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Research Article

CYTOTOXIC ACTIVITY OF METHANOL AND DICHLOROMETHANE EXTRACTS FROM MARINE SPONGES

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ABSTRACT

The study was aimed to evaluate the anticancer activity of the Blue Hard Neopetrosia sps on the HeLa cell line. The Blue Hard Neopetrosia sps methanol and Dichloromethane extracts were tested for its inhibitory effect on HeLa cell line. The percentage viability of the cell line was carried out by using colorimetric method. The cytotoxicity of Blue hard Neopetrosia on HeLa cell was evaluated by MTT assay. Both the methanol and Dichloromethane extract has significant cytotoxic effect on HeLa cell line in concentration range between 0.1µg/ml to 100µg/ml by using MTT assay. R2 value of Blue Hard Neopetrosia on HeLa cell of methanol extract was 0.9712 and for dichloromethane extract was o.7065 and percentage cell inhibition for methanol extract 58.12% and Dichloromethane extract was 72.32%.

From the performed assay both the extracts of the sponge shows greater activity on HeLa cell lines and that means it can be used as anti cancer activity.

Keywords: Marine sponges, Cytotoxicity, MTT assay, HeLa cell lines.

INTRODUCTION

Sponges (phylum Porifera) are most primitive of the multicelled animals that have existed for 700–800 million years. Of the approximately 15,000 sponge species, most occur in marine environments. Only about 1% of the species inhabits freshwater (Belarbi 2003). They inhabit every type of marine environment, from polar seas (Dayton 1974) to temperate and tropical waters (Reiswig 1973, Wenner 1983), and are often more abundant and diverse in the tropics than stony and soft corals (Targett & Schmahl 1984). These animals are frequently exposed to intense predation and /or tissue infection by microorganisms (Faulkner 2000; Newbold 1999;).

Sponges feed on bacteria (Bergquist 1978) and are constantly exposed to large populations of water-borne microbes, including opportunistic pathogens and fouling microorganisms. Despite these constant threats and a lack of the complex morphological and cellular defense mechanisms used by higher animals to combat bacterial pathogenesis (Simpson 1968, Zhuravleva 1970), sponges are highly successful members of the benthos and suffer few obvious bacterial infections.

The sponge class Demospongiae is known to produce the largest number and diversity of secondary metabolites isolated from marine invertebrates (Faulkner 1998). Although the functions of these secondary metabolites are largely unknown, there is some evidence that they provide chemical defenses against predators (Pawlik 1995, Chanas 1996). It has also been suggested that sponge secondary metabolites may provide defenses against fouling and infection (Amade & Chevolot 1982, Thompson et al. 1985, Pawlik 1993); however, this possibility has not been adequately explored.

A HeLa cell is an immortal cell line used in medical research. The cell line was derived from Cervical cancer cells taken from Henrietta Lacks, who died from her cancer in 1951. Initially, the cell line was said to be named after a "Helen Lane" in order to preserve Lacks's anonymity. The objective of this study is to investigate cytotoxic activity of methanolic and Dichloromethane extracts from sponges on HeLa cell lines using MTT assay.

MATERIALS AND METHODS

Collection of sponges

Blue Hard Neopetrosia species of marine demosponges were collected from the areas of the Rameswaram and Gulf of Mannar. Sponge samples were collected during September and October 2009. The sponges were collected at 1 to 27 m depth by snorkeling and SCUBA. Sponge sample was immediately frozen after collection and maintained at -20°C prior to extraction.

Chemicals & Reagents

MTT assay kit (Sigma chemical Co, USA), Dichloromethane and Methanol (In-house), All the chemicals used for the biochemical analysis were procured from Sisco research laboratory, India. HeLa cell lines were collected from Department of Biotechnology, Sri Ramachandra University, Chennai.

Extraction

Sponge specimen was allowed to thaw, cut into small pieces, and then taken in to a graduated Cylinder containing 1000 m1 of dichloromethane and in another cylinder with 1000ml of methanol and covered the cylinder with aluminum foil. And kept in cool and dry place for 15 days. Sponge tissue and solvent were transferred to capped containers and agitated for 24 h. After extraction, the sponge tissue was removed from the container and solvents squeezed from the tissue. The extract was collected by using vaccum separator. And the solution containing the extract at the volumetric concentration of the original tissue.

Cell lines and culture condition

The cytotoxicity of the Dichloromethane and methanol extracts was tested against HeLa cell lines. Cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 Ag/ml streptomycin, and 100 U/ml penicillin, and were incubated at 37^0C in a humidified atmosphere of 95% air and 5% CO_2.

Cytotoxic Bioassay

The proliferation rates of HeLa cells after treatment with essential oils were determined by the colorimetric 3-(4, 5-dimethyl-2-thiozolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble purple formazan, depending on the viability of cells.

HeLa cells were plated in 96-well plates (105 cells/well for adherent cells or 0.5_105 cells/well for suspended cells in 100 Al of medium). After 24 h, the extract (2–125 Ag/ml) dissolved in distilled water was added to each well and incubated for 3 days (72 h). Control groups received the same amount of distilled water. Tumor cell growth was quantified by the ability of living cells to reduce the yellow dye 3-(4, 5-dimethyl-2-thiozolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a purple formazan product. At the end of the incubation, the plates

were centrifuged and then, the medium was replaced by fresh medium (200 Al) containing 0.5 mg/ml MTT. Three hours later, the MTT formazan product was dissolved in 150 Al DMSO, and absorbance was measured using a multiplate reader (Spectra Count, Packard). Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm.

