Research Article

Anticancer Activity of *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* on MCF-7 Cell Line

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ABSTRACT

Natural products have served as an important source of drugs since ancient times. Marine natural products isolated from molluscs have been tested for an extensive range of biological activities. Marine compounds are known to have a serious potential as anticancer drugs. The present study aims to assess the anticancer activity of three marine gastropods *Turbo brunneus, Cypraea annulus* and *Babylonia spirata*. The cytotoxic effects of experimental organisms were performed using MTT assay on MCF-7 cell line. The percentage of cell viability was found to be decreased with increasing concentration of the samples. The results of the present study revealed that *T. brunneus, C. annulus* and *B. spirata* showed potent cytotoxic activities against MCF-7 cell lines with IC_{50} values of 135.590 µg/ml, 412.2 µg/ml and 222.918 µg/ml respectively.

Key words: *T. brunneus, C. annulus, B. spirata*, Cytotoxicity assay, MCF-7 cell line.

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INTRODUCTION

Cancer is a class of disease characterized by out-of-controlled cell growth. A report released by WHO has projected the cancer related deaths to about 12 million by 2030, while American Cancer Society has projected 27 million new cancer cases leading to 17.5 million deaths by 2050.[1] Although cancer accounts for around 13% of all death in the world, more than 30% cancer deaths can be prevented by modifying or avoiding key risk factors.[2] Use of chemicals through tobacco and alcohol, unhealthy food habits, physical inactivity, harmful radiations are some of the causes of the disease. The incidence of breast cancer has been increasing worldwide for many decades with asian countries attaining highest incidence rate.[3] In recent years, marine natural bioprospecting has yielded a considerable number of drug candidates. Research into the ecology of marine natural products has shown that many of these compounds have anticancer function.[4] Therefore, investigations for finding new anticancer compounds are imperative and interesting. After taking into consideration the immense side effects of synthetic anticancer drugs, many researchers are making concerted efforts to find new and natural anticancer compounds. The problems of systematic toxicity and drug resistance in cancer chemotherapy urge the continuing discovery of new anticancer agents. However, almost all chemotherapeutic drugs currently in the market cause serious side effects. Natural products and their derivatives represent more than 50% of all the drugs in clinical use of the world. Almost 60% of drugs approved for cancer treatment are of natural origin.[5] Although marine compounds are underrepresented in current pharmacopoeia, it is anticipated that the marine environment will become an invaluable source of novel compounds in the future.[6] Molluscs represent an important and highly diverse group of animals. Many species are of particular interest to humans as food and medicine. Marine organisms are rich source of bioactive compounds with remarkable impact in the field of pharmaceutical, industrial and biotechnological product developments. In this scenario there is much scope for future drug discovery within this phylum, exploring novel compounds with newer mode of action. Hence an

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attempt has been made to study the anticancer activity of three marine gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata*.

MATERIALS AND METHODS

Collection of experimental animals

In the present study the gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* were collected from the Thoothukudi ($8^0$45'N; $78^0$46'E) coastal region. *Turbo brunneus*, a benthic marine animal belonging to order archaeogastropoda was collected from the rocks and dead corals in the coastal area of Tuticorin. The mesogastropod *Cypraea annulus*, was collected by hand-picking along the sea shore and rocks of Hare Island region. The neogastropod *Babylonia spirata* was collected from the landed by-catch from fishing trawlers operated for crabs and prawns along the Hare Island region. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and then dried in hot air oven at 56°C for 48 hr and used for further studies.

In vitro cytotoxicity assay

The cytotoxicity assay was performed, following the method of Mosmann.[7] To determine the cytotoxic effects of the chloroform extract of experimental animals, MTT 3- (4, 5- dimethyl thiazol - 2- yl) - 2, 5 - diphenyl tetrazolium bromide assay was performed using MCF-7 (Breast carcinoma) cells. MCF-7 (Breast carcinoma) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 U/ml), Streptomycin (100 µg/ml) and amphotericin B (5µg / ml), in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt Ltd., Kolkata, India).

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4,5 dimethyl thiazole-2-yl) -2-5-diphenyl tetrazolium bromide (MTT) into a blue-coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells.

