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EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE EXTRACTS OF SELECTED MARINE MICROALGAE AGAINST HUMAN PATHOGENS

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ABSTRACT

The present study was designed to screen the biological activities of marine micro algal samples collected along the South India coastal belt. For that the marine water samples were collected from different coastal locations such as Colachel, Kadiapattinam and Muttom of Kanyakumari District, South India. The collected sea water sample were filtered, serially diluted, supplemented with Walne's medium and subjected to light incubation under laboratory conditions for two weeks for optimum growth and the algal species were identified and isolated based on their morphological characteristics with the help of light microscope. Totally 10 isolates, 5 from the Muttom seawater collection, 3 from Kadiapattinam and 2 from Colachel collections were identified. 5 isolates from the Muttom seawater collection was evaluated further. In this evaluation, the growth pattern of isolated algal species was studied and their mass production of was done. The cultured algal species were subjected to centrifugal separation and solvent extraction by using three solvents such as ethyl acetate, methanol and water. The supernatants collected were evaluated for the antimicrobial activity against six human pathogenic bacteria viz., *Bacillus subtilis, E. coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus* and *Shigella boydii* and three fungal pathogens viz., *Aspergillus niger, Candida albicans and Mucur* sp. In the antimicrobial evaluation, the methanol extract of S–2, S–3, S–4 and S–5 samples showed a significant antimicrobial activity against the tested bacterial and fungal culture. Our further studies directed toward the identification algal species, phytochemical screening and various detailed pharmacological screening may give more valuable results.

KEYWORDS: Marine microalgae, isolation, cultivation, extraction, antimicrobial activity.

INTRODUCTION

Usage of natural products for medicinal purpose is time immemorial, among these the terrestrial plants are the major source especially in traditional medicinal system. About twenty five percentage of present day drugs are derived from higher plants and about twelve percentage are microbially produced natural products and over fifty percentage of them are derived from natural sources either directly or indirectly or produced synthetically using natural products as templates.[1,2,3] In the last fifty years, the terrestrial bacteria and fungi are well screened and as a result, number of bio-active molecules are isolated and now in usage as important medicines. However, the rising requirement of novel molecules to control the resistant and newly emerging infections, nowadays, the researchers' attention focused on unconventional novel sources.[2]

In the biosphere, ocean is the unique environment and not only the habitat for numerous organisms, but also, a massive source for food, minerals, energy and also the pharmaceuticals.[2] The marine environment possess an extensive bio-diversity which is incomparable to the terrestrial environment because of its extreme differences in thermal, pressure and nutrient range and the presence of different photic and non-photic zones. Despite this fact, the search for novel pharmaceuticals form marine resources is still in its infancy in several countries in the world, particularly the developing countries.[1,4] New diving techniques and remote controlled machines developed in the last decades make the collection of marine samples from shallow waters to even below 900m as possible.[2] Recently, a revival can be observed in marine pharmacology. The search for new biomedical molecules resulted in the isolation of approximately 10000 metabolites, many of which revealed therapeutic properties including antibiotic, antifungal, antiviral, cytotoxic, neurotoxic, etc.[5]

Microalgae, the photosynthetic eukaryotes that forms a major part of freshwater and marine phytoplankton,[2] has gains more attention in life sciences because of their variety of phytomolecules with different types of chemical structures and biological activities.[7] With this view, the marine microalgae was selected for our research and the present study was focused on its

isolation and *in vitro* antimicrobial evaluation. The outcome of this study would provide a good platform for further researches.

MATERIALS AND METHODS

Isolation of microalgae

Marine water samples were collected from the different coastal locations such as Colachel, Kadiapattinam and Muttom of Kanyakumari District, South India. The samples collected in the special collection bottles were aseptically brought in to the laboratory for the isolation of microalgal species. The isolation and identification of algae was done in reference with the previous literature.[8]

Maintenance of stock culture

The cultivation and extraction of isolated algae was done in accordance with the previous literature[9] with slight modification. About 10ml of isolated inoculam in the growing phase was inoculated in the 100ml of sterilized sea water containing appropriate quantity of Walne's medium (55µl of solution–A, 50µl of solution–B, and 25µl of solution–C of Walne's medium/100ml of sterilized sea water). The inoculated conical flasks were kept in front of two tube lights (1000lux) and about 31°C temperature was maintained. The growth of the culture was checked daily by measuring the optical density and cell count and appropriate dilutions were given when required. After two weeks, when the maximum exponential phase was reached the light was reduced and allowed for further growth.

Inoculam development

50ml of stock culture of isolated microalgae was inoculated in the 450ml of sterilized sea water containing appropriate quantity of Walne's medium. The inoculated conical flasks were kept in front of two tube lights (1000lux) and about 31°C temperature was maintained. The growth of the culture was checked daily by measuring the optical density and cell count and appropriate dilutions were given when required. After two weeks, when the maximum exponential phase was reached, the mass production of isolated algae was started.

