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Extraction of Actinomycetes (*Streptomyces* sp.) Pigment and Evaluation of its Anticancer Propertyon HeLa Cell Line

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ABSTRACT

The antibacterial activities of the microbial pigment of the actinomycetes were carried out against the bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Salmonella typhi. The E. coli (8 mm) more susceptible than other microbial pathogens. Whereas remaining species showed negative result. The antifungal activity of the microbial pigment of the actinomycetes were carried out against the four different fungi such as Candida albicans, Aspergillus niger, Trichoderma viride, Rhizopus microporous. The actinomycetes pigment were failed to inhibit the pathogenic fungi. There is no antifungal activity against the all tested pathogenic fungi. Fourier Transform Infra-Red (FTIR) spectrum revealed the NH and NOH stretching for the actinomycetes pigment. In vitro cytotoxic assay on Human Cervical Adenocarcinoma (HeLa) cell line. The actinomycetes pigment prodigiosin, tripyrrole ring, was found to exhibit antiproliferative property against the (HeLa cell line. The pigment was found to be effective against the HeLa cell line. The IC_{50} value of the pigment was 62.50 µg/ml against HeLa cell lines respectively. From the results obtained it has been concluded that the pigment isolated from Streptomycetes could be used an antiproliferative agent. However it cannot be used as an antibacterial or antifungal agent.

Keywords: Antibacterial activity, Actinomycetes, Pigments, Antiproliferative

INTRODUCTION

Cancer (Medical term: Malignant neoplasm) is a class of disease in which a group of cells display uncontrolled proliferation, intrusion on and destruction of adjacent tissues (Invasion) and sometimes (Metastasis) spread to other locations in the body via lymph or blood). These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, and do not invade or metastasis. Most cancers form a tumor but some, like leukemia, do not. Cancer may affect people at all ages, even foetuses, but the risk for most varieties increases with age. Cancer causes about 25% of all human deaths and a major public health problem in many parts of the world [1].

Actinomycetes are Gram-positive filamentous bacteria which possess many important and interesting features. Actinomycetes having the high G+C content (>55%) in their DNA. The name Actinomycetes was derived from the Greek word Aktis (a ray) and Mykes (fungus). They are producing more than 70% of all currently known antibiotics. Among Actinomycetes, *Streptomyces* produce over two-thirds of the clinically useful antibiotics of natural origin found predominantly in soil and decaying vegetation. Some species of actinomycetes are known to produce spores and some do not. Actinomycetes are widely distributed in terrestrial environments. Actinomycetes produce branching mycelium which may be of two kinds *viz*, substrate mycelium and aerial mycelium [2]. Pigments are compounds with characteristics of importance to many industries. In the food industry they are used as additives, color intensifiers, antioxidants, etc. Pigments come in a wide variety of colors, some of which are water- soluble. Microorganisms are associated with all the foods that we eat and are responsible for the formation of certain food products by the process of fermentation and can also be used as a source of food in the form of single cell proteins and food supplements in the form of pigments, amino acids, vitamins, organic acids, and enzymes. In this way the pigments from microbial sources are a good alternative. Microorganisms are known to produce a variety of pigments; therefore they are promising source of food colorants [3]. Actinomycetes are found to produce pigmented colonies during their normal growth. Many genera of actinomycetes produce pigments like red, orange, yellow, violet, brown etc. A family of natural red pigments called prodigiosins is synthesized from different bacteria including Actinomycetes, *Streptomyces* and *Serratia marcescens;* it has more therapeutic values [4].

Pigment production in these bacteria is influenced by factors such as composition of the media, pH, extent of oxidation and temperature among others. For example, aerobic conditions and low temperatures boost pigment production. Pigmentation in actinomycetes is linked to respiratory mechanisms, defense mechanisms and UV protection [5]. Background HeLa cells are the most widely used cancer cell lines in the world. These cells were taken from a lady called Henrietta Lacks from her cancerous cervical tumor in 1951 which today is known as the HeLa cells. These were the very first cell lines to survive outside the human body and grow. Both GFP and blasticidin-resistant genes are introduced into parental HeLa cells using lentivirus [6].

In the past 50 years, different methods have been developed for selecting anticancer compounds including human tumor stem cell assay, human tumor *in vitro* cell line screening, hollow fiber assay and human tumor xenografts. The microbial pigment prodigiosin using in the anticancer activity. The prodigiosin induces the apoptosis in cells derived from other human cancers. The Prodigiosin cellular effects include oxidative DNA damage, induction of mitochondria – mediated apoptosis, inhibition of phosphatases and disruption of the pH gradient in various cellular compartments and DNA intercalation cell cycle arrest in late G1 and caspase activation [7]. To produce microbial pigment from actinomycetes and evaluate its anticancer property.

MATERIALS AND METHODS

Cleaning of glassware

The glassware used in this investigation were first soaked in chromic acid cleaning solution (10% potassium dichromate solution in 25% sulphuric acid) for 3 h and then washed thoroughly in tap water further it was soaked in commercial detergent for half an hour and washed thoroughly in tap water. Finally rinsed in distilled water and dried in the hot air oven.

Sterilization

Media and glassware were sterilized in autoclave at 121°C for 20 min. All sugars and extract were sterilized by using millipore membrane filter.

