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THE ROLE OF RED PIGMENT PRODIGIOSIN FROM BACTERIA OF EARTHWORM GUT AS AN ANTICANCER AGENT

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ABSTRACT

Earthworms are the most ancient invertebrate animals on earth which can be used as a good source of pharmaceutical compounds. A study was carried out to find out the distribution of microorganisms in the gut of earthworm, *Eudrilus eugeniae*. Significant number of microbial populations in the gut of earthworm was observed and it was gradually increased from the initial day to final day of composting. Pigmented colonies of bacteria from earthworm gut were selectively isolated, the pigment was extracted from the culture broth and a presumptive test was carried out for the confirmation of prodigiosin. The pigment component was separated using thin layer chromatography and the structural elucidation of the compound was performed using U.V. spectroscopy. The inhibitory effect of prodigiosin on bacterial pathogens was studied and the results confirmed the antibacterial activity against gram positive bacteria. The anticancer activity of the prodigiosin pigment was evaluated under *in vitro* conditions against the breast cancer cell lines and it was observed that prodigiosin induced the apoptosis in MCF-7 cell lines in a dose dependent manner. Then the potential isolate was subjected to morphological and biochemical analysis and it was confirmed that the colonies were of *Serratia marcescens*. The results obtained from the present study indicated that earthworm gut is promising and could be a vital source of habitat possessing antimicrobial and anticancer activity.

Keywords: *Eudrilus eugeniae*, prodigiosin, apoptosis, *Serratia marcescens*, MCF-7 cell line

INTRODUCTION

Earthworms are the most ancient invertebrate animals on earth and they play a major role in soil biology by providing ideal conditions for the growth of microorganisms. It was proposed that earthworms derive more of its energy and nutrients from gut specific micro biota than from micro biota already present in the ingested soil (**Sampedro *et al.*, 2006**). Earthworms are voracious feeders of organic wastes and they consume only a small portion of these wastes for their growth and excrete a huge quantity of wastes consumed in a half digested form. Earthworms intestine contains a wide range of microorganisms, enzymes and hormones which aid in rapid decomposition of half-digested material transforming them into vermicompost in a short time (neary 4–8 weeks) (**Nagavallemma *et al.*, 2004**). To understand the potential of earthworms in medicine, the role of intestinal microorganisms of earthworm must be accurately defined.

Microbial products are recently been widely used for therapeutic treatments and such products are called secondary metabolites or bioactive compounds which include pigments, enzymes, steroids and antibiotics. **Prasanna *et al.* (2014)** Isolated the antimicrobial substances producing bacteria, *Pseudomonas stutzeri* from gut of earthworm (*Eisenia foetida*) and studied their role against some phytopatogens while promoting plant growth. **Aruna *et al.* (2008)** isolated a new strain of *Streptomyces tritolerance* from earthworm gut (*Eisenia fetida*) and studied their antagonistic activity against plant pathogenic bacteria and fungi.

Microbial pigment production is now one of the promising fields of research. Pigment production from microorganisms have many advantages which include stability of the pigments, easy and fast growth in the cheap culture medium throughout the year, independence from weather conditions and colors of different shades. Prodigiosin is one of the studied biopigments of microbial origin. Cancer is one of the leading causes of death worldwide and nowadays synthetic drugs are the only option for cancer chemotherapy. However, most synthetic drugs kill not only tumor cells, but also normal cells and most have severe side effects. Natural anti-tumor drugs derived from organisms have also proven effective and less toxic for cancer therapy **(Cragg *et al.*, 2009)**. Prodigiosin, a tripyrrole ring pigment synthesized by *Serratia marcescens*, is a promising drug owing to its reported characteristics of having antibacterial, antimycotic and immunomodulatory activities (**Patricia *et al.*, 2000**). Prodigiosin also has the therapeutic use as potential antimalarial, antiprotozoal and anticancer drug. Interestingly prodigiosin has no marked toxicity in nonmalignant cell lines (**Campas *et al.*, 2003**).

The mechanism of action of Prodigiosin molecules is reviewed by **Perez-Tomas *et al.* (2003)**. Four possible mechanisms are suggested attributed to prodigiosins 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 cancer. It is also proposed that induction of DNA double breaks would be one mechanism and another being neutralization of pH gradient leading to apoptosis (**Pandey *et al.*, 2009**).