Mass production

300ml of inoculam was added to 2L of sterilized sea water containing appropriate quantity of Walne's medium. The inoculated Hoffkins flasks were kept in front of two tube lights (1000lux) and about 31°C temperature was maintained. Aeration was provided to prevent the sedimentation. The growth of the culture was checked daily by measuring the optical density and cell count and the exponential phase of the algal cultures were determined.

Centrifugal separation

Algal cells in the exponential growth phase were recovered from culture by batch centrifugation at 300rpm for 10min. The isolated cells were repeatedly washed in normal saline and centrifuged at low speed. The pellets of each isolated algal species were dried at room temperature and after completed drying, the cells were collected, weighed and stored under cooling.

Solvent extraction

About 0.4gm of each algal cell were mixed with 20ml of different solvents (Ethyl acetate, Methanol and Water), shaken well and each mixture was subjected to sonication for 15min. After that they were subjected to centrifugation at 3000rpm for 10min. and the supernatants were collected and stored.

In vitro antibacterial activity

The antibacterial activity of prepared algal extracts were tested against six pathogenic bacteria viz., *Bacillus subtilis, E. coli, Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus* and *Shigella boydii*. The cultures were procured from MTCC, Chandigarh, India. The inoculam were prepared from 24h old culture in N. broth. The Muller Hinton agar plates were prepared. A swab of test culture was inoculated in the agar surface so as to make a lawn. Sterile paper discs (Whattmann No.1 filter paper) were made, impregnated in the test extracts for 10min and placed in each plate. The inoculated plates were incubated at 37°C for 24hrs. Zones of inhibition were measured in mm and recorded.

In vitro antifungal activity

The antifungal activity of prepared algal extracts were tested against three fungal pathogens viz., *Aspergillus niger, Candida albicans and Mucur* sp., The test cultures were procured from MTCC, Chandigarh, India. The Sabouraud Dextrose Agar plates were prepared. A swab of test culture was inoculated in the agar surface so as to make a lawn. Sterile paper discs (Whattmann No.1 filter paper) were made, impregnated in the test extracts for 10min and placed in each plate. The inoculated plates were incubated in room temperature for 48h. Zones of inhibition were measured in mm and recorded.

RESULTS AND DISCUSSION

In the present study, seawater samples were collected from different coastal regions of Knayakumari district such as Colachel, Muttom and Kadiapattinam. The collected sea water sample were filtered, serially diluted, supplemented with Walne's medium and subjected to light incubation under laboratory conditions for two weeks. For the isolation of microalgal species, the samples were morphologically characterized under light microscope using bright field and differential interference contrast. The samples identified with the properties of microalgae including filamentous, spherical shape cells were preserved for further studies. In this manner, totally 10 isolates, 5 from the Muttom seawater collection, 3 from Kadiapattinam and 2 from Colachel collections were identified. In the present study, the isolates named as S–1 to S–5 from Muttom collection was selected for further evaluation.

Culturing of isolated algal species of S–1 to 5 was done in laboratory conditions. It had taken about 2 weeks for reaching the exponential growth phase. The growth of the culture was checked daily by means of optical density and cell count by using Neubauer chamber. All the five species showed a minimum growth initially and a constant rise in subsequent observations. On the 16th day observation, the maximum exponential phase of growth was reached (Table 1 & 2).

Table 1: Growth rate of isolated marine micro algal species by cell count.

Days	No. of cells/20µl				
	S–1	S–2	S–3	S–4	S–5
1st day	123	85	113	105	97
2nd day	146	94	128	116	115
3rd day	167	99	153	137	127
4th day	178	110	175	152	156
5th day	205	125	183	169	175
6th day	213	151	204	183	184
7th day	236	173	220	197	203
8th day	241	190	235	208	219
9th day	270	204	254	219	233
10th day	302	212	272	228	253
11th day	315	225	291	253	265
12th day	336	253	305	267	279
13th day	357	267	320	280	305
14th day	363	305	337	293	319
15th day	375	315	345	316	327
16th day	379	325	353	325	332

Table 2: Growth rate of isolated marine micro algal species by means of optical density.