Sample collection and processing

Soil samples were collected from less river sediments and well soil in Guindy, Chennai, India. The samples were collected in sterile containers and transported to laboratory under controlled conditions. Serial dilution up to 10-8 dilutions was done for all the soil samples and 100 µl from each dilution was spread plated on to starch casein agar. The plates were incubated at 37°C for 3-7 days. All the plates were morphologically observed for the actinomycetes colonies. Suspected actinomycetes colonies based on the morphology and colors of pigmentation including diffusible pigments were purified and stored in starch casein agar plates for further studies.

Isolation of pigment from actinomycetes

The natural pigment producing actinomycetes colonies obtained in the present study were inoculated in 250 ml of starch casein broth and incubated at 37°C, in an orbital shaker with 500 rpm for about 5-7 days to obtain the extracellular pigments. *Streptomyces* sp., grown in broth culture was centrifuged at 10,000 rpm for 15 min and cell pellet was extracted with methanol and the extraction was centrifuged at 10 000 rpm for 15 min and the pink pellet was recovered and the supernatant was discarded.

Identification of actinomycetes

The presumptive actinomycetes colonies were then subjected to Gram staining and a series of biochemical tests such as Indole, Methyl Red-Voges- Proskauer (MR-VP), Citrate, Urease, Coagulase and Catalase tests.

Preparation of inoculum

In the present study starch casein agar was used for isolation of actinomycetes from soil samples. These slants were inoculated from the stock culture and incubated at 28°C for 7-10 days for maximum sporulation. Spore suspension was prepared by transferring a few loopful of spores from these slants into sterile distilled water and shaken thoroughly. Fresh spore suspension was prepared for each test. For gelatin liquefaction, starch hydrolysis and casein hydrolysis, a loopful of spores taken from the stock culture was used for inoculation. For all other tests spore suspensions prepared as above, were used employing equal volumes of the suspension in each case.

Methods of identification

The isolated colonies were confirmed using staining methods and biochemical tests and compared with standard strain of Actinomycetes Microbial Type Culture Collection (MTCC) Number.

Microscopy

Typical colonies from MSA plates were identified and performed according to Gram [8] and the results were recorded.

Biochemical tests

The biochemical tests as Indole test [9], Methyl red test (MR) [10], Voges-Proskauer (VP) test [10], Urease test [11], Nitrate reduction test [11], Catalase production test [12] and Citrate utilization test [13] were used to identify the Actinomyces.

Results

In Simmon's citrate agar slants which were inoculated with the isolate, it was observed that there is no change in the color of the medium. It was confirmed that the isolate was a citrate-negative where the medium remains green.

Antibacterial activity assay and Antifungal activity assay

FTIR analysis

The FTIR spectra of sample were recorded in order to characterize the presence of functional groups in isolated bioactive [14]. Two mg of the isolated pure compound was added in 200 mg of potassium bromide (KBr-FTIR grade) and prepared as dry pellet. All measurements were carried out in the range of 400-4000 cm^{-1} at resolution of 4.0 cm^{-1}.

Evaluation of anticancer activity of pigment MTT assay

***In vitro* assay for anticancer activity (MTT assay)**

Cells (1×10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition [15]. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µl/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% Cell Viability = A570 of treated cells/A570 of control cells × 100

Graphs are plotted using the % of cell viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Biochemical characterization of actinomycetes

Table 1 represents morphological and biochemical tests for Actinomycetes. It has been observed that the organism is non-motile, rod and Gram-positive. Figure 1 represents the biochemical tests for actinomycetes and organism was found to be positive for nitrate reduction test and indole test and negative for MR-VP, citrate, urease and catalase tests. The actinomycetes isolates was observed using coverslip method. The isolate was gram-positive, filamentous and short spiral filaments break into coccoids. Result indicated that they belong to genus *Streptomyces.* The actinomycetes isolate found to degrade the citrate and gelatin. The indole test showed the positive result [16].

Streptomyces rochei and *Salmonella subtilis* were isolated on starch casein agar medium is Gram-positive, very long, rod shaped, produces yellow pigments and possessing an earthy odour characteristic of Actinomycetes. The biochemical tests like nitrate reduction test was positive, but Voges-Proskauer test, gas production from glucose, casein hydrolysis, starch hydrolysis, H_2S production were negative [17]. The microscopic studies and staining properties of selected *Streptomyces* sp., showed the Gram-positive. The biochemical test result showed the positive results for nitrate reduction and there was negative response in Voges-Proskauer test [18].