MATERIAL AND METHODS

Dissection of earthworms

The earthworm species *Eudrilus eugeniae* used for the present study was collected from the Vermiculturing Unit maintained by the Department of Microbiology, NASC, Coimbatore. A healthy sexually mature, clitellated worm was taken, washed with tap water and were then cleaned externally with 70% ethanol (**Mohammed *et al.*, 2014**). The fine edge of a dissecting scissor was inserted into the ventral surface of the earthworm at the region of the clitella; an incision was made longitudinally along the earthworm. Sterile dissecting pins were used to hold the earthworm down on a board. The gut was then freed from surrounding blood vessels and nephridia and separated into three sections: foregut, mid gut and hindgut.

Enumeration of microbes

The enumeration was carried out by following "serial dilution plate count technique" for each gut sections and was individually plated on to nutrient agar, actinomycetes isolation agar and sabouraud's dextrose agar for bacteria, actinomycetes and fungi respectively. Enumeration process was carried out at regular intervals up to 60th day.

Isolation and Purification of Pigment Producing Bacteria

The pigmented colonies of bacteria were selectively isolated and transferred to nutrient agar medium with the help of loop inoculum method. The plates were kept for incubation at 37ºC for 24 hours in an inverted position to screen for the pigment producing strains. The pigmented colonies were purified by pure culture techniques. The isolates thus obtained was then subjected to morphological and biochemical analysis for identifying the organism. The colonies were refrigerated in nutrient agar slants by frequent sub culturing for further studies.

Extraction of Prodigiosin

The nutrient broth inoculated with the isolated organism was kept in a shaker at 120 rpm for 16-18 h at 30°C and was observed for the pigment production. The culture broth was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded and the pellet was suspended in 95% methanol to extract the pigment from the cells. The mixture was vortexed and suspended pellet was centrifuged at 10,000 rpm for 10 minutes. Debris was removed and the supernatant was transferred in two test tubes. The content of one of the test tube was acidified with a drop of concentrated hydrochloric acid and the other was alkalinized with a drop of concentrated ammonia solution. A red or pink color in the acidified solution and a yellow or tan color in the alkaline solution indicated a positive, presumptive test for prodigiosin (**Gerber and Lechevalier, 1976**). Extracted prodigiosin was stored in a sterile container.

Estimation of Prodigiosin

The absorption pattern over various wavelengths was initially checked and it was found that the absorption maxima were at 499 nm where prodigiosin also absorbs maximally. At this wavelength the absorptions were recorded. The bacterial cell absorption prior to extraction was noted at every step. Isolated prodigiosin was estimated using the following equation (**Mekhael and Yousif, 2009**):

$$\text{Prodigiosin unit/cell} = \frac{[OD\ 499 - (1.381\ x\ OD\ 620)]x\ 1000}{\text{OD } 620}$$

Where,
OD 499 – pigment absorbance,
OD 620 – bacterial cell absorbance
1.381 – constant

UV/Visible absorption of extract

The structure elucidation of the compound was performed by using U.V. Spectroscopy. The UV-visible absorption spectrum of the extract was tested by using Shimadzu UV-2550 spectrophotometer at 400-700 nm to determine the λ maximum of the band.

Separation and Purification of Prodigiosin

The pigment component was separated using thin layer chromatography. Methanolic extract of prodigiosin was separated by the solvent system containing methanol, ethyl acetate and chloroform in the ratio of 6:3:1 (v/v). 10 µl of methanolic extract of prodigiosin was loaded on to the silica gel slides and run against the solvent till the solvent front reaches 2/3rd of the slide. After the development of the chromatograms, slides were removed and dried. The retardation factor (Rf) values of the chromatogram were calculated.

Evaluation of antimicrobial activity of prodigiosin

Agar-Well Diffusion Method

Antibacterial test was performed using the agar well diffusion method (**Collins *et al.*, 1995**). Three wells of 5 mm in diameter were made on MHA using a sterile cork borer. The methanolic extract of prodigiosin (80 µl) was added to the wells. The positive (Tetracycline) and negative controls (95% methanol) were also maintained. The plates were incubated at 37°C for 24 hours. All the determinations were performed in triplicates. The zone of inhibition was recorded.

***In vitro* Cytotoxicity Assay**

The anticancer activity of the prodigiosin pigment of *S. marcescens* was performed on three breast cancer cell lines MCF-7, MDAMB231 and T47D respectively according to **Mosmann (1983) and Monks *et al*. (1991)**.

