

# Antiproliferative and Proapoptotic Activities of Marine Sponge *Hyrtios erectus* Extract on Breast Carcinoma Cell Line (MCF-7)

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## ABSTRACT

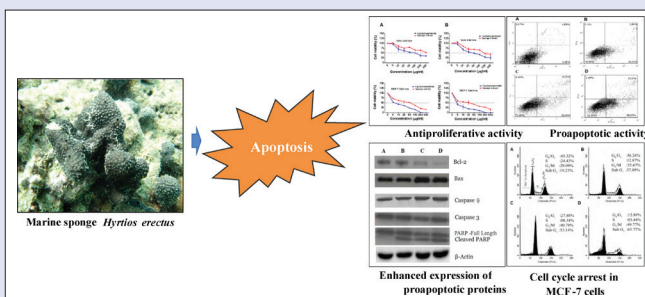
**Background:** Marine sponge is a rich natural resource of many pharmacologically important compounds. **Objective:** Marine sponge *Hyrtios erectus*, collected from North Bay, South Andaman Sea, India, was screened for potential antiproliferative and proapoptotic properties on a breast adenocarcinoma cell line (MCF-7). **Materials and Methods:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to test the antiproliferative and cytotoxicity effects of the sponge extract. Analysis of apoptosis and cell cycle stages were done by flow cytometry. The expression of several apoptotic-related proteins in MCF-7 cells treated by the extract was evaluated by Western blot analysis. Various analytical techniques including Fourier transform infrared spectroscopy, gas chromatography-mass spectrometry, and nuclear magnetic resonance were employed to determine the identity of the active compounds in the sponge extract. **Results:** *N*-Hexane extract of the sponge inhibited proliferation of the MCF-7 cell line in a dose- and time-dependent manner. Exposure of the sponge extract triggered apoptosis of the MCF-7 cells, induced DNA fragmentation, and arrested the cells in G<sub>2</sub>/M phase. Treatment of the sponge extract induced downregulation of antiapoptotic Bcl-2 protein and upregulation of Bax, caspase-3, caspase-9, and fragmented poly(ADP ribose)polymerase proteins in MCF-7 cells. Five bioactive compounds have been identified in the extract. **Conclusion:** The antiproliferative and proapoptotic activities of the tested extract suggested the pharmacologic potential of the identified compounds. Further characterization of the identified compounds are in progress.

**Key words:** Antiproliferative, apoptosis, breast carcinoma cells, *Hyrtios erectus*, marine sponge

## SUMMARY

- The *N*-hexane extract of the marine sponge *Hyrtios erectus*, collected from North Bay, South Andaman Sea, India, showed potential antiproliferative and proapoptotic properties against a breast adenocarcinoma cell line (MCF-7).

- The sponge extract retarded the growth of breast carcinoma cell line MCF-7 cells in a time- and dose-dependent manner.
- The sponge extract induced apoptosis of breast cancer cell line MCF-7 and arrested cells in G<sub>2</sub>/M phase.
- The sponge extract induced downregulation of Bcl-2 protein in MCF-7 cell line and upregulation of Bax, caspase-3, and cleaved PARP. Five bioactive compounds have been identified in the extract.



**Abbreviations used:** GC-MS: Gas chromatography-mass spectrometry; FT-IR: Fourier transform infrared spectroscopy; NMR: Nuclear magnetic resonance; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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## INTRODUCTION

The marine ecology, due to its phenomenal biodiversity, is a rich natural resource of many biologically active compounds. Marine organisms have shown to be potential sources of pharmacologically important bioactive compounds.<sup>[1]</sup> Among all marine organisms screened, marine sponges are the richest source of structurally diverse natural products and represent the single best source of marine bioactive compounds.<sup>[2]</sup> These compounds are secondary metabolites produced by the sponges and often serve defensive roles to protect the sponges from predators.<sup>[3,4]</sup> A broad spectrum of biologic activities for these compounds, including anticancer, antiviral, antibacterial, antifungal, antiprotozoal, anti-inflammatory, immunosuppressive, neurosuppressive, and neuroprotective activities, have been reported.<sup>[5]</sup> In recent years, several

anticancer compounds derived from marine sponges have entered preclinical and clinical trials.<sup>[6-8]</sup> One marine-derived compound, named

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eribulin mesylate, isolated from marine sponge *Halichondria okadae* has achieved phase III clinical trial<sup>[9]</sup> and has been approved by US Food and Drug Administration.

