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ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXICITY ACTIVITY OF PIGMENT PRODUCED FROM *SERRATIA MARCESCENS*

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ABSTRACT
Pigments from *Serratia marcescens* were characterized by GC-MS. *Serratia marcescens* pigment showed good antibacterial activity against *Clostridium sp, E.coli, Citrobacter ferundii, Calvibater sp,* and *Enterobacter amingences*. These pigments showed strong antioxidant activity by DPPH radical scavenging assay. The pigment was found to exhibit cytotoxicity activity against the Breast cancer cell line MCF-7. Cytotoxicity was determined by MTT assay. Thus on increasing the concentration of the pigment extract, the percentage of the cell viability was found to be reduced. These properties of pigment can be used in the development of drugs for cancers.

KEYWORDS: Pigments, GC-MS, DPPH method, MTT assay, Antimicrobial activity, Antioxidant activity, Cytotoxicity activity.

INTRODUCTION
Serratia sp is an aerobic or facultative anaerobic, motile, spore forming, Gram negative and enteric saprophytic rod shaped bacterium (Mody *et al*., 1990). Prodigiosin and prodigiosin like pigments are example for bioactive compound produced by many microorganisms. Prodigiosins are red pigment naturally occurring as (tripyrrylmethane structure) linear tripyrrolering, undecyl prodigiosin, cycloprodigiosin, meta cycloprodigison, dipyrroly dipyromethane depending on various organism. These are emerging broad spectrum of compound having distinct biological activites like antibacterial, antifungal, antiprotozoal (Croft *et al.*, 2002) cytotoxic (Nikashima *et al*., 2005) antitumour (Castro 1967, Perez – Tomes *et al*., 2003) antimalarial, antidialets, antioxidants, non steroidal anti – inflammatory drugs, dyeing of silks and wools. Thus the present study is carried to reveal the pigment of *Serratia marcescens* possessed antioxidant as well as cytotoxic properties.

MATERIALS AND METHODS
Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
The pigment was extracted and analyzed by GC-MS. The GC column dimension used was 30 X 0.25mm X 0.5mm AB-35MS fused silica capillary column. For GC the injector temperature was about 250°C, column temperature isothermal at 100°C then programmed to rise up to 250°C at 6°C/min and be held at this temperature for 10 minutes. The ion source temperature was 200°C and the interface temperature was 250°C. Helium gas was engaged as a carrier gas at the rate of 1ml/min. The spectra were obtained in the EI mode (values obtained from duplicate measurements) with 70V ionization energy. The compounds were identified by comparing with the mass spectrum and matched with the inbuilt library (NIST'08 software).

Antimicrobial studies against pigments
Antibacterial and antifungal activities of pigment were tested by agar well diffusion method. For antibacterial activity *Clostridium sp, E.coli, Citrobacter ferundii, Enterobacter amingences* and *Calivibacter sp* were used. For antifungal activity *Aspergillus niger, Aspergillus flavus, Tercoderma sp, Rhizopus sp* were used. Then the bacterial and fungal culture was swabbed onto the nutrient agar plates with the sterile cotton. A well with a diameter of 0.5cm was made in the nutrient agar plate using cork borer. The pigment extracts 20µl, 40µl, 60µl, 80µl were loaded in the well and incubated for 24 hours at 37°C. After incubation, the diameter of the incubation zone was measured (Rajasulochona *et al.*, 2009).

Antioxidant Assay (Mothana *et al.*, 2008)
The pigment extraction was mixed with 95% methanol to prepare the stock solution (10mg/10ml). The test samples were prepared from stock solution by diluting with methanol to attain a concentration of 600µg/ml, 500µg/ml, 400µg/ml, 200µg/ml, 50µg/ml respectively.

