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Screening of Some Marine Plants from the Indian Coast for Biological Activity

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Abstract

Extracts of twenty five seaweeds from Indian coast have been put through a broad biological screen which includes tests for antiviral, antibacterial, antifungal, antiprotozoal, antifertility activities and a wide range of pharmacological activities. Significant biological activity was obtained in 13 seaweeds, the most promising activity being 100% anti-fertility (anti-implantation) activity observed in 3 species.

Introduction

The marine organisms represent a potential source of materials including drugs (Marderosian 1968, Baslow 1969). Only limited studies have, however, been done to obtain biologically active compounds from this source (Ruggieri 1976, Grant and Mackie 1977). Antiviral, antifungal and antibacterial activities have been observed in the extracts of marine organisms (Su *et al.* 1973, Burkholder and Sharma 1969). Extracts of a large number of marine animals have also been recently screened for cardiovascular activities (Kaul *et al.* 1977). Some biodynamic substances have been isolated both from marine flora and fauna and their chemical and biochemical status has been reviewed (Bhakuni and Silva 1974).

As the first phase of our project on the isolation and characterization of biodynamic compounds from marine flora and fauna, we decided to test the extracts prepared from marine organisms for biological activity. The present paper presents the results of the screening of extracts from marine plants, mainly seaweeds belonging to the families Rhodophyceae, Chlorophyceae and Phaeophyceae and two species of sea grasses.

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Materials and Methods

Marine plants used for the study were collected from Goa, Maharashtra and Tamil Nadu coasts during premonsoon and postmonsoon periods depending upon their seasonal availability. Fresh material was washed free from extraneous matter, air dried, ground to a fine powder and extracted with 50% aqueous ethanol, as described by Bhakuni *et al.* (1969), except in the case of *Corallina* sp. which, owing to its calcareous nature, was extracted with absolute alcohol. Typically, air dried powdered material (300 g) was percolated thrice with 50% aqueous ethanol. The solvent from the combined alcoholic percolate was removed at reduced pressure below 40 °C. The alcoholic extractive so obtained was dried in vacuum desiccator. Almost all the extracts were tested for antibacterial, antifungal, antiviral, antifertility and a wide range of other pharmacological activities. The details of these biological tests have been described elsewhere (Dhar *et al.* 1968, Bhakuni *et al.* 1969, 1971) but they are briefly described below.

A. Antibacterial activity

The activity was tested against two Gram positive *Bacillus subtilis* (A₁) and *Staphylococcus aureus* (A₂) and three Gram negative – *Salmonella typhi* (A₃), *Escherichia coli* (A₄) and *Agrobacterium tumefaciens* (A₅) – strains of bacteria in peptone broth at 37 ± 1 °C by the two fold serial dilution technique. The observation period was 24 hours and extracts completely inhibiting the

growth in a concentration of 25 µg/ml were considered active.

B. Antifungal activity

The strains used were: *Candida albicans* (B₁), *Cryptococcus neoformans* (B₂), *Trichophyton mentagrophytes* (B₃), *Microsporum canis* (B₄) and *Aspergillus niger* (B₅). The basic schedule of testing was similar to that of antibacterial testing but Sabouraud's broth at 28 ± 1°C was used as the medium and the observation period was 24 hr for B₁ and B₂, 48 hr for B₅ and 96 hr for B₃ and B₄.

C. Effects in anaesthetised cats or rats

Adult cats were anaesthetised with pentobarbitone sodium (30 mg/kg i. v.) and extracts administered intravenously. The drug effect was assessed on respiration (C₁), blood pressure (C₂), nictitating membrane contraction in response to electrical stimulation of preganglionic sympathetic nerve (C₃) and heart rate. The rats were anaesthetised with and only C_{1,2} and 4 were recorded. C₁₋₃ were recorded on a kymograph with appropriate levers etc. and C₄ was determined from an 'Encardionite' heart rate metres. Extracts devoid of activity upto a dose of 50 mg/kg were considered inactive.

D. Diuretic activity

This was tested in male rats hydrated with 5 ml/100 g normal saline via a stomach tube. The extract (1/4 x LD₅₀) was given orally and extracts producing at least 75% of diuresis compared to 100 mg/kg chlorthiazide p. o. in a 4 hr period were considered active.

E. Antiviral activity

This was tested against Semliki Forest virus (SFV) *in vivo* (E₁) and Ranikhet Disease (E₂) and Vaccinia virus (E₃) grown on Chick allantoic membrane (CAM) culture.

