AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Evaluation of cytotoxic and anticancer activity of prodigiosin produced by *Serratia Spp.*

Phatake Y.B[1]*, Dharmadhikari S.M[2]

1.Department of Microbiology, Vidya Pratishtans School of Biotechnology, Baramati, Maharashtra.

2.Department of Microbiology, Government Institute of Science, Aurangabad.

ABSTRACT

Red pigment of *Bacillus prodigiosus*, now known as *Serratia marcescens*, were first time investigated and named in 1902. The major component was first isolated in pure form in 1929 and in next 5 years investigators elucidated the main structural features of Prodigiosin and culminated in the assignment of structure. In the present study, potent pigment producing bacteria were used which were previously isolated from soil samples collected from different regions of Baramati, Maharashtra, India. The large scale production of Prodigiosin was achieved by using optimized parameters (time of incubation, temperature of incubation, pH of the medium, speed of Agitation, carbon and nitrogen source used etc.). The effect of red pigment on normal cells was assessed by toxicity studies using trypan blue assay. It shows that pigment having very less or no toxic effect on normal cells at various concentrations (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml). The effect of various concentrations (7.8, 15.6, 31.25, 62.5, 125, 250 µg/ml) of pigment on human cancerous cells (Lymphocytes) was also determined by using flow cytometry. Apoptosis was quantified by a reduction in cell forward scatter and increase in cell side scatter in flow cytometric analysis. Study revealed that the produced red pigment may be used for production of effective anticancer drug in future.

Keywords: Prodigiosin, Serratia marcescens,ccytotoxic activity, Anticancer activity, FACS.

*Corresponding Author Email: phatakeyogmicro@gmail.com

Received 11 May 2016, Accepted 04 June2016

Please cite this article as: Phatake YB *et al*., Evaluation of cytotoxic and anticancer activity of prodigiosin produced by Serratia Spp.. American Journal of PharmTech Research 2016.

INTRODUCTION

From *Bacillus prodigiosus* red pigment was isolated and named "Prodigiosin" by Kraft in 1902. Prodigiosin is a multifaceted important secondary metabolite produced in large quantity by *Serratia marcescens*, *Pseudomonas magneslorubra*, *Vibrio psychroerythrous* and other bacteria, fungi and actinomycets. Which is a linear tripyrrole. Through a series of isotope incorporation experiments, it has been possible to show that this pigment is a pyrrolic compound, it is not closely related to the porphyrins biosynthetically. As it has been demonstrated that 8-aminolevulinic acid, a specific precursor of porphyrins, is capable of penetrating the membrane of *Serratia marcescens* but it is not used in the formation of Prodigiosin.

Pigment production is highly variable among species and depends on many factors such as species type, incubation time, pH, carbon and nitrogen sources and inorganic salts. Although Prodigiosin has no defined role in the physiology of the strains in which it is produced, it has antifungal, antibacterial and antiprotozoal activities, and thus may have potential clinical utility. Both Prodigiosin and its synthetic derivatives have potent and specific immunosuppressive activity, with new targets that are clearly distinct from those of other drugs. (Casullo de Araujo, H. W. *et. al.*, 2010).

In recent it has been found that Prodigiosin will be used as potent anticancer agent which could induce apoptosis of several cancer cell lines in vitro including hematopoietic cancer cells, colon cancer cells, B-cell and chronic lymphocytic leukemia cells. Many researches have been done to improve Prodigiosin production. Prodigiosin was formed via a bifurcated pathway which depended upon a number of genes coding for the enzymes involved. There are different fermentation skills that have been applied for the high production of Prodigiosin. Feng *et. al.* enhanced the Prodigiosin secretion by adding SDS into the cultures.

Cancer is characterized by unlimited growth, invasion and metastasis of cells whereas benign tumors are self-limiting, non-invasive, and non-metastasizing. Most clinical symptoms are accompanied by weight loss, poor appetite, fatigue, unusual lumps, bleeding, pain, enlarged lymph nodes and neurological symptoms. The unique properties of cancer cells include non-regulated proliferation, immortalization, metastasis and angiogenesis. Tumor angiogenesis is the proliferation of blood vessels that supply nutrients and oxygen and that remove metabolic waste from tumors. Tumor angiogenesis involves the interaction of tumor cells, endothelial cells, phagocytes and their secreted factors which may promote or inhibit angiogenesis. Therefore, inhibiting tumor angiogenesis is a major goal of cancer treatment.

Metastasis is one of the major causes of mortality in cancer patients. However, the treatment to metastasis is still far from satisfactory. Invasion of tumor cells involves multiple processes that depend on specific cell to cell and cell to ECM (extracellular matrix) interactions. These interactions are mediated directly by specific adhesion receptors and indirectly by extracellular proteinases that mediate degradation of the ECM. Several reports have indicated that RhoA protein, an important signal molecule, is required for cell adhesion and consequently influences many aspects of cell shape and movement. In addition, matrix metalloproteinases (MMPs), a well known family of zinc-binding enzymes, plays an important role in the process of cleaving ECM components. (Zhang, J. *et. al.*, 2005).

Recently, Prodigiosin-induced apoptosis in gastric cancer cell led to the discovery that Prodigiosin produced by *Serratia marcescens* triggered apoptosis in different cancer cell lines, behaving as a rapid, potent and selective drug (Montaner *et. al.*, 2000; Montaner and Perez-Tomas, 2001). The fact that the antiproliferative effect of Prodigiosin is p53-independent (Montaner *et. al.*, 2000) makes Prodigiosin an interesting new anti neoplasic candidate. (Diaz-Ruiz, C. *et. al.*, 2001).