Diluted test samples (1ml) were added to 1ml of a 0.004% methanol solution of DPPH and mixed. The solution was then kept in dark for 30mins for reaction to occur. The absorbance was measured at 517nm using colorimeter. Ascorbic acid was used as a standard. Methanol (1ml) with DPPH solution (0.004% 1ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula:

$$\% \text{ inhibition of DPPH activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} X100$$

Cytotoxicity of the pigment on Human breast cancer cell line MCF-7

The Cytotoxic effect of pigment against Breast cancer cell line MCF -7 was assayed by MTT {3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide} assay.Cells were plated at 37°C for 24 hours on 24 well plate at a density of 10^3-10^4cells per well, with Minimal Essential Medium (MEM) supplemented with 10% Fetal Calf Serum (FCS). Penicillin (100 units/ml), Streptomycin (100µg/ml) and Amphotericin B (5µg/ml) were added to the medium and maintained in a humidified atmosphere (5% CO_2) at 36°C. After 24 hours the cells were exposed to different concentration of pigment, 100µg/ml, 200µg/ml, 300µg/ml, and 400µg/ml which were prepared by diluting from stock solution of 5mg/ml. The cells were incubated at 36°C in humidified incubator with 5% CO_2 for a period of 72 hours. Morphological changes of the cell culture were examined using an inverted microscope. After 72 hours cell viability was determined. Cytotoxicity of pigment extraction was determined by plotting percentage cell viability against concentration of pigment.

RESULTS AND DISSCUSION

GC-MS Analysis of the compound present in the Pigment of *Serratia marcescens*

The components present in pigment were characterized by using GC-MS. The pigment extraction obtained from 5g *Serratia marcescense* were lyophilized to obtain 0.25mg. The GC-MS analysis with the chromatogram and m/z spectrum, were analyzed with the hit compounds by comparing with the inbuilt library, with the help of NIST'08 software. The component present in the pigment extraction of *Serratia marcescens* was determined, Octadecanoic acid (rt-1.58), Hexadecanoic acid (rt-4.58), Phenol (rt-13.61), 4(1H)-Pyrimidinone (rt-16.78), N-Ethylformamide (rt-18.21), Methane (rt-20.22), Pyridine2-Aminopyridie (rt-22.12), 2-Butyn-l-ol (rt-26.33), Napthalene (rt-29.77). The area-percentage, chemical formula and the molecular weight of the compounds are shown in Table1. Pigment has been shown to produce an array of low and high molecular weight compounds with antimicrobial, antifungal and various pharmaceutically-relevant activities. Compounds formed include toxic proteins, polyanionic exopolymers, substituted phenolic and pyrrole-containing alkaloids, cyclic peptides and a range of bromine substituted compounds (John *et al*., 2007).

Table 1: GC-MS analysis of the compounds present in the pigment of *Serratia marcescens*

S. No	Retention time	Compound name	Formula	Molecular weight	Area (%)
1	1.58	Octadecanoic acid	$C_{18}H_{36}O_2$	198	3.80
2	4.58	Hexadecanoic acid	$C_{17}H_{34}O_2$	390	6.70
3	13.61	Phenol	C_6H_6O	390	8.52
4	16.78	4(1H)-Pyrimidinone	$C_4H_6N_4O$	334	7.20
5	18.21	N-Ethylformamide	C_3H_7NO	119	8.56
6	20.22	Methane	C_2H_3NO	307	2.10
7	22.12	Pyridine	C_5H_5N	128	9.52
8	23.22	Aminopyridine	$C_5H_6N_2$	123	7.50
9	26.33	2-Butyn-1-ol	C_4H_6O	143	5.23
10	29.77	Naphthalene	$C_{10}H_8$	307	4.20