Mice were used in groups of 10 and given 0.5 or 1 mg of the extract intraperitoneally and challenged with 100 LD₅₀ dose of Smithburn and Haddow strain of SFV subcutaneously 24 hr later. The number of animals dying following hindlimb and forelimb paralysis during the next two weeks in control and treated animals was used to calculate % protection.

CAM culture was prepared by the method of Babbar (1961) and the extract (0.05 mg/ml) and virus (0.064 haemagglutination unit/ml E₂ and 50 pox forming unit/ml E₃) were added to the culture and incubated at 37°C for 48 (E₂) or 72 (E₃) hours and viral titre measured in 6 replicates. Activity was considered significant only after 75 (E₂) or 100 (E₃) % reduction in viral progeny.

F. Anti-inflammatory activity

Oedema was induced by injecting 0.025 ml of 1% carrageenan solution into subplantar region of left paw one

hour after oral administration of the extract (1/5 x LD₅₀). The animals were killed 4 hr later and the difference between weight of the two paws between treated and control animals was the measure of anti-inflammatory activity. A reduction of 20% was considered significant.

G. Anti-fertility activity

Anti-implantation (G₁) activity was tested by feeding the extract to a group of 5 female rats on days 1-5 post coitum and laparotomising them on day 10 to note the presence of implantation sites and corpora lutea. The presence of spermatozoa in the vaginal smear was considered evidence of coitus. Complete absence of implantation was taken to indicate antifertility activity.

H. CNS effects

These were studied in groups of 5 mice each at 1/4 - 1/2 LD₅₀ dose. The observation of gross effects (H₁) extended for 2 hr and was directed at detecting signs of CNS stimulation or depression and autonomic effects. The rectal temperature (H₂) was monitored with a thermocouple 1/2, 1 and 2 hr after drug administration. Analgesia (H₃) was tested by Haffner's tail clip method while anticonvulsant activity (H₄) was judged by the ability of the extract to abolish the tonic extensor component of supramaximal electroshock seizure induced by 48 ma current for 0.2 sec delivered via ear electrodes.

The anorexigenic activity was evaluated in fasted mice and percent reduction in consumption of sweetened milk was measured over a 15 minute observation period 30 min after the administration of the extract. For the potentiation of barbital hypnosis test (H₆) 200 mg/kg barbital sodium was administered to control and drug treated groups of 10 mice each and number of animals in which righting reflex was lost was noted. In control animals this dose abolished the reflex in only 3 out of 10 animals. For the rota-rod test (H₇) mice were trained to remain on a rotating rod for a period of 2 min. The test was repeated after administration of the extracts and number of mice falling out earlier noted. For antagonism of amphetamine hyperactivity (H₈) the drug treated and controls animals were injected with 2.5-5 mg/kg amphetamine i.p. and the activity of two groups compared with the help of an actophotometer 15, 30, 60, 90, 120, 150 and 180 min later. For the antiserpine test (H₉) the extracts were administered 3 1/2 hr after i.p. administration of 2.5 mg/kg reserpine and ptosis, sedation and hypothermia compared with a control group 1/4, 1/2, 1, 1 1/2, 2 1/2, 3 and 20 hr later.

J. Effect on isolated tissues

Guinea pig ileum (J₁) preparation was set up in an isolated organ bath using aerated tyrode at 35°C and contractions induced with acetylcholine (3 x 10⁻⁸ g/ml), histamine 3 x 10⁻⁸ g/ml, 5-HT (6 x 10⁻⁷ g/ml) or nicotine (6 x 10⁻⁷ g/ml) were recorded kymographically before and after addition of the extract (50, 250 µg/ml). If the extract

itself caused contraction, efforts were made to antagonise it with atropine (1×10^{-7} g/ml) or mepyramine (5×10^{-8} g/ml). Similarly isolated uterus (J_2) of stilboesterol (1 mg/kg) treated female rats was set up in aerated de Jalon's fluid at 30°C and its response to above mentioned concentrations of the extracts recorded.

LD_{50} was determined by giving graded doses of the extract to groups of 4–5 mice each by the i.p. route and recording the mortality during the next 24 hr. The highest dose used was 1000 mg/kg.

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

Results and Discussion

In Table I the names of seaweeds are arranged classwise in alphabetical order. The place of collection and the code of biological tests carried out are also recorded. The last two columns of the Table give the activity observed and the intraperitoneal LD_{50} 's in mice.