Over the last decade, different marine samples have been screened for a variety of compounds with different biologic activities. However, there is negligible report on the screening of sponge species from North Bay of South Andaman Sea, India. The marine sponge *Hyrtios erectus* [Figure 1], belonging to phylum Porifera, class Demospongiae, order Dictyoceratida and family Thorectidae, is blackish and attached to the sea bottom by means of masses of sand-filled fibers.<sup>[10]</sup> To the best of our knowledge, this sponge species collected from South Andaman Sea water has not been investigated before. In the present study, *N*-hexane extract of the sponge species from South Andaman Sea has been screened for its antiproliferative and proapoptotic activities on a breast adenocarcinoma cell line (MCF-7). A preliminary investigation of the sponge extract suggested the presence of five bioactive compounds and further studies are in progress.

## MATERIALS AND METHODS

### Reagents

All chemicals were purchased from Sigma-Aldrich (USA), unless stated otherwise. Cell culture plastics were from Midsci, USA.

### Collection of marine sponge

The marine sponge *H. erectus* was collected through scuba diving at depth varying from 10 to 15 m in and around North Bay of South Andaman Sea, India, in the month of December 2013. The taxonomy details were studied and a voucher specimen was deposited to Division of Aquaculture and Fisheries Sciences, Central Inland Agricultural Research Institute (ICAR), Andaman and Nicobar Islands, India, with a registration number PB5985.

### Preparation of sponge extract

Freshly collected sponge samples were cut into small pieces and freeze dried. The dried sponge samples (1 kg) were macerated with methanol for 24–48 h. After maceration, the solution was filtered and evaporated to dryness on a rotary vacuum evaporator (STRIKE 202, Germany) set at a maximum temperature of 40°C. This constituted the crude extract (45 g), which was dissolved in distilled water and partitioned subsequently with

*n*-hexane, ethyl acetate, and *n*-butanol. The extracts were weighed and stored at -20°C until used. Initial cytotoxicity screening showed that *n*-hexane fraction, among the three fractions mentioned above had the highest antiproliferative activity against the MCF-7 cell line and was chosen for this study. The extract was dissolved in Dimethyl sulfoxide (DMSO) (10 mg/mL) and further diluted in medium to get the final testing concentrations.

### Cell lines and cell culture

The cell lines MCF-7 (breast adenocarcinoma) and Vero (nontumorous cells) were obtained from National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 mU/mL penicillin, and 100 µg/mL streptomycin. Cell cultures were incubated in a humidified atmosphere of 5% CO<sub>2</sub> in air and 37°C, and upon reaching 80% confluence, were passaged with a solution of 0.25% trypsin-EDTA. Exponentially growing cells were used for all the experiments.

### *In vitro* cytotoxicity assay

The *in vitro* cytotoxicity of the sponge extract was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded in a 96-well plate at a density of 10<sup>4</sup> cells/well and after 24 h of adherence, the cells were treated with different concentration (0–500 µg/mL) of the sponge extract and incubated for 24 and 48 h at 37°C in an atmosphere of 5% CO<sub>2</sub> in air. Untreated cells were used as the control. After the incubation period (24–48 h), the medium was removed, 200 µL of PBS was used to wash the cells, and 100 µL MTT solution (1 mg/mL) was added to each well followed by incubation for 4 h under the condition mentioned above. Then the MTT-containing medium was removed and the formazan crystals were dissolved with 100 µL of DMSO. Absorbance was measured at 570 nm using a microplate reader (Spectra Max Plus, Molecular Devices, USA). The cell viability percentage was calculated using the following formula

$$\text{Viability (\%)} = \frac{[A570 (\text{treated cells}) - (\text{background})]}{[A570 (\text{untreated cells}) - \text{background}]} \times 100.$$

The concentration that gave a 50% reduction in the number of living cells (IC<sub>50</sub>) was estimated. Based on the *in vitro* cytotoxicity assay, extract in concentrations 25, 50, and 100 µg/mL were used for further experiments.

### Determination of cell cycle stages

Cell cycle stages were analyzed by flow cytometry as previously described.<sup>[11]</sup> MCF-7 cells were treated with different concentrations (25, 50, and 100 µg/mL) of the sponge extract for 24 h. Control and treated cells were fixed by treatment of 70% ethanol, washed twice by PBS, and cell concentration adjusted to 1 × 10<sup>6</sup> per mL. One hundred microliters of cell suspension was treated with 200 µL propidium iodide (PI) at 4 °C for 30 min and analyzed by using a flow cytometer (FAC Scan, Becton Dickinson). Cell cycle histograms were analyzed using Cell Quest software.

### Apoptosis assay

To determine the effect of the sponge extract on apoptosis, MCF-7 cells were cultured in DMEM with 10 % FBS in the presence of different concentrations (25, 50, and 100 µg/mL) of the extract. After 48 h, the cells were harvested, washed in PBS, and stained with Annexin V-FITC and PI according to the manufacturer's instruction (Invitrogen, Life Technologies, Indiana, USA). The cells were incubated at room temperature for 15 min and acquired using a flow cytometer (FAC Scan, Becton Dickinson). The data were analyzed using FCS Express 4 software (www.denovosoftware.com).



