

Anticancer Activity Of *Telescopium telescopium* On He-La Cell Lines**A. Jenivi¹ and R. D. Thilaga²**

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ABSTRACT

Nature is an attractive resource of novel therapeutic compounds as a remarkable chemical diversity is found in millions of plants and animals species. Cancer is the most widespread cause of human death worldwide. Conventional anticancer therapies, including chemotherapy and radiation, are associated with severe side effects and toxicities as well as low specificity. After taking into consideration the immense side effects of synthetic anticancer drugs, various researchers are in search of novel natural anticancer compounds. Marine molluscs are very promising source for wide array of bioactive compounds. With this backdrop, the present investigation focuses on the anticancer activity of methanol extract of *Telescopium telescopium* on human cervical cancer cell line through MTT assay. The results reveals that the percentage of cell death increased with increasing concentrations i.e (14.06% at 5µg/ml), (24.7% at 10 µg/ml), (30.24% at 15µg/ml),(38.27% at 20µg/ml) respectively. Therefore our findings suggest that, *T. telescopium* will take part in an essential role to develop new drug formulation for cancer treatment and further research should be done on isolation and characterization of the active anticancer principles so that better, safer and cost effective drugs can be developed for treating cancer.

Key Words: Gastropod, *Telescopium telescopium*, Anticancer activity, MTT assay, Cervical cancer cell line.

Introduction

Marine ecosystem covers more than 70% of the earth's surface but represents 95% of the biosphere (Ellis, 2001). Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species and more than double that of the land (Anand et al.,1997). Mollusca is one of the largest phyla of invertebrate animals with around 1,60,000 species recorded all over the world. The research on marine natural products in the last three decades has also brought to the discoveries of many chemically and biologically interesting

molecules, that have become indispensable tools in biochemical research and played significant roles in the recent advancement of life sciences (Umayaparvathi et al.,2012).

Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterised by cells in the human body continually multiplying with the inability to be controlled or stopped. Consequently, forming tumours of malignant cells with the potential to be metastatic. Current treatments include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health. Therefore, there is a focus on using alternative treatments and therapies against cancer. In recent years, more and more researchers have come to the realization that marine organisms hold immense potential as a source of novel molecules and new anticancer agents.

Molluscs represent good candidates for anti-cancer natural products research considering their evolutionary and ecological significance. Currently, natural products isolated from molluscs and their structural analogues are particularly well represented in the anticancer compounds in clinical trials (Simmons et al.,2005).

Mangrove snail (*Telescopium* sp.) is a water gastropod, generally inhabiting muddy areas feeding on organic material and algae on the surface. They are rich in organic matter, nearly tidal areas and able to withstand high salinity. Mangrove snail often found in abundant quantities in aquaculture areas close to mangrove forests (Hamsiah *et al.*, 2002). The empirical experience in coastal communities from Mentawai Island that mangrove snails (*Telescopium* sp.) were used as food, and believed to increase stamina. The species is highly delicious food for the local tribal people, *Munda* (Zaman *et al.* 2011).

Visualizing gastropods as the treasure house of bioactive substances, presently an attempt has been made to assess the anticancer property of *Telescopium telescopium*. It is hoped that, it would provide basic insight in the field of pharmaceutical discoverers from molluscs in order to battle with the growing cancer issues in new millennium.

Materials and Methods

Collection of Specimens

Fresh specimens of *Telescopium telescopium* (Linn.) used in the present study were collected from Punnaikayal mangrove ecosystem, Thoothukudi district, Tamil Nadu which is situated on the southern coast of India. This coast contains a rich biological diversity of flora

and fauna largely due to diversified microhabitats such as mangroves, corals, seaweed beds, sea grasses, sandy, rocky and muddy shore etc. The faunal diversity is also well pronounced with reference to different molluscan groups. In the present study whole body tissue extract of *T. telescopium* was used for the anti-cancer assay. The freshly collected samples were 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 hours and used for further studies.