(i) Cell preparation

The human breast cancer cell lines were obtained from National Centre for Cell Science (NCCS), Pune. MCF-7 and MDAMB231 cells were grown in Eagles Minimum Essential Medium (EMEM) and for T47D cells Rosewell Park Memorial Institute medium (R.P.M.I) was used. The media was supplemented with 10% fetal bovine serum (FBS) and antibiotics. The cells were maintained at 37ºC, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passage weekly and the culture medium was changed twice a week.

(ii) Cell treatment procedure

The monolayer cells were detached with trypsin- ethylene diamine tetra acetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37ºC, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted in the required final sample concentrations. Following drug addition, the plates were incubated for an additional 48 h at 37ºC, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

(iii) MTT assay

3- [4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48 h of incubation, 15 µl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and measured the absorbance at 570 nm using micro plate reader.

The % cell viability and inhibition was determined using the following formula.

% Cell Inhibition = 100 - Abs (sample) / Abs (control) × 100
% Cell Viability = Abs (sample) / Abs (control) × 100

Nonlinear regression graph was plotted between % cell inhibition and log concentration and IC_{50} was determined using GraphPad Prism software.

Statistical Analysis

SPSS-16 statistical computer program was used to evaluate the results. The results were expressed as Mean ± SEM, where $p<0.05$*, $p<0.01$** and $P<0.001$*** were taken as statistically significant. Equal variances between treatments were measured using Levene's test for equality of variance. A two-tailed P-value less than 0.05 was considered statistically significant.

RESULTS

Enumeration of Microbes from Earthworm Gut

The total microbial count present in various gut sections (fore gut, mid gut and hind gut) of earthworm *E. eugeniae* are tabulated in tab 1. It was observed that there was a gradual increase in their population from the initial day to final day of composting. The maximum bacterial population was found in the fore gut region than in mid gut and hind gut region. Fungal population was highest in the hindgut region. Actinomycetes population was predominant in the mid gut than fore gut and hind gut.

Table 1 Enumeration of microbes from earthworm gut

Micro-Organisms	Sampling Time (Days)														
	Foregut					Midgut					Hindgut				
	0	15	30	45	60	0	15	30	45	60	0	15	30	45	60
Bacteria (CFU × 10^6/ml)	12.0 ± 1.53 *	52.0 ± 1.53 ***	77.0 ± 1.53 ***	91.0 ± 1.53 ***	136.0 ± 1.73 ***	32.0 ± 1.53 ***	40.0 ± 1.53 ***	50.0 ± 2.08 ***	55.0 ± 0.58 ***	81.0 ± 1.53 ***	44.0 ± 1.53 ***	53.0 ± 1.53 ***	65.0 ± 1.73 ***	80.0 ± 1.53 ***	98.0 ± 1.53 ***
Actinomycetes (CFU × 10^5 /ml)	8.00 ± 2.08 *	10.0 ± 1.53 ***	11.0 ± 1.53 ***	13.0 ± 0.58 ***	20.0 ± 1.53 ***	13.0 ± 1.53 ***	15.0 ± 1.53 ***	15.0 ± 2.19 ***	17.0 ± 1.53 ***	32.0 ± 2.08 ***	11.0 ± 1.53 ***	12.0 ± 1.53 ***	10.0 ± 1.53 ***	14.0 ± 1.53 ***	15.0 ± 1.53 ***
Fungi (CFU × 10^4/ml)	15.0 ± 1.16 *	19.0 ± 1.53 ***	26.0 ± 1.53 ***	26.0 ± 1.16 ***	33.0 ± 1.53 ***	14.0 ± 2.08 ***	18.0 ± 1.53 ***	21.0 ± 1.53 ***	23.0 ± 1.16 ***	32.0 ± 1.53 ***	20.0 ± 1.53 ***	23.0 ± 1.53 ***	30.0 ± 0.58 ***	30.0 ± 0.58 ***	34.0 ± 1.53 ***
F value	4.63	209.6	513.0	1310	1 580	38.1	79.9	89.5	313.0	266.8	124.7	1930	410.3	711.2	810.4

CFU- Colony forming unit, Values are presented as the mean ± SEM (standard error of mean); n = 3 for all groups. Experimental group were compared with control, ***P<0.001, *P<0.05, considered significant.