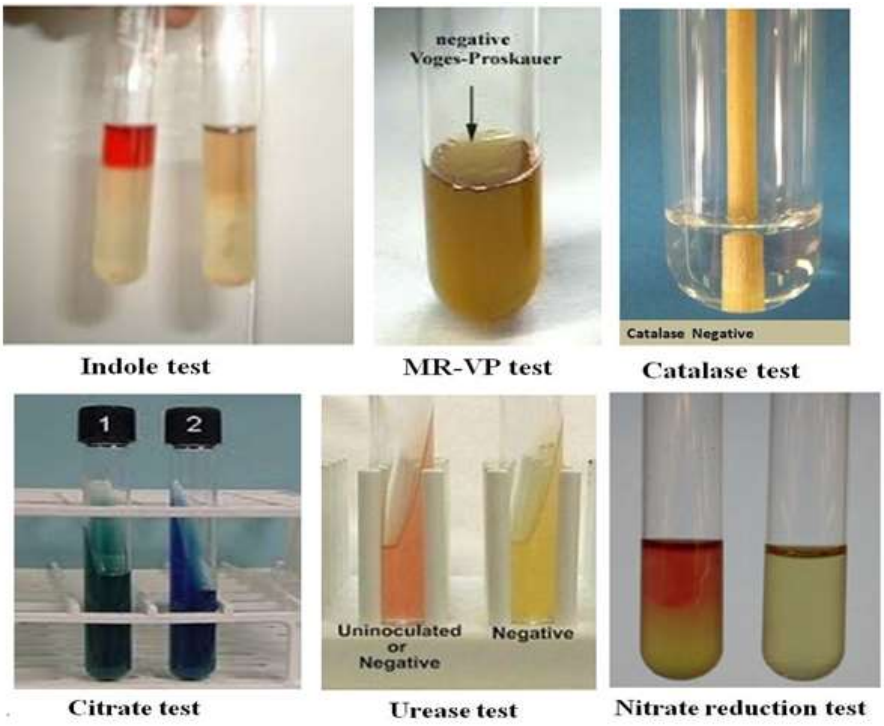

Figure 1: Biochemical tests

Table 1: Morphological and biochemical characterization of actinomycetes

Characteristics	Results
Motility	Non –motile
Gram's reaction	
Shape	Rod
Organism	Gram's positive
Biochemical test	
Nitrate reduction test	Positive
Indole test	Positive
MR-VP test	Negative
Citrate utilization test	Negative
Urease test	Negative
Catalase reduction test	Negative

Antibacterial activity of Actinomycetes pigment

Table 2 shows antibacterial activity of actinomycetes pigment against human pathogenic bacteria. Maximum zone of inhibition was found with *E. coli* followed by *B. subtilis, S. typhi,* whereas *P. aeruginosa* was found to be resistant with the pigment. Figure 2, the represents the zone of inhibition of actinomycetes pigment and it was less active against *E. coli* (8 mm), *P. aeruginosa* (6 mm), *B. subtilis* (6 mm), *S. aureus* (7 mm) and *S. subtilis* (7 mm). The actinomycetes exhibited antagonistic effect against the tested bacterial pathogens.

An antimicrobial substance produced by *Streptomyces* sp., was partially purified and studied for its antibacterial activity against several species several species. *E. coli* was found to be highly susceptible than the other microbial pathogens. Whereas remaining species showed negative result for it. Results of antimicrobial activity of the pigment is in accordance with the previous studies [19]. The active isolates when subjected to submerged culture showed different activity from that of primary screening in agar medium. The 80% of the actinomycetes isolates were found to exhibit antibacterial activity while there 20% isolates did not exhibit activity in broth culture. The antibacterial activity of *Streptomycetes* strains against *E. coli* (2). The 30% of the Actinomycetes strain had lost their inhibition potential perhaps due to the inconvenient liquid growth medium. The preliminary antibacterial assay of the isolates by perpendicular streak method exhibited that *Streptomyces* isolate showed the no inhibition on *B. subtilis, S. aureus, S. subtilis*. The isolate showed the less inhibition on the *E. coli* [20].

Out of 55 actinomycetes subjected for primary screening process, only 17 isolates showed the activity against test organisms. Of the 17 isolates, 05 were active against only Gram-negative organism, 06 against gram positive organisms and 09 against both Gram-positive and Gram-negative organisms. Among them, 15 of the isolates were active against *B. subtilis,* 05 against *Pseudomonas* sp., 07 isolates against *Enterobacter aerogenes,* 09 against *E. coli,* 03 against *Klebsiella* sp., 06 against *Proteus* sp., 08 against *S. typhi,* 03 against *Shigella* sp., and 11 against *S. aureus* [21].

