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Research Article

EFFICACY OF BIOACTIVE COMPOUNDS EXTRACTED FROM MARINE SPONGE *HALICLONA EXIGUA*

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ABSTRACT

Objective: To evaluate the biopotential activity of secondary metabolites in marine sponge *Haliclona exigua* (*H. exigua*) collected from the Gulf of Mannar. **Methods**: Soxhlet extraction method was used to extract the secondary metabolites and various assays were carried out. **Results**: *H. exigua* showed potent antibacterial, antioxidant, anti-inflammatory and anticancer activities. The secondary metabolites were characterized by Gas Chromatography analysis. **Conclusion**: Based on the present study it can be inferred that the bioassay guided fractionation and purification of *H. exigua* may come up with potent bioactive drug.

Keywords: Marine sponge, GC analysis, antibacterial, anti-inflammatory, anticancer

INTRODUCTION

Earth is a biosphere sizzling with activities of all terms of life. It is essentially a water planet, two thirds of which being covered with water especially marine water. The marine realm is an exceptional reservoir of biota and bioactive natural products, which has so far produced hundreds of novel structures with unique biological properties. The ocean environment is massively complex, consisting of extreme variations in pressure, salinity, temperature and biological habitats. The potential of marine life as a source of novel molecules is immense and has been barely investigated. Because of their longer evolutionary history, marine organisms are likely to possess a greater molecular diversity than do their terrestrial counterparts. The rapid growth in the chemistry of marine organisms over the last 15 years has led to the discovery of a large number of new structures, many of which have no precedence among structures of terrestrial origin and possess previously unknown pharmacological and toxicological propertied [1]. In comparison with the other marine life forms, bioactive compounds have been detected especially in sponges. These sponges are the most primitive of the multicelled animals that have existed through a long evolutionary history of 700-800 million years. Sponges can provide potential drugs against many major world wide occurring diseases. Thus far, it is not fully understood why, when, where and how these metabolites are produced in sponges. Many of these metabolites have been found to be cytotoxic or to processes other other biological activities[2-4] . However, little thorough cytotoxicity research has been performed on this sponge.

METHODOLOGY

Collection and identification of sample

Sponge samples were collected as entangled specimens from a bottom trawl fish net operated off Manoli and Hare Islands of Mandapam group of Islands, Gulf of Mannar. Samples were collected in bulk depending on the abundance of individual organisms and washed with freshwater to remove adhering debris and associated biota. Collected samples were stored in a refrigerated box and transferred to the lab. Further, the sponge samples are labeled properly and stored at -70°C. The taxonomic identification of the organisms was done using spicules separated using nitric acid digestion following standard identification keys [5,6].

Bioactive compound extraction

For the extraction of crude bioactives, 100g of powdered material was exhaustively extracted with 200ml of ethyl acetate using soxhlet apparatus and evaporated under reduced pressure to yield viscous dark gum. The extract was stored at 4^0C in air- tight plastic vials for further studies.

Anti-bacterial activity

Invitro antibacterial assay was done using disc diffusion [7] against human pathogens *Eschericia coli, Staphylococcus aureus, Klebsiella sp., Proteus* and *Pseudomonas by* agar well diffusion method. Nutrient Agar plates were prepared and wells were made using gel puncture. Test culture was swabbed aseptically and inoculated on the surface of the Nutrient Agar so as to make a lawn. This was allowed for 5 min for the agar surface to dry before making the wells. The extracts and respective solvent was added to the wells and the plates were incubated for 16 to 18 hrs at 37^0C. The efficacy of extracts against bacteria ws compared with the broad spectrum antibiotic erythromycin (positive strain). The zone of inhibition was observed around the well.