The identification of novel targets and the development of more specific chemotherapy agents are two of the most important goals of research in cancer therapy. Apoptosis is a process of cell death in which cells actively participate in their own destruction processes in which DNA fragmentation is initiated and propagated by single stranded breaks that result in double-stranded (ds) fragments, which are then further digested to oligonucleosomal ladders. Chromatin processing may proceed by two pathways. The first is caspase-dependent and involves caspase-activated DNase (CAD), responsible for oligonucleosomal DNA fragmentation and advanced chromatin condensation. The second is caspase independent and involves apoptosis-inducing factor (AIF), which leads to large-scale DNA fragmentation and peripheral chromatin condensation (Susin *et. al.*, 2000). However, some anti-cancer drugs can directly induce DNA damage via molecular targets.

Topoisomerases have been established as effective chemotherapeutic targets. These enzymes modulate DNA super helicity and act by introducing single (type I) or double (type II) DNA breaks. They are involved in DNA repair, replication, transcription, and chromosome segregation during mitosis. For topoisomerase-directed agents, resulting DNA damage can lead to cell cycle arrest and/or cell death by apoptosis (Montaner, B. *et. al.*, 2005).

The mechanism of action of Prodigiosin was reviewed. Four possible mechanisms are suggested attributed to Prodigiosin as pH modulators, cell cycle inhibitors, DNA cleavage agents and mitogen activated protein kinase regulators. These molecules when combined with

some other anticancer agents can greatly help in fighting with the cancer. (Kamble, K. D. and Hiwarale, V.D. 2012).

To reduce the risks of cancer, chemotherapeutic agents including antioxidants and non-steroidal anti-inflammatory drugs (NSAIDs), are used as immune modulators or promoters of cell differentiation or apoptosis. Nevertheless, surgery is the only option available to patients with teratocarcinoma, and adjuvant chemotherapy is applied to decrease recurrence and thereby metastasis. Apoptosis malfunctions can have health implications like in cancer. A variety of morphological and biochemical studies can be used to identify the apoptotic process, which is fundamentally different from necrosis or degenerative cell death. The morphology of cells during the development of apoptosis is associated with compaction and margination of nuclear chromatin, condensation of the cytoplasm, and partition of nucleus and cytoplasm into membrane bound-vesicles, many containing nuclear fragments. (Montaner, B. and Perez-Tomas, R. 2001).

MATERIAL AND METHOD

In this study bacterium *Serratia marcescens* previously isolated and characterized from soil sample were used **(**Phatake Y. B and Dharmadhikari S. M. 2015) for large scale production of Prodigiosin. The produced pigment was characterized qualitatively by using chromatographic and spectroscopic study (Davraj *et. al.*, (2009), Song *et. al.,* (2005) and Phatake Y. B and Dharmadhikari S. M. 2015). The quantity of produced pigment was determined by standard formula of calculating red pigment in Unit/cell. (Mekhael and Yousif, 2009)

Large Scale Production

Large scale production of the red pigment was carried out in lab scale fermenter (ESEDRA PLUS-4) of three liter capacity. The parameters previously optimized by classical method of optimization were used for large scale production, which include Time of incubation, Temperature of incubation, pH of the medium, speed of Agitation, Carbon source, Nitrogen source etc. For the production process, Peanut broth was used which found to give maximum yield of pigment.

Extraction of the Pigment

For the extraction of the Prodigiosin 500 ml fermented broth containing pigment was mixed with equal volume of methanol and kept the mixture in rotary shaker for 20 to 30 min. Then this mixture was pour into the centrifuge tube and shacked vigorously using a vortex mixture. Then centrifuge at 10000 rpm for 10 min. The supernatant was collected and filtered through Wattman filter paper number 1 (0.2µm).The filtrate was concentrated using a rotary evaporater and later

extracted with 3.0 M chloroform. The chloroform phase was collected and reconcentrated using rotary evaporator to obtained crude powder (Davaraj Naveen Raj *et al.,* 2009)

Toxicity Studies

For toxicity studies Dulbecco's Modified Eagle Medium (DMEM) was prepared, Different concentration of Prodigiosin (5, 10, 15, 20, 25, 30, 35, 40, 45, 50µg/ml) was prepared by dissolving 1 mg of pigment in 100µl 3% DMSO. Then each concentration was added in DMEM. Followed by transfer of lymphocyte cell line in it with addition of 10% fetal bovine serum, 1% penicillin and streptomycin solution (to avoid bacterial and fungal contamination).Each culture flask ($T_{12.5}$) then incubated at 37^{o}C with 5%CO_2 for four days (96 hrs.).

After incubation viable cell count was determined at regular interval of 24 hrs. by using trypan blue assay.

Trypan Blue Dye Exclusion Assay

0.4% Trypan blue was prepared in distilled water. 100µl medium along with cells was taken from the culture flasks aseptically and mixed with equal volume of trypan blue dye. A fix amount is then placed on hemocytometer and observed under compound microscope under 10 x magnifications to observe viable and dead cell (Davaraj Naveen Raj *et al.*,2009).

Evaluation of Anticancerous Activity

For evaluation of anticancer activity of produced red pigment Dulbecco's Modified Eagle Medium (DMEM) was prepared, Different concentration of Prodigiosin (7.8, 15.6, 31.25, 62.5, 125.0, 250.0µg/ml) was prepared by dissolving 1mg of pigment in 100µl of 3%DMSO. Then each concentration was added in DMEM. Followed by transfer of blood leukemia cell line in it with addition of 10% fetal bovine serum, 1% penicillin and streptomycin solution (to avoid bacterial, fungal contamination). Each culture flask ($T_{12.5}$) then incubated at 37^{o} C with 5% CO_2 for three days (72hrs). The control set was run by addition of normal human blood cell instead of leukemia cells. In control all other parameter kept constant. The activity was evaluated by using flow cytometry.

