

Bioactivity and Chemical Ecology of Some Intertidal Animals

A.H. Parulekar and Prita Shirvoiker

National Institute of Oceanography, Goa

Some marine organisms from intertidal regions of the Indian Coast have shown promising activities. Marine sponges, coelenterates, molluscs, and echinoderms are found to possess rich sources of novel chemical compounds. The ecological and biological significance in the production of these chemicals and the nature and magnitude of activity shown by sponges, zooantharians, amphineurans, opisthobranchs, and holothurians is discussed in detail.

INTRODUCTION

Many marine organisms, especially in shallow tropical environments, are known to produce bioactive compounds. Studies on habit, habitat, and associations often provide vital clues for understanding seasonal and temporal variations in bioactivity.

Some marine invertebrates collected from intertidal regions of the Indian coast for biological screening have shown promising activities. This paper deals with seasonal variation in distribution, abundance, and bioactivity of some of the intertidal invertebrates in relation to environmental variations.

STUDY AREA

The intertidal biotopes from the Gulf of Kutch (22° 28' N, 69° 05' E) to Mandapam (9° 15' N, 79° 15' E) were surveyed for collection of marine organisms (figure 1). The study area harbored diverse ecosystems such as coral formations, sandy and rocky shores, seagrass beds, mangrove vegetation, and estuarine embouchers.

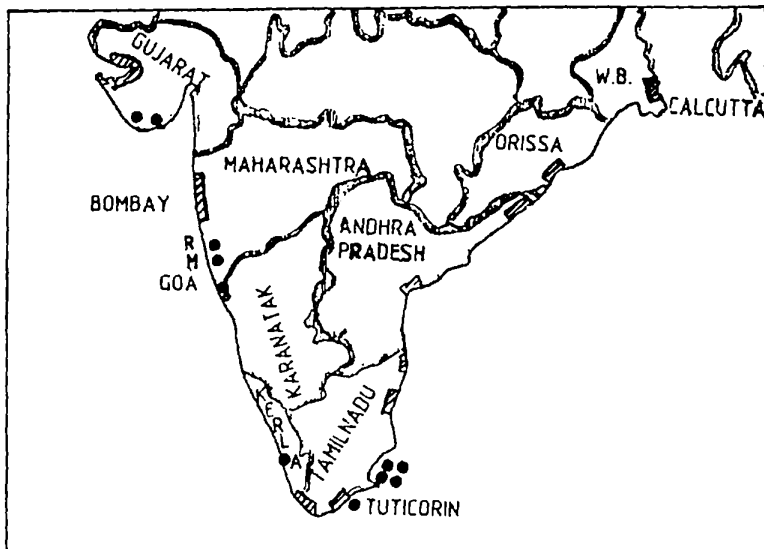


Figure 1. Map of India showing sampling sites (●).

MATERIALS AND METHODS

Marine organisms were collected from areas between tide marks from eight major stations from the western and southeastern coasts of India. Collections were made during low tide and ecologically significant organisms, which were widespread along this region, were collected. Detailed observations were made on their ecology and distribution. Alcoholic extracts of these organisms were tested for a wide range of pharmacological activities. Details of these biological tests have been described elsewhere (Dhar et al. 1968; Bhakuni et al. 1969, 1977).

RESULTS AND DISCUSSION

Of the 125 species of marine organisms collected for chemical investigation, sponges, soft corals, zoanthids, molluscs, and echinoderms were found to possess a rich source of novel chemical compounds.

Sponges are relatively simple multicellular animals. In spite of their sedentary habits and passive nature, they thrive well in their environment and account for a large portion of total biomass. Research on sponges has indicated that the group possesses a wealth of biologically active chemicals (Minale 1978). Many species of sponges from the shallow water environ-

ment along the Indian coast have shown promising but diverse activities (table 1).