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed once and different test concentrations of test drugs were added on to the partial monolayer in microtitre plates to obtain final concentrations of 100, 200, 300, 400 µg/ml. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere and microscopic examination was carried out and observations were noted every 24h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The untreated cells were used as the control. The effect of the samples on the proliferation of MCF-7 breast carcinoma cells was expressed as the percentage cell viability using the following formula:

$$\% \text{ of viability} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

% of toxicity = 100 - % of viability

RESULTS

Anticancer activity of chloroform extracts of *T. brunneus*, *C. annulus* and *B. spirata* on MCF-7 breast carcinoma cell line are shown in the Figure 1. In the present study, 4 concentrations (100, 200, 300 and 400 µg/ml) of chloroform extracts of samples were tested for anticancer activity (Figure 1). The percentage of cell viability was decreased with increasing concentration. In *T. brunneus* the percentage of cell viability was 35.07% at 100 µg/ml, 31.98% at 200 µg/ml, 26.34% at 300 µg/ml and 17.74% at 400 µg/ml concentration. The percentage of toxicity was observed as 64.93% at 100 µg/ml, 68.02% at 200 µg/ml, 73.66% at 300 µg/ml and 82.26% at 400 µg/ml concentrations respectively (Graph 1).

In *C. annulus*, the maximum percentage of cell viability with 63.92% was observed at 100 µg/ml, 61.44% at 200 µg/ml, 59.01% at 300 µg/ml and minimum viability of 48.06% at 400 µg/

ml. Maximum toxicity of 51.94% was observed at 400 µg/ml, 40.99% at 300 µg/ml, 38.56% at 200 µg/ml and minimum toxicity of 36.08% at 100 µg/ml concentration (Graph 2).

In *B. spirata* the cell viability ranged between 60.88% and 28.16%. The percentage viability at 100 µg/ml concentration was 60.88%, at 200 µg/ml was 58.34%, at 300 µg/ml was 40.02% and at 400 µg/ml was 28.16%. The percentage of toxicity varied from 39.12% to 71.84%. Maximum toxicity of 71.84% was observed at 400 µg/ml, 59.98% at 300 µg/ml, 41.66% at 200 µg/ml and minimum toxicity of 39.12% at 100 µg/ml concentration (Graph 3). The results of this study revealed that *T. brunneus*, *C. annulus* and *B. spirata* showed potent cytotoxic activities against MCF-7 cell lines with IC_{50} values of 135.590 µg/ml, 412.2 µg/ml and 222.918 µg/ml respectively (Table 1).

DISCUSSION

A lot of structurally and pharmacologically important substances have been isolated with novel antimicrobial, antitumour and anti-inflammatory properties.[8] In the areas of cancer and infectious disease, 60 and 75% of new drugs originate from natural sources. The majority of the compounds from marine organisms are being developed in the hopes of treating cancer, tumour growth and leukaemia. Over 67% of compounds isolated from marine origins have cytotoxic activity.[9] MTT assay is a well-established *in vitro* model used to test cytotoxicity of compounds against cancer cell lines. It was used as one of the conventional methods for screening of compounds with potential anti-tumour properties and to test the effectiveness of carcinostatic agents.[10] In this assay, viable cells convert the soluble yellow tetrazolium salt, the MTT compound to an insoluble purple coloured formazan precipitate by the mitochondrial enzyme succinate dehydrogenase whereas the dead cells cannot. Therefore, the untreated wells show higher absorbance than the treated wells at 570 nm due to the formation of formazan, which can be correlated with the number of viable cells in the culture. The incubation of cell lines for 24 h with ethyl acetate extract resulted in cell death indicated by the decrease in absorbance at 570 nm.[11]

Table 1: IC_{50} values of different sample extracts for study in cytotoxicity assay.