Preparation of Crude Extract

Methanol extract of the whole body tissues was prepared by following the slightly modified method given by Thilaga (2005). Dried tissues were soaked in 100% A.R grade of methanol for 10 days at room temperature. After filtration with Whatman No.1 paper, the methanol extract was concentrated by vacuum evaporation. This extract was dried and solubilized in deionized water and stored at 0°C for further studies.

Anticancer Activity

The anticancer activity of crude methanol extract of *T. telescopium* was performed on human cervical cancer cell lines obtained from National Center for Cell Science, Pune, India and has been maintained further in Center for Bioscience and Nanoscience Research Laboratory, Echanari, Coimbatore, Tamilnadu. The cell viability was tested using MTT assay (Kung *et al.*, 2004). MTT stands for (3[4,5-dimethylthiazol-2-y)-2,5- diphenyltetrazolium bromide). MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells were then solubilized with an organic solvent (isopropanol), solubilized formazan reagent was measured spectrophotometrically. The cells were grown in 96 well plate in DMEM medium (Delbucco's Minimum Essential Medium) (Hi Media) supplemented with 10% foetal bovine serum (Gibco laboratories) and antibiotics (*Streptomycin*, *Penicillin G*, *Canomycin* and *Amphotetracin B*). About 1ml cell suspension (1.2×10^4 cells/ ml) was seeded in each well and incubated at 37°C for 48 hrs in 5% CO₂ for the formation on confluent monolayer. The monolayer of the cells in the plate was exposed to various concentrations (5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml) of the methanol extract of *T. telescopium*.

The cell viability was analyzed using MTT assay. After 4 hours, the medium was discarded and 100 µl of DMSO was added to dissolve the formazan crystals. The tetrazolium salt is metabolically reduced by viable cells to yield, a blue insoluble formazan product which

was measured at 570 nm spectrophotometrically (Kang *et al.*, 2004). Controls were also maintained (untreated well as cell control). The assay was performed in triplicate in each concentration extracts. The mean cell viability values were calculated and compared to the control to determine the effect of extract on cells and the percentage of cell death was calculated. After taking the reading, percentage of cell death was calculated using the following formula.

$$\% \text{ of cell death} = \frac{\text{Control OD} - \text{treated OD}}{\text{control OD}} \times 100$$

RESULTS

Cancer is a multi-mechanistic second largest disease in the world and requires a multidimensional approach for its treatment, control and prevention. There are various types of tumors such as sarcoma, lymphoma, carcinoma and leukemia. During recent years, extraordinary attentiveness has been paid to investigate the bioactivity of natural products, due to their incredible pharmacological accomplishments. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in human. Nature has always been a great contributor towards this goal.

In our study, 4 different concentrations (5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) of methano extract of *T. telescopium* were tested for its anticancer activity on Human Cervical Cancer cell line. The percentage of cell death increased with increasing concentrations i.e. 14.06% at 5 µg/ml, 24.7% at 10µg/ml, 30.24% at 15 µg/ml, 38.27% at 20µg/ml concentration respectively (Table 1). The MTT assay of methanol extract of *T. telescopium* revealed a dose dependent cell death on Human Cervical Cancer Cell Line.

Table 1 Anticancer Activity of Methanol Extract of *T. telescopium* on HE-LA Cell Lines

Concentration of the sample (µg/ml)	% of cell death
5	14.06
10	24.7
15	30.24
20	38.27

DISCUSSION

Cancer is one of the most dreaded diseases of the 20th century and spreading further continuously with increasing incidence in the 21st century. It is the world's second killer after cardiovascular disease. Cancer is a major public health burden in both developed and developing countries. New cancer patients in India are estimated between 7 to 9 lakhs. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism). The failure of conventional treatment modules including chemotherapy and radiation therapy poses a magnitude of severe side effects and in this regard, animal origin therapy/pharmaceuticals of animal origin are emerging as a promising field in cancer therapy.

Now-a-days, cancer is one of the most lethal diseases in human beings. Therefore, imperative and interesting investigations are carried out for finding new anticancer compounds. 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 (Keivan Zandi et al., 2007). Therefore, marine organisms, because of their accessibility and their therapeutic applications for many other diseases, are of interest. Identification and isolation of some compounds with anticancer and antitumour activity were reported in some marine organisms (Krishnakumar and Yogamoorthi, 2013; Westley et al., 2013).