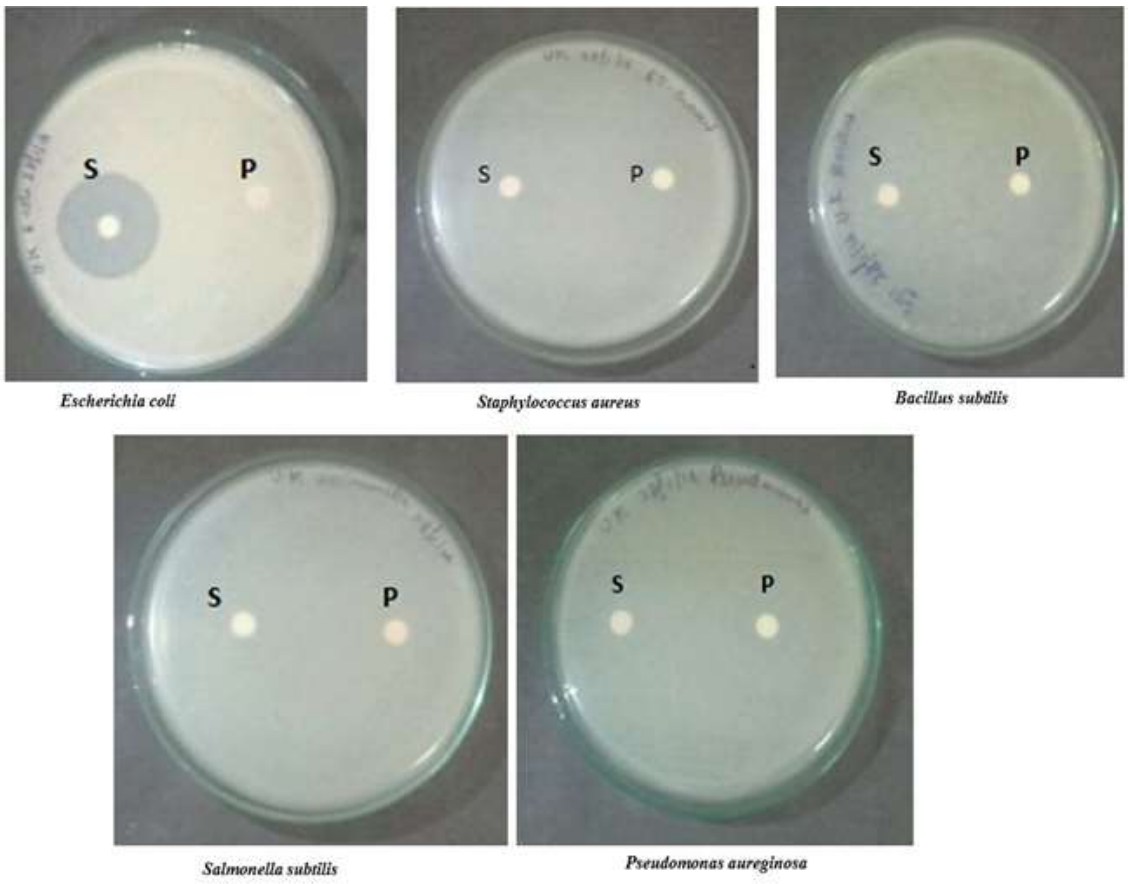

Figure 2: Antibacterial activity of actinomycetes pigment against pathogenic bacteria
S- Standard (Ampicillin); P-Pigment (Actinomycetes)

Table 2: Antibacterial activity of actinomycetes pigment

Microorganisms	Zone of inhibition (mm)	
	Standard (20 µg)	Sample (20 µg)
Escherichia coli	12	8
Pseudomonas aeruginosa	7	6
Staphylococcus aureus	7	6
Bacillus subtilis	8	7
Salmonella typhi	8	7

Antifungal activity of Actinomycetes pigment

As shown in Table 3, the cultures filtrate of actinomycetes pigments were tested against four pathogenic fungi. The actinomycetes pigments failed to inhibit the pathogenic fungi. There is no significant antifungal activity against the all tested pathogenic fungi. From the plate 3, showed the minimum level of inhibition zone on the four pathogenic fungi was observed. The *C. albicans* (7 mm), *A. niger* (6 mm), *T. viride* (6 mm), *R.*

microporous (6 mm) showed the negative result it.

The antifungal activity of pigment of *Streptomyces olivaceiscleroticus* showed maximum inhibitory activity against *Candida albicans*, *A. niger*. The minimum inhibitory activity was observed with *R. microporous* [22]. Totally 312 isolates only 69 showed the antifungal activity evaluated the antifungal *Actinobacteria* from atlas mountain soil, Sabarta sand, dung slage, river, sea water and sediments. Actinobacteria was determined against two pathogenic fungi. Only one species was showed the maximum inhibition remaining species showed the negative result. The culture filtrates of Actinobacerial isolates were tested against two pathogenic fungi. The culture filtrate were failed to inhibit the pathogenic fungi. Only *Streptomyces griseoflavus*, *Streptomyces cyaneus*, *Streptomyces exfoliates*, *Streptomyces albus* was maximum level of inhibition zone towards the *A. niger*, where remaining species showed negative result for it (Figure 3) [19].

Table 3: Antifungal activity of actinomycetes pigment

Microorganism	Zone of inhibition (mm)	
	Standard (20 µl)	Sample (20 µl)
Candida albicans	6	7
Aspergillus niger	7	6
Trichoderma viride	8	6
Rhizopus microporous	7	6

