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RED PIGMENT EXTRACT OF SERRATIA MARCESCENS INHIBITS CELL PROLIFERATION AT THE SCRATCH OF METASTATIC PROSTATE CANCER CELL LINES DU145 AND PC3.

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ABSTRACT

Purpose: The aim of the study was to evaluate the effect of Red Pigment Extract (RPE) of Serratia marcescens on cell proliferation at scratch for two prostate cancer cell lines DU145 and PC3. **Methods:** The effect of RPE of *Serratia marcescens* on DU145 and PC3 in scratch assay was checked at inhibitory concentration-5(IC5) at periodic time intervals namely 0, 8, 24, 30 and 48hrs for DU145 and PC3 respectively. In case of PC3 time interval was extended further to 54hrs.The width of scratch was measured in µm at each time point by "IS Capture" software. **Results:** Our results indicated that RPE has a strong effect on cell proliferation at the scratch for both DU145 and PC3 exhibiting % inhibition of 97.5% and 60.3% at the end of 48 and 54hrs respectively. Percentage inhibition of RPE was found to be significantly different ($p<0.001$) compared to Cisplatin and Sunitinib. **Conclusion:** Findings of our study indicate that RPE of *Serratia marcescens* has a strong potential to inhibit cell proliferation at scratch of metastatic prostate cancer cell lines DU145 and PC3.

KEYWORDS: Red Pigment Extract (RPE), *Serratia marcescens*, scratch assay, PC3 and DU145.

INTRODUCTION

Bacteria such as species of *Actinomycetes*, *Streptomyces* and *Serratia* have been reported to synthesize prodigiosins (Red pigment).[1] These prodigiosins have exhibited anticancer activity on various cancer cell lines[2,3] and no toxicity in non-malignant cell lines.[4]

Earlier, we have reported a higher antitumor activity of Red Pigment Extract (RPE) of *S. marcescens* on a panel of prostrate cancer cell lines compared to that of breast.[5] Recently, we have also reported higher activity of RPE on CD^{44} cell surface marker on metastatic prostrate cancer cell lines DU145 and PC3[6] which corresponds to Cancer Cell Stemness.[7]

In our present study, we were interested in understanding the role of RPE in preventing cancer cell proliferation using scratch assay. Scratch/wound healing assay has been widely adapted and modified to study the effect of drug on cell proliferation and migration.[8] We adopted this method to understand the effect of RPE in preventing cell proliferation and migration at the scratch made on the confluent monolayers of DU145 and PC3.

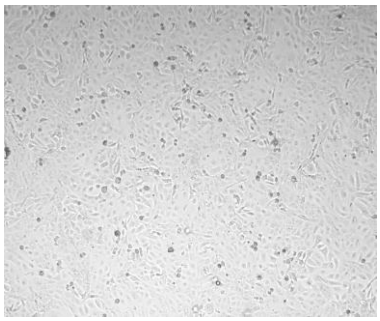
MATERIALS AND METHODS

Scratch Assay was performed in 12 well adherent Tissue Culture (TC) plates. Briefly, $1.75x10^5$ cells/well of DU145and PC3 cells (cultured in DMEM and R.P.M.I respectively), were plated in each well of the plate. The cells were incubated at 37^0 C, 5% CO_2 for 72hrs and were allowed to form a confluent monolayer with no gaps in entire monolayer. A scratch at 0hr was made using a sterile 1ml tip. The width of the scratch was immediately recorded in micrometers using "IS Capture" software. To compare the effect of RPE on scratch healing standard drugs like Cisplatin and Sunitinib were also set up in the assay. Briefly, Inhibitory concentration 5 (IC5) for each of the drugs, Cisplatin, Sunitinib and RPE were selected for experimental purpose.

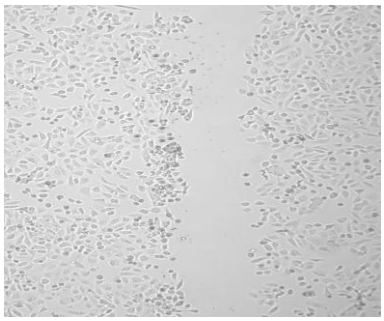
IC5 of RPE and Cisplatin was calculated as reported in our earlier study.[5] Dilutions of the drugs were prepared in Dimethyl Sulfoxide (DMSO). Suitable controls like Growth Control (GC), Growth Control with DMSO (GCD) and Media Control (MC) were set up during the experiment. The experimental plates were incubated at 37^0 C and 5% CO_2 and the width of the scratch was measured (n=5) at regular intervals 0, 8, 24, 30 and 48hrs respectively for DU145 and for PC3 the width was further measured at 54hrs.