**Figure 1 :** Marine sponge *Hyrtios erectus*. Morphologically, it is blackish and attached to the sea bottom by means of masses of sand-filled fibers

## DNA fragmentation assay

The MCF-7 cells ( $1 \times 10^6$  cells) were incubated with media containing different concentrations (25, 50, and 100  $\mu\text{g}/\text{mL}$ ) of the sponge extract for 24 h. After cells were washed with PBS and DNA was isolated using Blood and Cell Culture DNA Mini Kit, Qiagen, as per manufacturer's protocol. The DNA fragments were separated by electrophoresis in 2% agarose gel and visualized in the presence of 0.5  $\mu\text{g}/\text{mL}$  ethidium bromide.

## Nuclear morphology assessment

The breast cancer MCF-7 cells were seeded at a density of  $5 \times 10^4$  cells/well into a six-well tissue culture plate. After achieving 80% confluence, the cells were incubated in media containing different concentrations (25, 50, and 100  $\mu\text{g}/\text{mL}$ ) of the sponge extract for 24 h. The cells were then washed with PBS, fixed in methanol:acetic acid (3:1) for 10 min and stained with 50  $\mu\text{g}/\text{mL}$  of PI for 20 min. Nuclear morphology of the cells was examined under a fluorescent microscope and images were acquired with excitation and emission wavelength of 488 and 550 nm, respectively. At least  $1 \times 10^3$  cells were counted.

## Western blot assay

MCF-7 cells were incubated with media containing different concentrations (25, 50, and 100  $\mu\text{g}/\text{mL}$ ) of the sponge extract for 24 h. Cells were washed three times with PBS, lysed in cell lysis buffer containing 0.05 mol/L Tris-HCl (pH 7.5), 0.15 mol/L NaCl, 0.001 mol/L PMSF phenylmethylsulfonyl fluoride, 0.001 mol/L EDTA (pH 8.0), 1% Triton X-100, 0.1% SDS, and 2  $\mu\text{g}/\text{mL}$  Leupeptin, and centrifuged at 12,000 rpm for 5 min at 4°C. The protein concentrations of the samples were determined by a colorimetric assay using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Twenty-five micrograms of protein sample were separated by 13% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride (PVDF) membrane. Then, the membrane was blocked with 5% skim milk powder in TBS-Tween buffer (0.12 M Tris-base, 1.5 M NaCl, 0.1% Tween 20) for 1 h at room temperature and incubated overnight at 4°C with the appropriate primary antibodies treated with TBST containing 5% skimmed milk at room temperature for 1 h followed by incubation with primary antibodies Bcl-2, Bax, caspase-3, caspase-9, PARP, and  $\beta$ -actin at 4°C overnight. After incubating with anti-mouse peroxidase-conjugated secondary antibody, the signal was visualized by Enhanced Chemiluminescence (Amersham Pharmacia Biotech).

## Chemical analysis of the sponge extract

The methanol extract of *H. erectus* sponge was subjected to silica gel column chromatography followed by purification through high-pressure liquid chromatography (HPLC) to identify the bioactive compounds. Various analytical techniques were employed to determine the identity of these constituent compounds. The Gas chromatography-mass spectrometry (GC-MS) spectrum was taken on GC-MS (Thermo Scientific GC-Trace Ultra Ver. 5.0, USA) system equipped with a quantitative analysis by SIM mode detector as described.<sup>[12]</sup> For the MS detection, the electron ionization mode with ionization energy 70 eV was used, with a mass range at  $m/z$  50–650. ADB5-MS Capillary Standard Non-Polar Column (30 m  $\times$  0.25 mm, film thickness 0.25  $\mu\text{m}$ ) was used for GC-MS. Helium gas was utilized as carrier gas at a flow rate of 1.0 mL/min. Compounds were identified by their retention times and mass fragmentation patterns using data of standards at Wiley 7.0 library. To analyze the functional group of the bioactive compounds, the Fourier transform infrared spectroscopy (FT-IR) was carried out as described<sup>[13]</sup> on Shimadzu 8201 PC (4000–400  $\text{cm}^{-1}$ ) by making pellets with KBr (potassium bromide) in the diffuse reflectance mode at a

resolution of 4  $\text{cm}^{-1}$  in KBr pellets. Two milligram dried purified sample were used for FT-IR measurements. Nuclear magnetic resonance (NMR) measurements were done at Central Drug Research Institute, Lucknow, India. Sponge-purified fraction dissolved in DMSO-*d*<sub>6</sub> solvent was used for NMR spectroscopy. <sup>1</sup>H and <sup>13</sup>C spectra were recorded at Bruker Avance DRX 400 NMR spectrometer.