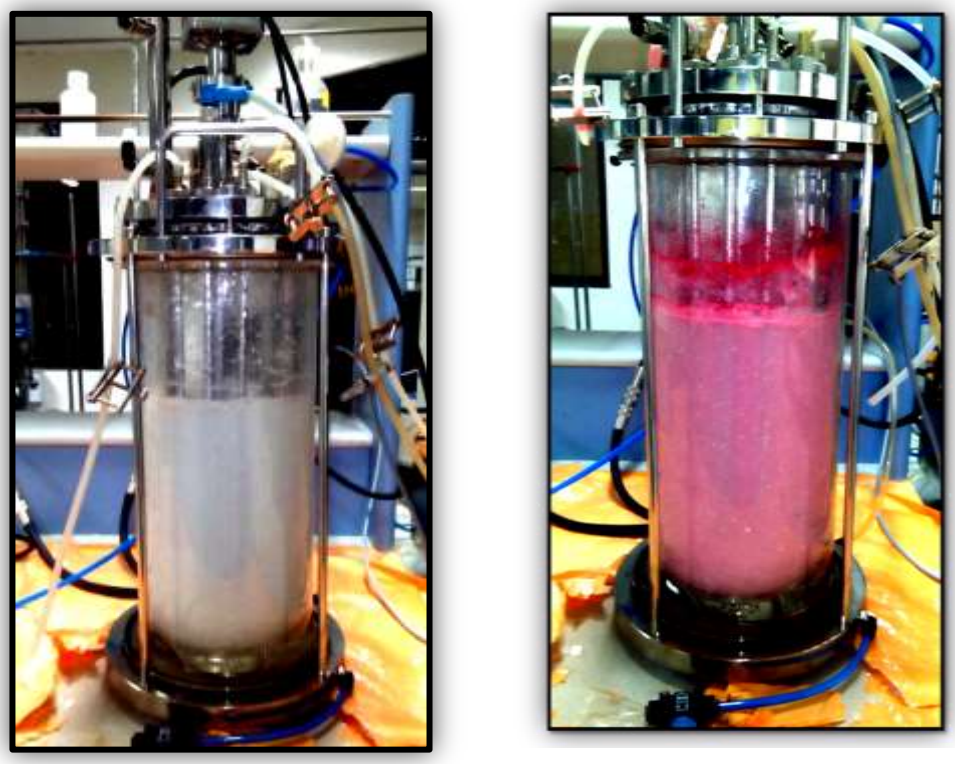
Flow Cytometry Assay

500µl medium along with cells was taken from the culture flasks and was centrifuged at 2500 rpm for 5 min. In the pellet freshly prepared 500µl PBS was added for flow cytometry analysis. The instrument used was(BD FACS Caliber).The anticancer activity in folds was also determined for each concentration of pigment. The values obtained from calculations were plotted by bar diagram.

RESULTS AND DISSCUTION

Large Scale Production

For large scale production of Prodigiosin, selected strain of *Serratia marcescens* was used. The process is carried out in lab scale fermenter (ESEDRA PLUS-4). The previously optimized parameters were used for large scale production, which include Time of incubation 72 hrs., Temperature of incubation 28^{o}C, pH of the medium 7, Agitation 100 rpm, carbon source maltose and Nitrogen source Ammonium nitrate etc. Peanut broth was used as a production medium.



BEFORE INCUBATION AFTER INCUBATION

Figure 1: Large scale production of Prodigiosin

TOXICITY STUDIES

The effect of different concentration of red pigment Prodigiosin on the viability human lymphocyte cell line was studied. Cells were incubated for 24 hours with several doses of pigment, ranging from 5 to 50 µg /ml dissolved in 3% DMSO and cell viability was then determined by Trypan blue assay. A no significant dose-dependent decrease in the number of viable cells was observed when sample was checked at regular time interval. (24, 48, 72 and 96 hrs.).The number of viable cells and dead cells were calculated by observingunder10 x objectives.

Table 1: Total cell count

Time	Cell	Control	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml	35 µg/ml	40 µg/ml	45 µg/ml	50 µg/ml
24 hrs.	Live	132	120	136	108	128	148	116	132	129	132	120
	Dead	1	2	5	6	3	6	4	6	4	2	3
48 hrs.	Live	180	174	192	164	204	200	180	176	200	188	168
	Dead	3	4	5	5	6	7	6	7	8	4	5
72 hrs.	Live	220	212	228	204	236	244	220	208	232	232	212
	Dead	8	8	9	8	6	9	9	9	10	7	6
96 hrs.	Live	232	252	264	232	268	272	244	232	264	260	244
	Dead	8	7	10	11	8	10	11	11	11	9	8

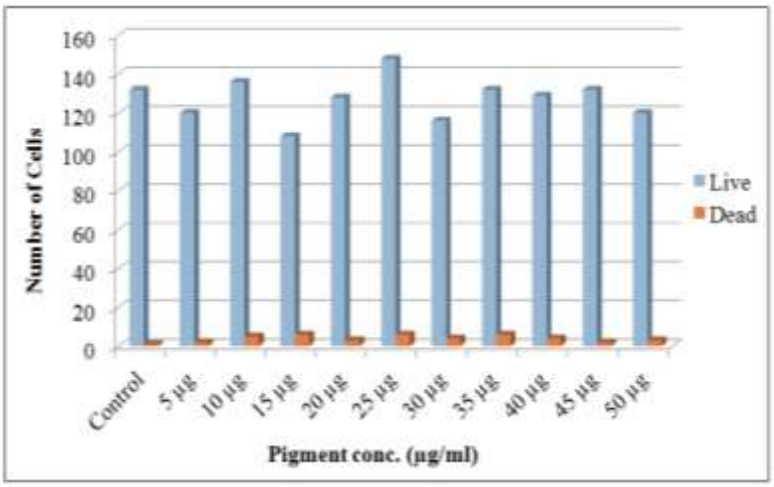


Figure 2: Total cell count at 24 hrs.