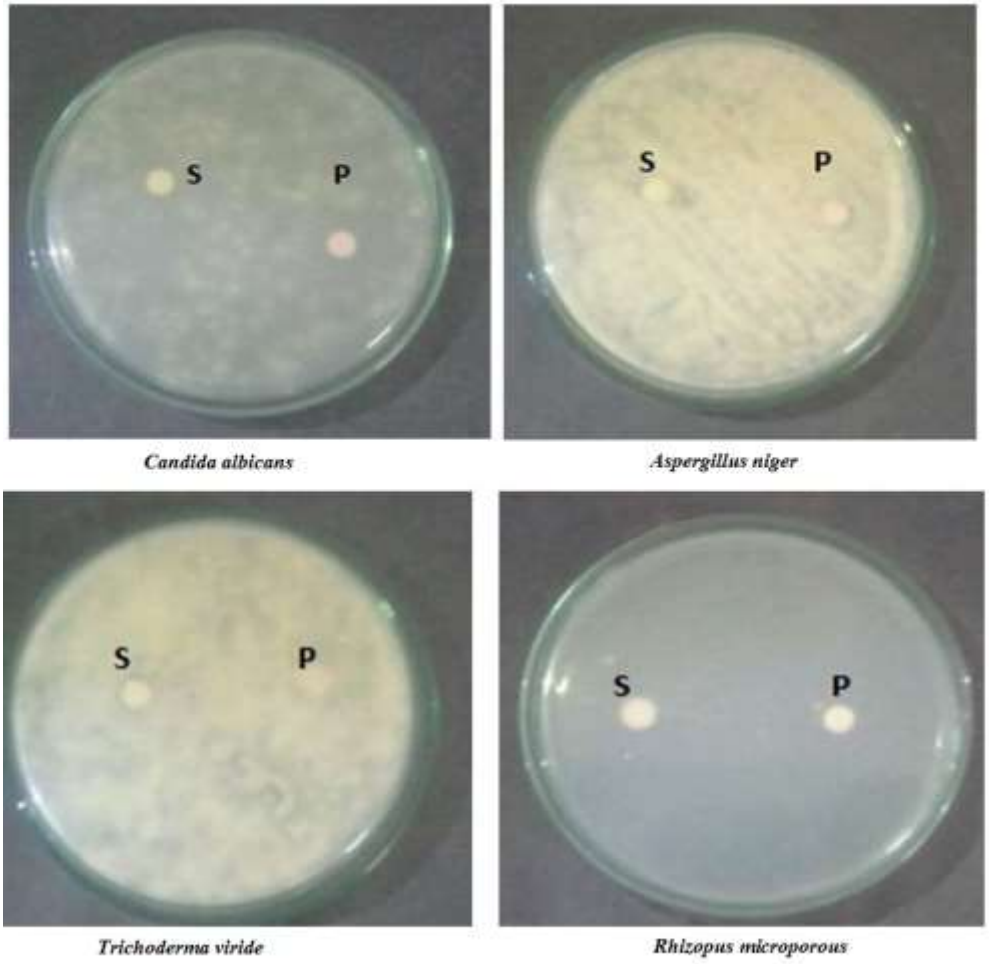

Figure 3: Antifungal activity of actinomycetes pigment

Antimicrobial activity of the *Streptomyces* sp., in soil. Antagonistic activity of *Streptomyces* was tested by diffusion plate method. It was grown on agar media of starch casein, yeast and malt extract, and oat meal and Sabouraud-dextrose agar. *Streptomyces* sp., showed no antimicrobial activity against *C. albicans* but it showed different activities against other test organisms. This is line with our findings [23].

Out of 37 isolates, only 21 showed antimicrobial activity against test microorganism in primary screening process by spot inoculation technique on agar medium. These 21 isolates were then subjected to submerge culture and there antimicrobial activity was evaluated. Out of these 21 isolates, 12 were found to be active against the test microorganisms like *Klebsiella pneumoniae*, *Micrococcus luteus*, *C. albicans* etc, [24]. The antimicrobial substances of Actinomycetes were extracted with ethyl acetate from isolate-inoculated Kuster's broth fermented for 7 days at 28°C by solvent extraction method. It has showed the significant MIC against dermatophytic fungi. However, our results showed no significant antifungal activity as compound with previous studies of antifungal activity with different species [25].

Anticancer cancer effect of actinomycetes pigment on HeLa cell line

Anticancer activity of the isolated actinomycetes pigment against HeLa cell line is represented in Figure 4. The pigment was found to be effective against the HeLa cell line. The IC_{50} value of the pigment was 62.50 µg/ml against HeLa cell lines. The actinomycetes pigment

prodigiosin had a potent apoptosis activity against HeLa cells. The assessment of the cell viability was based on the data from the MTT assay. MTT dye reduction assay was done to assess the antiproliferative activity. Decrease in proliferation of treated cells was observed when compared to the untreated controls. Actinomycetes pigment prodigiosin induced apoptosis in HeLa cell lines in a dose dependent manner.

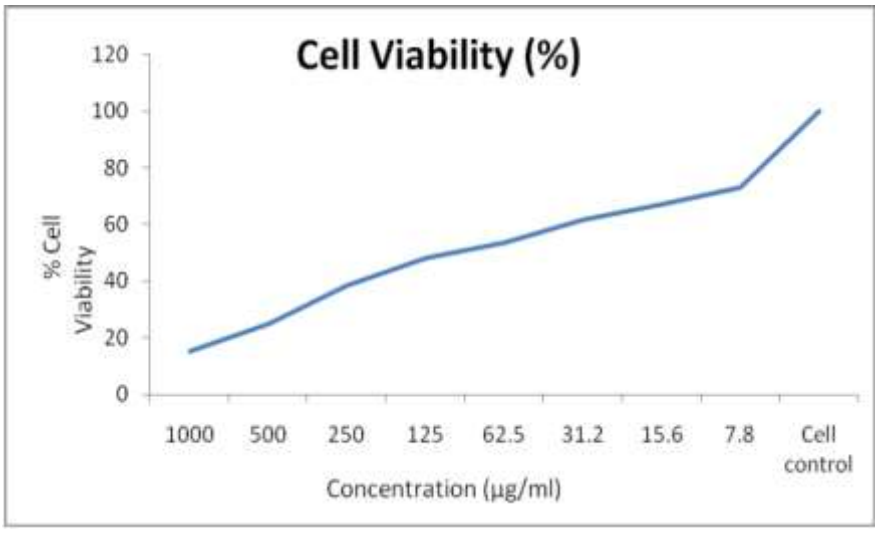

Figure 4: Anticancer effect of *Actinomycetes* on HeLa cell line graph

Figure 5 represents HeLa cell lines with the Actinomycetes extract at different concentration a) HeLa cells without pigment, b) HeLa cells with pigment at 500 µg/ml, c) HeLa cells with 62.50 µg/ml and d) HeLa cells with 7.8 µg/ml. It has been observed that the cells treated with less Actinomycetes showed lesser cell death whereas more cell death with more concentration of actinomycetes. A dose dependent response with actinomycetes was observed in HeLa cells. Table 4 is the representation of MTT assay on the HeLa cells.