## Statistical analysis

All the experimental data were expressed as mean  $\pm$  standard deviation. Statistically significant differences among means were assessed by one-way analysis of variance (ANOVA) with Dunnett's posttest using Graph Pad Prism version 6.00, Graph Pad Software (San Diego, California, USA). Data with *P* less than 0.05 was considered statistically significant.

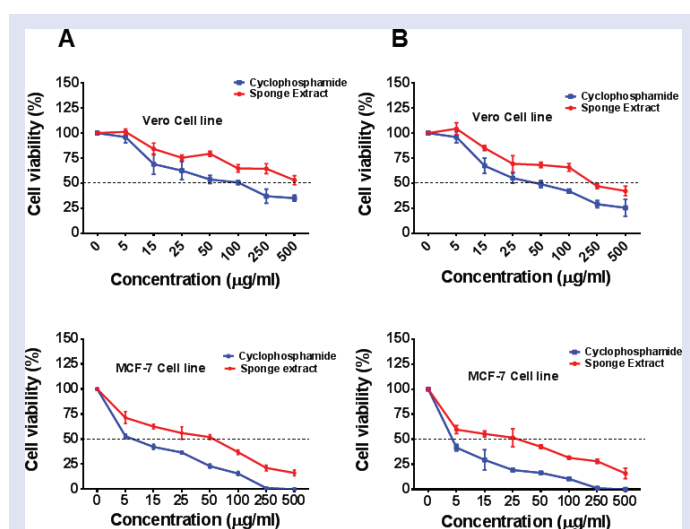
## RESULTS

### The sponge extract inhibited the proliferation of breast adenocarcinoma (MCF-7) cell line

To test the therapeutic use of the sponge extract on breast cancer, we examined the cytotoxic effect of the sponge extract on the proliferation of breast adenocarcinoma cell line (MCF-7). The effect of different concentrations (0–500  $\mu\text{g}/\text{mL}$ ) of the extract on cell viability of MCF-7 cell line is presented in Figure 2. The sponge extract showed marked cytotoxic effect on the MCF-7 cell line. The cell viability was reduced gradually with treatment of increasing concentrations of the sponge extract [Figure 2]. The determined half-maximal activity concentrations ( $\text{IC}_{50}$ ) of the extract on the MCF-7 cell line were 50 and 25  $\mu\text{g}/\text{mL}$  for 24 and 48 h, respectively. On the other hand, the  $\text{IC}_{50}$  values on noncancerous Vero cell line were 500 and around 250  $\mu\text{g}/\text{mL}$  for 24 and 48 h, respectively. Overall, the cytotoxicity assay depicts that the sponge extract inhibited the proliferation of the breast carcinoma cell line (MCF-7) in a dose-dependent manner, whereas it had minimal cytotoxic effect on the normal cell line (Vero).

### The sponge extract arrested the MCF-7 cells at $G_2/M$ phase

To explore the intrinsic mechanisms by which the sponge extract regulates growth inhibition in MCF-7 cells, we analyzed the effect of the



**Figure 2:** Cytotoxic effect of the sponge extract on MCF-7 cell line. MCF-7 and Vero cells were exposed to the extract for 24 h (A) or 48 h (B). MTT assay was done to evaluate the cytotoxic effect of the sponge extract. The values and error bar represent average and standard deviations of three independent set of experiments

extract on cell cycle progression. We found that MCF-7 cells cultured in DMEM and 10% FBS showed distribution of cells in  $G_0/G_1$ , S, and  $G_2/M$  phase [Figure 3]. Addition of the extract for 24 h resulted in a dose-dependent cell cycle arrest of MCF-7 cells. The extract decreased  $G_0/G_1$  and S phase and increased  $G_2/M$  phase [Figure 3]. In control

