

COMPARITIVE IN-VITRO CYTOTOXICITY OF RED PIGMENT EXTRACT OF *SERRATIA MARCESCENS* ON BREAST AND PROSTRATE CANCER CELL LINES

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ABSTRACT

The anticancer activity of the red-pigment extract of *S.marcescens* was evaluated on a panel of three breast cancer viz. MCF-7, MDAMB231, T47D and three prostate cancer viz. PC3, LNCaP and DU145 cell lines respectively by MTT assay. MTT assay showed an increase in the % viability of the tested cells against the decrease in concentration of the extract. IC50 of the extract was found to be 40.76, 110.53 and 113.81 µg/ml for MCF-7, MDAMB231, T47D and 43.79, 37.66 and 23.64 µg/ml for PC3, LNCaP and DU145 respectively. The activity of the extract was found to be almost similar for MCF-7 and PC3 but a very good activity on LNCaP and PC3 was obtained compared to that of MDAMB231 and T47D (p<0.05). Overall the study showed that the extract has a strong anticancer potential especially towards the prostate cancer cells.

Keywords: *Serricia marcescens*, MTT, IC50, Anticancer, Breast, Prostrate

INTRODUCTION

Cancer is a leading cause of death worldwide. Most of the commercially available anticancer drugs can be classified by origin as either chemical synthetic drugs (e.g., alkylating agents and antimetabolites) or natural drugs derived from organisms (eg. Taxol, Camptothecin and Trabectedin) [1,2]

Most of the times synthetic drugs are the only option for cancer chemotherapy [2,3,4]. However, most synthetic drugs kill not only tumor cells, but also normal cells and most have severe side effects [5]. Natural anti-tumor drugs derived from organisms have also proven effective and less toxic for cancer therapy [5,6]. In nature, colour-rich and pigment producing microorganisms (fungi, yeasts and bacteria) are quite common [7]. Microorganisms produce various pigments like carotenoids, melanins, flavins, quinines, prodigiosins and more specifically monascins, violacein or indigo [7].

For several decades, a family of natural red pigments called, prodigiosin, has been known to be a natural compound showing a broad range of cytotoxic activity [8]. Prodigiosin is a tripyrrole ring pigment synthesized by *Serratia marcescens* (a gram-negative bacillus shaped bacteria, belonging to the family Enterobacteriaceae). Prodigiosin is reported to have characteristics of antibacterial, antimycotic and immunomodulatory activities. [9]

Antitumor activity of red pigment extract from *S.marcescens* on human cervix carcinoma cell lines has been reported [10]. The anticancer activity of this pigment has also been reported in breast cancer cell lines [11,12] but till date the activity of this pigment has not been reported on a panel of prostate cancer cell lines. Present study reports the comparative *in-vitro* anticancer activity of the red pigment extract of *S.marcescens* on a panel of three breast cancer cell lines viz. MCF-7, MDAMB231, T47D and prostate cancer cell lines viz. PC3, LNCaP and DU-145 respectively.

MATERIAL AND METHODS

Microbial Culture

The culture of *S. marcescens* was surface spread on Nutrient Agar plates. These plates were incubated at room temperature for 72hrs in dark till a bright red pigment was formed.

Preparation of red pigment extract

The matt growth with the red pigment formed on the nutrient agar plates was scrapped off with a sterile scraper, avoiding taking agar. The weight of the culture obtained was 250mg. The pigment was then extracted from the culture with 5ml of acetone by thoroughly vortexing the culture for half an hour and subsequently centrifuging it. The supernatant was then carefully removed and allowed to

evaporate under sterile conditions. The weight of the air dried extracted pigment was taken and was found to be 10mg. The extract was dissolved in 200 µl of sterile Dimethyl Sulfoxide (DMSO). Subsequent dilutions of 250 µg/ml, 50 µg/ml, 5 µg/ml and 0.5 µg/ml were prepared in DMSO from the stock and were further taken for testing anticancer activity on breast and prostate cancer cell lines.

