In vitro Anticancer Activity of Marine Bacteria Isolated from Andhra Pradesh and Tamil Nadu Coastal Regions

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***Abstract*—** Marine microorganisms such as bacteria are well-known for being a source of bioactive natural products with the advantage of sustainable production of secondary metabolites. In order to evaluate their bioactivity against breast cancer cell lines, we have proceeded to the isolation of five marine bacteria present in sea water from Ongole and Tamil Nadu coastal areas. The anticancer property of the marine bacterial isolates was carried out by tryphan blue assay. Cells were treated with various concentrations of KMB-1 (Kothapatnam marine bacteria 1), KMB-2, KMB-3, CMB-1 (Chennai marine bacteria 1) & CMB-2 and incubated for 48hrs. After 48 hrs percent of inhibition was calculated by tryphan blue assay. KMB-1 and CMB-1 & 2 showed dose dependent inhibition of cell proliferation on MCF-7 cells but not in MDA-MB-231. Activity was not observed in KMB-2 & 3 in both cell lines. These results suggested that these isolates producing the secondary metabolites which are having anticancer and apoptosis activity at concentration of 100 µg against human breast cancer cell lines. Identification of these bacteria is in progress. Our work may lead to the finding of new bacterial species and/or new therapeutic agents.

***Keywords*----** Marine isolates, MDA-MB-231 breast cancer cell lines, MCF-7 (Michigan cancer foundation) cell line, Tryphan blue assay.

I. INTRODUCTION

CANCER is a disease in which a cell, or a group of cells represents uncontrolled growth invasion (intrusion on and distortion of adjacent tissues), and metastasis (spread from one part to another part in the body through lymph or blood). These three malignant properties differentiate cancer from benign tumors, which are self-limited and do not invade or metastasize while the malignant tumors are not self limited and metastasize. Most of cancers occur form a tumor; the oncology is deals with the study, diagnosis, treatment, and prevention of cancer and it's a branch of medicine. Cancer is a human tragedy that affects people at all ages with the risk in most types increasing with age. Cancers are primarily an environmental disease with 90-95% of cases due to modification in lifestyle and environmental factors and 5-10% due to genetics Cancer is caused.

Cancer still remains one of the most serious human health problems and breast cancer is the second most universal cause of cancer deaths in women. Therapeutic methods for cancer treatment are surgery, radiotherapy, immunotherapy and chemotherapy [7] and these techniques are individually useful in particular situations and when combined, they offer a more efficient treatment for tumor. Many of the antitumor compounds from marine drugs are derived from algal metabolites [3] and these metabolites play an important role in identification of new pharmaceutical compounds [12]. Actinomycetes are one of the most important sources for new bioactive compounds such as antibiotics and enzymes [16,14 &17] which have diverse clinical effects against many pathogenic organisms viz. bacteria, fungi and parasite etc. in fact, more than 60% of approved drugs from natural compounds [11] in that, 50 % of the natural antibiotics are produced from actinomycetes, among them marine actinomycetes have been reported to have antifungal, antibacterial and anti inflammatory activities [13]. But, studies related with the biopotential activities of anticancer metabolites from marine bacteria based drug discovery are too limited. In this connection, the present study was made an attempt to find out the anticancer property from marine bacteria isolated from sea water against two different breast cancer cell lines.

II. MATERIALS AND METHODS

A. Sample collection

Three different sea water samples were collected from Ongole (Kothapatnam) (59.12′E, 6.28′N), Nellore (48.29′E, 5.15′N), and Chennai (35.04′E, 5.45′N), Tamil Nadu, India at 20 m depth. Samples were collected in sterile plastic containers and shifted to the laboratory for further analysis.

B. Isolation and Characterization of isolates

The sea water samples were taken separately for serial dilution. One ml of sea water sample was suspended in 9 ml of distilled water. The suspension was considered as 10^{-1} dilution. About that 0.1 ml of the serially diluted sample was spread over the marine agar medium. After incubation, the plates were incubated in inverted position for 7-10 days at 28+2^{o}C.

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After incubation, colonies appeared on the agar medium were restreaked in the same agar medium and further subjected for the identification by using the conventional methods [4].

