

BIOACTIVITY OF THE EXTRACTS FROM SOME MARINE ANIMALS OF THE INDIAN COAST

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ABSTRACT

Twenty six species of marine animals from Indian coast were extracted with aqueous ethanol and the extracts were subjected to a broad-based biological screening including the tests for antibacterial, antifungal, antiviral, antifertility and pharmacological activities. Of these, fifteen were found to show significant biological activity.

INTRODUCTION

The record of success in isolating new drugs from terrestrial organisms is moderately impressive. Considering that about 80% of earth's animal life lives in the oceans and that the biotoxins are found throughout the entire phylogenetic series of marine animals (Halstead, 1965, 1967, 1968), the potential array of marine biologically active compounds should be even more impressive. Unfortunately, due to several reasons, especially difficulties in the procurement of the material very little attention has been paid to search for new drugs from the sea. Of all biotoxins from the sea, perhaps less than 1% have been examined so far for pharmacological activity. Probably fewer than two dozens have been fully evaluated as to their chemical and pharmacological properties (Halstead, 1965).

Recent interest in the marine flora and fauna as a potential source of new drugs led us to initiate a programme which involves the biological screening of the extracts and the chemistry of active constituents. The present paper gives the results of biological screening of extracts from 26 species of marine animals belonging mainly to the phyla Echinodermata, Porifera and Coelenterata.

MATERIALS AND METHODS

Marine organisms were collected from Goa, Maharashtra, Tamil Nadu coasts and from Laccadives and Andaman islands during premonsoon and post-monsoon periods depending upon their seasonal availability. Fresh material was washed with water to free it from extraneous matter, cut in small pieces and left immersed in

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95% alcohol at room temperature for 3 days or more. The insoluble material was filtered and the extract evaporated under reduced pressure below 40°C. The alcoholic extractive was finely dried in vacuum desiccator.

Corals were extracted with absolute ethanol and the extractive was fractionated into ether soluble and ether insoluble fractions. For the isolation of holothurin fraction from the body wall of sea cucumber, fresh pieces of body wall were boiled with water and filtered. Absolute ethanol was added to the concentrated filtrate so that the resulting mixture contained 60-70% ethanol. The precipitate was filtered off and the holothurin fraction precipitated from the filtrate by the addition of absolute ethanol to 94%. The saponin fraction from cuvierian glands was obtained by refluxing the glands with benzene for 4 hours followed by the extraction of defatted material with hot water.

Preliminary fractionation involved re-extraction of the alcoholic extractive with benzene followed by partition of the residue in *n*-butanol and water or ethylacetate-methanol water. The fractions so obtained were subjected to confirmatory tests.

The extractives have been tested for antiviral, antibacterial, antifungal, anti-fertility and a wide range of pharmacological activities. The details of the biological tests have been described in our earlier communication on marine plants (Naqvi, Solimabi, Kamat, Fernandes, Reddy, Bhakuni and Dhawan, 1981).

RESULTS AND DISCUSSION

Table I gives the names of species examined and arranged classwise in alphabetical order, the place of collection and code of biological tests carried out. The last two columns of the Table give the activity observed and the intraperitoneal LD₅₀'s in mice.

Out of 26 species of marine animals examined (Table I) two showed hypotensive activity (*Pocillopora damicornis* and *Gemmaria* sp. I), one exhibited anti-implantation activity (Cuvierian glands of *Holothuria* sp.); two were found to be spasmogenic (*Melitodes ornata* and *Suberitus carnosus*); five corals showed promising antifertility activity (*Porites lichen*, *Porites lutea*, *Acropora corymbosa*, *Lobophyllia corymbosa* and *Coscinarea monile*). Two of the extracts (*Holothuria* sp. and *Haliclona pigmentifera*) were found to be diuretic. The extracts of *Gemmaria* sp. II were extremely toxic to mice, the LD₅₀ being only 20 µg/kg by the intraperitoneal route. The oral LD₅₀ was, however, 147 mg/kg (unpublished observations) suggesting either very poor oral absorption or inactivation in the gut.