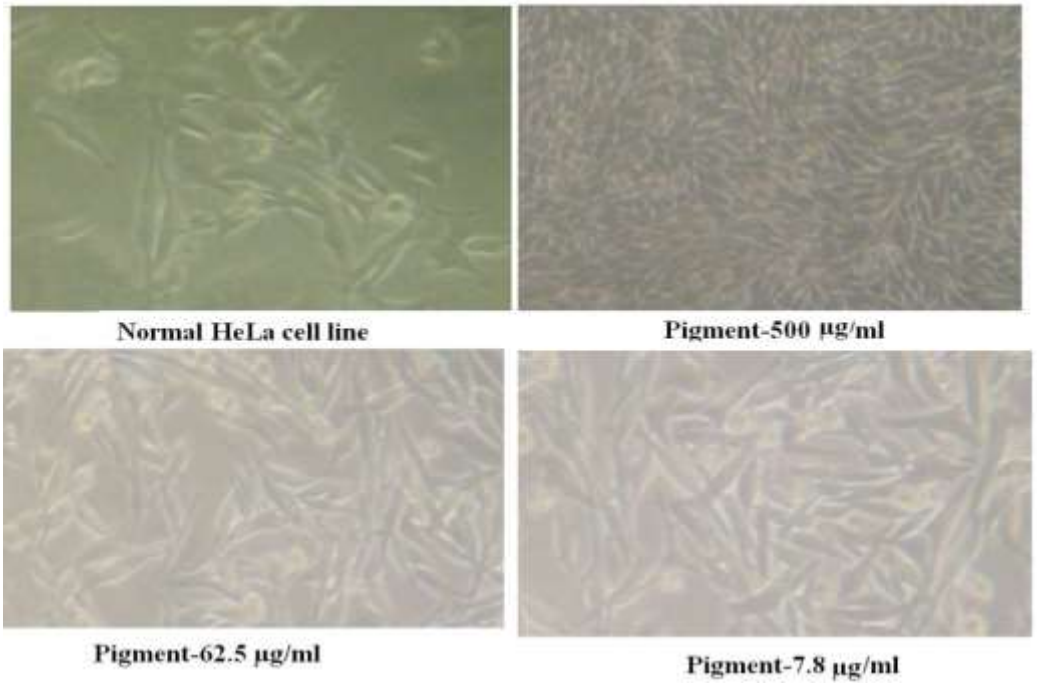

Figure 5: Anticancer effect of actinomycetes on HeLa cell line

Table 4: Anticancer effect of actinomycetes pigment on HeLa cell line

S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.08	15.38
2	500	1:1	0.13	25.00
3	250	1:2	0.20	38.46
4	125	1:4	0.25	48.07
5	62.5	1:8	0.28	53.84
6	31.2	1:16	0.32	61.53
7	15.6	1:32	0.35	67.30
8	7.8	1:64	0.38	73.07
9	Cell control	-	0.52	100

The Sporal Polyketide (GSP) pigment was isolated from *Streptomycetes* sp., and it used as the anticancer therapy. The pigment was purified by HPLC-PDA detector system. The GSP pigment tested for anticancer activity by MTT assay. The GSP showed potent cytotoxic activity against Hep2 and HeLa cell lines *in vitro* [26].

The *Serratia marcescens* produces the red pigment against the cancer activity. Prodigiosins is synthesized from different bacteria including Actinomycetes, *Streptomyces* and *S. marcescens.* Prodigiosin also has a therapeutic use as potential anticancer drug. Prodigiosin induces apoptosis in various human hematopoietic cancer cell lines [27]. Our results of antiproliferative activity on HeLa cells are in accordance with [28,26]. The strain Sm6, showing anticancer activity was with an IC_{50} value of 5. 5 µg ml^{-1} and a sequence similarity of 99. 87% to *Bacillus.*

Although this type of bacterium can be found in almost any substrate, it can be suggested that because of its association with *Sargassum muticum*, it seems to have acquired the ability to synthesize a compound able to inhibit colon cancer cells (HCT-116). *Sargassum muticum* has been found to show low antibacterial activity against some species of marine algae [29]. Isolation of prodigiosin from tannery solid waste fleshing and evaluation of its pharmacological effects. Optimization of fermentation conditions required for the maximum prodigiosin concentration were achieved at time 30 h, temperature 30°C, pH 8 and 3% substrate concentration. Cancerous cells are more susceptible to the prodigiosin at lower concentration [30].

