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# Anti Cancer Compounds of *Calappa calappa* L. (1758)

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## ABSTRACT

Marine invertebrates are constantly exposed to high concentrations of microorganisms. In crustaceans, the defense system against microbes rests largely on cellular activities performed by hemocytes such as adhension, phagocytosis, encapsulation, nodule formation and melanisation. The potential of marine crabs as a source of biologically active products is largely unexplored. In the present study, anticancer activity of the hemolymph of brachyuran crab *Calappa calappa* was assayed with standard MTT colorimetric procedure against a range of human cell lines viz., MCF -7, HepG2, HT-29, Rhabdomyosarcoma, A549. Cell Viability was evaluated by 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cytotoxicity was assessed by the morphological characteristics were analyzed through phase contrast microscope. The results showed remarkable cytotoxicity against all the cells. The results obtained from the MTT assay showed that the tested samples were significantly reduced the viability of all the tested cell lines in a dose-dependent manner upon 48 h of exposure, the results were recorded in $IC_{50}$. The present results revealed that the crab *C. calappa* might be a potential alternative agent for human cancer therapy.

**Key words:** Brachyuran crabs, human cell lines, bioactive compounds

## INTRODUCTION

The Ocean which is called the 'mother of origin of life', is also the source of structurally unique natural products that are mainly accumulated in living organisms. The chemical and biological diversity of the marine environment is immeasurable and therefore, is an extraordinary resource for the discovery of new drugs. Hundreds and thousands of humans have died over a disease. Available treatments for infectious diseases have always been limited. In recent years, a number of researchers have focused on identifying novel natural product as anticancer drugs (Simmons *et al*., 2005). Anticancer peptides have characteristics of multi-function, high sensitivity, stability (Leng *et al*., 2005). One of the major obstacles which are opposed to the success of anticancer treatment is the cell resistance that generally develops after administration of commonly used drugs. Zhang and Wu (2006) reported anticancer glycopeptides from Meretix meretrix and its inhibitory rate affecting the KB (human Caucasian/epidermal carcinoma) cell line was 69% at 200 μg $mL^{-1}$ (Zhang and Wu, 2006). Relatively strong anticancer peptides were also found from *M. meretrix* with $IC_{50}$ of 10 μg $mL^{-1}$. In addition, dolastatin-10, extracted from the sea hare *D. auricularia*, has entered into clinical trials. Dolastatin-10 is a pentapeptide with four of the residues being structurally unique. It is the most potent anti-proliferative agent known

with an ED50 of $4.6\times10^{-5}$ μg $mL^{-1}$ against murine PS leukemia cells (Pettit *et al*., 1987). Antimicrobial peptides from crabs exhibit numerous activities that make them promising candidates for therapeutics (Ravichandran *et al*., 2010; Imjongjirak *et al*., 2011; Noga *et al*., 2011). There had been an investigation showed cytotoxic activity from crab and anticancer peptide also described (Doyen *et al*., 2011). Crustaceans are studied for its antimicrobial (Priya and Chandran, 2014; Priya *et al*., 2014) but the anticancer properties of these crabs are poorly studied. Hence, the present study was carried out to study for anticancer activity of the brachyuran crab *C. Calappa*.

## MATERIALS AND METHODS

**Hemolymph collection:** Hemolymph was collected by cutting walking legs of the crab *D. dehaani* with a fine sterile scissor. To avoid hemocyte degranulation and coagulation, the hemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, v/v). Equal volume of physiological saline (0.85%, NaCl, w/v) was added to it. To remove hemocytes from the hemolymph it was centrifuged at 2000 rpm for 15 min at 4°C. Supernatant were collected by aspirating and stored at 4°C until use.

**Cell line and culture:** The MCF-7, HepG2, HT-29, Rhabdomyosarcoma, A549 cell lines purchased from the National Center for Cell Science and grown as monolayer in minimal essential medium supplement with 10% FCS, 3% glutamine Penicillin (100 U $mL^{-1}$) and Streptomycin (100 μg $mL^{-1}$) at 37°C for 5% $CO_2$ atmosphere. Stocks were maintained in 25 $cm^2$ tissue culture flasks.