The different phyla examined include Porifera, Echinodermata, Coelenterata and Annelida. Several members of these groups have been reported to possess a broad spectrum of biological activities (Bhakuni and Silva, 1975). Though a few species tested here have also been tested by other workers (Burkholder, 1968), in most of the cases different results have been obtained. This, however, is not unexpected since the activity observed may be dependent upon ecological factors prevailing at the time of collection and also upon the growth stage. For instance, it was observed that the extract of *Gemmaria* sp. I, collected in the month of October, showed hypotensive activity. However, when the material was again collected in June, the extract was found

Table I. Result of biological screening of extracts of marine animals.

Class-Zoological names	Place and month of collection (a)	Biological tests (b)	Activity observed	LD ₅₀ mg/kg
<i>COELENTERATA</i>				
1. <i>Acropora Indica</i> (ether soluble)	Mandapam, T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	681
<i>Acropora Indica</i> (ether insoluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	681
2. <i>Acropora intermedia</i> (ether soluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	681
<i>Acropora intermedia</i> (ether insoluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	681
3. <i>Anthopleura pannikarsii</i>	Malvan, M, 12	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	562
4. <i>Antkopleura pannikarii</i> (sedentary polychaetes on)	Anjuna, G, 12	A ₂₋₄ ; B ₁₋₅ ; C ₁₋₄ ; D; E ₂ ; H ₁₋₄ ; J ₁	Toxic	68·1
5. <i>Gemmaria</i> sp. I	Baga, G, 10	A ₂₋₄ ; B ₁₋₅ ; C ₁₋₄ ; H ₁₋₄	Hypotensive	187·5
6. <i>Gemmaria</i> sp. II	Malvan, M, 12	A ₂₋₄ ; B ₁₋₅ ; C ₁₋₄ ; E ₃ ; F; G ₁ ; H _{1-4,9} ; J ₁	Very toxic	0·02
7. <i>Melitodes ornata</i>	Champion island Andaman, A, 04	J ₁	Spasmolytic	—
8. <i>Montipora digitata</i> (ether soluble)	Mandapam, T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	>1,000
<i>Montipora digitata</i> (ether insoluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	>1,000
9. <i>Montipora diverticata</i> (ether soluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	Spasmolytic	>1,000
<i>Montipora diverticata</i> (ether insoluble)	„ T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	>1,000
10. <i>Turbinaria</i> sp.	Malvan, M, 12	A ₂₋₄ ; B ₁₋₅ ; C ₁₋₄ ; E ₁₋₃ ; F; G; H ₁₋₄ ; J ₁	—	>1,000
11. <i>Pocillopora damicornis</i>	Mandapam, T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	Hypotensive	681
12. <i>Porites lichen</i>	Malvan, M, 09	G ₁	100% Antifertility	>1,000

(Continued)

Table I. (Continued)

Class-Zoological names	Place and month of collection (a)	Biological tests (b)	Activity observed	LD ₅₀ mg/kg
13. <i>Acropora corymbosa</i>	Lakshadweep, L, 02	G ₁	100% Antifertility	>1,000
14. <i>Lobophyllia corymbosa</i>	Lakshadweep, L, 02	G ₁	100% Antifertility	>1,000
15. <i>Coscinarea monile</i>	Malvan, M, 04	G ₁	100% Antifertility	>1,000
16. <i>Porites lutea</i>	,, M, 04	G ₁	80% Antifertility	>1,000
ECHINODERMATA				
17. <i>Astropecten</i> sp.	Colva G, 02	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; D; E ₂ ; H ₁₋₄ ; J ₁	Spasmogenic	681
18. <i>Holothuria</i> sp. (body wall)	Anjuna, G, 11	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; H ₁₋₄ ; J ₁	Diuretic	15.63
<i>Holothuria</i> sp. (Cuvierian glands)	,, G, 11	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; G ₁ ; H ₁₋₄ ; J ₁	Anti-implantation	15.63
19. <i>Ophiotrix</i> sp.	Malvan, M, 12	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	681
20. <i>Loligo</i> (ink glands)	Mondovi, G, 10	A ₂₋₅ ; B ₁₋₅	—	—
PORIFERA				
21. <i>Chroteila australiensis</i>	Anjuna, G, 03	A ₁₋₄ ; B ₁₋₅ ; C _{1,2,4} ; D; E ₂ ; H ₁₋₄ ; J ₁	—	681
22. <i>Cinachyra cavernosa</i>	Mandapam, T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	261
23. <i>Haliclona pigmentifera</i>	Malvan, M, 04	C ₁₋₄ ; D; H ₁₋₄ ; J ₁	Diuretic	681
24. <i>Prostylyssa foetida</i>	Cabo de Rama, G, 02	A ₁₋₄ ; B ₁₋₅ ; C _{2,4} ; E ₂ ; H ₁₋₄ ; J ₁	—	825
25. <i>Spirastrella inconstans</i>	Mandapam, T, 03	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	—	316
26. <i>Subreitus carnosus</i>	Anjuna, G, 01	A ₁₋₄ ; B ₁₋₅ ; C _{1,2,4} ; D; H ₁₋₄ ; J ₁	Spasmogenic	681