Anticancer activity of the red -pigment extract on breast cancer cell lines

The anticancer activity of the red-pigment extract of *S.marcescens* was performed on three breast cancer cell lines MCF-7, MDAMB231 and T47D respectively. These cell lines were procured from NCCS, Pune, India.

Anticancer activity was checked by MTT (3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma cat no - M5655) assay and DMSO.

Briefly, MCF-7 and MDAMB231 cells were grown in 96 well adherent plate in Dulbecco's Minimum Essential Medium (D.M.E.M) and for T47D cells Rosewell Park Memorial Institute medium (R.P.M.I) with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was used. The cell culture media was procured from HiMedia Mumbai. The media was supplemented with 10% Fetal Bovine Serum (F.B.S, Gibco) and antibiotics (Penicillin and Streptomycin, Gibco). 100 µl of the cells with cell count of 7500, 10,000 and 20,000 respectively were seeded in each well and incubated at 37°C for 24 hrs in 5% CO₂ to obtain a log-phase culture (Fig 1a). The monolayered cells were then exposed to various dilutions of the extract. One molar concentration of the drug Cisplatin (with known anticancer activity) was used as Drug Positive Control (DPC). After drug addition the cells were further incubated at 37°C for 48hrs in 5% CO₂ and the assay was terminated by centrifuging the plates at 3000 r.p.m for 3mins. The supernatant was then removed followed by addition of 100 µl of (5mg/ml MTT) solution. The plates were incubated for 4hrs at 37°C in 5% CO₂. During this incubation period the MTT (a tetrazolium salt) gets metabolically reduced by viable cells to yield a blue insoluble formazan product measured at 570nm spectrophotometrically. Controls such as growth control (GC), growth control with DMSO (GCD), DPC and MC (Media Control i.e. only media for ensuring sterility) were run for each set of cell line. The assay was performed as six data points (n=6) for each dilution as well as controls. The mean of the cell viability values was compared to the GCD to calculate the % viability for each dilution. A X-Y graph of log % viability was then plotted against log drug concentration. IC50 (drug concentration inhibiting the growth of 50% of cell population) values of the extract for each cell line were then calculated by regression analysis.

Anticancer activity on prostate cancer cell lines

The anticancer activity of the red-pigment extract of *S.marcescens* was performed on prostate cancer cell lines PC3, LNCaP and DU145 respectively. These cell lines were procured from NCCS Pune, India. Fig 1b shows the photograph of the log-phase culture of these cell

lines. PC3 and LNCaP cell lines were cultured in R.P.M.I 1640 and DU145 in D.M.E.M medium respectively. The plating efficiencies used were 10,000 cells/well for PC3 and LNCaP and 5000cell/well for DU-145. Controls were maintained throughout the experiment. MTT assay was then performed as described above. Regression analysis was used to calculate the IC50 values for each cell line.