Anti-oxidant activity

The ability of the extract to scavenge DPPH radicals were determined by the method of *Gyamfi et al* with minor modifications. 20µl of test extract at different concentrations in methanol was mixed with 0.5 ml of 100 mM methanolic solution of DPPH. After 30 min of incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm and the percentage inhibition was calculated using the following formula: Percentage inhibition = (Abs control – Abs sample) X 100/ Abs control

Cytotoxic assay

Cytotoxicity of extracts at various concentrations (15- 1000 µg/ml-) was assessed for Hep2 and MCF7 using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) but with minor modification, following 72 h of incubation. Assay plates were read using a spectrophotometer at 520 nm. Data generated were used to plot a dose-response curve of which the concentration of extract required to kill 50% of cell population (IC_{50}) was determined.

Cell viability (%) = Mean OD/ control OD × 100

Anti-inflammatory activity

Blood was collected from healthy volunteer and mixed with equal volumes of Alsever solution. The blood was centrifuged at 3000 rpm and the packed cells were washed with isosaline and 10%v/v was made with isosaline. 0.5ml of this was taken in a tube and to this was added 1ml of PBS, 2ml of Hyposaline and 0.5ml of the extract. Indomethacin was used as a reference drug [8]. Instead of hyposaline 2ml of distilled water was used in the control. All the assay mixture were incubated and centrifuged. The haemoglobin content in the supernatant was read at 540 nm. The percentage of haemolysis was calculated by assuming the haemolysis produced in

the presence of distilled water ad 100%. The percentage of protection was calculated using the formula,

% Protection= 100- Optical Density of drug treated sample × 100

Optical Density of control

GC-MS Analysis

Gas chromatograph analysis was carried out on a Shimadzu (QP2010) equipped with a VF-5 ms column (diameter 0.25 mm, length 30.0 m, film thickness 0.25µm) mass spectrometer (ion source 200^{0}C; EI -70 eV), programmed at temperature 40-650^{0}C with a rate of 4^{0}C/min. Injector flow rate was 200^{0}C; carrier gas was He 99.9995% purity, column flow rate1.51ml/min, injection mode - split.

RESULTS AND DISCUSSION

Approximately 10,000 sponges have been described in the world and most of them live in marine waters. A range of bioactive metabolites has been found in about 11 sponge genera. Three of these genera (*Haliclona*, *Petrosia* and *Discodemia*) produce powerful anti-cancer, anti-inflammatory agents, but their cultivation has not been studied [9]. Marine sponge, *Theonella* spp. [10], which show *in vitro* cytotoxity and *in vivo* antitumour activity in many leukemia and solid tumour model systems [11].

In the present study, the collected sponge sample was identified as *H. exigua* by spicules separated by nitric acid digestion. In the search for bioactive compounds, the extract of *H. exigua* were tested for antibacterial, antioxidant, anti-inflammatory and cytotoxic activities.

Antimicrobial activity of extracts were examined and found to exhibit good antibacterial activity for crude extract at 100,125 µl/disc dose level against most of the gram positive and gram negative organisms which has been depicted in Table 1 for sponge crude extract respectively. Among the test organisms the extract showed good antimicrobial activity for all the bacterium species.

Secondary metabolites of sponges exhibited significant bactericidal activity (Table 1). The sponge exhibited broad spectrum antibacterial activity and exhibited the growth of all the test bacteria. Most of the available reports on antibacterial property of sponges revealed their activity on gram positive bacteria. Various studies have confirmed the predominance of gram negative producers in the marine environment [12]. In a study on antibiotic production in marine bacteria [13], have reported that 36% of the strains were gram negative rods. In our study, gram positive as well as gram negative bacteria was more or less equally represented in the producers encountered. The discovery of new classes of antibiotics is necessary due to the increased incidence of multiple resistances among pathogenic micro organisms to drugs that are currently in clinical use [14].

Antioxidantive property was assessed using DPPH for measuring radical scavenging activity. We have noted the ethylacetate extract showed 78 % of DPPH radical scavenging effect at concentration of 1 mg/ml. The compound manoalide from a Pacific sponge has spawned more than 300 chemical analogs, with a significant number of these going on to clinical trials as anti-inflammatory agents. To date reports concerning antioxidant metabolites from sponge extract are very rare. This study demonstrates that the sponge are an excellent source for the discovery of novel antioxidant.

