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Anticancer Activity of Marine nudibranch *Plakobranchus ocellatus* Extract on hepatocellular carcinoma cell line (Hep G2) and human breast cancer cell line (MCF-7)

Priyanka Devi[1] and Revathi. K[2]*
[1]Research Scholar, [2]Professor and Head, Department of Zoology, Ethiraj College for Women, Chennai-8, Tamilnadu, India.
*Corresponding author: *reva63@rediffmail.com*

Abstract

Drug development using marine bioresources is limited even though the ocean occupies about 70% of the earth and contains a large number of biological materials. From the screening test of the Marine nudibranch extracts, we found *Plakobranchus ocellatus* collected from South Andaman, India. In this study, the *P. ocellatus* extract was examined for anticancer activity against hepatocellular carcinoma cell line (Hep G2) and human breast cancer cell line (MCF-7). The *P. ocellatus* extract dose-dependently inhibited viability in both cell lines. Our results indicated that *P. ocellatus* extract suppressed cell growth in both cell lines, but MCF-7 cell was more sensitive to the cytotoxic effects of *P. ocellatus* extract than Hep G2 cells. Hence the *P. ocellatus* extract possess excellent anticancer potential that may be used for therapeutic purpose of cancer treatment with proper evaluation procedures.

Keywords: Nudibranchs, *Plakobranchus ocellatus*, anticancer activity, MTT Assay.

Introduction

Cancer is a devasting disease with tremendous negative implications at the personal, health care, economical and social level (Ullah and Aatif, 2009). It is one of the leading causes of death in the World, aflicting an estimated 7.9 million people in 2007 *World Health Organization* (WHO), and this number continues to increase almost 80 million per year (Maxwell, 2001). Conventional cancer treatment can be done in several ways: surgery, radiotherapy, chemotherapy, or in some cases, it is necessary to combine more than one method for treating the cancer. Several distinct biological strategies might prove effective in eliminating established tumors or preventing the maintenance of its progression (Devita and Chu, 2008). Derivative compounds of bioactive natural products have specific targets and have no side effects (Wei et al., 2007).

Marine natural sources as potential anticancer agents were reviewed in 2011 which mentioned 39 marine-derived potential anticancer agents and among them 18 compounds from molluscs with different mechanisms of action (Bhanot *et al*., 2011). Interestingly, from the 16 marine natural products that are currently under preclinical trials as new drug candidates, most are derived from invertebrates. The Nudibranchia is a large group of marine molluscs (Gosliner *et al*., 2008). They are often colourful and display several forms of crypsis, special resemblance, aposematic coloration and mimicry (Gosliner and Behrens,

1990; Tullrot, 1998). The Gulf of Mannar has very diverse habitats and is therefore a good region for finding nudibranchs. In our ongoing research, we investigated the bioactivity of marine molluscs before classifying and isolating their active compounds. Crude extracts were made from marine nudibranch, *Plakobranchus ocellatus* collected from the South Andaman and investigated for anticancer effect.

Materials and Methods

The tumor cell lines hepatocellular carcinoma cell lines and MCF-7 (human breast cancer), and the non-tumor cell line HaCaT (spontaneously immortalized human keratinocytes) were grown in DMEM medium (4.5 g/l glucose) supplemented by 10% (v/v) FBS, 2 m ML-glutamine, 100 µg/ml penicillin and 100 µg/ml streptomycin at 37ºC, 5% CO_2. These cells were routinely cultured in 75 cm^2 culture flasks and were trypsinized using trypsin-EDTA when the cells reached approximately 80% confluence.

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$$\% \text{ Cell viability} = \frac{\text{Absorbance at 570 nm of treated cells}}{\text{Absorbance at 570 nm of control cells}} \times 100$$

From the values thus obtained, the IC_{50} for the respective extracts, and for the respective durations of treatment, that is, 24 and 48 h, was deduced from the curves obtained by plotting percentage inhibition against concentration.

Lymphocytes from human anti-coagulated peripheral blood from healthy donors were isolated by means of sequential sedimentation in dextran 2% in saline and subsequent centrifugation in Histopaque-1077. The residual erythrocytes were removed with cold water and lymphocytes washed (PBS pH 7.4, twice) and resuspended in the same buffer (1 mg/mL glucose, 0.4 mm Mg^{2+}, and 1.20 mm Ca^{2+}). Cell viability was determined with the aid of the trypan blue dye exclusion method. The cytotoxicity of compounds (200 µg/ml) in human polymorphonuclear (2.5×10^6) cells was determined by the MTT assay [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide], after incubation (37 °C for 3 h in PBS pH 7.4). For the control negative (100% viability) was used PBS only. The formazan produced by the cells was dissolved in sodium dodecyl sulfate (at 10% in 0.01 M HCl) and incubated overnight. Cytotoxicity was evaluated at 570 nm with a 630 nm-reference filter and chlorpromazine was used as a positive control.