Isolation, Screening and Identification of Pigment Producing Bacteria

The promising isolate which showed prodigiosin production on the basis of presumptive test was selected for the present study. Identification and characterization was done based on its morphological, cultural and biochemical tests. The presumptive test for prodigiosin shows the red and yellow color in acidic and alkaline condition respectively.

The colony characteristics of the selected isolate were round, smooth margins, convex, and red pigment on the nutrient agar (Figure 1). The microscopic examination showed gram-negative rods in gram staining and were actively motile. The bacterium was rod shaped with smooth edged red colonies. The pigment production was observed only after 24-48 hrs of incubation. The results of various biochemical tests are presented in tab 2. The strain has fermented the glucose, sucrose, fructose, and did not fermented lactose. Pigmentation of the colonies and Gram's staining results followed by standard biochemical characterization confirmed that the colonies were of *S. marcescens.*

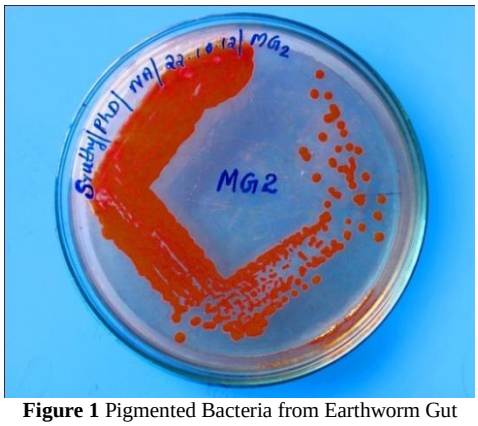

Figure 1 Pigmented Bacteria from Earthworm Gut

Table 2 Biochemical characteristics of isolate

S. No.	Name of the Test	Properties
1.	Indole	-
2.	Methyl Red	-
3.	Voges *Proskauer*	+
4.	Citrate Utilization	+
5.	Nitrate Reduction	+
6.	Urease	-
7.	Catalase	+
8.	Oxidase	-
9.	Hydrogen sulfide production	-
10.	Triple Sugar Iron Agar	K/A

Estimation of Prodigiosin

The strain produced 230.42 unit/cell Prodigiosin.

UV/Visible Absorption spectrum

The spectrum scan results showed that the prodigiosin molecule in acidic conditions showed an absorption maximum at 535 nm.

Separation and Purification of Prodigiosin

In TLC, the methanolic extract of prodigiosin produced a single band with Rf value of 0.88 was obtained.

Evaluation of Antimicrobial Activity of Prodigiosin

Agar-Well Diffusion Method

The antibacterial activity of prodigiosin was found maximum against gram positive bacteria when compared to gram negative. Prodigiosin possesses significant antibacterial activity against *S. aureus* with a zone of inhibition of 17.67 ± 0.882 mm (Tab 3, Figure 2).

Table 3 Antibacterial activity of prodigiosin against clinical pathogens

S. No.	Bacterial Pathogens	Zone of Inhibition (mm)	
		Prodigiosin	Tetracycline
1.	*S. aureus*	17.67 ± 0.882***	19.67 ± 0.882**
2.	*Bacillus* sp.	14.33 ± 0.882***	15.00 ± 0.577**
3.	*Enterococcus* sp.	12.00 ± 0.577***	NA
4.	*E. coli*	-	14.67 ± 0.882**
5.	*P. aeruginosa*	-	14.33 ± 0.882**
6.	*Klebsiella* sp.	10.67 ± 0.882***	17.00 ± 0.577**
	F value	14.111	8.296

(Values are presented as mean ± SEM (standard error of mean); (n = 3).
***P<0.001, **P<0.01 considered statistically significant

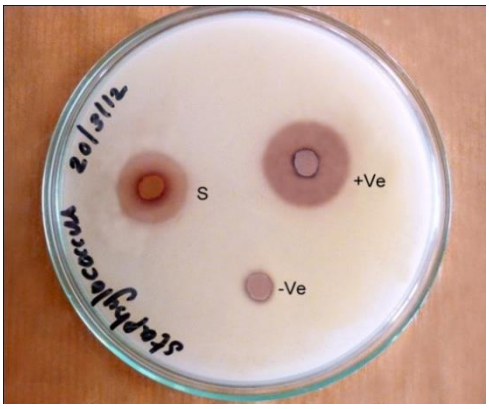

Figure 2 Antibacterial Assay – *S. Aureus*

***In vitro* Cytotoxicity Assay**

MTT dye reduction assay was done to assess the anti proliferative activity of prodigiosin. The cytotoxicity of the prodigiosin pigment was studied on human breast cancer cells (MCF-7, MDAMB231 and T47D) by exposing the cells to various concentrations of 18.75, 37.5, 75, 150 and 300 µg for 24 hours. The reduced MTT formazan was dissolved in DMSO and the absorbance was read in 96- well-plate reader (Tab 4). The graph was plotted as % of inhibition (absorbance at y- axis) against the concentration of the drug (x-axis) (Figure 3).