Extracts	IC_{50}(µg/mL)
Turbo brunneus	135.590
Cypraea annulus	412.2
Babylonia spirata	222.918

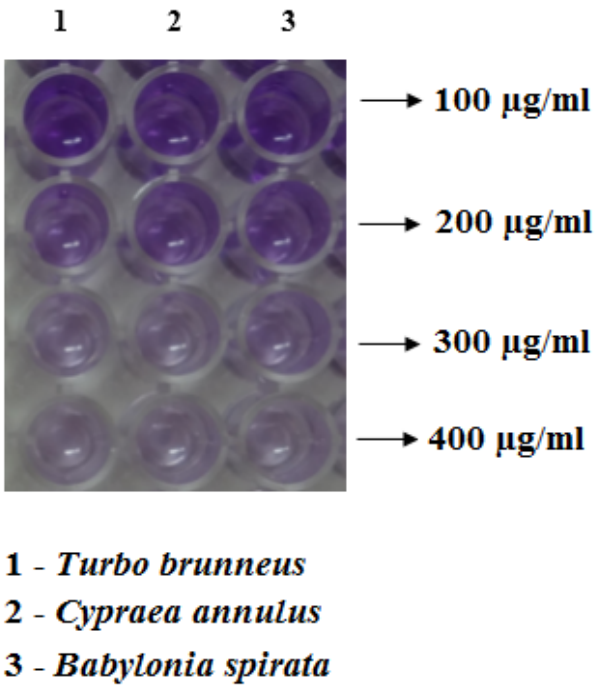
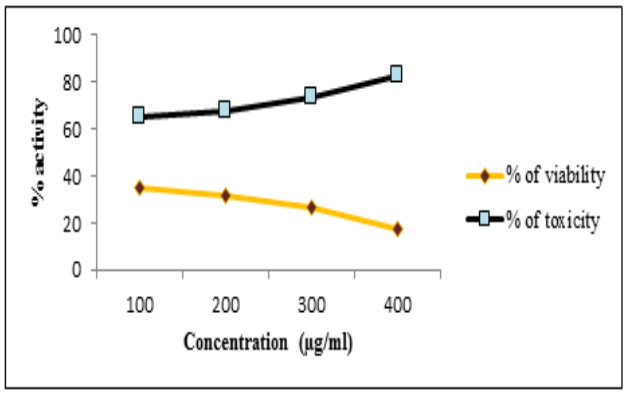
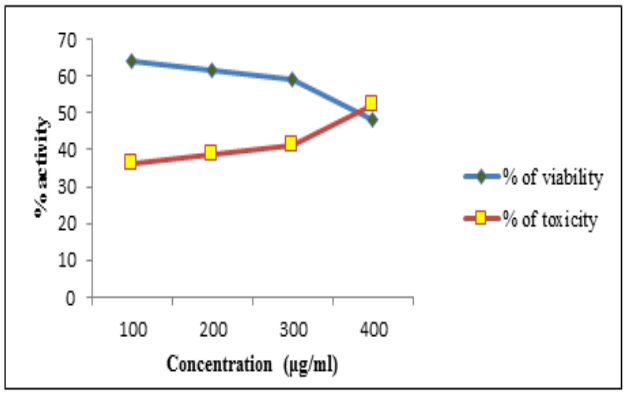


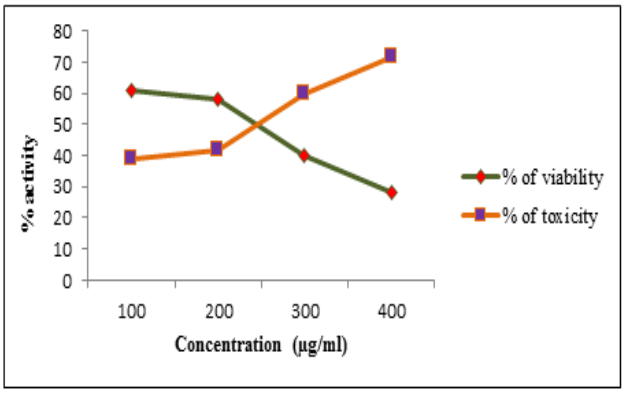
Figure 1: *In vitro* cytotoxicity assay of *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata*.



Graph 1: Anticancer activity of chloroform extract of *Turbo brunneus* on MCF-7 cell line.



Graph 2: Anticancer activity of chloroform extract of *Cypraea annulus* on MCF-7 cell line.



Graph 3: Anticancer activity of chloroform extract of *Babylonia spirata* on MCF-7 cell line.

It is believed that a rich source of anticancer drug candidates could be obtained from marine organisms or their metabolites. Interest in the pharmacological effects of bioactive compounds on cancer treatments and prevention has increased dramatically over the past twenty years.[12] Breast cancer is the second leading cause of cancer-related death in women. Current anti-estrogen medicine, tamoxifen, is widely used in the prevention and treatment of estrogen receptor positive breast cancer.[13] However, a significant number of patients develop tamoxifen resistance and experience severe side effects. Thus, it is imperative to search for new alternatives to breast cancer prevention agents.