**Cytotoxicity and MTT assay:** The *in vitro* cytotoxicity of the antimicrobial peptide on cell lines, were examined using a modified MTT assay (Plumb *et al*., 1989). The trypan blue exclusion test was used for detection of cell viability (Morgan and Darling, 1992).

**Data analysis:** All data were analyzed by the software of SPSS (SPSS Science Inc.).

## RESULTS

The hemolymph of the crabs was tested to evaluate their cytotoxic potential against the cancer cells. Cytotoxicity was assessed by the morphological characteristics of the cells such as rounding of the cells, shrinkage, aggregation and cell death was observed through Phase Contrast Microscope. Then they were screened by using MTT assay. These results showed remarkable cytotoxicity against all the cells in a dose dependent manner. The hemolymph showed high cytotoxicity on vero cells at concentrations viz., 400, 350, 300, 250, 200, 150, 100-50 and 10 μg $mL^{-1}$. The 50% of cytotoxicity ($IC_{50}$) was observed at the concentration of 200 μg $mL^{-1}$. The observations of the cell viability count to determine anticancer activity against different cell line is represented graphically in Fig. 1. In the case of Anticancer activity of *C. calappa* against MCF-7 cell lines showed high cytotoxicity at various concentrations and 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 75 μg $mL^{-1}$. In the HepG2 cell lines 50% of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$. In the A549 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 95 μg $mL^{-1}$. In the Rhabdomyosarcoma cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$. In the HT-29 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 95 μg $mL^{-1}$.

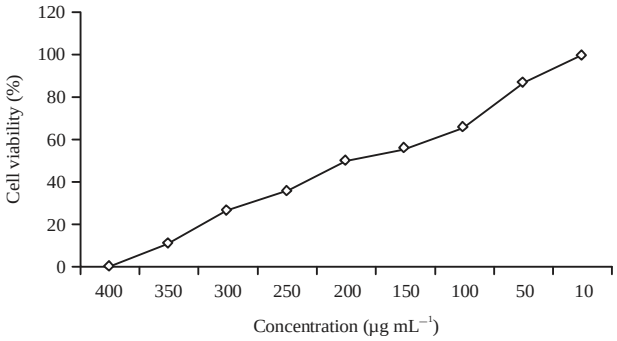


Fig. 1: Cytotoxicity of vero cells after treating with various concentration of *Calappa calappa*

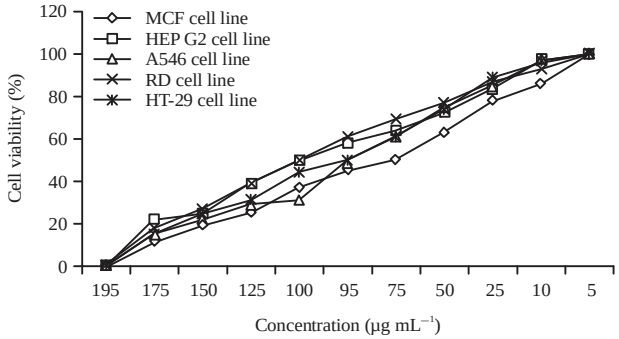


Fig. 2: *In vitro* anticancer activity of *Calappa calappa* after treating with various concentration of hemolymph

## DISCUSSION

Nature has supplied several active anticancer agents which have significantly improved the management of many types of human cancers. One can speculate that these organisms require potency and rapid penetration of cellular membranes for protection against predators, since their aquatic environment will rapidly dilute their poisons.

Cytotoxic compounds are one of the most important classes of drugs used for cancer treatment. There have been several researches to get new cytotoxic agents. In this regard antimicrobial peptides isolated from marine organisms showed considerable promises. In the present study, MTT assay was used for evaluation of cytotoxic activity of *C. calappa*.