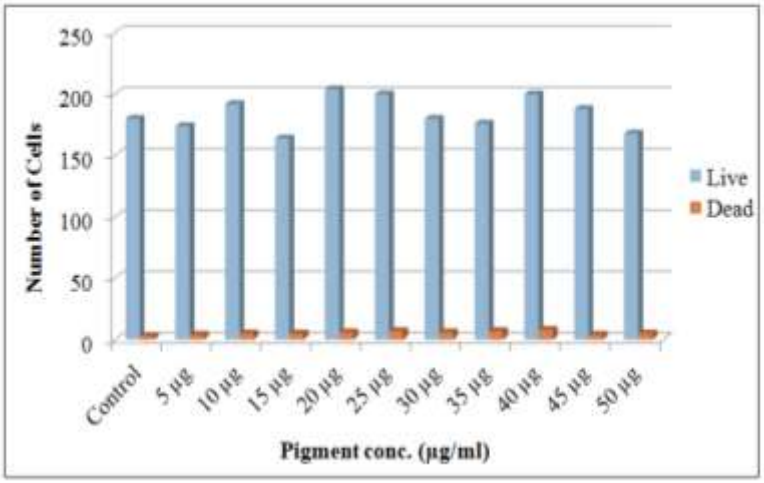


Figure 3: Total cell count at 48 hrs.

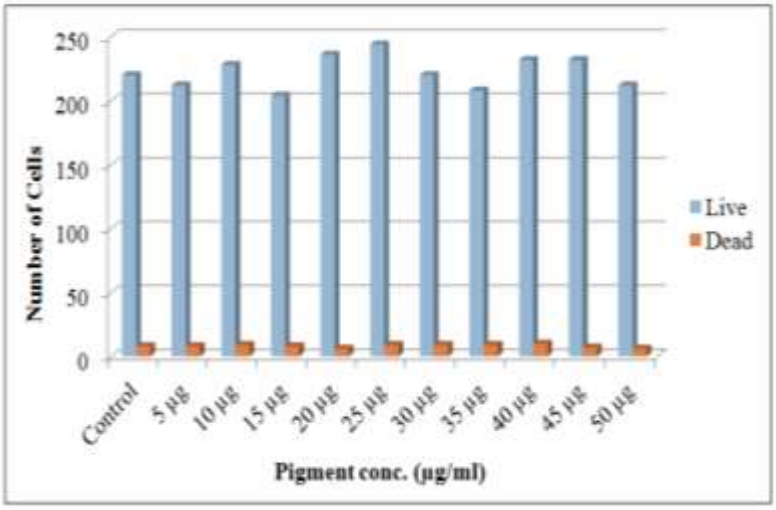


Figure 4: Total cell count at 72 hrs.

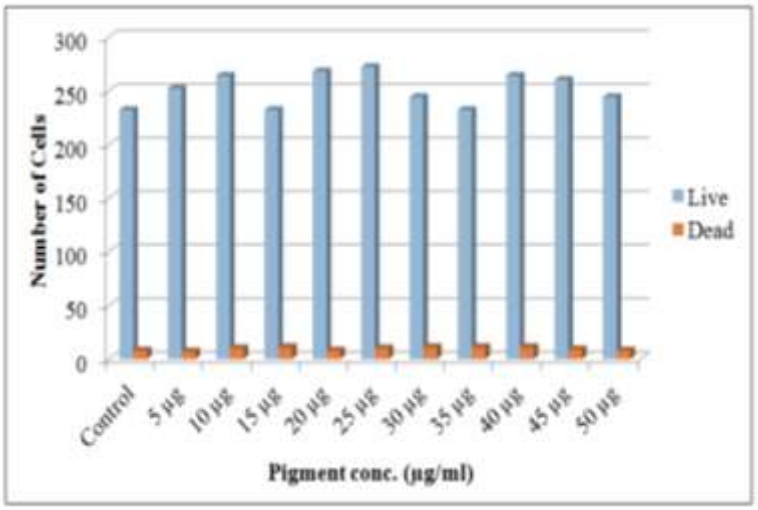


Figure 5: Total cell count at 96 hrs.

The effect of different concentrations of Prodigiosin (5, 10, 15, 20, 25, 30, 35, 40, 45, 50 µg/ml) on the viability of normal lymphocyte cell line was studied. It was found that there was no harmful effect of this pigment on the growth and viability of cell at various concentrations. Prodigiosin neither kill lymphocyte nor inhibit there growth. The growth pattern of cells in each flask when compared with the control shows similar results. The Davraj, *et. al.*, (2009)used different concentrations of Prodigiosin as (5, 15, 25, 50mg/ml)for cytotoxicity testing by dissolving in DMSO and cell viability was then determined by the MTT assay, They also found no cytotoxic effect of Prodigiosin on normal human cells.

Diaz-Ruiz. C. *et al.*,(2001) also reported that red pigment produced by *serratia marcescens* having apoptotic effect on several cancer cell line like Jurkat, NSO,HL-60 etc. but not on non malignant cells like NIH-3T3,Swiss-3T3 and MDCK. Williamson N.R, *et.al.*,(2007)found that Prodigiosin

showing potent activity against a broad range of cancerous cell lines and against multidrug-resistant cell line, with little effect on normal cells.

EVALUATION OF ANTICANCER ACTIVITY

For evaluation of anticancer activity of Prodigiosin, human blood leukemia cell line was prepared. Simultaneously a normal lymphocyte cell line was also prepared as a control. Various concentrations of test compound were prepared in DMSO and then cells were exposed to it. After incubation each sample was analyzed by Flow cytometry. Results obtained are shown in fig. 6, 7 and 8.