Of the twenty five species of algae examined (Tab. I) thirteen have shown significant biological activities. Diuretic activity was observed in the extracts of *Hypnea musciformis*, *Enteromorpha* sp. and *Trichodesmium erythraeum*; hypotensive activity in *Caulerpa racemosa* and *Chondria armata*; spasmogenic activity in *Corallina* sp. and *Padina tetrastromatica*; spasmolytic activity in *Stoechospermum marginatum*; antiviral activity in *Codium elongatum* and CNS depressant activity in *Sargassum tenerrimum*. Three extracts (*Acantophora specifera*, *Padina tetrastromatica* and *Gelidiella acerosa*) showed 100% antifertility activity. Diuretic activity observed in the fractions of *Hypnea musciformis* has been confirmed in benzene and ethyl acetate fractions. The results obtained with fractions of other active extracts will be reported later.

The different seaweeds examined in the present investigation include red, brown and green algae. Several members of these groups have been reported to possess a broad spectrum of biological activities. Some of the species examined here were also tested earlier by other workers (Starr *et al.* 1962, Aguilar-Santos and Doty 1968, Boyd *et al.* 1966) but none of the activities observed by us have been reported. In some cases, the activity expected on the basis of earlier work has not been observed in the present work. For instance, extracts from *Chondrus crispus* and *Gelidium cartilagineum* have been found to be active against influenza B and mumps virus (Garber *et al.* 1958). This has been attributed to the presence of galactan unit in the polysaccharides, agar and carrageenan, present in both the species. Based on this, one may expect *Hypnea musciformis*, *Gelidiella* sp., and *Gracilaria corticata* which are well known sources of

these polysaccharides to exhibit similar activity. Unfortunately, their extracts could not be tested against the above virus. However, they were found to be ineffective against those tested by us (*Ranikhet* disease virus, *Semliki Forest* virus and *Vaccinia* virus). The absence of activity may also be attributed to the varying solubility behaviour of the sulphated polysaccharide, responsible for the activity, in aqueous ethanolic solutions (Agol and Chumakova 1963) or to the well known variation in phytoconstituents depending upon the ecological factors prevailing at the time of collection and upon the growth stage of the plant. The last explanation could also be applicable to the differences in effects of two different samples of *Enteromorpha* sp. and *Hypnea musciformis* collected at different places.

The extract of *Sargassum tenerrimum* was not found to have any chemotherapeutic activity though the species of this genus have been reported to possess antibiotic effects due to the presence of antibiotic substances, Sarganin A and Sarganin B. This activity, however, seems to be seasonal and has been reported to decrease directly with plant vigour as a function of latitude (Conover and Sieburth 1964).

Acute poisoning from seaweeds has not been reported in the literature but mention has been made that *Caulerpa*, one of the most popular edible algae in the Philippines is considered toxic during the rainy season. This toxicity has been attributed to the presence of caulerpicin which has been found to have neuro-tropic effects (Bhakuni and Silva 1974). Unfortunately, the test for this activity could not be performed for the extract from *Caulerpa racemosa* which was collected during pre-monsoon season. Since caulerpicin is not reported to possess hypotensive activity, the effect observed by us in this species seems to be due to some other constituent. This inference is further supported by the absence of similar activity in *Caulerpa sertularioides* considering that caulerpicin is a common constituent of both the species (Aguilar-Santos and Doty 1968).

The most promising activity observed by us is perhaps the anti-implantation activity observed in three species viz., *Padina tetrastromatica*, *Acantophora specifera* and *Gelidiella acerosa*. Preliminary experiments conducted on mice have shown that these extracts are 100% active. The occurrence of such activity in seaweeds has not been reported in the literature hitherto. These and other active extracts have been taken up for detailed chemical studies the results of which will be published later.

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Tab. I. Results of biological screening of extracts of seaweeds