C. Extraction of bioactive compounds

The selected antagonistic marine bacterial isolates (KMB-1, KMB-2, KMB-3, CMB-1 & CMB-2) were inoculated into the nutrient broth separately and incubated at 37°C for seven days. After incubation the culture broth was centrifuged at 3000 rpm for 15 min to obtain a clear supernatant. Components were extracted successively dissolved with chemical solvent such Methanol followed by vacuum evaporation of these extracts to obtain the dry extracts by vacuum rotary evaporator. [1]

D. Cell culture

The human breast cancer cell lines MCF-7 and MDA-MB-231 were collected from ATCC. Cells were routinely maintained in DMEM medium, supplemented with 5% inactivated FBS, at 37°C in a humified atmosphere containing 5% CO_2. The effect of vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments. Each concentration triplicates were maintained.

E. Tryphan blue Cytotoxicity assay

Human breast cancer cell lines MCF-7, MDA-MB-231 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were seeded into 24-well plates at a density of 1.0×10^5 cells/well and the dilutions (1:500 and 1:1000) of the isolates were made in DMEM in the volumes of 10µl and 25µl were added. After an incubation period (24, 48 h), the growth media was removed and the cells were trypsinized and counted in a haemocytometer with tryphan blue *(Freshney, 2006)*. Percentage of inhibition was calculated by using the following formula.

% of inhibition: No.of cells in Control- No.of Cells in test x100

No of cells in control

III. Results

Tryphan blue growth inhibition assay was taken as in vitro measure of anticancer activity of marine bacterial extracts by using breast cancer cell lines. The anticancer property of the marine bacterial isolates against breast cancer cell lines revealed that, the cytotoxic index of all the five isolates on breast cancer cell lines (MCF-7 and MDA-MB-231) exhibited does dependent growth inhibition (Fig 3-5). In negative control inhibition was observed in both the cells that are below 10%.

Out of the five isolates KMB-1, CMB-1 and CMB-2 isolates showed inhibition at the concentrations tested both cell lines. But inhibiton was not observed in KMB-2 and KMB-3 isolates in both the cell lines (Fig. 3-5).

KMB-1 isolated showed 10.2 % inhibition at 100 µg on MCF-7 cells in case of MDA-MB-231 cells 8.9 % inhibition at 100 µg . CMB-1 showed 47.9 % inhibition in MCF-7 cells and 15.2 % inhibiton was observed in MDA-MB-231 cells at 100 µg concentration. CMB-2 showed 25.9 % inhibition was observed in MCF-7 cells and 11.6 % inhibition in MDA-MB-231cells at 100 µg concentrations.

At 50 µg concentration less activity was observed in KMB-1, CMB-1 and CMB-2 but that inhibition was equal to negative control. No activity was observed in KMB-2 and KMB-3 at the tested concentrations in both cell lines.

IV. Discussion

Cancer is considered to be the public health problem in developed countries, among which breast cancer is the second most universal cause of cancer deaths in women. Despite the intense efforts to develop treatments, effective agents are still not available. In this regard, natural products extracts continue to be the most promising source of new drugs for cancer [5]. The present study was made an attempt to find out the anticancer compounds from the marine environment. In the study the marine isolates CMB-1 and CMB-2 showed nearly 50 % inhibition at 100 ug /ml in breast cancer cell lines can be considered as promising for anticancer drug development [8].

The anticancer property of cell free extracts from marine bacterial isolates might be due to the presence of the active secondary metabolites such as alkaloids and quinine [15]. Alkaloids are one of the major physiologically active nitrogenous compounds derived from many biogenetic precursors. Alkaloids are microtubule interfering agents which can bind with beta tubulin, thus preventing the cell from making mitotic spindle fibres necessary to move the chromosome around as the cell divides, inhibiting toposiomerase [6], mitochondrial damage and inducing the release of cytochrome C and apoptosis inducing factor [12]. Moreover quinine derivatives Viz. driamycin, daunorubicin, mitomycin C, streptonigrin, and lapachol can interfere the DNA and RNA replication and mitochondrial oxidative pathways or the formation of super oxide, peroxide and hydroxide radicals as toxic products in the ell line.

Several compounds of anthroquinone families (parimycin, trioxacarcins and gutingimycin) showed antitumor activities [9,10 &2]. The cytotoxic effect of marine bacterial isolates seems to be the induction of the cell apoptosis, partial cellular differentiation, and degradation of fusion transcripts, antiproliferaion and inhibiton of angiogenesis [12]. Our results suggest that the compounds from these five marine bacterial isolates could be candidates for developing apoptosis specific anti-tumor agents with lower toxicity.

