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ANTICANCER ACTIVITY OF CRUDE EXTRACTS OF TWO SPONGES, *FASCIOSPONGIA CAVERNOSA* DOC VAR. BROWN & *FASCIOSPONGIA CAVERNOSA* DOC VAR. YELLOW

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ABSTRACT

This study reports the in vitro screening of two marine sponges (Porifera) collected from the coastline of Bay of Bengal, Visakhapatnam Coast, in the search for novel pharmaceuticals. In this study, the anticancer activity exhibited by crude sponge extracts from *Fasciospongia cavernosa* doc var. brown and *Fasciospongia cavernosa* doc var. yellow from Visakhapatnam coast of Bay of Bengal were reported. Antitumor activities were determined by a cell growth assay with three different tumor cells lines, HeLa (Human cervical cancer), A-549 (Human lung cancer cell line) and HEK-293 (Human kidney cell line) using SRB method. Among the two varieties of sponges *Fasciospongia cavernosa* doc var. brown displayed a high anticancer activity. *Fasciospongia cavernosa* doc var. brown marine sponge exhibited strong cytotoxic activities against all tumor cell lines. These extracts are currently undergoing further analysis to identify the active constituents.

KEY WORDS: **Marine sponge; Tumor Cell lines; Antitumor activity; SRB assay**

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INTRODUCTION

Natural products have served as important chemical prototypes for the discovery of new molecules, and continue to be the most promising source of drug leads, especially in the anticancer field. There is a high risk of cancer morbidity worldwide. The most common cancers in the world are prostate, colorectal, bowel, breast, melanoma and lung cancer, which are leading causes of death. In the Cancer treatment there is rate of failure with exceeding side effects from the use of chemotherapy. Additionally, the present treatment lacks the alternative anticancer drug resources, such as macrolides, porphyrins, terpenoids, aliphatic cyclic peroxides and sterols[10].In the last decades researchers of natural products chemistry focused their research in a wide variety of bioactive compounds from very fertile field for the discovery of bioactive natural chemical substances with respect to the diversity of their primary and secondary chemical components and metabolites. Natural products and their analogs or molecules derived there of comprise approximately 50% of the drugs presently used for the clinical purposes. Regarding anticancer drugs, 63% of them fall into this category[3,4] . Marine sponges have a bright potential in anticancer drug discovery as they represent a major source of new antitumor and anticancer drugs. During the last decade, hundreds of new compounds have been identified with significant bioactivities, such as anti-angiogeneic, anti-tumor and anti-inflammatory activities. In recent years marine organisms have been screened for a variety of compounds with different biological activities. Among all organisms screened, sponges represent one of the most promising sources of marine bioactive compounds particularly for pharmaceutical leads. Sponges are among the most studied zoological groups by marine chemists and pharmacologists, while showing the highest rates of cytotoxic molecules. Several studies also describe antitumor activity [13,6,14]. The present study is designed to screen the anticancer activity of two sponges collected from Bay of Bengal near Visakhapatnam Coast against human tumor cell lines.

2. MATERIALS AND METHODS

2.1. Sponge collection

The two sponges *Fasciospongia cavernosa* doc var. brown (Figure 1) and *Fasciospongia cavernosa* doc var. yellow (Figure 2) were collected by SCUBA diving at depths of 3–20 m in the Bay of Bengal near Visakhapatnam Coast (GPS: 24˚21.432 N; 28˚72.725 E) of Andhra Pradesh, INDIA[16]. The sponge samples soon after collection were transferred to a sterile polyethylene bag and transported under frozen condition to the laboratory for the identification of anticancer activity of the sponges.

2.2. Extract preparation

The sponge specimens were extracted in different solvents like Hexane, Ethyl acetate, Ethanol, Methanol etc. Among all the solvents Methanol showed good extraction of the compounds and continued further with methanol. The sponges were extracted in methanol four times by maceration in methanol (0.3 g/mL), over 4 days. The methanolic extract was reserved each of these days. After the fourth day, the solutions were blended, filtered, concentrated in a rotary evaporator and dried in a Speed Vacuum evaporator.

2.3. Cell culture

The cell lines HeLa (Human cervical cancer), A-549 (Human lung cancer cell line) and HEK-293 (Human kidney cell line) were obtained from American type culture collection Manassas, VA, and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine and 100 µg/mL Penicillin-Streptomycin incubated at 37^0C , humidified atmosphere with 5% CO_2.