Programmed cell death is primarily mediated by Fas receptor signaling and shows DNA fragmentation by BMAP-27. The BMAP-28-treated U937 cell line revealed that some AMPs are endowed with cytotoxic mechanisms also involving triggering of cancer cell suicide by apoptosis (Rizzo *et al*., 1998). The results of the present study demonstrate.

The observations of the cell viability count to determine anticancer activity against all the tested cell lines viz., MCF -7, HepG2, HT-29, Rhabdomyosarcoma, A549, represented graphically in Fig. 2. The sample also showed high cytotoxicity against all the cell lines at concentrations and 50% of cytotoxicity. In the cytotoxicity of the *C. calappa* in vero cell line the $IC_{50}$ value was found in the concentrations of 100 µg mL$^{-1}$. In the Anticancer activity *C. calappa* produced the half inhibitory concentration ($IC_{50}$) was 75 µg mL$^{-1}$ against MCF-7 cell lines. In the HepG2 cell lines the $IC_{50}$ was 100 µg mL$^{-1}$. In the A549 cell lines the $IC_{50}$ was 95 µg mL$^{-1}$. In the Rhabdomyosarcoma

cell lines the $IC_{50}$ was 100 μg $mL^{-1}$. In the HT-29 cell lines the $IC_{50}$ was 95 μg $mL^{-1}$. In the HepG2 cell lines the $IC_{50}$ was 75 μg $mL^{-1}$. In the A549 cell lines the $IC_{50}$ was 100 μg $mL^{-1}$. In the Rhabdomyosarcoma cell lines the $IC_{50}$ was 125 μg $mL^{-1}$ In the HT-29 cell lines the $IC_{50}$ was 100 μg $mL^{-1}$. In the case of Anticancer activity of *C. calappa* against MCF-7 cell lines showed high cytotoxicity at various concentrations and 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 75 μg $mL^{-1}$. In the HepG2 cell lines 50% of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$. In the A549 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 95 μg $mL^{-1}$. In the Rhabdomyosarcoma cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$. In the HT-29 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 95 μg $mL^{-1}$. In the HepG2 cell lines 50% of cytotoxicity was observed at the concentration of 75 μg $mL^{-1}$. In the A549 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$. In the Rhabdomyosarcoma cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 125 μg $mL^{-1}$. In the HT-29 cell lines 50% ($IC_{50}$) of cytotoxicity was observed at the concentration of 100 μg $mL^{-1}$.

It was also observed an enhanced scavenging effect at the low concentration of combined selenium and Ge-132 (Wu *et al*., 2001). Antitumor mechanisms of carboxyethyl-germanium sesquioxide (Ge-132) in mice bearing Ehrlich ascites tumors were reported. The Ge-132 showed its *in vivo* antitumor effect partly due to its inducing the antitumor immunity of the host (Zhang *et al*., 2009). Regarding toxicity of organogermanium derivative (Ge-Vit), when cell viability was measured by the MTT method using HaCaT cells, Ge-Vit showed no effect on it within the given concentration range (up to 50 mM), suggesting that the compound is not cytotoxic to cells (Lim *et al*., 2010).

Marine organisms are potential in terms of their ability to produce secondary metabolites which can be utilized as lead component in drug discovery (Jimeno *et al*., 2004). There are plenty of works related to antitumor and antimicrobial activity (Natarajan *et al*., 2010) carried out in ascidians which made the chemist to isolate the active principle responsible for the actions as they are the viable source for drug discovery process (Arutuso, 1997). Green *et al*. (2003) demonstrated the molecular weight of protein from hemolymph (43 kDa) of the solitary ascidian, *Styela plicata* from Australian waters. The endoderm specific alkaline phosphate protein with molecular mass of 86 and 103 kDa were reported by Kumano *et al*. (1996) from the ascidian *Halocynthia roretzi*. From these results it can be concluded that *C. calappa* might be great source for the anti-cancer drug developmental research areas.

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