Antimicrobial activity of the pigment

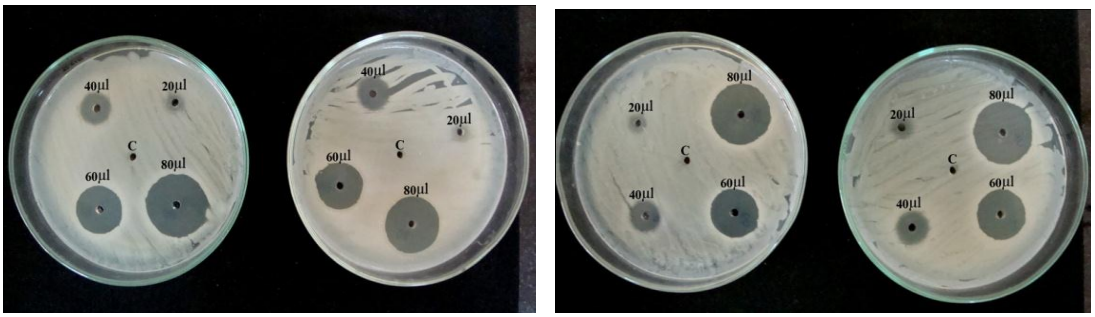
The Antimicrobial activity of the pigment isolated from *Serratia marcescens* was determined by agar well diffusion method using 5 bacterial and 4 fungal strains. The bacterial pigment with different concentration namely 20µl, 40µl, 60µl and 80µl was used for the antimicrobial activity analysis. The bacterial pigment extraction had shown the ability to inhibit the growth of microorganisms such as *Clostridium sp, E.coli, Citrobacter ferundii, Calvibater sp, Enterobacter amingences*, and no inhibition was observed in *Aspergillus niger, Aspegillus flavus, Rhizopus sp* and *Trichoderma sp.* The zone of inhibition of pigment extract result that the pigments has more potential antimicrobial activity as shown in table 2 and 3. All the concentration shows antimicrobial activity, among that 80µl of the pigment showed maximum zone. In the present study species of plant pathogenic fungi in addition to *Aspergillus niger, Aspegillus flavus, Rhizopus sp* and *Trichoderma sp* were found to be sensitive to pigment.

Table 2: Antibacterial activity of the pigment from *Serratia marcescens*

S.NO	Concentration	*Clostridim sp* (mm)	*Citrobcter ferundii* (mm)	*E.coli* (mm)	*Calvibater sp* (mm)	*Enterobacter amingences* (mm)
1	20µl	0.1	0.0	0.2	0.1	0.0
2	40µl	0.4	0.3	0.4	0.1	0.3
3	60µl	0.5	0.7	0.7	0.7	0.7
4	80µl	1.6	0.8	0.8	0.7	0.8

Table 3: Antifungal activity of the pigment from *Serratia marcescens*

S.NO	Concentration	*Aspergillus niger* (mm)	*Aspegillus flavus* (mm)	*Rhizopus sp* (mm)	*Trichodoma sp* (mm)
1	20µl	0.0	0.0	0.0	0.0
2	40µl	0.0	0.0	0.0	0.0
3	60µl	0.0	0.0	0.0	0.0
4	80µl	0.1	0.1	0.1	0.1



A.Clostridim sp *B. Citrobacter ferundi* *C. E.coli* *D.Calvibater sp*

E. Enterobacter amingences

Figure 1 Antibacterial Zone of Inhibition

Antioxidant property of the pigment by DPPH Assay

The antioxidant property of the pigment was revealed by DPPH radical scavenging assay. *Serratia marcescens* pigments were used in various concentration ranges from 200 - 800µg/ml. Among these concentrations 600µg/ml of pigment extraction showed the IC_{50} value to be 94.28µg/ml which indicates strong antioxidant activity of the pigment from *Serratia marcescens.* The result indicates that the antioxidant activity of pigment extraction is higher than that of standard ascorbic acid. Percentage inhibition was calculated and represented as graph in Figure 2.