Table 1. Results of biological screening of marine sponges

Species	Pharmacological Activity	Habitat
<i>Tedania anhelans</i>	Spasmogenic	Sandy
<i>Suberites</i> spp.	Antiviral	Sandy
<i>Sigmadocia fibulata</i>	Antiviral, toxic	Sandy, grows in association with seaweed <i>Cirratodictyon spongosum</i> :
<i>Spongia</i> sp.	N.S. stimulant	Sandy
<i>Callyspongia</i> sp.	Antiviral	Rock pools in association with <i>Testes</i> sp.
<i>Ircinia ramosa</i>	Antiviral, CNS stimulant	Rock pools
<i>Dysidea herbacea</i>	Diuretic	Rocks
<i>Hemna fortis</i>	Spasmolytic	Sandy
<i>Placospongia carinata</i>	Antimicrobial (<i>Bacillus</i> , <i>Pseudomonas</i>)	Sandy, spreading

In sponges, bioactivity depends greatly on habitat, population density, and its biological associates. The red sponge *Tedonia anhelans* when growing exposed in sandy areas exhibits spasmogenic activity while the same species from a rocky intertidal region is found to be inactive.

Many tropical species with well developed defense mechanisms are dominant by biomass, number, or both (Bakus et al. 1986). This is true with marine sponges. In a sponge-dominated area on Hare Island (Tuticorin), two species, namely *Sigmadocia fibulata* and *Suberites* sp., showed overall dominance. Both were found to be biologically active forms.

Many species are known to harbor a number of other marine organisms, both plants and animals. The toxic sponge *Sigmadocia fibulata* is found in association with the seaweed *Cirratodictyon spongosum*. The branches of this seaweed are completely embedded in the body of the sponge and impart a greenish color to its host. Although the sponge can lead an independent existence, there is so far no evidence that seaweeds can do so.

Another interesting association was found between the keratose sponge (*Spongia* sp.) and the boring pelecypod (*Pholas* sp.). Almost every available chamber space inside the sponge was found to be occupied by these bivalves. But the means by which this association helps its host in production of the active component are not well understood.

The discovery of antibiotics in sponges is credited to Nigrelli et al. (1959) who isolated a white crystalline material from ethyl ether extracts

of the red beard sponge *Microciona prolifera*. Antibiotic substances were also reported from other sponges (Jakowska and Nigrelli 1960, Nigrelli et al. 1961). Of the four species of sponges tested for antimicrobial activity, the extract from *Placospongia carinata* yielded antimicrobial activity against *Bacillus* and *Pseudomonas* species. This extremely hard sponge was found spreading in a sandy substratum and was relatively free of epiphytes.

The toxicity of marine sponges to animals has been reviewed by Baslow (1969). Ichthyotoxicity tests conducted on six species of sponges found along the Goa coast showed lack of toxicity in these sponges. Most of these species are found in crevices and are always in an unexposed condition.

Coelenterates are well known for their stinging cells, the nematocysts and cnidoblasts. Every member of this group possesses venomous organs for defence and predation. Of these, the zoantharians containing palytoxin are reportedly among the most toxic marine organisms known (Moore and Scheuer 1971, Moore and Bartolini 1981). Zoanthids are found in abundance in wave-beaten rocky shores all along the Indian coastline. Of the five different species of zoanthids collected, four were found to be highly toxic while one species from the Goa coast was found to be nontoxic. There was a marked seasonal fluctuation in the amount of active substances produced by these colonial organisms.

In molluscs, the most promising activity—antifertility—was observed in the amphineuran *Ischnochiton comptus*. Preliminary experiments conducted on mice have shown that the extract is 100% active. Activity in this species is found to be in close harmony with maturation and spawning behavior. Most of the chitons, collected earlier, had ripe gonads. Some fluctuations were found in activity during a later survey. Accordingly, it was noticed that most of the specimens had spent gonads indicating that spawning had already taken place. Detailed chemical investigations are required to confirm this.