One of the main problems in cancer therapy is the tumour cells which are heterogeneous in nature. They develop multiple cell population and one more aspect is the development of resistant phenotype which includes cytotoxic resistance to anticancer compounds. Apart from all these factors the prime aim of the drug is to control tumour cells. The natural products isolated from molluscs and their structural analogues are particularly well represented in the anticancer compounds in clinical trials (Simon et al., 2006). The use of certain molluscs, such as terrestrial pulmonates, in medicinal remedies dated to ancient Rome (Baily, 1929). Molluscs also feature in a number of traditional medicines from South Africa, India and China (Herbert et al., 2003; Prabhakar and Roy, 2009). Several molluscan derived therapies are listed on the homeopathic Materia Medica (Rothman, 1984). In many cultures molluscs are used as therapy in ancient Greek and more recently have been subjected

to several patents as a source of antimicrobial and antiviral peptides (Praveena and Kaneez Fathima, 2015). In this context an attempt is made to assess the anticancer property from the marine gastropod *T. telescopium*.

Cancer is abnormal or unregulated cell growth occurring due to uncontrolled cell division thereby forming tumour masses. Hence any compound that can slow down or impede cell division in rapidly growing cells has potential as an anticancer therapeutic (Queensley Eghianruwa et al., 2019). Cancer is mostly treated by surgery, or in some cases combined with chemotherapy and radiotherapy (American Cancer Society, 2015). However, such therapies often are associated with deleterious effects caused by drug-induced damage to healthy cells and tissue (Hubenak et al., 2014).

In the present study, the gastropod *Telescopium telescopium* has been screened for cell death property on Human cervical Cancer cell line through MTT assay. The *Telescopium telescopium* showed 38.27% cell death in Human cervical Cancer cell lines at 20 µg/ml concentration. Several reports have been recorded on the possession of anticancer properties in molluscs in general and especially the gastropods. Sherief et al. (2004) reported the increased antitumor activity with increasing purification of the different peptidoglycan fractions of *Sepia pharaonis* Ehrenberg ink on Dalton's Lymphoma Ascites (DLA) bearing mice. Zhang et al., 2005 analyzed Human leukemia HL-60 and human lung cancer A-549 in *Rapana venosa* and *Thais clavigera*. Subhasis Roy et al., 2010 observed that the spermatheca extract from the marine *Telescopium telescopium* also exhibited significant antitumour activity on human cervical cancer cell which is similar to the present study. Edwards et al. (2014) noted JAr and human granulosa cells in *Dicathais orbita*. A previous study reported that crude extract of *Meretrix meretrix* and *Meretrix casta* exhibited potential anticancer activity against hepatoma cell line HepG2. The study revealed that mollusc extract exerted anticancer activity through LDH leakage, depletion of glutathione, DNA damage & increased the expression of apoptosis stimulating factor such as caspases (Sugesh et al., 2014).

Similar to the present study, Babak Esmaeelian et al. (2017) found out the efficacy of methanol extract of the marine mollusc *Dicathais orbita* on colon cancer.

Queensley Eghianruwa (2019) investigated the most promising antiproliferative activity of methanol extracts of marine molluscs *Typanotonus fuscatus* and *Pachymelania aurita*. The anticancer activity of methanol extract of *T. telescopium* in the present study also agrees well with the above findings. In the present study molecular mechanism of anticancer

activity of extract and compounds responsible for cytotoxicity is not clearly known, the result suggested that methanol could act as efficient solvent to extract the bioactive structurally unique pharmacological substances.

CONCLUSION

The present investigation validate the use of whole body tissue of *T. telescopium* as a animal origin drug and confirming its anticancer potentiality. Here, the information investigated will be useful for further pharmacological and therapeutical evaluation along with the standardization of plant material. Further it is planned to identify more precisely the lead component responsible for different activities and to unveil the molecular mechanism behind its therapeutic action.

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