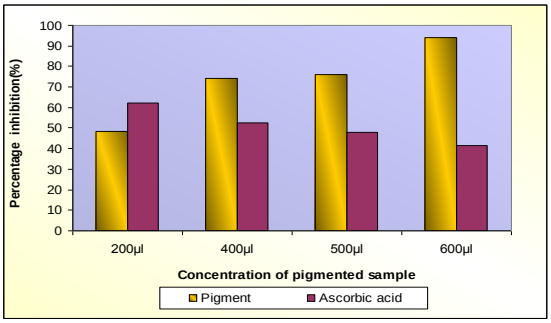


Figure 2: DPPH ASSAY

Analysis of cytotoxicity activity of the pigment on breast cancer cell line MCF – 7

The Cytotoxicity activity of the lyophilized pigment *Serratia marcescens* were analyzed by treating the sample with Breast cancer cell line MCF -7 with different concentrations namely 100µg, 200µg, 350µg, and 400µg by using MTT assay. Before carrying out the Cytotoxicity assay, 100µg of the sample was dissolved in 200µl of DMSO (dimethyl sulfoxide) and filtered through 0.45µg syringe filter. The cell viability was gradually decreased from different concentration and showed high anticancer property plotted in Figure 3.

MTT assay of the pigment *Serratia marcescens* were shown in fig 4.The extracted pigment was found to exhibit maximum cytotoxicity at 400µg concentration. As the concentration of the sample increases the cell viability of the cancer cell decreases. Prodigiosin has cytotoxic activity against numerous cancer cell line (Williamson *et al*., 2006).

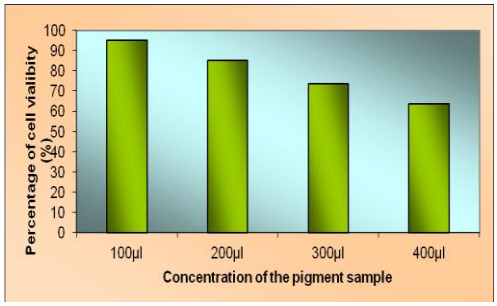
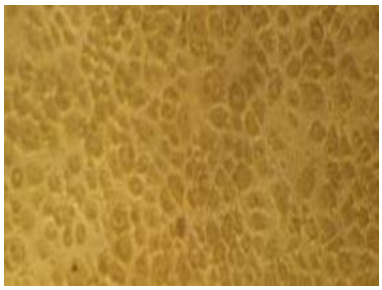


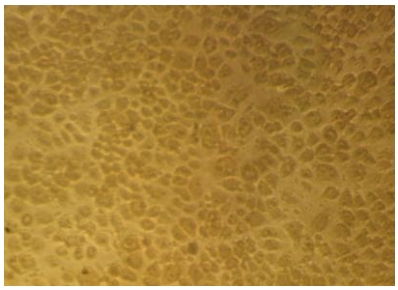
Figure 3. Cytotoxity effect of the pigment *Serratia marcescens* against Human breast cancer cell line MCF-7

Figure 4 MTT Assay of the pigment *Serratia marcescens* against Human Breast Cancer cell line MCF -7

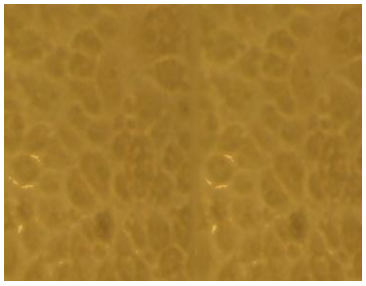
Control



Conc. (100µg/ml)



Conc. (200µg/ml)



Conc. (300µg/ml)

Conc. (400µg/ml)

CONCLUSION

The present study was carried out to characterize the components in the pigment of *Serratia marcescens*, and to evaluate its antimicrobial, antioxidant and cytotoxicity property. The compounds present in the pigment were revealed by GC-MS. The compounds were considered to have more antibacterial, antioxidant and cytotoxicity property. The study may further extend to identify the components and pathways of the pigment that have given rise to its antioxidant and cytotoxic properties.

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