Days	Optical density				
	S–1	S–2	S–3	S–4	S–5
1st day	0.04	0.03	0.02	0.02	0.04
2nd day	0.07	0.06	0.04	0.03	0.07
3rd day	0.10	0.07	0.04	0.05	0.07
4th day	0.13	0.07	0.07	0.07	0.09
5th day	0.15	0.09	0.09	0.09	0.13
6th day	0.22	0.12	0.13	0.15	0.18
7th day	0.25	0.14	0.16	0.23	0.25
8th day	0.31	0.17	0.21	0.31	0.29
9th day	0.35	0.21	0.25	0.34	0.33
10th day	0.40	0.25	0.29	0.47	0.37
11th day	0.42	0.31	0.33	0.53	0.44
12th day	0.45	0.35	0.37	0.57	0.47
13th day	0.51	0.39	0.42	0.59	0.51
14th day	0.55	0.42	0.47	0.61	0.57
15th day	0.58	0.45	0.47	0.66	0.60
16th day	0.59	0.48	0.49	0.69	0.65

The algal cells isolated by centrifugation from the exponential phase culture was dried and extracted with different solvents such as Ethyl acetate, Methanol and Water by sonication and centrifugation. The supernatants collected were used for antibacterial and antifungal evaluation. The result of antibacterial and antifungal evaluation is shown in Table 3 & 4.

Table 3: Antibacterial activity of algal extracts.

Isolated algae	Bacterial culture						
	Extracts	1	2	3	4	5	6
Zone of inhibition (Diameter/mm)							
S–1	Ethyl acetate	Nil	**13**	**10**	Nil	Nil	**15**
	Methanol	Nil	Nil	**12**	Nil	14	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
S–2	Ethyl acetate	**11**	Nil	Nil	**10**	Nil	Nil
	Methanol	**16**	Nil	**11**	Nil	**13**	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
S–3	Ethyl acetate	Nil	**14**	Nil	Nil	Nil	Nil
	Methanol	Nil	Nil	**15**	Nil	**12**	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
S–4	Ethyl acetate	Nil	Nil	Nil	**13**	Nil	Nil
	Methanol	Nil	Nil	**10**	Nil	**12**	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil
S–5	Ethyl acetate	Nil	**10**	Nil	Nil	**12**	Nil
	Methanol	**14**	Nil	Nil	**15**	Nil	Nil
	Water	Nil	Nil	Nil	Nil	Nil	Nil

1–Bacillus subtilis; 2– E. coli; 3–Klebsiella pneumoniae; 4–Proteus mirabilis; 5–Staphylococcus aureus; 6–Shigella boydii

In the antibacterial evaluation, it was observed that the methanol extracts of all the algal species showed maximum activity against the tested cultures comparing with other extracts. Water extracts of all the tested species had no activity. It was found that the methanol extract of S–1 sample showed maximum activity against *P. mirabilis*. In case of the sample S–2, the methanol extract showed a maximum inhibition of *B. subtilis*. The *K. pneumoniae* culture was highly inhibited by the methanol extract of S–3 sample. In case of the methanol

extract of S–4 and S–5 sample, the tested culture *S.aureus* and *P. mirabilis* were highly inhibited respectively. In anti fungal evaluation also the methanol extract showed a significant activity. All the tested fungal organisms were inhibited by the tested extracts except water extract. All the isolated algal species showed activity. From the results it was found that the *mucur* sp., was highly inhibited, particularly by the methanol extract of S–3 sample followed by S–2 and S–4 sample. Despite the methanol has antimicrobial property, from these results it was clear that the solvent methanol brought a maximum extraction that may be due to its high polarity and this extract may contain a promising antimicrobial molecules.

Table 4: Antifungal activity of algal extracts.

Isolated algae	Fungal culture			
	Extracts	1	2	3
Zone of inhibition (Diameter/mm)				
S–1	Ethyl acetate	**11**	**10**	**14**
	Methanol	**13**	**15**	**12**
	Water	Nil	Nil	Nil
S–2	Ethyl acetate	**14**	**12**	**13**
	Methanol	**11**	**15**	**16**
	Water	Nil	Nil	Nil
S–3	Ethyl acetate	**10**	**14**	**10**
	Methanol	**13**	**12**	**18**
	Water	Nil	Nil	Nil
S–4	Ethyl acetate	**10**	**10**	**12**
	Methanol	**14**	**13**	**16**
	Water	Nil	Nil	Nil
S–5	Ethyl acetate	**11**	**10**	**13**
	Methanol	**14**	**15**	**15**
	Water	Nil	Nil	Nil

1–Aspergillus niger; 2– Candida albicans; 3–Mucur sp.;

CONCLUSION

In the present study, totally 10 algal samples were isolated from the sea water samples collected from the coastal region of Kanyakumari District such as Colachel, Muttom and Kadiapattinam. Among these, the 5 samples isolated from the Muttom seawater collection was evaluated now. Initially, the growth pattern of the isolates were observed and then subjected to mass production and followed by extraction and antimicrobial evaluation in which the methanol extract of S–2, S–3, S–4 and S–5 samples showed a significant antimicrobial activity against the tested bacterial and fungal culture. Our further studies directed toward the identification algal species, phytochemical screening and various detailed pharmacological screening may give more valuable results.

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