At low concentration (18.75 µg), the viability was not effected by prodigiosin. However at greater concentrations such as 37.5, 75, 150 and 300 µg, the cell death were 18.3%, 36.4%, 82% and 98% respectively and the result of the same can be seen in the table 5 below. Decrease in proliferation of treated cells was observed when compared to the untreated controls. Out of 3 cell lines tested in the MTT assay, prodigiosin pigment was most active on MCF-7 cell lines which induced apoptosis in MCF-7 cell lines in a dose dependent manner (Figure 4a-f).

A good regression coefficient (R^2>0.90) was obtained for breast cancer cell line. The IC_{50} concentration was determined by regression analysis (drug concentration that is required to reduce half of the cells by the total population) and was found to be 86.25 µg/ml. The result indicates that the prodigiosin shows good anticancer activity against MCF-7, human breast cancer cell line.

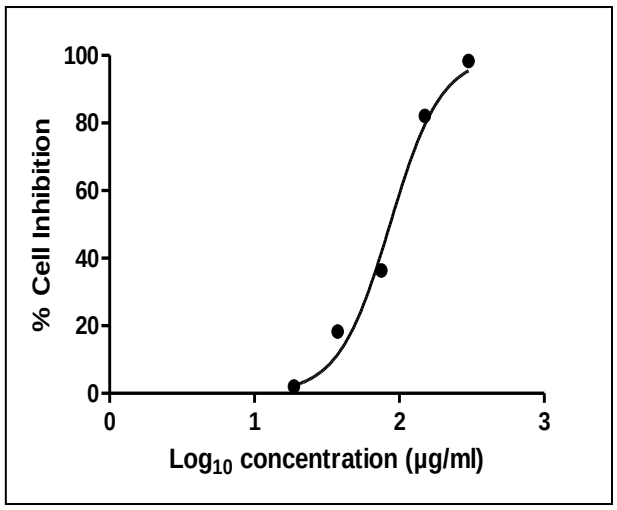

Figure 3 Anticancer activity of prodigiosin on breast cancer cell line MCF-7

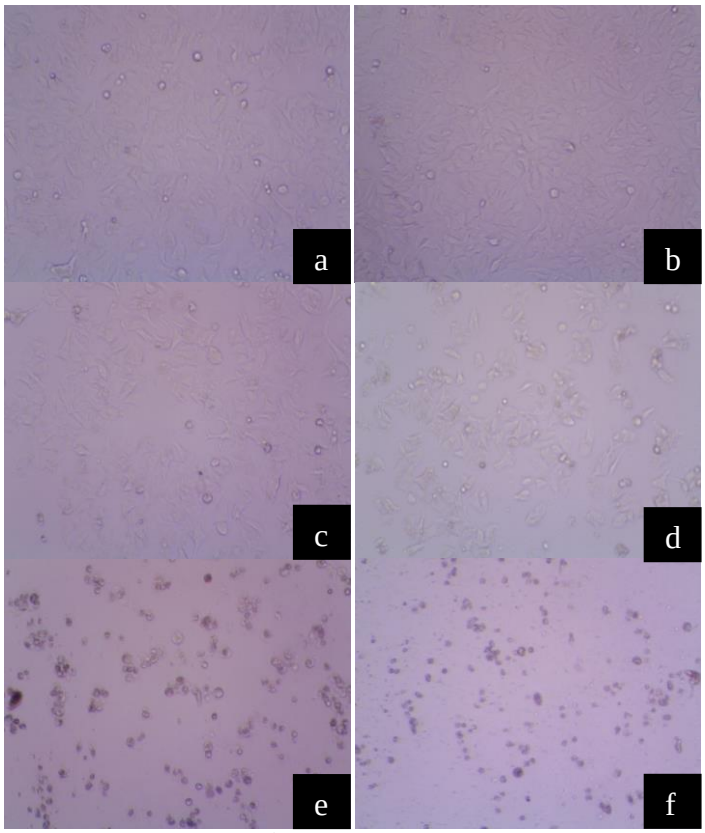

Figure 4 Anticancer activity of prodigiosin on breast cancer cell line MCF- 7 by MTT assay, (a) Control, (b) 18.75 µg (c) 37.5 µg (d) 75 µg (e) 150 µg (f) 300 µg

