

To 1ml of the extract few drops of Bradford's reagent (Coomassie Brilliant Blue G 250) and observed for formation of blue color[8].

Fehling's Test

1ml of Fehling's reagent was added to the filtrate of extract in distilled water and heat in steam bath and observed for brick red precipitation.

Molisch's Test

5% α-naphthol in ethyl alcohol was prepared and few drops of it was added to the 2ml of the extract, then 1ml of concentrated sulphuric acid along the sides of tube was added and observed for the appearance of reddish violet ring at the junction of two layers.

Ferric chloride Test

To 2ml of the extract, 2ml of the ferric chloride was added and observed for deep bluish green solution.

Salkowski Test

Ethanolic extract was dissolved in chloroform and shake with an equal volume of concentrated sulphuric acid and observed for rose red color which quickly changes through blue to green.

Sodium bicarbonate Test

To few ml of ethanolic extract few drops of sodium bicarbonate was added and observed for the formation for the honey comb.

Mayer's Test

To 1gm of the sample 10ml of 5% HCl was added and heated in boiling water bath for 10 minutes and filtered. To the filtrate 5ml of dilute ammonia and 5ml of chloroform, an aqueous layer formed and to it few drops of Mayer's reagent was added and observed for formation of creamy layer.

Braemer's Test

To 0.5gm of ethanolic extract 10ml of water was added. It was boiled and filtered. To the filtrate few drops of 10% ferric chloride was added and observed for dark green, blue or brown color.

Antibacterial activity

The antibacterial activity of the isolated microaglae was assessed by disc diffusion method. Activity was tested against the following bacteria *Salmonella typhi, Klebsiella pneumonia, Escherichia coli, Serratia marcescens,* and *Bacillus subtilis.* Gentamycin was used as positive control[9].

Antifungal activity

The antifugal activity of the extracts against *Penicillium sp.* and *Metarhizium anisopliae* was evaluated by disc diffusion method.

Results and Discussion

Isolation of microalgae

The growth was observed in the serially diluted test tube after 7 days in 5 test tubes. The cells were observed under the microscope. The cells with filamentous, spherical morphology was observed and 3 diatoms were observed in separate test tubes. The cells with the spherical and motile characteristic was taken and labelled as Si for the further study.

The preliminary phytochemical analysis revealed the presence of amino acid, carbohydrates and cholesterol in the extracts (Table 1).

Table 1- preliminary phytochemical analysis

Name of the phytochemical test	Isolated microalgae Si	
	Acetone Extract	Ethanol Extract
Bradford's Test	+	+
Fehling's Test	_	_
Molisch's Test	+	+
Ferric chloride Test	_	_
Salkowski Test	+	+
Sodium bicarbonate Test	_	_
Mayer's Test	_	_
Hager's Test	_	_
Braemer's Test	_	_

Antibacterial activity

The antibacterial activity of the ethanolic extract of the isolated microalgae was found to be activity against all tested microorganism (Fig 1) and the maximum activity was observed to be against the *Bacillus subtilis* with the zone of inhibition of 2.3cm. the acetone extract has shown activity against the tested microorganism and the maximum activity was observed to be against the *Bacillus subtilis* and *Klebsiella pneumonia* with the zone of inhibition of 2.9cm (Fig 2).