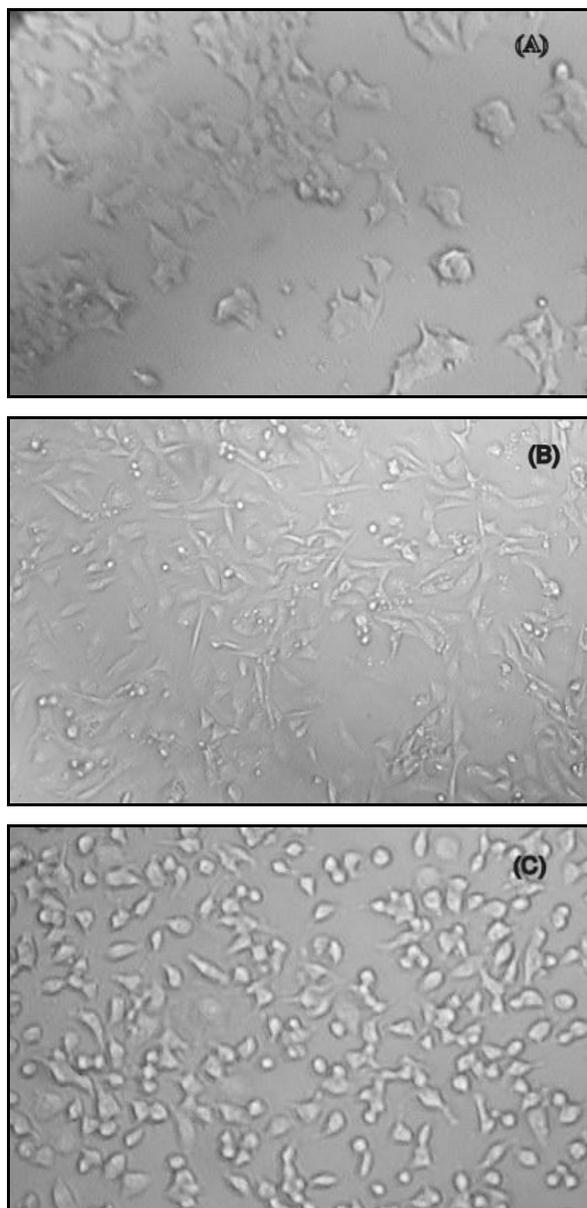


Fig1 (a): Photograph of log-phase culture of breast cancer cell lines (A).MCF-7 (B) MDAMB231 (C) T47D cultured in 96-well plates.

Statistical Analysis

Six data points (n=6) were considered for analysis for each concentration. The data was expressed as mean % viability \pm s.d. The significance of difference among the varied treated groups and control group were analysed by one-way ANOVA. The level of significance was set up at $p < 0.05$. IC50 was estimated using linear regression of the log% of cell viability against the log concentration of the tested compound using Microsoft Excel Software Programme.

RESULTS AND DISCUSSION

The average % viability (n=6) for these cell lines obtained for the respective concentration of the red pigment extract taken has been shown in Table 1. There is a gradation in % viability observed with a

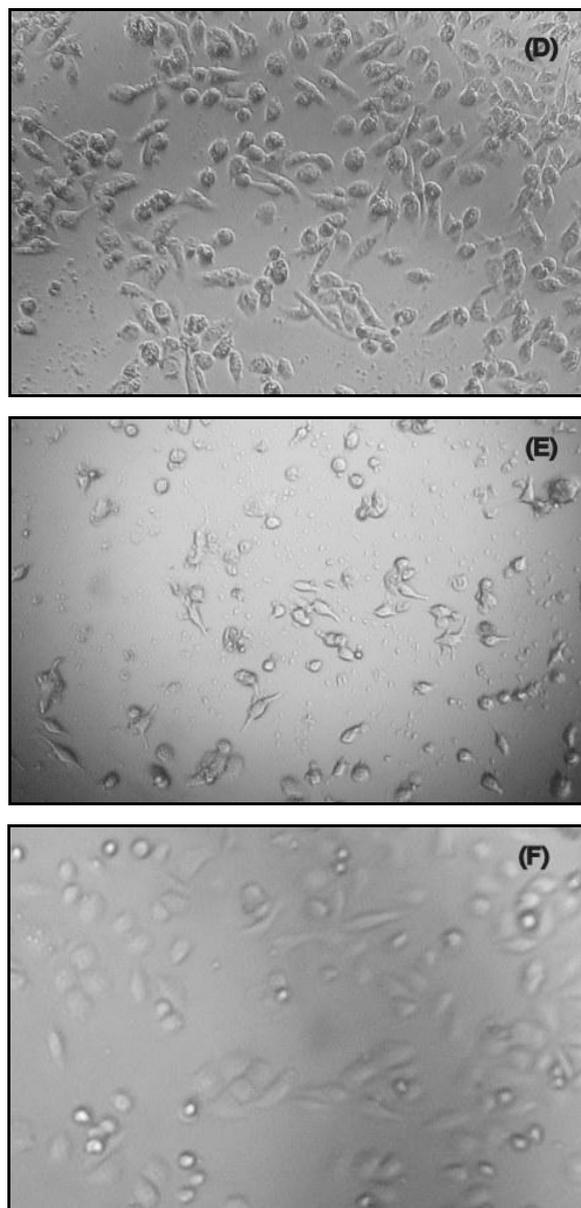


Fig1 (b): Photograph of log-phase culture of prostate cancer cell lines (D).PC3 (E) LNCaP (F) DU145 cultured in 96-well plates.