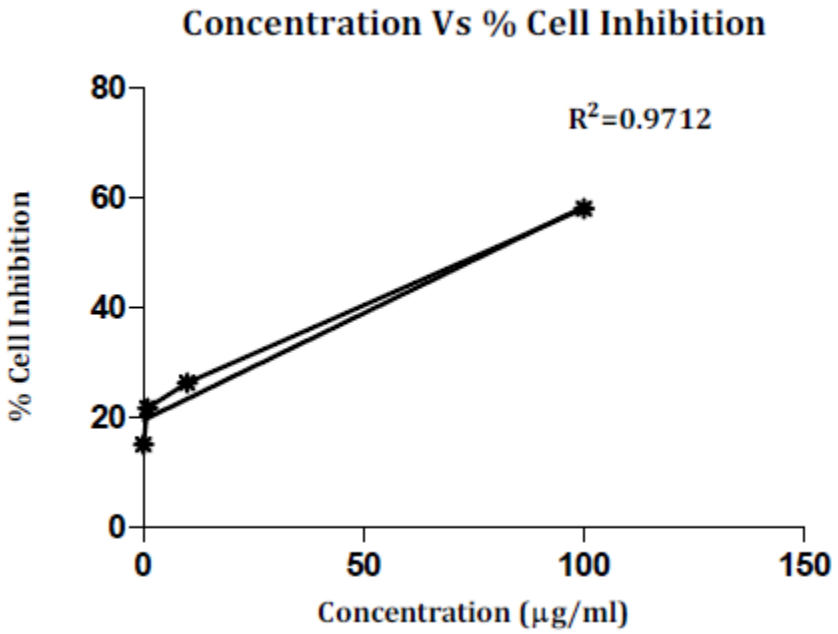

Fig. 1: Methanol Extract of Blue Hard Neopetrosia species for HeLa cell line by MTT assay

Table 1: Determination of cytotoxicity for Methanol extract by MTT assay.

Conc. (µg/ml)	% Cell Inhibition	% Cell Survival
0.1	15.25	84.75
1	21.81	78.18
10	26.43	73.56
100	58.12	41.88

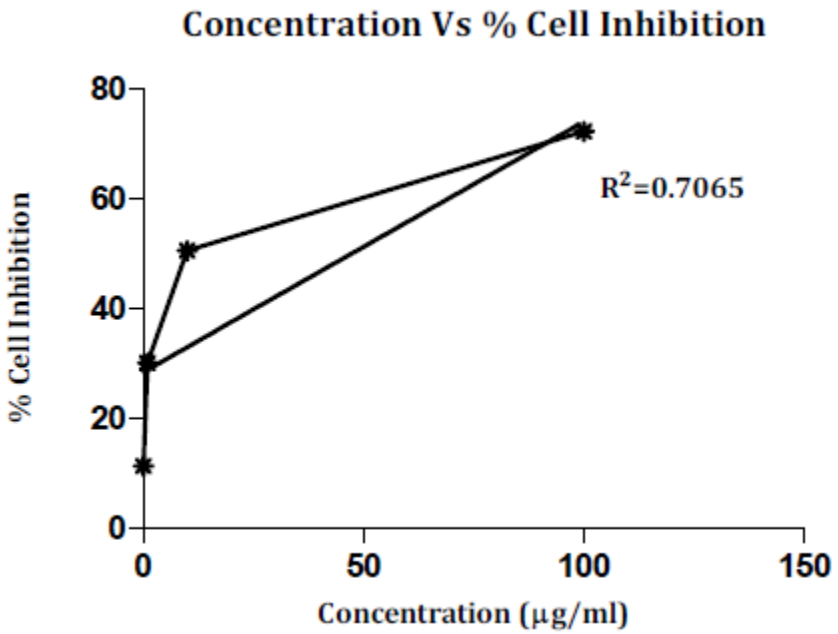

Fig. 2: Dichloromethane Extract of Blue Hard Neopetrosia species for HeLa cell line by MTT assay

Table 2: Determination of cytotoxicity for Dichloromethane extract by MTT assay

Conc. (µg/ml)	% Cell Inhibition	% Cell Survival
0.1	11.51	88.49
1	30.21	69.79
10	50.66	49.34
100	72.32	27.68

RESULTS & DISCUSSION

Percentage cell viability of cell lines was carried out by using colorimetric method. The cytotoxic activity of methanol extract and Dichloromethane extract is presented in table 1&2 and in graph 1&2. It was found that the cell inhibition increases with increase in concentration steadily up to 100µg/ml on HeLa cell line. Percentage cell inhibition of methanol extract is 58.12% and Dichloromethane extract is 72.32%. And R2 value for methanol is 0.9712 and Dichloromethane is 0.7065.

Now over all study evaluate the sponge has potential activity on HeLa cell. So these extracts have considerable for anticancer activity.

CONCLUSION

In conclusion, our study can be considered as the first report on the cytotoxic property of Blue Hard Neopetrosia species. The results of the cytotoxic activity against HeLa cell lines in this study are very promising with regards to possible antineoplastic chemotherapy and from a very sound basis for future research.

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