In the present investigation the HRBC membrane stabilizing potency of sponge extracts of was carried out. From the results obtained for the *invitro* studies it was observed that the extracts exhibited excellent membrane stabilizing activity. Among all the concentrations, 100µl and 200µl exhibited excellent membrane stabilizing activity. Flavonoids and steroids are known to show remarkable anti-inflamatory activity by inhibiting the cox and lox systems [15,16]. Some works showed the correlation between presence of flavonoids and their membrane stabilizing ability [17].

Table 1:Antibacterial activity of (crude extract) at different concentrations.

Bacterial Pathogen	Zone of Inhibition (mm)				
	Standard	50µl	75µl	100µl	125µl
Proteus vulgaris	9	10	15	17	18
Klebsiella pneumonia	12	12	15	15	18
Pseudomonas aeruginosa	14	12	13	16	18
Staphylococcus aureus	9	11	13	15	17

Table 2:Anti cancer activity *against* MCF7 and Hep2 cell lines

S.no	Concentration(mg/ml)	Dilutions	Cell viability	
			MCF7	Hep 2
1	5	Neat	12.24	5.88
2	2.5	1:1	18.36	17.64
3	1.25	1:2	34.69	25.49
4	0.625	1:4	42.85	33.33
5	0.3125	1:8	51.02	54.90
6	0.156	1:16	65.30	62.74
7	0.078	1:32	75.51	70.58
8	0.039	1:64	91.83	86.27
9	Control	–	100	100

MTT assay was conducted to examine the relationship between concentrations of the extracts and the cytotoxicity of MCF7 and Hep2 cells. Cells were treated with extracts at increasing concentrations for 18 hours, and the percentage of cell viability was analyzed. The extracts were dissolved in DMSO, and a parallel experiment demonstrated that the final concentration of DMSO in the medium (0.1%) did not produce any impact on MCF7 and Hep2 cell cytotoxicity (data not shown). As revealed in Table 2 the extracts inhibited MCF7 and Hep2 cell growth in a dose-dependent manner. The concentrations of extracts causing 50% and 80% cell growth inhibition (IC50 and IC80) were determined and are presented in Table 2. The IC50 of *H. exigua* was approximately 0.31mg/ml for MCF7 and Hep2. The cell cytotoxicity assay demonstrates that the extract exhibited the highest potency in inhibiting cell growth (Fig.1a &b).

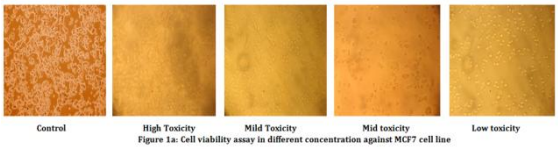

Figure 1a: Cell viability assay in different concentration against MCF7 cell line

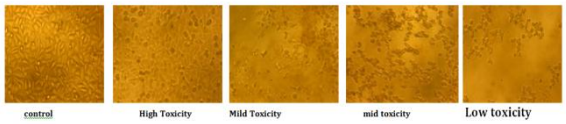

Figure 1b: Cell viability assay in different concentration against Hep2 cell line

The active fraction on the basis of spectral data by GC MS were found to be mixture of fatty acids which were observed on retention time as presented in Fig 2. The chromatogram *Haliclona exigua* active fraction found that the main constituent showed antibacterial, antioxidant, anti-inflammatory and anticancer compounds.

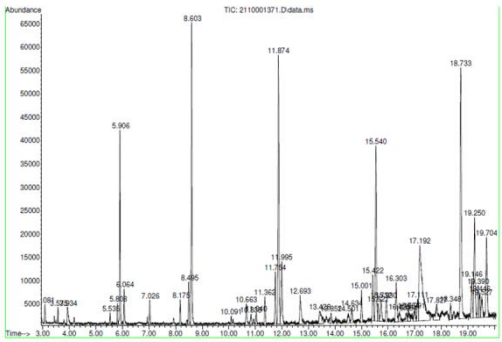

Figure 2: GC MS Spectral analysis

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