RESULTS

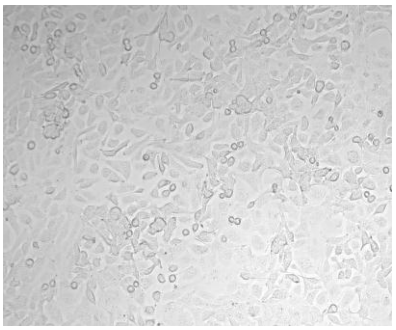
Image 1: Scratch Assay of DU145.



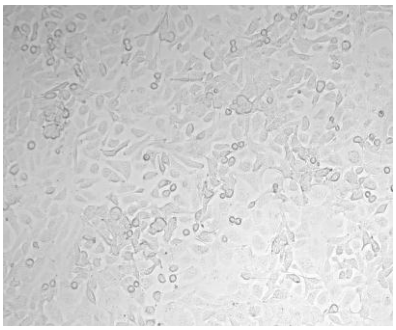
i) Before scratch



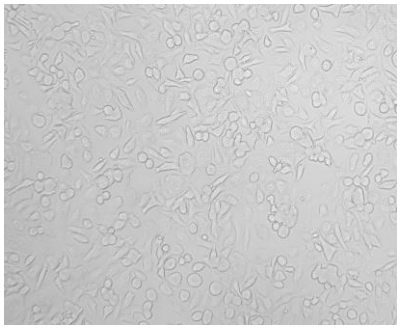
(ii) 0hrs



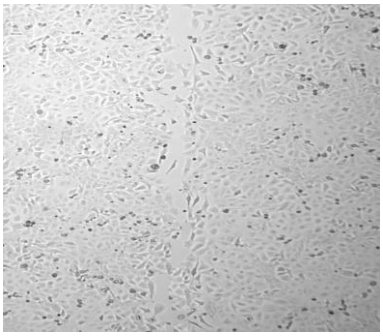
(iii) GC - 48hrs



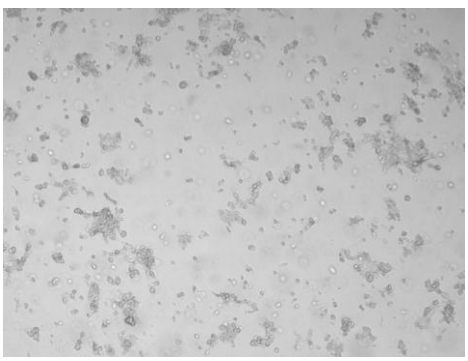
(iv) GCD - 48hrs



(v) Cisplatin- 48hrs

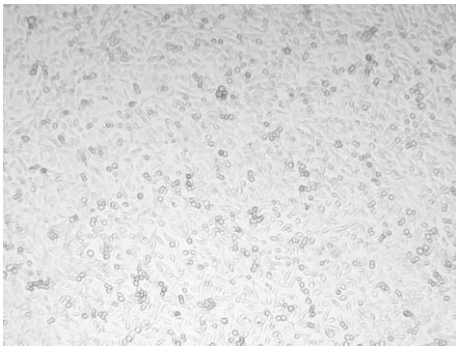


(vi) Sunitinib-48hrs

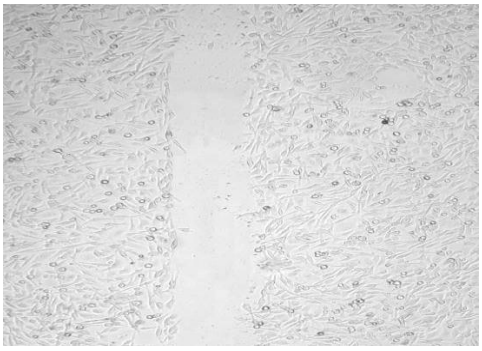


(vii) RPE-48hrs.

