the correlation co-efficient values for various combinations of body as well as shell characters taken for the study from immature, male and female clams are significant ($P < 0.001$). Allometric relationships in all combinations showed that all these factors are significantly related to each other as observed by earlier workers (George John, 1980; Kalyanasundaram, 1982).

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ANTIMICROBIAL ACTIVITIES OF MARINE ALGAE FROM THE INDIAN COAST

ABSTRACT

Thirteen species of matured marine algae, collected from the intertidal regions around Mandapam, Okha and Malvan Coasts, were extracted with chloroform-methanol and the extracts were subjected to microbial screening for antibacterial and antifungal activities. Most of the extracts inhibited the growth of *Escherichia coli, Bacillus subtilis* and *Candida albicans*. The extract from *Chnoospora fastigiata* showed pronounced antifungal activity against *Saccharomyces cerevesciae* and *Candida albicans*, whereas *Entermorpha flexuosa* exhibited marked activity against *E. coli*. The Minimum Inhibitory Concentration (MIC) in µg/ml was determined in each case.

Introduction

THE SEAS and oceans provide an abundant resource of marine organisms which have been attracting the attention of biologists as well as chemists in their search for compounds of pharmacological interests. Extracts of marine organisms have shown antiviral, antifungal and antibacterial activities (Burkholder and Sharma, 1969; Su *et al.*, 1973). Bhakuni and Silva (1974) have reported some biodynamic substances from marine flora and fauna. Biologically active compounds have also been obtained by Ruggieri (1976) and Grant and Mackie (1977). Using different modes of screening, the biological activities of algal extracts have been reported by Fenical *et al.* (1973) and by Mynderse *et al.* (1977). Extracts of marine animals have been screened for cardiovascular activities by Kaul *et al.* (1977). The results of biological screening of about 140 marine organisms collected from the Indian Coast by the National Institute of Oceanography, Goa (India) in collaboration with the Central Drug Research Institute, Lucknow (India) under an Indo-US Project have been reported (Naqvi *et al.*, 1980; Kamat *et al.*, 1981; Naik *et al.*,

1989; Kamat *et al.*, 1991). The biological activities of the extracts of another 50 marine plants have been recently published by Bhakuni *et al.* (1992).

In this paper we report the antimicrobial activities of 13 marine algal extracts. Samples of Chlorophyceae, Phaeophyceae and Rhodophyceae were collected from the coasts of Mandapam (Tamil Nadu), Okha (Gujarat) and Malvan (Maharashtra).

The authors' grateful thanks are due to Dr. K. Subbaramaiah, CSMCRI Marine Algal Research Station, Mandapam for his help in the collection and identification of algae. The algae collected at Okha were identified by the scientists in the marine algal discipline, CSMCRI, Bhavnagar. The identification of some samples was checked by Professor V. Krishnamurthy, Department of Botany, Presidency College, Madras. Financial assistance received from the Department of Ocean Development, Government of India, New Delhi, in the form of a sponsored project is gratefully acknowledged.

Materials and methods

Collection and extraction of algae : Different species of algae after matured growth were collected during the low and negative tides off Mandapam Coast, Tamil Nadu (09° 17′ N — 79° 09′E), Okha Coast, Gujarat (22° 28′ N — 69° 05′E) and Malvan Coast, Maharashtra (16° 03′ N — 73° 28′ E).

The fresh alga was washed with sea water to remove extraneous matter, dried in shade and brought to laboratory. The alga was washed in tap water, air-dried in shade, coarsely powdered and extracted at ambient temperature with $CHCl_3$-MeOH (2 : 1) mixture. The extract was filtered and the solvent was removed on a rotary evaporator under reduced pressure at temperature below 40°C. The residue was dried in a vacuum desiccator.

Antimicrobial assay

Each extract was tested on three species of Gram positive and two species of Gram negative bacteria and two species of yeasts.

The microorganisms were obtained from the typed culture collection of National Chemical Laboratory, Pune and all strains were subcultured on commercially prepared Nutrient Agar and Saboraud's Agar from Difco Laboratories. The Minimum Inhibitory Concentration of each algal extract found active against each strain was determined on the agar medium.