2.4. Growth inhibition of human tumour cell lines

The effects of crude sponge extracts on the growth of human tumour cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye Sulforhodamine B (SRB) to assess the cell growth. This colorimetric assay estimates cell number indirectly, by staining cellular protein with the protein-binding dye SRB. Each cell line was plated at an appropriate density (5.0 x 10^3 cells/well) in 96-well plates and allowed to attach for 24 hours [17]. Exponentially growing cells were further exposed for 48 hours to five serial concentrations (150; 75; 37.5; 18.75; 9.37 µM) of crude sponge extracts. Following this incubation period, the adherent cells were fixed with 10% trichloroacetic acid (final concentration), washed with 1% acetic acid, and stained with SRB. The bound stain was solubilized with 10 mMTris and the absorbance was measured at 492 nm in a micro plate reader (BIORAD 680 model). The concentration that inhibited cell growth in 50% (GI_{50}) was calculated[11]. Viability of cells was quantified as a percentage compared to that of control. The experiment was carried out in triplicate and the data were expressed as mean from these three sets of experiments.

3. RESULTS AND DISCUSSION

The sponge specimens collected from Visakhapatnam coast of Bay of Bengal were represented in Figure1 and Figure 2. The associated microorganism isolated from two sponges has shown good antibacterial and antifungal activity against various bacteria and fungi [16]. The associated isolates also showed good amylolytic and proteolytic activity[16]. There are several reports on the antibacterial and antifungal activity of the associated microorganisms from sponges[2]. In the present study the crude methanolic extracts of the two sponges , *Fasciospongia cavernosa* doc var. brown and *Fasciospongia cavernosa* doc var. yellow to inhibit the proliferation of human HeLa, A-549, and HEK-293 cell line were investigated using the protein-binding dye SRB

method. The ability of the compounds in the results from cell counting showed that the compound, at all the doses tested, inhibited the proliferation of cells. After incubation for 48 hours with crude sponge methanolic extracts, the effect of extracts on the *in vitro* growth of HeLa, A-549, and HEK-293 cell lines were evaluated by the SRB method. The Results were represented in Table-1. As shown in Table 1, both the sponge extracts were active against the cancer cell lines. Among two sponge extracts *Fasciospongia cavernosa* doc var. brown sponge extract exhibiting a moderate growth inhibitory effect against HeLa, A-549, and HEK-293 cell lines. *Fasciospongia cavernosa* doc var. yellow sponge extract exhibited a weak growth inhibitory effect against HeLa, A-549 cells and inactive against HEK-293 cell line at the highest concentration tested (GI_{50}>150 µM). The anticancer property of cell free extracts from sponge isolates might be due to the presence of the active secondary metabolites such as alkaloids and quninine[7]. Alkaloids are microtubule interfering agents which can bind with beta tublin, thus preventing the cell from making the mitotic spindle fibers necessary to move the chromosome around as the cell divides[15], inhibiting topoisomerase[5], mitochondrial damage and inducing the release of cytochrome C and apoptosis inducing factor. Moreover, quinine derivatives viz., driamycin, dauno- rubicin, mitomycin C, streptonigrin and lapachol, can interfere the DNA and RNA replication and mitochondrial oxidative pathways or the formation of super oxide, peroxide and hydroxide radicals as toxic products in the cell line. Several compounds of anthro-quinone families (parimycin, trioxacarcins and gutingimycin) showed antitumor activities [2,8,9]. Presently we are underway in purification of the anticancer compounds in the sponge extracts based upon the Thin Layer Chromatography studies.

Table 1
Growth inhibitory effect of compounds 1 to 3 in three human tumour cell lines GI_{50} (µM)

GI_{50} (150 µM)			
Sponge	**HeLa**	**A-549**	**HEK-293**
Fasciospongia cavernosa doc var. brown	49.2±2.1	54.3±1.5	51.1±1.5
Fasciospongia cavernosa doc var. yellow	102±1.4	132±1.1	>150

Figure1
Fasciospongia cavernosa doc var. brown

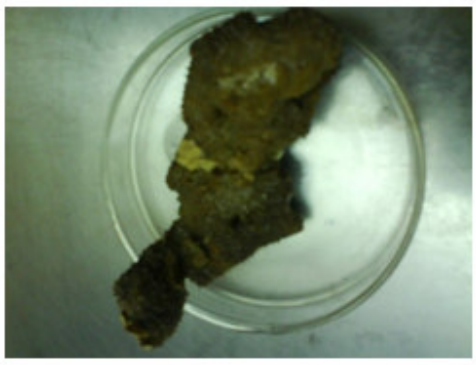

Figure 2
Fasciospongia cavernosa doc var. yellow

4. CONCLUSION

The cytotoxic nature of the compound inhibited the proliferation of tumor cells tested using SRB method. In the present study experiments were carried using HeLa, A-547 and HEK-293 cell lines. The crude extracts of the two sponges were able to inhibit the cell proliferation significantly. In the present study it is concluded that the methanolic crude extracts of the *Fasciospongia cavernosa* doc var. brown is significantly rich with anticancer compounds compare to *Fasciospongia cavernosa* doc var. yellow against the human cell lines. Further studies in purification of the anticancer compounds from *Fasciospongia cavernosa* doc var. brown is underway using thin layer chromatography studies.

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