(a) G—Goa; M—Maharashtra, T—Tamil Nadu; A—Andamans; L—Lakshadweep.

(b) Code for biological tests:

A, antibacterial (A₁, *Bacillus subtilis*; A₂, *Staphylococcus aureus*; A₃, *Salmonella typhi*; A₄, *Escherichia coli*; A₅, *Agrobacterium tumefaciens*); B, antifungal (B₁, *Candida albicans*; B₂, *Cryptococcus neoformans*; B₃, *Trichophyton metagrophytes*; B₄, *Microsporium canis*; B₅, *Aspergillus niger*); C, effects on respiration, cardiovascular system and nictitating membrane in anaesthetised cats (C₁, respiration; C₂, blood pressure; C₃, nictitating membrane contraction and C₄, heart rate) or rats (C_{1,2,4} only); D, diuretic activity in hydrated rats; E, antiviral (E₁, Ranikhet disease virus; E₂, Semliki Forest virus; E₃, Vaccinia virus); F, anti-inflammatory activity in mice; G, anti-fertility activity (G₁, anti-implantation activity in rat); H, CNS effects in mice (H₁, gross effects; H₂, effect on body temperature; H₃, analgesia; H₄, supramaximal electro-shock seizure pattern test; H₅, anorexigenic activity; H₆, barbital hypnosis test; H₇, rotarod test; H₈, amphetamine hyperactivity test and H₉, antireserpine activity); J, effect on isolated tissues (J₁, isolated guinea pig ileum; J₂, isolated rat uterus). The LD₅₀ values (mice) are by the i.p. route.

to be inactive. This shows that there are distinct seasonal variations in the activity and hence the various activities reported here stand for the period during which the sample was collected. Interesting activities have been observed in many organisms not investigated earlier.

Holothurins from sea cucumber have been found to be toxic to various animal species including mammals (Nigrelli and Zahl, 1952; Ruggieri and Nigrelli, 1960) and devoid of antibiotic activity. While confirming these results, the present investigation has also unmasked their diuretic activity. The saponin fraction from sea cucumber *Stichopus japonica* has been reported to possess antifungal activity against *Trichophyton asteroid* and *Candida albicans* and other fungal species *in vitro* (Shimada, 1969). This activity was, however, not observed in the species examined by us. Some of these saponins are reported to have sperm immobilising properties and cause induction of egg and sperm shedding effects (Kanatani, 1964). It was observed that saponin fraction of the cuvierian glands of the species examined had anti-implantation activity but it is not possible to suggest the mechanism of this effect on the basis of available data.

Extracts from sponges have been reported to show antibacterial and antifungal properties (Yakowska and Nigrelli, 1960). But none of the species examined showed similar activity. However, it may be added here that the absence of activity may be due to varying solubility behaviour of antimicrobial substance from sponges in aqueous alcoholic solutions (Sharma, Vig and Burkholder, 1968).

The most promising activity observed is perhaps the antifertility (anti-implantation) activity found in five species of corals. Preliminary experiments conducted on mice have shown that these extracts are 100% active. Three species of seaweeds have been found to exhibit similar activity (Naqvi, Solimabi, Kamat, Fernandes, Reddy, Bhakuni and Dhawan, 1981). These findings are of considerable importance in that they open new possibilities for the development of antifertility drugs from the sea.

Marine organisms showing promising activities have been taken up for detailed chemical studies.

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