The effect of different concentrations of Prodigiosin on the viability of normal lymphocyte cell line as well as blood leukemia lymphocyte cell line was studied. The results indicate that mean viable cell count were affected dramatically in cancerous cells when compared with normal cell. The viable cell count of blood leukemia lymphocyte cell line was decreased drastically as compared to normal lymphocyte cell line that means Prodigiosin has potent apoptic activity against cancerous

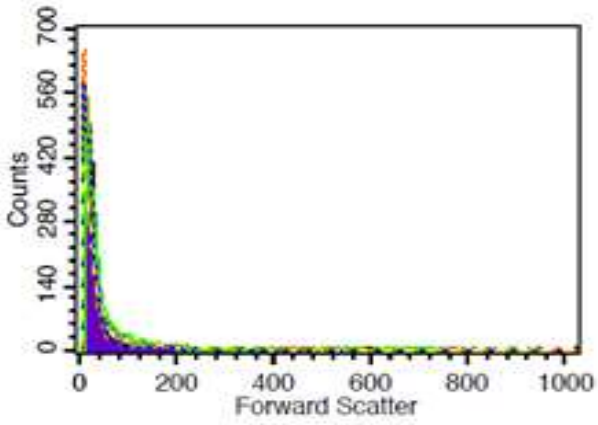
cells.

Davraj, *et al*., (2009)observed a significant dose-dependent decrease in the number of viable cells when HeLa cells were exposed to Prodigiosin for different time periods. Similar results were observed by Venil, C. K. and Lakshmanaperumalsamy, P.(2009). They investigated the cytotoxic potency of Prodigiosin in the standard 60 cell line panels of human tumor cells derived from lung, colon, renal, ovarian, brain cancers, melanoma and leukemia and they observed inhibition of cell proliferation as well as induction of cell death in these cell lines.

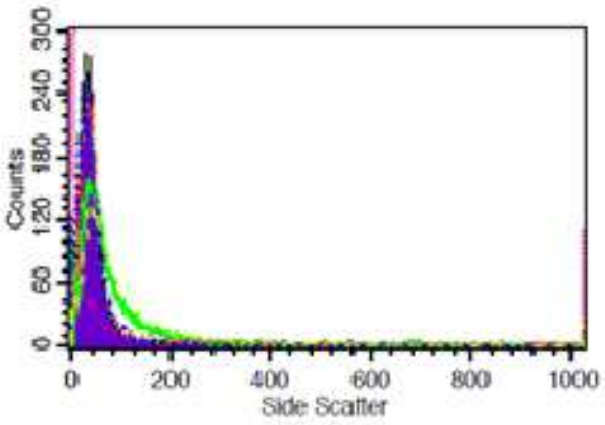
The mechanism of action of Prodigiosin on cancerous cell focus on its interaction with DNA and its ability to inhibit both topoisomerase I and topoisomerase II resulting in the DNA damage induced in cancer cell lines. Prodigiosin-DNA intercalation can analyzed using a competition dialysis assay with different DNA base sequences. Topoisomerase I and II inhibition can be studied in vitro by a cleavage assay, and in cultured cells, by analysis of its ability to form covalent complexes.

Prodigiosin has a potent apoptosis activity against cervix carcinoma cells and against many different types of human cancerous cells. In all this type of assay the assessment of the cell viability was based on the data from MTT assay, Trypan blue assay, NRU assay etc. These methods were employed for determining the antiproliferative activity of compounds in culture as well as for determining cell viability.

CANCER SAMPLE (24 HRS.)



Forward Scatter 24 hrs.



Side Scatter 24 hrs.

File: Control.001

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	37.41	28.33	139.28	25.00

File: 7.8 ug/ml.002

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	55.44	37.84	120.95	31.00

File: 15.6 ug/ml.003

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	49.43	36.60	109.97	31.00

File: 31.2 ug/ml.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	30.00	25.89	89.75	24.00

File: 62.5 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	33.30	26.55	126.02	24.00

File: 125 ug/ml.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	30.36	26.68	76.70	25.00

File: 250 ug/ml.007

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	48.62	36.84	93.64	32.00

File: Control.001

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	47.73	34.34	148.96	34.00

File: 7.8 ug/ml.002

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	83.95	53.20	140.82	51.00

File: 15.6 ug/ml.003

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	46.79	5.42	318.97	3.00

File: 31.2 ug/ml.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	39.43	30.26	126.56	33.00

File: 62.5 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	51.49	39.14	132.35	40.00

File: 125 ug/ml.006

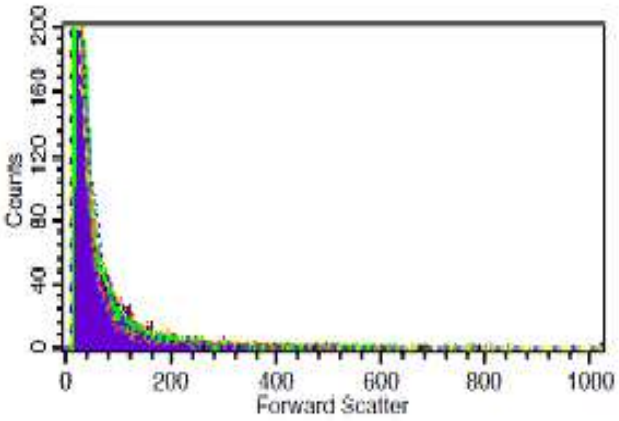
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	43.33	32.48	112.85	36.00

File: 250 ug/ml.007

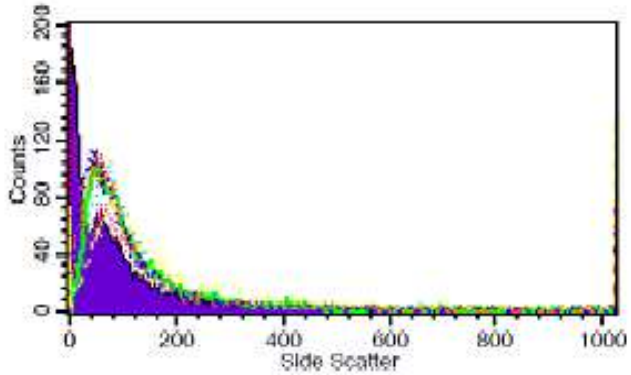
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	96.48	69.00	107.32	68.00

Figure 6: FACS analysis 24hr (Cancer)

CANCER SAMPLE (48 HRS.)