V. Conclusion

We concluded from the present study that, the CMB-1 and CMB-2 from the marine water Chennai could be used for the development of anticancer drugs. The bacterial isolates are to be purified further for obtaining the active compounds responsible for anticancerous property.

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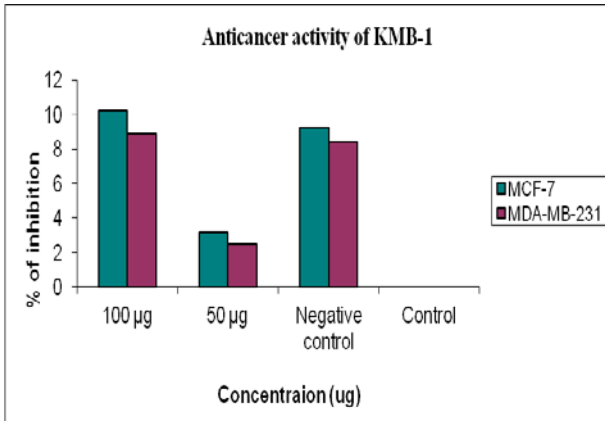
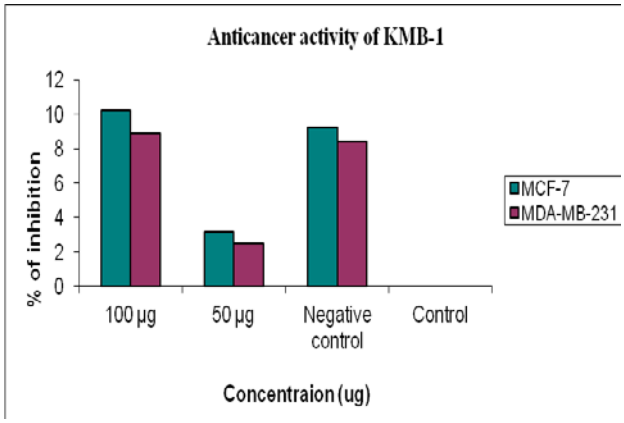
TABLE I
PHYSICAL CHARACTERISTICS OF MARINE ISOLATES

Name of the Isolate	pH	Temp range	Growth in NaCl (w/v)
KMB-1	7.8	40-45	6
KMB-2	8.2	42	6
KMB-3	8.0	40	6
CMB-1	8.7	40	7
CMB-2	7.5	40	6



Graphical representation of KMB-1 isolate % of inhibition on MCF-7 & MDA-MB-231 cells



Graphical representation of CMB-1 isolate % of inhibition on MCF-7 & MDA-MB-231 cells.

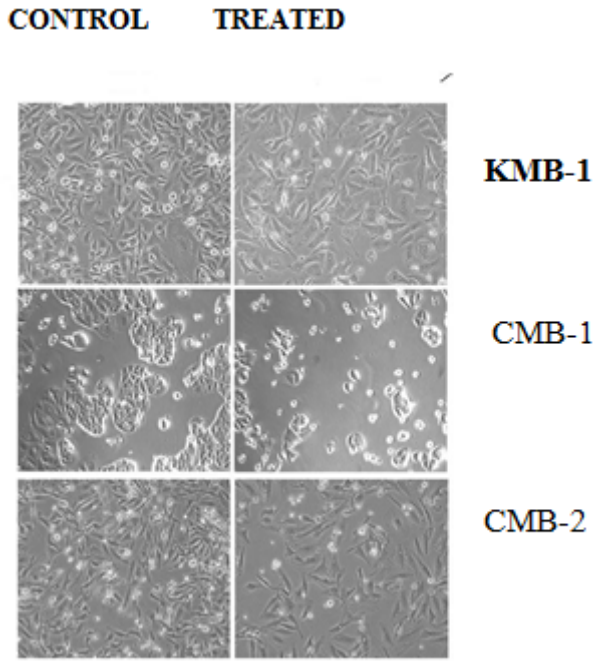


Fig. 1: Differentiation cell number on treatment of KMB-1, KMB-2 & KMB-3 (100**µg**) and control on MCF-7 cells.