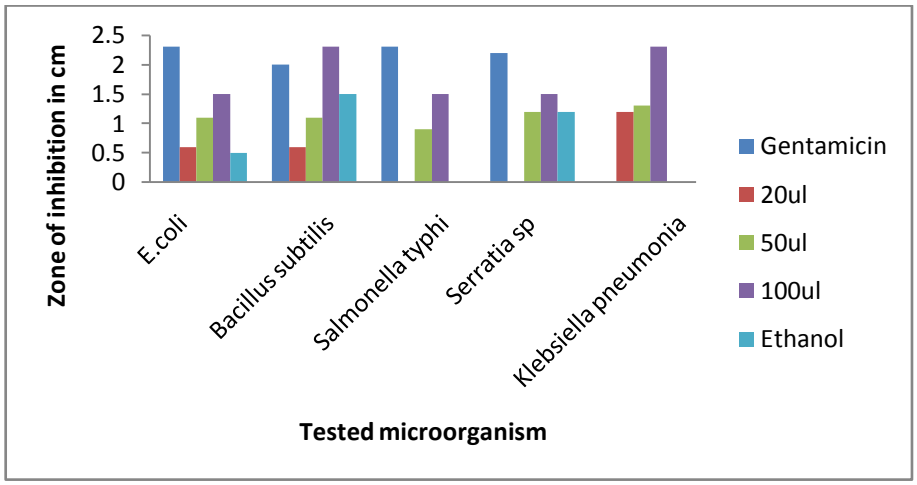

Fig 1 Antibacterial activity of ethanolic extract of the isolated microalgae

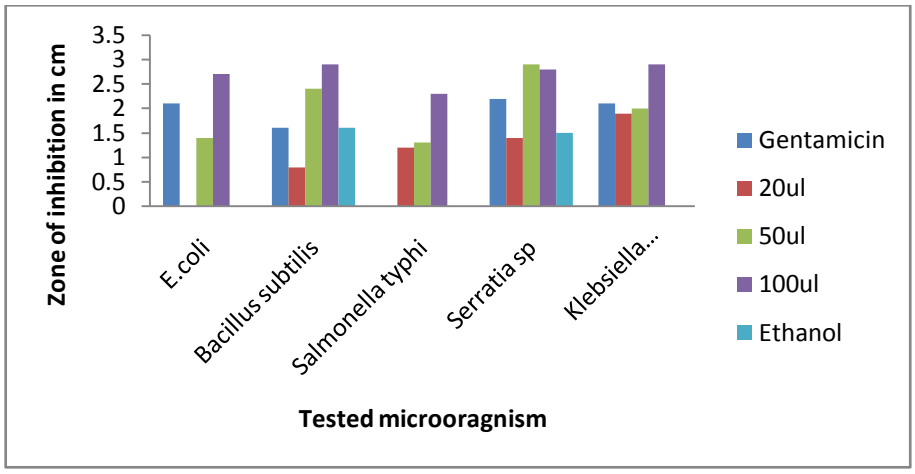

Fig2 Antibacterial activity of Acetone extract of the isolated microalgae

The extracts has shown antifungal activity tested two fungal species. The maximum activity of the ethanolic extract was found to be against *metarhizium anisopliae* with the maximum zone of inhibition was 3.5cm (Fig 3). For the acetone extract the maximum aciivty was observed to be against the both *Penicillium sp* and *Metarhizium anisopliae* with zone of inhibition of 3cm (fig 4).