Results and Discussion

The search for new anti-cancer drugs is one of the most prominent research areas of natural products. For the first time, the potential of cytotoxic activity in extracted compounds of *P. ocellatus* a sacoglossans collected from the Andaman Islands were investigated. To investigate the cytotoxic potential of extracts were prepared according to the traditional use of molluscs in traditional medicine for the treatment

of various diseases such as cancer, inflammation or infectious diseases. The nudibranch sample *Plakobranchus ocellatus* was collected in order to screen them for possible cytotoxic activity against lymphocytes cells.

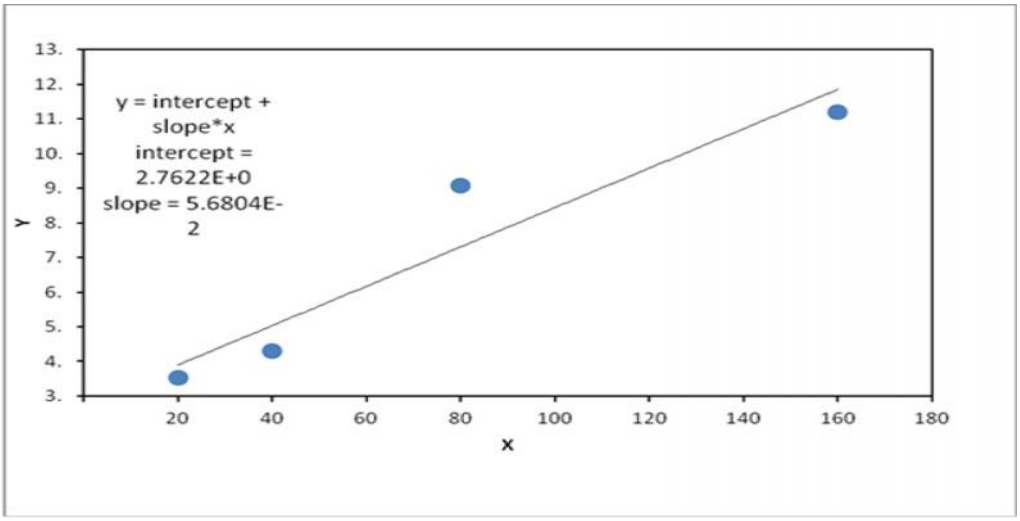
Anti-cancerous drug doxorubicin was used as a positive control in this study. The IC_{50} values for *in vitro* cytotoxicity activity of animal crude extract in hepatocellular carcinoma cell line (Hep G2) and human breast cancer cell line (MCF-7) are expressed as mean ± SD of three replicate experiments. There was a significant difference in cell inhibition percentage at different dose levels of 20µg/ml, 40µg/ml, 80µg/ml and 160µg/ml respectively. The anti-cancer activity displayed by extract of this Marine molluscs *P. ocellatus* was found 11..24 ± 0.69% at 160 µg concentration against the hepatocellular carcinoma cell line (Hep G2) and the standard drug Doxorubicin value was recorded as 0.67 ± 0.21% at 160 µg/ml. The values are expressed in table 1and figure 1.

Table 1: **IC_{50} (µg/mL) values for the *in vitro* cytotoxic activity of nudibranch crude extracts heptocellular carcinoma cell lines**

Tested extracts	IC50 (Mean ±SE)			
	20µg/ml	40µg/ml	80µg/ml	160µg/ml
Nudibranch	3.52 ± 0.21*	4.34 ± 0.31	9.07 ± 0.41	11.2 ± 0.69*
Doxorubicin	0.69 ± 0.21	1.10 ± 0.30	0.7 ± 0.12	0.67 ± 0.21

Results are expressed as mean ± SD of three replicate experiments. There was a significant difference in cell inhibition in nudibranch extract and cell line ($P<0.05$).

Fig 1: **IC_{50} (µg/mL) values for the *in vitro* cytotoxic activity of nudibranch crude extracts heptocellular carcinoma cell lines**