decrease in the concentration of the extract. Cisplatin is an age old anticancer drug and is used in many in-vitro assays as positive control [13]. The average percentage viabilities for the DPC and GCD for each cell line have also been expressed. The DPC for MCF-7, MDAMB231 and T47D shows that 1M Cisplatin inhibits the growth of these cells. The % viability of the results obtained in GCD indicate that the drug solvent (DMSO) does not interfere with the cell viability. Other controls like MC and GC were also run for each set of experiment for each cell line. IC50 values for each cell line were calculated by regression analysis after plotting a graph of log % viability vs. log drug concentration. A good regression coefficient ($R^2 > 0.90$) was obtained for each of the cell line. IC50 values obtained for the extract for MCF-7, MDAMB231 and T47D were 40.76, 110.53 and 113.81 $\mu\text{g/ml}$ respectively.

This indicates that the extract shows anticancer activity for all the three cell lines but is most active on MCF-7.

Table 2 indicates the average % viability (n=6) of these cell lines for the respective concentration of the red pigment extract taken. There is a good gradation seen in the % viability obtained with the decrease in concentration of the extract for all the three cell lines tested. GCD for each cell line shows 100% viability indicating no adverse effect of DMSO on the cell lines tested. Other controls like MC and GC run along with the assay showed good sterility and cell growth respectively throughout the experiment. The IC50 values for each cell line were calculated by regression analysis after plotting a graph of log % viability vs log drug concentration for each cell line. A good regression coefficient ($R^2 > 0.95$) was obtained for each cell line. IC50 values of the extract obtained for PC3, LNCaP and DU145 were 43.79, 37.66 and 23.66 $\mu\text{g/ml}$ respectively. The values indicate

that the extract shows anticancer activity on all the three cell lines and is most active on DU145.

Fig 2 shows the comparative anticancer activity of the extract on the breast and prostate cancer cell lines. It can be observed that the *red pigment extract* showed almost similar activity on MCF-7 and PC3 but when the activity of the extract on MDAMB231 and T47D was compared with that of LNCaP and DU145 a significant difference was observed ($p < 0.05$). PC3 and DU145 are "classical" human prostate cancer cell lines. DU145 cells have moderate metastatic potential compared to PC3 cells which has high metastatic potential [14]. LNCaP is an androgen sensitive cell line of human prostate adenocarcinoma cells commonly used in the field of oncology [15].

In short, the "extract" is showing very good activity on all prostate cancer cell lines.

Table 1: Anticancer activity on breast cancer cell lines:

Cell line	Concentration of red pigment extract $\mu\text{g/ml}$ Expressed as % viability ($\pm\text{s.d}$)				DPC % viability ($\pm\text{s.d}$)	GCD % viability ($\pm\text{s.d}$)	IC50 ($\mu\text{g/ml}$)
	250	50	5	0.5			
MCF-7	0 (± 0.0)	37.19 (± 0.015)	98.17 (± 0.0056)	108.53 (± 0.020)	0.76 (± 0.001)	100% (± 0.017)	40.76
MDAMB231	0.45 (± 0.002)	53.59 (± 0.05)	87.37 (± 0.0311)	105.74 (± 0.02)	0.6 (± 0.0010)	100% (± 0.043)	110.53
T47D	1.79 (± 0.0025)	65.83 (± 0.0191)	93.23 (± 0.0115)	93.66 (± 0.0088)	2.82 (± 0.005)	100% (± 0.0066)	113.81

Values are the mean of six replicates (n=6)