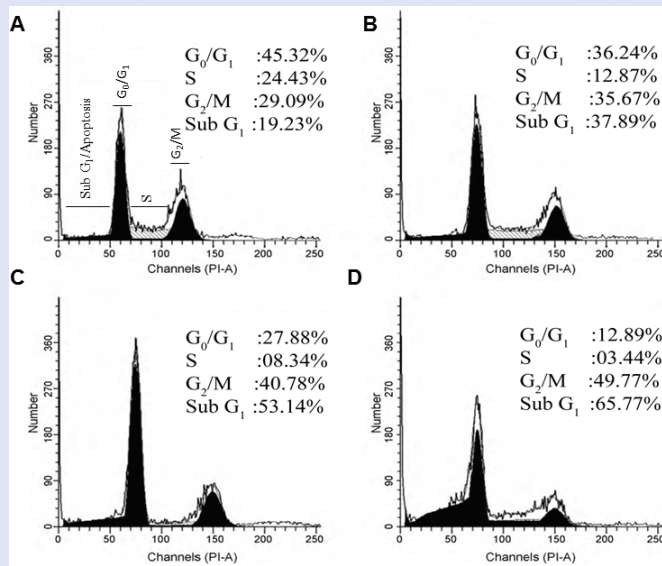
group, 45.32% cells were in  $G_0/G_1$  phase and 29.09% cells were in  $G_2/M$  phase, whereas in 100  $\mu\text{g}/\text{mL}$  sponge extract group, only 12.89% cells were in  $G_0/G_1$  phase and 49.77% cells were in  $G_2/M$  phase [Figure 3], suggesting that the sponge extract induced cell cycle arrest in  $G_2/M$  phase. It was also interesting to find that, incubation of MCF-7 cells with different concentrations of the extract for 24 h significantly increased the proportion of cells in sub- $G_0/G_1$  phase or apoptosis sub- $G_1$  phase compared with control (37.89, 53.14, and 65.77% at 25, 50, and 100  $\mu\text{g}/\text{mL}$  sponge extract concentrations vs. 19.23% in control). Reduced DNA content in sub- $G_0/G_1$  phase is indicative of apoptosis with loss of cells in  $G_1$  phase.

### The sponge extract induced apoptosis of MCF-7 cells

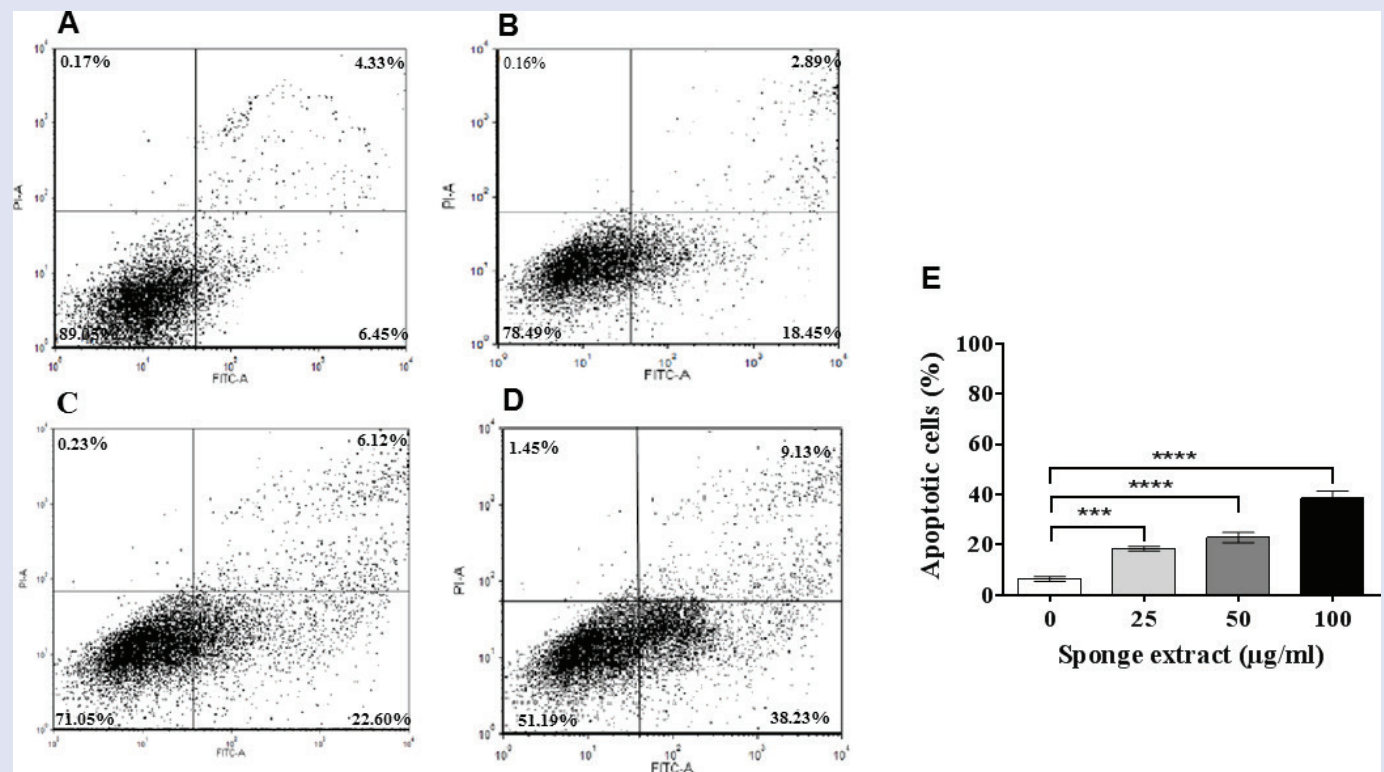
To further determine the mechanisms by which the extract regulates the cytotoxicity of MCF-7 cells, we examined the effect of the sponge extract on apoptosis. We found that apoptotic cells increased dose dependently following addition of the extract in culture media [Figure 4]. MCF-7 cells cultured in the absence of the extract showed 6.45% Annexin-V positive apoptotic cells, which increased to 18.45, 22.60, and 38.23% following addition of 25, 50, and 100  $\mu\text{g}/\text{mL}$  of the extract, respectively [Figure 4].