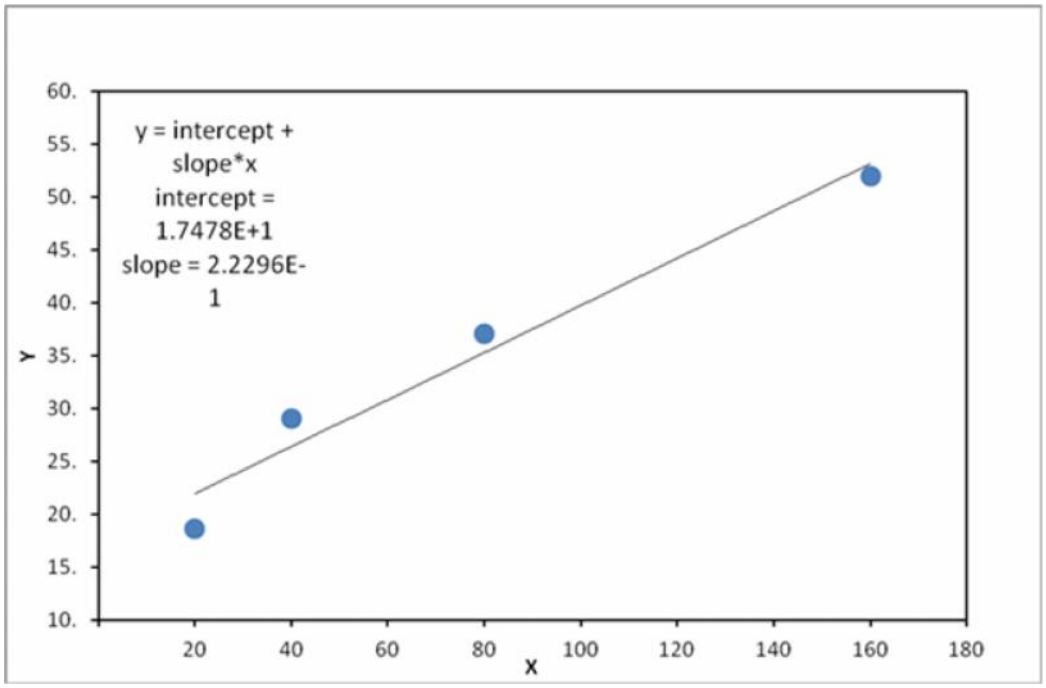
The higher anticancer activity of *P. ocellatus* was found 52 ± 0.53% at 160 µg/ml concentration against the human breast cancer cell line (MCF-7) and the standard drug Doxorubicin value was recorded as 2.10 ± 0.56% at 160 µg/ml. The values are expressed in table 2 and figure 2. The results obtained in this study were in agreement with the previous reports by Archana *et al.,* 2015. They reported that the methanol extract of *Spongia tosta* has a potent cytotoxic effect on MCF-7. Human breast adeno carcinoma cell line in concentration department manner. Morphological studies also confirmed that the methanol extract of *Spongia tosta* has got potential cytotoxic effect.

Table 2: IC50 (µg/mL) values for the *in vitro* cytotoxic activity of nudibranch crude extracts MCF-7 cell line

Tested extracts	IC50 (Mean ±SE)			
	20µg/ml	40µg/ml	80µg/ml	160µg/ml
Nudibranch	18.6 ± 4.85*	29.1 ± 0.64	37.1 ± 0.18	52 ± 0.53
Doxorubicin	0.55 ± 0.18	0.80 ± 0.11	1.30 ± 0.14	2.1 ± 0.56

Results are expressed as mean ± SD of three replicate experiments. There was a significant difference in cell inhibition in nudibranch extract and cell line (P<0.05).

Fig 2: IC50 (µg/mL) values for the *in vitro* cytotoxic activity of nudibranch crude extracts MCF-7 cell line



Metabolic activity can be evaluated by measuring the activity of a mitochondrial enzyme succinate dehydrogenase using MTT test. MTT is designed to be used for the quantification of both cell proliferation and cell viability in cell population using 96-well plate format. This test is widely used in the *in vitro* evaluation of the biosafety of animal extracts. In the present study, the MTT test was applied to evaluate the biosafety of cytotoxic effect of animal extract on lymphocyte cells. Therefore, cancer and normal cells were exposed to increasing concentrations of the aqueous of animal extracts for 24 h. Following removal of the extracts from each well, cells were washed in phosphate-buffered saline, and the MTT assay was carried out as described. The MTT assays data are presented respectively and the corresponding IC_{50} are summarized in Table 3. Percentage cell viability of both of cells was carried out by using trypan blue dye exclusion technique. The results show dose dependent response. The extract showed different anti proliferative profiles regarding extract concentrations. There were different inhibitions produced by different concentrations at 24-hours incubation. On the other hand, there was no difference in the level of inhibition produced by concentrations lower than 0.4 mg. The inhibition level of each concentration (7.5 and 10 mg) was significantly different from those in lower concentrations ($P < 0.05$ and $P < 0.001$ respectively) Table 3. There was however no significant difference between the inhibition produced by 7.5 and 10 mg.

Table 3: Determination of Cytotoxicity by MTT Assay from nudibranch using lymphocyte cells

Molluscan sps	Concentration mg/ml	Absorbance	% inhibition	IC_{50} mg/ml
Nudibranch	Control	0.419	-	5.59±0.94*
	0.1	0.401	13.21	
	0.4	0.352	24.91	
	3	0.291	49.12	
	7.5	0.139	92.01	
	10	0.0041	92.91	

*Data presented are the mean ± SEM. $p<0.05$

In this study, we investigated anticancer effects of *P. ocellatus* extract on Hep G2 and MCF-7 cell line. Our results indicated that *P. ocellatus* extract suppressed cell growth in both cell lines, but MCF-7 cell was more sensitive to the cytotoxic effects of *P. ocellatus* extract than Hep G2 cells. *P. ocellatus* is a natural marine molluscs that could be a good candidate for cancer treatments.

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