Forward Scatter 48 hrs Cancer



Side Scatter 48 hrs. Cancer

File: Control.011

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	19.63	11.19	149.73	12.52

File: 7.8 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	69.17	25.14	423.61	37.18

File: 15.6 ug/ml.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	17.66	9.52	319.72	11.29

File: 31.2 ug/ml.007

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	25.76	14.04	148.69	14.33

File: 62.5 ug/ml.008

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	19.12	8.71	206.67	11.24

File: 125 ug/ml.009

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	20.70	12.75	125.60	14.20

File: 250 ug/ml.010

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	1000	100.00	100.00	24.41	12.05	152.36	13.22

File: Control.008

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	89.89	28.92	172.33	45.00

File: 7.8 ug/ml.002

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	126.28	86.31	112.43	82.00

File: 15.6 ug/ml.003

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	106.19	44.34	138.34	67.00

File: 31.25 ug/ml.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	127.54	94.51	103.60	93.00

File: 62.5 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	102.95	71.63	110.50	74.00

File: 125 ug/ml.006

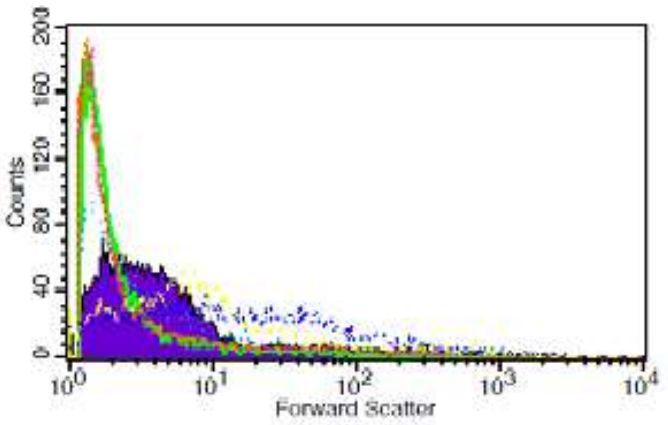
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	99.97	70.49	105.66	72.00

File: 250 ug/ml.007

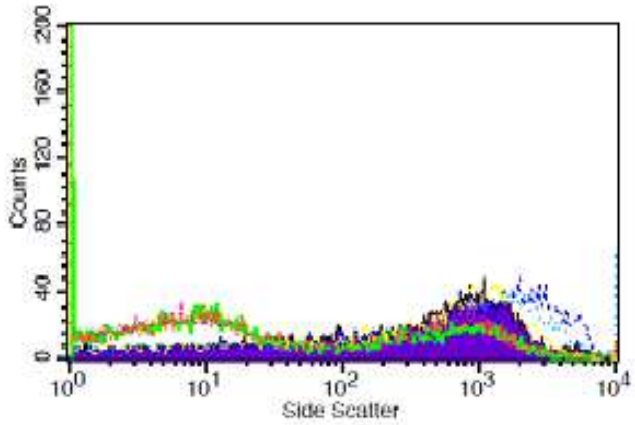
Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	0, 1023	10000	100.00	100.00	188.10	128.92	100.85	124.00

Figure 7: FACS analysis 48 hr (Cancer)

CANCER SAMPLE (72 HRS.)



Forward Scatter 72 hrs. Cancer



Side Scatter 72 hrs. Cancer

File: Control.008

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	15.57	4.19	487.90	3.31

File: 7.8 ug/ml.001

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	5.13	2.15	468.63	1.64

File: 15.6 ug/ml.002

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	5.69	2.22	464.41	1.64

File: 31.25 ug/ml.003

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	9.53	3.33	597.45	2.46

File: 62.5 ug/ml.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	5.31	2.23	437.38	1.64

File: 125 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	45.49	13.10	280.93	10.94

File: 250 ug/ml.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	31.92	7.78	417.92	6.58

File: Control.008

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	640.60	146.59	131.43	368.47

File: 7.8 ug/ml.001

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	276.02	25.71	243.66	12.75

File: 15.6 ug/ml.002

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	319.67	31.58	224.63	15.26

File: 31.25 ug/ml.003

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	1186.59	307.78	128.16	716.92

File: 62.5 ug/ml.004

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	303.40	30.70	227.65	15.54

File: 125 ug/ml.005

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	1413.31	362.76	103.98	973.38

File: 250 ug/ml.006

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median
All	1, 9910	10000	100.00	100.00	699.18	182.81	113.16	457.25

Figure 8: FACS analysis 72 hrs. (Cancer)

ANTICANCER ACTIVITY OF PIGMENT IN FOLDS

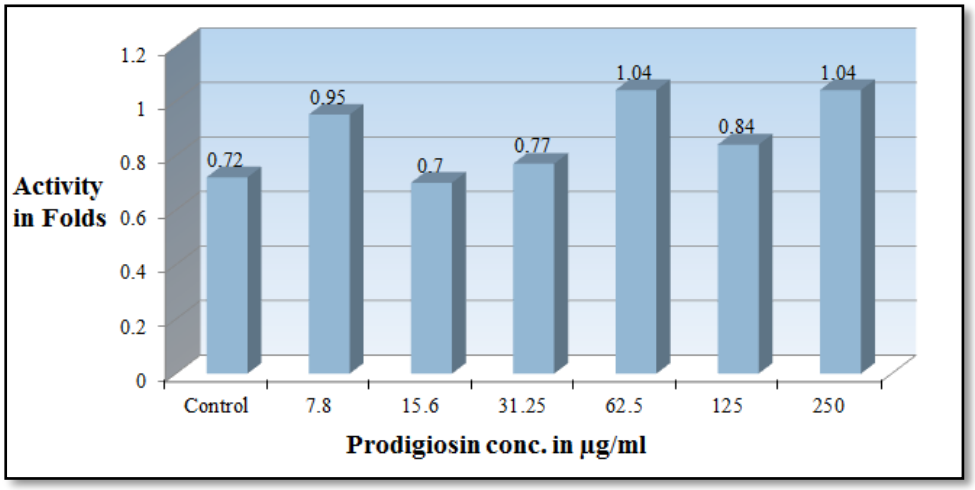


Figure 9: Prodigiosin Activity in folds (24 hrs.)