Class – Botanical names	Place and month of collection	Biological tests	Activity observed	LD ₅₀ mg/kg
	(a)	(b)	(c)	
CHLOROPHYCEAE				
1. <i>Caulerpa racemosa</i>	Malvan, M, November	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; G ₁ ; H ₁₋₄ ; J ₁	Hypotensive (90 mm, 50 mg/kg) 10 min	93.75
2. <i>Caulerpa sertularioides</i>	Malvan, M, November	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; D; H ₁₋₄ ; J ₁	–	> 1,000
3. <i>Chaetomorpha media</i>	Vengurla, M, May	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; G ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
4. <i>Cladophora pinnulata</i>	Baga, G, December	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁₋₃ ; F; H ₁₋₄ ; J ₁	Toxic	8.25
5. <i>Codium elongatum</i>	Cabo de Rama, G, February	A ₁₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁ ; G ₁ ; H ₁₋₄ ; J ₁	Antiviral (50%, 1 mg)	681
6. <i>Enteromorpha intestinalis</i>	Chapora, G, February	A ₁₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁ ; G ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
7. <i>Enteromorpha sp.</i>	Cabo de Rama, G, March	A ₁₋₅ ; B ₂₋₅ ; C ₁₋₄ ; D; H ₁₋₄ ; J ₁	Diuretic (112%, 170 mg/kg)	681
8. <i>Ulva fasciata</i>	Baga, G, December	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁₋₃ ; F; G ₁ ; H ₁₋₅ ; J ₁	–	46.4
CYANOPHYTA				
9. <i>Trichodesmium erythraeum</i>	Aguada, G, April	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; H ₁₋₄	–	> 1,000
10. <i>Trichodesmium erythraeum</i>	Mandovi, G, March	A ₁₋₅ ; B ₂₋₄ ; C ₁₋₄ ; D; H ₁₋₄ ; J ₁	Diuretic (104%, 170 mg/kg)	681
PHAEOPHYCEAE				
11. <i>Chnoospora implexa</i>	Mandapam, T, March	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	–	681
12. <i>Dictyota bartayresiana</i>	Anjuna, G, January	A ₁₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
13. <i>Stoechospermum marginatum</i>	Dona Paula, G, December	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; G ₁ ; H ₁₋₄ , 6-8; J ₁	Spasmolytic (50%, 50 µg/ml)	> 1,000
14. <i>Padina tetrastratica</i>	Baga, G, January	A ₁₋₅ ; B ₁₋₅ ; C ₁₋₄ ; D; E ₁ ; G ₁ ; H ₁₋₄ ; J ₁	Spasmogenic (50 µg/ml) & anti-fertility (100%, 200 mg/kg)	464
15. <i>Sargassum tenerrimum</i>	Baga, G, December	A ₁₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁₋₃ ; F; G ₁ ; H ₁₋₄ , 6-8; J ₁	CNS depressant (89 mg/kg, H ₁ , H ₂ , H ₆)	178
16. <i>Spatoglossum asperum</i>	Baga, G, December	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	–	681
RHODOPHYCEAE				
17. <i>Acantophora spicifera</i>	Baga, G, February	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; D; E ₁ ; G ₁ ; H ₁₋₄ ; J ₁	Antifertility (100%, 200 mg/kg)	> 1,000
18. <i>Chondria armata</i>	Baga, G, December	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁ ; H ₁₋₄ ; J ₁	Hypotensive (40 mm, 1 mg/kg) 200 min	17.8
19. <i>Corallina sp.</i>	Anjuna, G, January	A ₂₋₅ ; B ₁₋₅ ; C _{1,2,4} ; D; E ₁ ; G ₁ ; H ₁₋₄ ; J ₁₋₂	Spasmogenic & oxytocic (50 µg/ml)	681
20. <i>Gelidiella sp.</i>	Baga, G, November	A ₂₋₅ ; B ₁₋₅ ; C _{1,2} ; D; G ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
21. <i>Gracilaria corticata</i>	Baga, G, November	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; D; G ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
22. <i>Hypnea musciformis</i>	Baga, G, December	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₃ ; E ₁ ; H ₁₋₄ ; J ₁	Diuretic (130%, 250 mg/kg)	> 1,000

Tab. I. (Continuation)

Class – Botanical names	Place and month of collection	Biological tests	Activity observed	LD ₅₀ mg/kg
	(a)	(b)	(c)	
23. <i>Hypnea musciformis</i> (var. <i>cervicornis</i>)	Malvan, M, November	A ₂₋₅ ; B ₁₋₅ ; C ₁₋₄ ; E ₁ ; H ₁₋₄ ; J ₁	–	> 1,000
24. <i>Gelidiella acerosa</i>	Eravali, T, December	G ₁	Antifertility (100%, 100 mg/kg)	–
SEA GRASSES				
25. <i>Diplanthera uninervis</i>	Mandapam, T, March	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	–	681
26. <i>Thalassia hemprichii</i>	Mandapam, T, March	A ₂₋₄ ; B ₁₋₅ ; C _{1,2,4} ; H ₁₋₄ ; J ₁	–	681

(a) G – Goa; M – Maharashtra; T – Tamilnadu; A – Andaman

(b) See methods for details of biological tests.

(c) Figures in parentheses indicate effect observed, dose or concentration used and in the case of hypotension, duration of effect.

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