Table 4 Anticancer activity of prodigiosin on breast cancer cell line MCF-7

Concentration	18.75 µg	37.5 µg	75 µg	150 µg	300 µg	Control
ABS	0.279	0.248	0.184	0.06	0.006	0.291
	0.281	0.231	0.18	0.045	0.006	0.293
	0.29	0.23	0.188	0.05	0.002	0.284
Average	0.283**	0.236**	0.184**	0.051**	0.004**	0.289**

Values are presented as the average of three replicates; n = 3 for all groups.
**P<0.01, considered extremely significant.

Table 5 Percentage inhibition of breast cancer cells by prodigiosin

Concentration	18.75 µg	37.5 µg	75 µg	150 µg	300 µg
Cell Inhibition (%)	2.073733	18.31797	36.40553	82.14286	98.3871
IC_{50}	86.25 µg/ml				
R^2	0.9871				

Linear regression analysis was used to calculate IC_{50} value

DISCUSSION

In the present study, it was observed that earthworm gut harbors a large number of bacteria followed by fungi, whereas the actinomycetes population was least in the earthworm gut. The maximum bacterial, fungal and actinomycetes population were found in the fore gut, hindgut and mid gut respectively. This incorporates with the findings of the researchers proving that earthworms include microorganisms in their substrates as a food source and can digest them selectively (**Edwards and Bohlen, 1996**).

Increase in microbial populations might be due to the environmental conditions prevailing and nutritional status in the gut of earthworm as reported by **Edwards and Bohlen (1996)**. Microorganisms constitute an important nutritional component of the earthworm diet (**Edwards and Bohlen, 1996**). It was observed that there are variations in the population of microorganisms in the foregut, midgut and hindgut of earthworm. **Monroy *et al.* (2009)** observed that the hindgut is able to extract the organic matter from the inorganic matrix and hence there is decrease in the molecular weight of the organic matter. Microbial community in the gut is responsible for the digestion of the matter but how do they participate and carry out this activity is still not known (**Fadaee, 2012**). The findings of the present study thus confirm the concept that the earthworm gut might be a specialized microhabitat of enhanced microbial activities in soils (**Karsten and Drake, 1995**).

The pigments from microbial sources are potentially good alternative ones to synthetic pigments. Prodigiosines have recently received renewed attention for their reported antibacterial, antifungal, antimalarial, immunosuppressive and anticancer properties. In the present study the bacterial colonies showing red pigment production were isolated from the earthworm gut and were screened for the production of prodigiosin on the basis of presumptive test. The pigment production was observed only after 24-48 hrs of incubation and the intensity of the color increased upon prolonged incubation which exactly matches with the results of previous study done by **Giri *et al.* (2004)**.

Harris *et al.* (2004) reported that prodigiosin, a typical secondary metabolite is appearing only in the later stages of bacterial growth. The presumptive test for prodigiosin shows the red and yellow color in acidic and alkaline condition respectively which coincides with the study of **Chandni *et al.* (2012)**. According to **Antony *et al.* (2011**) the optimal condition for pigment production was found in nutrient broth at 28ºC and pH 7. **Jissa *et al.* (2008)** reported that the pigment produced by *Serratia* sp. BTWJ8 is water insoluble and methanol was found to be an ideal solvent for the maximal extraction of the pigment among the different solvents studied. In the present study also the pigment was purified by extraction with methanol and the dry pigment was obtained by evaporation of the solvent at 40ºC. In this study, the organism was found to produce 230.42 unit/cell prodigiosin in nutrient broth. The colony characteristics of the selected isolate were round, smooth margins, convex, and red pigment on the nutrient agar. Pigmentation of the colonies, gram's staining results, carbohydrate fermentation tests followed by standard biochemical characterization confirmed that the colonies were of *Serratia* sp. **Gerber (1975)** reported that *S. marcescens* are the major producers of prodigiosin. **Maheswarappa *et al.* (2013)** extracted the pigment prodigiosin from S. *marcesens* of termite gut. **Sundaramoorthy *et al*. (2009)** isolated new strains of *Serratia marcescens* that effectively produce prodigiosin.