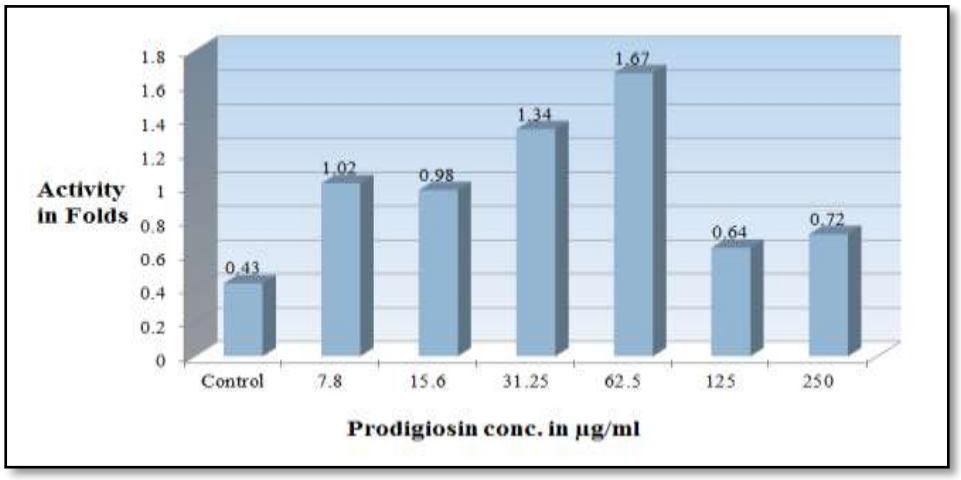


Figure 10: Prodigiosin Activity in folds (48hrs.)

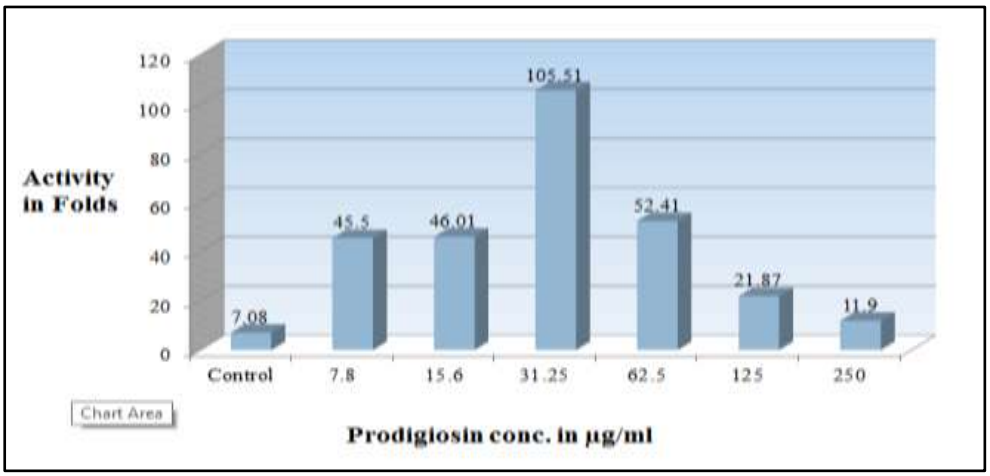


Figure 11: Prodigiosin Activity in folds (72 hrs.)

The effect of different concentrations of Prodigiosin on the viability of normal lymphocyte cell line as well as blood leukemia lymphocyte cell line was studied and the anticancer activity in folds was also determined. The best activity was observed after 72hr of incubation in 31.25 µg/ml concentration of Prodigiosin indicating the given concentration (31.25µg/ml) is optimum for maximum activity of the pigment. Effect of pigment was increases with time but not with the concentration. Very less (7.8 µg /ml) or very high (250µg/ml) dose of the pigment do not show significant activity.

CONCLUSION

The results obtained in this study suggest that red pigment Prodigiosin produced in large quantity by selected strain of *Serratia marcescens* when peanut broth is used with optimum condition of production. The produced pigment was subjected to cytotoxic activity determination which shows that the pigment not have any harmful effect on the normal human blood cells when mixture of cells and different concentration of pigment (5 to 10 µg /ml) was incubated for four days. Apoptosis was quantified by a reduction in cell forward scatter and increase in cell side scatter in flow cytometric analysis. In this case mean value of cell side scatter was found to be more for all doses of pigment than the forward scatter, which is the indication of induction of apoptosis of abnormal lymphocytes by Prodigiosin. The study reveal that red pigment form *Serratia marcescens* having no effect on normal blood lymphocytes but it having notable effect against cancerous cells. So in future this pigment can be used as promising lead compound for development of drug against leukemia.