Seahares, opisthobranch molluscs, are probably the most extensively investigated marine organism for chemical defense substances. Most of these substances are low molecular weight compounds derived from an algal diet (Faulkner et al. 1973, Kato et al. 1975). The seahare *Aplysia benedicti* found along the Indian coastline prefers to feed on the green algae *Ulva fasciata* and *U. lactuca*. However, the feed preference varies in different localities. While *A. benedicti* along the Goa coast prefers *U. fasciata*, the same species at Port Okha feeds exclusively on *U. lactuca*. Accordingly, there are changes in the secondary metabolites produced by this species.

Members of the family *Onchidiaceae* are known to possess peripheral epidermal organelles described as "repugnatorial" glands (Ireland and Faulkner 1978, Craig et al. 1986). The milky exudate from these glands has been reported to deter predators. While the *Onchidium verruculatum* found along the intertidal regions of rocky shores possess these glands,

some of them inhabiting mangroves do not. Despite this, they do protect themselves from potential predators. When different fractions of the crude extract of this species were tested for feeding deterrent properties, activity was found to be restricted to the petroleum ether. Food pellets treated with this fraction (100 $\mu\text{g}/\text{mg}$) were rejected by test fishes (table 2).

Table 2. Standard goldfish (*Carassius carassius*) antifeedant bioassay results (mangrove mollusc *Onchidium verruculatum*).

Conc Compound	50 $\mu\text{g}/\text{mg}$	100 $\mu\text{g}/\text{mg}$
Crude extract	+	+
Petroleum ether	+	-
Chloroform	-	+/-
Ethyl acetate	+	N.T.

- Pellets rejected

+/- Pellets partially eaten

+ Pellets eaten

N.T. Not tested

Marine nudibranchs, despite the lack of protective armor, are rarely eaten by predatory fishes (Thompson 1969). Along the Indian coast nudibranch fauna are diverse but abundance is relatively poor. Of the two species collected for chemical investigations, both showed the presence of active compounds. *Melilbe rangi* is an extremely peculiar and interesting nudibranch having a soft gelatinous body. This species has a characteristic smell produced by a glandular secretion from the body wall. A very interesting symbiotic association is found in this animal. The numerous tufted tubercles present in the body wall contain groups of digital papillae having colorless transparent walls containing threads of brown cell rows arranged at the periphery. These threads belong to bluegreen algae living in partnership with its host. Unlike dorid nudibranchs, which feed on sponges, this species feeds on free-swimming prey (zooplankton) with its oral hood.

In holothurians, the activity forms an integral part of the chemical defense mechanism (Bakus 1968). Five species of holothurians, *Holothuria atra*, *H. scabra*, *H. leucospilota*, *H. hilla*, and *H. cinerascens*, were most commonly encountered in the intertidal biota of the study area. Most of them were found exposed. Their abundance indicates that predation is not very common in this species. The presence of Cuverian glands and high

saponin content in the body wall have helped these organisms survive. Pharmacological activities exhibited by these seacumbers are given in table 3.

Table 3. Result of biological screening of extracts of marine molluscs and echinoderms.

Species	Pharmacological Activity	Habitat
<i>Ischnochiton comptus</i>	Toxic, hypoglycemic	High littoral rocks
<i>Cellana radiata</i>	Antifertility	High littoral rocks
<i>Aplysia benedicti</i>	Hypoglycemic	Sandy (seasonal)
<i>Melbe rangi</i>	CNS stimulant	Rock pools (rare)
<i>Asteropecten indica</i>	Toxic	Sandy
<i>Holothuria leucospilata</i>	Toxic, antiimplantation	Rock pools exposed
<i>Holothuria atra</i>	CMS stimulant	Sandy exposed
	Antiviral	
<i>Holothuria hilla</i>	Diuretic	Sandy exposed

SUMMARY

Observations on more than 18 species of sponges, zoanthids, amphineurans, opisthobranchs, and holothurians have clearly indicated the role of temporal variations both in ecological and life cycle characteristics on the quality, intensity, and quantity of bioactivity. A chemical ecological approach for understanding the magnitude of interactions between the environmental variability and the feeding, breeding, and defense strategies of a particular organism greatly helps in understanding and deciphering the mechanism of bioactivity.