In the present study the spectrum scan results showed that the extracted pigment in acidic conditions showed an absorption maximum at 535nm suggesting that this pigment is prodigiosin. **Mohammed *et al*. (2012)** reported that prodigiosin molecule in acidic conditions showed an absorption maximum at 534 nm. A single band with an Rf value of 0.88 was obtained after thin-layer chromatography of prodigiosin. **Antony *et al.*(2011)** extracted the pigment prodigiosin from *S. marcescens*, isolated from urine sample and subjected for TLC. The pigment prodigiosin was extracted from *S. marcescens*, isolated from urine sample and subjected for TLC. The Rf value of the fraction obtained was 0.87. The *in vitro* antimicrobial potency of the pigment prodigiosin was studied and was observed that the antibacterial activity of prodigiosin was found

maximum against gram positive bacteria when compared to gram negative bacteria used in the test. **Ramina and Samira (2009)** reported that the prodigiosin antibacterial activity was higher against gram positive bacteria as compared with gram negative bacteria. The antibacterial activity of prodigiosin (PG) is the result of their ability to pass through the outer membrane and to their capacity for inhibiting target enzymes, such as DNA gyrase and topoisomerase IV, which inhibit the cell growth (**Berlanaga and Vinas, 2000**). Since, the antibacterial activity of a compound may depend on the destruction of the structure or the inhibition of metabolic reaction in a microorganism, the presence and the level of antibacterial activity of the prodigiosin pigment varied significantly with the type of bacteria used.

In the present study cytotoxicity of the prodigiosin pigment was studied on human breast cancer cells – MCF-7, MDAMB231 and T47D by exposing the cells to various concentrations of 18.75, 37.5, 75, 150 and 300 µg for 24 hours. Prodigiosin was most active on MCF-7 cell line and it induced apoptosis in a dose dependent manner. The IC_{50} concentration was found to be 86.25µg/ml. The result indicated that the prodigiosin showed good anticancer activity against MCF-7, human breast cancer cell line. The popularity of MCF-7 is largely due to its exquisite hormone sensitivity through expression of oestrogen receptor (ER), making it an ideal model to study hormone response (**Levenson and Jordan, 1997**).

Maheswarappa *et al.* (2013) explained the dose dependent cytotoxicity and apoptotic property of the prodigiosin pigment extracted from *S. marcescens* bacteria isolated from termite gut by using Vero cell line. **Antony *et al*. (2011)** reported the cytotoxic activity of prodigiosin extracted from S. marcescens isolated from urine sample by using Vero cell line and cancerous cell line Hep 2. The IC_{50} value of isolated prodigiosin against Vero cells was found to be 2.5 mg/ml and against cancerous cell line, Hep2 was 0.625 mg/ml. It was found that least concentration of prodigiosin was found to possess anticancer activity. **Davaraj *et al.* (2009)** reported the cytotoxic activity of prodigiosin extracted from *S. marcescens* (MTCC 97*) isolated from soil by using HeLa cell line and it was found that the IC_{50} value was 35 µg/ml for 48 hours. **Beatriz and Ricardo (2001)** mentioned that the crude methanol extracts of prodigiosin which was analysed by GC-MS shows the presence of alkaloids as the major compound and are of high therapeutic importance. These alkaloids and quinoline compounds from other sources have been studied earlier for antimicrobial properties and potent anticancer property.

The therapeutically important secondary metabolites produced by *S. marcescens* reflect a new area of interest in chemical and pharmaceutical research and should be produced in large quantities to meet the future demands. For the best of our knowledge and literature review, this is the first study to report the production of prodigiosin from *S. marcescens* which was isolated from earthworm gut.

CONCLUSION

This study clearly indicates that earthworms can be used not only in environmental monitoring but also in the acquisition of novel molecules for human therapeutic purposes. The most important outcome of this result will be the development of novel bioactive compounds from microbes of earthworm gut. Prodigiosin showed good anticancer activity against MCF-7, human breast cancer cell line and it will be the better therapeutic agent for the treatment cancer in near future. The exact cytotoxic potential of the prodigiosin pigment need to be confirmed also by *in vivo* methods.

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