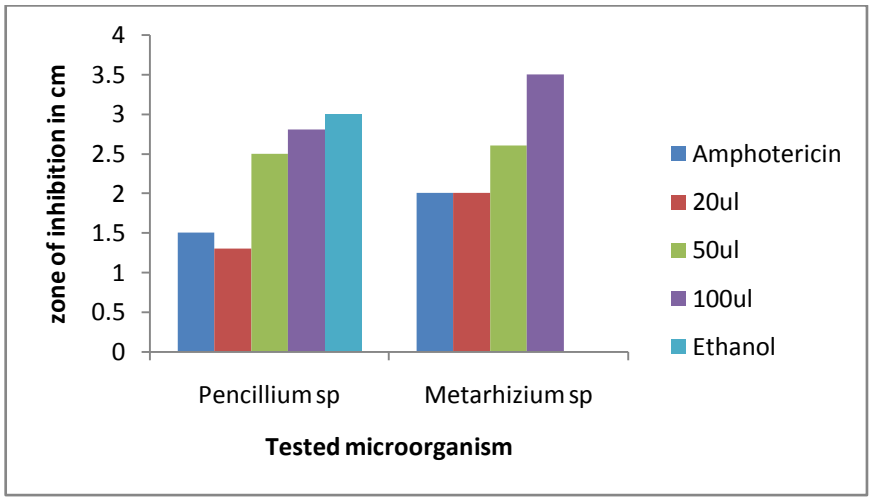

Fig3 Antifungal activity of Ethanolic extract of the isolated microalgae

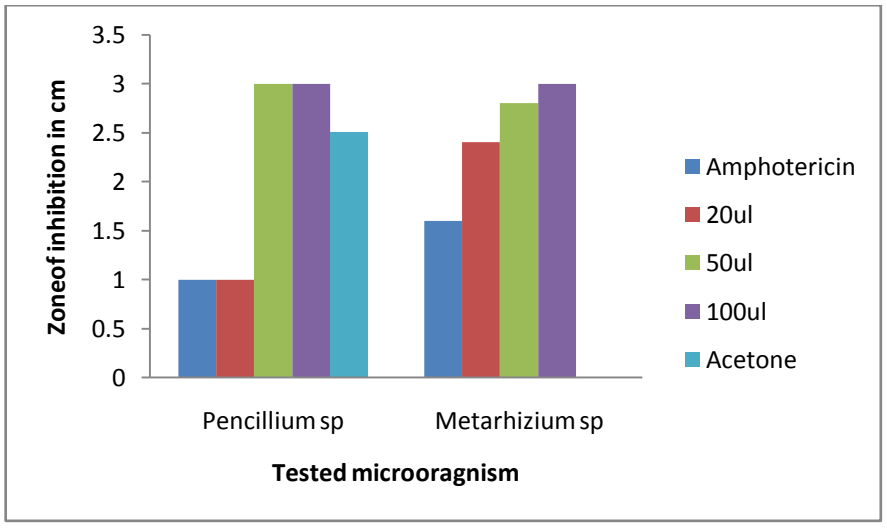

Fig4 Antifungal activity of Acetone extract of the isolated microalgae

Discussion

In this study the antibacterial activity and phytochemical analysis of the extracts of the iosalted microalgae was studied. The phytochemical analysis showed the presence of the carbohydrate, aminoacid and cholesterol. The exopolysaccharides of unicellular red algae *Porphyridium cruemtum* and *P. aerugineum*, and other *Porphyridium spp*. have been studied[10]. Microalgae protein and lipoprotein found to have antibacterial, antifungal, antiviral acivity[11]. The ethanolic extract of isolated microalgae has shown activity against maximum zone of inhibition of 1.5cm. Zone of inhibition of 2.3cm was observed against the *Bacillus subtilis* and *Klebsiella pneumonia* whereas the positive control, gentamicin has shown 2cm. against the serratia sp zone of inhibition was observed to be 1.5cm (Fig 1).

The acetone extract has shown maximum zone of inhibition than the positive control, gentamicin. The maximum zone of inhibition against the *Bacillus subtilis and Klebseilla pneumonia* was found to be 2.9cm. The second maximum zone of inhibition of 2.8cm was found to be against *Serratia sp.* Against *E.coli*, *Salmonella typhi* it was found to be 2.7cm and 2.3cm respectively (Fig 2).

The antifungal activity of the ethanolic extract was found to be 3.5cm and 2.8cm against the *Penicllium sp.* and *Metarhizium anisopliae.* Whereas the acetone extract has shown 3cm against both the tested fungal species. When compared to the positive control amphotericin the maximum zone of inhibition of observed against the fungal sp (Fig 3 and Fig 4). further study is required to identify and isolate the potential antimicrobial active compound from the microalgae isolate.

Conclusion

In this study, marine microalgae have been isolated and the preliminary phytochemical analysis was done. Antibacterial activity was tested against the bacteria by disc diffusion method. The preliminary phytochemical analysis revealed the presence of carbohydrates and amino acids. The acetone extract has shown more activity than the ethanolic extract of the microalgae isolate. The maximum activity was found to be against *Bacillus subtilis and Klebsiella pneumonia.* Against the fungi it was found to be of 3cm against *Penicillium sp* and *Metarhizium anisopliae* . Further study is required to identify and extract the antibacterial potential compound from the microalgae isolate.

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