An observed fact is the unpredictability in the distribution and abundance of marine organisms having promising bioactivities. Our studies, especially on *A. benedicti*, revealed the magnitude of environmental effects on the occurrence of intertidal organisms. For the sustainable availability of research material, one cannot solely depend on the animals occurring in their natural habitat. A reliable approach is to grow the desired organism under partial environmental and simulated conditions. Our efforts, especially in studying the horseshoe crab as a source of lysate amoebocyte, have generated good results when using this approach.

ACKNOWLEDGEMENTS

The work was carried out under the PL-480 aided Indo-U.S. collaborative project on "Bioactive Substances from the Indian Ocean".

LITERATURE CITED

- Bakus, G.J. 1968. Defensive mechanism and ecology of some tropical holothurians. *Mar. Biol.* 2(1): 23-32.
- Bakus, G.J., N. Targett, and B. Schulte. 1986. Chemical ecology of marine organisms: an overview. *J. Chem. Ecol.* 12: 951-987.
- Baslow, M.H. 1969. Porifera. Pages 86-99 in *Marine Pharmacology*. William and Wilkins, Baltimore, Maryland.
- Bhakuni, D.S., M.L. Dhar, M.M. Dhar, B.N. Dhawan, and B.N. Mehrotra. 1969. Screening of Indian plants for biological activity. Part II. *Indian J. Exp. Biol.* 7: 250-262.
- Bhakuni, D.S., M.L. Dhar, B.N. Dhawan, and B.N. Mehrotra. 1977. Screening of Indian plants for biological activity. Part VI. *Indian J. Exp. Biol.* 15: 208-219.
- Craig, M. Y., P. G. Greenwood, and C. Powell. 1986. Ecological role of defensive secretion in the intertidal pulmonate *Onchidella borealis*. *Biol. Bull.* 171: 301-404.
- Dhar, M.L., M.M. Dhar, B.N. Dhawan, B.N. Mehrotra, and C. Ray. 1968. Screening of Indian plants for biological activity. Part I. *Indian J. Exp. Biol.* 6: 232-247.
- Faulkner, D.J., M.O. Stallard, J. Fayos, and J. Clardy. 1973. (3R, 4S, 7S)-trans, trans-3, 7 dimethyl-1, 8, 8, tribromo 3, 4, 7-trichloro-1, 5-octadiene, a novel monoterpene from the seahare *Aplysia californica*. *J. Am. Chem. Soc.* 95: 3413-3414.
- Ireland, C., and D.J. Faulkner. 1978. The defensive secretion of the opisthobranch mollusc *Onchidella huxleyi*. *Boorg. Chem.* 7: 125-131.
- Jakowska, S., and R.F. Nigrelli. 1960. Antimicrobial substances from sponges. *Ann. N.Y. Acad. Sci.* 90: 913-916.
- Kato, Y., and P.J. Scheuer. 1975. *Pure Appl. Chem.* 41: 1.
- Minale, L. 1978. Terpenoids from marine sponges. Pages 175-240 in P.J. Scheuer, ed. *Marine Natural Products Chemical and Biological Perspective*. Vol. I. Academic Press, New York.
- Moore, R.E., and P.J. Scheuer. 1971. Palytoxin: a new marine toxin from a coelenterate. *Science* 172: 495.
- Moore, R.E., and G. Bartolini. 1981. Structure of palytoxin. *J. Am. Chem. Soc.* 103: 2491.
- Nigrelli, R., S. Jakowska, and I. Calrent. 1959. Ectyonin an antimicrobial agent from the sponge *Microciona prolifera*. *Verril. Zoologica* (N.Y.) 44: 173-176.
- Nigrelli, R.F., M. Baslow, and S. Jakowska. 1961. Further characterization of antimicrobial substances from the Bahamian sponges *Haliclona viridis* and *Tedania ignis*. First Interscience Conference on Antimicrobial Agents and Chemotherapy. *Am. Soc. Microbiol.* 83-84.
- Thompson, T.F. 1969. Defensive adaptation in opisthobranchs. *J. Mar. Biol. Assoc. U.K.* 39: 123-134.