Inocula of these species were prepared by picking colonies of each microorganism after overnight growth on Nutrient Agar or Saboraud's Agar plate and resuspending in the liquid medium (either Nutrient Broth of Saboraud's Broth) to a concentration of 10^6 cfu (per ml) approximately. The inocula were applied to plates containing the algal extracts in a serial twofold dilution and the plates were incubated overnight. The concentration of the algal extracts ranged from 25-500 μg/ml. The MIC was defined as the lowest concentration of an algal extract in μg/ml that inhibited growth of the microorganisms after 18-24 hours of incubation.

The culture media used for the bacteria and yeast were Nutrient Agar and Saboraud's Agar respectively. The bacteria were incubated at 37°C, whereas the incubation temperature for the yeasts was 28°C. The duration of incubation was 18-24 hours. The MIC values for the bio-active species are shown in Table 1.

Results and discussion

Our observations on the antibacterial activity of the algal extracts show that the activity cannot be co-related in the Gram-character of the bacteria, since the most susceptible bacteria namely *Bacillus subtilis* and *Escherichia coli* are dissimilar in Gram character.

The MIC values against *Bacillus subtilis* were generally lower than *Escherichia coli*

(Table 1) except in the case of *Enteromorpha flexuosa*. The Gram positive bacteria *Sarcina lutea* was inhibited by *E. flexuosa* and *Caulerpa taxifolia*, and did not show any susceptibility to other algal extracts. The MIC values against *Staphylococcus aureus* were equal in the case of *Hypnea musciformis* and *Caulerpa taxifolia* and it did not show any susceptibility to other algal extracts. None of the extracts showed any activity against *Salmonella typhi*. The extracts of *Caulerpa racemosa* and *Cladophora* sp. were inactive.

Among yeasts, *Saccharomyces cerevesciae* was generally resistant to more algal species as compared to *Candida albicans* (Table 1). *Saccharomyces cerevesciae* was inhibited by *Chnoospora fastigiata, Hypnea musciformis, Jania adherens* and *E. flexuosa*, and in the case of the first three algal species MIC values were lower than those for *Candida albicans.*

TABLE 1. *Minimum inhibitory concentration (MIC) μg/ml of marine algal extracts*

Alga	Collection		Bacteria Yeast					Yeast	
	Place	Manth	EC -ve	ST -ve	BS +ve	SL +ve	SA +ve	SC	AC
CHLOROPHYCEAE									
Caulerpa racemosa	Okha	Feb.	—	—	—	—	—	—	—
Caulerpa taxifolia	Okha	Feb.	>500	—	500	200	>500	500	500
Cladophora sp.	Okha	Dec.	—	—	—	—	—	—	—
Enteromorpha flexuosa	Okha	Feb.	25	—	>500	100	—	100	100
Ulva fasciata	Okha	Feb.	—	—	—	—	—	—	250
PHAEOPHYCEAE									
Chnoospora fastigiata	Mandapam	June	>500	—	200	—	—	—	5075
RHODOPHYCEAE									
Champia indica	Okha	Dec.	100	—	100	—	—	—	100
Gelidiella acerosa	Mandapam	June	>500	—	50	—	—	—	100
Gracilaria corticata	Mandapam	June	500	—	50	—	—	—	200
Gracilaria crassa	Mandapam	June	200	—	50	—	—	—	>500
Halymenia venusta	Okha	Feb.	500	—	—	>500	—	—	—
Hypnea musciformis	Malvan	Jan.	—	—	—	—	>500	50	>500
Jania adhaerens	Mandapam	June	250	—	50	—	—	75	200

EC = *Escherichia coli* ST = *Salmonella typhi* BS = *Bacillus subtilis* SL = *Sarcina lutea*
SA = *Staphylococcus aureus* SC = *Saccharomyces cerevesciae* CA = *Candida albicans*

Conclusions

The distribution of antimicrobial activities against three classes of microorganisms showed that *Bacillus subtilis* and *Escherichia coli* were more susceptible and among the yeast species *Candida albicans* was inhibited by more species of algae. *E. flexuosa* possessed greater antimicrobial activity than other algal species examined.

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