### The sponge extract induced DNA fragmentation of MCF-7 cells

The effect of the sponge extract on DNA fragmentation and nuclear morphology of MCF-7 cells was investigated. It was found that the extract induced DNA fragmentation of MCF-7 cells. Following treatment, the



**Figure 3:** Effect of the sponge extract on cell cycle stages of MCF-7 cells. Cell cycle stages were analyzed by flow cytometry (A) control (0  $\mu\text{g}/\text{mL}$ ), (B) 25  $\mu\text{g}/\text{mL}$ , (C) 50  $\mu\text{g}/\text{mL}$ , and (D) 100  $\mu\text{g}/\text{mL}$  of sponge extract



**Figure 4:** Effect of the sponge extract on apoptosis of MCF-7 cells. Apoptotic cells were stained by Annexin-V and detected by flow cytometry. Concentration-dependent increase in percentage of apoptotic cells was found: (A) Control (0  $\mu\text{g}/\text{mL}$ ), (B) 25  $\mu\text{g}/\text{mL}$ , (C) 50  $\mu\text{g}/\text{mL}$ , (D) 100  $\mu\text{g}/\text{mL}$  of sponge extract. (E) The histogram shows the mean Annexin-V positive MCF-7 cells (mean  $\pm$  SD) of three experiments. ANOVA followed by Dunnett's posttest was performed to find out significant difference among treatments. \*\*\* $P \leq 0.001$ . \*\*\*\* $P \leq 0.0001$

DNA fragments of MCF-7 cells showed a ladder-like pattern [Figure 5A]. Nuclear morphology changes of MCF-7 upon treatment of different concentrations of the sponge extract is presented in Figure 5B–D. Loss of DNA, a hallmark of apoptosis, occurred as a result of diffusion of degraded DNA out of the cells after endonuclease cleavage and after staining with PI [Figure 5B–D].

### The sponge extract induced downregulation of Bcl-2 and upregulation of Bax, caspase-3, caspase-9, and cleaved poly (ADP ribose) polymerase proteins

The effect of the sponge extract on the expression of Bcl-2, Bax, caspase-3, caspase-9, and cleaved poly (ADP ribose) polymerase (PARP) in cultured MCF-7 cells was investigated by Western blotting. Treatment of MCF-7 cells with different concentrations (25, 50, and 100 µg/mL) of the sponge extract downregulated the expression of antiapoptotic Bcl-2 protein and upregulated the expressions of proapoptotic proteins, i.e., Bax, caspase-3, caspase-9, and cleaved PARP [Figure 6].

### Chemical analysis of the sponge extract

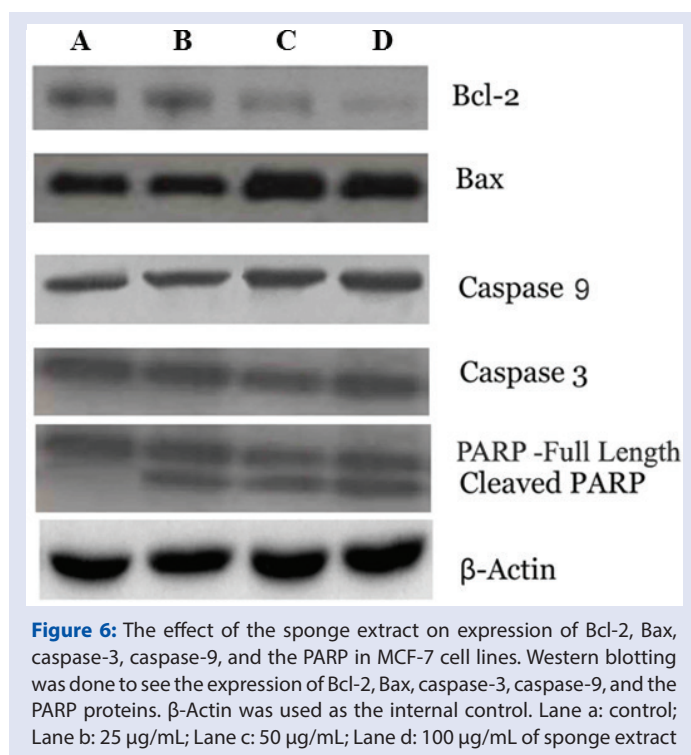
From the chromatographic purification and spectroscopic analysis, five major alkaloid compounds were identified from the marine sponge extract. Among them, the structures of four compounds, i.e., 5-hydroxy-3-(2-hydroxyethyl) indole (Compound 1), 1,6-dihydroxy-1,2,3,4-tetrahydro-β-carboline (Compound 2), hyrtiosin B (Compound 3), and hyrtiosulawesine (Compound 4), were previously reported<sup>[10]</sup> (Supplementary Figure S1). One new compound was also isolated as white solid and identified as 2-chloro-6-phenyl-8H-quinazolino[4,3-b]quinazolin-8-one (Compound 5) based on the GC-MS, FT-IR, and NMR data (Supplementary Figure S1).