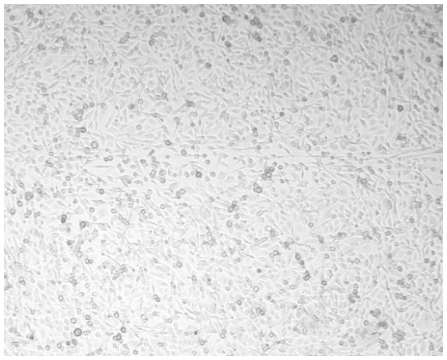
Image 2: Scratch Assay of PC3.



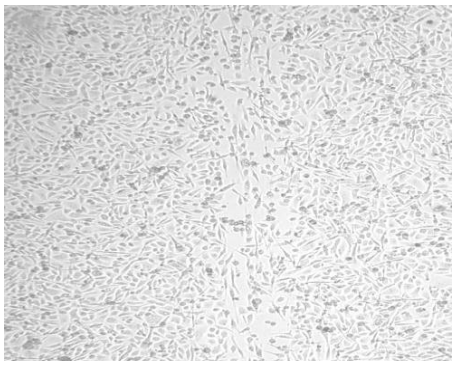
(i) Before scratch



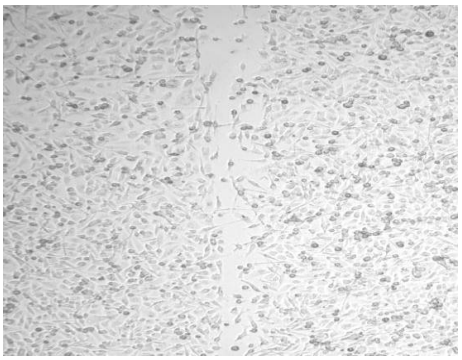
(ii) 0hrs



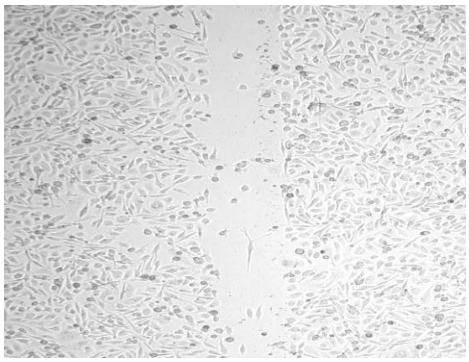
(iii) GC - 48hrs



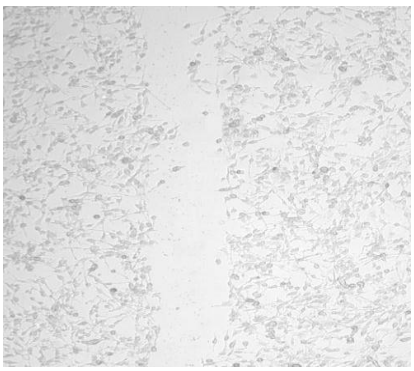
(iv) GCD - 48hrs



(v) Cisplatin 54hrs



(vi) Sunitinib 54hrs



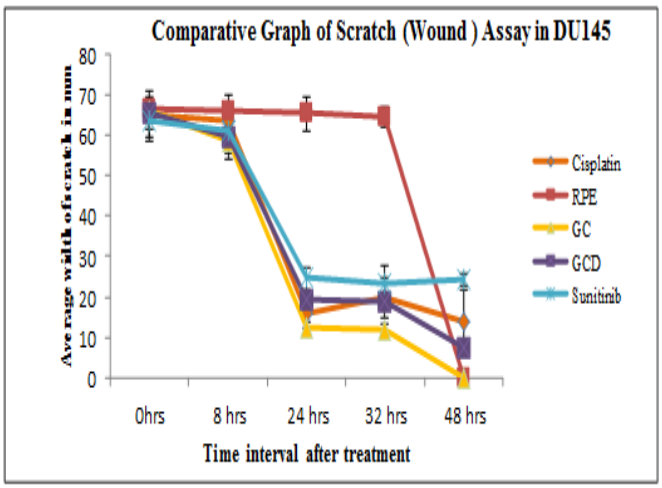
(vii) RPE 54hrs.

Table 1: Average width of scratch of DU145 at various time intervals when exposed to different compounds.