In the present study, the gastropod *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* have been screened for cytotoxic property on MCF-7 cell line through MTT assay. Of all the three species, *C. annulus* showed the maximum percentage of cell viability with 63.92% at 100 µg/ml and maximum toxicity of 82.26% was observed at 400 µg/ml in *T. brunneus*. *T. brunneus*, *C. annulus* and *B. spirata* showed potent cytotoxic activities against MCF-7 cell lines with IC_{50} values of 135.590 µg/ml, 412.2 µg/ml and 222.918 µg/ml respectively.

There are several reports on the possession of anticancer properties in gastropods. To cite few of those, cytotoxic glycoprotein (antitumour) aplysianin E, isolated from *Aplysia kurodai,*[14,15] julianins[16] from *A. julianin* and dolabellanin A from *Dolabella auricularia.*[17-19] Chattopadhyay and Pattenden[20] reported cytotoxic activity of *Hexabranchus sanguineus* against L1210 murine leukaemia cells. Lopez-Macia *et al.*[21] isolated Kahalalide F from Hawaiian mollusc *Elysia rufescens* is found to be good anti-cancer agent. A depsipeptide namely Turbostatins 1-4, derived from the marine mollusc *Turbo stenogyrus* found to have potent anticancer property.[22] Keivan Zandi *et al.*[23] reported anti-proliferative effect of *Aplysia dactylomela* on human cancer cell lines NB4 cell line. Tyrindoleninone and 6 - bromoisatin with anticancer properties have been isolated from gastropod *Dicathais orbita.*[24,25]

Sreejamole and Radhakrishnan[11] observed the cytotoxic activities of ethyl acetate extract of *Perna viridis* on MCF-7 cells with a percentage inhibition of 53.4% and 72.6% at 50 and 100 µg/ml respectively. Very similar to the present study molluscs with antitumour property have been reported by Santhi *et al.*[26] Jemma Hermelin Jesy Diaz,[27] Krishnamoorthi and Yogamoorthi,[28] and Subavathy *et al.*[29] Fahmy and Soliman[30] reported the cytotoxic activities of *S. officinalis* ink and *C. aegyptiaca* with IC_{50} value of 76 and 49.24 µg/ml respectively. Ramesh *et al.*[31] showed the cytotoxicity of *C. amadis* venom extract. Pati *et al.* (2015) reported marine molluscs as a potential drug candidate offering excellent opportunity to isolate anti-cancer compounds. Arun Kumar *et al.*[32] studied *in vitro* antioxidant potential of polyherbal formulation of three different herbal drugs. The above results, indicates that the gastropods *Turbo brunneus*, *Cypraea annulus* and *Babylonia spirata* possess good anticancer properties.

CONCLUSION

It can be concluded from the results of the present study that; three marine gastropods exert antiproliferative effect on MCF-7 cell line. The results of the present study is providing baseline data for the future researchers in this line of work and is also throwing more light on the use of marine gastropods, by the pharmaceutical technologists for the extraction of useful drugs or the active principals or functional units for the synthesis of anticancer drugs in future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MTT: 3- (4, 5- dimethyl thiazol - 2- yl) - 2, 5 - diphenyl tetrazolium bromide; **MCF-7:** Michigan Cancer Foundation 7 (Breast Cancer Cell Line); **NCCS:**

National Centre for Cell Sciences, Pune; **DMEM:** Dulbecco's Modified Eagle Medium; **FBS:** Fetal Bovine Serum; **TPVG:** Trypsin Phosphate Versene Glucose; **EDTA:** Ethylenediamine tetraacetic acid; **PBS:** Phosphate Buffered Saline; **CO_2:** Carbon-di-oxide; **h:** hour; **C:** Celcius; **µl:** micro litre; **nm:** nano meter; **IC_{50}:** Inhibitory Concentration.

SUMMARY

The present study describes the anticancer activity of marine gastropods *Turbo brunneus, Cypraea annulus* and *Babylonia spirata*. The marine gastropods have the good anticancer property which could be formulated as drug producing agent in near future.

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