### DISCUSSION

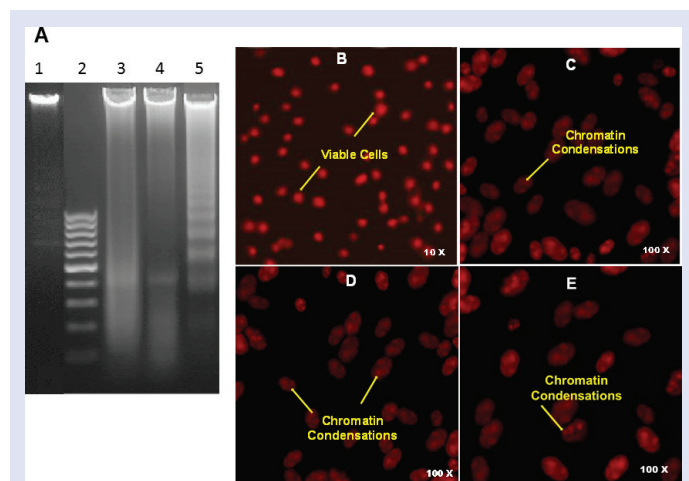
Breast cancer is the most common malignancy globally, with approximately one third of the women with breast cancer developing metastases and ultimately dies of the disease.<sup>[14]</sup> Despite an extensive

research over several decades and better understanding of the mechanisms of the breast cancer, treatment of this devastating disease is unsuccessful in most of the cases. There is a critical need for development of more effective drugs against breast cancer. Marine organisms, especially marine sponges, are the hotspots of many pharmacologically important bioactive compounds. Extracts from marine sponges have shown cytotoxic activities against different types of cancers.<sup>[15,16]</sup> In the present paper, antiproliferative and proapoptotic activities of marine sponge *H. erectus* extract on a breast carcinoma cell line (MCF-7) were evaluated.

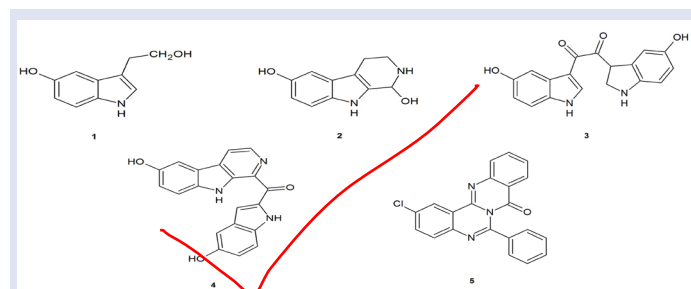
Breast adenocarcinoma cell line MCF-7 has become a prominent model system for the study of breast cancer.<sup>[17]</sup> The MTT assay showed that the sponge extract could inhibit the proliferation of breast cancer MCF-7 cells in a dose- and time-dependent manner [Figure 2]. The IC<sub>50</sub> value of the extract on the MCF-7 cell line was 25 µg/mL for 48 h, whereas that on noncancerous Vero cell line was around 250 µg/mL. It was found that the sponge extract induced apoptosis of the MCF-7 cells and arrested the cells in G<sub>2</sub>/M phase [Figures 3 and 4]. The basic apoptotic



**Figure 6:** The effect of the sponge extract on expression of Bcl-2, Bax, caspase-3, caspase-9, and the PARP in MCF-7 cell lines. Western blotting was done to see the expression of Bcl-2, Bax, caspase-3, caspase-9, and the PARP proteins. β-Actin was used as the internal control. Lane a: control; Lane b: 25 µg/mL; Lane c: 50 µg/mL; Lane d: 100 µg/mL of sponge extract