DU145	Average width of scratch in µm (± s.d) n=5				
	Treatment with			Controls	
Time interval	Cisplatin	Sunitinib	RPE (IC5)	GC	GCD
0hrs	65.22 (±5.70)	63.61 (±5.12)	66.35 (±2.74)	65.97 (±1.81)	65.62 (±3.90)
8hrs	63.60 (±2.50)	61.11 (±2.80)	66.02 (±3.75)	58.40 (±4.37)	59.54 (±3.98)
24hrs	15.63 (±3.53)	24.53 (±2.59)	65.37 (±4.20)	12.45 (±1.52)	19.08 (±2.37)
30hrs	19.78 (±5.09)	23.05 (±4.92)	64.65 (±2.50)	11.93 (±1.54)	18.84 (±6.02)
48hrs	13.95 (±7.90)	24.25 (±1.50)	Dead and floating cells	0	7.31 (±4.12)

Table 1 indicates the average width of scratch of DU145 at different time intervals when exposed to Cisplatin, Sunitinib and RPE respectively.

Graph 1: Scratch healing in DU145 with respect to time.



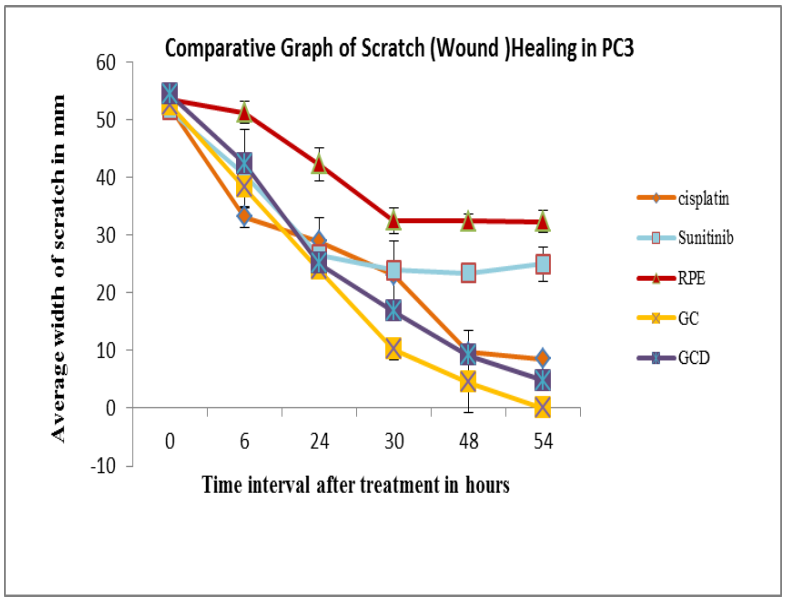
Graph 1 indicates time wise scratch healing in DU145 cells when exposed to different compounds.

Table 2: Average width of scratch of PC3 at various time intervals when exposed to different compounds.

PC3	Average width of scratch in µm (± s.d) n=5				
	Treatment with			Controls	
Time interval	**Cisplatin**	**Sunitinib**	**P extract (IC5)**	**GC**	**GCD**
0hrs	52.21 (±1.49)	51.69 (±1.60)	53.60 (±0.99)	52.63 (±0.78)	54.40 (±2.36)
8hrs	33.17 (±1.33)	40.54 (±7.85)	51.22 (±1.88)	38.31 (±1.83)	42.39 (±3.5)
24hrs	28.94 (±1.34)	26.63 (±1.44)	42.28 (±2.94)	24.03 (±4.18)	25.10 (±1.10)
30hrs	23.10 (±2.82)	23.95 (±1.30)	32.53 (±2.26)	10.28 (±5.98)	16.78 (±1.91)
48hrs	9.730 (±3.70)	23.37 (±1.33)	32.47 (±1.15)	4.46 (±3.80)	9.16 (±5.21)
54hrs	8.49 (±1.19)	24.96 (±2.98)	32.30 (±1.90)	0	4.84 (±1.05)