FTIR analysis for Actinomycetes pigment

Figure 6 appearance of absorbance peaks at 3947.49 cm^{-1}, 3936.27 cm^{-1} which has been assigned to NH group stretching. Similarly 3417-2853 cm^{-1} has been attributed to oxime group (=NOH) which appears as wide and broad peak in the spectrum (Table 5). Appearance of absorbance peaks at showed the absorbance at 1484.29 cm^{-1}, 1325.15 cm^{-1} which has been assigned to ketenes group (C=C=O) bond stretching, (N-H) 2¡-amide group. The absorbance at 1109.12 cm^{-1}, 497.66 cm^{-1}, 441.72 cm^{-1} which refer to (C-C-C) Ketones group, disulfide group(S-S) bond stretching.

The FTIR spectrum of antimicrobial substances produced from *Streptomyces* KGL-13 isolate was showed absorbance at 3529-3468 cm^{-1} which refer to amino group, furthermore, there is sharp bond at 1687 cm^{-1} which attributed to C=N bond [21]. Actinomycetes sp., certain remarkable chemical changes were evident. The absorption band observed at 3144 cm^{-1}, 2840 cm^{-1}, 2617 cm^{-1}, 1456 cm^{-1}, 1304 cm^{-1} and 940 cm^{-1}, reflect C-H bond stretching, 2582 cm^{-1} to O-H bond stretching and 2256 cm^{-1} and 2173 to C=C bond stretching [31].

Table 5: FTIR analysis for actinomycetes pigment

Range (1/cm)	Intensity	Group
3947.49	Strong	C=N Amino group
3926.27	Strong	C=N Amino group
3417.04	Strong	=NOH oxime
2853.81	Strong	CH_2=CH_2 (Alkanes)
2321.43	Medium	C≡N (nitriles)
2146.86	Medium	C=C=O (ketenes)
1634.74	Medium	C=C (Arenes)
1541.19	Medium	N-H (2¡-amide)
1484.29	Medium	CH_2 & CH_3 (Alkanes)
1450.53	Strong	S=O (Sulfate)
1444.75	Strong	S=O (Sulfate)
1416.78	Strong	α-CH_2 (aldehyde)
1325.15	Strong	S=O (Sulfones)
1109.12	Strong	C-C-C (Ketones)
1019.42	Weak	P-H (phosphine)
497.66	Weak	S-S (disulfide)
441.72	Weak	S-S (disulfide)

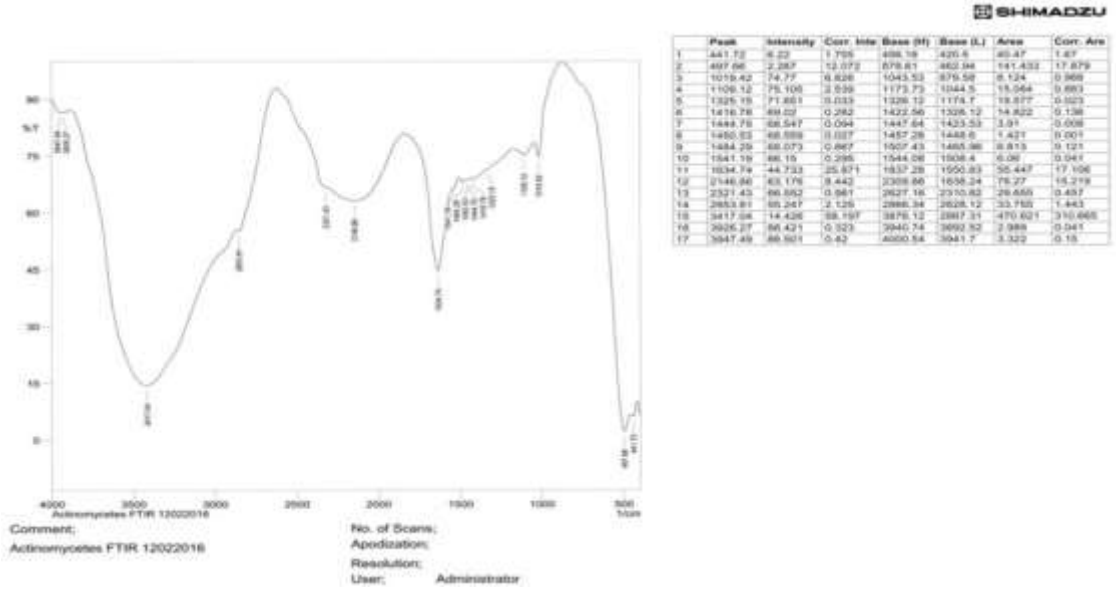

Figure 6: FTIR analysis for actinomycetes pigment

CONCLUSION

The antibacterial activity of the microbial pigment of the actinomycetes were carried out against the bacteria such as *S. aureus, P. aeruginosa, B. subtilis, E. coli, S. typhi.* The *E. coli* (8 mm) more susceptible than other microbial pathogens. Whereas remaining species showed negative result. The antifungal activity of the microbial pigment of the actinomycetes were carried out against the four different fungi such as *C. albicans, A. niger, T. viride, R. microporous.* The actinomycetes pigment were failed to inhibit the pathogenic fungi. There is no antifungal activity against the all tested pathogenic fungi. FTIR spectrum revealed the NH and NOH stretching for the actinomycetes pigment. *In vitro* cytotoxic assay on HeLa cell line. The actinomycetes pigment prodigiosin, tripyrrole ring, was found to exhibit antiproliferative property against the human cervical adenocarcinoma (HeLa) cell line. The pigment was found to be effective against the HeLa cell line. The IC_{50} value of the pigment was 62.50 µg/ml against HeLa cell lines respectively. From the results obtained it has been concluded that the pigment isolated from *Streptomycetes* could be used an antiproliferative agent. However it cannot be used as an antibacterial or antifungal agent.