**Figure 5:** Effect of the sponge extract on DNA fragmentation and nuclear morphology of MCF-7 cells. (A) Gel electrophoresis result showing effect of sponge extract on DNA fragmentation of MCF-7 cell line. Lane 1: control; Lane 2: marker; Lane 3: 25 µg/ml extract-treated cells; Lane 4: 50 µg/ml extract-treated cells; Lane 5: 100 µg/ml extract-treated cells. (B–E) Nuclear morphology changes of MCF-7 cells at different concentration of sponge extract treatment: (B) control (0 µg/ml), (C) 25 µg/ml, (D) 50 µg/ml, and (E) 100 µg/ml of extract-treated cells



**Supplementary Figure 1:** The chemical structures of secondary metabolites isolated from the marine sponge *Hyrtios erectus*. (1) 5-hydroxy-3-(2-hydroxyethyl) indole, (2) 1,6-dihydroxy-1,2,3,4-tetrahydro-β-carboline, (3) hyrtiosin-B, (4) Hyrtiosulawesine, (5) 2-chloro-6-phenyl-8H-quinazolino[4,3-b]quinazolin-8-one

pathway includes extrinsic signaling pathway and intrinsic signaling pathway. The intrinsic signaling pathway, also called the mitochondrial pathway, is strictly controlled by the Bcl-2 family of proteins.<sup>[18]</sup> Overexpression of Bcl-2 inhibits apoptosis by preventing cytochrome C release from the mitochondria and by inhibiting caspase-3 activity.<sup>[19]</sup> Bax, belonging to the proapoptotic family members, is crucial for inducing permeabilization of the outer mitochondrial membrane and the subsequent release of apoptogenic molecules, which leads to caspase activation.<sup>[20,21]</sup> The Bcl-2 interacts with Bax as homodimers leading to the tumor development, and the ratio of Bcl-2/Bax has been shown to be very critical in determining the susceptibility of cells to apoptosis. From [Figure 6], it is evident that downregulation of antiapoptotic Bcl-2 caused an increase in the proapoptotic Bcl-2/Bax ratio in the MCF-7 cell line after exposure to the sponge extract, suggesting that Bcl-2 participated in the sponge extract-induced apoptosis in breast cancer cells. These findings clearly demonstrate that the sponge extract induced apoptosis in MCF-7 cells, which might account for its anticancer activities.

Highly condensed chromatin in a fragmented nucleus are characteristics of apoptotic cells.<sup>[22]</sup> The morphology of sponge extract-treated MCF-7 cells showed shrunken cytoplasm, condensed and fragmented nuclei with features of apoptosis [Figure 5B–E]. One of the hallmarks of apoptosis is the digestion of genomic DNA by endonuclease, generating a ladder of small fragments of DNA.<sup>[23]</sup> In the present experiment, similar findings were observed [Figure 5A]. Thus, the morphologic observations along with DNA fragmentation and flow cytometry analysis confirmed the apoptotic mode of cell death for MCF-7 cells induced by the sponge extract.

Chemical analysis of the sponge extract revealed presence of five major alkaloid compounds, i.e., 5-hydroxy-3-(2-hydroxyethyl) indole (Compound 1), 1,6-dihydroxy-1,2,3,4-tetrahydro- $\beta$ -carboline (Compound 2), hyrtiosin B (Compound 3), hyrtiosulawesine (Compound 4), and 2-chloro-6-phenyl-8H-quinazolino [4,3-b] quinazolin-8-one (Compound 5). A number of biologic activities including antioxidant and anticancer are associated with quinazoline.<sup>[24-26]</sup> Most of quinazolines exert their antitumor activity through inhibition of PARP-1 enzyme, which is involved in a variety of physiologic functions including DNA replication and repair.<sup>[26]</sup> The functional characterization of the bioactive compounds will clarify the mechanisms of antiproliferative activity of the sponge extract.

Increasing reports on discoveries of novel bioactive compounds from marine ecosystems pose a great threat to seabed and coral reefs due to overexploitation of the bio resources. Moreover, climate change specially increased sea surface temperature has negative consequence on marine ecosystems. Massive coral bleaching and destruction of seabed in the recent past has been reported throughout the world, including Andaman Sea.<sup>[27-29]</sup> For the protection of the sea life, it is high time to seek possibilities of artificial harvesting of marine resources including marine sponges. Sea sponge aquaculture and sponge farming have shown promising results.<sup>[30,31]</sup> As production of bioactive compounds depend on marine microenvironment, artificial harvesting of the marine-derived compounds remains to be a great challenge. Although report on artificial harvest of marine sponge *H. erectus* is negligible, exploring the possibilities of its aquaculture may open up new windows for future research.

## CONCLUSION

The screening of the marine sponge *H. erectus* from North Bay of South Andaman Sea, India, suggests that this species accumulated compounds that induce a series of responses associated to apoptotic death in the

breast carcinoma cell line MCF-7. Five compounds have been identified in the extract, which most likely contributes to the observed bioactivities, and further functional characterization of the identified compounds is currently underway.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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