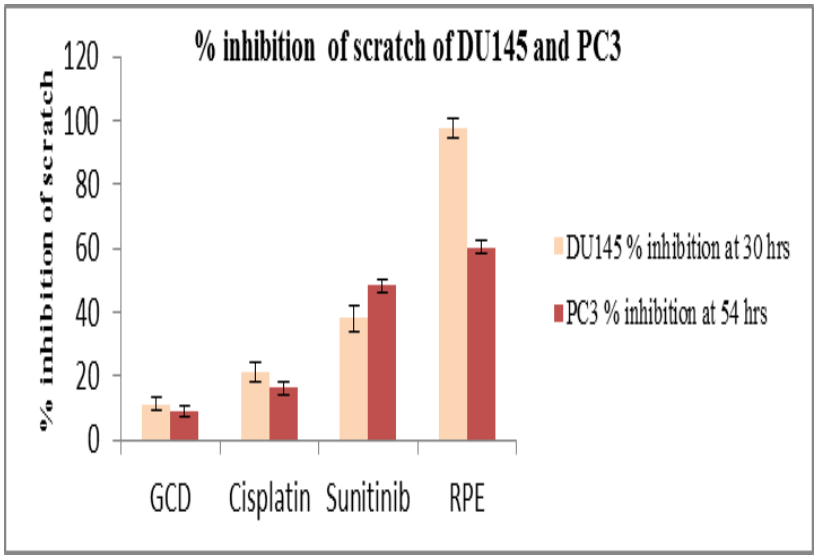
Table 2: Indicates the average width of scratch of PC3 at different time intervals when exposed to Cisplatin, Sunitinib and RPE respectively.

Graph 2: Scratch healing in PC3 with respect to time.



Graph 2: indicates time wise scratch healing in PC3 cells when exposed to different compounds.

Graph 3: Comparative proliferative inhibition of DU145 and PC3 at scratch.



Graph 3: Indicates % inhibition of scratch of DU145 and PC3 by different compounds. RPE exhibits significant % inhibition at scratch ($p<0.001$) for both DU145 and PC3 compared to Cisplatin and Sunitinib.

DISCUSSION

Image 1 (i) indicates the confluent monolayer formed by DU145, (ii) indicates the scratch made at 0hrs, (iii) and (iv) indicate almost complete healing of scratch of GC and GCD samples in the absence of any drug at 48hrs whereas images (v), (vi) and (vii) indicate the effect of Cisplatin, Sunitinib and RPE respectively. It can be clearly seen that, in case of Cisplatin there is healing of gap compared to Sunitinib, but in case of RPE almost all cells had become dead and were floating at the end of 48hrs. Similarly, in case of Image 2 (showing scratch assay of PC3), it can be clearly seen that the wide gap of scratch of PC3 cells is higher in RPE (vii), followed by Cisplatin (v) and Sunitinib (vi) at 54hrs.

Table 1 indicates the width of scratch at different time intervals i.e. 0, 8, 24, 30 and 48hrs respectively for DU145 for Cisplatin, Sunitinib and RPE at IC5. Cisplatin is standard chemotherapeutic drug used in various types of Cancers[9] whereas Sunitinib[10] is target drug therapy and is a tyrosine kinase inhibitor.[11] A close observation of Table 1 reveals that in the presence of Cisplatin, there is a marked reduction in the width of the scratch from 65.22 µm to 13.94 µm demonstrating an active cell growth in the area. In case of Sunitinib the average width of scratch had become 24.248 µm whereas in case of RPE there was no significant reduction in the width of the scratch at the end of 30hrs and by the end of 48hrs all the cells were dead and floating, indicating a potent effect of RPE on cancer cell proliferation compared to Cisplatin and Sunitinib. Table 2 indicates the scratch healing process of PC3 in the presence of Cisplatin, the cells were still able to proliferate and the width of the scratch was reduced from 52.2 µm to 8.48 µm indicating % inhibition of only 16.25% whereas in case of Sunitinib and RPE there was an increase inhibition observed i.e. 48.29 and 60.3% respectively.

A close observation of Graph 3, reveals that RPE exhibits highest % inhibition at scratch compared to Cisplatin and Sunitinib ($p<0.001$) in case of both DU145 and PC3.

Scratch or wound Assays are also used to check the anticancer stem cell effect of a drug.[12] Recently, we have demonstrated that RPE is able to make a shift in $CD44^{high}$ cells, making them $CD44^{low}$ $CD^{24\ high}$.[6] Our present study supports, the role of RPE in preventing cell proliferation and probably acting on the cancer stem cells at the scratch.

We have obtained a single peak of RPE at 539nm, by U.V spectrum which corresponds to the peak of prodigiosin.[13] This further proves that RPE has a major component of prodigiosin,

which could be used as a natural anticancer agent in future. However, RPE needs to be further purified and verified for its activity before it can be used as a natural chemotherapeutic agent.

CONCLUSION

Our present study has substantiated the role of RPE to inhibit cell proliferation of metastatic prostate cancer cell lines DU145 and PC3. Further, its activity needs to be confirmed by in-vivo methods be used as an approved natural chemotherapeutic drug.

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