Experimental groups were compared with GCD (vehicle control) $p < 0.05$

Table 2: Anticancer activity on prostate cell lines

Cell line	Concentration of red pigment extract $\mu\text{g/ml}$ Expressed as % viability ($\pm\text{s.d}$)				DPC % viability $\pm\text{s.d}$	GCD % viability $\pm\text{s.d}$	IC50 ($\mu\text{g/ml}$)
	250	50	5	0.5			
PC3	0.4273 (± 0.002)	42.8 (± 0.008)	93.16 (± 0.014)	104.98 (± 0.023)	1.566 (± 0.002)	100 (± 0.014)	43.79
LNCaP	0 (± 0)	34.03 (± 0.027)	91.01 (± 0.025)	100.07 (± 0.07)	4.42 (± 0.007)	100 (± 0.017)	37.66
DU145	2.60 (± 0.001)	14.85 (± 0.006)	66.21 (± 0.030)	90.022 (± 0.0566)	0.22 (± 0.002)	100 (± 0.027)	23.66

Values are the mean of six replicates (n=6)

Experimental groups were compared with GCD (vehicle control) $p < 0.05$

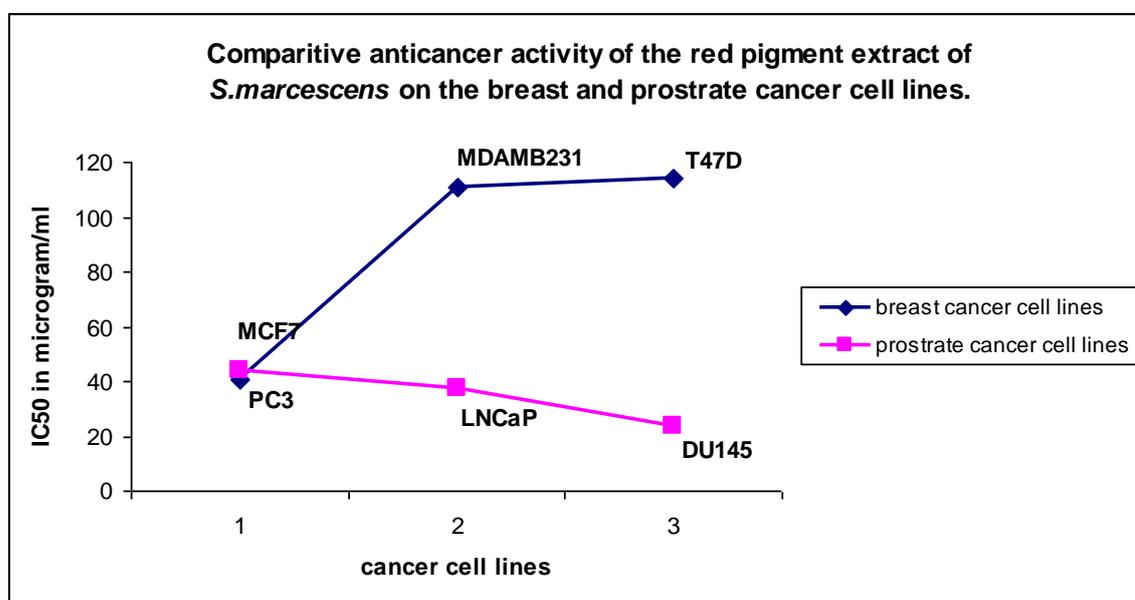


Fig. 2:

Fig 2: The plot of the cancer cell lines v.s. their IC50 values in (µg/ml) shows that the extract shows almost similar activity on MCF-7 and PC3 but there is a significant difference observed ($p < 0.05$) when the activity of the extract on MDAMB231 and T47D was compared with that of LNCaP and DU145.

CONCLUSION

The results of the study show that the *red-pigment extract* of *S.marcescens* has a strong anticancer activity on prostate cancer cell lines compared to that of breast cancer cell lines MDAMB231 and T47D. The extract showed almost similar anticancer activity on MCF-7 and PC3. Prodigiosin is one of the major components present in the red pigment of *S.marcescens* and is known to possess anticancer activity. There is a need to further check the purity, analyse isolate this active compound from the extract and verify its activity in the pure state. The results of the study need to be confirmed also by in-vivo methods.

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