REFERENCES

1. Campas, C., Dalmau, M., Montaner, B., Barraga, M., Bellosillo, B., Colomer, D., Pons, G., Perez-Tomas, R. and Gil, J. Prodigiosin induces apoptosis of B and T cells from B-cell chronic lymphocytic leukemia. *Leukemia* 2002;17, 746–750.
2. Carbonell, G. V., Amorin, C. R. N., Furumura, M. T., Darini, A. L.C., Fonseca, B. and Yano, T. Biological activity of *Serratia marcescens* cytotoxin. *Brazilian Journalof Medical and Biological Research* 2003; 36: 351-359.
3. Casullo de araujo, H. W., Fukushima, K. and Campos Takaki, G. M. Prodigiosin production by *serratia marcescens* UCP 1549 using renewable- resources as a low coast substrate. *Molecules* 2010; 15:6931-6940.
4. Chang, C., Chen, W., Ho, T., Wu, H. and Hongwei, Y. Development of natural anti-tumor drugs

by microorganisms. *Journal of bioscience and bioengineering* 2011; 111(5), 501–511.

5. Darshan, N., Manonmani. H.K. Prodigiosin and Its Potential Applications, *Association of Food Science and Technology* 2015.

6. Davaraj, N. R., Dharumaduari, D., Noorudin, T. and Annamalai P. Production of Prodigiosin from *serratia marcescens* and its cytotoxicity activity. *Journal of pharmacy research* 2009; 2(4):590-593.

7. Diaz-Ruiz, C., Montaner, B. and Perez-Tomas, R. Prodigiosin induced cell death and morphological changes indicative of apoptosis in gastric cancer cell line HGT-1.*Histology and Histopathology* 2001;16:415-421.

8. Francisco, R., Perez-Tomas, R., Gimenez-bonafe, P., Soto-Cerrato, V., ,Gimenez-xavier, P. andSabrosio, S. Mechanisms of Prodigiosin cytotoxicity in human neuroblastoma cell lines. *European journal of pharmacology* 2007; 572, 111–119.

9. Hertle, R., Hilger, M., Kocher, S. W. and Walev I. Cytotoxic action of *Serratia marcescens* hemolysin on human epithelial cells. *Journal of Infection and Immunity* 2007; 817-825.

10. Hong, B., Prabhu, V., Zhang, S., Heuvel, P., Dicker, D., Kopelovich, L. and El-deiry, W. Prodigiosin rescues deficient p53 signaling and antitumor effects via upregulating p73 and disrupting its interaction with mutant p53. *Cancer res* 2014; 74(4).

11. Kavitha, R., Aiswariy, S. and Ratnavali, C. M. G. Anticancer activity of red pigment from *serratia marcescens* in human cervix carcinoma. *International journal of pharmtech research* 2010; 2(1):784-787.

12. Khanafari, A., Assadi, M. M. and Fakhr, F. A. Review of prodigiosin, pigmentation in *Serratia marcescens*. *Online Journal of Biological Sciences* 2006; 6(1):1-13.

13. Maheswarappa, G., Kavitha, D., Vijayarani, K. and Kumanan, K. Prodigiosin as anticancer drug Produced from bacteria of termite gut.*Indian Journal of Basic and Applied Medical Research* 2013; 3(1): 257-266.

14. Malik, K., Tokkas, J.and Goyal S. Microbial pigment: A review. *International journal of microbial resource technology* 2012; 1(04): 361-365.

15. Montaner, B., Navarro, S., Pique, M., Vilaseca, M., Martinell, M., Giralt, E., Gil, J. and Perez-Tomas, R. Prodigiosin from the supernatant of *serratia marcescens* induces apoptosis in haematopoietic cancer cell lines. *British journal of pharmacology* 2000; 131:585-593.

16. Montaner, B. and Perez-Tomas, R. Prodigiosin-induced apoptosis in human colon cancer cells. *Life sciences* 2001; 68, 2025–2036.

17. Montaner, B., Avila, W., Martinell, M., Ollinger, R., Aymami, J., Giralt, E. and Perez-Tomas, R. DNA interaction and dual topoisomerase i and ii inhibition properties of the anti-tumor drug prodigiosin. *Toxicological sciences* 2005; 85, 870–879.

18. Pandey, R., Chander, R. and Sainis, K. Prodigiosins : a novel family of immune suppressants with anticancer activity. *Indian journal of biochemistry and biophysics* 2007; 44, 295- 302.

19. Perez-Tomas, R. and Vinas, M. New insights on the antitumoral properties of prodiginines. *Current medicinal chemistry* 2010; 17,2222-2231.

20. Perez-Tomas, R., Montaner, B., Llagostera, E. and Soto-Cerrato, V. The prodigiosins, proapoptotic drugs with anticancer properties. *Biochemical pharmacology* 2003; 66, 1447–1452.

21. Raj, D., Dhanasekaran, D., Thajuddin, N. and Panneerselvam, A. Production of Prodigiosin from *Serratia marcescens* and its cytotoxicity activity.*Journal of pharmacy research* 2009; 2(4), 590-593.

22. Regourd, J., Al-sheikh Ali, A. and Thompson, A. Synthesis and anti-cancer activity of c-ring-

functionalized Prodigiosin analogues.*J. Med. Chem* 2007; 50, 1528-1536.

23. Samrot, A. V., Chandana, K., Senthilkumar, P. and Narendra kumar, G. Optimization of Prodigiosin production from *serratia marcescens* and evaluation of its bioactivity. *International research journal of biotechnology* 2011; 2(5):128-133.

24. Soliev, A. B., Hosokawa, K. and Enomoto K. Bioactive pigment from marine bacteria: Application and physiological roles. Hindawipublication corporation 2011.

25. Venil, C. K. and Lakshmanaperumalsamy, P. An insightful overview on microbial pigment, Prodigiosin. *Electronic journal of biology* 2009; 5(3):49-61.

26. Williamson, N. R., Fineran, P. C., Gristwood, T., Chawral, S. R., Leeper, F. J. and Salmond, G. P. C. Anticancer and immunosuppressive properties of bacterial prodiginines. *Future microbial* 2007; 2(6):605-618.

27. Zhang, J., Shen, Y., Liu, J. and Wei, D. Antimetastatic effect of Prodigiosin through inhibition of tumor invasion.*Biochemical pharmacology* 2005; 69, 407–414.