REFERENCES

[1] WHO, Cervical cancer screening in developing countries, Report of the World Health Organization consultation Consulted, **2003**.
[2] J. Berdy, *J. Antibiot.*, **2005**, 58, 1-26.
[3] A.A. Aberoumand, *World J. Dairy Food Sci.*, **2011**, 6(1), 71-78.
[4] V. Karuppiah, Ecology, Diversity and Bioactivity of Marine *Actinobacteria* of the Gulf of Mannar Biosphere reserve, Ph.D. Thesis, Annamalai University, India, **2011**.
[5] N. Rajendra Prasad, A. Karthikeyan, S. Karthikeyan, B. Venkata, B. Reddy, *Mol. Cell. Biochem.*, **2011**, 349, 11-19.
[6] E.W. Knight-Jones, *Marine Biological Association of the United Kingdom*, **1951**, 30(2), 201-222.
[7] B. Montaner, R. Prez-Tom, *Current Cancer Drug Targets.*, **2003**, 3(9), 57-65.
[8] C. Gram, *J. Orofacial Ortho.*, **1884**, 2, 185-189.
[9] A. Sanchez-Marroquin, M. Zapta, *Appl. Microbial.*, **1954**, 4(4), 240-248.
[10] M. Zapta, A. Sanchez-Marroquin, *Appl. Microbiol.*, **1954**, 4(4), 240-248.
[11] F. Mavituna, M. Elibol, *Process Biochem.*, **1998**, 33, 307-311.
[12] E. Kuster, *J. Bacteriol*., **1959**, 78(4), 528-538.
[13] S.A. Waksman, E. Kuster, *J. Bacteriol*., **1959**, 78(4), 528-538.
[14] G.F. Gauze, L.P. Terekhova, T.S. Maksimova, M.G. Brazhnikova, G.B. Fedorova. *Antibiot. J.*, **1983**, 28(4), 243-245.
[15] D.A. Scudiero, R.H. Shoemaker, K.D. Paul, *Cancer Res.*, **1988**, 48, 4827-4833.
[16] H. Tsujibo, T. Kubota, *Appl. Environ. Microbiol*., **2003**, 69(2), 894-900.
[17] T.J. Mincer, P.R. Jensen, P.G. Williams, W. Fenical, Antonie van Leeuwenhoek, **2002**, 87, 43-48.
[18] T. Antonieta, M.J. Rodriguez, *J. Microbiol. Res*., **2006**, 161, 222-231.
[19] S.K. Augustine, S.P. Bhavsar, B.P. Kapadnis, *Indian J. Med. Res.*, **2005**, 121, 164-170.
[20] A. Kavitha, *Z. Naturforsch*., **2010**, 65, 528-531.
[21] M. Goodfellow, L.A. Maldonado, W. Fenical, P.R. Jensen, C.A. Kauffman, T.J. Mincer, *Int. J. Sys. Evol. Microbiol.*, **2002**, 55, 1759-1766.
[22] M.K. Sharma, A. Alexander, *Int. J. Pharm. Sci. Res.*, **2011**, 2(6), 1569-1571.
[23] K. Ochi, Y. Okamoto-Hosaya, S. Okamoto, *J. Appl. Environ. Microbial*., **2003**, 69, 4256-4259.
[24] L.S. Singh, I. Baruah, T.C. Bora, *Biotechnol.*, **2006**, 5, 217-221.
[25] T.P. Yongchun Youjiu, H. Yi, *Plant Protection.*, **2011**, 37(1), 120-123.
[26] R. Venkatesan, E. Ji, S.Y. Kim, *BioMed. Res. Int.*, **2015**, 814068, 22.
[27] P.D.S. Melo, *Toxicol. Lett.*, **2000**, 16, 237-242.
[28] J. Melo, P. Adragao, J. Neves, M. Ferreira, A. Timóteo, T. Santiago, R. Ribeiras, M. Canada, *Eur. J. Cardiothor. Surg.*, **2000**, 18(2), 182-186.
[29] D.M. Shin, R. Liu, I. Klich, J. Ratajczak, M. Kucia, M.Z. Ratajczak, *Leukemia*., **2010**, 24(8), 1450-1461.
[30] D.N. Herrmann, R. Horvath, J.E. Sowden, M. Gonzalez, A. Sanchez-Mejias, Z. Guan, R.G. Whittaker, J.L. Almodovar, M. Lane, B. Bansagi, A. Pyle, V. Boczonadi, H. Lochmüller, H. Griffin, P.F. Chinnery, T.E. Lloyd, J.T. Littleton, S. Zuchner, *Ame. J. Human Genetics*., **2014**, 95(4), 472.
[31] S. Umamaheswari, M. Murali, *Elixir Chem. Eng*., **2013**, 64, 19159-19164.